This year marks a milestone as the Department of Orthopaedics at Case Western Reserve School of Medicine and University Hospitals Case Medical Center celebrates our 100th anniversary. Today, a century since we opened our doors, we continue to provide a tradition of innovation, excellence in practice, and quality care for our patients. This is reflected in our leadership in NIH funding and our ranking among the nation’s leading centers for orthopaedic care according to US News & World Report. For more information, visit www.UHhospitals.org.
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References:
1. Stryker test data RD-03-041 and RD-04-027

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We are excited to bring you the fourth annual edition of the *Case Orthopaedic Journal*. With each successive issue, we strive to make improvements to make this one of the preeminent orthopaedic department journals. This has been another great year for the Department of Orthopaedics in Case Western Reserve University. In this journal, we will attempt to encapsulate the tremendous accomplishments of the faculty and residents from the past year. As in past years, we have had the honor and privilege of hosting world renowned visiting professors who have further enriched the resident experience at Case.

This issue is dedicated to Dr. Clyde L. Nash, professor emeritus in the Department of Orthopaedics at MetroHealth Medical Center. Although he has retired as Chairman of the Department, he remains a passionate educator and philanthropist. Most of the current orthopaedic residents know him as the tireless professor sharing his wisdom and knowledge at our grand rounds and spending countless hours developing the Orthopaedic Learning Center. As residents, we have been very fortunate to benefit from his unremitting commitment to orthopaedic resident education.

It has been an extraordinary privilege working on this journal. I would like to thank the editorial staff and numerous other contributors for dedicating their valuable time to the production of this journal. The Department of Orthopaedics at Case Western Reserve University continues to strive to maintain the highest standards in clinical excellence and innovative research. Hopefully, this journal reflects the immense pride we have in our program. Finally, I would like to thank the faculty for their mentorship in inspiring and preparing me for a career in academic orthopaedics.

Jerry Huang, M.D.
100 YEARS OF ORTHOPAEDIC SURGERY AT UNIVERSITY HOSPITALS CASE MEDICAL CENTER AND CASE WESTERN RESERVE UNIVERSITY SCHOOL OF MEDICINE

This year marks the 100<sup>th</sup> anniversary of orthopaedic surgery at both University Hospitals Case Medical Center and Case Western Reserve University School of Medicine. Our department is one of the oldest orthopaedic practices in the United States. Even the American Academy of Orthopaedic Surgeons was not created until 1933, followed one year later by the founding of the American Board of Orthopaedic Surgery in 1934.

1907 to 1910

The Orthopaedic Service at Lakeside Hospital began in 1907 when Dr. Dudley P. Allen, the Surgeon-in-Chief at the hospital, assumed the care and supervision of orthopaedic surgical cases. Dr. Allen had received his medical training at Harvard Medical School and the Massachusetts General Hospital, where he studied under Dr. Henry J. Bigelow, who authored the first American book on orthopaedic surgery. Dr. Allen had received further training in Europe with Dr. Von Lagenbeck in Germany and Dr. William MacEwen in Scotland. The first in our Orthopaedic Service, Dr. Allen was described as a surgeon with “natural surgical skill” who had studied with some of the most prominent early orthopaedic surgeons in the world.\(^1\)

1910 to 1911

Dr. Allen remained in charge of Orthopaedics at Lakeside Hospital until 1910, when Dr. Henry Becker was appointed Surgeon-in-Charge of the Clinic for Fractures and Dislocations. Working in Dr. Becker’s Orthopaedic Division were Dr. Henry O. Feiss (Assistant Surgeon-in-Charge of Orthopaedic Surgery) and Dr. Gordon N. Morrill (Assistant, Orthopaedic Surgery). Dr. Feiss had received his medical education at Harvard Medical School and had studied under Joel Goldthwait, one of the early orthopaedic surgeons in Boston. Dr. Feiss joined the staff of Lakeside Hospital in 1904 and was active in both basic and clinical research, publishing 48 articles in orthopaedic surgery. His basic science research included work on nerve regeneration. During his career, he studied in Madrid and at the Pasteur Institute in Paris, as well as at the University of Edinburgh, where he received a Doctor of Science Degree. During World War I, Dr. Feiss served as a medical officer in the United States Army in France. His family and friends, following his death, established the first endowment fund in the Orthopaedic Department at Case Western Reserve.

1911 to 1920

In 1911, Gordon N. Morrill, who had worked with Dr. Feiss in Dr. Becker’s Orthopaedic Division, was appointed Director of Orthopaedic Surgery at Western Reserve School of Medicine and Lakeside Hospital. In 1919, during Dr. Morrill’s tenure, Dr. Clarence Heyman returned from service in World War I in the armed forces and became actively involved in the crippled children’s program in Northeast Ohio. Dr. Heyman had trained with Dr. Royal Whitman in New York City and was one of the first orthopaedic surgeons in our country to specialize in pediatric orthopaedics. He was a founder of the Ohio Society for Crippled Children, an organization eventually responsible for establishing the International Society for Crippled Children. He also founded the Crippled Children’s outlying clinics throughout Northeast Ohio, many of which are still active to this day. Dr. Heyman became the first Clinical
Professor of Orthopaedic Surgery at Western Reserve School of Medicine. He was an attending surgeon at Rainbow Hospital and later became President of the American Board of Orthopaedic Surgery (1952-1953).

1920 to 1924
George I. Bauman, M.D., a graduate of Western Reserve Medical School, became Director of Orthopaedic Surgery at Lakeside Hospital and Western Reserve University in 1920. Dr. Bauman had trained in orthopaedic surgery in both England and Europe, published multiple papers on orthopaedic subjects and served as an editor of *The Journal of Bone and Joint Surgery*. While Dr. Bauman was Director of Orthopaedics, Dr. Louis Sterin became the first orthopaedic resident in 1922, to be followed by a long line of outstanding orthopaedic surgeons who received their training here at Case.

1924 to 1953
In 1924, Dr. Maxwell Harbin, who had trained under Dr. Harvey Cushing at the Peter Bent Brigham Hospital in Boston, was recruited to become Chief of Orthopaedic Surgery at Lakeside Hospital, Rainbow Hospital and Western Reserve University School of Medicine. In 1926, Lakeside Hospital, Babies and Children’s Hospital and the maternity hospital formed an amalgamation and were incorporated as University Hospitals of Cleveland. Lakeside Hospital moved to its current University Circle location, and Rainbow Hospital moved to its new campus on South Green Road in South Euclid. The orthopaedic staff consisted of Dr. Harbin, Dr. Clarence Heyman, Dr. John Murphy, Dr. Glen Barber and Dr. Wilbert McGaw.

In 1947, Dr. Charles H. Herndon was recruited to join the orthopaedic staff of University Hospitals. Dr. Herndon, a graduate of Harvard Medical School, had completed his internship in Cleveland at University Hospitals and then volunteered to join the American Hospital in Oxford, England. Following World War II, he completed his training at the Hospital for Special Surgery in New York.

1953 to 1982
In 1953, Dr. Charles Herndon became Chairman of Orthopaedic Surgery at University Hospitals and Western Reserve University School of Medicine. Under Dr. Herndon’s leadership, Orthopaedics at Case and University Hospitals flourished. He built a robust clinical and research program that attracted outstanding resident applicants from across the country. Dr. Herndon also recruited Drs. Victor Frankel and Albert Burstein, who in 1967 founded the Orthopaedics Biomechanics Laboratory, which became internationally recognized for its contributions to musculoskeletal care. In the early 1970s, Dr. Herndon went to England to study joint replacement surgery under John Charnley, FRCS, and became one of the first surgeons in the United States to perform hip replacement surgery—in specially constructed laminar flow operating rooms at University Hospitals. Dr. Herndon was elected President of the American Board of Orthopaedic Surgery (1964-1966) and President of the American Academy of Orthopaedic Surgeons (1967-1968). In 1961, Mr. and Mrs. George Humphrey established the Rainbow Professorship in Orthopaedics at the School of Medicine, and Dr. Herndon was appointed to this Chair.

In 1978, Orthopaedics became a separate department in the School of Medicine and quickly became one of pre-eminent orthopaedic departments in the United States. Under Dr. Herndon’s tenure, the full-time faculty increased from 3 to 17 orthopaedic surgeons, with an additional 9 basic scientists. The orthopaedic endowment funds increased 10-fold, and, upon Dr. Herndon’s retirement in 1982, Case Western Reserve University established the Charles H. Herndon Professorship in Orthopaedics.

1982 to 1988
On July 1, 1982, Dr. Kingsbury G. Heiple became the first holder of the Charles H. Herndon Professorship at Case Western Reserve University and Chairman
of the Department. Dr. Heiple had completed his orthopaedic residency at Case Western Reserve University and was recognized as an excellent technical surgeon and brilliant clinical scientist. Under his leadership, the department continued to grow its clinical and research divisions. During Dr. Heiple’s tenure, the department sub-specialized and increased its national recognition through its excellent faculty and residency program. Dr. Heiple pioneered many new and innovative orthopaedic operations and implants. These included procedures to correct congenital bone defects of the pelvis in children, artificial finger joints and intramedullary nails to improve the treatment of long-bone fractures. Dr. Heiple served as President of the American Board of Orthopaedic Surgery (1984-1985) and served as Chairman of the department until 1988.

1989 to 2002

In 1989, Dr. Victor M. Goldberg was appointed the Charles H. Herndon Professor and Chairman of the Department of Orthopaedics. He received his training at Case Western Reserve University, at the Hospital for Special Surgery and in England. Under Dr. Goldberg’s leadership, the department became internationally recognized in orthopaedic research and led the United States for many years in funding from the National Institutes of Health. His research has led to improvements in the design of both hip and knee implants that have had a direct impact on the care of patients with arthritis throughout the world. Dr. Goldberg is an internationally recognized expert in joint replacement surgery and was elected President of the Knee Society in 1996 and Chairman of the Orthopaedic Research and Education Foundation (2003-2005) and served as Chairman of the Department through 2002.

2003 to the Present

In 2003, Dr. Randall E. Marcus became the third holder of the Charles H. Herndon Professorship and the Chair of the Department of Orthopaedics, having trained at Case Western Reserve University, Oxford University in England, the University of Basle in Switzerland, and the University of Washington in Seattle. As the 9th surgeon to lead Orthopaedics at University Hospitals Case Medical Center and Case Western Reserve University, he has the privilege of leading an outstanding orthopaedic and basic science faculty and residency program. During the last 5 years, the department has recruited an additional 10 clinicians and 3 basic scientists and continues as a leading recipient of NIH funding among orthopaedic departments in the country. Additionally, the department has dramatically increased its endowment, and its number of endowed professorships has grown from 1 to 8. The department currently receives over 500 applications per year for its 6 residency positions. As in previous decades, the department has been honored nationally by the election of its Chairman to leadership positions (President of the American Board of Orthopaedic Surgery and President of the Association of Bone and Joint Surgeons).

In its 100th year, Orthopaedics at University Hospitals Case Medical Center has a continuing tradition of excellence in service to patients, innovative research and outstanding educational programs for residents, fellows and students.

REFERENCES
It was 1951 when the young Shaker Heights graduate began his journey at Amherst College where he majored in economics with the expectation of joining the family business upon completing his degree. While at Amherst, he played two varsity sports, captained the ice hockey team, was President of his fraternity and class, and was elected to the senior honorary society. Some 7 years later, after graduating from Amherst, marrying his wife of 52 years, and spending 2 years in the military in Heidelberg, Germany, he was found among Dr. Caughey’s “bent arrows” at Western Reserve Medical School, class of 1962.

In 1969, after 10 years of medical school (AOA), internship, and residency at University Hospitals, as well as one year as the Rainbow Fellow (the first ever and only) in scoliosis studying in Minnesota, California, and Helsinki, Finland, he began his orthopaedic practice with Drs. Charles Herndon, George Spencer, Kingsbury Heiple and Victor Frankel. In his early years, he practiced general orthopaedics with a specialty in scoliosis. In 1972 he founded the first scoliosis center at University Hospitals (The University Youth Spine Center) and instituted the statewide school screening program. His career focused on patient care, clinical research, and teaching. His academic appointments began as a Senior Instructor and culminated in his appointment as Professor of Orthopaedic Surgery. His research in scoliosis led him to active participation in the Scoliosis Research Society, an international organization, where he became President in 1982.

He returned to get his degree in medical education from Case Western Reserve University (CWRU) School of Medicine in 1975. He was Chair of the Musculoskeletal Committee and Director of Surgical Education at the School of Medicine. He also served for many years on the Faculty Senate, working with Bylaws, Promotions, Tenure, and the Committee on Medical School Affiliations. He served as President of the Alumni Association of CWRU School of Medicine, Chair of the Medical School Alumni Fund, and President of the Pasteur Society.

He and his colleague, Dr. Richard Brown, a bio-medical engineer, worked together in the operating room to design a spinal cord monitoring system that became an international model for spine surgeons. They traveled all over the world speaking and sharing their new technology. During those years, he was frequently a guest lecturer and held interviews on both radio and television. His presentations to national medical and scientific societies, national workshops, instructional courses, as well as published bibliographies, abstracts and book chapters number almost 200.

In 1982 Dr. Nash moved to St Luke’s Hospital where he became Director of the Department of Surgery, Director of Orthopaedic Surgery, and founder of the St Luke’s Spine Center. He worked with greats such as Fred Cross, Jimmy Jones, and Russ Trusso. During his years at St. Luke’s, a partnership with Metro General emerged under the leadership of Henry Manning. Dr. Nash subsequently straddled the river and ultimately joined the staff as Chairman of Orthopaedics and Interim Senior Vice President for Medical Affairs under Terry White, President and CEO. In that capacity he was Interim Associate Dean at CWRU School of Medicine.

During his medical years, he managed to rear a family of 3 children, all of whom married and then expanded the family to its current number of 16. Two of them graduated from CWRU School of Medicine in 1986. Integral to his professional life has been his secretary, Jackie Shafer, who has worked with him since the beginning of his career. She is known and loved by all of his patients. Dr. Nash’s by word has always been “call Jackie!” When not practicing medicine, he skied, played golf, raced a Tartan 10, followed his love of music with the Cleveland orchestra and Opera, pruned his shrubbery and gardened in his spare time. His extra-curricular life included involvement with all of his alma maters- CWRU School of Medicine,
Amherst College, and leadership roles as President of the Cleveland Opera, Leadership Cleveland 1982, Associate Editor of the Journal of Bone and Joint Surgery, Editor of SPINE and many memberships in honorary and professional societies, including being a Fellow in the American Academy of Orthopaedic Surgery and American College of Surgery.

Special Honors over the years include Physician of the Year, Visiting Nurse Association, Shaker Heights Alumni Hall of Fame, Clifford J. Vogt Alumni Service Award at CWRU School of Medicine. His Visiting Professorships included travel to Japan, Sweden, Finland, Switzerland, Toronto, Maine Medical Center, University of Vermont, and Quebec City. He was also chosen as the group leader to lead a delegation of physicians to China in 1984 and again as the leader of the Traveling Fellows in Europe in 1994.

Finally, in 1999, he retired from the surgery part of his practice. Because actually that is all he stopped doing!!!. Today he continues a very active, contributing life. In the years post retirement, he sees patients once a week, provides medical counsel to friends (at least once a day), edits medical articles for journals, teaches medical students at CWRU, does clinical research on bone density, chairs college and medical school reunions, is President of the Brittingham Library, serves on the Board of the Allen Memorial Library, has served on the MetroHealth Foundation Board, attends weekly spine conferences, has designed and spearheaded the Orthopaedic Learning Center at Metro Health, raises funds for Amherst College and chairs his 55th reunion, has served on the Board of Habitat for Humanity, He is currently a director on the Board of Apollo’s Fire and a local planning and zoning rep originally for Hunting Valley and now Gates Mills. He plays golf 3 times a week, serves as handyman and gardener, tends to his original wife and two family dogs by daily watering and feeding and when the muse shows up — he writes poetry. He makes most of us TIRED!!!

– Deborah Nash

Words from Dr. Nash’s Children

MY FATHER’S PATIENT

Having admired my father’s success as a top orthopedic surgeon focused on scoliosis, I have always wondered what it would be like to one of his patients. Knowing my father, his patients shared a common experience that included: 1) Getting up early as he was always committed to going to the hospital around 5am, including the weekends, 2) A sense of humor that had the ability to put one’s mind at ease while producing a smile in the most tense of situations, 3) A unique balance of emotional compassion for the patient’s needs blended with medical stoicism required for complex surgery. In the end, I envision that being a patient of my father was probably one of those life changing experiences that one does not easily forget and will always appreciate…and for that, being a patient of my father sounds very familiar.

– Doug, Kelly, and Liz
The year 2007 marks the 100th anniversary of Orthopaedics at Case Western Reserve University School of Medicine and University Hospitals Case Medical Center. Beginning with the first Chief of Orthopaedics, Dr. Dudley Allen in 1907, Orthopaedics at this medical center has been characterized by excellence in practice and quality care for our patients. In this year’s Case Orthopaedic Journal, an article on the history of Orthopaedics here at Case highlights many of the wonderful achievements in the clinical, research and educational realms of our department.

The Department of Orthopaedic Surgery here at Case Western Reserve University consists of our three medical centers, our Case Research Institute and, most importantly, our outstanding faculty, scientists, staff, residents and fellows. Our medical centers include:

- University Hospitals Case Medical Center, which includes Rainbow Babies & Children's Hospital,
- MetroHealth Medical Center, our level 1 trauma hospital, and
- Louis Stokes Veterans Administration Medical Center, here on our Case campus.

Our basic science laboratories are located:

- in the School of Medicine, with our Molecular Biology Division in the Biomedical Research Building,
- in the Case School of Engineering, in the Musculoskeletal Mechanics and Materials Laboratories, and
- at MetroHealth Medical Center and the Veterans Administration Medical Center, where our Functional Electrical Stimulation laboratories are located.

Additionally, our Anatomic Research Laboratory resides at the Cleveland Museum of Natural History, the site of the Hamann-Todd bone collection.

Departmental Achievements
The Department’s excellence in clinical activities was once again recognized by U.S. News & World Report. Our excellence in research was recognized by our continued high ranking (8th) as one of the top-funded orthopaedic departments in the United States by the National Institutes of Health (NIH). Our residency program received 518 applications for our six residency positions, and the Department once again matched six of our top selections. We welcome to the program: Dr. Michael Abdulian from Tulane University, Dr. Kasra Ahmadiania from the University of Michigan, Dr. Zachary Gordon from Case Western Reserve University School of Medicine, Dr. Ari Levine from the University of Cincinnati, Dr. Troy Mounts from the University of Tennessee and Dr. Erik Schnaser from the University of Nevada. In addition, we welcome our two spine fellows, Dr. Paul Gause from the University of Pittsburgh and Dr. Sheeraz Qureshi from Mt. Sinai Medical Center in New York. Our pediatric fellow for 2007-2008 is Dr. Hadeel Abaza from Wayne State University, and our MetroHealth Medical Center Trauma Fellow is Joshua Niemann, MD, who completed his orthopaedic residency at the University of Missouri-Kansas City. Our Adult Reconstructive Fellow is Dr. Aasis Unnanuntana from Mahidol University and Institute...
Sriraj in Bangkok, Thailand. Aasis’ father is an alumnus of this program and is the Emeritus Professor and Chairman of Orthopaedic Surgery at Mahidol University Medical School and the Institute Siriraj in Bangkok. Two Allen Research Fellowships were awarded this year to Dr. Ryan Garcia and Dr. Patrick Messerschmitt. This fellowship allows two of our residents to spend a year in one of our basic science research labs.

Congratulations to Faculty Members and Residents
On September 13, 2006, the Board of Trustees at Case Western Reserve University, upon the recommendation of the Dean of the School of Medicine and the President of University Hospitals Case Medical Center, appointed Matthew J. Kraay, MD, the inaugural holder of the Kingsbury G. Heiple and Fred A. Lennon Professorship in Orthopaedics. Matt Kraay is the Director of our Joint Replacement Center here at University Hospitals Case Medical Center and has earned this well-deserved honor for both the excellence in care he has provided to patients at our medical center and his contributions to the field of joint replacement in orthopaedic surgery.

Dr. Henry Bohlman was honored this year by the North American Spine Society (NASS). He received the Leon Wiltse Award for his great contributions to the art and science of spinal disorder management. University Hospitals Case Medical Center completed the funding for the Bohlman Professorship in Spine Surgery. This Professorship, funded by Dr. Bohlman’s former residents and fellows as well as many of his patients, honors his contributions to University Hospitals Case Medical Center in the field of modern spine surgery.

Dr. George H. Thompson, our Chief of Pediatric Orthopaedics and the Rainbow Babies & Children’s Foundation Chair for Excellence, is the President of the Scoliosis Research Society, an organization comprised of many of the world’s leading spine surgeons.

Clare Rimnac, PhD, Director of the Orthopaedic Musculoskeletal Mechanics and Materials Laboratories at Case Western Reserve University, was awarded the Wilbert J. Austin Professorship of Engineering Chair. This tremendous honor from Case Western Reserve University recognizes Dr. Rimnac’s many contributions to the fields of orthopaedic surgery and engineering. Her insights into the basic knowledge of musculoskeletal mechanics and materials have directly benefited our abilities to improve the care of patients with musculoskeletal problems.

In June, Dr. Joe Son-Hing joined our Department in the Division of Pediatric Orthopaedic Surgery. Before joining our faculty, Joe completed two fellowships: a pediatric orthopaedic fellowship here at Case Medical Center and a pediatric spine surgical fellowship at Washington University in St. Louis. Joe had received his BSc degree from McGill University, majoring in neurobiology, and his MD degree from the University of British Columbia. He completed his orthopaedic residency at the University of British Columbia, where he was awarded his Fellowship in the Royal College of Surgeons of Canada. Over the course of Dr. Son-Hing’s medical education, he received five scholarships for academic achievement, as well as the Parke-Davis Prize in Medicine, the Appleton and Lange Medical Publications Prize and the John S. Monteith Prize in Family Medicine. During his postgraduate medical training, Joe received the International Pediatric Orthopaedic Symposium Scholarship and the Edward Kampschuur Award from the Division of Orthopaedic Trauma at the University of British Columbia. Dr. Son-Hing has authored six scientific papers and has been invited to present his work on numerous occasions at local, regional and national meetings.

This spring, Eben Alsberg, PhD, joined our Department as a member of the Department of Biomedical Engineering at Case Western Reserve University. A postdoctoral fellow at Harvard Medical School before joining the faculty at Case, Dr. Alsberg received his BSE in Biomedical Engineering and MSE in Mechanical Engineering and Materials Science at Duke University. He then received a MSE in Biomedical Engineering and a MSE in Mechanical Engineering from the University of Michigan, followed by a PhD in Biomedical Engineering at the University of Michigan. His interests are in musculoskeletal cell biology and regeneration from an engineering perspective.

Further Department honors this year included Dr. Christopher McAndrew’s receiving the AO North America Dr. Kathryn Cramer Memorial Award for his research, “Timing and Method of Analgesia and Anesthesia for the Reduction of Pediatric Forearm Fractures. A Prospective Randomized Study of Three Treatment Protocols.” Drs. Clayton Dean, Josue Gabriel (Ohio State), Ezequiel Cassinelli, Michael Bolesla (University of Texas Southwestern) and Henry Bohlman received the Lewis A. Goldstein Award for the Best Clinical Paper presented by the Scoliosis Research Society at its recent meeting.

Dr. Reuben Gobezie was recognized by the European Society for Surgery of the Shoulder and Elbow (ESSSE), with
his coauthors, for his DVD presentation of “Suprascapular Nerve Arthroscopic Release” at this year’s meeting in Athens, Greece. A manuscript by Drs. Jason Eubanks, Michael Lee and Nicholas Ahn, “A Natural History of Lumbar Disc Degeneration and Facet Arthrosis: A Postmortem Specimen Study,” was recognized at this year’s North American Spine Society (NASS) meeting as one of the six best research papers of the year. Dr. Patrick Messerschmitt won first place in the 2007 Cleveland Orthopaedic Society Residents’ Essay Contest with his study, “Osteosarcoma: Decreasing Metastasis by Inhibiting Tyrosine Kinase Signaling.” This paper also won first place in the Ohio Orthopaedic Society’s Resident Research Competition.

Dr. Matthew Smith was awarded a position in the American Academy of Orthopaedic Surgeons/Orthopaedic Research and Education Foundation Clinical Scientist Development Program for 2007. Dr. Brian Victoroff, Director of our Shoulder Section, was reunited with the U.S. Short Track Speedskating Team this winter when their national competition was held in Cleveland, Ohio; he had served as team doctor for the 2005 World Cup Circuit traveling to China, Korea, the Netherlands and Italy.

Dr. Victoroff is an avid speedskating fan who also dabbles in the sport.

In April, the Department of Orthopaedics here at Case celebrated, jointly with the Department of Mechanical & Aerospace Engineering, the 40th anniversary of the Musculoskeletal Mechanics and Materials Laboratories at Case Western Reserve University. This celebration of 40 years of interdisciplinary research and discovery was highlighted by the return of Albert H. Burstein, PhD, to the laboratories he founded here at Case in 1967. Many of Dr. Burstein’s colleagues also returned for the 2-day scientific and social program.

This year’s chief residents, who graduated in June, were another outstanding class. They are all advancing on to fellowships in their subspecialty areas of choice, and we welcome them into the Case-Herndon Alumni Association and wish them all of the best in their future careers:

Dr. Sam Akhavan, Sports Medicine Fellowship, Cleveland Clinic

Dr. Mahi Durbhakula, Hand Surgery Fellowship, Hospital for Special Surgery

Dr. Lutul Farrow, Sports Medicine Fellowship, Cleveland Clinic

Dr. Jerry Huang, Hand Fellowship, UCLA Medical Center

Dr. Casey Jenkins, Hand Fellowship, Duke Medical Center

Dr. Bret Kean, Sports Medicine Fellowship, University of Utah Medical Center

Once again, it has been a privilege to lead this fabulous Orthopaedic Department, and I hope you will enjoy this volume of the Case Orthopaedic Journal, which highlights the outstanding work that typifies the faculty and staff of this Department.
UPDATE FROM METROHEALTH

Brendan Patterson, MD
Chief of Orthopaedics, MetroHealth Medical Center
Associate Professor, Orthopaedics Department, Case School of Medicine

The MetroHealth faculty are overjoyed with the editorial decision to dedicate the Case Orthopaedic Journal to Dr. Nash. Les has had a profound effect on the Case Western Reserve Department of Orthopaedics and his legacy as an educator resonates in the program. His academic progeny at University Hospitals and MetroHealth continue the teaching tradition he helped to establish and he so thoroughly enjoys. Dr. Nash’s career and commitment to education were recently acknowledged by the establishment of the Nash Professorship in Orthopaedics. The Nash Professorship assures in perpetuity his legacy of teaching, mentoring and community service. I am personally grateful to all of those who contributed to the campaign for the Nash Professorship. And for those who have not yet pledged…we know who you are and we are coming for the next chair in the next year!

What else is new? We have added a few new faces to the same old crowd. Dr. Kevin Malone has joined the staff at MetroHealth as a full time member of the faculty. Dr. Malone completed his residency at Beaumont Hospital and his hand fellowship at the University of Washington. Yes. Can you believe yet another Harborview connection? Who would have known what Jack Wilber started not so long ago? We remain heavily concentrated in trauma, hand and spine—some things just refuse to change. Dr. John Feighan has also decided to sign up as a part-timer. As many of you know Dr. Feighan is a graduate of the program and has an established Foot and Ankle practice in Northeast Ohio. Dr. Feighan will serve as Chief, Foot and Ankle Service and we welcome his expertise as not all of those hindfoot fractures turn out as well as we tell you they do.

Dr. Jack Wilber is as busy as ever especially given his role as President of AO North America. Jack remains an active leader in orthopaedic traumatology. He continues to lead the educational programs provided by AO. Dr. Roger Wilber has been on the circuit as well, providing insight on the finer points of acetabular reconstruction. Dr. John Sontich was elected President of the Limb Lengthening Research Society and will be spearheading their educational and research efforts over the next year. We intend to host the first annual Trauma Alumni Meeting at the OTA in Boston this year and count over 25 former residents and fellows who are engaged in traumatology across the nation including two in Israel.

The FES program continues to expand under the tutelage of Drs. Keith, Hoyen, Peckham, Kirsch and Kilgore. No it is not a law firm but it is a brain trust. Harry is running as always and I understand that “The House” is nearing completion. However Harry’s house may be like his dictation…(incomplete) Sorry Harry it was a line too good to let pass. Dr. Vallier has built a substantial clinical research organization and the publications are just entering the piping. The Orthopaedic Learning Center is alive and on the Web, browse at your convenience. Drs. Eppig and Moore are making the world a safer place for patients with spine injuries. The “Old Guard” continues to practice as new faculty are added. Drs. Nash and Makely are retired yet continue to see patients in the office. Drs. Lacey and Cooperman are threatening to join the “Old Guard” and Dr. George Thompson prefers to be Middle Guard at the time of this writing.

Emigda still has a smile that lights the office.

We are blessed.

We hope you; the collective you; those of you who have passed through the portals of Mother Metro on the journey that is your life, are blessed as well. We have toiled together in the service of humanity and it is this noble work that brings the blessing. Please keep in touch.
The Orthopaedic service at the Cleveland Veterans Affairs Medical Center (VAMC) continues to thrive and expand its service and educational activities. During the 2006-2007 academic year, we performed 482 operations (with nine different attending surgeons) and 6,420 outpatient evaluations. This represents a 29% increase in office visits and an 85% increase in surgical cases from last year. The VAMC service provides tremendous clinical experience for the four residents and spine fellows that are here for their four-month rotations.

The service is divided into the Sports/Spine team and the Arthroplasty/Upper Extremity team. Each team consists of one chief resident, one PGY-3 resident and various attendings with a spine fellow on the spine team. Attendings actively involved at the VA include Nicholas Ahn, MD (spine), J. Robert Anderson, MD (upper extremity), Patrick Getty, MD (orthopaedic oncology and general orthopaedics), Richard Grant, MD (arthroplasty), Randall Marcus, MD (foot/ankle and amputation), Thomas McLaughlin, MD (sports medicine and general orthopaedics) and William Petersilge, MD (arthroplasty).

The tradition of funded research continues with the ongoing functional electrical stimulation program for spinal cord injury patients under the direction of Ron Triolo, PhD. Richard Grant, MD is heading our current clinical research project studying Joint Replacement Utilization Disparities in the VA patient population.
2007 was a very strong year for the Division of Pediatric Orthopaedics at Rainbow Babies and Children's Hospital. Our faculty was increased in this year by the addition of Jochen Son-Hing, M.D. Dr. Son-Hing is from Vancouver, BC. He did his residency at the University of British Columbia Orthopaedic Residency Program. He did a fellowship in Pediatric Orthopaedics at Rainbow Babies and Children's Hospital. When it was elected to have him stay on the faculty, he did another half-year of fellowship here followed by three months at St. Louis Children's Hospital and Shrine's Hospital for Children in St. Louis under the supervision of Dr. Larry Lenke. His focus will be primarily in spinal deformity surgery, but with special expertise in spinal osteotomies.

Notable activities this year has been the continuation of the Pediatric Level I Trauma Center. This occupies a considerable amount of time for the faculty. Each year the number of trauma patients steadily increases.

Our outlying clinics had previously been under the direction of the Bureau for Children with Medical Handicaps. However, the state no longer funds outreach clinics in each specialty. Fortunately, our Sandusky and Loudenville clinics have been converted to private clinics. Ashtabula and Geauga are being combined into a single clinic in Geneva, Ohio.

Our academic productivity remains very high. Publications, presentations, and grants by members of the Division are listed in the “Faculty Publications” section of this journal. We have a monthly staff meeting for the faculty, as well as a monthly research meeting. All research is coordinated and all projects are discussed at this meeting. Computerized print-outs are used to track the progress of each study.

The conference schedule is basically unchanged with the exception of a Wednesday evening Spine Conference. Spinal deformity cases are presented and discussed. We continue to have our didactic conference every other Friday morning followed by our Preoperative and Postoperative Conference. Additional pediatric orthopaedic education is provided through the Monday morning Fracture Conference and the Wednesday morning Grand Rounds.

Over the past decade, we have created a very extensive Pediatric Orthopaedic Spine Deformity Database. This now has over 1500 patients with complete preoperative, intraoperative, and postoperative data. It is a cornerstone of our spine academic program.

We continue to have strong leadership, nationally and internationally in Pediatric Orthopaedics. I am currently serving as the President of the Scoliosis Research Society and Chairman of the Scientific Committee for the International Federation of Pediatric Orthopaedic Societies. I also serve on the Shrine Medical Advisory Board.
I am pleased that this issue of the Case Orthopaedic Journal (COJ) is dedicated to Dr. Les Nash. I have always been struck by Les’ strong interest and support of academics in the department. His role in the development of the Orthopaedic Learning Center at Metro further demonstrates that he not only talks the talk but walks the walk. I trust that this year’s COJ dedication will remind us of the importance of that legacy and encourage us to endeavor to strengthen all of our academic pursuits.

Each year, two of our residents are selected as Allen Fellows, who join a research lab for a full-time, year-long, experience. The 2006-2007 Allen Fellows were Ryan Garcia, who worked with Matt Kraay and Clare Rimnac (Mechanical Engineering) on analysis of retrieved implants, and Patrick Messerschmitt, who worked with me and Patrick Getty on tyrosine kinase signaling in osteosarcoma. As described in more detail elsewhere in this issue of the COJ, Ryan and Patrick have already made numerous presentations on their research and have received a number of local and state-wide resident research awards. We congratulate both of them for having done excellent jobs during their Allen Fellowship year and look forward to multiple publications from each of them based on their Fellowship research. The two Allen Fellows for the 2007-2008 year are Dan Master, who is working with Harry Hoyen and Bob Kirsch (Biomedical Engineering), and James Murphy, who is working with Matt Kraay and Clare Rimnac.

This year we also formed the Allen Fellows Society, which consists of all current and former Allen Fellows. The Society will help recruit the two Allen Fellows from each new class of residents and it will host the Allen Fellows Society Visiting Professor each fall. The 2006 Allen Fellows Society Visiting Professor was Fred Kaplan, MD, from the Department of Orthopaedic Surgery at the University of Pennsylvania. Fred presented a Grand Rounds Talk and a Research Seminar on his long-time research effort to understand fibrodysplasia ossificans progressiva and his lab’s recent breakthrough in discovering that FOP is due to an activating mutation in one of the BMP receptors. In addition to the talks, Fred had a dinner meeting with the Allen Fellows Society, where it became clear what a wonderful role model he is for residents. I would like to thank Steve Fitzgerald for suggesting that we invite Fred and for arranging the visit. We look forward to the 2007 Allen Fellows Society Visiting Professor, who will be Randy Rosier, MD, from the Department of Orthopaedic Surgery at the University of Rochester.

Last year in this column, I mentioned that the basic scientists in the Department of Orthopaedics had prepared a revised application for the CWRU Musculoskeletal Training Grant. I’m happy to say that the grant was approved and that funding began in May 2007. We are delighted to welcome the following trainees:

- Lindsay Bonsignore, PhD student with me in Pathology
- Sarah McBride, PhD student with Melissa Knothe-Tate in Biomedical Engineering
- Mike Sobeiraj, MD/PhD student with Clare Rimnac in Mechanical Engineering
- Ingrid Tomanova-Soltys, post-doctoral fellow with Nora Singer in Pediatric Rheumatology
Loran Vieregge, PhD student with Eben Alsberg in Biomedical Engineering

Karen Warden, post-doctoral fellow with Dwight Davy in Mechanical Engineering.

Finally, I would like to congratulate Clare Rimnac on having been named both the Chair of the CWRU Department of Mechanical and Aerospace Engineering and the 2007-2008 Program Committee Chair for the Orthopaedic Research Society. I am confident that she will do an excellent job juggling these responsibilities. Dr. Rimnac also arranged a 40th Anniversary celebration of orthopaedic biomechanical research at CWRU last spring, which is described in more detail elsewhere in this issue of the COJ.
BASIC SCIENCE FACULTY

Eben Alsberg  Dwight Davy  Jim Dennis  Edward Greenfield

Thomas Hering  Christopher Hernandez  Joseph Mansour*  Shunichi Murakami

P Hunter Peckham  Clare Rimnac  Ronald Triolo  Guang Zhou

* Modified copy of image [Source] property of Case Western Reserve University Archives.
THOMAS MCLAUGHLIN, MD
CASE WESTERN RESERVE UNIVERSITY
TRAINING ROOM DEDICATION

On Saturday, September 22nd, Dr. Thomas C. McLaughlin was honored by Case Western Reserve University for his 34 consecutive years as Varsity Team Physician for the CWRU Athletic Department. During his tenure, Dr. McLaughlin attended hundreds of athletic events and rendered care to thousands of CWRU athletes. The President of Case Western Reserve University, Barbara Snyder, honored Dr. McLaughlin at a reception prior to the Saturday night CWRU football game and announced that the Athletic Training Room will be named in honor of Dr. McLaughlin. Dr. McLaughlin received a replica of the plaque that will be on this new training facility, as well as an autographed football from the CWRU team. He was also named honorary Co-Captain for the game.

Dr. Shana Miskovsky has been named the new CWRU Head Team Physician and she will be assisted by Drs. Amanda Weiss-Kelly and Susannah Briskin.

(left to right): Catherine Keating, M.D., Sr. Vice President, University Hospitals Case Medical Center; Randall E. Marcus, M.D., Charles H. Herndon Professor and Chairman, Department of Orthopaedics, Case Western Reserve University; Thomas C. McLaughlin, M.D.; and Barbara Snyder, President of Case Western Reserve University.
This year, the 5th annual Clinician Scientist Development Program was held in Asheville, North Carolina on June 11-12, 2007. This program was created to help guide orthopaedic residents with an interest in both clinical practice and scientific research navigate through challenges like grant writing, research funding, establishing a mentor, and balancing it all. Participants in the program were given the opportunity to meet and interact with many highly successful clinician scientists. Participants were also paired with selected faculty to establish an on-going dialog and mentorship. Thirteen participants were selected from a nationwide pool of applicants. Matthew V. Smith, M.D was selected from our department this year.

This year’s 120th Annual Meeting of the American Orthopaedic Association was held in Asheville, North Carolina, at The Grove Park Inn. From June 12-14, residents from across the country were gathered at this beautiful location for the AOA-OREF-Zimmer Resident’s Leadership Forum. Among other leadership topics presented in this conference, the primary focus of discussion for this year’s forum concentrated on the fellowship match.

Jason David Eubanks, MD, a chief resident at Case, and a future spine fellow at the University of Pittsburgh 2008-2009, was elected as this year’s representative from the Department of Orthopaedics at Case Western Reserve University. The discussion at this year’s forum centered on the necessity of implementing a unified match program for fellowships. While there was no resounding consensus on the topic at meeting’s end, it was generally agreed that more uniformity was necessary, either through the implementation of a match or through specified and regulated dates.
2007 – “Top Docs” as rated by other MDs from Cleveland Magazine

Dr. Henry H. Bohlman, University Hospitals Case Medical Center

Dr. Patrick J. Getty, University Hospitals Case Medical Center

Dr. Victor M. Goldberg, University Hospitals Case Medical Center

Dr. Donald B. Goodfellow, University Hospitals Case Medical Center

Michael W. Keith, MetroHealth Medical Center

Dr. Matthew J. Kraay, University Hospitals Case Medical Center

Stephen H. Lacey, UHCMC

Dr. Brendan M. Patterson, MetroHealth Medical Center

Dr. John H. Wilber, University Hospitals Case Medical Center

Dr. Roger G. Wilber, MetroHealth Medical Center
Patrick Messerschmitt

1st Place – 2006-2007 Cleveland Orthopaedic Society, Resident Essay Contest
“Osteosarcoma: Decreasing metastasis by inhibiting tyrosine kinase signaling”

BARRY FRIEDMAN ORTHOPAEDIC AWARD 2007

Patrick Messerschmitt

2nd Place – 2006-2007 Barry Friedman Award, Resident Paper Contest at the Reich Lectureship
“Tyrosine kinase inhibitors reduce motility by osteosarcoma cells”

BOHLMAN LECTURESHIP RESIDENT ABSTRACT CONTEST

Ryan Garcia

Co-winner – 2006-2007 Bohlman Lectureship, Resident Abstract Contest
"An Evaluation of Information on the Internet of a New Device: The Lumbar Artificial Disc Replacement”

Patrick Messerschmitt

Co-winner – 2006-2007 Bohlman Lectureship, Resident Abstract Contest
“Does narcotic use prior to lumbar decompression surgery predict functional outcomes or duration of postoperative narcotic use?”
RESIDENT’S PAPERS CONTEST

Patrick Messerschmitt

1st Place – 2006-2007 Ohio Orthopaedic Society, Resident Paper’s Contest
“Osteosarcoma: Decreasing metastasis by inhibiting tyrosine kinase signaling”

MEMBER’S PAPERS CONTEST

Christopher Furey, M.D.

2nd Place – Ohio Orthopaedic Society, Members Paper’s Contest
“Surgery vs. conservative management in the treatment of sciatica”

SCOLIOSIS RESEARCH SOCIETY AWARD

Clayton Dean, M.D.

Best clinical paper – 2006 Scoliosis Research Society: Louis A. Goldstein Award
“Degenerative Spondylolisthesis of the Cervical Spine: A Long Term Follow-Up Analysis.”
Authors: Clayton Dean, Ezequiel Cassinelli, Josue Gabriel, Michael Bolesta, and Henry Bohlman.

Takehiko Matsushita MD, PhD

Young Investigator Award –
29th Annual Meeting of the American Society for Bone and Mineral Research
PUBLICATIONS


GRANTS

AO North America Kathryn Cramer Memorial Award - $15,000

Principal Investigator: Christopher McAndrew

“Timing and Method of Analgesia and Anesthesia for the Reduction of Pediatric Forearm Fractures- A Prospective, Randomized Study of Two Treatment Protocols.”
FACULTY RESEARCH GRANTS

Kathy Bogie, Ph.D
VA Rehabilitation and Research Development Service
Merit Review Award. 2007-2010
“Implanted gluteal stimulation system for pressure sore prevention.”

National Institute on Disability and Rehabilitation Research Task Leader collaborating with DM Brienza DM, University of Pittsburgh. 2007-2012
“Effects of weight shifting on pressure ulcer risk status.”

Jim Dennis, Ph.D
Defense Advanced Research Projects Agency (DARPA)
Sub-project Sponsor: Cell Targeting, LLC
1 year, “Cell Targeting for the Treatment of Injured Tissues”

Reuben Gobezie, M.D.
Minority Faculty Career Development Award, 2007-2010
Case Western Reserve University/University Hospitals of Cleveland
“Radiosteriometric Analysis in Anatomic Total Shoulder Replacement”

Edward Greenfield, Ph.D
NIH/NIAMS
Type: R21 (2 years)
Principle Investigator: Edward Greenfield, Ph.D
“In vivo regulation of cAMP/PKA signaling by PKIγ”

NIH/NIAMS
Type: T32 (5 years)
Principle Investigator: Edward Greenfield
“Training program in musculoskeletal research”

Thomas Hering, Ph.D
NIH
Type: R01
Principle Investigator: Diane M. Snow, Ph.D, University of Kentucky
Co-investigator: Thomas Herring, Ph.D
“Designer PGs for Central Nervous System Injury”

Chris Hernandez, Ph.D
NIH/NIAMS
Type: R21
Principle Investigator: Chris Hernandez, Ph.D
“Three-Dimensional Dynamic Bone Histomorphometry”

CWRU Presidential Research Initiative
Principle Investigators: Chris Hernandez, Victor Goldberg and Ezequiel Cassinelli
“An In Vivo Model of Damage and Repair of Cancellous Bone”

Matthew Kraay, M.D.
NIH, 2005-2010
“Mechanics and Performance of Traceable UHMWPE Implants”

Zimmer, Inc.
Principle Investigator: Matthew Kraay
Co-PI: Clare Rimnac
“Investigation of in-vivo oxidation in historic, conventional and highly cross-linked UHMWPE knee components.”

Shun Murakami, M.D., Ph.D
NIH, 2006-2008
“Roles of the MAPK pathway in cranial base development”

March of Dimes Birth Defects Foundation, 2006-2009
“Roles of ERK1, ERK2 and Fgfr3 in growth plate chondrocytes”

George H. Thompson, M.D.
Rainbow Board of Trustees

Heather Vallier, M.D.
Orthopaedic Trauma Association, 2006 to present.
Principal Investigator
“Timing of orthopaedic surgery in the multiply injured patient: Development of a Protocol for Early Appropriate Care”

Ruth Jackson Orthopaedic Society/Zimmer Research Award, 2006 to present.
Co-investigator
“Childbirth after Pelvic Fracture”

John Wilber, M.D.
Orthopaedic Trauma Association, 2006 to present.
Co-investigator
“Timing of orthopaedic surgery in the multiply injured patient: Development of a Protocol for Early Appropriate Care”
CLINICAL FACULTY PUBLICATIONS

Nicholas Ahn, M.D.


Douglas Armstrong, M.D.


Henry Bohlman, M.D.


Ezequiel Cassinelli, M.D.


Daniel Cooperman, M.D.


Christopher Furey, M.D.


Furey CG and Emery SE. Decompression and Instrumented Fusion for Management of Degenerative Lumbar Scoliosis.
FACULTY PUBLICATIONS

The Spine Journal. 2006. 6(5S): 32S.


Allison Gilmore, M.D.


Reuben Gobezie, M.D


Victor Goldberg, M.D.


Donald Goodfellow, M.D.


Matthew Kraay, M.D.


Randall Marcus, M.D.


**Brendan Patterson, M.D.**


**Jochen Son-Hing, M.D.**


**John Sontich, M.D.**


**George Thompson, M.D.**


**Heather Vallier, M.D.**

Vallier HA, Sontich JK, Patterson BM. Letter to the Editor, regarding: Failure of LCP Condylar Plate fixation in the distal part of the femur: A report of six cases, Journal of


Brian Victoroff, M.D.


John Wilber, M.D.


BASIC SCIENCE FACULTY PUBLICATIONS

Fadi Abul-Karim, M.D.


Emerson RE, Wang M, Liu F, Lawrence WD, Abdul-


Kathy Bogie, Ph.D


Bogie KM, Ho CH. Multidisciplinary approaches to the pressure ulcer problem. Ostomy Wound Management. 53(10), 2007 In press

Ho CH, Bogie KM. Integrating Wound Care Research into Clinical Practice Ostomy Wound Management. 53(10), 2007. In press

Jim Dennis, Ph.D


Weidenbecher M, Henderson JH, Tucker HM, Baskin JI, Awadallah A, Dennis JE. Hyaluronan-Based scaffolds to tissue-engineer cartilage implants for laryngotracheal reconstruction. Laryngoscope (On line/In press)


Edward Greenfield, M.D.


Chen X, Song IH, Dennis JE, Greenfield EM. , Endogenous PKIg inhibits the anti-apoptotic effects of PTH and b-adrenergic agonists in osteoblasts, J Bone Min Res, 2007. 22:656-64.

Taki N, Tatro JM, Lowe R, Goldberg VM, Greenfield EM. Comparison of the roles of IL-1, IL-6, and TNF-α in cell culture and murine models of aseptic loosening, Bone, 2007. 40:1276-83.

Clare Rimnac


Olsen KW, Rimnac CM, Ferrell DW, Garrett CE. Fatigue Crack Growth Analyses of Aerospace Threaded Fasteners; Part II: Material/Stress State and Bolt Strength. ASTM (2007) STP #1487, Structural Integrity of Fasteners.


Thomas Hering, Ph.D

Shun Murakami, M.D., Ph.D

Guang Zhou, Ph.D
The recent discovery of human mutations in the Mitogen-activated protein kinase (MAPK) pathway has highlighted the importance of this signaling cascade in human skeletal development. In this review, we summarize the current understanding of the roles of the MAPK pathway in chondrocyte, osteoblast, and osteoclast regulation. Further identifying the roles of the MAPK pathway in each cell lineage will lead to new therapeutic approaches for treating various human skeletal disorders.

I. Human syndromes caused by mutations in the MAPK pathway

Recently, a number of human mutations that affect several systems - including the musculoskeletal system - have been identified in the molecules in the MAPK cascade (Table 1). Missense activating mutations in KRAS, BRAF, MEK1, and MEK2 have been identified in Cardio-facio-cutaneous syndrome (1). KRAS mutations have been also identified in Noonan syndrome (2). HRAS mutations causes Costello syndrome (3) and loss-of-function mutations in RSK2 cause Coffin-Lowry syndrome (4). All of these syndromes present with various skeletal manifestations, including short stature and craniofacial and limb abnormalities (5-9), underscoring the importance of the MAPK pathway in human skeletal development.

II. ERK1/ERK2 MAPK in chondrocytes

A number of in vivo and in vitro studies have implicated the MAPK pathway in chondrocyte differentiation and bone growth. We previously found that the master transcription factor Sox9 is upregulated by FGF in a MAPK-dependent manner in primary chondrocytes (10). To examine the role of the MAPK pathway in chondrocytes, we further generated transgenic mice that express a constitutively active mutant of MEK1 in chondrocytes (11). These mice showed a dwarf phenotype similar to human achondroplasia and thanatophoric dysplasia. The growth plate is characterized by a reduced zone of hypertrophic chondrocytes and smaller than normal hypertrophic chondrocytes. Because ERK1 and ERK2 are the only known substrates of MEK1, these observations strongly suggest that ERK1 and ERK2 have profound effects on hypertrophic chondrocyte differentiation.
inhbhibits chondrocyte proliferation and reproduces the dwarf phenotype (17-23). In contrast, the lack of FGFR3 in mice causes skeletal overgrowth with a wider zone of hypertrophic chondrocytes in the growth plate (24,25). These observations indicate that FGFR3 inhibits longitudinal bone growth through the regulation of proliferation and differentiation of growth plate chondrocytes.

**ii) ERK1/ERK2 MAPK in Fgfr3 signaling in vivo**

 Intracellular signaling pathways that mediate the action of FGFR3 have been a topic of keen interest. Our in vivo and in vitro observations have strongly suggested that the MAPK pathway is a major mediator of FGF signaling in chondrocytes. We showed an increased phosphorylation of MEK1 in growth plate chondrocytes of mice expressing a human achondroplasia mutant of FGFR3, providing in vivo evidence that activating mutations of FGFR3 result in increased MAPK signaling (11). We also showed that the expression of a constitutively active mutant of MEK1 in the chondrocytes of FGFR3-deficient mice inhibited skeletal overgrowth, strongly suggesting that the regulation of bone growth by FGFR3 is mediated at least in part by the MAPK pathway (11). These observations strongly suggest that the MAPK pathway in chondrocytes plays an essential role in the regulation of bone growth.

**III. ERK1/ERK2 MAPK in osteoblasts**

Several lines of evidence suggest that ERK1 and ERK2 also have a role in osteoblast differentiation. ERK1/ERK2 has been shown to phosphorylate and activate a number of molecules implicated in osteoblast differentiation. Runx2, a master transcription factor for osteoblast differentiation, is directly phosphorylated by activated recombinant ERK2 (26). In addition, 32P metabolic labeling studies demonstrated that expression of a constitutively active mutant of MEK1 enhanced the phosphorylation of Runx2 in intact cells, while a dominant negative mutant MEK1 decreased its phosphorylation.

Furthermore, transgenic mice that express a dominant negative form of MEK1 under the osteocalcin promoter showed delayed calvarial mineralization and delayed formation of primary ossification centers in the long bones. In contrast, accelerated trabecular bone formation was observed in transgenic mice that express a constitutively active form of MEK1 under the osteocalcin promoter (27), suggesting a positive role of ERK1 and ERK2 in osteoblast differentiation. In humans, the inactivation of Rsk2, a downstream kinase of ERK1/ERK2, causes Coffin-Lowry syndrome, an X-linked mental retardation condition associated with progressive skeletal abnormalities (4). Rsk2-null mice showed a widening of cranial sutures at birth and decreased bone mass postnatally, indicating that Rsk2 plays a critical role in osteoblast differentiation (28,29). Rsk2 in turn phosphorylates Atf4, a transcription factor implicated in osteoblast differentiation. Since phosphorylation by ERK1/ERK2 is essential for the complete activation of Rsk2 (30,31), these observations suggest the possibility that ERK1 and ERK2 play a critical role in osteoblast differentiation.

**i) FGFR2 and craniosynostosis syndromes**

FGFR2 is expressed in preosteoblasts and osteoblasts in the cranial vault; in perichondrium, periosteum, and osteoblasts in the growing long bones; and in prechondrogenic mesenchymal condensation in the developing limbs (32,33). Activating mutations in FGFR2 cause skeletal syndromes characterized by craniosynostosis, a condition in which cranial sutures close prematurely (34-36). These include Apert, Pfeiffer, Jackson-Weiss, and Crouzon syndromes. In addition to craniosynostosis, these syndromes show variable degrees of limb abnormalities. The most severe hand and foot anomalies are associated with Apert syndrome. Characteristically, patients with Apert syndrome present with cutaneous and osseous syndactyly and fusion of various bones. Crouzon syndrome shows various facial anomalies, while the hands and feet are unaffected. Although there is a wide variability in phenotypic expression, the tendency to have bone fusions is a common feature among FGFR2-related skeletal syndromes. The expression of activating FGFR2 mutants in mice reproduces craniosynostosis, which is associated with enhanced osteoblast proliferation and differentiation (37,38). In contrast, inactivation in mice of Fgfr2IIIc, the alternatively spliced mesenchymal variant of FGFR2, causes a delay in ossification (39). Furthermore, the inactivation of a floxed FGFR2 allele in mesenchymal condensations results in mice with skeletal dwarfism and decreased bone density (40). These observations indicate that FGFR2 signaling positively regulates osteoblast differentiation and bone formation.

**ii) ERK1/ERK2 MAPK in cranial suture closure**

In mice, the targeted inactivation of Dusp6, a gene encoding an ERK-specific MAPK phosphatase MKP3, caused craniosynostosis, strongly suggesting that the MAPK pathway regulates the closure of the cranial sutures (41). In addition, in an organ culture system of mouse calvariae, a MEK inhibitor PD98059 inhibited the FGF2- and TGF-beta-induced
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<th>Syndrome</th>
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<td>Noonan syndrome</td>
<td>Superior pectus carinatum</td>
<td>Broad Forehead</td>
<td>Pulmonary stenosis</td>
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<td>Inferior pectus excavatum</td>
<td>Downward slanting palpebral fissures</td>
<td>Hypertrophic cardiomyopathy</td>
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<td>Radioulnar synostosis</td>
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<td>Sparse and friable hair</td>
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<td>Costello syndrome</td>
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<td>Delayed bone age</td>
<td>Papillomata</td>
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<td>Coffin-Lowry syndrome</td>
<td>Broad and soft hand with Stubby and tapering fingers</td>
<td>Prominence of the forehead</td>
<td>Delay in closure of the anterior fontanelle</td>
<td>Mental retardation</td>
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<td></td>
<td>Short horizontal crease in the hypothenar region</td>
<td>True orbital hypertelorism</td>
<td>Delayed bone age</td>
<td>Congenital hypotonia</td>
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<td></td>
<td>Intervertebral space narrowing</td>
<td>Slanting fissures</td>
<td>Short stature</td>
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<td></td>
<td>Irregular endoplates</td>
<td>Flat nasal bridge</td>
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<td></td>
<td>Anterior wedging</td>
<td>Midface hypoplasia</td>
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<td></td>
<td>Kyphoscoliosis</td>
<td>Full everted lip</td>
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</table>

**Table 1**

Clinical manifestations of Noonan, Cardio-facio-cutaneous, Costello, and Coffin-Lowry syndromes.
acceleration of suture closure, further suggesting a role for the MAPK pathway in regulating the closure of the cranial sutures (42,43). While the role of the MAPK pathway in FGFR2 signaling in skeletal tissues remains to be investigated, these observations strongly suggest that the MAPK pathway mediates accelerated cranial suture closure in FGFR2-related craniosynostosis syndromes.

IV. ERK1/ERK2 MAPK in osteoclastogenesis
Osteoclasts develop from the monocyte/macrophage lineage. Osteoclast formation is supported by RANKL-expressing mesenchymal cells, including osteoblasts and bone marrow stromal cells (44,45). Growth plate chondrocytes also express RANKL, suggesting their roles in supporting osteoclastogenesis, which in turn would regulate cartilage resorption (46,47). Indeed, the conditional inactivation of Vitamin D receptor (VDR) in chondrocytes resulted in a reduced number of osteoclasts, providing direct in vivo evidence that growth plate chondrocytes support osteoclastogenesis during skeletal development (48).

Several lines of evidence suggest that the MAPK pathway is involved in supporting osteoclastogenesis. In a co-culture system of osteoblasts and spleen cells, FGF2 strongly promotes osteoclast formation (49-51). Consistent with this observation, FGF2 upregulates RANKL expression in osteoblasts. Interestingly, an increased number of osteoclasts has been reported in mouse models of achondroplasia and thanatophoric dysplasia, suggesting a link between FGFR3 signaling and osteoclastogenesis (20,21,52). Given that the MAPK pathway is a major mediator of FGF signaling, it is possible that the MAPK pathway in osteoblasts and chondrocytes support osteoclastogenesis through regulation of RANKL.

V. Genetic manipulation of molecules in the ERK MAPK pathway in mice
To examine the role of the MAPK pathway, various mutant mice have been generated. Inactivation of Rsk2, a downstream kinase of the MAPK pathway, caused a widening of cranial sutures at birth, similar to delayed closure of fontanelles in patients with Coffin-Lowry syndrome (28,29). These observations indicate that Rsk2 plays a critical role in osteoblast differentiation. In contrast to Rsk2-null mice, inactivation of ERK, MEK, and Raf family members has provided little information regarding skeletal development. ERK1-null mice are viable and fertile and develop normally without obvious skeletal abnormalities, suggesting that ERK1 is dispensable for skeletal development (53,54).

In remarkable contrast, ERK2-null mice show early embryonic lethality at E6.5, precluding the analysis of skeletal development (55,56). Mek1-null embryos die at E10.5 due to placental defects, while Mek2-null mice develop normally without any obvious abnormalities (57,58). Araf-null mice show neurological and gastrointestinal defects, but do not show an obvious skeletal phenotype (59). Braf-deficient embryos die at midgestation due to vascular defects, precluding the analysis of skeletal development (60). Craf-null mice show placental defects and die at around E10.5-12.5 on the C57BL/6 and 129 backgrounds. On the outbred CD1 background, two-thirds of embryos reach term and die soon after birth. These surviving embryos show a mild delay in ossification; however, it is not clear whether the observed skeletal phenotype is primarily caused by Craf deficiency in the skeletal tissues (61). These observations suggest that...
members of the ERK, MEK, and Raf family are functionally redundant, while some of the tissue-specific functions are not fully compensated by other family members. To circumvent early embryonic lethality caused by the systemic inactivation of the target gene, tissue-specific inactivation in the skeletal tissues would be essential. Furthermore, inactivation of multiple family members may be necessary to uncover the roles of ERK, MEK, and Raf family members in skeletal development.

SUMMARY

The recent discovery of human mutations in the MAPK pathway has highlighted the critical roles of the MAPK pathway in human skeletal development. A number of in vitro experiments and genetic manipulations of FGF and MAPK signaling in mice have provided compelling evidence that MAPK plays an important role in the regulation of osteoblasts, chondrocytes and osteoclasts, proper functions of which are essential for skeletal development. However, systemic inactivation of ERK, MEK, and Raf family members in mice has provided little information regarding skeletal development due to the early embryonic lethality or possible functional redundancy among the family members. Tissue-specific inactivation of the target family member and generation of double mutant mice may be required to better understand the roles of the MAPK pathway in skeletal development. Elucidation of the roles of the MAPK pathway may lead to new therapeutic approaches to treat various skeletal conditions.

ACKNOWLEDGEMENTS

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LAMINOPLASTY VERSUS MULTILEVEL CORPECTOMY IN THE TREATMENT OF CERVICAL SPONDYLYTIC MYELOPATHY: A PROSPECTIVE, NON-RANDOMIZED STUDY

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ABSTRACT
The purpose of this study was to compare the clinical outcomes of patients with cervical spondylytic myelopathy treated with either multi-level corpectomy and fusion or laminoplasty. 44 patients (20 who underwent corpectomy and fusion, 24 who underwent laminoplasty) were followed prospectively for an average of 3.2 years (range 2.0-4.0). The choice of surgical technique was non-randomized and was based on degree of cervical lordosis, evidence of instability, and degree of axial neck pain. Duration of symptoms, severity of myelopathy as defined by the Nurick score, and number of medical co-morbidities were similar for the two cohorts. Average improvement in pre-operative Nurick score was similar for the two cohorts. Subjective improvement of gait and dexterity, relief of extremity pain and parathesias, and relief of axial neck pain were slightly better in the laminoplasty cohort. Sagittal alignment improved an average of 5 degrees in the corpectomy cohort and in no patient of the laminoplasty cohort. We conclude that both multi-level corpectomy with fusion and laminoplasty are effective in the treatment of cervical spondylytic myelopathy. Laminoplasty appears to be better tolerated by patients and is associated with less complications and a lower re-operation rate.

INTRODUCTION
Cervical spondylytic myelopathy (CSM) can cause disabling symptoms and lead to severe neurologic dysfunction. Degenerative changes affecting the cervical spinal column (spondylosis) may result in marked spinal canal stenosis causing spinal cord compression. The natural history of cervical spondylytic myelopathy is one of a slow, steady neurologic deterioration over time, with occasional exacerbations of increased severity (1,2). With established spinal cord compression, there is a risk of sudden, catastrophic spinal cord injury occurring even with subtle trauma, which can lead to irreversible paralysis. As such, surgery is generally recommended as the treatment of choice for most patients exhibiting clinical evidence of cervical spondylytic myelopathy (3,4). Surgical intervention has been shown to effectively relieve symptoms, improve neurologic function, and lessen the likelihood of further neurologic deterioration (5,6).

Several distinct surgical options exist for the management of CSM. Anterior surgical approaches include multilevel discectomy with fusion and single or multi-level corpectomy with strut graft fusion. Posterior surgical approaches include laminoplasty or laminectomy with fusion. Laminectomy alone has largely been abandoned because of the high incidence of post-laminectomy kyphosis and the resulting pain, deformity, and risk of additional spinal cord injury (7). The decision to perform an anterior or posterior approach (or even a combined approach) is based on several factors, including the number of levels of cord compression present, the source of cord compression, the alignment of the vertebral column, the presence of instability, the degree of axial neck pain, and the experience of the individual surgeon.

Distinct advantages and disadvantages of both anterior and posterior approaches exist. Anterior approaches afford the surgeon the ability to directly address the compressive pathology by removing disc material and osteophytic spurs. As well, with the accompanying...
fusione, sagittal plane deformity may be improved and a patient’s axial neck pain may be decreased. With a solid fusion, motion at one or more levels is eliminated and this can protect a previously compromised spinal cord. Disadvantages include the risk of iatrogenic soft tissue injury during an extensive anterior cervical exposure, the potential for significant post-operative dysphasia, the possibility of nonunion, the development of adjacent level disease, and the need for post-operative immobilization. There is also the potential morbidity from the harvest of an autograft, as well as the expense and risk of disease transmission that accompanies the use of allograft bone.

A posterior approach in the form of laminectomy and fusion allows for indirect spinal cord decompression and provides rigid support by eliminating motion like an anterior fusion. Laminoplasty also indirectly decompresses the spinal cord from a posterior approach, by lifting up (but not removing) the laminae as a unit and thus expanding the spinal canal. A benefit of laminoplasty is that without fusion there is no loss of motion, no risk of adjacent level disease, and no need for bone graft or lengthy post-operative immobilization. With a posterior approach, the risk of injury to the anterior soft tissue structures is eliminated and there should be no incidence of post-operative dysphasia or hoarseness. Potential disadvantages of laminoplasty include the possible development of instability and deformity. Also, with laminoplasty, there may be limited ability to address axial neck pain by not providing rigid support as occurs with a fusion.

Currently we present our experience comparing patients with cervical spondylotic myelopathy treated with either multilevel corpectomy and fusion or laminoplasty, in a prospective, non-randomized fashion.

**METHODS**

Forty-four consecutive patients with multilevel cervical spondylotic myelopathy treated with either multilevel corpectomy and fusion or laminoplasty from 2002 to 2004 were studied. The selection of the surgical technique was non-randomized and was based on surgeon discretion. The choice of the technique was based primarily on degree of cervical lordosis, the presence of instability, and degree of a patient’s axial pain. Patients with loss of normal cervical lordosis, evidence of instability with dynamic radiographs, or whose neck pain was described as more than mild were treated with corpectomy and fusion. In patients with maintenance of lordosis, with no evidence of instability and with minimal, if any, axial neck pain, laminoplasty was employed.

Twenty patients underwent multilevel corpectomy and fusion and 24 patients underwent laminoplasty. Average follow-up was 3.2 years (range 2.0 – 4.0 years). All patients had both subjective and objective evidence of myelopathy. No patient had undergone prior surgery. Each patient had evidence of spinal cord compression occurring at 3 or more levels on neuroimaging (either MRI or CT-myelogram).

The average age, duration of symptoms, and severity of myelopathy were similar between the corpectomy cohort and

| TABLE 1: Average Demographics of Laminoplasty and Corpectomy Cohorts |
|-----------------|-----------------|----------------|----------------|
|                 | Age            | Nurick Grade | Duration of Symptoms | # of Levels |
| Corpectomy Cohort | 55.4           | 3.2           | 11 months            | 3.1         |
| Laminoplasty Cohort | 58.2           | 3.6           | 12 months            | 3.6         |

| TABLE 2: Nurick Classification of Neurologic Dysfunction |
|----------------|----------------|
| Grade | Description             |
| 1     | Objective signs, but no functional symptoms |
| 2     | Slight difficulty walking, but employable |
| 3     | Difficulty with gait, ADL’s affected, functions independently |
| 4     | Requires assistance to walk |
| 5     | Wheelchair bound or bedridden |
Laminoplasty cohort (Table 1). Severity of myelopathy was defined by the Nurick criteria (Table 2), in which a numeric score of 1 to 5 is given for increasing severity of neurologic dysfunction (8). The frequency of congenital cervical stenosis was more common in the laminoplasty cohort. Twelve patients who underwent laminoplasty had a canal diameter : vertebral body diameter of less than 0.7, while no patients who underwent a corpectomy did.

Clinical evaluation was performed with Nurick grades pre-operatively and at the most recent follow-up. Additionally, patients were asked to specifically address improvement in gait, improvement in upper extremity dexterity, relief of radicular pain or parathesias, relief of axial neck pain, and their overall satisfaction with the surgery and its outcome.

Plain radiographs were obtained pre- and post-operatively. Instability was defined as greater than 3 mm of translation or 15 degrees of angulation occurring at adjacent levels with flexion and extension. Cervical lordosis was measured via a Cobb angle from C2 to C7 on a lateral film in the neutral position. CT scan was employed post-operatively only to assess the possibility of a pseudarthrosis. The pre-operative cervical lordosis measured an average of 5 degrees (range 3-12 degrees) in the corpectomy cohort and an average of 14 degrees in the laminoplasty cohort.

Figures 1a,b,c: Pre-operative radiograph and MRI and post-op radiograph following laminoplasty.

Figures 2a,b,c: Pre-operative radiograph and MRI and post-op radiograph following partial corpectomy C3, full corpectomy C4-5, and C3-6 fusion.
**Surgical Technique**

Spinal cord monitoring with somatosensory evoked potentials were employed in each case. Multilevel corpectomy and fusion was performed through a standard, left-sided approach to the anterior aspect of the neck. With the spinal column exposed, a discectomy was performed above and below each vertebra to be removed. This allowed adequate visualization of the uncovertebral joints and defined the appropriate lateral extension of the corpectomy. Traction was employed via Gardner-Wells tongs, generally between 20-30 pounds. Troughs were made within the endplates of the superior and inferior vertebra to be fused to allow appropriate docking of the fibular strut graft to ensure against graft migration or dislodgement. A fibular autograft was employed in each case and was harvested through a lateral approach to the mid-third of the calf. Anterior plate fixation was not used. Post-operatively, patients were immobilized in either a 2-poster brace or a rigid cervical collar for 6 weeks.

Laminoplasty was performed via a posterior midline approach to the cervical spine. Subperiosteal exposure was carried out to the edge of the lateral masses on each side. The laminoplasty was performed in an open-door fashion as described by Hirabashyi (17). A high speed burr created a trough on the opening side, which was completed with a microscopic Kerrison rongeur. The trough on the hinge side was also created with a burr, with care taken not to disrupt the anterior cortex of the lamina. The laminae involved were then carefully lifted as a unit, thus expanding the spinal canal. In 10 patients, spinous processes from C6 and C7 were used to prop open the laminae and secured with suture passed thru the lateral mass and the edge of the laminae on the opened side. In the other 14 patients, titanium plates were employed to support the laminae and were secured with screws to both the lateral mass and the edge of the laminae on the opened side. A soft cervical collar or hard collar was employed for 1-2 weeks post-operatively.

**RESULTS**

The average operative time was 1.9 hours in the laminoplasty cohort and 3.1 hours in the corpectomy cohort. The average estimated surgical blood loss was 375 cc in the laminoplasty cohort (range 100 – 600cc) and 600 cc in the corpectomy cohort (range 400 – 1200cc). The average hospital stay in the laminoplasty cohort was 2.3 days (range 2-5 days) and 3.5 days (range 3-6 days) in the corpectomy cohort. No laminoplasty patient required post-operative intubation. All 20 corpectomy patients (100%) remained electively intubated post-operatively. The standard protocol for patients undergoing multilevel corpectomy was ICU admission overnight for airway protection, followed by elective extubation the next day at the discretion of the Anesthesia/Critical Care specialist. Two of 18 patients (11%) required greater than 24 hours of intubation.

The Nurick grade in the laminoplasty cohort improved from a pre-operative average of 3.6 to a post-operative average of 1.3, an improvement of 64%. Eighteen of 20 patients (90%) in the laminoplasty cohort improved at least one Nurick grade. The Nurick grade in the corpectomy cohort improved from a pre-operative average of 3.2 to a post-operative average of 1.6, an average improvement of 50%. 20 of 24 corpectomy patients (83%) improved at least one Nurick Grade. The difference in the average improvement of Nurick grade between the cohorts was not statistically significant (p=0.073).

In the laminoplasty cohort, improvement in gait was excellent in 20 patients (84%) and good or fair in 4 patients (16%). In the corpectomy cohort, improvement in gait was excellent in 14 patients (70%), good or fair in 4 patients (20%), and not improved in 2 patients (10%). In the laminoplasty cohort, improvement in dexterity was excellent in 20 patients (84%), good or fair in 3 patients (12%), and not improved in 1 patient (4%). In the corpectomy cohort, improvement in dexterity was excellent in 12 patients (60%), good or fair in 6 patients (30%), and not improved in 2 patients (10%). In the laminoplasty cohort, improvement in upper extremity radicular symptoms was excellent in 21 patients (88%) and good or fair in 3 patients (12%). In the corpectomy cohort, improvement in upper extremity radicular symptoms was excellent in 14 patients (70%), good or fair in 3 patients (15%), and not improved in 3 patients (15%). In the laminoplasty cohort, of the 10 patients who complained of some degree of pre-operative axial neck pain, 5 described some improvement and 5 felt there had been no change. In the corpectomy group, of the 18 patients who described some degree of pre-operative axial neck pain, 12 felt there was considerable improvement and 6 felt there was no change. A statistically significant difference (p<0.05) in these clinical outcome measures was found only in the improvement in upper extremity radicular symptoms.

In the corpectomy cohort, the average post-operative lordosis was 8 degrees, an average increase of 3 degrees (60%). In the laminoplasty cohort, the average post-operative lordosis was 13 degrees, nearly identical to the pre-operative average.
In the corpectomy cohort, 4 patients (20%) underwent additional surgery. Two patients required a posterior fusion to treat a symptomatic pseudarthrosis. One patient with persistent dysphasia underwent surgery to reduce the prominence of a healed fibular strut graft. One patient developed symptomatic adjacent level disease which required an anterior discectomy and fusion below the prior fusion. No patient in the laminoplasty cohort required further surgery. Dysphasia lasting longer than 6 months after surgery was present in 6 patients (30%) in the corpectomy cohort. 4 patients (20%) complained of persistent pain at the fibular donor site 6 months following surgery. The only notable post-operative complication in the laminoplasty cohort was a superficial wound infection in one patient. No patient experienced nerve root palsy, as is often described following posterior cervical surgery.

DISCUSSION
Surgery is the accepted means of treatment for patients with cervical spondylytic myelopathy. Without surgical intervention, the majority of patients will experience a slow and steady worsening of neurologic function, while occasionally experiencing episodes of more acute deterioration (1,2). Over the past two decades, with improvement in neuroimaging and surgical techniques, the diagnosis and treatment of cervical spondylytic myelopathy has significantly evolved. Traditionally in this country, the favored surgical approach has been an anterior multilevel cervical corpectomy and fusion. Long-term results indicate favorable outcomes even in patients with far advanced neurologic dysfunction (5). More recently, posterior approaches, especially laminoplasty, has gained popularity as a means of effectively decompressing the spinal cord, while avoiding the morbidity associated with extensive anterior cervical procedures.

The pathoanatomy of spinal cord compression due to cervical spondylosis is usually anterior, in the form of disc protrusion and osteophytes. If cord compression occurs only at one or two disc levels, it may be adequately addressed with a one or two level anterior discectomy and fusion. More commonly with advanced myelopathy, there is compression at multiple disc levels and this compression occurs not only at the disc space but above and below as well, typically due to large osteophytes. This problem is more effectively treated with a corpectomy than a discectomy. Frequently it is necessary to perform multiple corpectomies to adequately address all levels of spinal cord compression. The accompanying fusion with a long strut can achieve improvement in pre-operative kyphosis and is effective in relieving axial neck pain and providing stability that can further protect the spinal cord.

A multilevel corpectomy and fusion is a technically-challenging procedure that can be associated with numerous well-documented complications (9-11). Complications with an anterior approach include soft-tissue injuries to the trachea or esophagus which, while catastrophic, are fortunately rare. Recurrent laryngeal nerve injuries can be minimized with a left-sided approach, but still may occur and can cause long-term disability with vocal cord paralysis and dysphonia. Injury to the vertebral artery during a corpectomy is a dreaded event which needs to be immediately addressed to prevent intra-operative morbidity and mortality (12). More common is the problem of post-operative dysphasia which is experienced by nearly all patients transiently post-op, but may be experienced by up to 15% of patients for two years following extensive anterior cervical surgery (13). Graft-related complications may occur with long level fusions, where there is a higher risk of graft displacement. Plate fixation which is often employed with one and two level discectomy is more difficult with a long level fusion spanning 3 or more vertebral levels. Additional posterior stabilization is often performed as the second stage of a combined approach for patients who undergo a multilevel corpectomy. Adjacent level disease following anterior cervical fusion is well-documented following anterior cervical fusion and is one main reason for the recent enthusiasm for motion-sparing anterior cervical procedures like total disc arthroplasty (14).

Laminoplasty was popularized in the Japanese spine community in large part due to the prevalence of ossification of the posterior ligament (OPLL) in the Japanese population (15,16). A posterior approach to the cervical spine is desirable in patients with OPLL, in whom anterior decompression of the ossified ligament is fraught with the problems of dural erosions and spinal fluid leaks. Several techniques for laminoplasty have evolved, all of which serve to expand the spinal canal by elevating the laminae, in contrast to removal of the laminae as would be accomplished with laminectomy. Hirabashyi was the first to describe the trap door laminoplasty technique, in which the laminae are lifted open as a unit on one side, turning on a hinge of the opposite side (17). By maintaining the integrity of the posterior boney elements and muscular attachments, the sequelae of instability and further deformity that occurs with posterior laminectomy kyphosis can be avoided.
Laminoplasty also avoids the loss of motion that occurs with fusion. It has been shown that motion is decreased by 30% following laminoplasty, which would be considerably less than multi-level fusion (18). Early techniques involved suturing of muscular and bone structures after the laminae were elevated to maintain the expansion of the canal, but were fraught with recurrence of stenosis as the bone elements settled (19). With the advent of small titanium plates secured to the lateral mass and lamina on the open side, maintenance of canal expansion is easier to achieve (20).

In our study, both corpectomy and laminoplasty were effective in improving neurologic function, relieving symptoms, and improving quality of life. Post-operative complications were clearly more serious and more common in patients who underwent multi-level corpectomy. Post-operative kyphosis was not found to be a noticeable problem in patients who underwent laminoplasty. Development or persistence of axial neck pain, a concern in patients who underwent laminoplasty, was not found to be a significant problem. Due to the ability to indirectly relieve spinal cord compression without creating the additional problems that accompany a major anterior cervical approach, laminoplasty must be considered as an option for the treatment of cervical spondylitic myelopathy. Multilevel corpectomy will continue to be a useful option for patients with instability or excessive kyphosis, but demands great attention to surgical detail to avoid peri-operative morbidity.

REFERENCES
Abstract

Amicar has previously been shown to be useful in decreasing perioperative blood loss in patients undergoing spinal fusion for Adolescent Idiopathic Scoliosis. This was a retrospective case-control study of 96 patients with Neuromuscular scoliosis who had a primary posterior spinal fusion (PSF) at Rainbow Babies and Children's Hospital between 1991 and 2006. 34 patients (Group 1) had not been treated with epsilon aminocaproic acid (Amicar) and 62 patients (Group 2) had received an intraoperative infusion of the drug during the PSF. There were no differences in number of vertebral levels fused, number of patients who had fusion to the sacrum and in operative times. Intraoperative blood loss was 2194 ± 1626 ml (range 450-7500) in Group 1 compared with 1125 ± 715 ml (range 165-3800) in Group 2. This difference was highly significant, p=0.0002. Postoperative suction drainage was not statistically different: 903 ± 547 (r 146-2310) in the controls compared with 695 ± 589 (r 0-3410) in Group 2 (p=0.08). The difference in total transfusion volume was highly statistically significant: 1548 ± 962 ml (0-4600) in Group 1 vs. 209 ± 269 ml (r 0-1469) in Group 2; p=0.003. Only 4 of the 34 patients in Group 1 did not receive allogeneic blood or blood products during their hospital stay, compared with 19 of 64 in Group 2 (p =0.047). The frequency of complications was similar between the 2 groups, none were due to the study drug. Our conclusion is that -aca is safe and is highly effective in prevention of bleeding and the need for allogeneic blood or blood products in pediatric Neuromuscular patients undergoing PSF.

Introduction

Spinal surgery for correction of deformity typically results in significant blood loss, especially in those patients who have neuromuscular conditions. In addition to the risk of disease transmission, transfusion with allogeneic blood or blood products increases the risk of postoperative infection, and can lead to transfusion reactions or transfusion-related lung injury. Anemia also increases risks and can result in prolonged hospital stays.

Therefore it is desirable to minimize the risk of blood loss and transfusion in these pediatric patients.

In previous studies at this institution have demonstrated the efficacy of the use of epsilon aminocaproic acid ( -aca) (Amicar) for patients undergoing spinal fusion for idiopathic scoliosis (IS). It is now clear that -aca is effective in decreasing perioperative blood loss and the transfusion requirements in IS patients.

Patients with Neuromuscular scoliosis can lose greater volumes of blood with spinal surgery and are more likely than those with Idiopathic Scoliosis to experience prolonged hospital stays and surgical complications.

Edler et al found that neuromuscular patients had an almost seven times higher risk of losing > 50% of their estimated total blood volume during scoliosis surgery. The propensity for increased bleeding has several causes and may be due at least in part to abnormal coagulation, poor nutrition, abnormal liver function, and anticonvulsants.

Brenn et al compared Cerebral Palsy patients with Idiopathic Scoliosis patients undergoing posterior spinal fusion. They found that there was a significant difference in clotting parameters at baseline, although both groups were within the norm. Once 15 % of blood loss had occurred in the CP patients however, PT, PTT became abnormal. In normal circumstances that amount of blood loss would be expected to induce a transient increase in clotting activity. Their findings suggested a functional abnormality of
platelet aggregation in CP patients.

Meert et al found that in neuromuscular scoliosis was the most important predictor of the need for transfusion and that those patients are 7.8 times more likely than IS patients to receive an allogeneic transfusion 31. Low preoperative weight and greater number of vertebrae fused were also significant independent predictors.

Since 1998, it has been the senior author's (GHT) practice to use Amicar in all spinal fusions unless there is a contraindication to it.

Our purpose for this study was to determine whether -aca has a beneficial effect in Neuromuscular patients undergoing posterior spinal fusion. We wished to examine its effect on total perioperative blood loss and to determine if it decreases the requirement for allogeneic blood and/or blood products during the patient's hospital stay.

**MATERIALS AND METHODS**

This was a retrospective case-control study conducted at Rainbow Babies and Children's Hospital. The study was approved by the Institutional Review Board of University Hospitals of Cleveland. The control group consisted of patients who had not received Amicar, the majority of whom had been treated from 1991 to 1998. Since 1998, -aminocaproic acid has been used routinely for spinal fusions at our hospital, thus the study group consisted of patients treated between 1998 and 2006.

Inclusion criteria were: all patients with Neuromuscular scoliosis who had a primary posterior spinal fusion using segmental spinal instrumentation; age at surgery 10 to 21 years old; a standard operative technique and standard postoperative care path.

No patients had prior spinal surgery or had an anterior spinal procedure. The indications for transfusion were hemoglobin of less than 7 gm/dl or cardiopulmonary considerations requiring higher hemoglobin.

Amicar was administered as previously described 11. A loading dose of 100 mg/kg of -aca was administered over 15 minutes at the time of skin incision then as a continuous infusion of 10 mg/kg/hr. The drug was discontinued at the time of skin closure. Amicar is contra-indicated in patients with hypersensitivity to the drug, or who have DIC or who are at risk for thrombosis.

At our institution the Anesthesiology service maintains intraoperative blood pressure between 60 and 70 mm Hg during spinal procedures.

Perioperative blood loss included estimated intraoperative blood loss and postoperative suction drainage. The hemovac drains which are used in every case are removed on the second postoperative day or when drainage is less than 25 ml per 8 hours. Cell-saved blood was calculated separately because its hematocrit is concentrated relative to circulating blood.

The following variables were included in statistical analysis: age, gender, weight, diagnosis of Cerebral Palsy, magnitude of major curve, number of vertebral levels fused, number of patients fused to the sacrum (Galveston technique), surgical time in minutes, total perioperative blood loss, amount of cell-salvaged blood, total transfusion requirement in ml, number of units of PRBCs transfused, exposure to allogeneic blood or blood products including fresh frozen plasma and platelets at any time during the hospital stay. We also examined the hospital stay and complications occurring within the first 6 weeks postoperatively.

Adequate data was available for 96 of the 100 patients who met the inclusion criteria and were treated between treated between 1/1/92 and 9/5/2006 at Rainbow Babies and Children's Hospital. All four of the patients who were excluded due to inadequate documentation had received Amicar during their operation.

There were 34 control patients in the Group 1 (those who did not receive -aca) and 62 patients in Group 2 (those who received -aca).

**Statistical analysis**

All statistical tests were two-tailed with p < 0.05 for significance. Chi-square tests were used unless at least one quarter of the cells had expected estimates of < 5, in which case Fisher's exact test was used.

**RESULTS**

There were no statistically significant differences in age, weight, number of patients with Cerebral Palsy, postoperative curve magnitude, number of vertebral levels fused and in number of patients who had fusion to the sacrum. Operative times were not significantly different although preoperative curve magnitude was significantly larger in Group 2 (-aca).

Intraoperative blood loss was 2194 ± 1626 ml (range 450-7500) in Group 1 (no Amicar) compared with 1125 ± 715 ml (range 165-3800) in Group 2. This difference was highly significant, p=0.0002. Postoperative suction drainage was not statistically different: 903 ± 547 (r 146-2310) in the controls compared with 695 ± 589 (r 0-3410) in Group 2 (p=0.08). The difference in total transfusion volume was highly statistically significant: 1548 ± 962 ml (0-4600) in Group 1 vs. 660 ± 589 (r 156-4540) in Group 2; p <0.001. There was also a significant difference
in the amount of blood returned to the patient from the cell saver: 487 ± 404 ml (r 0-2000) vs. 209 ± 269 ml (r 0-1469) in Group 2; p=0.003.

Only 4 of the 34 patients in Group 1 did not receive allogeneic blood or blood products during their hospital stay, compared with 19 of 64 in Group 2 (p =0.047).

The frequency of complications was similar between the groups. There were no permanent neurological injuries. One patient in Group 2 who had a holosyringomyelia preoperatively had a transient hemiparesis postoperatively. One patient with the diagnosis of spastic quadriplegia in the control group had massive intraoperative blood loss, developed Disseminated Intravascular Coagulopathy and subsequently expired 5 days postoperatively. One patient in Group 2 expired at home 1 month postoperatively, likely as a result of chronic respiratory compromise.

There were no complications attributable to the use of α-aca.

**DISCUSSION**

Various modalities have been used in an effort to reduce blood loss for patients undergoing surgical correction of spinal deformity.

In addition to the Hall-Relton

**TABLE 1: Results**

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (No Amicar)</th>
<th>Group 2 (Amicar)</th>
<th>P value</th>
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<tbody>
<tr>
<td>Patients</td>
<td>34</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>12.3 ± 2.8 (5-8)</td>
<td>14.0± (10-21)</td>
<td>0.009 (t-test)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>37.6 ± 14.4</td>
<td>42.8 ±15.2</td>
<td>0.11 (n.s.)</td>
</tr>
<tr>
<td>Males</td>
<td>27</td>
<td>36</td>
<td>0.04</td>
</tr>
<tr>
<td>Diagnosis CP</td>
<td>16 (47.1%)</td>
<td>35 (56.5%)</td>
<td>0.40 (n.s.)</td>
</tr>
<tr>
<td>Preoperative major curve (°)</td>
<td>55 ± 16 (15-90)</td>
<td>64 ±18(26-110)</td>
<td>0.02 (t-test)</td>
</tr>
<tr>
<td>Postoperative major curve (°)</td>
<td>20 ± 16 (0-57)</td>
<td>23 ±12 (0-58)</td>
<td>0.40 (n.s.)</td>
</tr>
<tr>
<td>Number levels fused</td>
<td>14 ± 2 (5-18)</td>
<td>15±2 (8-19)</td>
<td>0.06</td>
</tr>
<tr>
<td>Fused to sacrum (# patients)</td>
<td>17</td>
<td>38</td>
<td>0.39</td>
</tr>
<tr>
<td>Surgical time</td>
<td>337.7 ± 86 (195-540)</td>
<td>369 ± 84.3 (240-550)</td>
<td>0.09</td>
</tr>
<tr>
<td>Intraoperative EBL (ml)</td>
<td>2194 ± 1626 (450-7500)</td>
<td>1125 ± 715 (165-3800)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Postoperative suction drainage (ml)</td>
<td>903 ± 547 (146-2310)</td>
<td>695 ± 489 (57-2287)</td>
<td>0.08</td>
</tr>
<tr>
<td>Perioperative blood loss (ml)</td>
<td>3055 ± 1852 (800-7912)</td>
<td>1805 ± 940 (156 - 4540)</td>
<td>0.0006</td>
</tr>
<tr>
<td>Total Transfusion (ml)</td>
<td>1548 ± 962 (0-4600)</td>
<td>660 ± 589 (0-3410)</td>
<td>&lt;0.0001 (Wilcoxon)</td>
</tr>
<tr>
<td>Cell Saver (ml)</td>
<td>487 ±404 (0-2000)</td>
<td>209 ± 269 (0-1469)</td>
<td>0.003</td>
</tr>
<tr>
<td>Transfusion with allogeneic blood or products</td>
<td>30</td>
<td>43</td>
<td>0.047</td>
</tr>
</tbody>
</table>

**TABLE 1:**

- Patients: Group 1 (No Amicar) = 34, Group 2 (Amicar) = 62.
- Age: Group 1 = 12.3 ± 2.8 (5-8), Group 2 = 14.0± (10-21), p = 0.009 (t-test).
- Weight: Group 1 = 37.6 ± 14.4, Group 2 = 42.8 ±15.2, n.s.
- Males: Group 1 = 27, Group 2 = 36, p = 0.04.
- Diagnosis CP: Group 1 = 16 (47.1%), Group 2 = 35 (56.5%), n.s.
- Preoperative major curve: Group 1 = 55 ± 16 (15-90), Group 2 = 64 ±18(26-110), p = 0.02 (t-test).
- Postoperative major curve: Group 1 = 20 ± 16 (0-57), Group 2 = 23 ±12 (0-58), n.s.
- Number levels fused: Group 1 = 14 ± 2 (5-18), Group 2 = 15±2 (8-19), n.s.
- Fused to sacrum: Group 1 = 17, Group 2 = 38, n.s.
- Surgical time: Group 1 = 337.7 ± 86 (195-540), Group 2 = 369 ± 84.3 (240-550), p = 0.09.
- Intraoperative EBL: Group 1 = 2194 ± 1626 (450-7500), Group 2 = 1125 ± 715 (165-3800), p = 0.0002.
- Postoperative suction drainage: Group 1 = 903 ± 547 (146-2310), Group 2 = 695 ± 489 (57-2287), p = 0.08.
- Perioperative blood loss: Group 1 = 3055 ± 1852 (800-7912), Group 2 = 1805 ± 940 (156 - 4540), p = 0.0006.
- Total Transfusion: Group 1 = 1548 ± 962 (0-4600), Group 2 = 660 ± 589 (0-3410), <0.0001 (Wilcoxon).
- Cell Saver: Group 1 = 487 ±404 (0-2000), Group 2 = 209 ± 269 (0-1469), p = 0.003.
- Transfusion with allogeneic blood or products: Group 1 = 30, Group 2 = 43, p = 0.047.
frame which decreases abdominal pressure, other intra and postoperative modalities include preoperative donation of autologous blood with or without erythropoietin, the use of a cell saver, acute hemodilution, controlled hypotension, and reinfusion of drained blood.  

In our neuromuscular population, preoperative autologous donation was not feasible for most patients. Other investigators have found that the use of both pre- and intraoperative donation is not useful. Hypotensive anesthesia has been found to be useful for patients with Duchenne's muscular dystrophy. Neuromuscular scoliosis patients often have compromised cardiovascular systems and may be intolerant of hypotension. Hemodilution and hypotension have been associated with ocular complications in spinal surgery. Hypotension can also lead to spinal cord ischemia. Acute intraoperative hemodilution was not used in our patients.

Although some authors have questioned its cost-effectiveness, at our hospital salvage of shed red blood cells is routinely performed with the use of a standard cell-saver. The hematocrit is typically much greater that that of circulating blood so re-infusion of a relatively small volume may be helpful in avoiding transfusion.

Our study included all measurable blood loss and all blood and blood product transfusions during the hospital stay. Other authors have found that postoperative bleeding in patients with idiopathic scoliosis can be the main factor leading to transfusion. In a study of patients with idiopathic scoliosis, Guay et al found that postoperative bleeding could equal up to 77% of EBV. Total blood loss in that study correlated with the number of fused vertebrae and with the duration of surgery.

In a study of scoliosis patients undergoing spinal fusion Kannan, et al found that neuromuscular patients had significantly greater blood loss than idiopathic – in that group there was a median blood loss of 78% of estimated blood volume. Prothrombin time increased over time in both NM and AIS but was higher in the neuromuscular patients both preoperatively and postoperatively. Factor VII activity decreased in both groups but was lower in the neuromuscular group during surgery. This suggested that activation of the extrinsic coagulation pathway occurs in scoliosis surgery. Depletion of clotting factors occurs to a greater extent in patients with underlying neuromuscular disorders. One might therefore expect that in this population prevention of bleeding with medical could be more effective than in Idiopathic scoliosis.

We used a case control study to determine the effect of Amicar on blood loss in patients who had a primary posterior spinal fusion for neuromuscular scoliosis. We have shown that Amicar is an effective agent for reducing blood loss for these patients. The number of vertebral levels fused, number of patients fused to the sacrum, and operative times were comparable between the two groups. The average curve magnitude in the Amicar group was greater than the controls. However, compared with the control group, the Amicar group had half the intra-operative blood loss, less than half the volume of blood in the cell saver and required less than half the transfusion volume. All of these differences were highly statistically significant.

In prior studies at our institution involving patients with Idiopathic Scoliosis, Amicar was found to reduce the overall blood loss, mainly due to a decrease in postoperative drainage. This led to the conclusion that Amicar could potentially induce elevated fibrinogen levels, producing the observed decrease in postoperative suction drainage. In contrast, the present study found that the effect of the drug was entirely due to reduced intra-operative blood loss. Postoperative drainage was not significantly different between the two groups. The reason for the different effect of Amicar in different patient populations is not known.

The classification of patients into a single category of neuromuscular scoliosis inherently results in a heterogeneous study population. Most of the patients in our study had Cerebral Palsy and there was a similar distribution between the groups. One potential limitation of our study however was the greater number of patients with Duchenne's muscular dystrophy in the control group (10 of 34 compared with 5 of 62 in the Amicar group). This could have produced a bias toward greater blood loss, which is associated with that diagnosis. Nonetheless, a separate regression analysis of the data was performed leaving the Duchenne's patients out; the results were unchanged. Therefore, we believe that our conclusions are valid. Amicar has a highly significant effect on blood loss in surgery for Neuromuscular scoliosis, regardless of the diagnosis.

The overall frequency of complications was relatively low and despite the differences in bleeding, was similar between the groups. Pancreatitis, which has been associated with increased blood loss developed postoperatively in one patient in the -aca group, however he was found to also have gallbladder disease.
Other medications have been used to reduce blood loss in children and adolescents undergoing scoliosis surgery.

Prior studies and metanalyses have shown that there does seem to be a benefit to using antifibrinolytics in orthopedic surgery. Antifibrinolytics do not increase clotting but rather decrease clot dissolution.

Aprotinin has been used for several years in cardiac surgery and has also been found to be effective in reducing blood loss in pediatric and adult spinal surgery.

Khoshhal et al conducted a double-blind, randomized prospective clinical trial. Forty-three patients with idiopathic scoliosis underwent spinal fusion and instrumentation and were divided randomly into two groups. Fifteen patients received Aprotinin and 28 received placebo. The Aprotinin group had significantly less blood loss than the placebo group. Although the difference in transfusion rate was 46% less, it was not statistically significant. The authors felt that the benefit of aprotinin in reducing blood loss in spinal surgery for idiopathic scoliosis was consistent.

Cole et al also performed a prospective blinded, randomized controlled trial comparing Aprotinin to placebo in scoliosis surgery. Forty-four “high risk” patients were randomized; all had posterior spinal fusion with instrumentation. The criteria for selection included neuromuscular scoliosis or medical condition that might predispose to bleeding, or reoperation. All had fusion of 7 or more vertebral segments. Mild hypotensive anesthesia was included with the protocol. Blood loss was significantly reduced in the Aprotinin group compared with the controls: 545 ± 312 ml vs. 923 ± 772 ml.

Blood transfusions were fewer in the Aprotinin group. The authors concluded that Aprotinin is useful in selected patients who are at increased risk of bleeding. They also thought that it might be unnecessary to use both Aprotinin and the cell saver together. Interestingly, the majority of the patients in that study, 38 of 44 had neurological or neuromuscular causes for their scoliosis.

Kokoszka et al performed an extensive review of the use of Aprotinin in orthopedic and spinal surgery. They made a “grade-A recommendation” for use of a high-dose Aprotinin regimen in hip and spine surgery. Because of conflicting data, the low-dose Aprotinin therapy as well as the use of Aprotinin in patients with cancer could not be recommended. They felt that high-quality randomized trials are necessary to determine the optimal (and minimal) therapeutic dose of Aprotinin and the optimal time of Aprotinin administration during surgery.

Aprotinin carries some inherent risks and is considerably more expensive than Amicar. At our institution charges are more than $US 1200.00 per 100 ml compared with $ 5.51 per 5 gm for Amicar (about 10 gm would be used for a 6 hour PSF in a 50 kg patient). Aprotinin contains proteins which may cross-react with the components of tissue sealants. Anaphylaxis can occur in 1.8% of those who are exposed to Aprotinin and the risk has been reported to be 5% in patients who had recent previous exposure. It is therefore contra-indicated in patients who have been exposed to it within 12 months. Unfortunately, Aprotinin has recently been associated with increased morbidity and mortality in adult patients undergoing cardiac surgery. Because of these latter concerns in 2006 it was re-labeled by the US FDA as being approved only for use in high risk cardiac surgery.

Tranexamic acid (TXA) has a similar mechanism of action to EACA but is thought to be 6 to 10 times more potent.

TXA has been found to be useful in reducing the need for transfusion in patients undergoing total joint replacement.

Neilipovitz et al conducted a randomized trial of TXA vs. placebo in 40 children undergoing PSF for scoliosis at Children’s Hospital of Eastern Ontario. Mean Arterial Pressure was maintained at 55 ± 5 mm Hg. They found that the total amount of blood transfused in the perioperative period was significantly reduced – by 28% in the Tranexamic acid group. There was no significant difference in blood loss. Only intraoperative blood loss was accounted for and 25 of the 40 patients had secondary scoliosis. There was no difference between the groups in the number of patients not receiving allogeneic blood.

Sethna et al performed a randomized study of 44 pediatric patients undergoing PSF at Children’s Hospital of Boston. Twenty one patients had neuromuscular scoliosis and 22 had idiopathic scoliosis. Eight of the subjects had concurrent anterior and posterior surgery. Hypotensive anesthesia with MAP of 55 to 65 mm Hg was routine in all patients. They determined that Tranexamic acid reduced blood loss by 41% overall compared with placebo. In their study, the dose of TXA was 10 times that used by Neilipovitz et al: a loading dose of 100 mg /kg over 15 minutes followed by continuous infusion of 10mg /kg / hr until skin closure. Patients with secondary scoliosis clearly had benefit from TXA.
THE EFFICACY AND SAFETY OF EPSILON AMINOCAPROIC ACID

whereas those with Idiopathic scoliosis did not. Secondary scoliosis patients had significantly reduction in blood loss (48% less than placebo) and required significantly less transfusion volume. Interestingly, patients with Idiopathic Scoliosis had similar blood loss and transfusion volumes to those in the placebo group. There were no complications attributable to the study drug.

We have not used TXA at our institution, where hospital charges are higher than those for -aca at $162.56 per 1 gram.

Recently in a retrospective study performed in Europe, recombinant factor VIIa was found in a to be effective in reducing intraoperative blood loss in AIS 28. In the USA, the use of recombinant factor VIIa (“Novoseven”) is limited. Currently it is indicated for patients with hemophilia who have inhibitors and it may occasionally be used in cases of severe bleeding, such as might be associated with hepatic or renal failure.

Results of the use of Desmopressin (DDAVP) in patients having surgery for AIS and NM scoliosis have been mixed 27,29,39. A study by Letts, et al it was determined that there was 19% less bleeding with the use of desmopressin in NM patients 29. However, response to DDAVP was variable and it was not possible to predict which patients would benefit from administration of the drug.

CONCLUSIONS

Epsilon aminocaproic acid is highly effective in reducing blood loss in pediatric patients who require spinal surgery for Neuromuscular scoliosis. It reduces intraoperative blood loss and most importantly, the need for transfusion during the entire hospital stay. In our study there was significant reduction in exposure to allogeneic blood and /or blood components in those patients treated with Amicar. During more than 8 years and in over 600 pediatric and adolescent spine fusions, there have been no adverse events attributable to its use.

REFERENCES

2. Alany, A.; Acragoglu, E.; Ozdemir, O.; Ercelen, O.; Bulutcu, E.; and Surat, A.: Effects of desmopressin (DDAVP) in patients having surgery for AIS and NM scoliosis have been limited. Currently it is indicated for patients with hemophilia who have inhibitors and it may occasionally be used in cases of severe bleeding, such as might be associated with hepatic or renal failure.


The distal margin of the radius is formed by three distinct surfaces that articulate with the scaphoid, lunate and distal ulna. Literature largely depicts the volar aspect of the distal radius as smooth with the radial shaft. Volar plates used in the treatment of distal radius fractures have largely been designs following this concept. Surgical fixation of articular distal radius fractures with volar plating has shown loss of fixation of the lunate facet fragment resulting in carpal dislocation. Due to the attachments of the extrinsic ligaments including the radioscaphocapitate, short radiolunate and long radiolunate ligaments, maintenance of this fragment is vital to carpal stability.

Recent literature investigating the volar margin of the distal radius suggests that the lunate facet projects anteriorly with reference to the radial shaft. More detailed inspection of the distal radius articular surface suggests the lunate facet projects in the ulnar direction in conjunction with varying depths of the scaphoid, lunate and distal radio-ulnar joint (DRUJ) fossas. These variations may help to explain the difficulty in obtaining adequate fixation of volar distal radius fragments. The anatomy of the distal radius has been quantified in a small number of published studies.

This study was designed to further quantify the anatomic variations that exist with the volar lunate facet, DRUJ and radiocarpal articular surfaces with anatomic specimens. The major motivation was to further quantify the anterior and ulnar projections of the lunate facet along with the DRUJ anatomy. The hypothesis is that anatomic variations exist with respect to the volar lunate facet and DRUJ anatomy.

Materials and Methods
The Hamann-Todd Collection in the Cleveland Museum of Natural History includes more than 3000 human skeletons, collected in the late nineteenth and early twentieth centuries that have been used extensively for research. 100 adult radii were obtained from this collection for analysis. 50 adult male and 50 adult female specimens were analyzed; 56 Caucasian and 44 African-American. The specimen ages were distributed with a mean age of 60.3 and minimum and maximum ages 25 and 89, respectively.

Specimens were inspected and discarded if any signs of previous fracture or trauma were noted. Quantitative anatomic measurements were made using radial arc calipers and a high-precision digital caliper, accurate to 0.01mm. The dimensions analyzed included the lunate facet extension thickness(1), width(2), and ulnar projection(3); the lunate fossa length(4), width(5) and depth(6); scaphoid fossa length(7), width(8) and depth(9); DRUJ dorso-volar length(10) and depth(11); and radiocarpal articular width(12). (see Fig 1).
fossa length is defined as distance in dorso-volar plane and width as distance in radio-ulnar plane. The depth measurements were determined using radial arc calipers with geometric mathematical calculations. The mean and standard deviations were calculated for each measurement and rounded to the nearest 0.01mm.

RESULTS
The thickness (measurement 1) and width (measurement 2) of the volar lunate facet were 6.70 ± 1.34mm (range 2.00 – 10.13mm) and 14.63 ± 1.79mm (range 10.60 – 18.55mm), respectively. The lunate facet ulnar projection (measurement 3) was 4.95 ± 1.60mm (range 2.51-9.89mm). The average height, width and depth of the lunate fossa (measurements 4, 5 & 6) were 16.48 ± 1.46mm (range 13.2 – 20.32mm), 11.01 ± 1.51mm (range 8.60-16.11mm) and 3.93 ± 0.88mm (range 2.33 – 6.99mm), respectively. The mean scaphoid fossa height, width and depth (measurements 7, 8 & 9) were 13.58 ± 1.19mm (range 10.84 – 16.39mm), 15.90 ± 1.73mm (range 11.44 – 20.32mm), and 3.07 ± 0.59 (range 1.84 – 4.69mm). The radiocarpal articular length was 26.48 ± 2.12mm (range 22.78 – 32.55mm). The dorso-volar distance and depth of the DRUJ articulation were 14.74 ± 1.42mm (range 11.49 – 18.81mm) and 1.74 ± 0.75mm (range 0.22 – 3.36mm), respectively.

The ratio of lunate facet extension thickness (measurement 1) to the total lunate fossa length (measurement 4) was calculated at 0.41 ± 0.07 (range 0.12 – 0.59). Therefore, ~ 41% of the volar lunate facet projects anterior to the smooth volar radial shaft.

The measured data was stratified or sex and race in attempt to further determine population variations. When comparing females to males, all the measured dimensions were smaller with significant p-values < 0.05. This was to be expected due to females having smaller bone size relative to males. When the articular measurements were scaled relative to bone size by dividing by the total radiocarpal width, no significant differences between sexes were noted (p>0.05). With respect to race, no significant differences of the measured values were noted between Caucasian and African-American specimens (p>0.05).

DISCUSSION
The volar lunate facet was found to project on average 4.9mm in the ulnar direction and 6.7mm anterior relative to the volar radial shaft. Further evaluation did not find statistically significant differences relative to race or sex when the measurements were scaled. The population of distal radii evaluated should be adequate to quantitatively approximate the lunate facet and DRUJ anatomy. Both clinical experience and a literature review have shown the difficulty in fixation of the volar extension fragment. Plate configuration with an anterior bend and slightly ulnar placement would likely provide better capture of the volar lunate facet extension.

Distal plate edge configurations are largely flat in nature making a proper fit difficult when attempting to control the distal volar lunate facet extension. In addition, attachments of the vital radiolunate ligaments are present in this region. Placing a plate at the very distal aspect may cause an increased risk of joint penetration with screw fixation. Additionally, the DRUJ depth in the radio-ulnar plane was found to average 1.7mm. The most ulnar distal screw may need to deviate in the radial direction to avoid DRUJ infringement.

Various innovative implants attempting to provide an increased ability to capture the volar lunate facet extension are available with unclear success to date. Alternative fixation techniques are available to capture the lunate extension fragment including a suture loop through the short radiolunate ligamentous origin or the use of K-wires to capture the fragment. Techniques using arthroscopically assisted reduction and percutaneous fixation have also been described. The significant volar extension and close proximity to both the radiocarpal and DRUJ articular surfaces make sufficient capture of the volar extension difficult during volar plating procedures.

REFERENCES
THE EFFECTS OF AGE AND GENDER ON THE KINETICS OF FATIGUE CRACK PROPAGATION IN HUMAN CORTICAL BONE

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ABSTRACT

The objectives of this study were to examine the effects of age and gender on the kinetics of fatigue crack propagation (FCP) of human cortical bone.

There were four test groups: younger male, younger female, older male, older female. Longitudinal compact tension specimens were cyclically loaded wet at 37ºC with an R-ratio= 0.1 at 2 Hz. Fatigue crack growth rate, da/dN (m/cycle) versus the cyclic stress intensity, ∆K (MPa√m), was determined and evaluated by linear regression. An analysis of microdamage above and below the fracture plane was performed on all tested specimens to determine differences in microdamage patterns between groups.

All specimens exhibited stable crack growth. Fatigue crack propagation (FCP) resistance was comparable for the Younger Male and Younger Female bone, while the Older Male bone was significantly less resistant to FCP than the Younger Male bone. The FCP resistance of the Older Female bone was significantly reduced compared to all other groups. In addition, there was less microdamage in the older female group than in the other age-gender groups.

The results of this study suggest that resistance to fatigue crack growth in human bone may be diminished with age.

INTRODUCTION

Many factors have been shown to affect the monotonic mechanical properties of cortical bone, including orientation, composition, age, and gender (Burstein, 1976; Jepsen, 1996; Martin, 1998; Zioupos, 1998, 2000). It has been reported that the tensile elastic modulus, yield stress, ultimate stress, ductility, and work-to-fracture decrease while the post-yield slope increases with age of cortical bone for both genders. Also, it has been shown that the fracture toughness of human cortical bone is dependent on orientation, composition, and age (Norman et al., 1996; Phelps, 2000).

Monotonic loading is clinically relevant to overloads, however, repetitive loading is also clinically important. Many of the fractures in older adults occur from non-traumatic loading conditions related to the activities of daily living. Another health concern is stress fractures, which are partial or complete fractures in normal bone as a result of repetitive loading. These fractures occur in, among others, young military recruits and athletes who undergo strenuous and repetitive physical training regimens (Lee, 2000b). The kinetics of the growth and formation of the macrocracks that result in stress fractures are not well known.

The resistance to fatigue crack growth from a defect or stress concentration can be evaluated by conducting fatigue crack propagation tests in which the relationship between the cyclic fatigue crack growth rate (da/dN) and the cyclic stress intensity factor at the crack tip (ΔK) is determined (Hertzberg, 1996). Wright and Hayes (1973) demonstrated that stable fatigue crack growth occurred in bovine bone and that the Paris relationship could be used to describe fatigue crack growth at crack growth rates above Regime I. More recently, Shelton et al. reported on stable fatigue crack growth in equine cortical bone (Shelton et al., 2003). We have previously reported on the effect of gamma radiation sterilization on the fatigue crack propagation resistance of human cortical bone (Mitchell, et al., 2004).

The objective of this study was to further examine the fatigue crack propagation in human cortical bone using a fracture mechanics approach. Because age and gender have been shown to affect the monotonic mechanical behavior of human cortical bone (Carter, 1977; Currey, 1996; Hertzberg, 1996; Jepsen, 1996;
Norman et al., 1996; Yeni, 1997; Boyce et al., 1998; Phelps, 2000) it was hypothesized that age and gender would also influence the kinetics of fatigue crack propagation.

**MATERIALS AND METHODS**

Human femora pairs, with no known skeletal pathologies, were obtained from the Musculoskeletal Transplant Foundation (Edison, NJ) and the Anatomical Donations Program at the University of Michigan Medical School (Ann Arbor, MI). A subset of these donor groups had also been used in our study of the effect of radiation sterilization on fatigue crack propagation resistance (Mitchell, 2004). The donor groups were: Younger Female (A) (15yrs; 1 pair, n=5 specimens), Younger Female (B) (33yrs; 1 pair, n=5 specimens), Younger Male (A) (18yrs; 1 pair, n=7), Younger Male (B) (31yrs; 1 pair, n=5), Older Female (A) (75yrs; 1 pair, n=4), Older Female (B) (71yrs; 1 pair, n=5), Older Male (A) (61yrs; 1 pair, n=5), and Older Male (B) (60yrs; 1 pair, n=6), and Older Male (C) (61yrs; 1 pair, n=5).

Compact tension specimens (width = 14mm, thickness = 3mm, initial crack length = 3.75mm, based on recommendations from ASTM E399 (1990) and ASTM E647 (1996) were wet-machined with a CNC mill (Denford MicroMill 2000, Medina, OH) from the lateral and medial portions of the mid-diaphysis with the notch for crack growth parallel to the long axis of the femur. The tip of the notch was then razor sharpened in 5µm increments for a total distance of 250µm using a microtome (American Optical Co., Buffalo, NY). One surface was then polished using incrementally decreasing alumina solutions (finishing with a 0.05 µm solution) to result in a near mirror finish to aid in crack tip visualization. Specimens were kept wet frozen at -20 °C until testing.

The specimens were subjected to load controlled fatigue crack propagation testing in a servohydraulic-testing machine (Instron 8501M, Canton MA). The R- ratio (Pmin/Pmax) was 0.1 and the loading frequency was 2 Hz. The specimens were kept wet by a constant drip of distilled water at a physiologically relevant temperature of 37°C. All specimens were cycled to failure and kept wet frozen at -20 °C until preparation for histological and compositional analyses.

As the crack propagated, testing was stopped momentarily so that the crack length, a (mm), and the corresponding number of loading cycles, N, could be recorded. The coordinates were measured using a traveling microscope (Gaertner, Skokie, IL) with a lens objective of 10X that was mounted on an x-y stage. If there was crack branching or if there were two or three cracks ahead of the notch, the crack length was measured as the farthest crack tip from the load-line. The change in crack length per cycle, da/dN, was calculated using the backward difference method (Neter and Wasserman, 1974): 

\[
\frac{da}{dN} = \frac{(a_i - a_{i-1})}{(N_i - N_{i-1})}
\]

Based on crack length (a), specimen geometry, and cyclic stresses, \(\Delta K\) was calculated as: 

\[
\Delta K = \left(\frac{P_{\text{max}} - P_{\text{min}}}{B\sqrt{W}}\right) \left(\frac{2 + \frac{a}{W}}{1 - \frac{a}{W}}\right)^{1/2} \left(0.886 + 4.64 \left(\frac{a}{W}\right) - 13.32 \left(\frac{a}{W}\right)^2 + 14.72 \left(\frac{a}{W}\right)^3 - 5.6 \left(\frac{a}{W}\right)^4\right)
\]

where \(P_{\text{max}}\) and \(P_{\text{min}}\) are the maximum and minimum cyclic loads, respectively, B is the thickness of the specimen, W is the distance from the load line to the back edge of the specimen, and \(a\) is the average total crack length at that particular number of cycles.

The specimen data were pooled in a single test group for statistical comparison between test groups. Linear regression of \(da/dN = C\Delta K^m\) was conducted for each group and differences in the exponent, m, and the coefficient, C, were examined using the linear test method (Neter and Wasserman, 1974) with p<0.05 as significant (Minitab Release 13, Minitab Inc., State College, PA). For purposes of comparison of fatigue crack propagation resistance between groups, the coefficient, C, was of primary interest. A significant change in C would result in a shift in the log da/dN versus log \(\Delta K\) behavior. The relative fatigue crack propagation resistance between groups was considered with respect to differences in the coefficient (C) and the exponent (m).

The nature of fatigue crack propagation in human cortical bone was considered in light of intrinsic crack growth mechanisms that occur ahead of the crack tip and extrinsic mechanisms that act behind the crack tip (Ritchie, 2000). Additional compact tension specimens were machined from the anterior portion of the cortical rings from donors [YM(A), n=1 and OM(A), n=2] to be used for observing and recording crack growth behavior. No fatigue crack growth measurements were taken for these three specimens.
A video microscopy recording system was used to monitor and record crack growth behavior during testing.

Wet density, dry density, water content, ash content, and organic content were determined. Student’s t-test was used for comparison between test groups (p<0.05). All the data for each parameter were then pooled together and the mean and standard deviation was determined for each test group. The densities and contents were calculated using published methods (Akkus, 2000; Knott, 2000; Akkus, 2001).

A portion of each specimen from representative age and gender groups (one donor from each age and gender) remaining after tissue characterization studies was bulk stained in basic fuchsin and embedded in polymethylmethacrylate for sectioning in preparation for histological analysis following established methods (Lee, 2000a; Lee, 2000b). Microdamage analysis was conducted perpendicular to the plane of crack growth and parallel to the direction of crack growth using a Bioquant Advanced Image Analysis system (2000 R&M Biometrics, Inc.) and epifluorescence microscopy with a Texas red filter. The width of the zone of microdamage was measured every 0.1mm from the notch up to failure. For all test groups, only specimens from one donor were evaluated.

RESULTS

Stable cyclic crack growth occurred in every specimen that was tested. Generally, a fatigue crack grew in an alternating accelerating and decelerating manner (Figure 1). The decelerations appeared to be related to crack branching, to the crack tip encountering an osteon boundary, or to the crack tip encountering a vascular channel or other form of porosity. If there was crack branching or a discontinuity in the crack growth path, these appeared to combine together before catastrophic failure. A region of damage was visually observed at the crack tip just prior to crack advancement.

The four groups were compared to investigate possible influences of age and gender (Table 2). Younger Female
bone had similar exponent m and coefficient C values compared with Younger Male bone (Figure 2). So, the overall fatigue crack propagation resistance of these two groups was comparable. Comparison of the Older Female bone with the Older Male bone demonstrated a significantly higher C and no significant change in m (Figure 4), resulting in a reduced fatigue crack propagation resistance. Comparison of the Older Female bone to the Younger Female bone demonstrated C was significantly increased, resulting in a reduced fatigue crack propagation resistance compared with the Younger Female bone (Figure 5).

Comparing changes between test groups as a function of the variables of age and gender, the largest magnitude of difference in shifts in fatigue crack propagation resistance occurred when comparing male bone groups (YM versus OM) and female groups (YF versus OF) (Figure 6). That is, the female bone groups demonstrated a greater shift (change in coefficient C) in fatigue crack propagation resistance than the male bone groups with age.

At all fatigue crack growth rates, a region of deformation and/or damage was observed to develop ahead of the crack tip prior to crack tip advance. In this regard, the fatigue crack growth process was observed to occur in incremental jumps, rather than in a continuous manner. Crack branching was observed to occur around microstructural barriers such as osteons, and crack tip blunting at pores or vascular channels was also observed. The arrest or deviation of the fatigue crack tip at voids or osteon boundaries appeared to influence the alternating acceleration and deceleration fatigue crack growth behavior that was observed in human cortical bone. At intermediate fatigue crack growth rates (1x10^-7 m/cycle - 1x10^-4 m/cycle), new cracks were observed to form ahead of the original crack. This was observed

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Figure 3. Fatigue crack propagation behavior for Older Female (n=5+4) and Older Male (n=5+5+6) cortical bone.

Figure 4. Fatigue crack propagation behavior for Younger Male (n=7+5) and Older Male (n=5+5+6) cortical bone.
the original crack was temporarily arrested due to encountering an osteon or cement line. The consequence of the formation of new cracks ahead of
the original crack was that there were lamellar bridges of cortical bone tissue connecting these cracks behind the advancing crack tip. In some cases,

when the original crack was temporarily arrested due to encountering an osteon or cement line. The consequence of the formation of new cracks ahead of
when the original crack was temporarily arrested due to encountering an osteon or cement line. The consequence of the formation of new cracks ahead of

the lamellar bridges persisted during fatigue crack growth and were only disrupted by fast fracture (Figure 7). Near unstable fracture, fatigue crack growth was dominated by fast fracture mechanisms (e.g., multiple individual cracks) leading to catastrophic failure.

Overall, with the exception of the Older Female bone, there were few significant differences found in the measured compositional parameters between test groups (Mitchell, et al., 2004). There was no significant difference in any compositional measurement between Younger Female and Younger Male bone, and between Older Female and Older Male bone. Older Female bone was significantly lower in water content and dry density compared to Younger Female bone. Older Female bone was only significantly decreased in water content compared with the Younger Male bone.

Microdamage thickness increased significantly with increase in $\Delta K$ for the Younger Male (A), Younger Female (A), and Older Male (A) bone groups. For the Older Female (A) bone group, microdamage thickness did not change with increase in $\Delta K$.

**DISCUSSION**

There have been few studies to examine the kinetics of fatigue crack growth in human cortical bone (Mitchell et al., 2004, Vashishth et al., 1994). The exponents for the Paris relationship varied little between the age/gender groups, ranging from $m = 4.72$ to $5.45$. This finding may be reflective of similar fatigue crack growth mechanisms in the age/gender groups. The range of the exponent $m$ in this study is somewhat higher than that which has been reported for FCP behavior of bovine bone ($m = 2.8$ to $5.1$) and somewhat lower than that reported for equine bone ($m = 10.4$) (Wright and...
Hayes, 1976; Shelton et al., 2003). In the equine bone study, the fatigue cracks were grown transversely, as opposed to longitudinally, which was the crack growth orientation in this study and in the bovine bone study. Crack growth orientation relative to the bone microstructure will likely affect FCP behavior. In addition, there are microstructural differences in bovine, human and equine bone which may also influence FCP behavior.

The results of this study support the hypothesis that age and gender have a significant effect on the fatigue crack growth. Human cortical bone exhibited stable fatigue crack growth along the longitudinal axis of the femur in all age and gender groups examined. In this study, Older Male and Older Female human cortical bone had reduced fatigue crack resistance when compared to Younger Male and Younger Female human cortical bone.

A limitation of this study is that the fatigue crack growth data was assumed to have been collected primarily in the Paris regime. That is, it was not possible to definitively identify a threshold regime from the Paris regime for the individual data sets or for the pooled data sets (Hertzberg, 1996). Linear regression was therefore conducted on all the experimental human fatigue data in a data set for purposes of characterization of fatigue crack growth behavior and statistical comparison between groups. This approach allowed for relative comparisons between age/gender groups. Another limitation of this study was the small and unequal number of independent donors in the various tests groups (YF n=2, OF n=2, YM n=2, OM n=3). Thus, while significant differences in fatigue crack propagation resistance were seen between age and gender groups, the results must be validated with a larger number of donor femora per test group. This study also only evaluated fatigue crack growth behavior in the longitudinal orientation; fatigue crack growth in the transverse orientation may be more clinically relevant, particularly with respect to clinical stress fractures (Belkin, 1980).

Fatigue crack growth in human cortical bone can be considered in light of extrinsic damage and toughening mechanisms (Ritchie, 2000). In this study, development of a region of deformation in front of the crack tip during cyclic loading was observed. This was likely a region of microdamage in the form of microcracks and diffuse damage that accumulated in the bone tissue ahead of the crack tip during cycling. The next increment of crack growth thus likely occurred through this region of accumulated microdamage after it attained some critical level of damage. The histological findings of microdamage above and below the crack plane of the fatigue specimens following testing support this viewpoint.

Observations of a damage zone ahead of the crack tip in bone have

Figure 7. Series of microstructural bridging captured immediately before catastrophic failure.
Table 1. Exponent, coefficient, and correlation coefficient, $R^2$, for $da/dn$ vs. $\Delta K$ for each age and gender group.

<table>
<thead>
<tr>
<th>Test Groups</th>
<th>Exponent</th>
<th>Coefficient</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Younger Male</td>
<td>5.04</td>
<td>1.48E-8</td>
<td>0.384</td>
</tr>
<tr>
<td>Younger Female</td>
<td>5.30</td>
<td>1.50E-8</td>
<td>0.571</td>
</tr>
<tr>
<td>Older Male</td>
<td>5.45</td>
<td>3.37E-8</td>
<td>0.503</td>
</tr>
<tr>
<td>Older Female</td>
<td>4.72</td>
<td>1.67E-7</td>
<td>0.525</td>
</tr>
</tbody>
</table>

Table 2. P-values for comparisons of gender and age conditions for log $da/dn$ vs log $\Delta K$.

<table>
<thead>
<tr>
<th>Test Groups</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exponent</td>
</tr>
<tr>
<td>Younger Male vs Younger Female</td>
<td>0.401</td>
</tr>
<tr>
<td>Younger Male vs. Older Male</td>
<td>0.285</td>
</tr>
<tr>
<td>Younger Male vs. Older Female</td>
<td>0.436</td>
</tr>
<tr>
<td>Younger Female vs. Older Male</td>
<td>0.032*</td>
</tr>
<tr>
<td>Younger Female vs. Older Female</td>
<td>0.982</td>
</tr>
<tr>
<td>Older Male vs Older Female</td>
<td>0.039*</td>
</tr>
</tbody>
</table>

P values refer to a comparison of each age-gender test group.

*Significance taken at p < 0.05.

THE EFFECTS OF AGE AND GENDER

been made by other researchers under monotonic loading conditions (Zioupos et al., 1996; Vashishth et al., 1997; Zioupos, 1998; Ritchie, 2000; Nalla et al., 2003; Nalla et al., 2004). For example, Zioupos et al. (Zioupos et al., 1996) reported on observations of a “plastic” deformation zone at the crack tip in fracture toughness tests conducted on cortical bone from the mid-diaphyseal region of human femora. They attributed this plastic deformation zone to pre-initiation of microcracks in the vicinity of the notch.

The extent of microdamage above and below the fracture surface increased with an increase in $\Delta K$ (except for the Older Female bone). The Older Female group had significantly less damage compared with the other bone groups. Taken together with the finding that the Older Female group had the worst fatigue crack propagation resistance, these results suggest that the ability to generate damage at the crack tip is an important intrinsic mechanism which retards fatigue crack growth in human bone.

The crack branching of macrocracks in this study was observed around microstructural barriers such as osteons and crack tip blunting at pores or vascular channels. These findings are similar to previous research on microcracks that demonstrated deceleration and sometimes arrest of growth upon encountering microstructural barriers such as osteons or cement lines (Akkus, 2000).

The lamellar bridging observed to occur during cyclic loading of human cortical bone is another extrinsic toughening mechanism that has been observed under monotonic loading conditions in dentin and human bone (Nalla et al., 2003; Nalla et al., 2004). Microstructural parameters in engineering materials have been found to have a varying degree of influence on the extrinsic crack growth mechanisms (Ritchie, 2000). In this regard, the Older Female bone was observed to have noticeably less lamellar bridging compared with the other bone groups. The fact that this extrinsic toughening mechanism did not appear to be present in the Older Female bone group likely contributed to its reduced fatigue crack propagation resistance.

The results of this study suggest that the fatigue crack propagation resistance of human cortical bone is significantly affected by both age and gender. Fatigue crack propagation resistance was significantly decreased for Older Male and for Older Female bone, compared with their younger bone counterparts, in that the coefficient $C$ was significantly increased in older bone. These results are consistent with reports that older bone has a reduced resistance to fracture from a
stress concentration or defect in that the static fracture toughness ($K_I$) is reduced (Schaffer et al., 1995; Yeni, 1997; Zioupos, 1998). The magnitude of reduction in fatigue crack growth resistance between the Younger Female and the Older Female bone groups was significantly larger than the magnitude of reduction in fatigue crack growth resistance between the Younger Male and Older Male bone groups. Other compositional parameters not evaluated in this study, such as porosity and collagen content and quality, likely also contribute to the observed decreased fatigue crack propagation resistance with aging (Zioupos, 1999; Wang et al., 2000). The influence of age and gender related differences in ultrastructure and microstructure as well as their respective contributions to the fatigue crack propagation resistance of human cortical bone remains to be fully elucidated.

**ACKNOWLEDGEMENTS**

This study was supported by NIH Grant AG17171, the National Science Foundation Graduate Fellowship, Case Prime Fellowship Program, and the Allen Fellowship. We thank Mr. Jay Bensusan for his contributions in the experimental testing and custom fixture design. We also thank Adam Ratzel and Sara Gencur for their assistance in compositional measurements, and Teresa Pizzuto for her assistance with the histology.

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ABSTRACT

Fibroblasts are critical to scar tissue formation and strengthening. Thus, insufficient fibroblast number and activation are thought to play a significant role in the failure of healing in chronic wounds. The goal of our study was to determine the extent to which fibroblast proliferation might be accelerated by electrical stimulation, in addition to waveform optimization for wound treatment. Our initial objective was to develop a high-throughput stimulation platform to distribute electrical current through a cell culture in a non-uniform manner, representative of the situation found in vivo. To assess the relationship between various waveform types, including microcurrent and millicurrent waveforms, both balanced and unbalanced waveforms have been tested. In the current model, fibroblast proliferation did not appear to be stimulated by either millicurrent or microcurrent pulsed stimulation.

INTRODUCTION

Physiological wound healing is a continuous, ordered process, which is characterized by three overlapping phases: inflammation, tissue formation, and tissue remodeling, which includes scar formation. Each stage is governed by different cell types, soluble mediators, and extracellular matrix. Both dermal and epidermal components of skin must be repaired in cutaneous wounds. Fibroblasts along with the structural matrix components they secrete make up granulation tissue which eventually fills the wound cavitation. This serves as a temporary tissue bed, upon which reepithelialization can occur. In the early stages of tissue repair, during which cell migration into the wound cavitation is necessary, fibroblasts initially secrete proteins which form a provisional matrix upon which cells can adhere and migrate. These proteins include fibrin, fibronectin, and hyaluronic acid. In the later stages, as the more permanent tissue is formed, various types of collagen replace other matrix molecules, and ultimately become the main component of scar tissue. Fibroblasts play another unique role in that at about two weeks post-injury, they may assume another phenotype known as myofibroblasts. In this form, they possess some similarities to muscle cells and are thought to play a role in wound contraction (1).

Dermal wounds are typically classified as chronic when they persist for more than three months, and such wounds may never heal without proper treatment (2). It is reported that in the United States alone, 6.5 million people suffer from pressure ulcers, one class of chronic wound (3). Globally the cost of treatment is estimated to be $13 billion, with 7% growth annually (4). Significant factors leading to chronic wound development include prolonged pressure, shear force, excess moisture, and tissue ischemia (5). The combination of these factors can cause necrosis of skin and subcutaneous tissues. These complications are especially prevalent among those with limited mobility such as individuals with hip fractures, spinal cord injury (SCI), diabetes mellitus, or venous stasis. One study reported that individuals with hip fractures accounted for 47% of all incidental pressure ulcers (6). Currently treatment modalities for chronic wounds are limited, and include procedures such as debridement.

Electrical stimulation (ES) has been shown in-vitro to influence a variety of regenerative physiological processes relevant to dermal wound healing including vascularisation, angiogenesis, bacterial load reduction, collagen matrix production, and fibroblast cell proliferation and activation (7; 8; 9; 10; 11). In clinical settings, ES has not been widely implemented as a standard due to conflicting results, and limited understanding of the mechanisms by which ES may facilitate wound healing. Bourguignon’s group showed that high voltage pulsed stimulation (HVPC) could increase fibroblast proliferation (12). They also determined optimal voltage stimulus, which is specific to their model, and difficult to translate to...
clinical settings. In the current study, our goal was to develop a methodology to determine optimal parameters for fibroblast proliferation which could be translated to clinical wound therapy.

**MATERIALS AND METHODS**

**Cell culture**
Normal human dermal fibroblasts were obtained from adult tissue preps and expanded through typical cell culture techniques. These were then frozen and stored in liquid nitrogen. Once thawed, cells were cultured in DMEM with 10% FBS, 2% L-glutamine, and 1% pen-strep. Cells were used between passages two and six for all experiments.

**Outcomes measures**
Cells were thawed and synchronized by culturing until confluent, then plated at 5000 cells/well in the test platform. Cell number was indirectly measured with Alamar Blue (Fisher Scientific, Waltham, MA). Alamar Blue is non-toxic, allowing multiple time-points to be read for each culture. When placed in the media of metabolically active cells, the active component, resazurin is reduced to resorufin, which fluoresces at 590 nm (13).

Baseline readings were taken 24 hours after plating using a Genios Pro spectrophotometer (Tecan Systems, San

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**Figure 1.** Custom 24-well plate lid. Sterilizable custom lid design (a) and complete setup (b) allows reuse between experiments and delivery of ES to cultures while maintaining culture sterility.

**Figure 2.** Cell response to stimulus. Addition of bFGF to fibroblast cell cultures at 20 ng/ml increased Alamar Blue fluorescence 9%. N=3; ANOVA p<.01

**Figure 3.** Fibroblast cell counts with bFGF. Treatment of fibroblasts with bFGF at 20ng/ml increased the number of cells by more than 200%.
Jose, CA) and repeated at designated intervals post-stimulation, dependant on the specific test. The percentage increase in cellular metabolism compared to baseline was determined for each well, and treated wells were compared to untreated wells using one-way ANOVA analysis (Excel, Microsoft, Redmond, WA).

Electrical stimulation test platform
In order to make the assay high-throughput, 24-well dishes (Corning Incorporated, Corning, NY) were chosen as the test platform and a custom sterilizable lid was designed which allowed customized 316L stainless steel (EPI, New Berlin, WI) electrodes to pass through slots into the cell cultures. Three wells of each column were connected together in order to ensure an n=3 for each test parameter, as connecting the wells in series guaranteed that the same current would be delivered to each cell culture (Figure 1). Stimulation was monitored using an Urdaq data acquisition board (Cyberresearch, New Haven, CT), capable of sampling at up to 250 kHz.

Electrical stimulation parameters were derived from the literature (8). The NPS-IV stimulator (Case Western Reserve University’s Technical Development Laboratory) employed in the model can stimulate 16 independently programmable channels capable of delivering balanced-charge, microsecond pulses with amplitudes from 1 to 21 mA and frequencies between 12 and 25 Hz. In order to deliver current in the microampere range, shunt resistors were used across the anode and cathode to pass excess current around the well plate. Figure 4 shows the NPS-IV capabilities in the three variable dimensions, and the points tested covering the parameter space.

For unbalanced waveforms, a laboratory stimulator also developed by Case Western Reserve University’s Technical Development Laboratory was used. Nearly identical amplitudes as were tested with the NPS-IV were produced with the laboratory stimulator, and frequencies up to 100 Hz were tested.

Three wells containing cells were designated as positive controls and did not receive ES. Two wells were also left unseeded and used as negative controls. Both controls were identical to treated wells in all other ways.

Treatment groups were stimulated for 0.3, 2, 6, or 24 hours and readings were taken on both treatment and control groups after 12, 24, 48, or 96 hours.

Positive controls
In order to verify the ability of the cells to respond to stimulus, as well as the correlation of the Alamar Blue assay to cellular proliferation, cells were cultured with bFGF at 20 ng/ml.

In order to verify that Alamar Blue fluorescence correlates to cell number and therefore proliferation, the same comparison was carried out on a larger scale, in 10 cm culture dishes (Thermo Scientific, Waltham, MA). After treatment, cell counts were performed by hemocytometer.

RESULTS
On day 4, Alamar Blue fluorescence was significantly greater for cultures stimulated with bFGF (Figure 2).

In order to correlate the Alamar Blue metabolic assay to cell number, cell counts were performed in 10 cm dishes. Day five cell counts showed an increase of more than 200% with treatment of bFGF at 20 ng/ml (Figure 3), showing that Alamar Blue fluorescence correlates to cell number.

For all ES patterns tested, proliferation was statistically equivalent to controls for confidence levels of p<0.05.

DISCUSSION
In the current study our initial objective was to develop a high-throughput test platform for evaluation of the effects of ES on fibroblast proliferation. This platform could then be employed to determine the optimal stimulation parameters to maximize fibroblast proliferation. Fibroblast proliferation was chosen because of the crucial role of fibroblasts in physiological wound healing of wound contraction, and matrix synthesis.

Electrical stimulation has great potential for treating the significant complications of chronic dermal
wounds. Breach of the highly resistive keratinocyte layer produces an endogenous wound current as actively transported Na+/K+ ions (14) diffuse down established gradients. It is believed that such currents actively guide the healing process, and studies have shown both in the dermis and the cornea, that stimulating ion transport increases both the cell number and cell alignment at the wound edge, while blocking ion transport inhibits healing (14). Therefore, the rational for applying ES is to induce ion flow, recapitulating the endogenous current found in healing wounds.

ES parameters were chosen based on values reported in literature for in-vivo testing, as well as studies using voltage stimulation sources (8; 12). The current study showed that fibroblast proliferation was not accelerated by the selected waveforms. Our findings imply that fibroblast proliferation may not be the primary outcome of ES under in-vivo testing. However, it can not be conclusively stated that electrical stimulation does not influence proliferation or wound healing.

Our model may be limited by the monolayer design, since the culture medium is much less resistive than the cell membranes. This results in electrical current flow parallel to the monolayer, in which the majority of the current passes above the cells. Uniform field parameter optimization may, however, allow the elucidation of the mechanism by which ES effects proliferation. The multiwell platform developed will be modified to produce uniform electric fields by using hanging membrane inserts.

Models employing single cell types may not be directly translatable to in-vivo models or clinical applications, where current densities are complex functions of tissue and electrode geometry and properties. This finding is supported by Sullivan et al (15) who reported that only 57% of in-vitro wound healing studies showed concordance with the same interventions applied in humans. Therefore a more realistic in-vitro model based on the co-culture of human dermal microvascular endothelial cells and human dermal fibroblasts (16) will be investigated. Real-time PCR will be utilized to quantify the effect of ES on genes vital to wound healing, including Collagen IV.

REFERENCE
ENHANCED IN VIVO BONE FORMATION BY MESENCHYMAL STEM CELLS AFTER IN VITRO OSTEOSTTGIC INDUCTION.

In-Hwan Song1,2,3, Arnold I Caplan2, James E Dennis1,2

1Department of Orthopedic and 2Skeletal Research Center Case Western Reserve University Cleveland OH USA, 3Department of Anatomy College of Medicine Yeungnam University Daegu Korea

ABSTRACT

Bone formation with Mesenchymal Stem Cells (MSCs) is one of relevant clinical application. Bone grafting is the current standard of care for treatment of fracture non-unions, while alternative strategies such as the use of BMPs and bone marrow or concentrated bone marrow are also being used. It is likely that these marrow treatments rely on the presence of MSCs, and it is also known that dexamethasone (dex) treatment is known to induce osteogenic differentiation of MSCs in vitro. The current study attempts to determine the optimal duration of dex treatment for osteoblast differentiation of MSC in vitro and evaluate the effect of dex pretreatment for ectopic bone formation in vivo.

To determine the optimal dex treatment duration, MSCs were cultivated in the osteogenic media for 5 weeks with a varying dex withdrawal schedule such that MSCs were exposed to dex for either 0, 1, 2, 3, 4 and 5 weeks. During this period, weekly alkaline phosphatase, calcium, and DNA assays as well as von Kossa staining and morphological observations were recorded. One and two week dex-treated groups returned to control levels rapidly, whereas 3 and 4 weeks groups retained higher levels of differentiation markers with the 4 week group higher than the 3 week group. Based on these in vitro results MSCs (with and without dex) and a control fibroblast group were seeded into ceramic cubes, incubated for 4 weeks in medium, and then implanted into SCID mice, subcutaneously, and harvested 6 weeks for histologic evaluation of bone formation. There was no bone formation in fibroblast-seeded negative control (Cube Score 0), little bone formation in control (CS 1) and extensive bone formation (CS 3-4) in dexamethasone treated group.

These results indicate that MSCs with dexamethasone pretreatment produce more bone formation and untreated controls.

INTRODUCTION

Human bone marrow-derived mesenchymal stem cells (MSCs) are a rare population of undifferentiated cells that have the capacity to differentiate into mesodermal phenotypes, including osteocytes, chondrocytes, and adipocytes in vitro3,4, and have the capacity to form bone in vivo6,7,8. MSC cultures are suitable for use as an in vitro model to study osteoblast activity and differentiation and have the potential to be used for bone tissue engineering1,8. Tissue engineering of bone requires three primary components; osteogenic cells, factors that support osteogenic cell growth and differentiation, and an osteoconductive scaffold. The degree to which the factors and the scaffold contribute to osteogenesis is variable, while the cellular component is clearly essential.

Bone grafting is the current standard of care for treatment of fracture non-unions. Autograft has the limitation of available tissue mass and requires there is donor site morbidity. Allograft has the potential risk of immune rejection and pathogen transmission. Therefore, autologous MSC transplantation is a reasonable alternative strategy to bone graft. Few reports, however, have addressed methods to boost the osteogenic potential of MSCs for ectopic bone formation, and there is controversy as to whether direct stimulation of osteogenesis is the most effective means of forming more bone1 or if an increase in MSC number prior to differentiation, either by adding more cells or promoting MSC expansion, would be more effective. Still, others indicate that indirect effects on the tissue milieu, such as the secretion of cytokines to promote vascularization might be more effective. The current study attempts to determine the duration of dexamethasone treatment for osteoblasts differentiation of MSCs in vitro and test the effect of dexamethasone pretreatment on ectopic bone formation in vivo.
MATERIALS AND METHODS

Cell Culture
MSCs were isolated from aspirated iliac crest of normal human donors (age 17 to 58 years) after informed consent. Using methods modified from those described previously. Briefly, 10 ml of marrow was added to 20 ml of Dulbecco’s modified Eagle medium with low glucose (DMEM-LG; Sigma, St Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS, Gibco-BRL, Rockville, MD, USA) from selected lots of selected lots and centrifuged at 3.0 × 10^3 per cubic centimeter for continued passage. Second passage cells were used for experimentation.

An initial experiment was done over a 3 week (mid-term) time span, and a subsequent experiment was done over 5 weeks (long-term). Osteogenic supplements for mid-term culture (OS-1), included 100 nM dexamethasone (Sigma), and 50 µM ascorbate-2-phosphate (As-2-P, Wako Chemical, Osaka, Japan) and osteogenic supplements for long-term culture (OS-2), added another 10 mM beta-glycerophosphate (βGP, Sigma).

Three experimental groups were investigated: Basal (Bas), Control (Con), and dex treatment groups. Bas cells were cultivated cells with DMEM-LG with 10% FBS only, Con groups cultivated with DMEM-LG supplemented with As-2-P for mid-term culture, and As-2-P and βGP for long-term culture. Dex treatment groups were divided according to the duration of dex exposure, including 3 days, 6 days, 2 weeks, 4 weeks exposure to osteogenic supplements (OS-1 or OS-2) with a switch to normal medium for the remainder of the time. Media were changed 2-3 times a week and βGP was added 10 days after seeding.

Alkaline Phosphatase Assay
Alkaline Phosphatase (APase) activity was measured in quadruplicate cultures in 12 well plates. Measurements were made every 3 days in mid-term culture and every 7 days in long-term culture. Cells were washed twice with PBS then incubated with 5 mM p-nitrophenyl phosphate (pNP, Sigma) in 50 mM glycine, 1 mM MgCl₂, pH 10.5 for 4-12 min. APase activity was measured by absorbance at 405 nm on a plate reader (Tecan US, Durhan, NC, USA) and absolute amount of enzyme activity were calculated with p-nitrophenol standard (Sigma). Total enzyme activity of each well was expressed as nM of pNP/min/well and relative activity per cell was divided with result of DNA assay and expressed as nM pNP/min/ DNA.

DNA assay
MSC numbers and rate of growth was derived from DNA assays. DNA was measured in quadruplicate cultures on 12 well plates. After the APase assay (above), the cultures were washed 3 times in PBS, frozen and stored at -70°C. At the end of each experiment, all samples were collected, the plates were thawed and 500 µl 0.1 M sodium hydroxide was added, and the sample was refrozen and thawed. Next 500 µl neutralization buffer (5 M sodium chloride, 100 mM dibasic sodium phosphate, 2 mM EDTA, 0.1 N hydrochloric acid) was added. Aliquots of 100 µl were loaded into 96 well plates and equal volume of 1 µM/ml Hoechst dye (Sigma) was added, and DNA content was measured in a Geniospro fluorescent measurement plate reader (Tecan US, Durhan, NC, USA) at 360 nm excitation and 460 nm emission; DNA concentrations were derived from calf thymus DNA standards run at the same time (Amersham Biosciences, Piscataway, NJ, USA).

APase stain
APase staining was done using the ELF97 Endogenous Phosphatase Detection Kit from Molecular Probes (Eugene, OR, USA). Briefly, Cells grown on coverslips were fixed in 4% formalin for 10 min and treated 10 min in 0.2% Tween 20 in PBS. Enzyme was detected by incubating in substrate solution for 90 sec and the reaction was halted with stopping buffer. For counting, nuclei were stained with 100 nM DAPI (Sigma) in PBS for 5 min followed by washing in PBS.

Calcium assay
A Calcium Assay Kit from Biotron Diagnostics (Hemet, CA, USA) was used to determine calcium content from quadruplicate cultures. Cultures were stored dry at 4°C after fixing in 10% formalin for 30 min and washing three times distilled water. When all samples were collected, all wells were extracted by shaking overnight in 0.6 N HCl followed by centrifugation at 2,000 ×g for 10 min. The supernatant was used for calcium detection according to the manufacture’s instructions and read on a Tecan microplate reader (San Jose, CA) at 570 nm absorbance. DNA content was measured in parallel MSC cultures and the calcium deposition expressed as µg of calcium per mg DNA.

Von Kossa stain
Cell cultures were fixed with 10%
buffered formalin phosphate for 30 min. After rinsing three times with distilled water, cells were incubated in 2% silver nitrate (Sigma) for 10 min in the dark, washed 3 times with distilled water and exposed to bright light for 15 min. After washing thoroughly with distilled water, the samples were dehydrated with 100% ethanol and allow air dry.

**Cube implantation and cube test**
Preparation, loading, and implantation of ceramic cubes have been described previously 1,2. Briefly, biphasic porous tricalcium phosphate-hydroxyapatite (60:40) ceramic with mean pore size of 200 µm (Zimmer Corporation, USA) were cut into 3×3×3 mm cubes and autoclaved. Sterile ceramic cube were immersed in sterile Tyrode salt solution with 100 µg/ml human fibronectin (Collaborative Biomedical, USA) and dried overnight in a laminar flow hood. Control human dermal fibroblasts and MSCs were trypsinized, rinsed, counted and resuspended in serum-free medium at 5.0 × 10^6 cells/ml. Fibronectin-coated ceramic cubes were added to a tube containing suspended cells. The cells loaded ceramic cubes were incubated at 37º for 2 hr and transferred to culture dish. Media were switched to OS-2 and corresponding control medium then cultured for 4 weeks. After dex treatment, cubes were implanted subcutaneously on both sides of the dorsal surface of SCID mice (n=4). After 6 weeks, ceramic cubes were harvested, fixed for 24 hr with 10% neutral buffered formalin phosphate, washed overnight in tap water, decalcified in RDO (Apex Engineering, Plainfield, IL, USA), paraffin embedded and serial sectioned in 5 µm thickness. Every 8th section was collected and stained with toluidine blue. Each section was examined and evaluated a score of 0-4 based on a visual estimate of the percentage of bone or cartilage positive pores, as described previously 1. Briefly, score of zero means neither bone nor cartilage was formed. Scores of 1 through 4 mean trace to 25% (1), 26-50% (2), 51-75% (3) and 76-100% (4) of bone containing pores per all pores in a given section. All score of a cube were accumulated then divided by the number of sections observed (12-16).

**Electron Microscopy**
Samples were decalcified with 10% EDTA solution after fixation in 2.5% glutaraldehyde (polysciences, Niles, IL, USA) in 0.1 M PBS, pH 7.2. Followed by post-fixation in 1% osmium tetroxide (polysciences) in 0.1 M PBS, dehydrated in ethanol and embedded in Epon 812 (polysciences). Ultrathin sections were stained with uranyl acetate and lead citrate.

**RESULTS**

**In vitro differentiation**
The level of APase activity of each group in mid-term (up to 3 weeks) culture increased with time and correlated with length of exposure to

![Graph of APase activity and cell proliferation (DNA)](image)

**Fig. 1.** APase activity and cell proliferation (DNA) in MSCs grown in basal (Bas), OS-1 control (Con), and dex containing medium for 6, 9, 12, 15, 18, and 21 days (6D, 9D, 12D, 15D, 18D and 21D) then switched to control medium. Samples were harvested at 3, 6, 9, 12, 15, 18, and 21st day after culture and APase and DNA assaya were performed as described in methods. Top: APase activity in pNP nM/min/well; Mid: Total DNA/well; Low: divided APase activity with DNA content in each well. The results represent the mean ± SD of triplicate culture of one representative experiment.
OS-1 medium (containing 100 nM dexamethasone, 0.05 mM As-2-P). APase activity of each well increased with time to 21 days but the absolute level per cell, which is calculated by dividing corresponding well DNA amounts, decreased when samples were switched to control medium after a slight delay of a couple of days where it continued to rise. The relative level of APase per cell in Bas, Con, 3D, 6D, 9D, 12D, 15D, 18D, and 21D groups at 21th day were 1.0, 1.3, 1.9, 3.4, 5.7, 10.1, 13.7, and 15.0 pM PNP/min/mg DNA. The overall trend of APase activity per cell was to continuously increase with exposure to dex for a duration up to 3 weeks without peaking (Fig. 1).

The APase staining results support the biochemical assays wherein more and more intensely positive granules are observed with increasing dex pretreatment duration. It was not easy to determine all of the cell boundaries in APase stained samples after confluence because of overlap and file up of the cells, but it is clear that not all the cells show APase positive even though cultured in OS-1 medium for 21 days. The basal group only showed traces of positive reaction after the 6th day, and increased only slightly in reaction intensity and number of positive cell (Fig. 2).

We extended the osteogenic medium experiment duration to 5 weeks owing to the observation that APase activity had not peaked by 3 weeks in the previous experiment. The results of two APase assays form long-term experiments are presented in Figure 3. During the 5 weeks assay period, in MSCs exposed to OS-2 medium (containing 100 nM dexamethasone, 0.05 mM As-2-A, α-glycerophosphate) APase activity reached a peak between 2-3 weeks (we can see in 3W and 4W group) then decreased with time. APase level of the 1W group was much higher than Con or Bas groups during dex pretreatment but returned to control level rapidly after dex removal, and the 2W group approached near peak level during pretreatment but also decreased to control levels with time. 3W and 4W groups reached APase peak and remained higher level than the other groups. These trends were obvious in the 4W group, the gaps between the 3W and 4W groups were more pronounced than that between that the 3W and 2W, or the 2W and 1W groups.

Calcium assay showed that the deposition of calcium also correlated with dex exposure time and culture duration. Calcium deposition started after APase peak. The APase peak was observed at 2 to 3 week and calcium deposition per wells increased from 3rd week and showed some differences at the 4th week and even larger differences between groups at week 5. Calcium levels normalized to DNA also showed large differences between groups from the 4th week. Calcium levels of the 1W group were the same as controls, and the 2W group showed only a slight elevation at the 5th week, but 3W and 4W groups showed high levels by the 5th week. (Fig. 4).

Von Kossa staining confirmed the results shown by the calcium assay (Fig. 5), wherein the number and size of granules increased in correlation with dex treatment. Black granules were rarely observed in Bas and Con groups up to 5th week. In dex treatment groups, black granules increased correlated with OS-2 exposure duration and the amount in 3W and 4W was higher than that of 1W and 2W groups, with the 4W group being the highest (Fig. 5).
Examination of histological sections of the *in vivo* transplanted cubes showed that the cubes were enclosed by capsular structure and that all pores were filled with connective tissue but showed different osteogenic results depending upon *in vitro* culture conditions and cell types seeded. Fibroblasts seeded cubes contained only fibrous tissue and blood vessels but no bone was observed, whereas MSC seeded cubes showed bone formation with a significant difference between control and dex pretreatment groups. In the control group, only a small proportion of pores in peripheral region showed bone formation while extensive bone formation was observed in the dex pretreatment group (Fig. 6). There was no cartilage formation observed in any sample.

Bone formation was predominantly found in peripheral areas but some central cavities also filled with bone; some cavities filled up all cavity space with bone. Total cube scores for the fibroblast, control, and dex groups were 0, 1.0, 3.2, respectively (Fig. 7).
This study examined how induction towards the osteogenic lineage in vitro may effect osteogenesis in vivo.

In this study, it was shown that APase activity correlated with longer dex exposure in vitro. One of our interests was the change of APase activity in individual cells and also the change in the number of APase positive cells with dex treatment. In the APase staining assay, the intensity and number of positive granules in individual cells, as well as the number of APase positive cells, increased with dex exposure duration. These observations are supported by studies on single-colonies of MSCs has shown that the number of bone forming colonies increases with exposure to dex.

These results indicate that these preparations of MSCs are heterogeneous with respect to the stage of osteogenic differentiation. It is speculated that cells with more stem cell characteristics would need longer exposure to dex to enter the osteogenic lineage.

Other researchers have shown that osteoblasts have distinct stages in differentiation from mesenchymal stem cells to osteocytes, including proliferation, extracellular maturation, and mineralization stages. APase elevation is an early marker of extracellular maturation and APase negative cell population can form bone in vitro with dex treatment, whereas APase positive populations can form bone in vitro without dex.

Therefore, at least in this assay system, early expression of APase is a reliable predictor of osteogenesis and it was hypothesized that jump-starting MSCs down the osteogenic lineage would increase or accelerate bone formation in vivo.

In bone tissue engineering, the roles of transplanted MSCs can be inferred from this report. APase positive osteoprogenitor cells will differentiate
into osteoblasts and play a major role in bone formation, whereas APase negative MSCs are likely to play an alternative role, such as cytokine release or differentiation into other lineages, such as adipose or stromal support. Nevertheless little work has been done to measure the effects of dexamethasone (DEXA) pretreatment on bone formation in vitro to modulate MSC differentiation in vivo. In this experiment, MSCs were expected to be induced toward osteogenesis under influence of dexamethasone, but the effects of the duration of pretreatment was unknown, as were the effects on subsequent in vivo differentiation.

With the results we concluded that 3 weeks dexamethasone treatment may be insufficient for direct full osteogenic differentiation. If dexamethasone is removed from in vitro culture, a population of cells may regress toward a more undifferentiated state or differentiate along alternative pathways, such as adipogenic lineage. Therefore, a continuous dexamethasone presence is required to archive maximal osteogenic differentiation of MSC culture. With all in vitro results, we concluded that for full differentiation induction, at least 3 weeks’ continuous dexamethasone treatment is needed. With these results, we transplanted cubes after 4 weeks dexamethasone pretreatment. As expected there was no bone formation in fibroblast seeded cubes whereas dexamethasone pretreated MSCs made extensive bone compared to dexamethasone untreated MSCs. Generally, the osteogenic potential of MSCs after transplantation is dependent on number of transplanted MSCs and, and at the same time, the viability of MSCs after transplantation is relatively low. Therefore, this dexamethasone pretreatment method may be a useful method to enhance bone formation with smaller numbers of MSCs.

REFERENCES


INHIBITION OF THE PI3K-AKT SIGNALING PATHWAY REDUCES TUMOR NECROSIS FACTOR-α PRODUCTION IN RESPONSE TO TITANIUM PARTICLES IN VITRO

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**BACKGROUND**

Wear debris contributes to implant loosening after total joint arthroplasty, and few advances have been made in our ability to inhibit the biological response to wear particles. Bacterial endotoxins augment the effects of wear particles in vitro and in vivo. The cytokine, tumor necrosis factor-α (TNF-α), is produced by macrophages in response to bacterial endotoxins and wear particles, and it increases osteoclast activity resulting in bone resorption and implant loosening. The phosphoinositol-3-kinase (PI3K)-Akt intracellular signal transduction pathway contributes to cytokine production in response to soluble endotoxin. We investigated the role of the PI3K-Akt pathway in the production of TNF-α in response to wear particles with adherent endotoxin and so-called endotoxin-free wear particles.

**METHODS**

Cultured RAW264.7 murine macrophages were incubated with titanium particles with adherent endotoxin or with endotoxin-free titanium particles in the presence and absence of specific inhibitors of PI3K (LY294002) or Akt (SH-5). Akt activation was assessed with use of Western blot. TNF-α production was measured with use of enzymelinked immunosorbent assay. Cytotoxicity was determined by measuring lactic dehydrogenase release.

**RESULTS**

Titanium particles with adherent endotoxin increased Akt activation, whereas endotoxin-free titanium particles did not. The PI3K inhibitor reduced TNF-α production by 70% in response to titanium with adherent endotoxin without increasing cytotoxicity. Similarly, the Akt inhibitor reduced TNF-α production by 83% in response to titanium particles with adherent endotoxin without increasing cytotoxicity. High concentrations of endotoxin-free titanium particles resulted in a small delayed increase in TNF-α production that was completely blocked by the PI3K inhibitor.

**CONCLUSIONS**

Inhibition of the PI3K-Akt pathway reduces macrophage TNF-α production in response to titanium particles with adherent endotoxin and endotoxin-free particles in vitro.

**CLINICAL RELEVANCE**

In vivo studies are needed as these results suggest a possible pharmacological target to reduce wear particle-induced osteolysis and subsequent implant loosening.

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Joint replacement surgery is an effective way to treat patients with severe arthritis. Unfortunately, many joint replacements fail over time because of aseptic loosening. Loosening in the absence of clinical or microbiological signs of infection is, in part, caused by wear debris generated from the implants. Advances in biomaterials and surgical technique have been developed in an effort to reduce wear debris. The advent of improved bearing surfaces, such as cross-linked polyethylene, will likely reduce the rate of particle generation. However, this approach alone will be unlikely to eliminate aseptic loosening since it does not completely block particle generation from the bearing surfaces; leads to the production of smaller, more biologically active particles; and will likely have little effect on particles generated from third-body wear, backside wear, cement degradation, corrosion, fretting, malalignment, wear of the Morse tapers, or delamination of hydroxyapatite coatings. Understanding the biological effects of wear debris may provide for pharmacological treatments to reduce the prevalence of implant loosening.

Wear particles activate macrophages to release proinflammatory cytokines, such as interleukin-1 (IL-1), IL-6, and tumor necrosis factor-α (TNF-α). However, wear particles themselves may not be the sole stimulus of cytokine production in aseptic loosening. Rather, bacteria-derived endotoxins that are adherent to the wear particles may provide an increased inflammatory stimulus. Lipopolysaccharide from gram-negative bacteria as well as lipoteichoic acid and peptidoglycan from gram-positive bacteria induce cytokine production and have similar biological effects. There is evidence that these endotoxins may be important for the biological activity of wear debris. First, removal of endotoxin from titanium particles results in a substantial reduction of TNF-α, IL-1, IL-6, osteoclast differentiation, and osteolysis induced by titanium particles. Adding back adherent lipopolysaccharide to the so-called endotoxin-free particles restores their ability to induce these cytokines. In addition, the biological effects of the titanium particles depend on Toll-like receptor-4 (TLR-4), which is the primary receptor for lipopolysaccharide.

There is also clinical evidence that bacterial endotoxins may be important in the development of aseptic loosening. A study of >20,000 hip replacements in the Norwegian Arthroplasty Register showed that systemic antibiotics and antibodies in cement reduced the incidence of aseptic loosening. Also, a gram-negative bacterial biofilm has been found on many implants retrieved from patients with no clinical evidence of infection. This biofilm may be a source of local endotoxin. An additional source of local endotoxin may be the implants themselves, as adherent endotoxin (twelve to fifteen units of endotoxin per square meter) has been found on titanium discs sterilized, processed, and packaged by a major orthopaedic implant manufacturer in the same manner as their commercially available joint replacements. Additionally, endotoxin may accumulate from systemic sources as suggested by the fact that endotoxin-free titanium and polyethylene particles accrue endotoxin in the first seven days after implantation on mouse calvaria. Potential sources of accumulated endotoxin include minor infections, gut flora, and, in humans, dental procedures. Accumulation from these sources may explain why endotoxin exists in periprosthetic tissue from patients with aseptic loosening and inflammatory arthritis and why peptidoglycan exists in synovial tissue of patients with either rheumatoid arthritis or osteoarthritis. The clinical relevance of these potential local and systemic sources of endotoxin requires additional investigation.

The cell-signaling mechanisms by which wear particles induce macrophages to generate cytokines are incompletely understood. However, there is a substantial body of literature examining the macrophage response to lipopolysaccharide. Multiple signaling pathways, including the phosphoinositol-3- kinase (PI3K)-Akt pathway, mediate inflammatory cytokine production in lipopolysaccharide-stimulated macrophages. Several of these pathways, including the mitogen-activated protein kinases (MAPK) and nuclear factor-kappa B (NF-κB), have also been studied in response to wear particles and have been shown to contribute to their biological activity. Although the role of the PI3K-Akt pathway in response to wear particles has not been studied previously, it is involved in many important cell functions, including cell motility, cell survival, and inflammation. PI3K is activated in macrophages in response to lipopolysaccharide. PI3K activates Akt through a series of coordinated events resulting in phosphorylation at Ser473 and Thr308 (Fig. 1). Once activated, Akt has many downstream targets that mediate its regulatory functions. The purpose of our study was to determine whether titanium particles activate the PI3K-Akt pathway and to determine whether this pathway is important for titanium particle-induced TNF-α production.

**MATERIALS AND METHODS**

**Titanium Particle Preparation**

Commercially pure titanium particles were obtained from Johnson Matthey (number 00681, lot G11G04; Ward Hill, Massachusetts). Ninety percent of the titanium particles were <3.6
INHIBITION OF THE PI3K-AKT SIGNALING PATHWAY

μm in diameter, 75% were <2.4 μm, 50% were <1.6 μm, 25% were <1.2 μm, and 10% were <1.1 μm, as measured with a Coulter Counter (Multisizer 3; Beckman Coulter Particle Characterization Laboratory, Miami, Florida). Titanium particles sterilized in 70% ethanol contained substantial amounts of adherent gram-negative-derived endotoxin (36 U of endotoxin per 109 particles) as measured with the high-sensitivity version of the Chromogenic Limulus Amoebocyte Lysate assay (QCL-1000; BioWhittaker, Walkersville, Maryland) supplemented with a β-glucan blocking reagent (β-G Blocker; BioWhittaker)\(^{11,26}\). Virtually endotoxin-free titanium particles were prepared by removal of >99.9% of the adherent endotoxin without detectably altering the size or shape of the particles by alternating incubations in nitric acid and alkali ethanol as previously described\(^ {11}\). Titanium particles with adherent endotoxin and so-called endotoxin-free titanium particles were stored (4°C) until use at a concentration of 2 ×10^7 particles per milliliter in phosphate-buffered saline solution supplemented with penicillin (100 U/mL) and streptomycin (100 µg/mL). For experiments, the particle stock solution was diluted 1250-fold with serum-free culture media and was preincubated (5% CO2 at 37°C) for one hour. As a control, phosphate-buffered saline solution with antibiotics, but without particles, was equivalently diluted in serum-free media.

Cell Culture

The culture media used throughout was minimum essential media with Earle’s salts (Hyclone, Logan, Utah), supplemented with 2 mM L-glutamine (Mediatech, Herndon, Virginia), nonessential amino acids (Mediatech), 100 U/mL penicillin (Mediatech), and 100 µg/mL streptomycin (Mediatech). Unless otherwise specified, all culture media also contained 10% fetal bovine serum (Hyclone). Serum-free culture media contained 0.1% bovine serum albumin (Sigma, St. Louis, Missouri). Calcium and magnesium-free Dulbecco phosphate-buffered saline solution was obtained from Mediatech. All of these reagents were tested for endotoxin with use of the high-sensitivity version of the Chromogenic Limulus Amoebocyte Lysate assay (QCL-1000; BioWhittaker) and were from lots that contained the lowest concentration of endotoxin available.

The RAW264.7 murine macrophages (ATCC, Manassas, Virginia) were maintained by culture (5% CO2 at 37°C) in Petri dishes. Prior to the experiments, the RAW264.7 cells were plated (2.4 × 10^6 cells per 58 cm2 dish) in tissue-culture dishes and were cultured for eighteen to twenty hours to allow the cells to adhere to the plates. Cultures were supplemented with the diluted titanium particle suspensions described in the previous paragraph to a final concentration of 3.3 × 10^6 particles/cm^2 (approximately eighty particles per cell) or with the control media without particles. Previous studies have shown that this particle concentration produces a robust TNFα response by RAW264.7 cells\(^ {27}\). In selected experiments, RAW264.7 cells were cultured with titanium particles in the presence or absence of specific inhibitors of PI3K or Akt. For this purpose, cultures were supplemented with the indicated concentrations of LY294002 (Calbiochem, La Jolla, California), SH-5 (Calbiochem), or equivalent concentrations of

![Fig. 1](image-url)

**Fig. 1** Activation of the PI3K-Akt pathway. See text for details. PI3K = phosphoinositol-3-kinase, PIP2 = phosphatidylinositol,4,5-bisphosphate, PIP3 = phosphatidylinositol-3,4,5-triphosphate, and PDK1 = phosphatidylinositol dependent kinase-1.
dimethyl sulfoxide (DMSO; Sigma) vehicle during the incubation with titanium particles as well as during a preincubation period prior to the addition of the particles (one hour for LY294002 and two hours for SH-5). In all cases, the final cultures contained 12 mL of serum-containing media (added with the RAW264.7 cells) and 12 mL of serum-free media (added with the titanium particles and the inhibitors).

**Assays of TNF-α Production and Cytotoxicity**

After culture for the indicated periods of time, the media were harvested and centrifuged to remove cellular debris (twentyfive minutes times 6400 g). Aliquots were stored at −20°C for TNF-α measurement by enzyme-linked immunosorbent assay (ELISA) with use of capture and detection antibodies (AF-410-NA and BAF410; R and D Systems, Minneapolis, Minnesota) and Poly-HRP20-Streptavidin conjugate (RDIPHRP20-SA2; Research Diagnostics, Concord, Massachusetts) as previously described. Additional aliquots of media were stored for no more than three days at 4°C for cytotoxicity assessment with use of a lactate dehydrogenase (LDH) detection kit (Roche, Mannheim, Germany). The percent cytotoxicity was calculated by comparison with cultures lysed with 1% Triton X-100 as recommended by the manufacturer.

**Western Blot Analysis**

The RAW264.7 cells were washed twice with ice-cold phosphate-buffered saline solution containing 1 mM sodium orthovanadate and then were lysed in ice-cold buffer containing 1% Triton X-100, 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM β-glycerophosphate, 1 mM EDTA, 1 mM EGTA, 2.5 mM sodium pyrophosphate, 1 mM sodium orthovanadate, 1 ng/mL leupeptin, and 1 mM PMSF (phenylmethylsulfonyl fluoride). One protease inhibitor cocktail tablet (Complete Mini; Roche) was added to every 10 mL of lysis buffer. Cell lysates were sonicated four times for five seconds each on ice at a setting of 8 with use of a sonic probe (60 Sonic Dismembrator; Fisher Scientific, Pittsburgh, Pennsylvania). Sonicated lysates were centrifuged (ten minutes × 10,300 g) and stored at −80°C. Total protein was assayed with use of the BCA protein assay kit (Pierce, Rockford, Illinois). Aliquots of cell lysates containing 10 µg of protein were subjected to SDSPAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) and were electrotransferred to a polyvinylidene difluoride membrane (PVDF; BioRad, Hercules, California). In selected experiments, cell lysates containing 200 µg of protein were immunoprecipitated with use of an anti-Akt antibody that binds to all
three Akt isoforms (Upstate, Waltham, Massachusetts). The cell lysates and the immunoprecipitates were subjected to electrophoresis and electrotransferred to a polyvinylidene difluoride membrane as described above. After electrotransfer, polyvinylidene difluoride membranes were incubated for one hour with block buffer (Tris-buffered saline solution, 0.1% Tween-20, and 5% w/v nonfat dry milk), washed with Tris-buffered saline solution with 0.1% Tween-20 (TBS-T) buffer three times for seven minutes each and then probed with a 1:500 dilution of anti-phospho-Ser473 or 1:1000 anti-phospho-Thr308 (Cell Signaling Technology, Beverly, Massachusetts) in phosphate-buffered saline solution containing 5% bovine serum albumin overnight at 4°C.

Bound antibodies were detected with HRP (horseradish peroxidase)-linked anti-rabbit IgG (immunoglobulin G) secondary antibody (Cell Signaling Technology) and enhanced chemiluminescence reagents (Amersham Biosciences, Piscataway, New Jersey). The polyvinylidene difluoride membranes were stripped by washing with 0.2 M NaOH for fifteen minutes followed by three washes with TBS-T for seven minutes each. The stripped membranes were incubated for one hour in block buffer and then were reprobed with a 1:1000 dilution of Akt antibody (Cell Signaling Technology) with 5% bovine serum albumin in phosphate-buffered saline solution overnight at 4°C. Bound antibodies were again detected with HRP-linked anti-rabbit IgG secondary antibody and enhanced chemiluminescence reagents.

Statistics

All figures are representative of at least three experiments. All Western blots depict representative bands from triplicate cultures. All TNF-α and cytotoxicity data are presented as the mean and the standard error of the mean of triplicate cultures, each assayed in triplicate. Statistical analysis was performed by analysis of variance with use of SuperANOVA software (Abacus Concepts, Berkley, California). Bonferroni-Dunn (control) post hoc tests were used when a single control group was compared with all other groups (Figs. 3, 4, and 5, B). The Fisher protected least significant difference post hoc tests were used when multiple groups were compared with each other (Figs. 2, C and 5, A).

RESULTS

Activation of the PI3K-Akt Pathway by Titanium Particles with Adherent Endotoxin

To assess activation of the PI3K-Akt pathway, we examined Akt phosphorylation at the Ser473 and the Thr308 sites since phosphorylation at these sites is required for Akt activity23. Titanium particles with adherent endotoxin transiently increased Akt phosphorylation (Fig. 2, A, top two panels). Increased phosphorylation at the Ser473 site is first observed thirty minutes after stimulation, peaked at sixty minutes, and returned to baseline by ninety minutes (Fig. 2, A [top panel]) and Fig. 2, B [top panel]). Increased phosphorylation at the Thr308 site is maximal at forty-five minutes after stimulation and returned to baseline at ninety minutes (Fig. 2, A [middle panel]). Akt phosphorylation was also increased by incubation with lipopolysaccharide, which was included as a positive control (Fig. 2, A [top two panels]). Equivalent sample loading was demonstrated by stripping the phospho-Ser473 and phospho-Thr308 blots, then, reprobing for total Akt levels (Fig. 2, A [bottom panel]). In addition, Akt activation in response to endotoxin-free titanium particles was
compared with titanium particles with adherent endotoxin. Endotoxin-free particles did not increase phospho-Ser<sup>473</sup> Akt activation (Fig. 2, B [top panel]). Equivalent sample loading was again demonstrated by stripping phospho-Ser<sup>473</sup> Akt blots and reprobing for total Akt levels (Fig. 2, B [bottom panel]). TNF-α production did not increase after stimulation with endotoxin-free particles (Fig. 2, C). Moreover, Akt phosphorylation in response to titanium particles with adherent endotoxin preceded TNF-α secretion, which was first detectable at sixty minutes (Fig. 2, C).

**Effect of PI3K-Akt Inhibitors on the Production of TNF-α in Response to Titanium Particles with Adherent Endotoxin**

To assess the role of the PI3K-Akt pathway in the production of TNF-α in response to titanium particles with adherent endotoxin, we examined the effects of LY294002, which is a specific inhibitor of PI3K. For example, LY294002 does not inhibit any of the mitogen-activated protein kinase (MAPK) pathway kinases, like ERK1/2 or p38, which are activated in response to titanium particles with adherent endotoxin. SH-5 inhibited TNF-α production in a dose-dependent manner with 83% inhibition at 75 µM (Fig. 4, A). Akt phosphorylation at the phospho-Ser<sup>473</sup> site was also inhibited in a similar dose-dependent manner by SH-5 with a maximal effect at 75 µM (Fig. 4, A).

**Effect of LY294002 on TNF-α Production in Response to Endotoxin-Free Titanium Particles**

As shown in Figure 5, A, high concentrations (16.5 × 10<sup>6</sup> particles/cm<sup>2</sup>) of so-called endotoxin-free titanium particles induce TNF-α production. However, the production of TNF-α occurs much more slowly and to a lesser extent than the response to a fivetfold lower concentration of titanium particles with adherent endotoxin. To examine the role of the PI3K-Akt pathway in response to endotoxin-free titanium particles, a high concentration of endotoxin-free particles was used to stimulate RAW264.7 cells. Although there was no detectable increase in
Akt phosphorylation with endotoxin-free particles at any of the time-points measured between zero and 180 minutes (data not shown), LY294002 (100 µM) completely inhibited TNF-α production induced by these particles (Fig. 5, A). LY294002 increased cytotoxicity by a significant (p < 0.0001) but small amount that does not account for its inhibition of TNF-α production (Fig. 5, B).

**DISCUSSION**

Titanium particles with adherent endotoxin activate the PI3K-Akt signaling pathway in vitro to produce TNF-α Akt is activated prior to the onset of TNF-α production, and inhibitors of PI3K and Akt activity block titanium particle-induced TNF-α. Furthermore, higher concentrations of endotoxin-free particles over a longer incubation period produce a small but significant (p < 0.0001) increase in TNF-α that is also blocked by inhibition of PI3K. Although no Akt activation was detectable by Western blot in response to endotoxin-free particles at any time-point that we examined, it is possible that we missed the time-point within the threehour period that Akt activation increased. Alternatively, the magnitude of Akt activation may be low and thus difficult to detect by Western blot. Also, basal levels of Akt activity can exert potent downstream effects and may be required to generate a response to an external stimulus. Nonetheless, our results show that the PI3K-Akt signaling pathway contributes to the induction of TNF-α by wear particles, irrespective of the presence or absence of adherent endotoxin. We measured TNF-α as a marker for inflammation rather than other cytokines because of its well-documented role in wear particle-induced bone loss. Moreover, TNF-α is the first proinflammatory cytokine produced after macrophage stimulation with wear particles.

TNF-α then stimulates macrophages to increase production of other proinflammatory cytokines. Therefore, measuring TNF-α as it begins to increase provides a more accurate assessment of the direct particle effect on cytokine production than measuring the secondary effects of TNF-α as it stimulates other cytokines. Although polyethylene particles are the most prevalent type of particles in periprosthetic tissue, we used titanium particles in our experiments for three reasons. First, titanium particles are present in periprosthetic tissues from patients undergoing revision total joint arthroplasty. Second, titanium and polyethylene particles induce osteolysis by similar mechanisms. Third, polyethylene is difficult to use in cell culture because the particles tend to float in the culture media and, therefore, do not reach the macrophages adherent to the bottom of the culture plate. Thus, titanium particles serve as an appropriate stimulus in a cell culture model.

Although gram-positive bacteria have been found in the biofilm surrounding implants removed for aseptic loosening, we studied the effects of gram-negative endotoxins rather than lipoteichoic acid or peptidoglycan. As previously mentioned, gram-positive and gram-negative endotoxins have similar biological activity. However, the assays available to measure lipoteichoic acid and peptidoglycan are not sensitive and specific enough to quantify the amount of these bioactive molecules on particles or, more importantly, to document the removal of these molecules from particles. Assays to measure gram-

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**FIG. 5**

LY294002 inhibits TNF-α production induced by endotoxin-free titanium (Ti) particles (A) and causes a small increase in cytotoxicity that does not account for the inhibition of TNF-α production (B). Cells were pretreated with or without LY294002 (one hour) followed by stimulation with endotoxin-free titanium particles (16.5 x 10⁶/cm²) for three hours or titanium particles with adherent endotoxin (3.3 x 10⁶/cm²) for one hour in the continuous presence or absence of LY294002. All groups contained 1% DMSO (dimethyl sulfoxide) as the vehicle control. The pound sign denotes a significant difference (p < 0.0001) compared with the titanium with endotoxin group, and the asterisk denotes a significant difference (p < 0.0001) compared with the endotoxin-free titanium group without LY294002.
negative endotoxin are very sensitive and are more accurate for assessing the particle effect compared with the endotoxin effect on TNF-α production.

Other pathways, including nuclear factor-kappa B (NF-κB) and MAPKs, are activated in macrophages stimulated with lipopolysaccharide. Adherent endotoxin on titanium particles increases activation of NF-κB and all three major mitogen-activated protein kinases (ERK1/2, JNK, and p38). Of these, activation of NF-κB and ERK1/2 is required for TNF-α production and inflammation in response to wear particles. The interaction between NF-κB, ERK1/2, and the PI3K-Akt pathways is likely complex. Figure 6A summarizes these mechanisms of titanium particle-induced TNF-α production in macrophages. In addition, on the basis of what is known about macrophage stimulation with lipopolysaccharide, Figure 6B outlines a proposed mechanism by which the NF-κB, ERK1/2, and the PI3K-Akt pathways interact to increase TNF-α production. Studies are in progress in our laboratory to clarify the cross-talk between these pathways in response to particles. Nonetheless, the current evidence indicates that local or systemic inhibition of any of these pathways could result in the reduction of osteolysis and aseptic loosening.

Furthermore, in vivo studies are needed to clarify the consequences of inhibiting these pathways and to determine whether there is a resultant reduction of particle-induced bone loss. LY294002, the inhibitor that was used in our experiments, inhibits multiple isoforms of PI3K. This agent therefore is potentially lethal in vivo, since mice lacking the genes for either PI3Kα or PI3Kβ isoforms die during embryologic development. In contrast, mice lacking the PI3Kγ gene show no adverse phenotype and are protected from joint destruction related to rheumatoid arthritis. An oral drug that selectively blocks PI3Kγ and the resultant Akt phosphorylation, and thereby protects mice from joint inflammation and destruction in models of rheumatoid arthritis, has recently been developed. Since our data show that the PI3K-Akt pathway mediates the biological response to wear particles in vitro, this new drug provides an exciting avenue for further research into pharmacological inhibition of aseptic loosening.

REFERENCES


INHIBITION OF THE PI3K-AKT SIGNALING PATHWAY


THE COST COMPARISON BETWEEN CEMENTLESS AND HYBRID TOTAL HIP REPLACEMENT AT UNIVERSITY HOSPITALS CASE MEDICAL CENTER

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ABSTRACT

Introduction
Many studies showed long term success of both cemented and cementless femoral fixation. There is an assumption that cementless femoral components are more expensive than cemented femoral components, however, previous reports showed that the price of a cemented femoral component with cement accessories, was comparable to a cementless stem.

Material and methods
We collected the hospital’s cost for the prostheses from three different manufacturers. The total operative costs which included prosthesis costs, supply costs, and OR/PACU costs were also recorded. The data was reported as the price difference between cementless and hybrid total hip replacement, with and without cement accessories.

Results
The hospital’s cost for implanting a cementless femoral component was $1000-$1300 higher than that for a cemented implant. This price difference decreased to $650-$950 when cement accessories were added to the cost for hybrid total hip replacement.

Conclusions
Cemented femoral fixation remains an attractive option for total hip replacement in terms of total hospital cost.

INTRODUCTION
Total hip replacement (THRs) is among one of the most successful operations with excellent long term outcomes either using cemented or cementless fixation. Cemented fixation of the femur is a durable, reproducible and cost-effective technique. The literature clearly demonstrates that cemented femoral stems can routinely last 20-30 years (1,2,3,4,5,6,7,8). However, over the last few years, there has been a growing body of evidence to support the use of cementless femoral components. Many studies demonstrate long term results that are equal to any of the published results utilizing contemporary cemented fixation of the femoral prosthesis (9,10,11). Moreover, it has been proposed that cementless implants have other potential benefits to the patient in terms of reduced likelihood of fat embolism, risk of venous thromboembolic disease, and operative time (12,13,14,15).

With the current number of THRs done yearly and the expectation that increasing numbers of THRs will be done in the future, it is important to understand the costs associated with primary THRs. Many efforts should have focused more on lowering the patient’s expenses for this procedure such as using mini-invasive surgical technique which, theoretically, should reduce the hospital length of stay after the operation or the use of less costly implants. There is an assumption that cementless components are more expensive than cemented components due to their increased design and manufacturing complexity. However, implanting cemented stems requires the addition of cement and the necessary accessories needed for cement fixation techniques as well as potentially longer operative time.
This study aims to compare the price difference between cementless and hybrid total hip replacements (THRs) with and without cement accessories in 3 different manufacturers. The price difference was calculated as the cost of cementless THRs minus the cost of hybrid THRs with and without cement accessories.

**Figure 1** The price difference between cementless and hybrid THRs with and without cement accessories in 3 different manufacturers. The price difference was calculated by cost of cementless THRs – cost of hybrid THRs with and without cement accessories.

**Figure 2A** The actual operative cost for primary cementless THRs at the University Hospitals Case Medical Center which included prosthetic costs, OR/PACU costs and supply costs in the operating room.
determine the total operative costs and the hospital cost of the prostheses when all necessary materials used to perform the procedure are taken into consideration. The hypothesis of our study is that in our institution the cost for the use of a cemented stem even with cement accessories is still lower than the cost for a cementless femoral prosthesis.

**MATERIALS AND METHODS**
We collected data from University Hospitals Case Medical Center: 1) the hospital’s cost for prostheses from 3 different companies, that are used in our institution and 2) total operative costs which included operating room/post anesthetic care unit (OR/PACU) costs, supply costs and the hospital cost for the prostheses (prosthesis costs). This information is up to date as of August 2007.

The hospital cost was defined as the actual amount that the hospital paid to the manufacturer for the implant. The prices were obtained from the relevant companies in US dollars, excluding taxes. Our medical center performs over 1000 joint replacement procedures per year and therefore, has volume discount contracts with the manufacturers. Because of confidentiality clauses and contract policies with the manufacturers, we could not present the manufacturers’ name and the actual hospital cost for the prostheses. This information was shown as the price difference between cementless and hybrid total hip replacement. The cost of a cementless hemispherical acetabular component with one screw, 28 or 32 mm femoral head and highly cross linked polyethylene liner were utilized with both femoral components and included in the total prosthesis costs for either cementless or hybrid total hip replacement. Therefore, the only two differences in cost between cementless and hybrid total hip replacement were the femoral prosthesis and the addition of cement accessories for the hybrid total hip replacement. Third generation cement technique was used in all hybrid total hip replacements which included vacuum preparation of the cement. Three bags of bone cement were mixed in one cartridge. Other disposable equipment routinely used included the vacuum mixing cartridge,
cement pressurizer, canal plug, pulsatile lavage, proximal and distal cement centralizer, canal brush, and cement scrapers. All the equipment that was used for third generation bone cement technique was categorized as cement accessories.

The OR/PACU costs are those involved with an actual OR/PACU room cost which mainly depends on the operative time, monitoring costs and other costs in the PACU.

Supply costs are costs for various supplies used in the operating room such as sterile drapes, sterile gown, gauzes, sponges, suction drain, the cost for instrument sterilization, etc.

The prosthesis costs, OR/PACU costs and supply costs were included into the total operative costs.

RESULTS
The average cost differential to the hospital for implanting a cementless stem was $1000-$1300 higher than for a cemented stem. The total cost to the hospital for all accessories used to achieve third generation cement technique was approximately $364. If the cost for cement accessories was added to the cemented femoral prosthesis, the actual price difference between implanting a cementless and cemented femoral stem decreases to $650-$950 (Fig 1). The cost of the prostheses comprised about 20-30% of the total operative cost for primary THRs in both cementless and hybrid THRs as shown in Figure 2A and 2B respectively. The average cost for operative room/PACU was approximately the same in both methods of fixation ($9000).

DISCUSSION
The success of THRs which has lead to the increased frequency in which it is performed is largely due to the great improvement in prosthetic design. A meta-analysis which compared the survivorship between cemented and cementless fixation of the femoral component showed that there was no overall superiority in either mode of fixation as measured by a difference in survival rate (16). Due to this success in THRs, resource utilization has continued to escalate because of the increase in the number of patients undergoing hip arthroplasty. Based on Medicare and United States census data which was incorporated into a Poisson regression model to determine the projected economic burden through 2015, the annual hospital charges for primary THRs is likely to increase to 17.7 billion dollars (17). It is clear that the prosthetic costs are a major contributor to the overall costs for the operation. Barber and Healy showed that the cost of the prosthesis was the most rapidly increasing portion of the expenditure for hip arthroplasty (18).

The difference between the cost of cementless and hybrid THRs in our study ($1000-$1300) was slightly higher than the other previous reports which illustrated that the price of an average cemented stem was approximately $900-$1000 less than the average cementless stem (19). When including cost for cement accessories, the actual cost of the prostheses for cementless THRs in our institution was still higher ($650-$950) than for hybrid THRs. This data differs from earlier published studies which revealed that the actual cost of implanting a modern cemented stem including cement accessories was greater than for a corresponding cementless stem (19,20). However, in Barrack’s report (19) was a cost comparison of the equipment utilized in 1996 which is probably no longer relevant, while the other study (20) compared the cost of the prostheses that were used primarily in Europe. Moreover, the cost of cement accessories in our hospital is much lower than that previously reported by Barrack ($364 VS $710). This difference in cost for cement accessories can be explained by competition among manufacturers and volume discounts. Although the $650 to $950 cost differential per patient represented only a small portion of the total expenses for THR in our institution, it would be almost a million dollars savings for over 1000 patients.

Decreasing operative time by utilizing cementless implants is a theoretical advantage of cementless femoral fixation over cemented fixation. Although we have not reported the operative time, the OR/PACU costs which were a reflection of the operative time were approximately the same for both cementless and hybrid THRs. Therefore, we can imply that the operative time was not different between the two surgical procedures. This would suggest that in an uncomplicated primary THRs, an experienced surgeon would take the same operative time either implanting a cemented or cementless femoral stem.

The data from our hospital showed that the cost of the prostheses whether cementless or hybrid THRs comprises only 20-30% of the total operative cost (Fig 2A, 2B). It is also important to point out that in order to decrease the total costs for total hip arthroplasty, savings can be achieved not only from the decreased prostheses expenses, but also from decreased OR/PACU costs and ancillary supply costs. This can be done by utilizing the operative time more efficiently and avoiding open unused and unnecessary supplies. With these efforts, we believe that the overall operative costs can be reduced.

A major shortcoming in our study is
that our cost analyses captured only costs during peri-operative period. Professional charges, additional hospital component charges, and rehabilitation costs were not included. It is apparent that the length of hospital stay, postoperative nursing care, and cost after discharge play a major role in the overall expenses to the health care system for primary THR. However, there is no evidence that these costs would vary based on the types of femoral component fixation utilized in our study.

CONCLUSION
The results of this study revealed that cemented femoral fixation remains an attractive method of fixation that was less expensive than cementless femoral fixation for THRs at our institution.

REFERENCES

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Fracture of the Proximal Humerus with Injury to the Axillary Artery in a Boy Aged 13 Years

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Fractures of the proximal humerus with concomitant vascular injury are rare in children. We describe the presentation, diagnosis, and treatment of a fracture of the proximal humerus in association with an axillary artery injury in a child.

Several studies have described fractures of the proximal humerus in adults with concomitant vascular or neurological injury.1,2 However, the management of such fractures in children has not been described.2 To our knowledge, there are no specific descriptions of paediatric fractures of the proximal humerus with associated vascular injury. These type of fractures account for approximately 0.45% of all paediatric fractures, and approximately 3% of epiphyseal fractures.3

**CASE REPORT**

A right-handed 13-year-old boy with no previous medical or surgical history presented with pain in the right arm and an obvious deformity. He stated that a falling television set had struck the arm just above the elbow on the medial side, approximately one hour prior to presentation. He lay supine with the right arm abducted to 90°. He complained of pain and was unable to dorsiflex the wrist or extend the thumb. Sensation to light touch was intact. However, his radial, ulnar, and brachial pulses were not recordable by palpation or Doppler. The injury was closed and no ecchymoses were present.

Anteroposterior (axillary) and scapula Y views of the right shoulder (Fig. 1) showed a Salter-Harris type II fracture of the proximal humerus. The patient received ketamine sedation in the Emergency Department to allow reduction of the fracture (Fig. 2). However, following reduction the pulses did not return in the radial, ulnar, or brachial arteries. A firm, nonpulsatile haematoma in the axilla was noted after reduction and the advice of a vascular surgeon obtained. During examination by the vascular and orthopaedic surgeons, approximately 30 minutes after reduction, variable Doppler signals at the brachial, ulnar, and radial arteries were seen. However, they were not palpable or consistent, and an angiogram showed occlusion at the junction of the brachial and axillary arteries (Fig. 3).

The patient was taken to the operating theatre for percutaneous pinning of the right proximal humerus followed by reverse saphenous vein interposition grafting of the right brachial and axillary arteries. The intra-operative findings showed a haematoma and arterial contusion, but no active bleeding or laceration of the brachial or axillary arteries. There was no haematoma within the common sheath of the axillary artery and brachial plexus. The axillary artery itself was stretched and thrombosed (Fig. 4). The cords of the brachial plexus were intact on inspection. The patient’s post-operative course was uneventful. He was monitored for 48 hours in the paediatric intensive care unit where his radial and ulnar pulses remained palpable. He was given a sling and a volar splint in 15° of dorsiflexion to prevent a flexion contracture of the wrist. One week after the injury his motor function had returned and his pulses were stable. The percutaneous pins were removed three weeks after operation, and anteroposterior and scapular Y radiographs taken at three
months showed no deformity or epiphyseal damage.

**DISCUSSION**
Fractures of the proximal humerus with absent distal pulses are very rare in children. One case was included in a review of 57 proximal humeral epiphyseal fractures by Baxter and Wiley. Their case, like ours, had a completely displaced Salter-Harris type-II fracture and a cool, pulseless arm. Angiography revealed interruption of the brachial artery at the lateral border of the axilla. Another report of injury to the axillary artery in fractures of the neck of the humerus found the mean age of such patients to be 66 years. In 1968 McQuillan and Nolan described 37 cases of ischaemic limb injuries, five of which involved the brachial or axillary arteries. Among these five, there was one failure of treatment resulting in amputation. Overall, there were 12 failures attributed to extensive intimal damage, inadequate immobilisation, impaired venous return, thrombosis and delay in diagnosis or treatment.

These fractures heal quickly in children and have an excellent long-term prognosis even when left unreduced. A nine-year follow-up of 64 cases of fractures of the proximal humerus in children showed no sequelae except for four cases which had a decreased range of movement.

A series of 15 cases of fractures of the
proximal humeral epiphysis in children with a mean age of 12 years concluded that they heal well despite severe displacement.8 Six of their cases were Neer9 type 2, one was type 3 and eight were type 4.8

The patient in our report sustained a Neer type 4 fracture (a subclassification of the Salter-Harris type II fracture) which required reduction because of the associated vascular injury. This necessitated prompt percutaneous fixation in order to position the upper limb in abduction and external rotation for revascularisation. Injury to the growth plate is described more frequently when fractures of the proximal humerus are treated by operation.10 Our patient did require surgical stabilisation to facilitate vascular repair but we did attempt to minimise any damage to the epiphysis by using a single attempt at reduction with smooth pins for fixation. Other complications of operative fixation include re-fracture, infection, and shoulder impingement.11 After three months there was no radiological evidence of epiphyseal damage, infection, or impingement.

We believe his inability to extend the wrist and thumb was the result of a neurapraxia of the posterior cord of the brachial plexus. In adults with this injury, an enlarging haematoma within the common sheath may explain a progressive or delayed neurological deficit.12 However, our patient did not have an active axillary artery bleed or haematoma within the sheath. The case illustrates the importance of a thorough vascular examination in all cases of fracture and of co-operation between vascular and orthopaedic surgeons when such injury is suspected. The patient and his guardian both gave informed consent. No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

REFERENCES
Osteosarcoma affects approximately 400 children and adolescents each year in the United States, making it the most common bone sarcoma in this age group. Osteosarcoma tends to arise in areas of rapid bone turnover, with the most common primary site being the metaphysis of the distal femur, proximal tibia, or proximal humerus. Typically, patients present with activity related pain and are found to have had symptoms for several weeks. The fact that 20% of patients will have clinically detectable metastases on presentation demonstrates the aggressive nature of the disease. The remainder of patients are considered to have micrometastases, since prior to chemotherapy, 80% of patients with localized tumor still succumbed to metastases following amputation of the affected extremity. Current treatment strategies for osteosarcoma patients include preoperative chemotherapy, surgical resection with limb salvage, and postoperative chemotherapy. With advanced chemotherapy protocols currently in use, the 5-year survival rates have increased from below 20% to 70%. However, the remainder 30% of osteosarcoma patients not surviving the disease outlines the need for new therapeutic targets and more effective medications.

Tyrosine kinases are attractive targets in the development of new chemotherapeutic agents for many forms of cancer, including osteosarcoma. Cell-to-cell and intracellular signal transduction is critical for the progression of a tumor from proliferation to angiogenesis to metastasis. Polypeptide growth factors are the main signal mediators between, and within, tumor cells. Growth factor ligands bind to tyrosine kinase receptors activating a plethora of downstream, intracellular signaling cascades and gene transcription. Tyrosine kinases have been shown previously to participate in the regulation of tumor growth, proliferation, migration, angiogenesis, and apoptosis. Often in cancer cells, the pathways governed by tyrosine kinase signaling are altered. This dysregulation, such as overexpression of a specific growth factor, provides an opportunity for developing target-selective chemotherapies. The majority of current tyrosine kinase inhibiting agents under investigation are directed towards abnormally enhanced cell signaling.

At the present time, the most common chemotherapeutic agents prescribed for osteosarcoma patients include methotrexate, cisplatin, doxorubicin, and ifosfamide. Unfortunately, these traditional chemotherapies have an array of side effects that may plague patients undergoing treatment. Mild and nonspecific off-target effects can include emesis, anorexia, leucopenia, and thrombocytopenia. Potentially devastating side effects include acute liver failure from methotrexate, renal toxicity from cisplatin, cardiac toxicity or heart failure from doxorubicin, or sterility from ifosfamide. On the contrary, tyrosine kinase inhibitors in clinical use have proven to be well tolerated by patients. The target specificity provided by tyrosine kinase inhibition has generally led to less toxicity and fewer side effects than conventional chemotherapeutic agents. Since non-tumorigenic cells depend on a multitude of signaling pathways for survival, they are less sensitive to the inhibition of a particular tyrosine kinase.

Structurally, tyrosine kinases are categorized as receptor or non-receptor tyrosine kinases (Figure 1). Receptor tyrosine kinases transduce a signal from the surrounding environment into the cell while non-receptor tyrosine kinases function to amplify activated receptor signals inside the cell. Inhibitors of tyrosine kinases can consist of either monoclonal antibodies or small molecule inhibitors (Figure 2). Monoclonal antibodies, such as cetuximab, an EGF-R inhibitor approved for the treatment of advanced colorectal cancer, function by attaching to the tyrosine kinase receptor expressed on the surface of tumor cells and thereby prevent the binding of growth factor ligands. On the other hand, small molecule inhibitors permeate the cell membrane and function intracellularly by actively competing for the ATP-binding site of the receptor, which then renders the phosphorylation mechanism useless. Developmental strategies are underway to produce tyrosine kinase inhibitors of either variety.

Overexpression of tyrosine kinase receptors such as epidermal growth factor receptor (EGF-R), insulin growth factor receptor (IGF-R),
and met/hepatocyte growth factor receptor (HGF-R) has been observed in osteosarcoma. Additionally, overexpression of growth factors such as nerve growth factor (NGF), IGF-1, and EGF has been reported. Therefore, our project was designed with the hypothesis that activation of specific tyrosine kinases contribute to metastasis in osteosarcoma. To study the effects of tyrosine kinase inhibition, we utilized five human osteosarcoma cell lines with varying tumorigenic and metastatic potential to study cell motility and colony formation, which are two critical components of metastasis. Non-tumorigenic/non-metastatic parental cells: TE85 and SAOS-2; tumorigenic but non-metastatic cells: MNNG; and highly tumorigenic/metastatic cells: 143B and LM-7). Several small molecule tyrosine kinase inhibitors, of the receptor and non-receptor subtypes, were obtained from Calbiochem and tested at effective concentrations based on the literature.

Early results reveal that an inhibitor targeting EGF-R drastically reduced motility and colony formation in osteosarcoma cell lines. Accordingly, uncontrolled activation of the EGF pathway is known to cause cancer, and overexpression of EGF-R or the EGF ligand has been found in osteosarcoma cell lines. Additionally, the receptor for EGF has been found to be continuously phosphorylated in certain osteosarcoma cell lines, which leads to uncontrolled growth and anti-apoptosis signals. Signaling through the EGF receptor has also been linked with motility, as cells treated with epithelial growth factor ligand were found to have increased motility. Our results of EGF-R inhibition decreasing osteosarcoma cell motility are encouraging and may further support the development of CI-1033, which inhibits EGF-R and related receptor tyrosine kinases (Erb-2, Erb-3, Erb-4) and is currently in clinical trials for osteosarcoma.

Similar to our results seen with inhibition of EGF-R, an inhibitor targeting met/HGF-R significantly...
reduced motility and colony formation by MNNG osteosarcoma cells. These findings correlate with the fact that the MNNG cell line derivation is due to a chromosomal translocation resulting in overexpression of met. C-met is the receptor for hepatocyte growth factor (HGF), which can increase motility, proliferation, and invasion of osteosarcoma cells. Overexpression of c-met has been found in human osteosarcoma, and c-met overexpression can cause oncogenic transformation in primary osteoblast culture. Our results demonstrate that the MNNG cell line is a useful model for the study of c-met overexpression in osteosarcoma. Furthermore, osteosarcoma patients found to have overexpression of c-met would likely benefit from a chemotherapeutic regimen which includes a met inhibitor.

The results of this project support the hypothesis that specific tyrosine kinases regulate critical steps of metastasis in osteosarcoma cells. Investigations are underway to study the effects of inhibiting other tyrosine kinases, such as NGF, IGF, Syk (spleen tyrosine kinase), platelet derived growth factor receptor (PDGF-R), and the cytoplasmic Janus kinases. Additionally, the motility and colony formation assays utilized in this project are being used to screen a siRNA (single-stranded interference) library that individually targets all 88 tyrosine kinases in the human genome. The siRNA technology essentially provides an effective means of silencing the expression of a particular gene of interest. The data will hopefully support our results from small molecule tyrosine kinase inhibition and may provide other avenues for further pursuit.

In conclusion, a better understanding of the biology of osteosarcoma cells, including the intricate structure and activation mechanism of tyrosine kinases, will lead to the development of target-selective drugs for anti-cancer therapy. Patients suffering from osteosarcoma, who demonstrate a specific signaling overexpression, may benefit from a tailored chemotheraphy regimen which addresses the hyperactive pathway on which tumor survival is dependent. The ultimate potential of tyrosine kinase inhibition in the treatment of osteosarcoma remains to be discovered.

REFERENCES
In the United States, a physician earns certification in a medical specialty by meeting the qualifications predetermined by the appropriate specialty board. The American Board of Medical Specialties and one of its twenty-four specialty boards—the American Board of Orthopaedic Surgery (ABOS)—offer certification as a voluntary process for individuals who have completed their training in a residency program accredited by the Residency Review Committee for Orthopaedic Surgery. The mission of the ABOS is to establish educational standards for orthopaedic residents and to evaluate the initial and continuing qualifications and competence of orthopaedic surgeons. The Board "defines minimum educational requirements in the specialty, stimulates graduate medical education and continuing medical education, and aids in the evaluation of educational facilities and programs."1

SUCCESSFUL RESIDENCY COMPLETION
The ABOS is one of three sponsoring organizations represented on the Residency Review Committee for Orthopaedic Surgery; the other two organizations are the Council on Medical Education of the American Medical Association and the American Academy of Orthopaedic Surgeons.

An orthopaedic resident also serves on the committee. The Residency Review Committee functions autonomously under the direction of the Accreditation Council for Graduate Medical Education (ACGME). According to the ABOS, the goal of orthopaedic residency education is to prepare a resident to be a competent and ethical practitioner of orthopaedic surgery1. During their orthopaedic residency, applicants for certification by the ABOS must have received, and successfully completed, the following preparation1:

A. Education in the entire field of orthopaedic surgery, including inpatient and outpatient diagnosis and care as well as operative and nonoperative management and rehabilitation.

B. The opportunity to develop, through experience, the necessary cognitive, technical, interpersonal, teaching, and research skills.

C. The opportunity to create new knowledge and to become skilled in the critical evaluation of information.

D. Education in the recognition and management of basic medical and surgical problems.

E. An evaluation of ethical performance.

THE HISTORY OF THE ABOS AND ITS CERTIFYING EXAMINATION
The ABOS was founded on January 7, 1934, the year after the establishment of the Advisory Board for Medical Specialties, the American Academy of Orthopaedic Surgeons, and the certifying boards for ophthalmology, dermatology, and obstetrics and gynecology. The ABOS, founded to "serve the best interests of the public and of the medical profession," was created by the American Orthopaedic Association, the American Academy of Orthopaedic Surgeons, and the American Medical Association (Section on Orthopaedic Surgery).

In 1934, the ABOS initiated certification without an examination, which required ten years of orthopaedic surgery practice. In 1935, the ABOS introduced the first written examination for certification. In 1937, the Board became a member of the Advisory Board for Medical Specialties, now called the American Board of Medical Specialties.

The ABOS currently is composed of eighteen directors elected from diplomates of the Board and one public member. Directors are nominated by the American Orthopaedic Association, the American Academy of Orthopaedic Surgeons, and the American Academy of Orthopaedic Surgeons, and they serve a ten-year
term without salary. The Board consists of many standing committees, including the Written Examination Committee and the Oral Examination Committee. The mission of these certification committees is to produce the best possible examinations to fairly and accurately evaluate the competence of candidates for certification.

THE WRITTEN AND ORAL EXAMINATIONS
The voluntary certification examination is divided into two parts. Part I is a written examination taken after the successful completion of the ABOS educational requirements noted in the previous section. Part II is an oral examination taken after passing Part I, completing the twenty-two-month practice requirement, and undergoing an evaluation of one’s practice by the credentialing committee of the Board. The candidate must pass both the written and oral parts of the examination to be certified by the ABOS as competent to practice orthopaedic surgery.

After passing the written examination, candidates have five years to successfully complete the oral examination process. Candidates who do not pass the oral examination within those five years must retake and repass the written certification examination before again applying to take the oral examination. Time spent in fellowship education after passing Part I does not count as part of the five-year time limit. A candidate is only considered “Board Eligible” from the time of successful completion of the Part-I examination until the successful completion of the Part-II examination, for a maximum period of five years.

EDUCATIONAL REQUIREMENTS
An applicant seeking certification by the ABOS must satisfy the educational requirements that were in effect when he or she first enrolled in an accredited orthopaedic residency. On the completion of fifty-four of the sixty months of required education and on the recommendation of the program director, a candidate may apply to take the Part-I written certification examination. To be admitted to this examination, the candidate must complete the full sixty months of required education by June 30 of the examination year. An applicant who has received orthopaedic surgery residency education in Canada must have fulfilled the requirements of the ABOS and must have passed the orthopaedic surgery qualifying examination of the Royal College of Physicians and Surgeons of Canada.

Applicants who are in practice at the time they apply for Part I and all applicants for Part II must either possess a full and unrestricted license to practice medicine in the United States or Canada or be engaged in full-time practice with the United States federal government, for which licensure is not required.

THE PART-I WRITTEN CERTIFICATION EXAMINATION
Examination Construction
The Part-I written certification examination evaluates a candidate’s knowledge of general orthopaedics and the basic sciences of orthopaedics as well as a candidate’s ability to use this information for problem-solving in the diagnosis and treatment of patients. It is a secured examination, consisting of 300 to 330 multiple-choice questions, given over seven hours of testing divided into two sessions. The examination covers acute and chronic disease as well as disorders and injuries of the musculoskeletal system, including the diagnosis and management of congenital, developmental, infectious, inflammatory, neurological, vascular, metabolic, neoplastic, degenerative, and traumatic conditions affecting the limbs and the spine. The examination also covers the basic sciences of anatomy, pathology, physiology, biochemistry, genetics, embryology, microbiology, immunology, pharmacology, and molecular biology as they apply to the musculoskeletal system.

The questions are written by the ABOS Question-Writing Task Force, a group of forty orthopaedic surgeons consisting of both private and academic practitioners. The questions are then reviewed by a second group, the ABOS Field Test Task Force, consisting of twenty-six practicing orthopaedic surgeons who also represent both private and academic practices. Surgeons from across the United States are represented on these two task forces (Fig. 1).

The life of an ABOS written certification question begins in August when question assignments are made to the members of the Question-Writing Task Force. The task force members submit their questions, all with evidence-based references, to the National Board of Medical Examiners (NBME) in Philadelphia. The NBME is contracted by the ABOS to edit and review questions for technical flaws and to provide professional expertise in developing, printing, and evaluating the written examination.

The NBME edits and corrects grammatical and technical flaws in the submitted questions. All questions are then placed into a standard style and format to provide consistency for the examination. The questions are further divided into drafts on the basis of content: adult trauma, adult disease, basic and applied science, pediatric trauma, pediatric disease, and rehabilitation.
The ABOS Question-Writing Task Force meets in Philadelphia in the spring, and every question is reviewed for accuracy, content, and relevancy. The NBME then reedits the items and enters them into the ABOS question library. The examination drafts are then administered to the members of the ABOS Field Test Task Force and are scored by the NBME for question statistics. The Field Test Task Force then meets in the fall in Chicago to review all of the items selected for the examination. The questions are again reviewed for their relevancy and accuracy with the aid of the question statistics, which are based on how the questions actually performed when administered to the members of the Field Test Task Force.

The NBME then assembles the examination on the basis of the ABOS content domains and valid psychometrics; the content domains are set by the ABOS each year. The current content domains are shown in a table in the Appendix.

During the winter meeting of the ABOS, the Written Examination Committee reviews each question and makes final item selections. In the spring, the chairman of the Written Examination Committee and the executive director of the ABOS review page proofs and approve the final draft of the examination.

The examination is administered in July to the candidates.

**Annual Examination Key Validation**

After the examination, the NBME performs a key validation process on every question to identify any potentially defective items. Items can be identified as defective if they have a very high or extremely low percentage of correct values or if they have a statistically poor discrimination value. This means that candidates who scored in the upper percentiles of the overall examination tended to get the question wrong as opposed to candidates who scored poorly on the overall examination and tended to get the question correct. All of the defective items are reviewed by the ABOS Written Examination Committee chair, the ABOS executive director, and members of the NBME. Items determined to be defective are deleted from the final scoring.

After the final scoring of the examination, the ABOS Written Examination Committee reviews the results and sets the passing standard. The passing standard is based on a Rasch bank scale calculated in logits2. Test equating by the use of a question bank scale permits the examinees tested in different years to be evaluated on a common scale. This effectively eliminates variations in test difficulty as well as variations in the average proficiency of examinees. There has been and continues to be a fixed minimum passing standard based on specific content-based and compromised standard-setting procedures that a group of practicing surgeons (the Standard Setting Task Force) has determined must be known by qualified candidates. An indepth review of the passing standard is performed on a regular basis because, as the knowledge base in the practice of orthopaedic surgery continues to evolve, it is anticipated that the standard will also change.

The candidates are notified of their results in the fall.

The final cost to the ABOS to create, edit, categorize, review, print, administer, and statistically analyze each question is approximately $2000 per question.

**2006 Examination Psychometrics**

In July 2006, the written certification examination was administered to 741 examinees. There were 321 questions administered, and six questions were deleted in the key validation process because of defects; their deletion enhanced the test score validity. Therefore, 315 questions contributed to the total examination score.

The candidates consisted of 633 United States and Canadian medical school graduates taking the examination for the first time and eighty-nine United States and Canadian medical school graduates who were repeating the examination. Thirteen international medical school graduates took the examination for the first time, and six international medical school graduates repeated the examination.

The items on the examination were analyzed psychometrically by evaluating the mean discrimination index; this is the average point biserial correlation coefficient of item score with total score. In essence, this analyzes the questions to see how well items discriminated between those candidates who obtained high scores and those with low scores on the examination. The target for this value on an examination of this type is between 0.10 and 0.30. For the 2006 examination, this value was 0.16.

The questions were also analyzed for the internal consistency reliability coefficient \( \text{KR}_{20} \). This analysis reflects the consistency of the scores and calculates the standard error of measurement for the examination. It determines the precision of examination scores or how much an examinee’s score would vary across repeated testing with different questions on the same content. For the 2006 examination, the \( \text{KR}_{20} \) was 0.90.

The passing score was calibrated with use of the Rasch bank scale model,
which has been utilized by the ABOS since 1980. The Rasch bank passing score for the 2006 examination was 1.13 logits, the same score that has been utilized since 2000. It corresponded to a raw score of 211 and 67% correct. This logit score of 1.13 was then equated to a standard score by means of a linear transformation technique. With use of the standard score, a mean and standard deviation of the Rasch score for the reference group could be calculated. The mean standard score for the 2006 examination was 200, and the standard deviation was 20 standard score points for the reference group. The standard error of measurement was 9 standard score points, which meant a candidate’s true proficiency on the examination was ±9 standard score points. This means that, if a candidate took this examination on the same content with different questions in repeated testings, his or her score would vary ±9 standard score points. This allows a candidate to understand normative data as to where he or she stands relative to the mean score of the other candidates taking the examination. The Rasch bank passing score for the 2006 examination of 1.13 logits corresponded to a standard score of 170.

The results from the 2006 examination revealed that 87.3% of all of the candidates taking the examination passed. Table I reveals the pass rates for the individual subgroups taking the examination in 2006 and for the previous five years.

In October, the candidates received their individual performance report for the 2006 examination, which included the pass-fail decision, the minimum passing standard score for this year’s examination, their standard score, and their percentile rank. Last year, the candidates also received their individual performance report for the individual content areas (see Appendix).

The program directors also received a report on how their individual candidates performed on the examination as well as aggregate information about the performance of the first-time takers from their specific program (see Appendix).

THE PART-II ORAL CERTIFICATION EXAMINATION

The ABOS recognizes that knowledge is important for competence in our specialty, but that knowledge does not equal competence. Unlike the Part-I written examination, which tests exclusively orthopaedic knowledge, the Part-II oral examination tests the application of knowledge, diagnostic acumen, surgical techniques, outcomes, and ethics and professionalism. It tests the applicant’s ability to apply knowledge in a safe and appropriate way.

The present oral examination has been developed over the last seventeen years. Previous to 1993, the oral examination involved testing over four basic content areas: pathology, pediatrics, adult, and trauma, with use of standard cases for all candidates. In 1993, the first practice-based oral examination was administered. The board of directors at the time, as well as the ABOS today and during the interim period, believed that the best way to examine an orthopaedic surgeon is to evaluate his or her own practice. The oral examination of the ABOS is the only specialty board examination that is totally based on the candidate’s own practice. This oral examination evolved over the years to the present examination, for which the candidate submits a notarized case list collected over a six-month period beginning the July prior to the examination. The case list is collected with use of a software package entitled SCRIBE (Data Harbor Solutions, Hinsdale, Illinois), which has been used and constantly updated since 1999. Presently, it is an Internet-based collection system, and cases are entered with use of CPT (Current Procedural Terminology) codes. It generates the candidate’s practice profile and complication list. Directors of the ABOS and other oral examiners select twelve cases for the candidate. The candidate then selects ten of the twelve cases to present at the time of the oral examination. All pertinent information concerning these cases, including preoperative, postoperative, and follow-up notes as well as imaging studies, laboratory studies, intraoperative arthroscopy photographs, operative reports, and consultant notes, are required for each case. The ABOS stringently adheres to HIPAA (Health Insurance Portability and Accountability Act) regulations regarding privacy in the gathering and use of this information. Pass rates have ranged from 86% to 93% over the past decade, with 90% of the 656 candidates passing the oral examination in 2006.

The examination is one hour and forty-five minutes in length, divided into three thirty-five-minute segments with a five-minute break in between each segment. During each segment, the candidate is examined by two examiners who are matched to the candidate for areas of his or her stated expertise (Fig. 2). For example, if a candidate identifies his or her special area of practice as spine surgery, at least one of the two examiners is a practicing orthopaedist who dedicates a significant part of his or her practice to spine surgery. The examiners are provided the complete case list as well as a graphic analysis of the candidate’s practice profile and complications.

The decision on pass-fail is based on the candidate’s performance as assessed
The oral examination has incorporated, over the last several years, all of the core competencies outlined by the ACGME—to include communication and interpersonal skills, professionalism, ethics, patient care, knowledge, systems-based practice, and practice-based learning and improvement—in the scoring of the examination. Last year, the ABOS provided to the residency programs and candidates the rating definitions for the various categories by which candidates will be evaluated and graded. These are available to the candidates who are taking the examination and have been provided to all program and residency directors. They can also be obtained from the ABOS office. Several articles related to the oral examination have recently been published.

OVERVIEW

The ABOS certification process is a voluntary process that was developed to "serve the best interests of the public and of the medical profession" by "evaluating the initial and continuing qualifications and competence of orthopaedic surgeons." This process is
successful because of the dedication of numerous members of our profession who donate their time to produce the best possible examinations to fairly and accurately evaluate candidates for certification by the ABOS as competent to practice orthopaedic surgery.

APPENDIX
A table showing the 2006 written examination content domain and figures showing the performance reports are available with the electronic versions of this article, on our web site at jbjs.org (go to the article citation and click on “Supplementary Material”) and on our quarterly CD-ROM (call our subscription department, at 781-449-9780, to order the CD-ROM).

<table>
<thead>
<tr>
<th>Group</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total pass rate (% of candidates)</td>
<td>76.9</td>
<td>79.1</td>
<td>82.6</td>
<td>87.5</td>
<td>83.9</td>
<td>87.3</td>
</tr>
<tr>
<td>United States and Canadian medical school graduates</td>
<td>79.3</td>
<td>80.7</td>
<td>83.9</td>
<td>88.6</td>
<td>84.9</td>
<td>87.8</td>
</tr>
<tr>
<td>First-time takers</td>
<td>87.2</td>
<td>88.8</td>
<td>93.1</td>
<td>96.3</td>
<td>92.9</td>
<td>93.8</td>
</tr>
<tr>
<td>Repeaters</td>
<td>39.2</td>
<td>45.8</td>
<td>40.3</td>
<td>45.9</td>
<td>28.0</td>
<td>44.9</td>
</tr>
<tr>
<td>International medical school graduates</td>
<td>29.7</td>
<td>47.4</td>
<td>38.1</td>
<td>25.0</td>
<td>62.5</td>
<td>68.4</td>
</tr>
<tr>
<td>First-time takers</td>
<td>76.9</td>
<td>94.1</td>
<td>66.7</td>
<td>50.0</td>
<td>80.0</td>
<td>84.6</td>
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<tr>
<td>Repeaters</td>
<td>4.2</td>
<td>9.5</td>
<td>26.7</td>
<td>20.0</td>
<td>0.0</td>
<td>33.3</td>
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<tr>
<td>All first-time takers</td>
<td>87.0</td>
<td>88.9</td>
<td>92.9</td>
<td>96.1</td>
<td>92.3</td>
<td>93.7</td>
</tr>
<tr>
<td>All repeaters</td>
<td>33.6</td>
<td>41.2</td>
<td>38.9</td>
<td>43.8</td>
<td>25.8</td>
<td>44.2</td>
</tr>
<tr>
<td>Passing score</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correct answers (%)</td>
<td>69.8</td>
<td>67.8</td>
<td>69.8</td>
<td>67.1</td>
<td>68.6</td>
<td>67.0</td>
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<tr>
<td>Standard score* (points)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>171</td>
<td>170</td>
</tr>
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</table>

*2005 was the first year that standard scores were reported.

REFERENCES
### TABLE E-1 ABOS WRITTEN EXAMINATION CONTENT DOMAINS FOR 2006

<table>
<thead>
<tr>
<th>Category</th>
<th>Items (#)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Adult Trauma</td>
<td>60</td>
</tr>
<tr>
<td>2. Adult Disease</td>
<td>67</td>
</tr>
<tr>
<td>3. Pediatric Trauma</td>
<td>22</td>
</tr>
<tr>
<td>4. Pediatric Disease</td>
<td>30</td>
</tr>
<tr>
<td>5. Sports Adult/Pediatric</td>
<td>33</td>
</tr>
<tr>
<td>6. Rehabilitation</td>
<td>19</td>
</tr>
<tr>
<td>7. Diagnosis</td>
<td>105</td>
</tr>
<tr>
<td>8. Operative Management</td>
<td>103</td>
</tr>
<tr>
<td>9. Nonoperative Management</td>
<td>59</td>
</tr>
<tr>
<td>10. Basic Science</td>
<td>101</td>
</tr>
<tr>
<td>11. Neoplasm</td>
<td>28</td>
</tr>
<tr>
<td>12. Spine</td>
<td>51</td>
</tr>
<tr>
<td>13. Upper Extremity</td>
<td>27</td>
</tr>
<tr>
<td>(Clavicle, Scapula, Shoulder)</td>
<td></td>
</tr>
<tr>
<td>14. Upper Extremity</td>
<td>24</td>
</tr>
<tr>
<td>(Humerus, Elbow, Radius/Ulna)</td>
<td></td>
</tr>
<tr>
<td>15. Upper Extremity</td>
<td>26</td>
</tr>
<tr>
<td>(Wrist, Hand)</td>
<td></td>
</tr>
<tr>
<td>16. Lower Extremity</td>
<td>26</td>
</tr>
<tr>
<td>(Pelvis, Hip, Femur)</td>
<td></td>
</tr>
<tr>
<td>17. Lower Extremity</td>
<td>35</td>
</tr>
<tr>
<td>(Knee, Tibia/Fibula)</td>
<td></td>
</tr>
<tr>
<td>18. Lower Extremity</td>
<td>29</td>
</tr>
<tr>
<td>(Ankle, Foot)</td>
<td></td>
</tr>
</tbody>
</table>

The eighteen categories are not mutually exclusive. The item counts are after the key validation process and for actual scoring.
Table E-1: Written Examination Content Domains for 2006

<table>
<thead>
<tr>
<th>Category</th>
<th>Items (#)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Adult Trauma</td>
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</tr>
<tr>
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<td>67</td>
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<tr>
<td>3. Pediatric Trauma</td>
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<tr>
<td>4. Pediatric Disease</td>
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</tr>
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<td>5. Sports Adult/Pediatric</td>
<td>33</td>
</tr>
<tr>
<td>6. Rehabilitation</td>
<td>19</td>
</tr>
<tr>
<td>7. Diagnosis</td>
<td>105</td>
</tr>
<tr>
<td>8. Operative Management</td>
<td>103</td>
</tr>
<tr>
<td>9. Nonoperative Management</td>
<td>59</td>
</tr>
<tr>
<td>10. Basic Science</td>
<td>101</td>
</tr>
<tr>
<td>11. Neoplasm</td>
<td>28</td>
</tr>
<tr>
<td>12. Spine</td>
<td>51</td>
</tr>
<tr>
<td>13. Upper Extremity (Clavicle, Scapula, Shoulder)</td>
<td>27</td>
</tr>
<tr>
<td>14. Upper Extremity (Humerus, Elbow, Radius/Ulna)</td>
<td>24</td>
</tr>
<tr>
<td>15. Upper Extremity (Wrist, Hand)</td>
<td>26</td>
</tr>
<tr>
<td>16. Lower Extremity (Pelvis, Hip, Femur)</td>
<td>26</td>
</tr>
<tr>
<td>17. Lower Extremity (Knee, Tibia/Fibula)</td>
<td>35</td>
</tr>
<tr>
<td>18. Lower Extremity (Ankle, Foot)</td>
<td>29</td>
</tr>
</tbody>
</table>

The eighteen categories are not mutually exclusive. The item counts are after the key validation process and for actual scoring.

MANUSCRIPTS

Fig. E-1
A candidate’s individual performance report.
The American Board of Orthopaedic Surgery

Performance Report for the 2006 Part I Written Examination

Content Area Performance Profiles

<table>
<thead>
<tr>
<th>Content Area</th>
<th>Lower Performance</th>
<th>Borderline Performance</th>
<th>Higher Performance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult Trauma</td>
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<td></td>
</tr>
<tr>
<td>Adult Disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pediatric Trauma</td>
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<td>*</td>
<td></td>
</tr>
<tr>
<td>Pediatric Disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sports (Adult/Pediatric)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rehabilitation</td>
<td></td>
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<tr>
<td>Diagnosis</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Operative Management</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Operative Management</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basic Science</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neoplasms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper Extremity (Clavicle, Scapula, Shoulder Joint)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper Extremity (Humerus, Elbow Joint, Radius/Ulna)</td>
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<td>★</td>
<td></td>
</tr>
<tr>
<td>Upper Extremity (Wrist Joint, Hand)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower Extremity (Pelvis, Hip Joint, Femur)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower Extremity (Knee Joint, Tibia/Fibula)</td>
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</tr>
<tr>
<td>Lower Extremity (Ankle Joint, Foot)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The above performance profile is provided to aid in self-assessment. The shaded area defines a borderline level of performance for each content area; borderline performance is comparable to a high fail/low pass on the total test. Performance bands indicate areas of relative strength and weakness. Some performance bands are wider than others. The width of a performance band reflects the precision of measurement: narrower bands indicate greater precision. The bandwidth for a given content area is the same for all examinees. An asterisk indicates that your performance band extends beyond the displayed portion of the scale. Small differences in the location of the bands should not be over interpreted. If two bands overlap, the performance in the associated areas should not be interpreted as being significantly different. Because the ABOS Part I Written Examination is designed to be integrative, some items contribute to more than one content area. As a consequence, caution should be used when interpreting differences in performance across content areas. This profile should not be compared to profiles from other ABOS Part I Written Examination administrations.

Fig. E-2
A candidate’s performance report for content domains.
American Board Of Orthopaedic Surgery

Performance of First Takers from Your Program on 2005–2006 ABOS Written Certifying Examination Program:

Total Examinees: 14  Total Passing: 12  Total Failing: 2

- Adult Trauma
- Adult Disease
- Pediatric Trauma
- Pediatric Disease
- Sports (Adult/Pediatric)
- Rehabilitation
- Diagnosis
- Operative Management
- Non–Operative Management
- Basic Science
- Neoplasms
- Spine
- Upper Extremity (Clavicle, Scapula, Shoulder Joint)
- Upper Extremity (Humerus, Elbow Joint, Radius/Ulna)
- Upper Extremity (WristJoint, Hand)
- Lower Extremity (Pelvis, Hip Joint, Femur)
- Lower Extremity (Knee Joint, Tibia/Fibula)
- Lower Extremity (Ankle Joint, Foot)

The above graph provides information regarding the score distribution of U.S./Canadian medical school graduates taking the test for the first time from your program relative to the distribution for all U.S./Canadian medical school graduates in each score category. All scores are scaled in standard score units based on the performance of U.S./Canadian medical school graduate first-takers: the mean and standard deviation (SD) for this group are 0 and 1, respectively, for each content category. The mean performance of U.S./Canadian medical school graduate first-takers is represented by the vertical solid green line at 0.0. Roughly 68% of U.S./Canadian medical school graduate first-takers scored within one SD of the mean, between −1.0 and 1.0. The distribution of performance for U.S./Canadian medical school graduate first-takers from your program is represented by the red boxes and horizontal lines. The red box depicts the mean performance of U.S./Canadian medical school graduate first-takers from your program. The distance from the red box to one end of the red line indicates one SD for your program. The interval spanned by each red line represents your program mean plus/minus one SD; approximately 68% of your residents scored in this interval.

By comparing the locations of the red boxes, you can determine the score categories in which the performance of your residents was relatively strong or weak.

Fig. E-3
A program director's report of aggregate data illustrating the performance of the program's candidates who took the examination for the first time.
CONFLUENCE INDUCES APOPTOSIS IN HUMAN MESENCHYMAL STEM CELLS IN VITRO AND IS INHIBITED BY DEXAMETHASONE

In-Hwan Song1,2,3, Arnold I Caplan2, James E Dennis1,2

1Department of Orthopaedics and 2Skeletal Research Center, Case Western Reserve University, Cleveland OH, 3Department of Anatomy, Yeungnam University College of Medicine, Daegu, Korea.

INTRODUCTION

Cellular homeostasis in the body is maintained by a balance between stem cell renewal and cell loss. Human bone marrow-derived MSCs are a rare population of undifferentiated cells that have the capacity to self-renew and can differentiate into mesodermal phenotypes including osteoblasts, chondrocytes, myocytes, and adipocytes. While the self-renewal capacity of MSCs remains in question, it is clear that, at the very least, MSCs have the capacity to expand extensively and to differentiate into multiple mesenchymal phenotypes in culture. Induction of osteoprogenitor differentiation of MSCs by Dex is well known. Supplementation with Dex at 10^{-8}M has been reported to promote osteoprogenitor cell differentiation in marrow stromal cells. While self-renewal capacity of MSCs remains in question, it is clear that, at the very least, MSCs have the capacity to expand extensively and to differentiate into multiple mesenchymal phenotypes in culture. Induction of osteoprogenitor differentiation of MSCs by Dex is well known. Supplementation with Dex at 10^{-8}M has been reported to promote osteoprogenitor cell differentiation in marrow stromal cells. In addition, Dex is also a component of medium used for differentiation into chondroblasts, myocytes and adipocytes in vitro.

Dex has been extensively used as an anti-inflammatory agent because of its known immuno-suppressive effects. However, the exact mechanisms of this suppression is still unknown. Dex also has been shown to enhance cell survival or to promote cell death by modulating apoptosis in a cell-specific manner. For example, Dex treatment has been shown to induce apoptosis in thymocytes, lymphocytes, some tumor cells, and respiratory epithelium, whereas primary cultured hepatocytes, neutrophils and some solid tumors show reduced apoptosis after Dex treatment. Induction of apoptosis in immune system is one possible mechanism for reducing autoimmune and hyper-immune responses. Induction of apoptosis is also an important defense mechanism against emergence of cancer, and is frequently used as co-treatment for cancer because of its potential pro-apoptotic properties and for its ability to reduce side effects of chemotherapy or radiotherapy. In some solid tumors, pro-apoptotic effects of chemotherapy or radiotherapy regimens in patients with tumors might be strongly antagonized by the significant anti-apoptotic effects of Dex.

Clearly, the effects of Dex on apoptosis vary widely with the cell type in question. Interestingly, while Dex has been used extensively with MSCs as an inductive factor for multiple end-stage phenotypes, but few studies have assessed the effects of Dex on proliferation and apoptosis of MSCs. In the present study, higher levels of apoptosis were observed in cultured human bone marrow MSCs at high confluence in standard medium but not in Dex supplemented medium. Previous reports in our lab showed that Dex enhanced proliferation of MSCs, but this contrasts with general concept that proliferation capacity of stem cells decreased with differentiation. Accordingly, in this study we investigated the induction pattern of apoptosis in MSCs and assessed the independent effect of humoral factors, cell contact, and Dex supplementation as it relates to apoptosis of MSCs.

MATERIALS AND METHODS

Cell Culture

Mesenchymal stem cells (MSCs) were isolated from aspirated iliac crest from normal human donors (age 17 to 58 years) after informed consent using methods modified from those described previously. Briefly, 10 ml of marrow was added to 20 ml of Dulbecco’s modified Eagle’s medium with low glucose (DMEM-LG; Sigma, MO, USA) supplemented with 10% fetal bovine serum (FBS; Gibco-BRL/InVitrogen, Carlsbad, CA) from selected lots and centrifuged at 450
were stained with uranyl acetate and lead citrate.

Ultrathin sections were fixed in 1% osmium tetroxide (Polysciences, Warrington, PA) in 0.1 M PBS, dehydrated in ethanol and embedded in Epon 812 (Polysciences, Warrington, PA). After embedding, ultrathin sections were cut and counterstained with 100 nM DAPI (Sigma, St. Louis, MO). 

**Ultrathin sectioning**

For electron microscopy, cells were collected with a cell scraper and centrifuged in a 15 ml conical tube containing PBS. Pellets were fixed in 2.5% glutaraldehyde and permeabilized with cytonin. DNA fragmentation sites were labeled by biotinylated TdT solution and streptavidin-fluorescein conjugate was tagged to TdT. The nuclei of the cells were counterstained with 100 nM DAPI (Sigma, St. Louis, MO) in PBS for 5 min.

**Electron Microscopy**

Cells were collected with a cell scraper and centrifuged in a 15 ml conical tube containing PBS. Pellets were fixed in 2.5% glutaraldehyde (Polysciences, Warrington, PA) in 0.1 M PBS, washed in PBS, post-fixed in 1% osmium tetroxide (Polysciences, Warrington, PA) in 0.1 M PBS, dehydrated in ethanol and embedded in Epon 812 (Polysciences, Warrington, PA). Ultrathin sections were stained with uranyl acetate and lead citrate.

**Apoptosis and DNA Assays**

For the cell counting assay, MSCs were plated over cover slips inserted in 100 mm culture dishes at cell densities of 1×10^4 (1×), 3×10^4 (3×), 9×10^4 (9×) cells per square centimeter. Each set of cells was cultured with (D) or without 100 nM Dex (Sigma, St. Louis, MO) (C).

**DNA fragmentation ELISA**

DNA assay was performed with parallel prepared cultures. Briefly, all cultures were placed in -70°C after washing with PBS. When all samples were collected, the dishes were thawed and 500 µl 0.1 M NaOH was added to extract DNA. The sample was then refrozen, thawed and an equal volume of neutralization buffer (5 M sodium chloride, 100 mM disodium phosphate, 2 mM EDTA, 0.1 N hydrochloric acid) was added. Aliquots of 100 µl were loaded into 96 well plates and an equal volume of 1 µM/ml Hoechst dye (Sigma, St. Louis, MO) was added, and DNA content was measured in a GeniosPro fluorescent measurement plate reader (Tecan US, Durham, NC) at 360 nm excitation, 460 nm emission and absolute amount was calculated with parallel prepared calf thymus DNA standards (Amersham Bioaciences, NJ).

**Tunel stain and DAPI stain**

Apoptotic bodies were detected with TdT-Fluorescein TACS™ In Situ Kit (R&D Systems, Minneapolis, MN). Briefly, MSCs grown on coverslips were fixed in 2.5% glutaraldehyde and permeabilized with cytonin. DNA fragmentation sites were labeled by biotinylated TdT solution and streptavidin-fluorescein conjugate was tagged to TdT. The nuclei of the cells were counterstained with 100 nM DAPI (Sigma, St. Louis, MO) in PBS for 5 min.

**Apoptotic bodies**

Cells were collected with a cell scraper and centrifuged in a 15 ml conical tube containing PBS. Pellets were fixed in 2.5% glutaraldehyde (Polysciences, Warrington, PA) in 0.1 M PBS, washed in PBS, post-fixed in 1% osmium tetroxide (Polysciences, Warrington, PA) in 0.1 M PBS, dehydrated in ethanol and embedded in Epon 812 (Polysciences, Warrington, PA). Ultrathin sections were stained with uranyl acetate and lead citrate.

**Electron Microscopy**

Cells were collected with a cell scraper and centrifuged in a 15 ml conical tube containing PBS. Pellets were fixed in 2.5% glutaraldehyde (Polysciences, Warrington, PA) in 0.1 M PBS, washed in PBS, post-fixed in 1% osmium tetroxide (Polysciences, Warrington, PA) in 0.1 M PBS, dehydrated in ethanol and embedded in Epon 812 (Polysciences, Warrington, PA). Ultrathin sections were stained with uranyl acetate and lead citrate.
A cell-free, empty control (EMP), was also tested. Cell count, DNA fragmentation ELISA assay, and DNA assays were performed as described. A glass coverslip was inserted into each well for cell counting and 6 well plates with tissue culture insert were used for DNA quantification and for the DNA fragmented ELISA assay. Cultures were harvested and tested after 2 weeks in culture.

**Cell Density/Cell Contact Effects**

To determine the effect of cell contact on apoptosis, M/SCs were tested in directly mixed co-cultures. MSCs were plated at 3.0 × 10³ cells/cm² and, after 2 days, exchanged into medium containing 10 µM of BrdU (Sigma, MO, USA). After 5 days, the BrdU-labeled MSCs were trypsinized and plated into 6 well plates at a cell density of 3.0 × 10³ cells/cm² onto culture dishes containing glass cover slips. Parallel cultured human chondrocytes were added at zero (1:0), twice (1:2), and 8 times (1:8) the number of MSCs. After 2 weeks, cultures were harvested and fixed in -20°C methanol for 10 min. BrdU labeled MSCs were immunostained with anti-BrdU mouse IgG (Dako, Denmark) and anti-mouse FITC-conjugated goat IgG (Sigma, MO, USA) and were also counterstained with 10 nM DAPI in PBS.

At least 15 fields were randomly selected for each sample and two kinds of images in the same field were captured at 200× magnification: BrdU-positive cells and apoptotic bodies were imaged with a blue filter (480 nm), and DAPI counterstained nuclei were imaged under a UV filter (360 nm). Corresponding pictures were merged to assess the percentage of apoptotic bodies. BrdU-positive small bodies that were irregularly shaped and merged within a well-formed nucleus were counted as simply a dividing MSCs (Fig. 6), but small bodies that were rounded and didn’t merge as part of nucleus were counted as apoptotic bodies (Fig. 7). The results were expressed as the number of apoptotic bodies per BrdU positive MSCs.

**RESULTS**

**Histochemistry and electron microscopy**

MSCs showing fragmented and condensed nuclei, indicative of apoptotic bodies, were frequently observed in over-confluent MSCs stained with DAPI (Fig. 1). Tunel staining was used to confirm this interpretation and showed that the fragmented DNA observed in the DAPI-stained samples (Fig 2A) usually stained positively with the TUNEL stain (Fig. 2B). Over-confluent MSC preparations were also examined with electron microscopy and apoptotic cells showing segregation of chromatin and condensation of cytoplasm were observed (Fig. 2C). Based on these results, we concluded that cells with nuclei fragmented that were observed in DAPI stain preparations are apoptotic cells.

**Apoptosis Trends**

The general trend of cell expansion...
with cells seeded at different densities was mostly as expected: total cell numbers increased with time and higher density cultures had more cells, but one important point is that the difference between groups decreased with time, that is, the higher density cultures expanded more slowly than the lower density cultures. The number of cells cultured in medium with Dex was lower than in control medium at early periods, but surpassed the cells in control medium with time (data not shown). The ratio of apoptotic cells to total cells increased with time and correlated with seeding density (Fig 3). The percentage of apoptotic cells cultured with Dex was lower, at each comparable cell density and at each of the time points (Fig. 3). The count assay (DAPI staining counts) and the ELISA assay were in general agreement with each other with just occasional minor differences. There were significant differences with time in culture as well as with supplementation with Dex in both tests (p<0.01) while cell density results only showed a significant difference (P<0.05) with the ELISA assay, as determined by General Linear Model analysis.

**Reversibility of apoptosis**

The ratio of apoptotic to total cells increased more rapidly in control medium with culture duration, but it is not known if Dex treatment could reverse the process or not. To test this, MSCs were placed in control conditions and, after 1 or 2 weeks in control conditions, the culture medium was switched to Dex-supplemented conditions; continuous Dex conditions and continuous control conditions were also included (Fig. 4). It's clear from the 3 week data of either the direct count or the ELISA data that longer exposure to Dex resulted in less apoptosis. At the same time, there seems to be a delayed entrance into apoptosis that is not completely reversible with Dex supplementation. For example, looking at the samples that are switched at 1 or 2 weeks from control to Dex, there is still a significantly greater number of apoptotic cells in the switched samples after three weeks compared to the Dex control group. There were significant difference among groups in General Linear Model analysis (P<0.01).

**Soluble factors**

While increased cell density is known to correlate with apoptosis levels, it was not known if that effect was due to cell contact or through soluble factors. To test this, MSCs plated and then
co-cultured with inserts containing MSCs, fibroblasts or empty inserts to determine if soluble factors were responsible for the effect on apoptosis levels. As shown earlier, there was a significant difference in apoptosis levels between Dex and Con groups (Fig.5) in the count and ELISA assays. The apoptosis ratio in Con groups was about 1.3% while the Dex groups were lower than 0.06%. MSC, Fib, and Emp groups in the Con group had similar apoptosis levels with no significant differences among them as determined by General Linear Model analysis (P>0.05). The ratio of DNA fragmentation in the ELISA assay showed a similar trend to that with the counting assay (Fig. 5). These data indicate that cell number alone does not account for the density effects on apoptosis.

Cell Contact Effects
MSCs were labeled with BrdU to track the MSCs in co-cultures with chondrocytes and the number of apoptotic bodies per BrdU positive MSC was determined to see the effect of cell density when cells are in contact. The BrdU labeling index was more than 80% and it had a negative effect on MSC growth, with the number of BrdU positive MSCs being highest in the 1:0 condition and lowest in 1:8, with 18.1, 8.6, and 6.0 BrdU-positive MSCs per field in 1:0, 1:2, and 1:8.

CONFLUENCE INDUCES APOPTOSIS IN HUMAN MESENCHYMAL STEM CELLS

![Fig. 5. Ratio of MSCs apoptosis in co-culture with other cells separately in tissue culture insert. MSC: co-culture with MSCs; Fib: co-culture with fibroblasts; Emp: empty insert as control. Dexa: culture with dexamethasone; Con: culture without dexamethasone. Bar represents mean±SE. No significant difference among groups (p>0.05).](image)

![Fig. 6. Fluorescent micrographs of BrdU-labeled MSCs co-cultured with chondrocytes. Merged picture (A) shows BrdU-positive, irregularly shaped small bodies observed within rounded nuclei, indicating they are chromatin of dividing cells. DAPI counter-stain only shows both nuclei of MSCs and chondrocytes (B). BrdU only immunostained image (C).](image)
Con conditions. An example of the BrdU-labeled MSCs co-cultured with chondrocytes is shown in Figure 7. The ratio of apoptotic bodies per BrdU positive cells was 5.2, 22.3, and 18.8 in samples co-cultured with chondrocytes at a ratio of 1:0, 1:2, and 1:8 (MSCs: Chondrocytes). In Con sample (Fig. 8) there were significant differences between 1:0 and 1:2 or 1:8 conditions (P<0.05), but no significant difference between the 1:2 and 1:8 samples using General Linear Model analysis (P>0.05). Moreover apoptosis of MSCs correlated with total cell number in correlation analysis (P<0.05). Even in the Dex group, where apoptosis was again inhibited, there was a trend of increasing MSC apoptosis in the samples containing more chondrocytes. These data, along with the results shown in Figure 7, show that cell contact is required for cell density effects on apoptosis.

**DISCUSSION**

While the use of Dex to induce osteogenic differentiation of MSCs in culture has been well documented, the potential effects on apoptosis have not been elucidated. In this study, we examined the effects of Dex on MSC apoptosis and determined that Dex treatment decreases MSC apoptosis, that high cell density increases apoptosis and that the high cell density effect requires cell contact. These results reveal how culture conditions may effect not only cell differentiation along the osteogenic lineage, but may also effect the survival of subsets of cells within the MSC population. For example, Dex induces osteogenesis in human MSCs, but the fact that it also impedes apoptosis raises the question as to whether Dex may actually be allowing the survival and expansion of osteogenic MSCs which may disappear in culture conditions that do not contain Dex. These results also raise the question as to whether the MSCs that apoptose in the absence of Dex are a specific subset of the MSC population or if the apoptotic process is stochastic. This information may impact on whether all MSCs are identical, and may impact how Dex might be used to promote the selective survival of osteogenic MSCs.

The mechanism of the apoptotic protective effects of Dex is unknown and there are several pathways that need to be investigated. Since Dex has been shown to promote osteogenesis in MSCs, the possibility of how osteogenic factors effect MSCs needs to be investigated. In one study, it was shown that BMP-2 promotes apoptosis in human MSCs via binding to the BMP-2 receptor RIB. Interestingly, BMP-2 has been shown to decrease in its level of expression on day-6 human MSCs under the influence of...
CONFLUENCE INDUCES APOPTOSIS IN HUMAN MESENCHYMAL STEM CELLS

Fig. 8. Total cell number (MSCs plus chondrocytes; left), BrdU positive MSCs number (middle), and ratio of MSCs apoptosis to MSCs number in direct co-culture with human chondrocytes and MSCs. 1:0, 1:2, and 1:8 indicates seeding cells ratio of MSCs to chondrocytes. Bar represents mean±SE. Significant difference between a and b (P<0.05).

Dex 38 while, at the same time, Dex highly upregulates the expression of BMP-6 24,39. This might account for both the osteogenic properties of Dex and for its apoptotic inhibitory effects. Another possibility is that Dex may effect another regulator of apoptosis, such as Bcl-2, an inhibitor of apoptosis 40 or sclerostin, which has been shown to promote apoptosis in osteoblasts 41. Clearly, the effects of Dex on the multiple factors involved in the mitochondrial and death receptor apoptosis pathways should also be investigated (Reviewed by 42).

The fact that cell contact of MSCs is potentially apoptotic is potentially very important with respect to expansion of MSCs for clinical applications. Either MSCs should be kept at sub-confluent conditions throughout expansion or, perhaps, they can be allowed to reach confluence as long as there is Dex present. It would be interesting to determine how crowding effects the MSCs in primary cultures where the colonies are extremely dense. Is there a high level of apoptosis in the center of colonies and would this be alleviated by the addition of Dex? It may be also be advantageous that newly isolated MSCs should be passaged earlier to avoid overcrowding and potential apoptosis of early osteogenic progenitors, especially when they are in colonies and are especially crowded.

ACKNOWLEDGEMENTS

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REFERENCES


Leon Root

“The hip in cerebral palsy”

Mark Gebhardt

“Benign bone tumors in children”
“Evaluation and management of soft tissue sarcomas”
“Limb salvage in pediatric malignant bone tumors”
Dr. Harry Herkowitz

“Cervical radiculopathy and myelopathy”
“Evolution of spondylolisthesis”

Fred Kaplan

“Fibrodysplasia Ossificans Progressiva”

Dr. Fred Kaplan (left) and Dr. Steve Fitzgerald
Department of Orthopaedics Celebrates 40 Years of Interdisciplinary Research and Discovery in Orthopaedic Biomechanics

By Clare Rimnac, Ph.D. and Dwight Davy, Ph.D.

This past April, the Department of Orthopaedics, along with the Department of Mechanical and Aerospace Engineering, hosted a two-day symposium to celebrate 40 years of interdisciplinary research in orthopaedic biomechanics through the Musculoskeletal Mechanics and Materials Laboratories. The symposium featured lectures by current faculty and by former engineers and clinician alums who have pursued research projects in the MMM labs, including Victor Goldberg, M.D., Matthew Kraay, M.D., and Erika Mitchell, M.D. (2005). Current primary and related faculty of the MMM labs, Clare Rimnac, Ph.D., Dwight Davy, Ph.D., Joseph Mansour, Ph.D., Christopher Hernandez, Ph.D., and Melissa Knothe Tate, along with former graduate student Ozan Akkus, Ph.D. (2000) and post-doctoral fellow Karl Jepsen, Ph.D. (1996), also made presentations. The keynote speaker for the event was Albert Burstein, Ph.D., one of the founders of the program. Dr. Burstein spoke on the influence of knee mechanics and the role of design analysis in the development of total knee replacements.

The Musculoskeletal Mechanics and Materials Laboratory at Case Western Reserve University began its history with the arrival of Dr. Albert Burstein and Dr. Victor Frankel at Case Western Reserve University in 1967. Dr. Burstein, a Mechanical Engineer, teamed with Dr. Frankel and later with Dr. Kingsbury Heiple to pursue research in a number of areas related to orthopaedic biomechanics. The Orthopaedic Biomechanics Laboratory, as it was then known, rapidly became an internationally recognized contributor to musculoskeletal research. Collaborative research spanned such areas as kinematics and dynamics of musculoskeletal systems, design of prosthetic joints, in-vivo measurements of forces on implants, spinal cord monitoring during surgery, and fundamental properties of bone, cartilage, and other skeletal tissues.

Reflecting changing faculty research interests, the lab assumed the name Orthopaedic Engineering Laboratories in the 1970’s and in the 1990’s, assumed its present name. Today the lab’s activities reflect some of the most pressing issues facing the field of musculoskeletal medicine. Work includes characterizing and modeling damage accumulation in tissues and implant materials, failure analysis of prosthetic joints, tissue engineering and tissue regeneration, and tissue adaptation. Throughout its history, the Lab has served as a training ground for students, fellows, residents, and visiting faculty. In this 40th anniversary year, we celebrate the contributions of many to both research and education in engineering applied to orthopaedics.
John Callaghan

“Why did we leave the Charnley total hip replacement”
This summer Kitty and John Makley and later Deb and Les Nash traveled to nearby Kelleys Island to visit with Kay Herndon and some of her family. Kay travels to Kelleys Island from Galveston, Texas to enjoy the summer at the Herndon family compound on the East side of the Island. This has been in the family for generations and was a favorite retreat for Charlie Herndon including his retirement. It was here in his favorite apple orchard that Charlie was struck down by a fatal stroke. The family has recently memorialized him with a stone bench placed in that orchard.

Kay lives there with her son, Chuck, and his wife Cindy who are full time residents of the Island. Chuck is a well-recognized artist dealing mainly in stone and wood sculptures. His studio is located next to Kay’s House. It was Chuck who was recently in the national news for his daring nighttime rescue of a young boy from a plane that went down offshore shortly after taking off from the Kelley Island airstrip.

Kay is visited by her family and grandchildren during the summer and is a well-recognized member of the Kelley Island community. She remains her bright, humorous, intelligent self though slowed a bit by failing health. However, she has a number of friends and family who escort her around the island on her golf cart. Her memories and stories of Charlie are wonderful, laced with chuckles about one adventure or incident or another. The Makley and Nash time spent with her was particularly treasured as in many ways Kay was as interesting and vital as her renowned husband. When told of the fact that we might be doing this article, she asked that she and Charlie be remembered to all of the wonderful faculty and residents whose lives they were part of along with those that have come along since.

John Makley, M.D.
Les Nash, M.D.
Kay Herndon, her son Chuck and grandson Chris in Chuck’s studio on Kelleys Island.

Memorial stone bench dedicated by Dr. Herndon’s family in his favorite orchard.

Kay Herndon and grandson Chris on her favorite mode of transportation.
Dr. Carter, a retired Professor of Pathology and Orthopaedics, School of Medicine, Case Western Reserve University, died Aug. 26, 2007, at his home in Willoughby. Born April 21, 1917, in Buffalo, N.Y., he attended Hamilton College as a premedical student while continuing to cultivate a lasting love of classical and big-band music. After receiving his Bachelor’s of Science degree, he entered the Rochester School of Medicine and Dentistry where he earned his M.D. with honors in 1943. He served his internship and residency at the University of Iowa and returned to its faculty following a two-year tour of duty in the U.S. Navy.

Appointed Professor and Chairman of the Department of Pathology and Oncology at the University of Kansas Medical Center in 1960, he moved on to Case Western Reserve University six years later as Professor and Director of its Institute of Pathology.

Freed from administrative responsibilities with his retirement in 1981, he returned to his favorite field, orthopaedic pathology, in which he continued to teach and do research for 21 years.

During the course of a distinguished career, he contributed to the advancement of medical knowledge in more than 100 publications in the fields of blood coagulation and orthopaedic pathology. Additionally, he served on innumerable professional and governmental committees and chaired more than a few of them.

It was his contribution to teaching and education, however, that gave him particular satisfaction, and among the many honors and accolades he received, he rightly cherished most his teaching awards from generations of medical students and residents. His most recent (2004) award that touched him deeply was the first Howard T. Karsner Medal given as most reflecting the professional attributes of Dr. Karsner, found of the Institute of Pathology.

Later in life he embraced fly fishing as he had done earlier with archery.

He is survived by his wife of 64 years, Adelaide (nee Briggs); daughters, Marilyn A. Thompson of Dublin, Ohio and Jeanne C. Halpern of Princeton, N.J.; and granddaughter, Lily W. Halpern of Princeton, N.J.
On the 21st of March, 2007, in Madrid at the “Fundacion Jimenez Diaz” Clinic, where he practiced and developed his techniques in Orthopaedic Surgery, Dr. Miguel Ferrer Torrelles passed away surrounded by his family, his colleagues and friends, the victim of a rapid and mortal illness that did not impede him to exercise his activities until almost his last days.

With Dr. Ferrer there disappears a revealing figure in orthopaedic surgery in Spain, who has been, and will continue to be an obliged reference and permanent remembrance for those who had the privilege and fortune of being formed and worked at his side. With his example as a professional and an individual he transmitted a very special behavior that marks the trajectory of many of his men.

Dr. Ferrer graduated from Madrid’s Central University of Medicine, “San Carlos University,” at the age of 23 years. He studied under Professor Morales Pleguezuelo, a course on Bone Tumors, then, published two books on this subject. When Professor Jimenez Diaz founded his clinic “Fundacion Jimenez Diaz,” Dr. Ferrer was called to take part in the orthopaedic group. Soon after, in 1956, he left for the United States to complete his residency in Orthopaedics at Case Western Reserve University, in Cleveland, Ohio, under Dr. Charles Herndon, whom he admired and followed both professionally and personally. After having completed his studies he was asked to remain six months more to prepare a course on bone tumors to be added to future programs for orthopaedic residents.

During those years of learning he came in contact with personalities in Orthopaedic Surgery such as Dr. Austin Moore, McElroy, Moe, Winter, Sarmiento, Enneking, and companions of many others such as King Heiple, Dean McEwin, Bradford, Simmons, Hall, Ponseti, Salter, Tachdijan, Westin, Salvati, Cabanela, Roger Mann, George Mitchell, Merle D’Aubigne, etc., all important leaders in the field of Orthopaedic Surgery and with whom he participated in publications and scientific courses, nationally and internationally.

When he returned to Spain in 1960 he was named Chief of the Department of Children’s Orthopaedic Surgery at the “Fundacion Jimenez Diaz.” He also was a consultant to the Hospital at Madrid’s Torrejon Air Base of the United States Air Force.

His staff included Drs. Teresa Ceballos, Castillo Benitez Cano, Enrique Fdez. Paredes, and, his son, Antonio Ferrer Loewinsohn.

In time, the Orthopaedic Services at the “Fundacion Jimenez Diaz” became a well-known academic center in Spain and internationally, from where many graduates emerged with sound orthopaedic knowledge acquired under Dr. Ferrer’s training.

Dr. Ferrer also cultivated cultural aptitudes, especially in the Arts. His last presentation in the United States was given in Tampa, Florida, in 2006 to the Orthopaedic Community titled “Musculo-Skeletal Pathology in the paintings of Francisco de Goya.”

It has been a privilege to have known an alumnus from Dr. Herdon’s orthopaedic program. As well as his wife Margot and their children, Tono (Orthopaedic Surgeon), Carmen, Mike, Jaime, and Marga, following behind, his grandson Dr. Andres Vergara Ferrer, a resident in Orthopaedics, the third in the Ferrer tradition. Visiting their home felt like you never left your own, and sharing his family was like a continuity of your own.

F. Castillo-Benitez, M.D.
G.E. Vega, M.D.
Paul H. Curtiss Jr. MD, 87, of South Natick, Ma died Tuesday, September 25th 2007, at the Wayside Hospice in Wayland, Massachusetts. He was diagnosed with small cell lung cancer in July. He underwent one cycle of chemotherapy. Shortly after, he decided that hospice care was the most dignified way to live out his last few days of life. He was the beloved husband of Maria Elizabeth (Goldman) Curtiss, who passed away in 2001. Dr. Paul Curtiss was born in Kokomo, Indiana in June of 1920. He was the son of Paul H. and Georgia (Tanner) Curtiss. He was the brother of the late Col. George Wells. Dr. Curtiss had been a resident of South Natick for the past 29 years, previously living in Bexley, Ohio.

Dr. Curtiss was a graduate of the University of Wisconsin, receiving his medical degree in 1944. He had many achievements throughout his life, including Professor of Orthopedic Surgery at Western Reserve University School of Medicine in Cleveland, Ohio, from 1964-1965; Head of Orthopedics at Ohio State University in Columbus from 1965-1978. Dr. Curtiss was a lecturer on orthopedic surgery at Harvard Medical School and lecturer at the Massachusetts General Hospital from 1978-1995. Dr. Curtiss was the editor of The Journal of Bone and Joint Surgery from 1979-1985 and Editor Emeritus from 1985-1990. He was a member of the Shakespeare Club of Natick. He was also a member of the Cambridge Boat Club and president of the Cambridge Boat Club from 1990-1992. In addition he was an active member of the Eliot Church of South Natick. Dr. Curtiss was also a lecturer for many years at the Bacon Free Library and the Natick Historical Society.

He is survived by his son, Jonathan P. and his wife Diane Curtiss of Holliston and their children Tanner & Lauren; his daughter, Stacey A. Bown and her children Miles M., Rebeccah Roth, and Robert “Sean” Bown of Framingham.

Expressions of sympathy may be made in his name to Cheryl Chagnon Lymphoma Research Fund. PO Box 2355 Natick, Ma. 01760; or to the Parmenter Wayside Hospice, 266 Cochituate Road, Wayland, Ma. 01778.
**SKELETAL LIVES**  
*Lines from the Hamman-Todd Collection*

It is the fool  
Who says  
The dead never speak,  
Locked in some eternal silence—  
Easily forgotten?  
Everyone—  
Vanished without a trace?  
None—  
Some have left behind more than others,  
And these, 3,100 working-class lives  
From the Revolution’s dirty, city skies,  
Leave me their skeletal remains,  
Bodies disarticulated,  
Spread out in boxes  
Stacked from floor to ceiling,  
Reduced to a number, age, sex and race—  
It is enough  
With which to speak,  
Sotto voce,  
Through an anatomic braille  
My fingers decipher as they run  
The length of the dried spines,  
Reading the bumps of degenerative time,  
The total collapse of unrivaled infection,  
The anomalous wrinkles  
Of osseous creation.  
Who knew that in death  
These destitute lives would inform the world?—  
And me, one of the messengers  
Of these skeletal truths  
From beyond the grave.

– Dr. Jason David Eubanks

**I AM**

I am humbled and determined.  
I wonder how I will succeed.  
I hear the motivation.  
I see what awaits me.  
I want to overcome all obstacles.  
I am humbled and determined.

I pretend to play the game.  
I feel sweat crawl down my face.  
I grasp the goal to be earned.  
I worry about the past.  
I cry out with pain and agony.  
I am humbled and determined.

I understand the sacrifices.  
I talk with optimism.  
I dream of the satisfaction.  
I try to rebuild what was destroyed.  
I hope to one-day play again.  
I am humbled and determined.

– Rob Razzante
JOURNAL STAFF

(Back, left to right) - Parke Oldenburg, MD; Rob Anderson, MD; Sam Akhavan, MD; Mahi Durbhakula, MD; Anthony Skalak, MD; Randall Marcus, MD. (Front, left to right) - Shana Miskovsky, MD; Jerry Huang, MD; Matthew Smith, MD
EXITING RESIDENTS’ FUTURE PLANS

From left to right - Casey Jenkins, Bret Kean, Lutul Farrow, Sam Akhavan, Mahi Durbhakula, Jerry Huang.

**Dr. Sam Akhavan**, Sports Medicine Fellowship, Cleveland Clinic

**Dr. Mahi Durbhakula**, Hand Surgery Fellowship, Hospital for Special Surgery

**Dr. Lutul Farrow**, Sports Medicine Fellowship, Cleveland Clinic

**Dr. Jerry Huang**, Hand Fellowship, UCLA Medical Center

**Dr. Casey Jenkins**, Hand Fellowship, Duke Medical Center

**Dr. Bret Kean**, Sports Medicine Fellowship, University of Utah Medical Center
INCOMING INTERNS – CLASS OF 2012

Dr. Michael Abdulian, Tulane University

Dr. Kasra Ahadinia, University of Michigan

Dr. Zachary Gordon, Case Western Reserve University

Dr. Ari Levine, University of Cincinnati

Dr. Troy Mounts, University of Tennessee

Dr. Erik Schnaser, University of Nevada
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2. Submissions will only be accepted in electronic format via CD or e-mail.

2a. Figures
   • Figures and tables must be submitted separately from text with a separate page for legends
   • Illustrations and photographs must be submitted in TIFF, EPS or high resolution JPEG format in black and white

3. Title
   • Include degree and institutional affiliation with each author’s name

4. Abstract
   • Limit to 325 words

5. The Body of the manuscript should include:
   • Introduction: a brief review of the literature
   • Materials and Methods
   • Results
   • Discussion
   • Please limit document to 12 pages, double-spaced

6. References
   • References should be numbered and superscripted in the text and sequenced as they occur
   • Format should be as in the example below:


7. Paragraphs and Spaces
   • Do not indent paragraphs, put a return between paragraphs, but do not use a return within paragraphs
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8. If a manuscript has previously been published in or has been accepted to a peer-reviewed journal, permission must be obtained from the journal’s editor for publication in the Case Orthopaedic Journal. Proof of permission must be submitted with the manuscript.

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We are currently accepting manuscripts for the fourth issue of the Case Orthopaedic Journal. Submissions are accepted throughout the year but the due date for publication in the upcoming issue is March 1, 2008. Please read the following for instructions. Manuscripts should be sent in electronic format on CD to Matthew Smith 11100 Euclid Ave, Cleveland, OH 44106, or by e-mail to mvsmith23@gmail.com
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