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Upcoming Visiting Professors

Basic Science Visiting Professor
March 22-23, 2010: Richard Brand, MD, Editor, Clinical Orthopaedics And Related Reasearch

Bohlman Visiting Professor
April 2010: Frank Eismont, MD, Professor and Chairman, Department of Orthopaedics, University of Miami

Rainbow Visiting Professor
May 18-19, 2010: James Kasser, MD, Professor and Chairman, Department of Orthopaedics, Harvard Medical Center, Boston Children’s Hospital

Research Day Visiting Professor
June 22-23, 2010: Peter Stern, MD, Professor and Chairman, Department of Orthopaedics, University of Cincinnati School of Medicine
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**Resident Editors**

Andrew Islam, M.D.  
*Editor-in-chief*

Michael Chen, M.D.  
*Editor-in-chief Elect*

Anthony Skalak, M.D.  
Steven Fitzgerald, M.D.  
*Senior Editors*

Ryan Garcia, M.D.  
Patrick Messerschmitt, M.D.  
*Junior Editors, Advertising*

**Faculty Editors**

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J. Robert Anderson, M.D.  
Edward Greenfield, Ph.D.  
Shana Miskovsky, M.D.  
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*Secretary*

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We are pleased to present to you the sixth edition of the Case Orthopaedic Journal. We are proud to highlight the outstanding accomplishments of the faculty and residents from the past year. Award winners are highlighted and the many distinguished professors who visit each year are showcased. In addition, the events that the department holds each year are recorded.

This edition is dedicated to Dr. Victor M. Goldberg. As all of you are aware, Dr. Goldberg is a surgeon of international prominence due to his surgical abilities, leadership and research endeavours. However, his greatest accomplishment is probably the hundreds of orthopaedic residents he has played such an integral role in training. As one of his last residents, I am truly grateful for the guidance and support he has provided me. Dr. Goldberg will always be a revered member of the Case Orthopaedic Surgery Department.

The production of this journal is always a large undertaking. I would like to express my thanks to all who have made it possible including all the resident and faculty editors, contributors and advertisers. I truly hope this edition conveys the spirit that this department embodies, which made it such an excellent place to receive an orthopaedic education.

Thank you!

Andrew Islam, MD, MS
This 4th Volume of the Case Orthopaedic Journal is dedicated to Victor M. Goldberg, MD, past Chairman of the Department of Orthopaedics at Case Western Reserve University and University Hospitals Case Medical Center. Every graduate of our residency program over the past 25 years remembers well the time spent with Dr. Goldberg on the Joint Replacement Service at University Hospitals Case Medical Center, since for most of us, this represented our true “coming of age” as an orthopaedic surgeon.

Dr. Goldberg is a native of Brooklyn, N.Y. and attended New York University and the State University of New York, Downstate Medical Center where he received his medical degree from in 1964. His initial introduction to Cleveland came in 1964-66 where he completed an internship and residency in general surgery at University Hospitals of Cleveland. After serving two years as a Captain in the United States Air Force, he returned to his roots in New York City and began his residency in orthopaedic surgery at The Hospital for Special Surgery (HSS) in 1968. During this time, he developed an interest in the biology and immunology of bone allografts and transplantation which continued throughout his career. Following his residency at HSS, he completed a Post-Doctoral Fellowship in Transplantation Immunology with Eugene Lance in Middlesex, England.

Dr. Goldberg returned to Case Western Reserve University and University Hospitals in 1972 where he continued to pursue his research interests into bone allografts and transplantation. His intense and
Words from Dr. Goldberg’s Children

FOCUS

“Just show up” and “Stay focused”. These are two phrases that our father always says when there is a task at hand that needs to be completed. Whether it is an academic endeavor such as a thesis or an extracurricular activity like skiing he remains committed to this mission. It is through these simple phrases that he has approached his career. We will never forget the times that he lived up to this statement. Weeks after he fractured his navicular bone skiing he was back shaking hands with the president, George Bush, and then back in the operating room soon thereafter. He is the quintessential role model; a quintuple threat (the triple threat plus father and grandfather). The orthopedic residents affectionately refer to him as "Dad", a term of endearment and respect that he earns daily. He is our Dad and with this title he has earned our love and respect forever.

– Rebecca, Jonathan and Eden
I am delighted to introduce this year’s volume of the Case Orthopaedic Journal, which highlights the outstanding achievements of the Department of Orthopaedics at Case Western Reserve University School of Medicine. The Department continues its ranking as one of the top orthopaedic departments in the United States, which makes me extremely proud of the outstanding achievements and excellent work carried out during the past year by our clinicians, scientists, residents and staff.

The Department of Orthopaedics at Case Western Reserve University consists of our three medical centers, our research laboratories and, most importantly, the outstanding people who have earned our reputation for excellence. Our medical centers include:

• University Hospitals Case Medical Center, which includes Rainbow Babies and Children’s Hospital,

• MetroHealth Medical Center, our Level I 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 Laboratory, 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 in the Cleveland Museum of Natural History, the home 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 as one of the top 20 orthopaedic departments in the country. Our national leadership in musculoskeletal research is again confirmed by our continued ranking as one of the top 10 funded orthopaedic departments in the United States by the National Institutes of Health (NIH). Our residency program received over 500 applications again this year for our 6 residency positions, and the Department matched 6 of our top selections. We welcome to the program Dr. Jonathan Belding from Case Western Reserve University, Dr. Chad Fortun from Case Western Reserve University, Dr. Anna Wallace from the University of Tennessee, Dr. Jason Solomon from the University of Medicine and Dentistry of New Jersey, Dr. Jonathan Streit from the University of Michigan, and Dr. Ashraf Youssef from the University of Virginia. In addition, we welcome our two Pediatric Orthopaedic Fellows, Dr. Rachel Cuenca from Walter Reed Army Medical Center and Dr. Justin Kunes from the University of Kentucky Medical Center. This year’s Trauma Fellow is Dr. Lisa Blackrick from the University of Pittsburgh Medical Center. The two Allen Research Fellowships were awarded this year to Dr. Jonathan Macknin.
who will be working in our Wound Healing Laboratory under Dr. Kath Bogie, and Dr. Lorraine Stern, who will be working in our Musculoskeletal Mechanics and Materials Laboratories under Dr. Clare Rimnac and Dr. Christopher Hernandez.

**Congratulations to Faculty Members and Residents**

On February 9, 2009, Dr. John H. Wilber was appointed the inaugural holder of the Hansjoerg Wyss Professorship in Orthopaedic Traumatology at Case Western Reserve University. The Wyss Professorship was established by a generous donation from the Hansjoerg Wyss Foundation. Mr. Wyss is the former Chairman and CEO of Synthes USA and has been a strong advocate in protecting wilderness spaces and wild lands. He serves as an active board member of the Southern Utah Wilderness Alliance, the Grand Canyon Trust and the Wilderness Society. Dr. “Jack” Wilber is the Chief of the Musculoskeletal Trauma Center at MetroHealth Medical Center and the Director of Orthopaedic Trauma at University Hospitals Case Medical Center. He received his medical degree at Case Western Reserve University, where he also completed his orthopaedic residency. Dr. Wilber completed orthopaedic trauma fellowships both at the University of Washington Harborview Medical Center and in Chur, Switzerland, under Dr. Thomas Rüedi. On the faculty here at Case Western Reserve University for the past 25 years, Jack Wilber has an international reputation for his expertise in the management of complex fractures. He currently serves as president of AO North America and as a trustee of the AO Foundation. He is the recipient of many awards for his work, including the John R. Carter, MD, Teaching Excellence Award.

Dr. Richard Grant received the J. Robert Gladden Society’s 8th Annual Alvin H. Crawford Mentoring Award at this year’s annual meeting of the American Academy of Orthopaedic Surgeons. Dr. Grant also was selected by the Student National Medical Association of Case Western Reserve University to be their graduation speaker.

Dr. Henry H. Bohlman was selected to be the honored guest at the 15th annual Advanced Techniques in Cervical Spine Surgery in St. Louis, Missouri. Dr. William J. Petersilge received the John R. Carter, MD, Teaching Excellence Award from the orthopaedic residents at Case Western Reserve University. Dr. Shana N. Miskovsky received the Scholarship in Teaching Award from Case Western Reserve University School of Medicine.

Drs. Nicholas U. Ahn, Christopher G. Furey and Patrick J. Getty were elected to membership in the American Orthopaedic Association. This association, founded in 1887, is the oldest orthopaedic association in the United States, and membership is achieved by those who make significant contributions to the practice of orthopaedic surgery.

Dr. Amanda Weiss Kelly was elected to the Executive Council of the American Academy of Pediatrics’ Council on Sports Medicine and Fitness.

Dr. Allison Gilmore was elected to the Faculty Council of the Case Western Reserve University School of Medicine.

Shunichi Murakami, MD, PhD, received a $1,962,500 NIH grant for his work, “ERK Mitogen-activated Protein Kinases in Skeletogenesis.” Dr. Murakami also received a $125,000 grant from the Musculoskeletal Transplant Foundation for his project, “Effects of FGFR3 and MAPK Signaling on Bone Graft Healing.”

Dr. Ronald Triolo received a $4.75 million grant from the Veterans Administration for his Advanced Platform Technology Center. Dr. Eben Alsberg received the prestigious Ellison Medical Foundation New Scholar in Aging Award of $400,000. Dr. Alsberg received this award based on his project proposal, “Novel Microenvironmental Technology to Rescue the Chondrogenic Potential of Mesenchymal Stem Cells from Aged Individuals for Autologous Cartilage Tissue Engineering.” Dr. Alsberg also received a $125,000 grant from the Musculoskeletal Transplant Foundation for his project, “Timed Delivery of Multiple Growth Factors for Enhanced Bone Repair by Mesenchymal Stem Cells.” Dr. Guang Zhou’s research project, “Regulation of Craniofacial Skeletal Development by Jab1,” received a $236,000 grant from the NIH.

Dr. Clare Rimnac, the Wilbert J. Austin Professor and Chair, Department of Mechanical and Aerospace Engineering, received a $124,000 grant for her project, “Effect of Microscopic Tissue Damage on the Long-Term Viability of Cortical Bone Allografts.”

This fall, Jason D. Eubanks, MD, and Glenn D. Wera, MD, joined our Department. Dr. Eubanks will be joining our Adult Spine Division, having completed his Spine Fellowship training at the University of Pittsburgh Medical Center. Jason received his medical degree (AOA) and completed his orthopaedic residency here at Case Western Reserve University.

In addition to his 17 invited national and international presentations and 18 publications, he has authored two books, including one in poetry:
Rotations: A Medical Student’s Clinical Experience. His writing and poems have also been published extensively in many journals, including the Annals of Internal Medicine and the Journal of the American Medical Association, as well as The Plain Dealer newspaper.

Dr. Wera, a graduate of Stanford University (BA) and the University of Illinois (MD) (AOA), completed his orthopaedic residency here at Case Western Reserve University. He recently completed a Fellowship in joint reconstruction at Rush Medical Center in Chicago, Illinois, and will be joining our Division of Adult Reconstructive Surgery. He has 12 peer-reviewed publications and has been invited to present his work on over 15 occasions at national and international meetings. Glenn’s subspecialty is complex joint replacement surgery of the hip and knee, as well as the use of minimally invasive surgical procedures.

Further honors received by the Department include awards to our orthopaedic residents. Dr. Steven Fitzgerald was the honored with the American Orthopaedic Association/Orthopaedic Research and Education Foundation Leadership Forum Award, and he represented the Department at the 122nd Annual Meeting of the American Orthopaedic Association in Naples, Florida. Dr. Daniel Master and his coauthors Dr. Tom Cowan, Dr. Sreenath Narayan, Dr. Robert Kirsch and Dr. Harry Hoyen won this year’s Orthopaedic Rehabilitation Association’s Jacqueline Perry Award for excellence in research conducted by a resident or fellow. Additionally, their work also received the Mayfield Basic Science Award from the American Association of Neurological Surgeons at their annual meeting in Phoenix, Arizona. The title of their project was “Involuntary, Electrically Excitable Nerve Transfer for Denervation: Results from an Animal Model.” Dr. Master also won first place in the Cleveland Orthopaedic Society’s annual Resident Research Award for this project. Dr. Douglas Dickson was invited to present his research on “Retrospective Assessment of Distal Foundations in the Correction of Adolescent Idiopathic Scoliosis” at the 28th Annual European Paediatric Orthopedic Society (EPOS) in Lisbon, Portugal. Additionally, at the 36th Annual Meeting of the Cervical Spine Research Society, Dr. Dickson presented his project, “Does Cocaine Use Affect Post Op Recovery in Cervical Myelopathy?”

Dr. Frederick Oldenburg received a $5,000 grant from AO North America for his project on Iatrogenic Fracture Following Fixation of Subtrochanteric Femur Fractures. Dr. Ryan Garcia received 2nd place in the Barry Friedman Orthopaedic Resident Research Competition for his paper entitled, “Isolated Resurfacing of the Previously Unresurfacd Patella in Total Knee Arthroplasty,” and Dr. Daniel Master received 3rd place award in this competition for his research on “Nerve Transfer for Denervation: Results from an Animal Model.” Dr. Thomas Kean, a postdoctoral fellow in Dr. James Dennis’ lab, won the Young Investigator Award at the 37th Annual Midwest Connective Tissue Workshop for his project, “Development of a Peptide-targeted Cell for Delivery to Bone Marrow.”

Ms. Havalee Henry, a medical student at the University of Rochester School of Medicine, is the inaugural holder of the Timothy L. Stephens, Jr, MD, Orthopaedic Fellowship here in our Department. This fellowship, funded by a grant from the St. Luke’s Foundation, honors Timothy L. Stephens, Jr., MD, the first African-American orthopaedic surgeon in the State of Ohio and the first African-American orthopaedic attending physician in the Department of Orthopaedic Surgery at University Hospitals Case Medical Center. The clerkship and fellowship program provides exposure to musculoskeletal research, clinical investigation, community-based medicine and multiple subspecialty lectures, as well as orthopaedic clinical experience.

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 Orthopaedic Alumni Association and wish them all the best in their future careers:

- Andrew Islam, MD – Sports Medicine, Cleveland Clinic Foundation
- Raymond Liu, MD – Pediatric Orthopaedics, The University of San Diego Medical Center
- Christopher McAndrew, MD – Trauma, University of Washington Harborview Medical Center
- Michael Paczas, MD – Hand Surgery, University of Cincinnati Medical Center
- Anthony Skalak, MD – Trauma, Carolinas Medical Center
- Ben Smucker, MD – Sports Medicine, UHZ Sports Medicine Institute

Once again, it has been a privilege to lead this fabulous Orthopaedic Department in its 102nd year. I hope that you will enjoy this volume of the Case Orthopaedic Journal, which highlights the outstanding work that typifies the faculty, residents and staff of this outstanding Department.
The Orthopaedic section at the Cleveland Veterans Affairs Medical Center (VAMC) continues its efforts to enhance resident education and service to our veterans. During the 2008-2009 academic year, we performed 530 operations (with eight different attending surgeons) and 7,103 outpatient evaluations. The VAMC section provides this significant clinical experience for the two chief residents and two PGY-3 residents that are here for their four month rotations.

The section 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. Faculty actively involved at the VA includes 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). We performed 128 joint replacements, 94 upper extremity operations, 86 spine operations, 92 sports operations, 26 foot and ankle operations and 104 other (mostly trauma) operations.

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. Our current clinical research project, headed by Richard Grant, MD, is studying Joint Replacement Utilization Disparities in the VA patient population and is nearing completion.

We are extremely happy to report that Victor Goldberg, MD has joined the VA faculty. This will significantly enhance resident education both in the OR and during Monday morning Indications Conference. We all recognize Dr. Goldberg’s important contributions to the field of Orthopaedic Surgery as well as to the education of the Case Orthopaedic residents. We are delighted that he has agreed to continue his pursuit of excellence in teaching here at the VA.
I am pleased that this issue of the COJ is dedicated to Victor Goldberg. Victor and I have a long-term collaboration that started in the early 1990's shortly after he recruited me as a beginning Assistant Professor. He encouraged me to expand my research to osteolysis responsible for loosening of orthopaedic implants, which remains a major portion of my lab. Victor has always been extremely supportive of research in the department and I am deep indebted to him for his visionary guidance.

Each year, two of our residents are selected as Allen Fellows, who join a research lab for a full-time, year-long, experience. The 2009-2010 Allen Fellows are Lorraine Stern and Jon Macknin. Lorraine is working on polyethylene fatigue with Clare Rimnac in Mechanical Engineering and Jon is working with Kath Bogie on electrical stimulation of wound healing. Kath is a member of the Functional Electrical Stimulation Center and has had a long-standing interaction with our department. I’m happy to say that we’ve recently been able to formalize that interaction as Kath has become an Adjunct Assistant Professor in our department.

The 2009 Allen Fellows Society Visiting Professor was Jung Yoo, MD, from the Department of Orthopaedic Surgery at the Oregon Health & Sciences University. It was great to see Jung again. He presented a Grand Rounds Talk and a Research Seminar on his spine surgery research and the use of mesenchymal stem cells. In addition to the talks, Jung met with the Allen Fellows to discuss the roles that research can play in the careers of orthopaedic surgeons.

Havalee Henry has joined us as the inaugural Stephens Fellow. The Timothy L. Stephens Jr., MD, Orthopaedic Clerkship and Fellowship is a year-long program to prepare highly qualified minority medical students for competitive orthopaedic residency programs and musculoskeletal scientist faculty appointments. Richard Grant should be congratulated for designing and organizing this unique program and we are extremely grateful to the St. Lukes Foundation for funding the program.

Since last year’s COJ, we’ve been fortunate to welcome CJ Slyfield as a pre-doctoral trainee on the CWRU/NIH Musculoskeletal Training Grant. CJ is working to understand bone remodeling with Chris Hernandez in Mechanical Engineering.

I would like congratulate Ron Triolo on his well-deserved promotion to Full Professor. I would also like to congratulate Jim Dennis, Shun Murakami, and Guang Zhou. All three of them have recently obtained additional research funding from the NIH. It is extremely gratifying to see our colleagues develop their research programs.

Finally, I’d like to express my overwhelming gratitude to the Figgie Foundation for establishing the Harry E. Figgie III M.D. Professorship in Orthopaedics. This professorship honors Harry E. Figgie III, who was a very popular member of our faculty, and is a testament to his legacy. As the inaugural Figgie Professor, I am committed to living up to that legacy. I consider this an incredible honor not only for myself, but also for all the members of my lab, our collaborators, and everyone who contributes to the research program in our department. It’s the hard-work of all of these extremely talented individuals that makes research successes possible.
The MetroHealth faculty and staff continue to provide NorthEast Ohio with a premiere center for the treatment of injury and a superior venue for resident education. Dr. Jack Wilber still leads the “band-of-five” traumatologists. This group includes himself, Dr. Brendan Patterson, Dr. John Sontich, Dr. Heather Vallier and Dr. Roger Wilber. These five are master surgeons with diverse interests. Their presence at Metro, along with their backup (general and specialty surgeons, medical types, anesthesia, nursing, office staff) allows the institution to provide care for the severest injuries imaginable. Dr. Jack Wilber is busy in research, teaching and clinical care. He is still President of AO North America and is the North American representative on AO’s new Trauma Board, which will attempt to optimize Trauma care in the 21st century. Roger Wilber is active in teaching through the AO institute and is especially active in the care of patients with complex pelvis and acetabular pathology. Dr. John Sontich is the “go-to-guy” for infections, non-unions and malunions because of his mastery of both internal and external fixation techniques. Additionally, as President of the Limb Lengthening and Reconstruction Society, he is directing education and research in circular frames at a national level. Dr. Heather Vallier continues her active efforts in teaching, clinical care and especially research. She is pivotal in our efforts to participate in and complete prospective research projects, especially large multi-center studies. These are the kind of studies that are critical to our “evidence-based” future. Additionally, the Orthopedic Learning Center, which she and Dr. Les Nash created, provides learners with 24/7/365 access to everything a learner with a computer, an access code and curiosity could want to know about Trauma care. Dr. Patterson continues teaching the residents about long bone fractures, total joint arthroplasty and life. Also, he has recently become very involved in creating MetroHealth’s future, as the new director of surgical care at Metro. His job is to lead and coordinate Orthopedic Surgery, General Surgery and Oral Surgery. His charge is to fix the problems which hold us back presently and identify the synergies that will secure Metro’s ability to serve patients in the future. The Hand and Upper Extremity Service continues to provide exceptional trauma and reconstructive care. Drs. Michael Keith, Harry Hoyen, Kevin Malone and Stephen Lacey have seen it all and can do it all. Their patients get great care and the residents love working with the Hand Team. Additionally, under the leadership of Dr. Michael Keith and Dr. Harry Hoyen, and with the indispensable help of the basic science aces led by Dr. Hunter Peckham, the NeuroProsthetic Team is creating motion and function for patients with spinal cord injury. It is a wonder. Dr. Michael Keith had a little spare time on his hands recently and decided to get his mind around medical decision making. Presently, he is co-Chair of the AAOS committee charged with creating Evidenced Based Clinical Practice Guidelines for Orthopedic Surgeons. This is an essential task for our subspecialty and I am delighted that Dr. Keith is directing the effort. The Spine Team which is headed by Dr. Tim Moore and supported by Dr. Clyde Nash, sadly bid farewell to Dr. Michael Eppig this year. Dr. Eppig was offered a tremendous opportunity to develop programs at Hillcrest Hospital. It was too good to pass up. For the last twelve years, Dr. Eppig has served Metro’s patients who presented with complex spine problems. His expertise in reconstructive surgery and trauma care will be sorely missed. Dr.
Moore’s commitment and expertise in all aspects of spine trauma care leave us with a terrific talent to build our spine program around.

Drs. Dan Cooperman and George Thompson provide Pediatric Orthopedic care at Metro, as they have for 22 and 30 years, respectively. Dr. Thompson has recently increased his commitment to the scoliosis program here, with Dr. Eppig’s departure.

Dr. John Feighan is the Chief of the Foot and Ankle Service. His expertise and kindness are greatly appreciated.

Working at Metro continues to be a pleasure. We have a wonderful complementary group of physicians and staff, with a remarkably simple mission, help people get healthy. Under Dr. Patterson’s direction, we do that. We are often reminded that our mission is central. Thomas Jefferson wrote that all people have an inalienable right to life, liberty, and the pursuit of happiness. Jefferson also wrote: “Liberty is to the collective body, what health is to every individual body. Without health no pleasure can be tasted by man; without liberty, no happiness can be enjoyed by society.” How can one meaningfully pursue any individual definition of happiness if one is not as healthy as possible?

Of course, in 2009, we face challenges in our effort to provide the best possible care to everyone who presents to our hospital. Metro believes that overcoming the challenges is worth the effort.
HERNDON DINNER

Bob Derkash, Barry Samson, John Zachary, Bob Mack

Bang Hoang, Mike Archdeacon, Lynne Archdeacon

Carmelita Teeter, John Feighan, Andrea Young

Irwin Mandel, Melinda Elyaderani, Mehrun Elyaderani

Hosts Heidi and Ryan Grabow of Las Vegas

Rebecca Battersby, Brian Battersby, Matt Kraay

Randall Marcus, Mustafa Haque, Cheryl Petersilge, Will Petersilge

Tom McLaughlin, Adam Mirarchi, Roger Wilber
ANNUAL TROUT CLUB

Dr. Mclaughlin

Ryan Garcia watching his 300+ yard drive

Mike Paczas trying not to dislocate a shoulder

Dr. Goodfellow showing him how it’s done

Loraine Stern preparing for the lab year

John Macknin catching two beer bottles
Enjoying drinks and appetizers before dinner and the roast

Amar Mutnal and John Macknin

Drs. Marcus and Dennis with a successful catch

Brian Tonne and Ben Beecher

Rob Coale, Chris McAndrew, and Troy Mounts
GRADUATION DINNER AND DANCE

Brian Tonne, Anthony Skalak, and Ben Beecher

Zach Gordon, Jen Prusa, and Kas Ahmadinia

Amanda Escano and Jake Bosley

Rob Anderson, Adrianne Smosky, and Joe Son-Hing

Katie and Eric Schnaser

Andrew Islam, Mike Chen, and Mike Paczas

INTERN PICNIC
INTERVIEW NIGHTS

Ryan Garcia, Rob Gillespie, Dr. Victoroff

Amar Mutnal and Troy Mounts

Alissa Mounts, Katie Schnasser, Cynthia Gordon
YEAR IN REVIEW

INTERVIEW NIGHTS

Kas Ahmadinia, the organizer of the social event

Troy Mounts, Alissa Mounts, Eric Schnasser, Katie Schnasser, Kas Ahadinia, Zach Gordon

Amanda Escano, Jake Bosley, Katie Schnasser, Kas Ahadinia

Amar Mutnal, Rachel Cuenca, Justin Kunes
BASIC SCIENCE FACULTY

Eben Alsberg  Dwight Davy  Jim Dennis  Edward Greenfield

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.
AWARDS

HANSJOERG WYSS CHAIR OF ORTHOPAEDIC TRAUMATOLOGY

Dr. John H. Wilber was appointed the inaugural Hansjoerg Wyss Professor of Orthopaedic Traumatology at Case Western Reserve University and Metrohealth Medical Center. Dr. Wilber is the chief of the Musculoskeletal Trauma Center at MetroHealth Medical Center, Director of Orthopaedic Trauma at University Hospitals Case Medical Center, Professor of Orthopaedic Surgery at Case Western Reserve School of Medicine, and fellowship director of the Orthopaedic Trauma Fellowship at Metrohealth Medical Center.

Dr. Wilber received his medical degree from Case Western Reserve School of Medicine. He then completed his orthopaedic surgery residency at University Hospitals Case Medical Center. He then became one of the first fellowship-trained orthopaedic traumatologists in the United States by completing a fellowship at Harborview Medical Center in Seattle, Washington and an AO/ASIF fellowship in Switzerland.

Dr. Wilber has been recognized as one of the national leaders in orthopaedic trauma. He has served as the President of AO North America for many years and as a Trustee on the AO Foundation. He is also the North American representative on the AO Trauma Board. Dr. Wilber has taught basic and advanced AO courses nationally and internationally. He is also an active member of the Orthopaedic Trauma Association and has served on many national committees.

The Hansjoerg Wyss Foundation established the Wyss Professorship to focus support teaching and mentoring emerging leaders in orthopaedic traumatology. The Wyss Foundation was created by Hansjoerg Wyss, Chairman of the Board of Synthes, Inc., which manufactures orthopaedic implants. It is a nonprofit organization dedicated to improving the care of patients with musculoskeletal injuries and their sequelae through research, development, education, and quality assurance in the principles, practice, and result of fracture treatment.

Harry E. Figgie, III, M.D. Professorship in Orthopaedics

Dr. Edward M. Greenfield was confirmed by the Board of Trustees at Case Western Reserve University as the inaugural holder of the Harry E. Figgie, III, M.D. Professorship in Orthopaedics. Dr. Greenfield is Professor of Orthopaedic Surgery, with secondary appointments in Pathology, Physiology, and Biophysics.

Dr. Greenfield, who is the Director of Orthopaedic Research at Case Western Reserve University, joined the faculty in 1990. He is an internationally renowned scientist whose research has brought over $10 million of external funding to the University. He received his doctorate from the University of North Carolina, Chapel Hill. He then completed his postdoctoral fellowship at Washington University in the laboratory of Dr. Steven Teitelbaum.

Dr. Greenfield is the recipient of numerous local, regional and national awards for his research, including the William Harris Award from the Orthopaedic Research Society and the Kappa Delta Award from the American Academy of Orthopaedic Surgeons.

The Figgie Foundation, under Mr. and Mrs. Harry E. Figgie, Jr., made a generous donation in honor of their son, the late Harry E. Figgie, III, M.D., who was a former member of the Department of Orthopaedic Surgery. Dr. Figgie was a 1979 graduate of the School of Medicine and completed his orthopaedic surgery residency at University Hospitals of Cleveland.
The Cleveland Orthopaedic Society Resident Essay Contest took place on January 3, 2009. Eight papers were presented, featuring five by residents from our department. Daniel Master, M.D. won first place and a $1000 award.

**Long-term Outcomes After Arthroscopic Lateral Retinacular Release for Patellofemoral Pain and Chondromalacia**  
MICHAEL CHEN, M.D.

**Use of Somatosensory Evoked Potentials Alone in a Consecutive Series of Laminoplasty Procedures**  
RYAN GARCIA, M.D.

**Intravenous Versus Oral Antibiotic Therapy for Pediatric Acute Osteomyelitis**  
RAYMOND LIU, M.D.

**Involuntary, Electrically Excitable Nerve Transfer for Denervation: Results from an Animal Model**  
DANIEL MASTER, M.D.

**The Effect of Design-Induced Stress Concentration (Notches) on the Performance of Highly-Crosslinked Polyethylene**  
JAMES MURPHY, M.D.
ANATOMIC VARIABILITY OF 120 L5 SPONDYLOLYTIC DEFECTS

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ABSTRACT

Study Design. Osteological specimens were assessed for spondylytic defects and characterized.

Objective. To describe the anatomic variation in the location of L5 lytic defects of the pars interarticularis in 120 cadaveric specimens.

Summary of Background Data. Spondylolysis is a condition characterized by a defect in the pars interarticularis occurring in 6% of the population. The defects are believed to be the result of the sum of mechanical stresses through the pars interarticularis. The characteristics and variation of lytic pars defects, however, have not been significantly described.

Methods. Lumbar vertebrae from the Hamann-Todd Osteological Collection at the Cleveland Museum of Natural History were examined. One-hundred twenty specimens with L5 isthmic spondylolytic defects were identified from the 3100 specimens. Digital images were made of the spondylolytic vertebrae. Images were analyzed using image processing software. The distance of the pars defect in the sagittal plane in relation to the caudal aspect of the pedicle was analyzed. As a control for vertebral size, the distance of the defect to the inferior vertebral endplate was also analyzed.

Results. Defects were present in 6 females and 114 males, with a mean age at death of 49.43 years. There were 95 bilateral complete (BC), 16 unilateral incomplete (UI), 5 unilateral complete (UC), and 4 unilateral complete defects with an incomplete defect on the contralateral side (UCI). The mean distance of BC defects from the pedicle and inferior vertebral endplate was 4.03mm (range 0-10mm) and 4.88mm (range -2 to 13mm) respectively. The mean distance of the defect from the inferior endplate on the left and right sides were 5.31mm and 4.44mm respectively (p=0.001, correlation coefficient = 0.56). The mean distance of UI and UC defects from the inferior endplate was 6.38mm (range 2-10mm) and 2.6mm (range -3-6mm) respectively. UC defects were noted to be all on the right side, and the complete defect in UCI defects were also all on the right side.

Conclusion and Significance. L5 spondylytic defects were found in 3.87% of the sample. This large-scale description of isthmic spondylytic defects reveals that significant variability exists in the location of the defect. Although there are different types of defects, no significant differences in the location of the defect appear between groups. Unilateral defects appear to occur more commonly on the right side. We feel the anatomic location of the pars defect plays a role in the development of L5 nerve root compression and radiculopathy in this clinical scenario. A defect occurring more cranial or ventral may simply unroof the foramen while a more caudal or dorsal defect may produce more foraminal stenosis. Surgical options for this condition often involve decompression of the L5 nerve roots and L5-S1 fusion. Classifying these defects might allow surgeons to better identify those patients who might benefit from fusion alone without an extensive posterior decompression and longer recovery times.

INTRODUCTION

Spondylolysis is a condition characterized by a defect or abnormality in the pars interarticularis of vertebrae. It is a common condition that occurs in approximately 3 to 6% of Americans, and at higher frequencies in certain athletes and ethnic populations.1-5 There also appears to be a genetic predisposition for spondylolysis as relatives of index cases have a greater than fourfold increased incidence. The lytic defects of the pars interarticularis are most common at the L5 vertebrae, with decreasing incidence at the more cranial lumbar levels.1,6
The etiology of spondylolysis is poorly understood. Spondylolysis is a defect unique to humans. It appears to be acquired, as the fetal incidence has conclusively been shown to be zero. The primary lesion in spondylolysis is believed to be a stress fracture of the pars interarticularis that remains unhealed. This theory is supported by the fact that spondylolysis also has not been demonstrated in patients who have never ambulated. Other factors that have also been implicated in the etiology or susceptibility to spondylolysis include mechanical stress, anatomic variation, or repeated trauma that leads to a gradual dissolution of the pars. The result of hyperextension on the development of spondylolysis is also suggested by the high incidence of defects in gymnasts, swimmers, and other young athletes who undergo frequent lumbar hyperextension.

Most cases of spondylolysis are asymptomatic and it is often an incidental finding. The major sequela of spondylolysis is progression to spondylolisthesis. The most common presenting symptom is activity-related low back pain, while radicular symptoms are rare without the presence of severe spondylolisthesis. Most patients will respond to nonsurgical management. Failure of conservative management (intractable pain, progression of neurologic symptoms, progression of slippage, or segmental instability) is an indication for operative treatment. Many different surgical options are available. Direct repair of the defect may be possible through debridement, bone grafting, and compression across the defect with Scott wiring or Buck fusion. A Gill laminectomy has also been performed alone in the past, as removal of the lamina and inflammatory pars defect allows decompression of the nerve roots and possible removal of part of the inflammatory back pain generator. Current surgical options for this condition often involve decompression of the L5 nerve roots and L5-S1 fusion. Although the role of decompression with instrumentation remains controversial, laminectomy without fusion is not recommended. Conventional treatment usually consists of posterolateral fusion in situ with autogenous bone graft with or without decompression. Other options include interbody fusion through an anterior or posterior approach, or a combination with posterolateral fusion.

Although the orthopaedic literature regarding spondylolysis and spondylolisthesis is extensive, there is a paucity of information describing the nature of spondylolytic defects. Edelson and Nathan described the area of the spondylolytic defect as varying from 2 to 9 mm below the caudal aspect of the pedicle in a sample of thirty-four bony specimens. They also described the consistent finding of a hook-like projection of the proximal lamina at the inlet to the intervertebral foramen. Merbs has also characterized and described the asymmetry of spondylolytic defects.

In this study, we describe the anatomic variability of the lytic L5 pars defect in a large sample population. The age, sex, and race of all the specimens were recorded. Images were made of each L5 vertebra from a direct lateral view from both the left and right sides using a digital camera (Cybershot DSC-W7, Sony Corp, Tokyo, Japan). An image processing program (ImageJ, National Institutes of Health, Bethesda, MD), was used to measure distances. Images of each vertebra were made next to a ruler to allow the program to standardize for magnification. A single individual made all measurements. A consistent hook-like projection of the proximal lamina was seen in the spondylolytic specimens and the caudal tip is referred to as the level of the spondylolytic defect. The distance of the level of the spondylolytic defect below the caudal aspect of the pedicle in the sagittal plane was measured to the nearest millimeter. This was performed by drawing a horizontal line at the caudal aspect of the pedicle (most superior aspect of the neural foramen). The level from this line inferiorly to the level of the spondylolytic defect was then measured (Figure 1). As a control for vertebral size, the distance of the level of the spondylolytic defect to the inferior vertebral endplate in the sagittal plane was also measured to the nearest millimeter. A horizontal line representing the posterior continuation of the inferior vertebral endplate was drawn. In cases where the inferior vertebral endplate was asymmetrical, the line was based off the most posterior point of the endplate. The distance from this line to the most caudal part of the proximal lamina was then measured (Figure 2).

**Statistical analysis**

Histograms were made of all groups of specimens to determine whether there was a normal distribution. A paired
t-test was performed to compare side-to-side differences in the BC defect specimens. A two sample t-test was used to compare BC samples with the UI samples. A Wilcoxon rank sum test was used to compare all other groups because of non-normal distributions or small number of specimens. Correlation coefficients were determined to compare side-to-side differences. Analysis was performed using statistical software (Minitab 13, Minitab Inc, State College, PA).

Reliability and validity
For intraobserver reliability, the same individual made three sets of measurements on the left and right sides from seven different vertebrae at one-week intervals. For interobserver reliability, two different individuals who had experience with the ImageJ software made measurements from the same seven different vertebrae. The validity of the ImageJ software was tested by comparing values calculated by the program with those obtained from a more orthodox measuring tool (digital caliper, Mitutoyo Co., 0.01mm).

RESULTS
Relevance and Validity
Intraobserver reliability was found to be 97%. Interobserver reliability was found to be 96%. The validity of the ImageJ software was found to be 99%.

Study population
The study population consisted of 120 of the 3100 human skeletal specimens that were found to have a L5 spondylolytic defect, representing an incidence of 3.87%. Ninety-five of these specimens were found to have bilateral complete (BC) spondylolytic defects in the L5 lumbar vertebrae. Sixteen specimens had a unilateral incomplete (UI) defect. Five specimens had a unilateral complete (UC) defect. Four specimens had a complete defect on one side and an incomplete defect on the contralateral side (UCI). One hundred fourteen specimens (95%) were male, while 6 were female. Ninety-six specimens (80%) were white and 24 were black. The mean age was 49.43 years (range 18-105 years) at the time of death.

Bilateral Complete (BC) Defects
The mean distance (+ standard deviation) of the defect from the pedicle was 4.05 + 1.88mm (range 0-10mm). The mean distances for the left and right sides were 3.92 + 2.05mm and 4.18 + 1.70mm respectively, which were not significantly different (p=0.197), but demonstrated moderate correlation (correlation coefficient 0.46) within each vertebra. The mean distance of the defect from the inferior vertebral endplate was 4.88 + 2.51mm (range -2-13mm) (Figure 3). The mean distances for the left and right sides were 5.31 + 2.57mm and 4.44 + 2.41mm respectively, which was significantly different (p=0.0005), but also demonstrated a high correlation (correlation coefficient 0.56) within each vertebra.

Unilateral Incomplete (UI) Defects
UI defects occurred on the right side in 10 specimens, and on the left in 6. No significant differences were found between the level of the defect on the left and right sides in relation to the pedicle or the inferior vertebral endplate (p= 0.25 for both).

The mean distance of the defect from the pedicle was 3.88 + 0.89mm (range 2-5mm). No significant difference could be detected when all UI defects were compared with all BC defects (p=0.55). Because of a possible association between the left and right defects in each vertebra of BC defects as determined by the correlation coefficients, single sides from the BC group were compared to
the other groups. However, there was no significant difference when only left BC defects were compared with the UI defects (p=0.89) or when only right BC defects were compared with the UI defects (p=0.29).

The mean distance of the defect from the inferior vertebral endplate was 6.38 + 2.13mm (range 2-10mm). This was significantly greater (defect was located more cranially) when all UI defects were compared with all BC defects (p=0.016). The majority of this significance appears to be contributed from the right side of BC defects (p=0.01 when compared with all UI defects) than from the left side of BC defects (p=0.08 when compared with all UI defects).

**Unilateral Complete (UC) Defects**

All 5 UC defects occurred on the right side. The mean distance of the defect from the pedicle was 6 + 3.16mm (range 2-10mm). The mean distance of the defect from the inferior vertebral endplate was 2.6 + 3.97mm (range 2-6mm). No difference was found when UC defects were compared with the UI defects in relation to the pedicle (p=0.16). With the number of samples available, no difference relative to the inferior vertebral endplate was found between UC and UI defects, although the values did approach statistical significance (p=0.0546).

**Unilateral Complete with Unilateral Incomplete (UCI) Defects**

Of the 4 specimens with a complete defect on one side and an incomplete defect on the other side, the complete defect was always on the right side and the incomplete on the left. A very large inverse correlation appeared to exist between the complete and incomplete defect (correlation coefficient -0.83) within each vertebra. However, with the limited number of specimens available, this correlation was not significant (p=0.165).

The mean distance of the complete defect from the pedicle was 3.75 + 2.06mm (range 2-6mm). No significant differences were found when compared with the right sides of BC defects (p=0.68) or with UC defects (p=0.32). The mean difference of the complete defect from the inferior vertebral endplate was 4.5 + 2.38mm (range 2-7mm). No significant differences were found when compared with the right sides of BC defects (p=0.89) or with UC defects (p=0.53).

The incomplete defect was a mean distance of 2 + 0.82mm (range 1-3mm) from the pedicle. This distance was significantly less than UI defects (p=0.01). The mean distance of the incomplete defect from the inferior vertebral endplate was 5.25 + 1.26mm (range 4-7mm). There was no difference when compared with UI defects (p=0.25).

**DISCUSSION**

This study is the largest description of L5 isthmic spondylolytic defects. Our 3.87% incidence corresponds to that seen in population studies, even though we only studied L5 vertebrae. Because the Hamann-Todd Collection consists of approximately 85% male specimens, it is to be expected that a large portion (95%) of our defects occurred in males.

As previously described by Merbs, 24 several types of spondylolytic defects were found (Figure 4). The only type of defect not observed was a bilateral incomplete defect. As expected, the most common type observed were bilateral complete defects (the classic form of spondylolysis). The relative order in frequency of all the defects was also similar to what Merbs observed in a Canadian Inuit population. Unlike Merbs, all unilateral complete defects, which included the unilateral complete with unilateral incomplete defect group, in our study were observed on the right side. Overall however, unilateral defects were more frequently observed on the right side, which is similar to previous studies.24, 35.

Based on the mechanical stress theory where hyperextension causes repeated stress on the pars, leading to dissolution, one might assume...
that there would be equal stresses on the pars, leading to equal defects bilaterally. Asymmetry appears to be the general trend in spondylolytic defects though, as evidenced by the significant difference in the location of the spondylolytic defect, when standardized for vertebral body size. However, the defects do not appear to be completely independent of each other since a moderate to high correlation was observed between the location of the defects on the left and right sides in bilateral complete defects. Complete defects may lie more caudally compared to incomplete defects. When standardized for vertebral size, bilateral complete defects were significantly different from unilateral incomplete defects. There was also almost a statistically significant difference between unilateral complete and unilateral incomplete defects. There was no difference, however, when all types of complete defects were compared with each other.

Although unilateral defects are more common on the right side, no difference existed between left and right unilateral incomplete defects. When present in the same specimen however, the incomplete defects may be affected by complete defects. The incomplete pars defect was significantly more caudal in specimens with unilateral complete and unilateral incomplete defects compared to unilateral incomplete defects. There was also a very large inverse correlation between the level of the incomplete and complete defect in the unilateral complete and incomplete group. With our limited sample size though, this correlation was not significant.

Foraminal stenosis may be an important etiology of nerve root compression when it is associated with spondylolysis and spondylolisthesis. We believe that the anatomic location of the pars defect may play a role in the development of L5 nerve root compression and radiculopathy in this clinical scenario. A defect occurring more cranial or ventral may simply unroof and decompress the foramen with progressive spondylolisthesis as suggested in the past by Newman and Leger et al. A more caudal or dorsal defect, however, may produce further foraminal stenosis as the remaining part of the proximal lamina causes traction or compression on the nerve root with progression of spondylolisthesis as suggested by Edelson and Nathan. Our study demonstrates a normal distribution in the location of the pars defect in the bilateral complete group, which are the most likely to progress to spondylolisthesis as shown in natural history studies. Those defects at the extremes may represent patients who are the most and least likely to experience neurologic symptoms. Further clinical studies with CT or MRI in symptomatic patients will reveal the impact of the location of the pars defect.

The clinical significance of the location of the pars defect relates to the selection...
REFERENCES


SPECIFIC TYROSINE KINASE INHIBITORS REGULATE HUMAN OSTEOSARCOMA CELLS IN VITRO

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Dedicated to the memory of John R. Carter, MD


ABSTRACT

Inhibitors of specific tyrosine kinases are attractive lead compounds for development of targeted chemotherapies for many tumors, including osteosarcoma. This study was designed to determine whether inhibition of specific tyrosine kinases would decrease the motility, colony formation, and/or invasiveness by human osteosarcoma cell lines (TE85, MNNG, 143B, SAOS-2, and LM-7). An EGF-R inhibitor reduced motility of all five cell lines by 50% to 80%. In contrast, an IGF-1R inhibitor preferentially reduced motility by 42% in LM-7 cells and a met inhibitor preferentially reduced motility by 80% in MNNG cells. The inhibitors of EGF-R, IGF-1R, and met reduced colony formation by more than 80% in all tested cell lines (TE85, MNNG, and 143B). The EGF-R inhibitor reduced invasiveness by 62% in 143B cells. The JAK inhibitor increased motility of SAOS-2 and LM7 cells without affecting colony formation or invasiveness. Inhibitors of HER-2, NGF-R, and PDGF-Rs did not affect motility, invasiveness, or colony formation. These results support the hypothesis that specific tyrosine kinases regulate tumorigenesis and/or metastasis in osteosarcoma.

INTRODUCTION

Osteosarcoma, the most common bone sarcoma, predominantly affects rapidly growing bones in adolescents. Although only about 400 cases occur in the United States per year, osteosarcoma is the fifth most frequent malignancy in 15- to 19-year-olds. Prior to the development of chemotherapy regimens, long-term survival rates were 10-20% with surgical resection, usually amputation, as the only treatment available. During the 1970’s, initiation of chemotherapy protocols in combination with aggressive surgical resection resulted in long-term survival rates of 60%-70% in patients with localized disease. However, patients with metastatic disease still face 20-30% survivorship at 10 years following diagnosis. Thus, a greater understanding of the basic biology of osteosarcoma is needed to allow development of novel approaches to increase survival rates.

Reduced dependence on growth factors is a common mechanism in many cancers, usually due to autocrine production of the growth factors themselves or to overexpression or mutation of either growth factor receptors or downstream signaling molecules. Because many of the receptors and downstream signaling molecules are tyrosine kinases, inhibitors of these kinases are a majority of the most promising anticancer drugs. Although osteosarcoma has not been as well-studied as other types of cancer, overexpression in osteosarcoma has been reported for both growth factors and their tyrosine kinase receptors and overexpression of some of these molecules correlates with metastasis and poor survival in osteosarcoma patients.

The prognostic value of tyrosine kinases in predicting outcomes or response to treatment in osteosarcoma has yet to be finalized. For example, there are reports establishing an association between HER-2 expression and decreased overall patient survival, while other studies fail to confirm any significant association. However, this does not undermine the potential
benefit that inhibitors of tyrosine kinases may play in future treatment of osteosarcoma patients. Additionally, the vast majority of human tyrosine kinases have yet to be tested for correlation with long-term survival.

Current antiproliferative chemotherapies used to treat osteosarcoma patients may induce debilitating side effects, including hematological, liver, renal, cardiac, neurologic, and/or gonadal toxicity. These agents are also mutagenic and can cause secondary malignancies, most commonly leukemia, brain cancer, soft tissue sarcomas, and breast cancer. In contrast, therapies against specific targets, such as tyrosine kinases, would likely produce fewer side effects. Thus, such targeted therapies offer the hope of an improved quality of life as well as increased survival.

We tested the osteosarcoma cell lines to determine if in vitro differences in proliferation, motility, colony formation, and invasiveness correlated with their tumorigenic and metastatic potentials. We asked whether inhibitors of specific tyrosine kinases alter the motility of osteosarcoma cell lines. We asked whether inhibitors of specific tyrosine kinases alter colony formation by osteosarcoma cell lines. We also asked whether inhibitors of specific tyrosine kinases alter the invasiveness of osteosarcoma cell lines.

MATERIALS AND METHODS

Two families of genetically-related osteosarcoma cell lines were used. TE85, MNNG, and 143B cell lines were obtained from the American Type Culture Collection (Manassas, VA). SAOS-2 and LM-7 cell lines were obtained from Dr. E. Kleinerman, MD (Anderson Cancer Center, Houston, TX). Each family includes a parental cell line (TE85 and SAOS-2) isolated from human osteosarcoma tissue that exhibits little tumorigenesis or metastasis when implanted in immunodeﬁcient mice and a highly tumorigenic/metastatic cell line (143B and LM-7, respectively) derived from the parental cell line. The TE85 family also includes a tumorigenic but only weakly metastatic cell line (MNNG).

Unless otherwise specified, all cell cultures contained minimal essential medium (MEM) (Hyclone, Logan, Utah) supplemented with 10% fetal bovine serum (FBS, Hyclone), nonessential amino acids (Mediatech, Herndon, VA), sodium pyruvate (Invitrogen, Carlsbad, CA), 1-glutamine (Mediatech), and penicillin-streptomycin (Hyclone) and were maintained at 37°C in a humidified 5% CO2 atmosphere. All experiments were performed on cells harvested at the mid-log phase of growth.

The effects of small molecule inhibitors that are relatively specific for individual tyrosine kinases were used in tests of motility, invasiveness, or nonadherent colony formation. Small molecule inhibitors were obtained from Calbiochem (San Diego, CA) and the concentrations of small molecule inhibitors used were based on publications demonstrating the effective concentrations in intact cell assays (Table 1). Inhibitors were dissolved in dimethyl sulfoxide (DMSO; Sigma, St. Louis, MO) and aliquots were stored at -20°C.

Scrape motility assays were performed similarly to a previously described method. Cells (1.0 × 105 per 9.6 cm2 well) were cultured overnight to allow a confluent monolayer to form. Scrape, approximately 0.6 mm in width, were made using a 1 mL pipette tip. Media was changed to remove cellular debris and the cultures were incubated with tyrosine kinase inhibitors or 1% DMSO as a vehicle control. Each scrape was photographed immediately and at the indicated time points using the 10x objective on a Leica DM IRB inverted, phase contrast microscope (Leica Microsystems, Deerfield, IL). Scrape widths were measured with ImageJ (NIH). Motility was calculated by subtracting the scrape width at the indicated time points from the initial scrape width and dividing by two.

Colony formation assays were performed similarly to a previously described method. 8.0 × 103 cells were suspended in 160 µL of collagen gel, 1.69 mg/mL of rat tail Type I collagen (BD Biosciences, Bedford, MA), 0.8 x EMEM with 5% FCS (Hyclone, Bedford, MA), and 0.75% NaHCO3 (Invitrogen, Carlsbad, CA) overlying a lower layer of collagen gel (185 µL) in a 2 cm2 culture well. Gels were covered with 1 mL of media containing 10% FBS plus sufficient levels of the tyrosine kinase inhibitors or DMSO as a vehicle control to obtain the indicated concentrations after complete equilibration with both layers of collagen gel. Media and inhibitors were changed every 48 hours. Colonies (≥ 5 intact cells) were counted after 4 days (TE85, MNNG, 143B) or 7 days (SAOS, LM7) using phase contrast microscopy.

Basement membrane invasion assays were performed similarly to a previously described method using 96-well CultrexTM chambers (Trevigen, Gaithersburg, MD) as recommended by the manufacturer. Basement membrane layers (CultrexTM) were composed mainly of laminin, Type IV collagen, entactin, and heparin sulfate proteoglycan. 5 × 105 cells were placed in the upper chamber in serum-free media containing 0.1%
bovine serum albumin (Proliant Biologicals, Ankeny, IA). The lower chamber contained media with 1.0% FBS as a chemoattractant. Tyrosine kinase inhibitors or 1% DMSO as a vehicle control were added to both the upper and lower chambers. Cells that transversed the basement membrane layer within 24 hours were measured by fluorescence (GENios Pro, Multimode microplate reader, Tecan, Durham, NC) following incubation with calcein AM (Trevigen).

Statistical analysis of the differences in motility distance, quantification of colony formation, and quantification of invasiveness between the experimental groups with tyrosine kinase inhibitors and the control groups with vehicle only were determined by ANOVA with Fisher’s least significant difference post-hoc tests (SigmaStat, San Jose, CA). All groups of data demonstrated a normal distribution as assessed by the Kolmogorov-Smirnov Normality Test (SigmaStat). All figures illustrate mean ± standard error of the mean.

RESULTS
The results of our in vitro assays of motility, invasiveness, and colony-forming generally reflect the in vivo tumorigenic/metastatic potential of the osteosarcoma cell lines. The metastatic 143B cell line migrated 65% faster (p = 1.0 x 10-10, except at 4 hours, where p = 9.9 x 10-8 for the comparison between the 143B and TE85 cell lines) than the genetically related nonmetastatic TE85 and MNNG cell lines (supplemental Fig 1 & 2). Migration rates of the LM-7 cell line were similar to those in its genetically related nonmetastatic parental cell line SAOS-2 (p = 0.18) (supplemental Fig 1 & 2). The migration rates reflect motility rather than proliferation since they were not affected by aphidicolin, a DNA polymerase inhibitor that blocked cell growth (supplemental Fig 3).

The metastatic 143B cell line formed 47% to 50% more colonies than the nonmetastatic TE85 and MNNG cell lines (p = 0.016 and p = 0.022, respectively) (supplemental Fig 4 & 5). The 143B cell line formed numerous large colonies (supplemental Fig 5). The MNNG cell line formed smaller colonies that were more spread out (supplemental Fig 5). The parental TE85 cell line formed smaller and fewer colonies compared to the genetically related metastatic line (supplemental Fig 5). The metastatic MNNG (p = 0.013 and 143B (p = 0.030) cell lines were five- to sevenfold more invasive than the parental TE85 cell line (supplemental Fig 6).

Specific tyrosine kinase inhibitors (Table 1) reduced motility of the osteosarcoma cell lines tested as assessed by scrape motility assays. Motility was reduced (TE85 (p = 9.4 x 10-9), MNNG (p = 1.0 x 10-10), 143B (p = 1.0 x 10-10), SAOS-2 (p = 6.0 x 10-9), LM7 (p = 1.0 x 10-10), IGF-1R inhibitor: LM7 (p = 1.6 x 10-4); met inhibitor: MNNG (p = 1.0 x 10-10), JAK inhibitor: SAOS-2 (p = 3.6 x 10-8), LM7 (p = 2.8 x 10-5).

Fig 1. Specific tyrosine kinase inhibitors reduced motility by the osteosarcoma cell lines. Motility was measured four hours after scraping in the presence of the tyrosine kinase inhibitors listed in Table 1 or 1% DMSO as a vehicle control. Bars represent the means ± SEM of four to six independent experiments, each with four to six scrapes per group. Asterisks denote differences in motility compared to the control group without inhibitor [EGF-R inhibitor: TE85 (p = 9.4 x 10-9), MNNG (p = 1.0 x 10-10), 143B (p = 1.0 x 10-10), SAOS-2 (p = 6.0 x 10-9), LM7 (p = 1.0 x 10-10); IGF-1R inhibitor: LM7 (p = 1.6 x 10-4); met inhibitor: MNNG (p = 1.0 x 10-10); JAK inhibitor: SAOS-2 (p = 3.6 x 10-8), LM7 (p = 2.8 x 10-5).
assessed by the non-adherent collagen assays. Colony formation was reduced over 80% by the EGF-R (p = 9.2 x 10-9), IGF-1R (p = 9.2 x 10-9), and met inhibitors [TE85 cell line (p = 9.2 x 10-9); MNNG cell line (p = 9.9 x 10-7); 143B cell line (p = 1.04 x 10-8)] in all three cell lines (Figs 3 & 4). The inhibitors of HER-2, JAK, NGF-R, and PDGF-R did not detectably affect colony formation (Fig 3 & 4).

Specific tyrosine kinase inhibitors (Table 1) reduced invasiveness of the osteosarcoma cell lines tested as assessed by basement membrane invasion assays. Invasiveness was reduced 62% (p = 6.1 x 10-5) by the EGF-R inhibitor in 143B cells (Fig 5). The inhibitors of HER-2, JAK, NGF-R, and PDGF-R did not detectably affect invasiveness (Fig 5).

DISCUSSION

Tyrosine kinases are known to regulate several cellular processes such as proliferation, motility, differentiation, and apoptosis. In osteosarcoma, as well as many other cancer cells, these processes may go unregulated leading to tumorigenesis and/or metastasis. The goal of this study was determine if inhibitors of specific tyrosine kinases decreased the motility, colony formation, or invasiveness of human osteosarcoma cells in vitro.

A limitation of this study was the in vitro nature of motility, colony formation, and invasion assays. However, the in vitro behavior (proliferation, growth, colony formation) of the human osteosarcoma cell lines used in this study corresponds to their in vivo tumorigenic and metastatic potential (see Supplemental Figures 1-6). Nonetheless, future studies will be needed to determine whether the same tyrosine kinases also regulate tumorigenesis and metastasis in vivo.

A second limitation of this study is the potential for nonspecific effects of the small molecule inhibitors. Moreover, several of the currently available tyrosine kinase inhibitors are well-tolerated by patients in clinical trials, despite their nonspecific effects. Nonetheless, future studies will be needed to determine whether more specific approaches, such as siRNA or antisense, demonstrate similar effects as the small molecule inhibitors used in this study.

Our in vitro assays of motility, colony formation, and invasiveness reflect the tumorigenic and metastatic capacity of the human osteosarcoma cell lines used in this study, similar to data published by other groups. These results provide rationale, not only for testing the effects of tyrosine kinase inhibition, but also using these cell lines and in vitro assays for the initial screening of new therapeutic compounds.

Our data shows that specific tyrosine kinases regulate motility, colony formation, and invasiveness of osteosarcoma cells, all of which are critical components of tumorigenesis.

Fig 2. Photomicrographs showing the effects of specific tyrosine kinase inhibitors on motility. Images of the same microscopic field of the 143B cell line at the time of initial scraping (panels A-E) and after culture for 4 hours in the presence of 1% DMSO as a vehicle control (panel F), an inhibitor of EGFR (panel G), an inhibitor of HER-2 (panel H), an inhibitor of IGF-1R (panel I), and an inhibitor of met (panel J).
TYROSINE KINASE INHIBITORS

The EGF-R inhibitor substantially decreased motility, colony formation, and invasiveness of all tested cell lines. The receptor for EGF mediates growth of malignant cells and promotes cell survival, and uncontrolled activation of the EGF pathway causes many types of cancer. Overexpression of EGF and its receptor is present in osteosarcoma patients and causes continuous growth and antiapoptosis signals in osteosarcoma cells. Additionally, EGF stimulates motility, invasion, and tumor progression of other types of cancer cells. Our results further support the development of EGF-R inhibitors as a novel osteosarcoma therapy. For example, gefitinib, a specific inhibitor of EGF-R, is currently in clinical trials for osteosarcoma and is well tolerated by children. Also, CI-1033, which inhibits EGF-R and related receptor tyrosine kinases (HER-2, ErbB-3, ErbB-4), is currently in clinical trials for osteosarcoma.

The met inhibitor blocked colony formation by all tested cell lines and preferentially slowed the motility of the MNNG cell line. The preferential effect on motility reflects the known expression of the tpr-met oncogene by the MNNG cell line. Overexpression of HGF and met/HGF-R has been demonstrated in osteosarcoma patients and causes oncogenic transformation of primary osteoblasts. HGF increases motility, proliferation, and invasion of osteosarcoma cells.

Our data show that the met inhibitor affects not only the MNNG cell line, which expresses tpr-met, but also

![Fig 3. Specific tyrosine kinase inhibitors reduced colony formation by the osteosarcoma cell lines. Colony formation assays were performed in the presence of the tyrosine kinase inhibitors listed in Table 1 or 1% DMSO as a vehicle control. Bars represent the means ± SEM of three individual experiments, each with three wells per group. Asterisks denote a decrease in the colony count compared to the control group without inhibitor (p = 9.2 x 10^-9 for each asterisk except: 143B cells in the presence of the met inhibitor (p = 1.04 x 10^-8) and MNNG cells in the presence of the met inhibitor (p = 9.9 x 10^-7))](image)

![Fig 4. Photomicrographs showing the effects of specific tyrosine kinase inhibitors on colony formation. Images of the 143B cell lines were collected after culture for 4 days in the presence of 1% DMSO as a vehicle control (panel A), an inhibitor of EGF-R (panel B), an inhibitor of HER-2 (panel C), an inhibitor of IGF-1R (panel D), and an inhibitor of met (panel E). Panels D & E reveal air bubbles, which became trapped in the collagen gel.](image)
blocks colony formation by the TE85 and 143B cell lines, which do not express tpr-met. Inclusion of a met inhibitor in a chemotherapeutic regimen could therefore be potentially beneficial in osteosarcoma patients, even in the absence of tpr-met expression.

The IGF-1R inhibitor blocked colony formation by all tested cell lines and preferentially slowed the motility of the SAOS-2 and LM-7 cell lines. IGF-1R and its ligands are overexpressed in human osteosarcoma cell lines. IGF-1R overexpression mediates proliferation, provides apoptosis protection, and has been associated with increased invasiveness and metastasis. Furthermore, IGF-1R activation induces motility and protects tumor cells from apoptosis-inducing agents such as cytotoxic drugs, osmotic stress, and hypoxia.

Therefore, IGF-1R inhibition may provide tumor chemosensitization and enhance the effect of traditional chemotherapeutic agents when used in combination. Our results support the development and investigation of novel IGF-1R inhibitors.

The JAK family inhibitor preferentially increased motility of the SAOS-2 and LM-7 cell lines. This result is consistent with the finding that JAK-mediated activation of STAT1 suppresses metastasis of other types of other types of tumors. In contrast, JAK-mediated activation of STAT3 or STAT5 increases metastasis of other tumors.

The inhibitors that target HER-2, NGF-R, and PDGF-R did not detectably affect motility, colony formation, or invasiveness of any of the tested cell lines. These results should not be interpreted as showing that these receptors are unimportant in osteosarcoma patients. Rather, our data only shows that inhibitors of these receptors did not alter the malignant phenotype of the tested cell lines. The receptors may be overexpressed or activated in many osteosarcoma patients but not in the cell lines used in this study.

Unlike many types of cancer that are due to single genetic changes (translocations, mutations, deletions, etc), osteosarcoma is characterized by a large number of genetic changes in each patient and a great diversity of genetic changes between patients. It is therefore unlikely a single “magic bullet” therapy will be uniformly successful for osteosarcoma patients.

Thus, development of a series of novel therapies is needed as well as methods to allow selection of the most appropriate therapy or therapies for each patient. Tyrosine kinases are a promising class of potential targets for development of such therapies. However, the roles of most of the 90 tyrosine kinases in the human genome have not been studied in osteosarcoma or in other types of tumors. Ongoing studies in our laboratory are designed to identify novel tyrosine kinases that may contribute to tumorigenesis and/or metastasis in osteosarcoma. Discovery of new and interesting tyrosine kinases important in the malignant transformation of osteosarcoma will be the first step in developing new therapeutic agents. In vitro results will lead to experiments focusing on establishing cellular pathways and...
understanding mechanisms of action, with the goal of transitioning to an animal model and eventually human clinical trials.

This data demonstrate specific tyrosine kinases regulate motility, colony formation, and invasiveness of osteosarcoma cell lines. Therefore, tyrosine kinase inhibition provides a promising avenue in the evolving treatment of osteosarcoma.

ACKNOWLEDGMENTS

The authors wish to thank E. Kleinerman for providing the SAOS-2 and LM-7 cell lines, L. Licate and T. Egelhoff for advice on motility and invasion assays, H. Luu for advice on colony formation assays, and T. Matsushita for advice on microscopy.

REFERENCES

ABSTRACT
Testing of the recruitment properties and selective activation capabilities of a multi-contact spiral nerve cuff electrode was performed intraoperatively in 21 human subjects. The study was conducted in two phases. An exploratory phase with ten subjects gave a preliminary overview of the data and data collection process and a systematic phase with eleven subjects provided detailed recruitment properties. The mean stimulation threshold of 25 ± 17 nC was not significantly different than previous studies in animal models but much lower than muscle electrodes. The selectivity, defined as the percent of total activation of the first muscle recruited before another muscle reached threshold, ranged from 27% to 97% with a mean of 55%. In each case the muscle that was selectively activated was the first muscle to branch distal to the cuff location. This study serves as a preliminary evaluation of nerve cuff electrodes in humans prior to chronic implant in subjects with high tetraplegia.

INDEX TERMS
Functional Electrical Stimulation (FES), nerve electrodes, peripheral nerve; spinal cord injury, human subjects implantable electrodes.

INTRODUCTION
Functional electrical stimulation (FES) is used to elicit contractions in paralyzed muscles and increase the independence of people with impaired neurological function. Most existing neuroprosthetics consist of muscle based electrodes, where a single electrode activates a single muscle. However, as neuroprosthetic designs increase in complexity different types of electrodes will be required. One type of electrode that may simplify neuroprosthetic implementation is the nerve cuff electrode.

Nerve cuff electrodes wrap around the nerve and stimulate the axons leading to distal muscles. This method of stimulation has several potential benefits compared to electrodes implanted within, or on the surface of muscles: 1) All axons in the nerve can be stimulated together to completely activate broad or pennate muscles that are difficult to stimulate using muscle based electrodes. 2) Nerve electrodes require less current than muscle-based electrodes. Lower stimulating currents will reduce the power required and extend the life of an implanted battery. 3) Several contacts within one electrode can activate different portions of the nerve, potentially selectively activating different functions and/or muscles.

1-10. This could reduce the number of electrodes needed and decrease the length and complexity of surgery.

Many different types of nerve cuff electrodes have been developed and tested in animals 4, 11-13 but few have been transferred to clinical use. The most successful has been the Huntington Medical Research Institute (HMRI) helix. It has been implanted in over 30,000 patients for reduction of epilepsy and has been recently approved for treatment of depression. Since the HMRI helix has one channel of stimulation, it was not suitable for evaluating selective stimulation and therefore not used in this study.

The Case spiral nerve cuff electrode was chosen for initial clinical implementation because it has demonstrated selective and stable chronic nerve activation in cats 2, 5, 6, 14-18, and has been implanted on the human optic nerve 19. These electrodes are self-sizing coils with four contacts evenly distributed around the nerve. At its nominal diameter, the cuff wraps twice around the nerve to hold the electrode in place. The natural coiling of these electrodes results in an intimate fit between the nerve and the contacts without constricting blood flow.
The reported stimulation thresholds of nerve electrodes in animals (5 nC 2, 5, 18-40 nC 16) are lower than muscle-based electrode thresholds reported for humans (200-500 nC) 20. The only reported human nerve stimulation studies have been on the optic nerve (55-120 nC) to evoke visual sensations 19 and the peroneal nerve (222 nC) to correct footdrop 21. These do not accurately estimate motor nerve activation since the optic nerve was not eliciting muscle contractions and the electrodes used in the footdrop system were loosely wrapped around the nerve, which typically results in higher thresholds. One aim of this study was to determine the human motor nerve threshold using a snug-fitting cuff electrode.

Stimulators designed for muscle based electrodes typically produce currents of 2 mA and higher which are likely inappropriate for stimulation with nerve-based electrodes. Higher pulse amplitudes lead to steep recruitment, where increasing the pulse duration slightly from threshold results in complete activation of the muscles. Stimulators with lower pulse amplitude values will be required for gradual recruitment using nerve-based electrodes.

Prior to chronic implantation of nerve cuff electrodes in an upper extremity neuroprosthetic system, the electrodes were tested intraoperatively in this study to evaluate their recruitment properties, their selectivity and to refine the surgical implantation technique. Two hypotheses were tested. First, the threshold values of nerve stimulation in the human are not different from nerve stimulation in the cat. Second, individual muscles could be controlled selectively from a proximal nerve cuff electrode in human subjects.

METHODS

Subject Recruitment
Subjects were recruited from patients scheduled for upper extremity nerve repair surgery. The standard surgical procedure for these cases consisted of stimulation of the potentially injured nerves to assess their function. Typically, a hand-held, bipolar, stimulating probe was used to stimulate the nerves. The nerve function was evaluated by measuring evoked electromyograms (EMG). For this study the Case spiral nerve cuff electrode was used in addition to the traditional probe, and EMG recruitment data were collected. The experimental procedure added approximately twenty minutes to the length of the surgery. The MetroHealth Medical Center IRB approved the study and all subjects gave informed consent prior to participation in the study.

The study consisted of two phases. The first 10 subjects were used to show the feasibility of intraoperative testing using a commercially available data collection system (Epoch 2000, Axon Systems, Hauppauge NY). After demonstrating feasibility, we developed custom software to collect full recruitment curves which was not possible with the standard equipment. The next 11 subjects were used to fully characterize the recruitment properties of the nerve cuff electrodes.

Spiral Nerve Cuff
The spiral nerve cuff electrodes for this study (Fig. 1) were fabricated at the Technical Development Laboratory, part of the Cleveland FES VA Center of Excellence and Case Western Reserve University. Fabrication has been previously described by Naples et al. 14. Briefly, the cuff electrodes consist of four ovoid pieces of platinum foil connected to seven-strand, stainless-steel, PFA-coated wire between two layers of silicone sheeting. One layer of sheeting is stretched before being bonded to the unstretched sheeting. This unequal tension produces a spiral of a predetermined diameter with the four stimulation contacts spaced evenly about the circumference.

Fig. 1. Schematic of spiral cuff electrode. (A) Spiral electrode coiled, resulting in two full wraps. (B) Electrode unwrapped to show contact layout. The four independent contacts are located at 90° around the nerve. Notice tabs added to the leading edge of the cuff to facilitate implantation.

Exploratory Phase
The purpose of the exploratory phase of this study was to establish initial values for threshold and determine the design requirements of a system to collect more complete recruitment data. Spiral nerve cuff electrodes were placed around one or more nerves of each subject, distal to the site of the potential lesion where possible. A pair of needle EMG recording electrodes (27 gauge, 13 mm long, Axon Systems, Hauppauge NY) were placed about 1 cm apart within as many as four target muscles. A reference recording patch (2” by 4”, Nicolet-VIASYS, Madison WI) was placed on the shoulder of the uninvolved side. The EMG twitch data for subjects 1-10 were recorded using a commercially available clinical evoked potential system (Epoch 2000, Axon Systems, Hauppauge NY). Threshold values were obtained by increasing the
stimulation intensity until a response was recorded from any of the muscles.

**Systematic Phase**
The purpose of the systematic phase was to characterize the nerve cuff electrodes using recruitment properties such as threshold and selectivity. To maximize the data quality, the selection criteria was changed to require that subjects have positive preoperative stimulated muscle responses. An automated data collection system was developed to minimize the time required to collect full recruitment curves. As in the exploratory phase, the spiral nerve cuff electrodes were placed on upper extremity nerves while recording from up to four target muscles.

The data collection algorithm used a binary search routine to generate pulse width and pulse amplitude modulated recruitment curves. The inputs to the algorithm were subthreshold and supramaximal stimulation values. The algorithm compared the EMG responses at these two stimulation values and if the difference of any channel of EMG was greater than 30% of the maximum EMG, the next stimulation pulse was selected at the midpoint of the original two. This process was repeated recursively between all pairs of points until all differences were less than 30% of the maximum.

Twitch recruitment was used to minimize muscle fatigue and the duration of testing. The stimulation

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**Fig. 2:** Procedure for quantifying the EMG of the twitch responses. These twitches were produced by a 0.5 mA, 500 μs pulse on the radial nerve of subject 1A. The brachioradialis EMG response illustrates how the twitches were quantified. First the response was rectified and then the area under the curve was calculated over a range specified during data collection (usually 5-40 ms).

**Fig. 4:** Strength-Duration and Charge Duration relationships from the systematic study. (A) The experimental data follows the classic strength-duration relationship but with considerable variability. (B) The experimental charge-duration relationship. The filled in points in each plot correspond the nerve activation thresholds of the cat.
frequency was 4 Hz to minimize the experiment time without fusing of muscle twitches. To quantify each twitch, the area under the rectified EMG response was calculated between 5 and 40 ms post-stimulus (Fig. 2). The limits of 5 ms and 40 ms were chosen to eliminate stimulation artifact and any reflexes, respectively. Three twitch responses were averaged together for each point on the recruitment curve.

A schematic of the experimental setup is shown in Fig. 3. The EMG signal was preamplified (B&L Engineering, Tustin CA) with a gain of 350. Additional amplification, AC coupling and lowpass filtering at 1000 Hz were performed by CED amplifiers (Model 1902, Cambridge Electronic Design, Cambridge England). The amplifiers were set to clamp the signal during the stimulus pulse to prevent saturation of the amplifiers. The gain for each channel was set to maximize the size of a supra-maximal twitch response without saturation. The signals were acquired by a PCMCIA A/D card (DAQCard-6036E, National Instruments, Austin TX) in a Dell Latitude laptop PC at 2400 Hz. A custom computer controlled stimulator (Crishtronics, Cleveland OH) delivered charge balanced, biphasic pulses with an amplitude range of 0.02-5 mA (resolution of 0.005 mA up to 1.25 mA and 0.02 mA up to 5 mA), and pulse width range of 10-500 µs (resolution of 2 µs). A 13 mm, 27 gauge subdermal needle (Axon Systems, Inc) was used as the return. The data collection interface software was written in Matlab (Mathworks, Inc., Natick MA).

**Data Collection**

Recruitment curves were generated using both pulse width and pulse amplitude modulation. Most available implanted stimulators use pulse width modulation. Therefore, effort

<table>
<thead>
<tr>
<th>Subj Num</th>
<th>Injury/ Condition</th>
<th>Nerve</th>
<th>Threshold PW (µs)</th>
<th>Threshold PA (mA)</th>
<th>Threshold Q (nC)</th>
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<td>1</td>
<td>Brachial plexus avulsion</td>
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<td></td>
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<td>3</td>
<td>C4 SCI – nerve transfer</td>
<td>Upper Trunk</td>
<td>200</td>
<td>1</td>
<td>200</td>
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<tr>
<td>5</td>
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<td>No motor response</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
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<td>2.7</td>
<td>270</td>
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<tr>
<td>7</td>
<td>Median nerve compression</td>
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<td>100</td>
<td>1</td>
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<tr>
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<td>Ulnar nerve compression</td>
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<td></td>
<td></td>
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<tr>
<td>9</td>
<td>Radial nerve compression</td>
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<td></td>
<td></td>
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<tr>
<td>10</td>
<td>Brachial plexus stab</td>
<td>No motor response</td>
<td></td>
<td></td>
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</table>

Average Threshold (nC): 140 ± 80

**Table I:** Summary of Exploratory Phase Subjects. In subjects 2, 5, 8, 9 and 10, the nerve was severely damaged distally prior to the surgery and no motor response was seen. PW = Pulse width; PA = Pulse amplitude; Q = Total Charge injected (PA*PW).

<table>
<thead>
<tr>
<th>Subj Num</th>
<th>Injury/ Condition</th>
<th>Nerve</th>
<th>Threshold PW (µs)</th>
<th>Threshold PA (mA)</th>
<th>Threshold Q (nC)</th>
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<tr>
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<tr>
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<td>16.5</td>
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<td>100</td>
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<td>40</td>
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<td>39</td>
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<td>1A</td>
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<td>0.9</td>
<td>14.4</td>
</tr>
<tr>
<td>19</td>
<td>MVA</td>
<td>No data recorded</td>
<td></td>
<td></td>
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<tr>
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<td>Cuff Implant/C5 SCI</td>
<td>Radial</td>
<td>20</td>
<td>0.8</td>
<td>16</td>
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</tbody>
</table>

Average Threshold (nC): 25 ± 17

**Table II:** Summary of Systematic Phase Subjects. In subject 15 the nerve was never exposed and in subject 19 there was a recording system failure. Subjects 1A and 2A were receiving nerve cuff implants. PW = Pulse width; PA = Pulse amplitude; Q = Total Charge injected (PA*PW).
width modulation curves with gradual recruitment and high selectivity.

Two to five recruitment curves were generated for each channel of stimulation. First, recruitment curves using pulse amplitude modulation were generated with a fixed pulse width of 100 µs for each channel. Then, pulse width modulation recruitment curves were generated using a fixed pulse amplitude equal to the threshold of the pulse amplitude modulation curves.

Finally, several pulse width modulation curves with smaller pulse amplitudes were generated to determine the effect of pulse amplitude on recruitment slope and stimulation threshold.

Data Analysis

The EMG values for each muscle were normalized to the maximum EMG response measured for that muscle across all trials within each subject. The threshold for each channel was defined as 10% of the maximum activation. Full recruitment was defined as 90% of maximum activation [13]. Selectivity was quantified as the percent activation of one muscle before any other muscle reached threshold.

RESULTS

Exploratory Phase

Ten subjects participated in the exploratory phase (Table I). The average threshold was 140 ± 80 nC. The thresholds were significantly higher (p < 0.01, one-sided t-test) than values obtained from the cat (5 nC 2, 5, 18-40 nC 16) but significantly lower than the 200-500 nC required for muscle-based electrodes (p < 0.001 one-sided t-test).

Systematic Phase

Eleven subjects participated in the systematic phase. No data was recorded from two subjects, one because the nerve was never exposed and one because of a recording system failure. Fourteen nerves of the remaining nine subjects were stimulated using the custom data collection system. Three nerves innervated only a single muscle so these nerves were included in the threshold analysis but not the selectivity analysis. Subjects 1A and 2A were spinal cord injured subjects receiving chronic nerve cuff implants. The data collected intraoperatively from these two subjects were included in this analysis.

1) Threshold values: Stimulation thresholds ranged from 3 to 62.5 nC (table II). The average threshold was 25 ± 17 nC. The thresholds were not significantly different (p > 0.7, two-sided t-test) than values reported from the cat (5 nC 2, 5, 18-40 nC 16) but significantly lower than the 200-500 nC with muscle electrodes (p < 0.0001 one-sided t-test). The thresholds from the systematic phase were also significantly lower than the thresholds from the exploratory phase (p < 0.01 one-sided t-test).
the classic strength duration and charge-duration relationships (Fig. 4). There was, however, considerable variability. The filled-in points in each plot correspond to reported cat nerve thresholds 2, 5, 16. The cat data are within the range of the human data.

2) Selectivity: Full recruitment curves of multiple muscles were generated for a total of eleven nerves across nine subjects. On the ulnar nerve of subject 14, the hypothenar muscle was fully activated (97%) before any other muscle reached threshold (Fig. 5). The arrow indicates how the selectivity value was calculated. The selectivity on each nerve was calculated and ranged from 27% to 97% (Fig. 6). Out of the 38 contacts tested, 10, or 26%, had a selectivity above 30%.

On the radial nerve of subject 1A, two muscles could be selectively activated over 30% using two different contacts within the nerve cuff electrode (Fig. 7). Channel 1 selectively activated the triceps to 85% and channel 3 selectively activated the brachioradialis to 42%. This was the only nerve where multiple muscles achieved selectively greater than 30%.

**DISCUSSION**

This study is the first use of selective nerve cuff electrodes for stimulation in the human upper extremity. The Case spiral nerve cuff electrode was tested intraoperatively on 21 human subjects. Threshold values were found to be significantly smaller than muscle electrodes but not significantly different than nerve electrodes in the cat. The stimulation selectivity ranged from 27% to 97% of total activation with a mean of 55%.

1) Threshold values: The threshold values of this study were compared to similar studies on nerves in the cat. The exploratory phase data indicated that human stimulation thresholds were significantly smaller than muscle electrodes but not significantly different than nerve electrodes in the cat. The stimulation selectivity varied from 27% to 97% of total activation with a mean of 55%.

The just noticeable contraction level was recorded. The just noticeable contraction on the radial nerve corresponded to 6-9% activation. The just noticeable contraction on the suprascapular nerve corresponded to 28% activation. This is likely a consequence of the fact that the muscles innervated by the suprascapular nerve are deep and difficult to palpate and observe. The 10% activation level is a reasonable match to “just noticeable” contraction, but a more consistent method to measure threshold.

Stimulation in the cat was typically performed using a 10 µs pulse width and had stimulation thresholds of approximately 5 nC. Pulse amplitude modulation in the present study was performed at 100 µs. One study in the cat using a 100 µs pulse width reported threshold values ranging from 18-40 nC 2 (see dark circles in Fig. 5), in the range of human nerves.

Many published studies of nerve cuff electrode testing in animals define threshold as a “just noticeable” contraction 2, 5. To standardize this measure, we defined threshold as 10% of the maximum EMG signal recorded during each set of trials. In trials on two nerves, the “just noticeable” contraction level was recorded.

In the systematic phase, six paralyzed nerves were tested. In spinal cord injury, the lower motor neurons are uninjured. The average threshold of the six paralyzed nerves was 11 ±5 nC, significantly lower than the threshold of the eight voluntary but possibly injured nerves (p < 0.001, one-sided t-test). Therefore, the expected human nerve threshold using the spiral electrode is around 10 nC.
2) Selectivity: This study has shown 30% selective activation of a single muscle in 10 out of 11 nerves. Previous studies using the spiral nerve cuff electrode on the cat sciatic nerve showed that this electrode was capable of selectively activating two to four of the four possible functions of each nerve 22. In the present study, selective activation of more than one muscle from a single nerve was only seen on the radial nerve of a single subject. One possible explanation for this difference is based on the finding that animal nerves contain fewer fascicles than human nerves 23, 24. Anatomical dissections have shown that as the size of the nerve increases, the number of fascicles increases rather than the size of fascicles. More information is needed on the fascicular organization of the human upper extremity nerves as the arrangement of the fascicles may affect selectivity.

In all but one nerve, the muscle that was selectively activated was innervated by the first branch of the nerve distal to the nerve cuff electrode. It is reported 25 that branching axons remain closely grouped together proximal from the branch, sometimes for a considerable distance. This functional organization would enhance selectivity of the first muscle.

Selectivity was an estimated measure in some trials where EMG was not recorded from all muscles innervated by the stimulated nerve. This was unavoidable due to a limited number of recording channels and finite time during surgery. The muscles chosen were key functional and larger muscles identified by the surgeon.

3) Implications for Stimulator Design: Since nerve electrodes use less current than muscle-based electrodes, a higher resolution stimulator will be required to gradually modulate the muscle output. The implantable stimulator-telemeter 26 developed by the Cleveland FES Center uses pulse width modulation with a resolution of 1 µs but has a limited number of preset pulse amplitude values. The stimulation threshold even for a single pulse width value varied between subjects and nerves. This was expected since activation depends on the position of the electrode on the nerve, the amount of tissue between the fascicles and the contacts, and the position of the fascicles relative to the contacts. These factors vary extensively across nerves and across individuals. This suggests that it will be difficult to choose one appropriate amplitude value for pulse width modulation of all nerves in all individuals. If the pulse amplitude is too high, there is steep recruitment and possibly an on-off response. If the pulse amplitude is too low, some muscles will never reach full recruitment. The lowest stimulation amplitude that produced full recruitment in this study was 0.1 mA (long thoracic nerve in subject #2A). One nerve did not reach full recruitment with a stimulation amplitude of 1 mA (upper trunk of subject #17). Based on our experience, pulse amplitude values ranging from 0.1 to 3 mA with a logarithmic distribution is recommended for peripheral nerve stimulation with nerve cuff electrodes.

4) Other Applications: In the course of this study, the surgeons were able to qualitatively compare the function of the nerve cuff electrode with the hand-held stimulation probe as a tool for intraoperative monitoring. In most cases, the cuff electrode was easier to use because once it was placed around the nerve, it remained in place and allowed the surgeon the freedom to view the results of stimulating and interact with the technicians without having to continuously hold the electrode. It also enabled more repeatable comparison of data, since the stimulation parameters could be varied without varying the position of the stimulating contacts, as often happened when using the hand-held probe. Another advantage of the nerve cuff electrode was that it

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**Fig. 7:** Selective activation of two muscles from a single nerve. (A) Stimulation on channel 1 of the radial nerve activated triceps to 85% before other muscles reach threshold. Pulse Amplitude = 0.9 mA (B) Stimulation on channel 3 of the radial nerve cuff activated brachioradialis selectively to 42%. Pulse amplitude = 0.9 mA.
could stimulate even if there was fluid surrounding the nerve, which typically shunts the stimulation current of the standard hand-held probe.

The purpose of this study was to determine nerve stimulation thresholds, evaluate selectivity and develop clinical procedures and feasibility of the nerve cuff electrodes. The long-term goal is to implant cuff electrodes chronically for FES applications. These results do not indicate multi-muscle selectivity is possible with 4-contact spiral nerve cuff electrodes. Therefore, target applications will require limited or no selectivity.

Initial indications are for non-selective activation of the axillary, suprascapular, musculocutaneous, thoracodorsal, and long thoracic nerves with selective activation of triceps (the first muscle to branch) from the radial nerve.

**CONCLUSION**

This study is the first to demonstrate nerve stimulation by cuff electrodes on upper extremity human peripheral nerves. These preliminary results support use of spiral nerve cuff electrodes in clinical trials which require only limited selectivity. It is expected that chronically implanted nerve cuff electrodes will be able to selectively recruit a single muscle, typically the first to branch distal to the nerve cuff. Gradual recruitment could be obtained by varying the stimulation parameters. The low thresholds (compared to muscle electrodes) reduces the power requirements of an implanted system. Based on these data, it is projected that the spiral nerve cuff electrode can be used for functional restoration of shoulder and arm function in subjects with high tetraplegia.

**ACKNOWLEDGMENT**

The authors would like to thank Robert F. Kirsch for valuable guidance and support; Jim Uhlir and Karen Brooks for fabrication of the electrodes; Tim Crish for development of the stimulator; and George Pyatt for assistance with intraoperative monitoring.

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A NEW TOOL FOR STUDYING PERIOSTEUM: ISOLATION OF PERIOSTEAL CELLS USING THE PRX1CREER-GFP TRANSGENE

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ABSTRACT

Periosteum, essential to skeletal development and bone healing, still remains poorly characterized, partly due to the difficulty in isolating periosteal cells from small experimental animals. To develop a research tool to study these cells, we have recently established Prx1CreER-GFP transgenic mice that express green fluorescent protein (GFP) in the periosteum, and by fluorescence-activated cell sorting we have successfully isolated GFP-expressing periosteal cells. Our newly established method of isolating these cells will greatly facilitate the study of periosteum.

INTRODUCTION

Periosteum and perichondrium, which play essential roles in bone and cartilage formation, cover the outer surface of bone and cartilage. Histologically, periosteum consists of at least 2 layers, an outer fibrous layer and an inner cambium layer. While the outer fibrous layer contains fibroblasts, the inner cambium layer is believed to contain osteochondro progenitor cells. Importantly, osteochondro progenitor cells in the inner cambium layer are believed to give rise to chondrocytes and osteoblasts during normal development and under pathologic conditions.1,2 It has been well recognized that the absence of periosteum delays fracture healing. Other studies have also indicated that perichondrium negatively regulates chondrocyte proliferation and hypertrophy.3 Furthermore, periosteal and perichondrial cells express numerous cytokines and growth factors that have profound effects on bone and cartilage formation. These include multiple members of the FGF, BMP, and Wnt families.4-6

Despite the importance of periosteal cells, they remain poorly characterized, partly due to the lack of tools for molecular and cellular analyses. There are no ideal cell lines of periosteal cells. Additionally, the isolation of periosteal cells from small experimental animals is extremely challenging due to the animals’ small size. The presence of heterogeneous cell populations in the periosteum poses further challenge to the isolation of a homogeneous cell population from the periosteum.7 The development of a method for isolating a pure population of periosteal cells, therefore, will greatly facilitate the molecular and cellular analyses of periosteal cells.

Green fluorescent protein (GFP) is a protein originally discovered in jellyfish. GFP exhibits a bright green fluorescence when illuminated by blue or UV light. By expressing GFP in a particular cell type under the control of a tissue-specific promoter, it is possible to mark the specific cell type in transgenic animals. Such GFP-expressing cells can be isolated by fluorescence-activated cell sorting, a method to sort a heterogeneous mixture of cells based on the fluorescent characteristics of each cell. Chondrocytes, osteoblasts, and osteocytes have been successfully isolated from transgenic mice expressing GFP in each cell type.8-10

To develop a new research tool for the study of periosteal cells, we have recently established Prx1CreER-GFP mice that express GFP and CreER in the perichondrium and periosteum under the control of a 2.4 kb Prx1 promoter.11 The 2.4 kb Prx1 promoter drives the expression of the transgene in mesenchymal cells in the developing limb buds, and the promoter remains active in the perichondrium and periosteum after cartilage formation.12 The expression of CreER—a fusion molecule between Cre recombinase and the mutated ligand-binding domain of estrogen receptor—allowed us to determine the localization of transgene expressing cells in the periosteum. By using the ROSA26-LacZ reporter mice, and by inducing Cre recombinase activity by tamoxifen administration, we have shown that the Prx1CreER-GFP transgene is predominantly expressed in the inner cambium layer of the periosteum. Since the transgene also expresses GFP, these cells can be isolated by fluorescence-activated cell sorting. Indeed, we have successfully isolated GFP-expressing periosteal cells. Here we describe our method of isolating the periosteal cells from Prx1CreER-GFP mice.
Surgical Technique Materials and Methods

To isolate periosteal cells, the diaphyses of tibiae, radii, and ulnae were dissected out from 5- to 19-day-old Prx1CreER-GFP mice (Figure 1). The surrounding soft tissues, such as skin, muscle, and epiphyseal cartilage, were removed from each skeletal element prior to isolation. Because a small fraction of epiphyseal chondrocytes also expresses the transgene, epiphyseal cartilage was totally removed from the diaphyses. The diaphyses were placed in cold phosphate buffered saline (PBS) in a microfuge tube and centrifuged for 1 min to remove cells in the bone marrow. Then, the diaphyses were placed in a tissue culture flask, washed several times with PBS, and digested with 3 mg/ml collagenase B (Roche) in DMEM at 37°C for 1.5 h. At this stage, the dissociation of periosteal cells was incomplete, and numerous periosteal cells remained attached to the diaphyses. Then, the whole medium and diaphyses were transferred to a 50 ml conical tube and centrifuged to collect the released cells. The supernatant containing collagenase was discarded, and the pelleted cells and tissues were further digested in 0.25% Trypsin/EDTA (Invitrogen) at 37°C for an additional 1.5 h. By the end of the Trypsin/EDTA digestion, most of the periosteal cells were released into the Trypsin/EDTA solution. Then, the diaphyses were removed, and the Trypsin/EDTA solution was centrifuged to collect the released cells. The cells were resuspended in DMEM, 10% FCS, plated in a culture dish, and cultured overnight in a CO2 incubator. On the following day, cells were trypsinized, resuspended in 1 ml PBS, 5% FCS, and filtered through a 70 micrometer cell strainer. The cells were then sorted by fluorescence-activated cell sorting. Only cells with a strong GFP signal were isolated as GFP positive, and cells without a GFP signal were collected as GFP negative. Cells with an intermediate signal were not collected for experiments.

Results and Discussion

By fluorescence-activated cell sorting, we were able to isolate, on the average, approximately 9,000 cells/mouse from heterozygous mice and 25,000 cells/mouse from homozygous mice. GFP-positive cells constituted 0.3% to 9.2% of cells that were released from the diaphyses. The sorted GFP-positive cells would be predominantly periosteal cells, because very few cells express the transgene in the endosteum and bone marrow. The isolated GFP-positive cells were plated in a tissue culture dish and cultured in DMEM, 10% FCS. These cells showed a polygonal morphology, and all the cells retained GFP expression on the following day (Figure 2 and data not shown).

Characterization of sorted GFP-positive cells

The phenotype of sorted GFP-positive cells was characterized by RT-PCR. GFP-positive cells expressed a number of periosteal markers, including Prx-1, Fgf18, Tenascin-W, Alcam (CD166), Periostin, and Thrombospondin 2, indicating that the sorted GFP-positive cells are indeed periosteal cells. These cells expressed Sox9, Runx2, and Osterix, master transcription factors for chondrocyte and osteoblast differentiation.

Figure 1. Isolation of periosteal cells from Prx1CreER-GFP mice by sequential enzymatic digestion and fluorescence-activated cell sorting.
A NEW TOOL FOR STUDYING PERIOSTEUM

Furthermore, receptors for FGF, Ihh, PTH, and PTHrP were also expressed in the sorted GFP-positive cells, indicating that these cells are potential targets of these cytokines and hormones.

The phenotype of isolated cells can change during subsequent culture. We have observed a gradual decrease in the intensity of GFP fluorescence in the sorted-GFP positive cells. We have also observed that the tamoxifen-inducible Cre recombinase activity is lost in the sorted-GFP positive cells. These observations indicate that the expression levels of the transgene or the Cre recombinase activity is decreased in cell culture.

**Chondrogenic and osteogenic differentiation of sorted GFP-positive cells**

To examine chondrogenic and osteogenic potential, the sorted GFP-positive cells were further induced to differentiate into chondrocytes and osteoblasts. When the cells were cultured at high density in micromass, BMP2 treatment induced chondrogenic differentiation, as judged by alcian blue staining and Col2a1 and Aggrecan mRNA expression. In addition, when the cells were plated at 6000–8000 cells/cm² and treated with betaglycerophosphate and ascorbic acid, the cells showed osteogenic differentiation, as judged by alkaline phosphatase activity and matrix mineralization.

The osteogenic differentiation was further confirmed at the mRNA levels. The chondrogenic and osteogenic differentiation was much more pronounced in GFP-positive cells compared with GFP-negative cells. These observations strongly suggest that the sorted GFP-positive cells are enriched in osteochondro progenitor cells.

In summary, we have established a novel method of isolating a pure population of periosteal cells from Prx1CreER-GFP mice. These cells express multiple markers of the periosteum, and these cells can differentiate into chondrocytes and osteoblasts in vitro. This newly established method of isolating periosteal cells will offer novel approaches for the molecular and cellular analyses of periosteal cells. These cells may be also used for tissue engineering and bone regeneration studies, as the cells possess osteogenic and chondrogenic potential.

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ABSTRACT
Traditionally, bone has been considered to be a reservoir of minerals and a target of hormones such as vitamin D and parathyroid hormone (PTH). Our understanding of the regulatory mechanisms of phosphate homeostasis, though, has greatly improved during recent years. Recent studies on fibroblasts growth factor 23 (FGF23) have indicated that bone is an endocrine organ that coordinates mineral metabolism.

INTRODUCTION
Serum phosphate levels are regulated by excretion from the kidney, absorption from the intestine, and release from the bone. Reduced serum phosphate levels cause osteomalacia in adults and rickets in children. Traditionally, bone has been recognized as a reservoir of minerals and as a target organ of systemic hormones such as Vitamin D and PTH.

FGF23, secreted from osteoblasts and osteocytes, is a circulating factor that regulates phosphate and vitamin D metabolism. FGF23 is a 251 amino-acid polypeptide that is synthesized by osteocytes and osteoblasts and released into the circulation. FGF23 was first discovered as a mutated gene in autosomal dominant hypophosphatemic rickets (ADHR) by positional cloning in 2000. FGF23 in turn negatively regulates serum 1,25(OH)2D3 levels. Consistent with this observation, serum 1,25(OH)2D3 levels are elevated in FGF23-null mice. FGF23 decreases the synthesis of 1,25(OH)2D3 by inhibiting 1-alpha hydroxylase expression in the renal proximal tubule.

i) FGF23 regulates phosphate homeostasis
FGF23 synthesis is induced by high phosphate intake, hyperphosphatemia, and an increase in serum 1,25(OH)2D3 concentration. FGF23 administration in animals induces a rapid and marked inhibition of renal phosphate reabsorption, leading to severe hypophosphatemia and bone demineralization. Consistent with this observation, the targeted disruption of the Fgfr3 gene in mice leads to increased renal phosphate reabsorption and high serum phosphate levels. FGF23 decreases the expression of NPT2a and NPT2c, two sodium phosphate cotransporters expressed at the apical domain of renal proximal tubular cells. In addition, FGF23 inhibits NPT2b expression in the intestine, further reducing the intestinal absorption of phosphate.

ii) FGF23 and vitamin D metabolism
1,25(OH)2D3 treatment in animals induces FGF23 expression in bones leading to increased serum FGF23 levels. FGF23 in turn negatively regulates serum 1,25(OH)2D3 levels. FGF23 administration in animals reduces serum 1,25(OH)2D3 concentration. Consistent with this observation, serum 1,25(OH)2D3 levels are elevated in Fgfr3-null mice. FGF23 decreases the synthesis of 1,25(OH)2D3 by inhibiting 1-alpha hydroxylase expression in the renal proximal tubule. FGF23 also stimulates 24-hydroxylase, which inactivates 1,25(OH)2D3. In vitamin D receptor-null mice, FGF23 has no effect on intestinal sodium-dependent phosphate transport activity and
NPT2b protein levels, suggesting a mechanism that is dependent on vitamin D.\textsuperscript{29,30}

\textbf{iii) FGF23 and PTH}
Recent studies have indicated that FGF23 regulates PTH synthesis and secretion in the parathyroid glands. FGF23 inhibits \textit{PTH} gene expression in the parathyroid glands.\textsuperscript{31} In addition, FGF23 administration in animals rapidly inhibits PTH secretion through the MAPK pathway.

\textbf{iv) Posttranslational regulation of FGF23}
Gain-of-function mutations in FGF23 have been identified in autosomal dominant hypophosphatemic rickets (ADHR).\textsuperscript{7} The biochemical analyses of FGF23 mutants have led to the discovery of posttranslational regulation of FGF23 activity. The activity of FGF23 resides in the 32kDa intact protein, which normally circulates in the plasma. FGF23 is cleaved between the amino acids 176 and 179 by a still-unidentified enzyme, and this cleavage yields two biologically inactive polypeptides. Amino acid substitutions at 176 and 179 in ADHR prevent the proteolytic cleavage of the FGF23 protein, which results in an increased biological activity causing severe renal phosphate wasting.

\textbf{v) FGF23 and its cofactor Klotho}
FGF23 exerts its biological activity by interacting with its cognate FGF receptors (FGFRs). Unlike most other members of the FGF family, FGF23 specifically requires Klotho, a cofactor that facilitates the binding of FGF23 to FGF receptors. Klotho is a single-pass transmembrane protein whose extracellular domain carries sequence homology to beta-glucosidases. Klotho is expressed in the kidney, parathyroid gland, pituitary gland, brain, ovary, testis, skeletal muscle, duodenum, and pancreas.\textsuperscript{31-34} The restricted pattern of Klotho expression defines the tissue specificity of FGF23 activity.\textsuperscript{32,35} Interestingly, Klotho is expressed in

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\caption{FGF23 and the bone-kidney axis. FGF23 is synthesized by osteoblasts and osteocytes. Hyperphosphatemia and 1,25(OH)\textsubscript{2}D\textsubscript{3} induce FGF23 synthesis. FGF23 inhibits renal phosphate reabsorption and 1,25(OH)\textsubscript{2}D\textsubscript{3} synthesis. FGF23 also inhibits PTH secretion from the parathyroid glands.}
\end{figure}
the distal tubular epithelial cells in the kidney, and it is not expressed in the proximal tubule.\textsuperscript{36} Biochemical studies have shown that Klotho facilitates the binding of FGF23 to several FGFR isofoms, including FGFR1c, FGFR3c, and FGFR4.\textsuperscript{35,37} Only in the presence of Klotho, FGF23 transduces signal and phosphorylates FGFR receptor substrate and ERK1/2.\textsuperscript{35} The absolute requirement of Klotho in FGF23 signaling is indicated by a number of genetic observations in mice. Mice with an insertional disruption or a targeted disruption of Klotho exhibit a phenotype similar to that of Fgfr2-null mice.\textsuperscript{7,15,24,26,34,38} Klotho-deficient mice show hyperphosphatemia despite extremely high serum FGF23 levels.\textsuperscript{39} FGF23 does not exhibit phosphaturic effects in these mice.\textsuperscript{35} These observations clearly indicate the requirement of Klotho in FGF23 signaling.\textsuperscript{40}

\textbf{vi) FGF23 and PHEX}

Hypophosphatemia caused by excess FGF23 levels has been observed in X-linked hypophosphatemia (XLH), a dominant disorder caused by inactivating mutations of the gene encoding PHEX (the phosphate-regulating gene with homologies to endopeptidases on the X chromosome). PHEX is expressed predominantly in osteoblasts and osteocytes in the bones. Patients with XLH exhibit increased serum FGF23 levels, phosphaturia, and osteomalacia.\textsuperscript{3} The hypophosphatemia in the Hyp mice, a mouse model of XLH characterized by a deletion in the Phex gene, is reversed to hyperphosphatemia by the inactivation of the Fgfr2 gene, suggesting that increased FGF23 levels cause hypophosphatemia in Hyp mice and in XLH patients.\textsuperscript{16} The tissue-specific inactivation of the Phex gene in osteoblasts and osteocytes causes increased FGF23 expression leading to hypophosphatemia, renal phosphate wasting, and osteomalacia, indicating that the loss of Phex in osteoblasts and osteocytes alone can account for the Hyp mouse phenotype.\textsuperscript{41}

\textbf{vii) FGF23 and dentin matrix protein 1}

Expressed in osteoblasts, osteocytes, odontoblasts, and hypertrophic chondrocytes, dentin matrix protein 1 (DMP1) is an acidic noncollagenous extracellular matrix protein. It was originally identified from a rat incisor cDNA library and thus thought to have a primary function in the regulation of dentinogenesis. In 2006, homozygous mutations in the DMP1 gene were identified in patients with autosomal recessive hypophosphatemic rickets (ARHR), indicating a role for DMP1 in phosphate homeostasis.\textsuperscript{4} Both patients with ARHR and Dmp1-null mice show rickets and osteomalacia, hypophosphatemia, and elevated levels of circulating FGF23. The inactivation of Dmp1 in mice leads to a dramatic increase in Fgfr2 mRNA expression in osteocytes.\textsuperscript{6} When Fgf23 was inactivated in the Dmp1-null mice by crossing FGF23-null and Dmp1-null mice, serum phosphate levels were elevated similar to those in Fgfr2-null mice, indicating that increased Fgf23 plays a role in the hypophosphatemia in Dmp1-null mice.\textsuperscript{42,43} These data suggest that DMP1 regulates matrix mineralization at least in part through the regulation of osteoblast and osteocyte production of FGF23, which in turn regulates renal phosphate reabsorption and vitamin D metabolism.

\textbf{viii) Role of FGF23 in chronic kidney disease}

Chronic kidney disease has profound effects on bone metabolism. Recent evidence shows that FGF23, secreted from osteoblasts and osteocytes, also plays a role in the mineral metabolism in chronic kidney disease. The circulating concentration of FGF23 increases as renal function declines, thereby maintaining the serum phosphate levels within the normal range.\textsuperscript{44,45} In pre-dialysis patients, the elevated FGF23 levels correlate with those of phosphate and creatinine. In addition, serum FGF23 levels show a negative correlation with tubular reabsorption of phosphate and serum 1,25(OH)2D3 levels, as expected from the physiological function of FGF23. In contrast, in advanced stages of chronic kidney disease, the urinary phosphate excretion does not increase sufficiently, as the number of functional nephrons decreases. As a result, serum phosphate levels remain elevated, despite high FGF23 levels. The high serum phosphate levels and reduced 1,25(OH)2D3 levels further stimulate PTH secretion, leading to the development of secondary hyperparathyroidism, which in turn affects bone turnover.

\textbf{CONCLUSION}

Our understanding of the regulatory mechanisms of phosphate homeostasis has greatly improved during recent years. Since the discovery of disease-causing mutations in FGF23, PHEX, and DMP1 in hereditary forms of human rickets, a number of novel mechanisms that control renal phosphate reabsorption have been identified. These discoveries have also changed our view of mineral metabolism. Recent studies on FGF23 have indicated that bone is an endocrine organ that coordinates mineral metabolism. Osteoblasts and osteocytes play active roles in the control of renal phosphate reabsorption, PTH secretion, and vitamin D metabolism. FGF23 secreted from osteoblasts and osteocytes also plays a role in the pathogenesis of
secondary hyperparathyroidism in chronic kidney disease. Further analysis of the regulatory mechanisms of FGF23 will provide novel insights into the mechanisms of controlling mineral metabolism.

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ANEURYSMAL BONE CYST IN THE CERVICAL SPINE: CASE REPORTS OF 2 ADOLESCENTS

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INTRODUCTION

Aneurysmal bone cysts (ABCs) are rare, benign, but locally destructive bone tumors. ABCs often present with expansile growth in bones of children and adolescents, with a peak incidence occurring in the second decade of life.¹ The vast majority of aneurysmal bone cysts present before the age of 20 years and are slightly more common in females. Most aneurysmal bone cysts occur in the metaphyseal region of long bones, but as many as 30% involve the spine.² When present in the spine, ABCs uniformly involve the posterior elements, but they may also extend into the vertebral body.³ Spinal ABCs pose particular challenges because of the potential for compression of adjacent neural structures and the possible development of spinal deformity or instability. Surgical resection is the treatment of choice for aneurysmal bone cysts of the spinal column to afford pain relief, to protect neural structures, and to impart stability when necessary.

Currently, we present 2 adolescents with ABCs within the cervical spine. Both patients presented in a similar fashion with progressive neck pain due to expansile lesions of the lamina and spinous process. Despite compromise of the spinal canal in each case, neither patient had significant neurologic deficits at the time of presentation.

The treatment of each patient included pre-operative embolization followed by complete surgical resection of the involved bone. One patient required an instrumented fusion because of the magnitude of facet joint involvement and the subsequent extent of bone resection necessary. Each patient remained asymptomatic and showed no evidence of radiographic recurrence after two years.

CASE REPORT 1

A healthy, active 15 year old boy presented with a 7-month history of increasing neck pain and stiffness. His symptoms began after a direct blow to the back of his neck while playing football. He experienced transient parathesias in his arms and legs at the time of the injury, but was able to continue playing. Within several months though, the localized neck pain had continued and increased in severity. He was not experiencing any ongoing radicular pain, parathesias, or weakness.

On physical exam, he had normal gait and coordination. He had limited active range of motion of his neck and there was a prominent, mildly tender, subcutaneous mass in the midline of the posterior aspect of the cervical spine. He had mild weakness in the left biceps, triceps, and grip strength, all graded as 4 of 5. He had a normal sensation and reflex exam.

Plain radiographs of the cervical spine revealed an expansile, lytic lesion that had replaced the C5 spinous process (Figure 1a). The lesion had faint septae and a thin, irregular boney margin. There was slight anterior angulation of the C5 body on C6 in the neutral position, which did not change with flexion or extension. A CT scan confirmed the expansile nature of the lesion within C5 spinous process lesion and laminae (Figure 1b). An MRI revealed an expansile lesion of the C5 spinous process and lamina composed of multiple cyst-like structures (Figure 1c). The spinal canal was moderately narrowed as a result of the mass and the there was slight compression of the spinal cord.

The differential diagnosis following the advanced imaging was aneurysmal bone cyst versus a giant cell tumor.

The day prior to surgery, an arteriogram was performed via the femoral artery. The left ascending cervical artery provided the sole arterial supply to the lesion and this was effectively embolized with particulate matter (polyvinyl alcohol).

A posterior midline surgical approach was made to the mid-portion of the cervical spine. Spinal cord monitoring was employed with somatosensory evoked potentials (SSEP’s). A large mass was noted arising from (and obliterating) the C5 spinous process and lamina. The lesion had a thin osseous rim which was carefully dissected from the surrounding paraspinal musculature. The mass was dissected from the underlying spinal cord, to which it was slightly adherent. The lesion extended into and had replaced the majority of the left lateral mass of C5. The posterior elements of the C4 and C6 were not involved. A frozen section revealed a cystic lesion comprised of blood-filled spaces with
intervening septae and fibroblasts, giant cells, and new bone formation, consistent with the diagnosis of an aneurysmal bone cyst.

Because of the extent of bone and soft tissue involvement, particularly the entire left C5 lateral mass, there was not sufficient stability present following the resection of the lesion. Thus, an instrumented fusion was performed with lateral mass screws from C4 to C6; the left C5 lateral mass did not have sufficient bone to allow for screw placement. Iliac crest autograft was harvested and packed laterally on each side.

The patient was maintained in a hard cervical collar for 8 weeks postoperatively. He began isometric exercise immediately postoperatively. He was pain-free by 3 months. By 6 months he had returned to full activities, with the exception of football. At latest follow-up, 24 months following surgery, he is pain-free and remains neurologically intact. Radiographs reveal persistence of slight anterior angulation of C5 on C6, but a solid fusion and no instability (Figures 1d,e).

**CASE REPORT 2**

A healthy, active 13 year old boy presented with a 2 month history of progressive neck pain and limited motion. His symptoms began after getting hit on the left side of the head during a football game. At the time of the injury, he had no loss of consciousness, nor radiating arm or leg pain, but he admitted to brief tingling in the left index and ring fingers. With time, the neck pain intensified such it was present at all times.

On physical exam, he held his head slightly to the right. His gait and coordination were intact. There was no palpable mass or tenderness. He had very limited active motion of the cervical spine. His strength and sensation were intact in both upper and lower extremities and he had normal reflexes.

Plain films of the spine showed an expansile lesion within the C2 spinous process (Figure 2a). The osseous rim was thin and irregular, with suggestion of a vertical fracture thru the lesion. Cervical alignment was intact and no instability was noted on with bending films. A CT scan confirmed an expansile, lytic lesion arising from the C2 spinous process, without obvious soft tissue mass (Figure 2b). No involvement was noted with the lamina or facet joints.

The MRI revealed a lesion of the C2 spinous process that was predominantly hyperintense on T2 weighted images, with visualization of multiple fluid-fluid levels (Figure 2c). There was no soft tissue mass nor was there extension into the spinal canal or spinal cord compression.

The differential diagnosis following the advanced imaging was of aneurysmal bone cyst or less likely a giant cell tumor.

On the day of surgery, an angiogram revealed the arterial supply to the lesion originated from muscular branches of both the right and left ascending cervical arteries. Both sides were embolized with particulate matter (polyvinyl alcohol). A repeat contrast injection showed no further tumor blush, consistent with a successful embolization.

A posterior surgical approach to the upper cervical spine was made. Spinal cord monitoring was employed with SSEP’s. A subperiosteal dissection exposed the C2 spinous process which was markedly expanded and had a thin, delicate boney rim. There was no soft tissue mass extending from the mass. The mass involved, but did

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**Figure 1a**: Plain radiograph of Case 1 revealing expansile lesion of C5 spinous process.

**Figure 1b**: Sagittal CT image revealing expansile lesion of C5 spinous process.

**Figure 1c**: T2 weighted MRI image revealing C5 spinous process lesion and resulting canal compromise.

**Figure 1d**: Lateral radiograph at 24 months post-op.
not completely replace the C2 lamina, nor was there involvement of the lateral masses and the facet joints were preserved. Complete removal of the lesion necessitated a C2 laminectomy, although great care was taken to avoid excessive resection of the lateral masses and facets. The intra-operative pathology revealed a cystic lesion with blood-filled spaces surrounded by a compact layer of benign spindle cells and multi-nucleated giant cells, as well as abundant reactive woven bone, which was consistent with the diagnosis of an aneurysmal bone cyst. Given the absence of instability on the pre-operative radiographs and the preserved nature of the facet joints, it was decided to avoid a spine fusion.

The patient was kept in a soft cervical collar for two weeks until his surgical pain had resolved. He began cervical isometric strengthening immediately. He was cleared to return to full activities by 3 months. At two year follow-up he remains pain free and is fully active and continues to play competitive basketball. He was advised to avoid football and any diving activities. Plain radiographs reveal slight angulation of C2 on C3, but no evidence of instability on dynamic bending films (Figures 2d,e).

DISCUSSION

Aneurysmal bone cysts are uncommon lesions that may cause severe bone destruction. The term “aneurysmal” refers to both the radiographic appearance of the affected bone with its balloon-like expansion surrounded by thin cortical margins and to the gross histological appearance of large cystic, blood-filled spaces. However, an aneurysmal bone cyst is neither an aneurysm nor a bone cyst. According to the World Health Organization Classification of Tumors, an aneurysmal bone cyst is an expanding osteolytic lesion consisting of blood-filled spaces of variable sizes that are separated by connective tissue septa containing trabeculae or osteoid tissue and osteoclastic giant cells.\(^4\)

The etiology of aneurysmal bone cysts is not known. They are generally thought to be a benign condition and are not true neoplasms. It is postulated that the inciting event is a vascular disturbance such as a sudden venous occlusion or the development of an arteriovenous shunt.\(^5\) It is likely, although unproven, that when these types of dynamic vascular events occur in the vascular, newly formed parts of the immature skeleton, an aneurysmal bone cyst will arise. It is also suggested that that localized trauma (as occurred in both our patients) may be the precipitating factor for the subsequent vascular changes and bone expansion.\(^1\) Aneurysmal bone cysts may occur as a solitary lesion or can be found as a secondary lesion in association with other tumors, such as giant cell tumor, chondroblastoma, chondromyxoid fibroma, fibrous dysplasia, or osteosarcoma.\(^3\) The presence of a secondary ABC does not change the prognosis or treatment of the underlying primary lesion.\(^6\)

Aneurysmal bone cysts are relatively uncommon. Their prevalence is 1.4 cases per 100,000 individuals, and they constitute approximately 1% of all bone tumors.\(^7\) They are a condition seen most commonly in children and adolescents, with the vast majority occurring before the age of 20 years.\(^3\) Aneurysmal bone cysts may occur in any bone, but show a predilection for the metaphyseal end of the tubular long bones. In 20% of cases, the lesion is located in the spine, and most often in the posterior elements.\(^6\) On rare occasions an ABC may involve adjacent vertebrae and they have been described to involve three or more consecutive levels.\(^4\) Within the spinal column, almost 70% occur in the thoracolumbar region, and less than 25% within the cervical spine.\(^6\)

As is the case when located elsewhere in the skeleton, spinal ABC’s most commonly present with progressive, localized pain. If the lesions are of sufficient size, neural compression can occur resulting in radicular pain, paresthesias, and even myelopathic symptoms. Because the location of most ABC’s is within the posterior elements, extensive growth may lead to instability if there is sufficient involvement of the facet joint.
complexes. In rare instances, growth may extend into the vertebral body leading to pathologic fractures and vertebral plana. Growth of aneurysmal bone cysts is rapid and there is generally an interval of less than 6 months between onset of symptoms and the necessity for treatment.4

The diagnosis is be made with plain radiograph, CT, and MRI, but ultimately biopsy is necessary for histological confirmation. Radiographs demonstrate a radiolucent, expansile lesion within the metaphyseal regions of a long bone or the posterior elements of the spine.7 Within the osteolytic cavity of the lesion are irregular strands of bone which form the characteristic bubbly appearance of an ABC. The cortical rim of the lesion is eggshell thin and frequently blown out or absent. CT scans confirm the expansile lesion with the characteristic soap bubble appearance (due to its ballooning, multi-locular nature) and the thin, fragile-appearing walls which result from constant erosion and expansion of the cortex.8 The hallmark feature of an aneurysmal bone cyst on MRI scans are a double fluid densities, which have low signal intensity on T1 images and high signal intensity on T2 images. The finding of fluid-fluid levels within a multi-locular cysts is highly suggestive of an aneurysmal bone cyst.9

The differential diagnosis of ABC's include giant cell tumors, telangiectatic osteosarcomas, and unicameral bone cysts.4 Unicameral bone cysts generally do not have the aggressive appearance of distention and expansion of the ABC, nor the multiple loculations and fluid-fluid levels seen on MRI.3 Giant cell tumors and telangiectatic osteosarcomas may be difficult to distinguish radiographically from an ABC. Giant cell tumors produce rarefaction of the bone similar to an ABC, but the overlying cortex is thin and generally not thinned or “ballooned” as with an ABC. Telangiectatic osteosarcomas, while radiolucent on plain radiograph, typically have thick nodular tissue in a largely hemorrhagic or necrotic osseous lesion and frequently an associated soft-tissue mass not routinely seen with an ABC.4

Histologically, the term aneurysmal bone cyst is a misnomer. It is not an aneurysm, in that there is not one true vessel that exists within the lesion. Nor is an ABC a true cyst, as its cavity contains a dense cellular composition containing plump stromal cells, multinuclear giant cells, and thin-walled blood vessels, or a preponderance of fibrous tissue with enlarging vascular spaces.3 The most striking and essential feature in a well preserved specimen is the numerous dilated blood spaces surrounded by an irregular margin of small osteoclastic giant cells. These spaces have more or less delicate walls of loose-texture fibrous tissue containing scattered islets of osteoid. Elsewhere there may be a surprising amount of richly vascularized undifferentiated connective tissue with numerous giant cells, or areas showing poorly formed cartilage and osteoid.4

Surgical management is the treatment of choice for most aneurysmal bone cysts. For most metaphyseal lesions, aggressive curettage and bone grafting is effective in relief of symptoms and associated with relatively low recurrence rates (<10% in most reports).10 In the spine, complete surgical resection is necessary. As was the case in our 2 patients, most spinal lesions are large and have violated the cortical margins of the posterior elements. Thus, en bloc resection of the posterior spinal elements is necessary to effectively eradicate the lesion, prevent its recurrence, and to relieve pressure on the adjacent neural structures. It is also essential to provide stability if the extent of the lesion or the surgical resection has rendered the spine unstable. In general, if greater than 50% on the facet complex at one level (i.e. one entire facet or greater than half of both facets) are involved, it is necessary

Figure 2c: T2 weighted MRI image revealing hyperintense lesion within C2 spinous process.

Figure 2d: Lateral radiograph at 24 months post-op revealing slight angulation of C2 on C3.

Figure 2e: Lateral radiograph in maximal flexion revealing no instability.
to perform an instrumented fusion to avoid a kyphotic deformity and the potential of subsequent pain, instability or additional neurologic injury. Children, in particular for the development of post-laminectomy kyphosis in the cervical spine because of anterior wedging of the vertebral bodies is caused by compression of the cartilaginous endplates. In one of our cases, a C2 laminectomy alone was performed because of integrity of the facet joints and because a fusion may have necessitated extension proximally to the occiput, with subsequent significant reduction in the adolescent’s range of motion and function.

Radiotherapy, cryotherapy, and intralesional injection of a sclerosing agent are less likely to definitively eradicate the lesion and are generally reserved for small, minimally symptomatic lesions. Aneurysmal bone cysts are radiation-sensitive tumors, however the use of radiation therapy is precluded by the potential of growth arrest or induction of radiation sarcoma in metaphyseal lesions and injury to adjacent neural structures in the spine. Cryotherapy has been employed for cysts in long bones, but its use in the spine is contraindicated by the inability to control the liquid nitrogen and the zone of freezing which could damage the adjacent neural structures. The same is true of the use of sclerosing agents for ABC’s within the spine, which have been of some benefit when injected into metaphyseal lesions.

Preoperative embolization is effective in reducing the intraoperative blood loss and subsequent need for blood transfusions in patients being undergoing surgical treatment for aneurysmal bone cysts. In some cases, selective arterial embolization alone as the first treatment option for spinal aneurysmal bone cyst has been described as being as effective as intralesional surgical excision. However, this approach should be reserved for very small lesions, with minimal symptoms and no evidence of neurologic compression or the potential of instability. If employed as the sole treatment, careful surveillance for recurrence is essential.

CONCLUSION
While benign in nature, aneurysmal bone cysts may display aggressive expansion that leads to significant bone destruction. When present in the spine, along with the common symptom of localized pain, there is the potential for neural compression, deformity, and instability. Spinal involvement occurs in the posterior elements and the expansion of the lesion is generally rapid. Prompt recognition of these lesions is necessary and diagnosis can be made with plain radiographs and advanced imaging employing MRI and CT scans. Pre-operative embolization followed by complete surgical resection and when indicated, an instrumented fusion, is the treatment of choice. Results, as occurred with our two patients, are generally favorable with relief of symptoms and no local recurrence.

REFERENCES
ALIGNING INCENTIVES IN ORTHOPAEDICS

Opportunities and Challenges—the Case Medical Center Experience

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ABSTRACT

For 30 years, the orthopaedic faculty at Case Western Reserve University worked as an independent private corporation within University Hospitals Case Medical Center (Hospital). However, by 2002, it became progressively obvious to our orthopaedic practice that we needed to modify our business model to better manage the health-care regulatory changes and decreased reimbursement if we were to continue to attract and retain the best and brightest orthopaedic surgeons to our practice. In 2002, our surgeons created a new entity wholly owned by the parent corporation at the Hospital. As part of this transaction, the parties negotiated a balanced employment model designed to fully integrate the orthopaedic surgeons into the integrated delivery system that included the Hospital. This new faculty practice plan adopted a RVU-based compensation model for the physicians, with components that created incentives both for clinical practice and for academic and administrative service contributions. Over the past 5 years, aligning incentives with the Hospital has substantially increased the clinical productivity of the surgeons and has also benefited the Hospital and our patients. Furthermore, aligned incentives between surgeons and hospitals could be of substantial financial benefit to both, as Medicare moves forward with its bundled project initiative.

INTRODUCTION

From 1907 until 1971, the physicians who comprised the orthopaedic surgical facility at Case Western Reserve University (the “University”) and the orthopaedic medical staff at University Hospitals Case Medical Center (the “Hospital”) practiced in a collaborative affiliation with the University and the Hospital for research, academic, and clinical purposes. In 1972, after the promulgation of state legislation permitting the incorporation of professional associations, these orthopaedic surgeons formed an independent Ohio professional corporation with shareholders and an elected Board of Directors, and began doing business as University Orthopaedic Associates, Incorporated (UOAI). UOAI was one of approximately 21 independent physician groups that served as faculty practice plans for the University and Hospital. UOAI’s physicians were all appointed faculty at the University and were all credentialed on the medical staff of the Hospital. Its orthopaedic surgeons therefore comprised the Department of Orthopaedics for the University and the Hospital.

Over the next 30 years, UOAI’s clinical, academic, and research practice expanded and succeeded. By 2002, however, healthcare regulatory changes coupled with decreasing reimbursement for the provision of orthopaedic clinical care to patients placed economic and management stresses on UOAI’s ability to attract and retain the best and brightest orthopaedic surgeons for employment in its group practice. As a result, UOAI, along with its academic and hospital affiliates, endeavored to identify a physician practice and compensation paradigm that would allow for continued growth and recruitment, while simultaneously responding to the relentless economic and legal pressures present in the modern practice of medicine.

This report describes the challenges and opportunities of aligning incentives between hospitals and academic clinical departments, the 5-year results of our experience, and the future challenges that face hospitals and their orthopaedic departments.

The Challenges of Aligning Incentives with the Hospital in an Academic Setting

Medicare payments for the most
common orthopaedic surgical procedures have decreased dramatically since 1995, compared with the increase in the cost of living \(^1\) (Fig 1), and corresponding increases in physician compensation benchmarks. The decrease in Medicare payments for these procedures is closely linked to the decrease in reimbursement from most commercial insurance payors \(^2\).

In 1989, changes in healthcare law had considerable impact on the practice of medicine. In 1989, Congressman Fortney “Pete” Stark, representing California’s 13th congressional district, sponsored legislation (commonly known as the Stark Law) enacted by Congress that substantially affected the scope, structure, and nature of compensation and financial relationships between physicians and other types of healthcare providers such as hospitals \(^3\). The crux of the Stark Law is a prohibition against physicians referring Medicare patients for designated health services (or DHS such as referring patients to an offsite imaging facility in which the physician has ownership) to an entity with which the physician or member of the physician’s family has a financial relationship unless the relationship fits within a statutory or regulatory “exception” \(^4\). Similarly, if such a referral does not fit within a statutory or regulatory exception, the Stark Law prohibits the entity (such as a hospital) receiving the referral from billing the federal government for reimbursement for services provided to the Medicare patient. Subsequent amendments to the Stark Law in 1993 and 1994 expanded this prohibition by including additional categories of services in the definition of DHS and also included referrals of patients who are covered under the Medicaid program \(^5,6\). In 1995, 2001, 2004, 2006, 2007, and 2008, the Centers for Medicare & Medicaid Services (formerly known as the Health Care Financing Administration) issued, reissued, revised, and replaced a multitude of regulations concerning acceptable and prohibited arrangements under the Stark Law \(^7,8,9,10,11,12\). In 2003, the Social Security Act was amended to include an 18-month moratorium on physician referrals to certain specialty hospitals (specifically including orthopaedic hospitals) in which the referring physician maintained an ownership or investment interest \(^13\). In combination, this evolution of the Stark Law resulted in severe limitations on the ability of physicians, including UOAI, to own and/or share in revenue generated from clinical services performed ancillary to the physician’s treatment of his or her patients. As the Stark Law evolved and other healthcare regulatory changes took effect, the practice of medicine became increasingly complex. The effect of the increased legal scrutiny and regulation substantially restricted the ability of hospitals to support and effectively utilize the professional services of independent physicians’ practices, even if such practices served as the faculty practice plan for a hospital and/or its medical school affiliate.

Other critical federal regulatory schemes enacted and implemented about the same time as the Stark Law also materially impacted the business operations of medical practices. For example, the Health Insurance Portability and Accountability Act of 1996 (HIPAA) mandated standards for the privacy of individually identifiable health information \(^14\). HIPAA markedly increased the overhead expenses for independent medical practices such as UOAI, requiring medical practices to make substantial investments in
Orthopaedic practices affiliated with universities and teaching hospitals, most of which are tax-exempt pursuant to Internal Revenue Code Section 501(c)(3), faced additional challenges. A fundamental requirement of Section 501(c)(3) is that a charitable organization such as a tax-exempt hospital or university must ensure no part of its net earnings inures “to the benefit of private shareholders or individuals” such as an independent, for-profit physician group. Internal Revenue Service oversight and enforcement of the inurement prohibition added to the limitations on the ability of for-profit physician groups to partner with tax-exempt hospitals and/or medical schools15.

As the Stark Law evolved and the plethora of other healthcare regulations took effect and were applied and enforced in new and unexpected ways, the ability of academic medical centers to provide funding or loans to independent physician practices for the recruitment of new physicians necessary to support the medical center’s tax-exempt mission became extremely complex. Alternatives to academic medical center funding for independent physician practices, although limited, included funding recruitment out of declining practice revenues, seeking commercial loans, and/or increasing capital contributions from physician shareholders. All of these options created burdens on the ability of physician practices to maintain necessary operating revenue and margin, let alone equitable physician compensation arrangements in the face of ever-increasing competition.

By 2002, UOAI was struggling with the prospect of severely contracting its recruiting efforts as well as its programs and services, due in large part to the substantial changes in the healthcare environment. The Stark Law, HIPAA, and the prohibition on private inurement under the Internal Revenue Code put at risk UOAI’s ability to function as an independent professional corporation serving as the orthopaedic faculty practice plan for a major, tax-exempt academic medical center comprised of the University and the Hospital.

**Opportunities for Alignment Between the Hospital and the Academic Orthopaedic Department**

An independent analysis of UOAI’s orthopaedic practice revealed differences between the revenue generated by UOAI and the revenue generated by private orthopaedic practices. These differences were attributable to the fact that such private practices were not required to participate in the charitable activities of the Hospital and University, including teaching, research, and caring for the indigent and uninsured. In particular, unaffiliated private orthopaedic practices concentrated on private-pay patients and were also able to invest in equipment and capabilities to provide ancillary services (eg, imaging), surgical services (eg, ambulatory surgical facilities), and related services (eg, physical therapy). One estimate UOAI obtained from an outside consultant stated that for every $1 of professional revenue a practice generated, it also generated $6 to $10 of other revenue from these services.

It was obvious that to properly remain competitive while serving as the orthopaedic faculty plan with a charitably focused academic practice, UOAI would require subsidization from the University and/or Hospital. UOAI needed to identify an economically feasible means of setting, evaluating, and providing benchmark physician compensation in an increasingly competitive marketplace. In reviewing successful orthopaedic practice models, UOAI concluded there were three general ways of structuring compensation for its employed surgeons: cash-based, salary-based, and work relative value unit (wRVU)-based16,17. Each compensation model offered unique advantages and disadvantages.

A cash-based physician compensation plan is one in which a physician’s compensation is dependent on collections from patient care. Adoption of this compensation model would permit UOAI to remain legally independent from the University and the Hospital and would include an incentive compensation component based on productivity—an important component of equitable compensation in a private-practice model. On the other hand, due to the pressure to identify and capture additional revenue to maintain competitive market salaries, adoption of this compensation model could require UOAI to purchase, own, and manage the ancillary services that are most used in connection with the clinical treatment of patients with musculoskeletal disorders. Further, UOAI recognized the potential for such a model to create internal competition between surgeons based on the fact that the compensation model, if not properly structured, could provide disproportionate economic benefits to those physicians who cared for patients needing complex diagnostic and other ancillary services in conjunction with their higher acuity underlying clinical issues. Since UOAI physicians were also actively involved in substantial...
teaching and research efforts, the practice was concerned that adoption of the cash model would not encourage physicians to participate in academics and/or other programs designed to fulfill the charitable missions of the University and the Hospital.

UOAI considered a second model designed around a salary-based compensation program, in which physician compensation is standardized and does not change with productivity over the contract period. UOAI determined this model would allow the UOAI physician shareholders to maintain their corporate independence from the Hospital and the University while eliminating competition based on the nature of internal referrals and/or clinical acuity. Further, UOAI determined this model would facilitate funding for academic work and encourage its employed physicians to spend time providing clinical services to fulfill the Hospital’s mission to provide high-quality care to treat underserved, indigent, and other high-risk populations. This model, however, also could have necessitated UOAI’s purchase and management of certain ancillary services in order to maintain revenue required to fund otherwise unreimbursed and under-reimbursed activities of its physicians. On top of this, the model contained no entrepreneurial component or incentives for clinical productivity.

Throughout 2002, as UOAI surveyed successful orthopaedic practices across the country, it became clear that regardless of the type of compensation model, the universally accepted method for measuring clinical productivity was based on wRVU measurements. The advantages of a third model, the wRVU compensation model were substantial. Such a model included clear productivity incentives, eliminated the potential for internal competition for “select” patients, and favored entrepreneurial physicians who filled their schedules and provided access to all patients, and eliminated the potential for “adverse selection” of patients who have no insurance, insufficient insurance, or low acuity.

In analyzing how a wRVU model could work for UOAI, it became clear that the per-wRVU payment that was available based on UOAI’s actual collections would need to be increased in order to maintain market competitiveness. For example, UOAI’s analysis found that private groups that owned their own ancillary and related services were able to offer potential recruits a higher per-wRVU payment: in 2007, the MGMA 50th percentile for wRVUs generated by orthopaedic surgeons without ownership of ancillary services was $42.46, whereas during the same time period the value of wRVUs generated by orthopaedic surgeons owning their own ancillary services was $57.10, almost a $15 difference per wRVU.

It is important to note there is no correlation between orthopaedic physician compensation and any referrals made by such physicians to any ancillary services owned/provided by the Hospital (or any other entity). Moreover, but for the fact that the Faculty Practice Plan was structured to comply with state corporate practice of medicine doctrine, the Hospital and/or the University directly would have most likely employed the orthopaedic physicians directly. Since this option was not pursued, the corporate structure of the Faculty Practice Plan and its relationship to the Hospital was designed to permit the Hospital to indirectly subsidize the Faculty Practice Plan in order to facilitate its charitable mission. This in turn permits the Faculty Practice Plan to provide funding for various activities of the Department of Orthopaedics, including key recruitments and charitable clinical services and related programs. Funding provided to the Faculty Practice Plan is consistent with the Academic Medical Center exception under the Stark Law and other regulatory requirements as well as state corporate law. Key aspects of this structure include the facts that no individual orthopaedic physician compensation is linked directly to any subsidization, physician compensation does not vary based on the volume or value of any referrals or other business made by an orthopaedic physician to the Hospital (or any affiliated entity), and all physician compensation is evaluated for compliance with fair market value.

UOAI would need to work with the Hospital and University in order to design a compensation system that was both competitive and geared toward the fulfillment of the Hospital and University’s charitable missions. Fortunately, our Hospital recognized the continuing importance of the Department of Orthopaedics’ academic mission to pursue scholarly activities including research and education. Therefore, UOAI would be able to redistribute a portion of the compensation based on the surgeons’ academic contributions.

UOAI recognized, however, that in order to fully implement a wRVU model, it would need to cease operating its corporation and cede operations and governance of the orthopaedic service line to the Hospital and the University. Even more concerning to UOAI shareholders and physician employees was the requirement that all of the orthopaedic surgeons become
employees of an entity owned and controlled by the Hospital. Despite these drawbacks, UOAI recognized that in order to adjust to and comply with the ever changing healthcare regulatory and reimbursement landscape, UOAI would need to relinquish its independence.

From the perspective of the Hospital and the University, the advantage of securing a worldclass group of high-quality orthopaedic subspecialists as employees of a tax-exempt wholly owned faculty plan was clear. Such an arrangement would facilitate simpler, legally compliant funding of programs necessary to serve underserved populations, indigent and Medicaid patients, and provide the means for enhancing teaching, academic, and cutting-edge research activities. In addition, this arrangement would further integrate the orthopaedic surgeons into the clinical and economic structure of the academic medical center all the while aligning incentives among all three constituents: the physicians, the University, and the Hospital. For UOAI, this was the best possible solution.

**Hospital Issues in Aligning Incentives with an Academic Orthopaedic Department**

The same regulatory climate that posed challenges for UOAI also had a direct effect on the clinical, academic, research, teaching, and strategic activities of the Hospital and the University. The Hospital component of the academic medical center faced substantial financial challenges arising from decreasing reimbursement, increased competition, declining populations in primary service areas and the varied pace of integration of the Hospital’s affiliated continuum of healthcare services. For example, UOAI’s review of orthopaedic referral patterns revealed that approximately 70% of outpatient imaging, physical therapy, and outpatient surgery required by UOAI’s patients was performed at facilities that were not part of the Hospital’s integrated delivery system, resulting in the Hospital’s inability to exercise its quality assurance, utilization review, and risk management activities related to these postsurgical services. In addition, the corporate independence of UOAI inhibited the ability of the Hospital to control the use and purchase of the expensive implants used in both joint replacement and spine surgery. The failure of the Hospital and the orthopaedic surgeons who comprised the academic medical center’s orthopaedic faculty to collaborate on and set appropriate parameters for the purchase and use of medical supplies resulted in inefficiencies and increased cost, due to the loss of volume purchasing opportunities and the need to maintain stocks of too many device types. Similar failures to achieve a high level of collaboration contributed to the Hospital’s difficulties in obtaining comprehensive physician coverage and in recruiting orthopaedic physicians to provide for certain lower-reimbursed orthopaedic services such as pediatric orthopaedics and orthopaedic oncology.

In 2002, it became readily apparent to UOAI and to the Hospital, that a new clinical and financial structure was needed. To create this structure, UOAI agreed to sell its assets for an independently determined fair market value purchase price and move its assembled workforce of approximately 21 orthopaedic surgeons to a newly created nonprofit tax-exempt entity (the “Faculty Practice Plan”), wholly owned by the parent corporation of the Hospital. As part of this transaction, the parties negotiated a balanced employment model designed to fully integrate the orthopaedic surgeons into the integrated delivery system that included, at its hub, the Hospital.

The Faculty Practice Plan adopted a wRVU-based compensation model for the orthopaedic department with additional components that created reasonable incentives both for clinical practice and for academic and administrative service contributions. This model, which requires all compensation to be fair market value, eliminated private inurement concerns since: (1) the Faculty Practice Plan, as a tax-exempt organization, shared the same charitable mission as the Hospital; and (2) the UOAI compensation model was calibrated to offer fair salaries that were related to achievement of the nowshared charitable missions of the Faculty Practice Plan and the Hospital. It was relatively straightforward to establish the basis whereby the Faculty Practice Plan would augment the funds available to implement a per-wRVU compensation system for the UOAI physicians, as compared to only the funds available through professional collections. This process involved an analysis of the services performed by UOAI in furtherance of the charitable mission of the Hospital and University (teaching, research activities, clinical care for the indigent and uninsured, and administrative services), with a concomitant allocation of the fair value of such services to a pool of available compensation. The wRVU value would follow a fairly designated Medical Group Management Association percentile for orthopaedic surgeons who owned their ancillaries. The value based on national data...
may vary yearly, but the percentile would be set in the contract in order to avoid yearly negotiations. Under such a wRVU model, payor source and level of reimbursement are not factors in the calculation of compensation. Additionally, the compensation for our surgeons is guaranteed based on individual productivity and not at risk based on the Hospital’s economic performance or based on any referrals for any ancillary or other services or business generated by the physicians.

Through the new model, the Faculty Practice Plan’s employed physicians as well as the Hospital were able to enhance the quality and efficiency of the patient experience by ensuring that patients requiring other healthcare services could choose to secure such services from other providers within the integrated delivery system, thereby achieving a continuity of care that had not previously existed. Further, as the Hospital and the Faculty Practice Plan move towards the implementation of their electronic health record, along with the implementation of other critical technology, the benefits of this structure continue to be revealed. The alignment of incentives promoted the participation of the orthopaedic physicians in the management of necessary ancillary services as well as in quality and financial control of implant purchasing strategies. There is no correlation between revenue derived from ancillary services and referrals of patients to such ancillary service by Faculty Practice Plan physicians, including the orthopaedic physicians. Notwithstanding the foregoing, due to the enhanced clinical and economic integration of the physicians with the Hospital resulting from the Faculty Practice Plan structure, there are meaningful opportunities for orthopaedic physicians to provide clinical, management, business, and operational input with respect to various ancillary services provided by the Hospital or that are otherwise part of the academic medical center’s continuum of care. This alignment also further encouraged the Faculty Practice Plan surgeons and the academic medical center to invest jointly in three areas: (1) improved technology; (2) the expansion of orthopaedic practice sites to provide broader community access; and (3) the recruitment and retention of expert orthopaedic surgeons for the academic medical center.

The Hospital and the Faculty Practice Plan have developed a synergetic approach to operational and strategic decisions. For instance, while the orthopaedic surgeons are intimately involved in the quality and financial review of implants in order to establish purchasing policies that ultimately benefit the patient community, the Hospital is equally engaged in strategic decisions regarding the recruitment of new orthopaedic surgeons and in evaluating the success of the surgeons’ practices. Both constituencies now measure success in the same manner. They jointly evaluate the orthopaedic surgeons’ contribution to the shared charitable mission of both organizations and the enhancement of the quality and efficient delivery of orthopaedic services to the patient population. This includes an analysis of the individual productivity of the surgeons (including clinical, academic and research productivity) and the development and adoption of best practices, cutting edge technology and sufficiency of other required resources. They also share and review in a transparent manner the same orthopaedic surgery financial data, including profits and losses, revenue and expenses, and other key factors that sustain the ability of the academic medical center to achieve its mission.

Ultimately, the structure selected in 2002 and implemented in 2003 requires both the Hospital and the Faculty Practice Plan to share in each other’s successes and failures. This ability to share in the risks and the rewards was derived from a very simple principle—trust. In 2002, as UOAI was evaluating its next moves, and as the Hospital was undergoing its own transitions and strategic challenges, the parties committed themselves to negotiate in good faith and establish balanced contractual arrangements that served as the catalyst for the development of a lasting trust between all individuals involved, including new leadership and participants that would be added along the way.

The specifics of the Faculty Practice Plan’s current wRVU compensation plan include direct compensation to the physician, which is based 80% on wRVUs generated from the respective physician’s individual clinical practice. The remaining 20% is based on the respective physician’s academic and service contributions to the academic medical center. In essence, the Faculty Practice Plan takes 20% of all the funds generated by its clinical practice wRVUs and redistributes these funds to its surgeons based on a 25-point scoring system (Fig 2). Points are provided for: (1) scholarly work; (2) academic rank; (3) service to the Department of Orthopaedics, academic medical center, and orthopaedic profession; and (4) participation in the teaching and education of medical students. The academic points are reviewed and adjusted on a yearly basis in consultation with the Department Chair and corporate leadership of the
Faculty Practice Plan. Furthermore, there is no cross-subsidy of other departments or specialties by the Department of Orthopaedics. Payments made to the University by the Hospital ("Dean’s tax") on behalf of the Orthopaedic Department are not considered in the physician compensation formulation.

Results of Our 5-Year Experience in Aligning Incentives with the Hospital

For the last 5 years, this new practice model, incentive-based and transparent to all participants, has substantially increased the clinical productivity of the Faculty Practice Plan’s orthopaedic surgeons (Fig. 3) and has greatly benefited the academic medical center and the patients and communities served thereby. The patients have benefited by the more seamless management of their orthopaedic problems made possible by the enhanced clinical integration of the physicians with the Hospital. The alignment of incentives and vertical integration of the Faculty Practice Plan and the Hospital has resulted in enhanced use of the integrated delivery system when patients, insurance benefits, and/or legal requirements do not otherwise require the use of alternate providers. Additionally, the joint administration and governance of the Faculty Practice Plan has allowed the Hospital to lower costs by working with its orthopaedic physicians to jointly evaluate and update implant-purchasing strategies such as shelf pricing and kit-purchasing policies. Such activities would have been much more difficult, if not impossible, to implement without aligned incentives based on an employment structure and a foundation of collaboration and trust between the Hospital and the orthopaedic surgeons.

In the 5 years since UOAI made the decision to sell its assets and to have its physicians become employed by the Faculty Practice Plan (becoming the first of 21 independent, physician-owned and/or controlled medical practices to do so), additional benefits have resulted for the physicians. This critical mass of subspecialty faculty physicians aligned with both the Hospital and the University’s school of medicine enhanced the retirement and benefit packages available to faculty physicians. Moreover, the creation of a unified, tax-exempt faculty practice plan employing all of the specialty physicians who provide the clinical, academic, research, teaching, and strategic physician services for the academic medical center facilitated the consolidation of redundant front office and back office activities as well as the centralization of key corporate functions such as human resources, marketing, legal, compliance, and information technology. The integration of UOAI into the academic medical center has markedly decreased the complexities arising from the ever-evolving

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Fig 2. The 25-point academic scoring system provides incentives for scholarly work and contributions in education and service.
healthcare regulatory environment and has provided a legally permissible, compliant vehicle for the academic medical center to fund appropriate clinical programs and administrative positions, including directorships for pediatric orthopaedics, musculoskeletal cancer surgery, and the spine center18. Finally, by aligning incentives, the orthopaedic faculty practice can participate in and benefit from the Hospital’s robust institutional relations and development office, which has substantially increased the endowment for chairs in orthopaedic surgery over the last 5 years, as demonstrated by the progression from only one endowed chair to the Department of Orthopaedic’s current level of nine endowed chairs.

The orthopaedic surgeons in the Faculty Practice Plan expressed early that academic efforts should be recognized and encouraged. The Faculty Practice Plan physicians believed that scholarly work such as peer-reviewed publications and presentations not only benefit the department of orthopaedics, but the entire academic medical center and the patients it serves. By clearly defining expectations and rewards under the Faculty Practice Plan’s compensation program for participation in the academic points program, the surgeons have noted a marked increase in academic participation (Fig 4).

Future Challenges for Hospitals and Academic Orthopaedic Departments

Future challenges facing the profession of orthopaedic surgery include the continually evolving and ever-more complex healthcare regulations and related compliance issues that impact financial and compensation relationships between physicians, hospitals and other healthcare providers 7, 19, 20, 21. These changes, coupled with changes in federal and commercial reimbursement programs, including an increased focus on measuring and paying for quality and performance22, present opportunities that can be effectively captured under the Faculty Practice Plan model. If handled correctly, the new “Medicare-bundled payment schedule” can facilitate collaboration between hospitals and physicians so they can develop “best practices” and create legally permissible clinical integration models that permit hospitals to share operating room and other savings generated by enhanced efficiencies in surgical practice23. This approach, commonly known as “gainsharing”, is made easier when physicians and hospitals participate jointly in the governance, operations, and administration of the clinical departments and physician employment entities.

Risks and benefits exist when physicians, hospitals, and payors, including commercial payors, proceed with a bundling strategy. For instance, the cost of providing care on a case-by-case basis may exceed the net bundling payments received. Accordingly, the parties must ensure the transparent exchange of information concerning the equitable distribution of payments, quality benchmarks and patient satisfaction information so that all parties continue to work from the same set of factual assumptions towards the combined goal. Aligning incentives between all of the participants (the hospital, physician, and payor) in the bundling strategy can minimize risk and enhance the benefits for all involved. Most importantly, aligned incentives can improve the overall patient experience and clinical outcome while decreasing the cost of the service to the patient. The federal government is currently testing this concept 24.

**Fig 3.** The increase in work relative value units (wRVU) per FTE productivity, 2003-2007, illustrates the positive effect of incentivizing clinical productivity.

**Fig 4.** The academic points earned by faculty from 2003 to 2008 demonstrate the positive effect of incentivizing scholarly work and educational and service contributions.
Medicare is currently running a demonstration project called the Acute Care Episode (“ACE”) Demonstration, with Texas, Oklahoma, New Mexico, and Colorado as the pilot states. This demonstration requires the formation of a physician-hospital organization (“PHO”), and encompasses the bundling of 38 cardiac MS-DRGs and nine orthopaedic MS-DRGs. The nine orthopaedic MS-DRGs focus on procedures that are joint-related (hips and knees) 24.

The ultimate goal is to maintain or improve the quality of patient care while reducing costs to the patient and to the Medicare program. The participating hospitals and physicians are required to jointly develop the best practices in a cost-effective manner, and the physicians are permitted to participate in the cost savings through CMS-approved provider incentive programs (gainsharing). Provider incentive programs require the formation of a hospital committee consisting of administrative physicians and an independent patient advocate or consumer representative. The committee is responsible for the development and operation of the provider incentive program 25.

Within these programs, incentives to the physician must not induce the physician to reduce or limit services that are medically necessary to the patient. Additionally, incentives cannot be based on the volume or value of referrals to the hospital participant. Incentives must be based on net savings and must be linked to actions that improve overall quality and efficiency and result in cost savings for the episode of care. Payments to physicians may not exceed 25% of the amount that is normally paid to physicians for the cases. To qualify for financial incentive payments physicians must adequately meet quality performance targets set by the hospital committee 26.

Also, CMS is designating the providers in the demonstration project as Medicare value-based care centers, which enhances the centers’ community reputation 27. Medicare beneficiaries are provided financial incentives to receive care at the value-based care centers. CMS is also marketing the demonstration, which may lead to increased patient volume for participating facilities 27.

In response to the proposed Medicare bundling, if the hospitals and orthopaedic surgeons work together to align incentives and develop “best practices” that focus on patient care quality and cost-effectiveness, the bundling should represent minimal risk to the orthopaedic surgeon and may actually present an opportunity for the surgeon to participate in economic efficiencies or “gain share” and realize financial savings.

Participating surgeons would be instrumental in analyzing the effectiveness of the bundled procedures by utilizing recent patient cases as a baseline. The surgeons would conduct an internal peer review of all relevant cases. This analysis would focus on the quality outcomes to be achieved, the surgical procedures that will be the focus of the program, the types and best suppliers of implants, and other items used in developing the guidelines for best-practice cases.

In the bundling demonstration project, Medicare expects discounted base DRG payment amounts for the treatment of the patient, in addition to improved patient quality of care. The discounted payment, with the hospital and the physician components, is applicable to all of the bundled DRGs and is subject to the annual updates each October. In light of the bundling, the hospitals and physicians will be working to reduce costs while maintaining or increasing the quality of patient care. With diligence, the cost savings will be close to or exceed the reduction in payment associated with bundling (Table 1). The advantage therefore is that the cost savings in excess of the baseline may be shared with the physicians. Per Medicare, incentives must be based on quality measurements. If the measurements are met or surpassed, the physician may receive incentives up to 25% of what the physician would have been paid for treating the patient before bundling 28.

This program, if successful, has the potential to create powerful, unprecedented incentive compensation opportunities to physicians premised on two of the chief industry concerns in healthcare—cost reduction and improved quality.

**DISCUSSION**

The past two decades have witnessed dramatic changes in health-care regulatory law and decreased reimbursement as seen in this report. In order for our academic orthopaedic practice to continue to attract and retain the best and brightest surgeons, we created a new, nonprofit, tax-exempt entity wholly owned by the parent corporation at the Hospital. This new faculty practice plan was adopted to be a RVU-based compensation model with components that created incentives for both clinical practice and for academic and administrative service contributions.

Our practice plan model is one of several that have been described 28, 29, 30 to meet the challenges of supporting an academic department in an era of increased health-care regulatory legislation and decreased remuneration for service. However,
the basic tenet of these plans, in order to achieve success, is the ability to align incentives between the physicians and the hospital.

If incentives are properly aligned between medical centers and physicians, benefits will accrue to the hospitals, physicians and most importantly to the patients. Moving forward in our current healthcare environment, the success of the orthopaedic surgery profession will depend on the ability of hospitals and physicians to align incentives and provide opportunities for the physicians to actively participate in defining quality measurements, providing appropriate oversight, and reporting and communicating data and other information transparently. Only when the physicians are working together in close collaboration with their respective hospitals can they ensure that objective criteria to ensure cost and quality benefit goals are achieved.

ACKNOWLEDGMENT
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MINI-REVIEW: SCREENING FOR ACTIVATED RECEPTOR TYROSINE KINASES THAT ARE IMPORTANT TO THE TUMORIGENESIS AND METASTASIS OF OSTEOSARCOMA

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Osteosarcoma is the most common primary bone malignancy. It predominantly affects adolescents in areas of rapid bone growth, such as the distal femur or proximal tibia. Prior to the introduction of chemotherapy protocols, the treatment was limited to surgical resection of the tumor, usually by amputation. Only 10-20% of all patients experienced long-term survival, with 75% of patients succumbing within the first 2 years after diagnosis. In order to increase survival, chemotherapy was introduced in the 1970’s. Typical treatment includes neoadjuvant chemotherapy followed by surgical resection of the tumor and postoperative chemotherapy. With the current treatment protocol, the long-term survival rate increased from 10-20% to 60-70% for patients with localized disease.

There are several factors that indicate an extremely poor prognosis for osteosarcoma patients. The most common factor is the presence of metastases either at the time of diagnosis or as a result of recurrent disease. Approximately 20% of patients are diagnosed with detectable metastases and 30-40% of patients diagnosed with localized disease will relapse, often within the first 3 years following diagnosis. Metastases are primarily found in the lung, however other secondary sites include soft tissues and other bones. Most patients are believed to have subclinical micrometastases since prior to neoadjuvant chemotherapy, 80-90% of patients developed metastases despite surgical resection of the primary tumor.

Unfortunately, for patients with detectable metastases, the prognosis remains poor. Even with current chemotherapy protocols, only 10-20% of patients presenting with metastases survive to 10 years. These patients do not receive consistent treatment and the optimal disease management is not properly established. Importantly, survival rates have not improved over the last 30 years. Therefore, novel therapeutic and prognostic tools are needed.

The process of metastasis is composed of sequential, correlated steps, any of which can be rate limiting. Invasion is the initiating step and first involves invading the local surrounding tissue followed by invasion of the vascular or lymphatic walls in order to gain access to the circulation. This is achieved by changing cell adhesion to other cells and the extracellular matrix (ECM) as well as proteolytic degradation of ECM components. During invasion the tumor cells ultimately migrate to the bloodstream. This involves changes in the cell’s cytoskeletal structure and focal interactions with the ECM.

Once tumor cells have migrated to the bloodstream, arrested and extravasated into a secondary location, they proliferate and recruit blood vessels in order to form metastatic lesions. The microenvironment of the secondary site is thought to be an important factor in successful colonization.

Generally, sarcomas can be subdivided into two classes based on the genetic alterations associated with disease development. The first class is characterized by reciprocal chromosome translocations with balanced karyotypes, such as the EWS/FLI-1 chromosomal translocation characteristic of 85% of Ewing’s Sarcoma cases. The second class, which includes osteosarcoma, is defined by complex karyotypes with nonspecific alterations of many genes. Moreover in osteosarcoma, there is a large variation of mutations between patients. Therefore, osteosarcoma patients may benefit from individualized, targeted therapy. Additionally, targeted therapy would be less toxic to patients compared to chemotherapy, which can cause numerous debilitating side effects.

Transformation of a cancer cell is a multistep process that involves detrimental alterations of the genome. Cancer cells acquire characteristics that include uncontrolled, limitless cell proliferation due to self-reliance on...
growth signals, evasion of apoptosis, insensitivity to antigrowth signals, induction of angiogenesis, and the ability for tissue invasion and metastasis. Many of these processes involve tyrosine kinase signaling.

Protein tyrosine kinases are important signaling molecules with highly regulated activity. Their main function is to catalyze the transfer of the γ-phosphate of ATP to tyrosine residues on protein substrates. This creates binding sites for adaptor proteins and downstream signaling molecules leading to changes in cell proliferation, differentiation, migration, survival or other metabolic changes. In most situations, phosphorylation leads to increased enzymatic activity.

There are 90 protein tyrosine kinases encoded in the human genome. 58 are transmembrane glycoproteins known as receptor tyrosine kinases (RTKs), most of which undergo dimerization and autophosphorylation upon ligand binding. Many receptors for growth factors are included in the RTK family.

Since tyrosine kinases are involved in many signaling networks and dysregulation or mutation can lead to malignant transformation, inhibitors of these kinases have emerged in the development of targeted anticancer therapy. Unlike other cancers, the role of tyrosine kinases in osteosarcoma has not been as well characterized and therefore, the inhibitors have not yet been approved for treatment. Overexpression of specific RTKs and their ligands has been previously reported in osteosarcoma. These include EGFR, ErbB2, IGF-1R, met, NGFR, PDGFR, VEGFR and their cognate ligands. Specifically, overexpression of ErbB2, PDGFR, PDGFR, VEGF, and VEGFR has been correlated with metastasis and an overall poor prognosis. However, overexpression of an RTK does not always correlate with activation. For example, a small initial stimulus may elicit a large, amplified response. The RTK does not need to be overexpressed to have an effect on cell signaling. Therefore, we hypothesized that activation of receptor tyrosine kinases can contribute to the tumorigenesis and metastasis even in the absence of overexpression.

In order to study this hypothesis, the strategy depicted in figure 1 was employed. We used two established families of genetically related human osteosarcoma cell lines. Each family consists of a parental cell line (TE85 or Saos-2) created from tumor cells isolated from 2 different adolescent patients. When injected into immunodeficient mice, these cells are non-tumorigenic/non-metastatic. The tumorigenic/non-metastatic MNNG cell line and the highly tumorigenic/metastatic 143B and LM7 cell lines were directly derived from the parental cells.

Since phosphorylation events occur during the activation of most receptor tyrosine kinases, phosphoproteomic screening, as illustrated in figure 2, was performed to identify activated RTKs. This was done using antibody arrays that allow for the simultaneous detection of 42 individual phosphorylated receptor tyrosine kinases. This method is more time-efficient compared to others that allow for the analysis of only one tyrosine kinase at a time. Each array membrane is spotted in duplicate with capture antibodies specific for the extracellular portion of the individual receptor tyrosine kinases. Receptor tyrosine kinases that are present in the cell lysate, phosphorylated or not, bind to the specific capture antibodies while unbound material was washed away. However, only those receptor tyrosine kinases that are phosphorylated bind the secondary anti-phosphotyrosine antibody. Results from the screening identified 12 RTKs that are phosphorylated in one or both of the metastatic cell lines.

Functional genomic screening using
siRNA was performed in order to determine which of the activated RTKs are required for motility, invasion, colony formation and/or proliferation in vitro. siRNA is a powerful technique for reducing gene expression. This type of experiment is commonly referred to as gene knockdown to distinguish from gene knockout, where the gene is completely removed. Previous studies from our lab utilized small molecule inhibitors (SMIs) to target specific tyrosine kinases. However, siRNAs provide higher specificity than SMIs and are available against any identified tyrosine kinase in the human genome.

Preliminary results reveal that IGF-1R knockdown had the greatest effect of the 12 siRNAs tested in the metastatic LM7 cells, reducing motility and colony formation by over 50% compared to cells treated with control siRNA targeting luciferase. These results are consistent with recently published results from our lab demonstrating that a SMI targeting IGF-1R also inhibited motility. These results are further supported by previous reports showing that IGF-1R is required for the proliferation of osteosarcoma cells and is overexpressed in osteosarcoma tissue samples. Metastasis was inhibited in hypophysectomized mice, which are unable to produce IGF-1 and treatment with a neutralizing monoclonal antibody targeting IGF-1R increased the event-free survival in a murine xenograft model of osteosarcoma. Currently, there are phase I and II clinical trials studying the effects of IGF-1R monoclonal antibodies in multiple solid tumors, including osteosarcoma.

Eph receptors are the largest subgroup of RTKs, with 14 structurally related receptor family members. Their ligands, known as ephrins, are membrane bound, thus requiring cell-to-cell contact to induce signaling. Eph/ephrin interaction facilitates bidirectional signaling in which tyrosine phosphorylation may occur on the cytoplasmic domain of both the Eph receptor and the ephrin, initiating signaling cascades in both cells. Eph signaling is known to be important to cell segregation, cell attachment, shape and motility and has been implicated in the tumorigenesis and metastasis of many different cancers such as:

| Table 1. Two families of genetically-related human osteosarcoma cell lines. Each family consists of non-tumorigenic/non-metastatic parental cell lines derived from two separate osteosarcoma patients. The MNNG and 143B were directly derived from TE85 cell and the LM7 cells from the Saos-2 cell line. |
|--------------------------------------|----------|---------|
| Non – tumorigenic Non – metastatic   | TE85     | Saos-2  |
| Tumorigenic Non- metastatic          | MNNG     |         |
| Tumorigenic Metastatic               | 143B     | LM7     |
melanoma, lung and breast cancers. Interestingly, some Eph receptors have been reported to be both pro- and anti-oncogenic. This could be due to ligand-dependent and independent activities of the receptor.

In our siRNA screen, knockdown of EphB2 inhibited colony formation by 70% in LM7 cells. Interestingly, it was previously reported that ephrin-A5 and ephrin-B1, ligands for EphB2, are expressed in osteosarcoma specimens but not in normal bone. The same study indicated that expression of ephrin-B1 is an indicator of tumor progression.

In conclusion, these results suggest that IGF-1R and EphB2 may be valuable targets for the development of new treatments. Validation experiments are underway to identify additional target RTKs. Furthermore, a murine model of osteosarcoma will be established to determine which RTKs are important for tumor growth and/or metastasis in vivo. In order to identify potential prognostic factors, immunostaining for specific RTKs in patient tumor samples will be done to correlate expression/activation with patient outcome. Additionally, phosphoproteomic screening of frozen patient tumor samples will be performed to detect those receptors that are activated in human tumors. Ultimately, identification of activated RTKs by array-based technology could lead to similar approaches being used clinically to identify targets for individualized therapy.

REFERENCES
CHONDROGENIC DIFFERENTIATION OF HUMAN MESENCHYMAL STEM CELL AGGREGATES VIA CONTROLLED RELEASE OF TGF-β1 FROM INCORPORATED POLYMER MICROSPHERES

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ABSTRACT

Aggregate culture is a useful method for inducing chondrogenic differentiation of human Mesenchymal Stem Cells (hMSC) in a three-dimensional (3D) in vitro culture environment. Conventional aggregate culture, however, typically requires repeated growth factor supplementation during media changes, which is both expensive and time-intensive. In addition, homogenous cell differentiation is limited by the diffusion of chondrogenic growth factor from the culture medium into the aggregate and peripheral cell consumption of the growth factor. We have engineered a technology to incorporate growth factor-loaded polymer microspheres within hMSC aggregates themselves. Here we report on the system’s capacity to induce chondrogenesis via sustained delivery of Transforming Growth Factor-β1 (TGF-β1). Cartilage formation after 3 weeks in the absence of externally supplied growth factor approached that of aggregates cultured by conventional methods. Importantly, the inclusion of growth factor-releasing polymer microspheres in hMSC aggregates could enable in vitro chondrogenesis for cartilage tissue engineering applications without extensive in vitro culture.

INTRODUCTION

The tissue engineering approach to repair or regenerate damaged cartilage is often based on providing transplanted or host cells with signals to guide their behavior and induce the creation of new replacement tissue through manipulation of the cellular milieu. Human Mesenchymal Stem Cells (hMSCs) are a promising cell source for use in cartilage tissue engineering applications.1 These cells are pluripotent mesenchymal progenitor cells that can be readily obtained from donor bone marrow.2,3 They can be expanded in culture for several passages without losing their pluripotent capabilities, which permits the generation of clinically relevant numbers of undifferentiated cells. In addition, hMSCs show great utility for cartilage tissue engineering because they have the capacity to undergo chondrogenic differentiation under specific culture conditions.2,3

Aggregate culture is a commonly-used method of inducing in vitro chondrogenesis of hMSCs in a three-dimensional culture environment. This method has been used to study the in vitro chondrogenesis of rabbit4 and human5 bone marrow MSCs, and was recently modified for high-throughput aggregate formation6 in a 96-well format. In this modified format, aliquots of an MSC suspension are centrifuged in polypropylene wells, allowing the cells to form free-floating condensations through cell-cell mediated interactions. In the presence of soluble signaling factors including dexamethasone and Transforming Growth Factor-β1 (TGF-β1), the cells in these aggregates are capable of undergoing chondrogenic differentiation and produce cartilage-specific extracellular molecules such as glycosaminoglycans (GAG) and Type II collagen.4,5,6
Using these culture methodologies, however, the MSC aggregate is subject to limitations of the diffusion of chondrogenic factors from the surrounding medium. The outer layer of cells in an aggregate is exposed to the highest concentrations of soluble chondrogenic factors, while cells closer to the center of the aggregate are exposed to lower concentrations. The factors must penetrate the cell layers according to Fick’s Law of Diffusion, and the inner regions are exposed to lower levels of growth factors due to the increased time required for the factors to reach the central regions of the aggregate as well as consumption by the peripheral cells as the factor diffuses through the cell layers. This could result in a nonuniform spatial pattern of aggregate chondrogenesis. Due to these limitations, a higher concentration of TGF-β1 in the culture medium is required to achieve chondrogenic differentiation in the central region of the aggregate.4 Traditional chondrogenic aggregate culture requires repeated dosing of TGF-β1 during media changes, and aggregates are typically supplemented with 10 ng/ml of TGF-β1 every other day for a period of up to three weeks.4,6 This repeated growth factor supplementation during extended in vitro culture is both costly, due to the high concentrations of TGF-β1 required, and time-intensive.

To address issues of sustained local protein delivery, growth factor-encapsulating polymer microspheres are widely used as delivery vehicles in tissue engineering technologies. One polymer commonly utilized for microsphere-mediated protein delivery is poly(lactic-co-glycolide) (PLGA), a biocompatible, biodegradable polymer that breaks down hydrolytically into lactic acid and glycolic acid, which are natural metabolic by-products.7 Growth factor release from PLGA microspheres occurs via diffusion out of the microspheres and polymer degradation, and it can be controlled by varying polymer properties such as molecular weight, copolymer ratio, and degree of crystallinity.7,8 Through adjusting polymer formulation parameters, the protein release kinetics can be tailored to fit the requirements of a specific system.

To overcome the TGF-β1 diffusion limitations associated with conventional aggregate culture and potentially enable in vivo aggregate chondrogenesis without extensive in vitro culture, we report here on a new engineered technology in which growth factor-encapsulating polymer microspheres are incorporated within hMSC aggregates.9 In this system, chondrogenic growth factor is released from PLGA microspheres uniformly dispersed within the cell aggregates, which bypasses the problems of TGF-β1 diffusion from the culture medium. This allows for a more homogenous spatial pattern of chondrogenic differentiation within the aggregates, and permits a much lower amount of growth factor to be delivered to achieve a similar chondrogenic response compared to exogenous TGF-β1 supplementation. As the polymer microspheres degrade and the growth factor diffuses out, TGF-β1 is released throughout the interior of the aggregates in a controlled manner, eliminating the need for repeated TGF-β1 supplementation in vitro during media changes.

**MATERIALS AND METHODS:**

**Materials**

Poly(lactic-co-glycolic acid) (PLGA) 5050 2A (50:50, 0.18 dl/g inherent viscosity) was purchased from Lakeshore Biomaterials. Poly(vinyl alcohol) (PVA), papain, and chondroitin 6-sulfate were purchased from Sigma-Aldrich. Cali’Thymus DNA standard was obtained from Rockland Immunochemicals. TGF-β1 was from PeproTech, and Safranin O, Hoechst 33258, and Tween-20 were from Acros Organics. ITS’ Premix was from BD Biosciences, and dexamethasone was obtained from MP Biomedicals. Ascorbate 2-phosphate was purchased from Wako Chemicals USA. Sodium pyruvate and nonessential amino acids were obtained from Lonza. Ethyl acetate and other standard chemicals were obtained from Fisher Chemical. Polypropylene 96-well plates were purchased from Phenix Research Products. Statistical analysis software used was InStat V. 3.06 software for Windows from GraphPad.

**Polymer Microsphere Synthesis**

Polymer microspheres were synthesized by a double emulsion technique as previously described.10 Briefly, 250 mg of PLGA dissolved in ethyl acetate was combined with 100 µl of diH2O containing 5 µg TGF-β1 (or diH2O only for empty control microspheres) and sonicated to form the primary emulsion. The primary emulsion was combined with 1 ml of 5% (w/v) poly(vinyl) alcohol (PVA), and then vortexed to form the secondary emulsion. The mixture was immediately poured into a stirring extraction solution containing 0.3% PVA (w/v) in diH2O. The microspheres were stirred for 3 hours at room temperature to allow for solvent evaporation, collected by filtration, flash-frozen in liquid nitrogen, and lyophilized until dry. Microspheres were sterilized via a 10 minute exposure to ultraviolet (UV) light (TUV 30W/ G30T8; Philips) at a distance of 30 cm.
to examine the surface morphology before and after UV sterilization. In addition, the mean microsphere diameter was determined using ImageJ (NIH) image analysis software (N=1296 microspheres). Protein release profiles from the growth factor loaded microspheres were determined by placing 5 mg of microspheres into 1 ml of 0.02% Tween-20 in PBS at 37°C (N=4). Every 1 or 2 days, samples were centrifuged, the supernatant was removed, and 1 ml of fresh release medium was added to the samples. Supernatants from the release samples were stored at -80°C until analyzed. TGF-β1 release was quantified using an ELISA kit (R&D Systems).

**Microsphere-incorporated hMSC Aggregate Formation**

Sterilized microspheres were added at a concentration of 3.5 mg or 7.0 mg microspheres/ml to chemically defined medium containing DMEM-HG with 1% ITS+ Premix, 37.5 µg/ml ascorbate-2-phosphate, 10-7 M dexamethasone, 1% nonessential amino acids, and 1% sodium pyruvate. First passage hMSCs were trypsinized, suspended in this solution at a concentration of 1.25x10^6 cells/ml, and then dispensed into sterile V-bottom polypropylene microplates in aliquots of 200 µl per well. The plates were centrifuged at 500x g to form free-floating cell aggregates. Cell aggregates were transferred to fresh polypropylene microplates after 48 hours, and the aggregate medium was replaced every other day. The mass of unincorporated microspheres was measured by collecting the remaining medium after 48 hours to determine the microsphere loading efficiency into the aggregates. Media from 4 wells containing the unincorporated microspheres was combined into a single tube, and the microspheres were collected via centrifugation (N=3). The microspheres were rinsed three times with diH2O, flash-frozen in liquid nitrogen, lyophilized, and weighed.

**Aggregate Analysis**

After 3 weeks of culture, a minimum of 3 aggregates from each group were harvested for DNA and glycosaminoglycan (GAG) quantification, and 2 from each group were processed for histologic examination. For biochemical analyses, aggregates were digested in 200 µl of a papain solution for 2 hours at 65°C. 400 µl of 0.1 N NaOH was added to...
the digests, which were incubated for 30 min at room temperature and then neutralized with 400 µl of a solution containing 0.1 N HCl, 5M NaCl, and 100 mM NaH2PO4. A standard Hoechst 33258 dye assay was used to quantify total DNA in the aggregates.13 Fluorescence was measured on a Safire microplate reader (λex/λem=358 nm/452 nm; Tecan, Durham, NC, USA) and compared to calf thymus DNA standards. GAG content was quantified using a previously described dot-blot assay.14 Aggregates for histologic evaluation were fixed for 20 minutes in 10% neutral buffered formalin, paraffin embedded, and sectioned. Adjacent sections were stained for glycosaminoglycan content with Safranin O and counterstained with Fast Green. For statistical analysis, two-tailed unpaired t-tests were performed using GraphPad InStat software, with P < 0.05 considered significant.

RESULTS AND DISCUSSION
Prior to their inclusion into cell aggregates, the polymer microspheres were characterized to determine size, surface morphology, and growth factor release kinetics. Before in vitro cell culture use, the polymer microspheres were sterilized via exposure to UV light. We examined the microspheres using SEM before and after 10 minute UV sterilization to monitor any changes in surface morphology, as changes to the surface would be one indicator of UV damage. No change was observed, and the microspheres appeared smooth both before (Figure 1A) and after (Figure 1B) UV exposure. As determined from the SEM images, the average diameter of the microspheres was 6.8 ± 4.1 µm, which is similar to the size of the cells.15 This aids in creating a well-mixed suspension of cells and polymer microspheres, and results in cell aggregates with microspheres dispersed throughout. Protein release kinetics from growth factor-loaded microspheres show sustained release of TGF-β1 over the first 21 days, with a total cumulative release of 608 ± 24 pg per mg of microspheres after 3 weeks (Figure 1C).

After forming and culturing microsphere-incorporated hMSC aggregates for 3 weeks, their chondrogenic differentiation was assessed through biochemical assays for DNA and GAG content as well as histological examination. Aggregate histology indicated greater glycosaminoglycan content in aggregates formed with the 1.4 mg/well initial microsphere loading (Figure 2B and 2D) compared to those formed with the 0.7 mg/well initial microsphere loading (Figure 2A and 2C), as is qualitatively apparent from the area and intensity of Safranin O stain. Biochemical assay results confirmed that aggregates formed with the 1.4 mg/well initial microsphere loading produced larger quantities of GAG (Figure 3A) and GAG normalized to DNA (Figure 3B) per aggregate after 3 weeks by comparison to those formed with the 0.7 mg/well initial microsphere loading, but the difference between these two groups was not significant (P = 0.1953). The average GAG content of aggregates formed with 0.7 and 1.4 mg/well initial microsphere loading corresponds to 27% and 55%, respectively, of the values obtained by Penick et al. for aggregates cultured for 3 weeks in medium supplemented with 10 ng/ml TGF-β1. Additionally, DNA assay results showed no significant difference in DNA content between aggregates formed with the 0.7 or 1.4 mg/well initial microsphere loadings (Figure 3C), confirming that the polymer microsphere loadings had no differential effects on cell viability within the aggregates.
Most negative-control aggregates cultured with empty microspheres in the absence of TGF-β1 disintegrated over the 3-week culture period, and those that could be harvested produced no glycosaminoglycans as evidenced by histology (data not shown). Lack of GAG production in the negative controls indicated that the PLGA microspheres themselves were not promoting aggregate chondrogenesis. Positive-control aggregates formed with empty microspheres and cultured in medium containing TGF-β1 exhibited no significant change in DNA or GAG content by comparison to normal chondrogenic control aggregates containing no microspheres as was apparent from biochemical analysis and histologic examination (data not shown). This demonstrates that the empty microspheres did not adversely affect cell viability or chondrogenic differentiation in the presence of exogenously supplemented TGF-β1.

To determine the absolute quantity of growth factor incorporated into each hMSC aggregate, microsphere incorporation efficiencies were determined. The incorporation efficiencies for aggregates formed with initial loadings of 0.7 and 1.4 mg of polymer per well were 70.5 ± 9.2% and 59.8 ± 1.9%, respectively, resulting in actual microsphere incorporations of 0.49 ± 0.06 mg and 0.84 ± 0.03 mg of microspheres per aggregate for the two groups. Assuming that release profiles from the microspheres within the aggregates are similar to those obtained in the release study (Figure 1C), these microsphere masses correspond to 298 and 511 pg of total TGF-β1 released per aggregate over the 3 week culture period, or an average release of 65 pg/ml per day and 115 pg/ml per day for aggregates cultured in 200 μl of medium. Interestingly, chondrogenesis is limited to only the very outer regions of aggregates cultured by conventional methods with comparable levels of exogenous TGF-β1 supplementation. Johnstone et al. observed that culturing MSC aggregates in media containing TGF-β1 in concentrations below 10 ng/ml resulted in decreased aggregate chondrogenesis. When cultured in medium containing 1 ng/ml TGF-β1, chondrogenesis was limited to the outer third of the aggregates after 21 days of culture. When cultured in medium containing 500 pg/ml TGF-β1, chondrogenesis was even more limited, suggesting that transport problems of growth factor from the culture medium are restricting chondrogenesis to only the outermost regions of the aggregates.

We have shown that TGF-β1 loaded polymer microspheres incorporated within hMSC aggregates can induce chondrogenesis and cartilage formation after 21 days in the absence of externally supplied growth factor. Through the use of this system, it is possible to induce aggregate chondrogenesis with amounts of TGF-β1 much lower than those required by conventional aggregate culture methods, likely due to the ability of the growth factor released from the incorporated microspheres to overcome the transport problems associated with delivering growth factor from the culture medium into the central regions of the aggregates. The use of growth factor-loaded polymer microsphere incorporated hMSC aggregates eliminates the need for repeated TGF-β1 dosing over a multi-week in vitro culture period, and in vivo chondrogenesis could be enabled without extended prior in vitro culture. Studies are currently underway to systematically investigate the effects of varying growth factor release profiles and microsphere amount on aggregate chondrogenesis.

Figure 3. DNA and glycosaminoglycan (GAG) content for hMSC aggregates made with 0.7 mg/well (gray) or 1.4 mg/well (black) of growth factor-containing microspheres (avg ± SD). (A) GAG content. (B) GAG content normalized to DNA. (C) DNA content.
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CALCIUM PHOSPHATE - DNA NANOPARTICLE GENE DELIVERY FROM ALGINATE HYDROGELS INDUCES IN VIVO OSTEOGENESIS

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ABSTRACT

There is a significant need for improved therapy for bone regeneration. The delivery of recombinant bone morphogenetic proteins has been approved for clinical use to promote osteogenesis, but still has limitations such as expense, degradation of the proteins in vivo and difficulties retaining protein at the site of injury. Localized gene delivery is a promising alternative therapy, as it would allow sustained expression of specific osteoinductive growth factors by cells near the damaged site. We have engineered an injectable system for localized, sustained non-viral gene delivery from alginate hydrogels containing preosteoblastic cells and calcium phosphate – DNA nanoparticles. The nanoparticles utilized in this report are stable, on the order of 100 nm, and have a high DNA incorporation efficiency (>66%). When the nanoparticles were incorporated in alginate hydrogels, sustained release of DNA was observed. Furthermore, MC3T3-E1 preosteoblast cells exhibited the capacity to form bony tissue in as little as two and half weeks when mixed with DNA nanoparticles encoding for BMP-2 into the alginate hydrogels and injected subcutaneously in the backs of mice. This injectable, minimally invasive gene delivery system may be efficacious in bone regeneration applications.

INTRODUCTION

Treatment of bone defects caused by trauma or surgical resection remains a significant challenge in orthopedics. Currently, defects are typically treated by autologous or allogenic grafts. However, there is limited tissue availability for autologous grafts and they carry risks of donor site morbidity, while allogenic grafts carry the risk of disease transmission or host rejection. Another more recent therapy is the delivery of bone morphogenetic proteins (BMPs) to help promote bone regeneration. One of the BMPs shown to have a strong osteoinductive effect is BMP-2. This molecule is involved in osteoinductive signaling pathways and promotes the differentiation of mesenchymal stem cells into osteoblasts. The delivery of BMP-2 for bone regeneration holds much promise, but still suffers from the need to deliver large amounts of protein to elicit an effect, due to protein degradation in vivo and poor retention locally at the site of injury.

An attractive alternative is localized gene delivery to allow sustained expression of specific osteoinductive growth factors by host and/or transplanted cells near the defect site. Gene delivery can be achieved by several methods, both viral and non-viral. Although viral vectors are highly efficient delivery vehicles, there are major concerns with immunogenicity, toxicity, the risk of recombination, and possible extended expression of the protein beyond the timeframe required for the healing of the defect. As a result, much effort has been invested into the development of efficient non-viral methods, which include the creation of nanoparticles with encapsulated DNA using liposomes, polycations, or inorganic compounds. Of these, inorganic compounds, especially calcium phosphate (CaP), are attractive as they are widely available, inexpensive, and relatively easy to prepare.

An important consideration in using gene delivery in vivo is the retention of the DNA at the site of the injury to promote a strong therapeutic effect. Although many groups have performed direct injections of DNA for increased gene expression to promote osteogenesis, the duration of expression is difficult to control and often limited, and the timing of the
Injection is extremely important for therapeutic benefit. Biodegradable materials can be of great use in allowing a sustained delivery of DNA at the desired location. The choice of the material is a major consideration. It must be biocompatible, and ideally should degrade over time as new tissue is formed. Many scaffolds are formed ex vivo and then must be implanted. In contrast, hydrogels offer a minimally invasive alternative, as they can typically be injected directly into the site of injury. Another advantage is that it is easy to incorporate osteogenic cells throughout a hydrogel for subsequent transplantation.

Rather than delivering DNA at the site of interest and relying on uptake by surrounding cells, some groups have reported on ex vivo gene therapy of cells with subsequent transplantation of the engineered cells into the injured area within a scaffold. A major difficulty with this technique is that the amount of BMP produced by these pre-transfected cells is dependent on their survival during implantation and within the defect. In contrast, DNA delivery from a scaffold can provide a sustained supply of DNA for surrounding host cells or transplanted cells to take up and express. Enhancement of bone regeneration resulting from the release of DNA encoding for osteogenic factors from scaffolds with and without cell transplantation has been demonstrated. However, many of these studies utilized viral vectors, which have disadvantages previously discussed. In addition, many involved implantation of scaffolds, which requires invasive surgical procedures.

When considering delivery from a scaffold, it is important to ensure that the DNA maintains its ability to transfect cells over time, and that it can withstand any processing techniques used to incorporate it into the scaffold. DNA complexed with liposomes can be unstable with agitation or during freezing, and the electrostatic interaction between DNA and polycations such as chitosan, polyethylenimine (PEI), or poly-L-lysine can be disrupted in the presence of other charged compounds (for example, negatively charged polysaccharides, such as alginate). Calcium phosphate crystals complexed with DNA do not have problems with cytotoxicity at the concentrations used for transfection, but they can aggregate or grow over time, rendering the particles ineffective for transfection, as the optimal size for cellular uptake by endocytosis has been shown to be around 100 nm or less. Therefore, it is important to make DNA nanoparticles that remain stable over time when incorporated into a scaffold for transplanted or host cell uptake.

In this work, we present the ability to deliver osteogenic cells and DNA complexed with CaP from an injectable hydrogel to allow for localized delivery of the application of bone regeneration. We examine plasmid DNA (pDNA) encoding for lacZ as a measure of transfection efficiency, and we also use pDNA encoding for BMP-2 to enhance bone regeneration in vivo. As mentioned previously, the transfection efficiency of standard preparations of CaP-DNA can decrease over time as the inorganic crystals continue to grow and aggregate, becoming too large for endocytosis by the cells. Therefore, we have optimized CaP-DNA nanoparticles (NPs) to remain stable over time. These NPs are incorporated into an alginate hydrogel to allow for localized delivery of the complexes to both transplanted cells and host cells at a bone defect site. One recent study demonstrates that alginate can serve as an effective matrix for localized gene delivery. In our study, alginate modified with adhesion ligands containing the RGD peptide sequence was used as it has been shown to act as a synthetic ECM material which can promote cell attachment, proliferation, osteoblastic differentiation, and bone formation. It is possible to incorporate osteoblasts or osteoprogenitor cells within this hydrogel to provide a cell population capable of taking up the genetic material being delivered, and therefore to further promote osteogenesis. This nanoparticle-cell-alginate mixture can be injected, and as such it is a minimally invasive therapeutic option. We show sustained release of CaP-DNA from the alginate over time, the ability of the CaP-DNA NPs to transfect preosteoblast cells in vitro, and the combination of CaP-DNA NPs with cells in alginate to promote bone formation in vivo.

**MATERIALS AND METHODS**

**Materials**

Sodium alginate powders were a generous gift from FMC Biopolymers (Princeton, NJ). The plasmid pcDNA3.1/Hygro/lacZ and Quant-It PicoGreen dsDNA quantitation reagent were obtained from Invitrogen (Carlsbad, CA). The plasmid encoding for BMP-2 was a generous gift from Chris Evans, Harvard Medical School. MC3T3-E1 Subclone 4 (ATCC #CRL-2593) cells were obtained from American Type Culture Collection (Manassas, VA). Phosphate buffered saline (PBS) and α-Methyl Eagle’s Medium were obtained from Hyclone (Logan, UT). All other chemicals were obtained from Fisher Scientific (Fairlawn, NJ).

**Alginate Preparation**

Two different sodium alginate powders, with product names of 10/60 and
20/40, were lyophilized until dry, and the 20/40 alginate was subjected to gamma irradiation at 5 Mrad (Phoenix Lab, University of Michigan, Ann Arbor). The molecular weight was found to be 37,000 g/mol for irradiated 20/40 alginate, and 121,000 g/mol for non-irradiated 10/60 alginate as determined by SEC-MALS (FMC Biopolymers). The percentage of guluronic (G) and mannnuronic (M) acid for each alginate was determined by NMR (FMC Biopolymers); 10/60 was found to have 68% G-content and 32% M-content, and 20/40 5 Mrad was found to have 66% G-content and 34% M-content. The peptide glycine – arginine – glycine – aspartic acid – serine – proline (GRGDSP; Commonwealth Biotechnologies, Richmond, VA), which contains the RGD cell-binding domain, was covalently coupled to the irradiated alginate as previously described. Both types of alginate were then purified by dialysis for 4 days, subjected to activated charcoal treatment, and sterilized through a 0.22 µm filter.

**Preparation and Characterization of Calcium Phosphate DNA Nanoparticles**

Two types of calcium phosphate DNA nanoparticles were fabricated: calcium phosphate core with pDNA coating, and calcium phosphate – DNA core with BSA coating. The fabrication of the CaP core – DNA coated NPs was based on a method described by Sokolova, et al. Briefly, equal volumes of 18.7 mM CaCl₂ (pH 9) and 11.23 mM Na₂HPO₄ (pH 9) were added simultaneously to a tube with a magnetic stir bar. The solution was mixed for 30 seconds and 200 µg of pDNA was added to quench the crystallization by coating the crystals. CaP-DNA core – BSA coated NPs were created by first preparing calcium phosphate – DNA particles and subsequently adding a solution of bovine serum albumin (BSA) to coat the particles and prevent further crystal growth. Specifically, 120 µg pDNA was mixed with 100 µl 2 M CaCl₂ and the solution added dropwise to 1 ml of 2X HBS (pH 7) while stirring. Then, 780 µl of distilled water was immediately added, the mixture stirred at room temperature for 30 minutes, and 200 µg of BSA was added.

The size of the NPs was determined by transmission electron microscopy (TEM). NPs were freshly prepared (N=3 preparations for each type), and a sample of each was diluted 1:50 with distilled water, spotted onto a nickel formvar grid, and allowed to dry at 37°C. The remaining NPs were kept for two weeks at 4°C, and samples were taken at one and two weeks for imaging. Images were scanned into digital format, and from these images, particle diameters (N>200 NPs) were calculated using ImageJ software (NIH, Bethesda, MD).

The DNA incorporation efficiency of each particle type (N=3) was determined by centrifugation of the NPs at 17,000 rpm for 30 minutes, followed by spectrophotometric measurement of the pDNA in the supernatant using the PicoGreen dsDNA assay kit. Plasmid DNA at known concentrations was used to construct the standard curve.

**In vitro DNA Release from Alginate Hydrogels**

CaP NPs were freshly prepared, and mixed at a concentration of 10% v/v with a 2% 10/60 alginate solution reconstituted in PBS. The alginate was then crosslinked with a slurry of calcium sulfate (210 mg/ml in distilled water) at a ratio of 25:1 and cast between two glass plates spaced 0.75 mm apart. After 20 minutes, 10 mm diameter disks (N=8) were cut out and transferred to PBS containing calcium and magnesium. In the same manner, alginate disks containing naked pDNA and alginate disks without pDNA or NPs were created. The disks were incubated at 37°C under gentle agitation. Release samples were taken periodically by removing the PBS and replacing with fresh PBS. The released DNA was measured using the PicoGreen dsDNA assay kit.

**In vitro Bone Formation**

CaP NPs were freshly prepared and mixed into a 2% GRGDSP-alginate solution, followed by the addition of MC3T3 cells at a final concentration of 24x10⁶ cells/ml. Additionally, as a control, cells in the modified alginate without NPs or pDNA were examined to obtain a background level of bone formation due to the cells alone, if any. The alginate was crosslinked as described above with calcium sulfate, and kept in a syringe on ice until...
injection. 200 µl of each experimental condition was injected subcutaneously through an 18-gauge needle into the backs of anesthetized 5-week-old male C.B-17 SCID mice (Harlan, Indianapolis, IN). NIH guidelines for the care and use of laboratory animals (NIH Publication #85-23 Rev. 1985) were observed. Implants were harvested, fixed, and processed histologically at 2.5 and 6 weeks post-injection. Slides were stained with hematoxylin and eosin (H&E) or Goldner’s Trichrome.

RESULTS
Calcium Phosphate – DNA

Nanoparticles Characterization and In Vitro Transfection
The size of the CaP – DNA NPs was quantified to determine their stability over time. The size and morphology of both types of NPs were examined at 0, 1, and 2 weeks post-fabrication by transmission electron microscopy. Representative images are depicted in Figure 1. The NPs are relatively uniform in size with a round morphology. The particle sizes were determined, and as shown in Table 1, the NPs did not grow or aggregate over time. The average particle diameters remained close to or below 100 nm at all timepoints, which has been shown to be the optimal particle size range for cell uptake by endocytosis.37,38 Additionally, the DNA incorporation efficiency was determined to be 66.5% +/- 3.5% for CaP core – DNA coat NPs, and 79.5% +/- 16.2% for CaP-DNA core – BSA coat NPs.

The ability of these NPs to transfect preosteoblast cells in vitro in monolayer culture was examined using pDNA encoding for lacZ (Figure 2). We
used the MC3T3-E1 subclone 4 preosteoblast cell line from mouse calvaria, as they have exhibited high capacity for osteoblastic differentiation. The transfection efficiency was low; approximately 1% of cells stained positively for lacZ expression. However, we were able to verify that both particle types had the ability to transfect the preosteoblastic cells.

**Release of CaP-DNA NPs from Alginate Hydrogels**

The *in vitro* release of naked pDNA and both types of CaP-DNA NPs from alginate hydrogels over the course of 78 days was quantified. As seen in Figure 3, all conditions show sustained release of pDNA throughout the course of these 78 days. The CaP-DNA core – BSA coat NPs were released most rapidly, followed by naked pDNA, then by CaP core – DNA coat NPs.

**In vivo Bone Formation Study**

We next wanted to test the ability of this novel injectable gene delivery system to transf ect preosteoblasts and promote bone formation *in vivo*. For this *in vivo* study, we used low MW alginate with RGD modification. As shown previously, irradiated alginate degrades more rapidly *in vivo*, and the RGD amino acid sequence allows cells incorporated within the alginate to adhere to the hydrogel. Degradation of the hydrogel is important to allow the cells to proliferate and secrete extracellular matrix, and for the cells and extracellular matrix to have space to come together into the new tissue being formed. The addition of the RGD amino acid sequence allows cells to adhere to the hydrogel, promotes their proliferation, and has also been shown to increase the rate of bone formation when combined with preosteoblast or osteoblast cells. CaP-DNA NPs and preosteoblast cells were mixed into irradiated alginate modified with the cellular adhesive peptide, GRGDSP. These constructs were crosslinked with calcium and injected subcutaneously in the backs of SCID mice. Although we used pDNA encoding for lacZ in our *in vitro* studies to visualize the transfection, the pDNA in the NPs that we used *in vivo* encoded for BMP-2, to promote bone formation. The constructs were harvested at 2.5 and 6 weeks post-injection, and the histology was examined. In the samples with the CaP core – DNA coat NPs, bony tissue as shown in Figure 4 was found in some of the implants as early as 2.5 weeks post-injection, and also at our later time point 6 weeks post-injection. In the samples with cells only (i.e., no NPs or DNA), no bony tissue was seen at any time point. This was also the case for samples with CaP-DNA core – BSA coat NPs.

**Figure 4.** Histology of implants. (A) 2.5 weeks post-injection, alginate with MC3T3-E1 cells and CaP core – DNA coat NPs, H&E staining. (B) 6 weeks post-injection, alginate with MC3T3-E1 cells (no NPs), Goldner’s Trichrome staining. (C, D) 6 weeks post-injection, alginate with MC3T3-E1 cells and CaP core – DNA coat NPs. (C) H&E staining, (D) Goldner’s Trichrome staining. a = alginate, b = bone. Scale bar = 100 nm.

**Table 1.** Size stability of CaP-DNA NPs over 2 weeks as determined by analysis of transmission electron microscopy images (average diameter ± standard deviation).
DISCUSSION
In this study, CaP-DNA was delivered from alginate hydrogels to enhance bone formation of transplanted preosteoblasts. CaP-DNA NPs were created using two different methods, one with the pDNA serving as a coating to halt particle growth, and the other with BSA serving the same purpose. Both types of NPs were on the order of 100 nm in diameter, the optimal size for cellular endocytosis, and remained stable in size for at least two weeks. The pDNA incorporation efficiency was high, indicating that the majority of the pDNA was complexed to the CaP NPs, and therefore available for cellular uptake. Furthermore, both types of NPs were capable of transfecting cells in vitro, although at a low efficiency as expected. However, we theorized that it may only be necessary for a portion of transplanted cells to be transfected with the BMP-2 plasmid in this system to enhance bone formation, because the cells that are expressing this protein will secrete it and signal to other neighboring cells to differentiate into osteoblasts and then begin laying down extracellular matrix that will become bony tissue. Therefore, it should be possible to have a large population of cells differentiate into mature osteoblasts, even if only a select portion of them actually exhibit upregulated expressed of BMP-2 as a result of transfection.

To determine the amount of pDNA that is released from the alginate hydrogels over time due to diffusion of the pDNA out of the hydrogels or degradation of the hydrogels, we measured the release of pDNA from alginate for over two months. From this study we found that the pDNA is released very slowly from the alginate during this period. The CaP-DNA core – BSA coat NPs released faster than naked pDNA or CaP core – DNA coat NPs. The pore size of alginate (both low and high MW) has been reported to be on the order of 5 nm, which likely explains the slow release of the pDNA from the hydrogels, and suggests that the majority of the release that we measured was due to degradation of the hydrogels rather than diffusion through them. With this system, the large fraction of pDNA remaining in the hydrogels would be available to transfect incorporated cells, and the smaller fraction of pDNA released from the hydrogel would be capable of transfecting host cells locally surrounding the hydrogel.

Low molecular weight alginate produced by irradiation has been shown to degrade well in vivo. We hypothesized that incorporating preosteoblast cells within degradable alginate containing the CaP-DNA NPs would allow for sufficient NP release from the hydrogels and uptake of the NPs by the transplanted preosteoblast cells or surrounding host cells to improve bone tissue formation. Therefore, we proceeded with an in vitro study using low MW, RGD-modified alginate, MC3T3-E1 cells, and the CaP-DNA NPs with pDNA encoding for BMP-2. Evidence of bony tissue was found in some of the samples containing the CaP core – DNA coat NPs after as little as 2.5 and 6 weeks. No bony tissue was observed at these early time points in samples with MC3T3s alone (without NPs or pDNA) or, surprisingly, given our ability to transfect in vitro, in samples containing the CaP-DNA core – BSA coat NPs. It is possible that the CaP is interfering with cellular uptake in vivo; another group has reported decreased transfection with CaP-DNA NPs coated with BSA as compared to CaP-DNA NPs without BSA.49 In the future, the choice of a different protein coating, for instance one that promotes cellular uptake, may show more promising results. Other groups have shown increased pDNA NP uptake when they are coated with ECM molecules such as collagen or fibronectin. Such NP modifications might enhance the bone formation that was observed in both H&E and Goldner’s Trichrome stained histology in the CaP core – DNA coat NPs condition.

This injectable gene delivery system shows promise for bone regeneration applications. It is injectable, and therefore minimally invasive, it allows the inclusion of cells, and the CaP NPs provide an additional source of calcium and phosphate which are required for the formation of hydroxyapatite. Furthermore, the use of alginate hydrogels as the biomaterial delivery platform of the system allows for modification of the scaffold’s biochemical and physical properties, such as its cell adhesiveness, mechanical properties, and degradation profile. The CaP NPs may transfect both transplanted cells delivered within the alginates, as well as host cells that will surround the hydrogel. These transfected cells will produce and secrete BMP-2, which can cause surrounding cells to differentiate down the osteoblastic lineage. As the pDNA is delivered via a non-viral method, the expression of the protein will be short-term, ceasing when the transfected cells later senesce and die. Future directions include improving cellular uptake of these CaP-DNA NPs to increase transfection efficiency. Additionally, the use of different cell types, such as mesenchymal stem cells, preosteoblasts, or primary osteoblasts, may be studied to see which are more readily transfected in this system and provide for increased bone growth. It would also be informative to examine what effect varying the biochemical
and physical properties of the alginate used in the system has on gene uptake by cells within the 3D hydrogels and subsequent bone formation.

ACKNOWLEDGEMENTS

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CONFLICT OF INTEREST STATEMENT

No benefit of any kind will be received either directly or indirectly by the authors.

REFERENCES

28. Lee TC, Ho JT, Hung KS, Chen WF, Chung


4th Annual Bohlman Lecture  
April 7-8, 2009

Visiting Professor:  
**Todd Albert, M.D.**  
Professor and Chairman, Orthopaedic Surgery  
Thomas Jefferson University  
Philadelphia, PA

The annual Bohlman Lecture was given by Dr. Todd Albert. The two-day seminar included a presentation by Dr. Albert about the results of the SPORT trial and research presentations by the residents and spine fellow about the abundant spine research here at Case.

**Correlation of sacropelvic parameters with disc degeneration in spondylolytic cadaver specimens**  
Jason Tinley, M.D.

**Hip degeneration is increased in subjects with spondylolysis: a study of postmortem specimens**  
Michael Abdulian, M.D.

**Disc degenerate is increased in subjects with spondylolysis at L4 versus L5: a cadaveric study**  
Eric Schnaser, M.D.

**Postoperative wound infections after lumbar surgery in the morbidly obese**  
Douglas Dickson, M.D.

**Smoking increases blood loss and transfusion requirements following lumbar spine surgery**  
Eric Schnaser, M.D.

**Preoperative anticoagulant use and blood loss in lumbar surgery – when does blood loss normalize to expected levels?**  
Kasra Ahmadinia, M.D.

**Concurrent lumbar and cervical arthrosis: an anatomic study of cadaveric specimens**  
Daniel Master, M.D.

**Detection of nerve root and spinal cord deficits using somatosensory-evoked potentials alone in a consecutive series of cervical laminoplasty procedures**  
Ryan Garcia, M.D.

**Trabecular microfracture precedes cortical shell failure in the rat caudal vertebra under cyclic loading**  
Seetha Kumari

**Parathyroid hormone enhances bone formation after lumbar fusion in a destabilized rat spinal model**  
Eric Schnaser, M.D.

**Paralyzed nerve transfer for denervation in the setting of a spinal cord injury: results from an animal model**  
Daniel Master, M.D.
June 23-24, 2009

Visiting Professor:
**Thomas P. Sculco, M.D.**
Professor and Chairman, Orthopaedic Surgery
Hospital for Special Surgery
New York, NY

Dr. Tom Sculco was this year’s visiting professor for Resident Research Day. The chief residents’ presentations were especially exceptional since every one has been published in a peer-reviewed journal or presented at national meetings such as the Hand Society annual meeting, the Orthopaedic Trauma Association annual meeting, or the annual American Academy of Orthopaedic Surgeons meeting. Dr. Sculco also made a presentation on “New Technology in Arthroplasty: Can We Afford It?”

Papers:

**Particulate Bound LPS and LTA Do Not Induce Migration of Toll-Like Receptors 4 or 2 Into Lipid Rafts in Contrast to Soluble LPS and LTA**
Andrew Islam, M.D.

**The Relationship Between Femoral Neck Inclination and Femoral Version**
Raymond Liu, M.D.

**Incidence of Postthrombotic Syndrome in Patients Undergoing Primary Total Knee Arthroplasty for Osteoarthritis**
Christopher McAndrew, M.D.

**The Volar Extension of the Lunate Facet and DRUF Articulation of the Distal Radius: A Quantitative Anatomic Study in Skeletons**
Michael Paczas, M.D.

**Quantitative Characterization of Upper Sacral Segment Dysmorphism**
Anthony Skalak, M.D.

**Increasing Radiation Exposure in the Trauma Population**
Benjamin Smucker, M.D.
March 31-April 1, 2009

Visiting Professor:
Thomas A. Einhorn, M.D.
Professor and Chairman, Orthopaedic Surgery
Boston University School of Medicine
Boston, MA

Dr. Thomas Einhorn was the Distinguished Visiting Professor for Musculoskeletal Research this year. His visit included meetings with the many basic scientists in our department to discuss musculoskeletal research. Dr. Einhorn also made presentations on “The Cell and Molecular Biology of Bone Repair and Regeneration” and “New Technologies for the Enhancement of Skeletal Repair.”
November 18-19, 2008

Visiting Professor:

**Michael A. Simon, M.D.**
Professor and Chair, Orthopaedic Surgery
University of Chicago
Chicago, IL

Dr. Michael Simon was this year’s Carter / Makely Visiting Professor. The program included a vigorous discussion after Dr. Simon’s afternoon presentation on the “Diagnosis and Treatment of Subcutaneous Soft Tissue Sarcoma” and thoughtful insight about “How to Improve Orthopaedic Surgery Graduate Medical Education” during his Grand Rounds presentation.
June 16-17, 2009

Visiting Professor:
**Benjamin Stephens Richards, M.D.**
Professor, Orthopaedic Surgery
Texas Scottish Rite Hospital for Children
Dallas, TX

Dr. Steve Richards was the 2009 RBC Visiting Professor. The itinerary included research presentations by residents and case presentations. Dr. Richards’ discussed supracondylar humerus fractures in children and congenital pseudarthrosis of the tibia.

Dr. Richards comparing notes with Drs. Son-Hing, Gilmore, and Thompson

Drs. Marcus and Thompson present Dr. Richards with an honorary membership into the Herndon Society
This year’s visiting professor was Jung Yoo, M.D., Chairman of the Department of Orthopaedics and Rehabilitation at the Oregon Health and Science University. Dr. Yoo was a former resident, Allen Fellow, and faculty member. The program included two talks by Dr. Yoo about donor characteristics of stem cells and instability in spondylolisthesis. Troy Mounts, M.D. and Eric Schnaser, M.D. also presented their research from their Allen Research Fellowship year to Dr. Yoo.
On October 12, 2009, the department was one of the host sites for the AOA North American Traveling Fellows. The North American Traveling Fellowship promotes significant clinical and scientific exchange and fellowship. The tour is an intense introduction to the diverse ways that leaders address challenges facing orthopaedics today. The tour runs approximately five weeks in the fall of odd-numbered years and travels to regional orthopaedic centers on the East Coast/Eastern Canada, Midwest/Central Canada or West Coast/Western Canada.

The traveling fellows included Dr. Ryan Bicknell from Queen’s University, Dr. Robert Brophy from Washington University, Dr. Gregory Della Rocca from University of Missouri – Columbia, Dr. Wellington Hsu from Northwestern University, and Dr. Amanda Marshall from University of Texas Health Science Center in San Antonio.

Their visit included a full morning academic program featuring talks from the fellows as well as from our faculty, and case presentations by the chief residents.

Current faculty who are alumni of the fellowship include Dr. George Thompson, Dr. Heather Vallier, and Dr. Roger Wilber. Many other former residents and fellows have also participated in the traveling fellowship.
EXITING RESIDENTS’ FUTURE PLANS

Andrew Islam, M.D.
Sports Medicine Fellowship
Cleveland Clinic
Cleveland, OH

Michael Paczas, M.D.
Hand Surgery Fellowship
University of Cincinatti
Cincinatti, OH

Raymond Liu, M.D.
Pediatric Orthopaedics Fellowship
Rady Children’s Hospital / UC San Diego
San Diego, CA

Anthony Skalak, M.D.
Orthopaedic Trauma Fellowship
Carolina’s Medical Center
Charlotte, NC

Christopher McAndrew, M.D.
Orthopaedic Trauma Fellowship
Harborview Medical Center
Seattle, WA

Benjamin Smucker, M.D.
Sports Medicine Fellowship
UHZ Sports Medicine Institute
Miami, FL
INCOMING INTERNS – CLASS OF 2014

Johnathan Belding, M.D.

Chad Fortune, M.D.

Jason Solomon, M.D.

Johnathan Streit, M.D.

Anna Wallace, M.D.

Ashraf Youssef, M.D.
1. Manuscript admissions will be accepted throughout the year. This year’s deadline is September 1, 2010.

2. Submissions will only be accepted in electronic format via CD or e-mail. Text should be submitted in a text file such as MicroSoft Word. PDFs are not accepted.

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

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
   • Single space between sentences

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.

8a. Publication in the Case Orthopaedic Journal does not prohibit publication in a peer-reviewed journal, however, efforts should be made by the authors to submit an abridged version to the Case Orthopaedic Journal if submission to another journal is planned.

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**Case Orthopaedic Journal**

**CALL FOR MANUSCRIPTS**

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 September 1, 2010. Please read the following for instructions. Manuscripts should be sent in electronic format on CD to Ryan Garcia 11100 Euclid Ave, Cleveland, OH 44106, or by e-mail to ryangarcia78@gmail.com.
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