Clin Orthop Relat Res (2008) 466:1699–1704
DOI 10.1007/s11999-008-0269-5

ORIGINAL ARTICLE

Chitosan-coated Stainless Steel Screws for Fixation in Contaminated Fractures

Alex H. Greene BS, Joel D. Bumgardner PhD, Yunzhi Yang PhD, Jon Moseley PhD, Warren O. Haggard PhD

Received: 26 July 2007 / Accepted: 10 April 2008 / Published online: 29 April 2008

Abstract Stainless steel screws and other internal fixation devices are used routinely to stabilize bacteria-contaminated bone fractures from multiple injury mechanisms. In this preliminary study, we hypothesize that a chitosan coating either unloaded or loaded with an antibiotic, gentamicin, could lessen or prevent these devices from becoming an initial nidus for infection. The questions investigated for this hypothesis were: (1) how much of the sterilized coating remains on the screw with simulated functional use; (2) is the unloaded or loaded chitosan coating bacteriostatic and biocompatible; and (3) what amount and rate does an antibiotic elute from the coating? In this study, the gentamicin eluted from the coating at a detectable level during 72 to 96 hours. The coating was retained at the 90% level in simulated bone screw fixation and the unloaded and loaded chitosan coatings had encouraging in vitro biocompatibility with fibroblasts and stem cells and were bacteriostatic against at least one strain of Staphylococcus aureus. The use of an antibiotic-loaded chitosan coating on stainless steel bone screws and internal fixation devices in contaminated bone fracture fixation may be considered after optimization of antibiotic loading and elution and more expanded in vitro and in vivo investigations with other organisms and antibiotics.

Each author certifies that he or she has no commercial associations (eg, consultancies, stock ownership, equity interest, patent/licensing arrangements, etc) that might pose a conflict of interest in connection with the submitted article.

A. H. Greene, J. D. Bumgardner, W. O. Haggard (✉)
Department of Biomedical Engineering, University of Memphis, Memphis, TN, USA
e-mail: whaggrd1@memphis.edu

Y. Yang
Department of Biomedical Engineering, University of Tennessee Health Science Center, Memphis, TN, USA

J. Moseley
Wright Medical Technology, Arlington, TN, USA

Introduction

With orthopaedic surgery for disease or trauma, wound contamination is a major issue that can impact patient outcome. Studies have reported 65% to 70% of wounds are contaminated with microorganisms [2, 26]. Wound contamination that develops into an infection can delay recovery in some cases for as much as 6 weeks, increase healthcare costs at a reported $30,000, and affect patient morbidity and mortality [2, 15]. Internal fixation in these contaminated wounds, especially with bone fractures, can be beneficial and detrimental [2, 20]. Stable fractures with internal fixation have positive effects on bone healing, but the implanted biomaterials with these devices can be a source of protection for the contaminating bacteria from antibiotic treatment and patient immune responses [20]. A local approach to lessen this protective effect and prevent the internal fixation devices from becoming an initial nidus of infection is needed.

Chitosan is a biopolymer biomaterial that has many reported medical applications, including as an implant coating [1, 3, 4, 7, 8, 10, 13, 22]. This biopolymer biomaterial is a linear polysaccharide derived from crustacean shells and fungi cell walls and has known biocompatibility, drug delivery, and reported bacteriostatic properties [4, 7, 8, 13, 22]. This coating approach with chitosan on internal fixation device surfaces could lessen their participation in increasing bacterial resistance and contamination to infection progression.

Other surface coatings, hydroxyapatite (HA) and polymethylmethacrylate (PMMA), have been investigated on internal fixation devices to expand their clinical use in contaminated bone fracture sites and wounds [5, 16–19, 21]. Polymeric coatings with poly(lactic acid) (PLA) and poly(glycolic acid) (PGA), or a composite of both, also have been investigated on these devices [11, 25]. Although HA and PMMA are good candidates, bacterial adherence and the potential resultant increased bacterial resistance and infection are limitations for their application in many contaminated wounds [9]. The lack of an inherent bacteriostatic property for HA and PMMA coatings in comparison to a chitosan coating would restrict their effectiveness even in an antibiotic-loaded condition on completion of antibiotic release. For the PLA and PGA coatings, biocompatibility issues with these materials have been reported [12, 14, 23, 24]. These reported issues for PLA and PGA include high acidity and inflammation from their degradation products. In contrast, the chitosan coating has degradation products from enzymatic and hydrolytic processes that are saccharides and glycosamines used in the Krebs cycle [4, 8].

In this preliminary investigation, we sought to determine if a chitosan coating with or without an antibiotic on a stainless steel fixation screw could lessen the synergetic effects of contaminating bacteria and an internal fixation biomaterial, stainless steel. This limited study examined: (1) the coating robustness in simulated, functional bone screw fixation to a targeted objective of 80% coating adherence; (2) the biocompatibility and the presence or absence of a bacteriostatic effect of unloaded and antibiotic-loaded coatings against Staphylococcus aureus; and (3) the amount and rate of an eluted antibiotic, gentamicin, in comparison to reported inhibitory concentrations.

Materials and Methods

The goal of this investigation was to determine if a coating of chitosan, loaded or unloaded with gentamicin, on a stainless steel implant alloy has the potential to reduce or prevent devices from becoming an initial nidus for infection. To begin to test this hypothesis, we used randomized experimental designs to compare (1) the retention of chitosan coatings containing gentamicin with plain chitosan coatings during simulated bone fixation; (2) the compatibility of chitosan coatings without gentamicin with uncoated control specimen in osteoblastic and fibroblastic cell cultures; and (3) the antibacterial properties of the coatings with and without gentamicin with uncoated controls in the zone of inhibition tests using S. aureus. In vitro elution testing also was performed to determine the amount and rate of antibiotic release from coatings containing gentamicin.

Solution casting techniques, adapted from Bumgardner et al. [4], were used to bond coatings through silane-glutaraldehyde molecules on stainless steel bone screws (prototype, 2 cm length, 3.5 mm diameter; Wright Medical Technology, Arlington, TN) for use in coating retention, zone of inhibition, and elution studies. Flat stainless steel specimens, 1.22 × 1.22 × 0.3 cm, were coated for use in cell culture studies. Briefly, screws and flat specimens were roughened by grit blasting (Abrasive Blast Cabinet 101698G-A; Econoline, Grand Haven, MI) using silica beads (220 grit) to maximize surface area for bonding. Grit blasting was performed on all specimen surfaces in seven 2-minute intervals. Surface profilometry (Alpha-Step 500 Surface Profiler; KLA Tencor, San Jose, CA) was used to ensure all test specimens had similar surface roughness values (10–20 Ra value). Surfaces were silanated using 3-amino-propyl-triethoxy-silane (United Chemical Technologies, Bristol, PA) under acidic conditions. Glutaraldehyde was added to the amino end of the silane to provide a reactive aldehyde to form amide bonds with amine groups in the chitosan molecule. A 1 wt% chitosan (92.3%DDA; Vanson Halosource, Bothell, WA) in 1% acetic acid (Acros Organics, Geel, Belgium) solution was used to form coatings without gentamicin. Gentamicin (MP Biomedicals, Solon, OH) at 2 wt% of chitosan was used to form antibiotic-containing coatings. Sterilization of coated test specimens was performed using ethylene oxide (at 38°C for 4 to 5 hours).

Functional mechanical testing to simulate screw placement into bone was performed by inserting chitosan-coated bone screws with (n = 3) and without gentamicin (n = 3) into solid rigid polyurethane bone density foam (Sawbones; Pacific Ridge Laboratories, Vashon, WA) with a density of 0.32 g/cc (following ASTM standard specifications F-1839) [5, 18]. Each screw was photographed and weighed before implantation ($mass_{initial}$) and then reweighed postimplantation ($mass_{after}$). Three screws with gentamicin-containing coatings also were tested in an aqueous (phosphate-buffered saline [PBS]) environment to evaluate effects of wetting on coating adherence. The aqueous environment was established by submerging the polyurethane foam in PBS and placing under a vacuum for 1 hour at room temperature. The amount of coating that was retained during the implantation process was determined by visual observation and as percent retention [$100\ (1 - (mass_{initial}/mass_{after}))$]. Analysis of variance was performed on the percent retention of coating with the type of coating (plain chitosan, chitosan with gentamicin, and chitosan with gentamicin wet) as the major factor. Post hoc analyses used the Student Newman-Keuls multiple comparison technique.

In vitro compatibility of the chitosan coatings without gentamicin was compared with uncoated samples using the

flat specimen in cultures with Normal Human Dermal Fibroblasts (CC-2511; Cambrex, East Rutherford, NJ) and a preosteoblastic cell line (Human Embryonic Palatal Mesenchymal Cells; ATCC, Manassas, VA). Cells were seeded at 10^4 cells/cm^2 on the test specimen in 12-well culture plates, placed into an incubator at 37°C and 5% CO_2, and the number of cells (cells/mL) on the specimen was estimated (at 2, 4, and 6 days for the fibroblasts; 1, 3, and 5 days for the preosteoblasts) after double-trypsinization and counting in a Coulter counter (Z2 Coulter Particle Count and Size Analyzer; Beckman Coulter, Hialeah, FL). Triplicate samples of each specimen (chitosan-coated and uncoated) were evaluated at each time. For each cell line, Student's t-test was used to compare the number of cells on the chitosan-coated samples with the number on the uncoated samples at each time.

A zone of inhibition study was performed using an established protocol as an initial screening test for antibacterial properties of the chitosan coatings with and without gentamicin as compared with uncoated stainless steel bone screws [6]. S. aureus was cultured for 24 hours on Todd-Hewitt agar containing 0.2% yeast extract. Screws were placed individually on each plate and consisted of three uncoated stainless steel screws, three unloaded chitosan-coated bone screws, and three loaded chitosan-coated bone screws (with 2 wt% gentamicin). After 24 hours in the agar, measurements (in millimeters) were taken to determine the distance from the screw that the bacteria were inhibited. Analysis of variance was performed on the distance from the screw for each group as the major factor.

An elution study was performed on gentamicin-loaded, chitosan-coated bone screws ($n = 6$) to determine the amount and time course of antibiotic release in vitro. Each specimen was placed in a scintillation vial with 10 mL of PBS and kept in a water bath at 37°C. Samples of the eluent were collected at 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, and 146 hours. At each time, the screw was removed from the PBS solution and placed in new solution. Samples were drawn from the eluent and run in a fluorescence polarization immunoassay device (TDxFLx; Abbott Laboratories, Abbott Park, IL) and reported as micrograms per milliliter.

Results

The coating had initial biocompatibility similar to stainless steel and exhibited no inhibitory effect compared with stainless steel using two different cell lines. In testing with human dermal fibroblasts, cell count techniques revealed similar growth rates between the chitosan-coated stainless steel and the uncoated stainless steel at all times (2 days, $p = 0.249$; 4 days, $p = 0.484$; 6 days, $p = 0.252$) (Fig. 1).

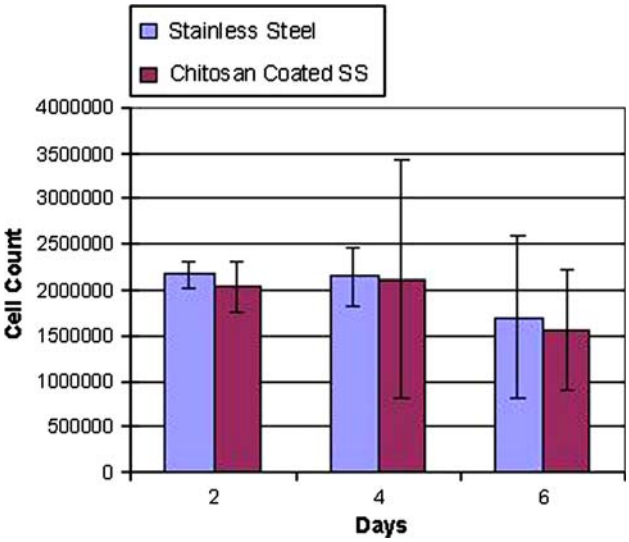

Fig. 1 Testing with human dermal fibroblasts revealed similar growth rates between the chitosan-coated stainless steel and the uncoated stainless steel at all times.

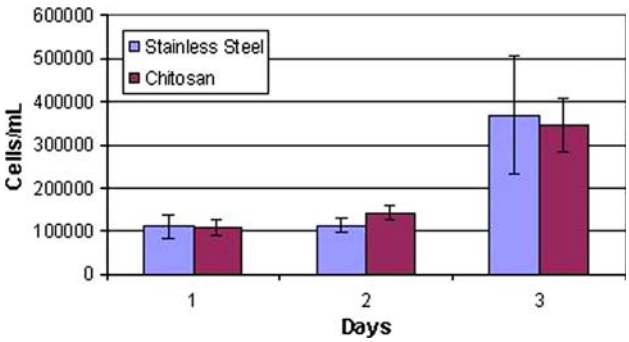

Fig. 2 Cytotoxicity testing with an osteoblastic precursor cell line revealed similar growth rates between chitosan-coated stainless steel coupons and uncoated stainless steel coupons at all times.

During cytotoxicity testing with an osteoblastic precursor cell line, similar trends were observed showing similar growth rates between chitosan-coated stainless steel coupons and uncoated stainless steel coupons at all times (1 day, $p = 0.258$; 3 days, $p = 0.177$; 5 days, $p = 0.457$) (Fig. 2).

The effect of the coatings in the presence of a typical contaminating bacterium was evaluated with zone of inhibition testing. A zone of inhibition was detected in loaded and unloaded chitosan-coated bone screws, whereas uncoated stainless steel bone screws showed no sign of bacterial inhibition. Unloaded chitosan-coated bone screws had a mean zone of inhibition of 18.6 ± 1.4 mm measured from the center of the screw (Fig. 3). Loaded chitosan-coated bone screws (with 2 wt% gentamicin) had a larger

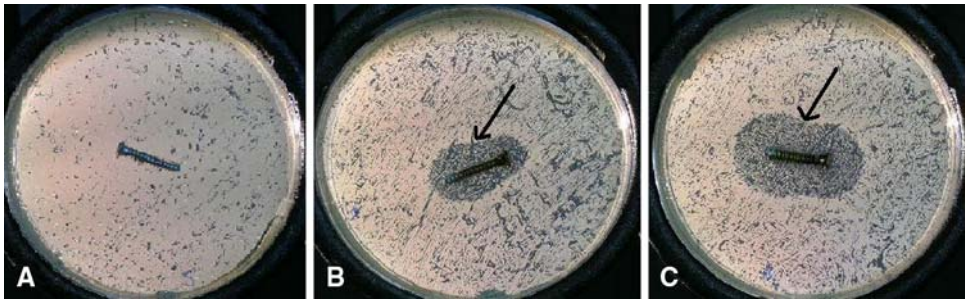

Fig. 3A–C Compared with the (**A**) stainless steel control, zone of inhibition testing confirmed the inhibition of bacteria in the presence of (**B**) chitosan and (**C**) gentamicin-loaded chitosan.

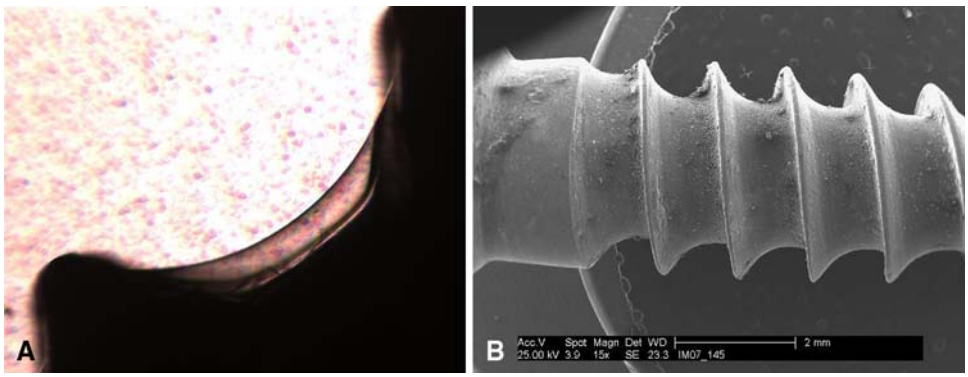

Fig. 4A–B (**A**) Light microscopy confirmed the establishment of a loaded chitosan coating on the surface of a stainless steel screw. (**B**) Scanning electron microscopy also confirmed a loaded chitosan coating covering the surface of the bone screw.

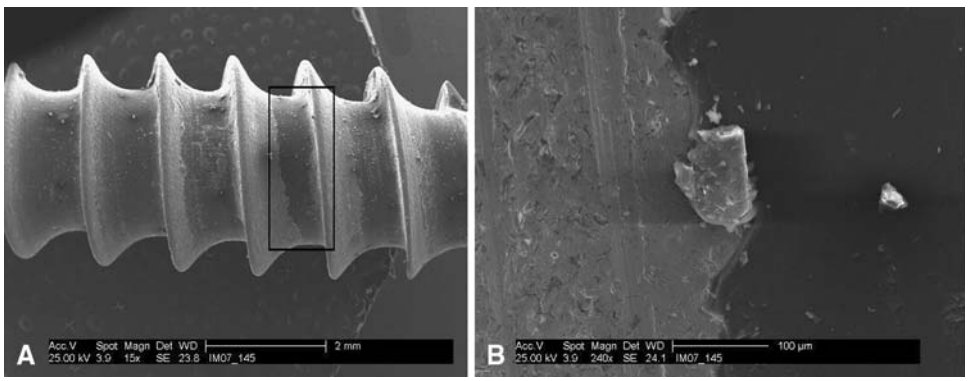

Fig. 5A–B (**A**) After functional bone simulation testing, scanning electron microscopy revealed some of the coating was lost during implantation. (**B**) Higher magnification scanning electron microcopy shows where the coating became separated.

($p = 0.001$) zone of inhibition with a mean inhibition diameter of 26.6 ± 1.0 mm.

Functional mechanical testing to simulate bone implantation of the coated screw had no visible effect on the coating, but scanning electron microscopy revealed minor coating loss (Fig. 4, preimplantation; Fig. 5, postimplantation). The percentage of coating remaining for the dry, loaded (gentamicin) chitosan-coated bone screws was $93.7\% \pm 4.1\%$ after implantation and $89.9\% \pm 9.8\%$ for the chitosan-coated bone screws in an aqueous environment. Testing of the dry unloaded chitosan-coated bone screws yielded a $90.9\% \pm 1.3\%$ coating remaining, indicating no statistical differences were seen among unloaded chitosan coatings, dry loaded chitosan coatings, and wet loaded chitosan coatings ($p = 0.689$) (Fig. 6).

Elution testing of the antibiotic released from the coating revealed a gentamicin release profile from the chitosan solution of 1 mg/mL at 1 hour to 0.05 mg/mL at 96 hours. This release profile was characterized by a burst release of gentamicin within the first 4 hours in solution followed by a steady decrease over 7 days (Fig. 7).

Discussion

Contamination from microorganisms is an established risk for many orthopaedic surgeries as a result of complex trauma and disease-related bone defects [2, 26]. This contamination has the potential to lead to infection, considerably delaying recovery and increasing the cost of

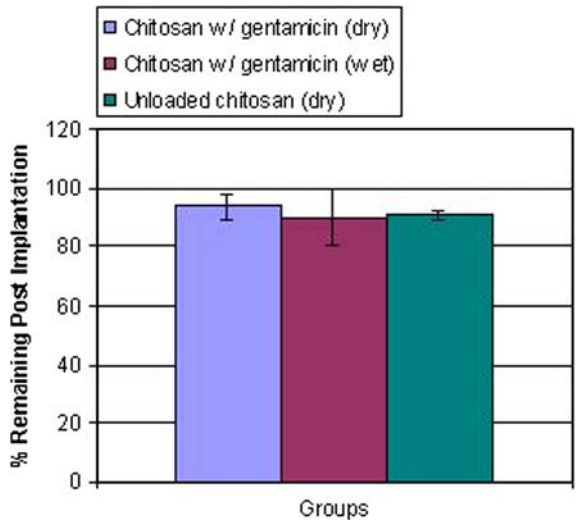

Fig. 6 No statistical differences were seen among unloaded chitosan coatings, dry loaded chitosan coatings, and wet loaded chitosan coatings, and all coatings were retained well above the desired 80% retention rate.

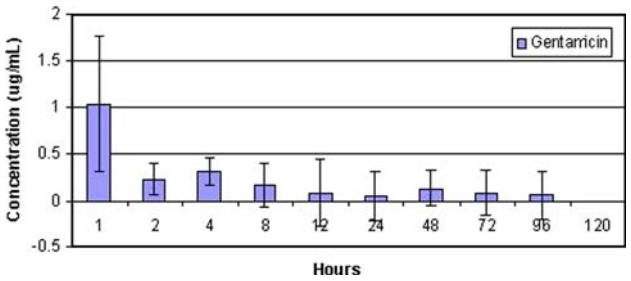

Fig. 7 Elution testing confirmed a measurable release of gentamicin up to 96 hours characterized by a burst release of antibiotic within the first 4 hours.

health care [2, 15]. Internal fixation devices help aid healing in many of these cases but can be detrimental by protecting bacteria from antibiotic treatment. For this reason, local approaches to combat infection have become more common, including antibiotic coatings [20]. This preliminary study sought to determine if a chitosan coating with or without an antibiotic on a stainless steel fixation screw could lessen the effects of contaminating bacteria and a stainless steel internal fixation device.

This preliminary investigation was pursued to investigate chitosan as a potential alternative to current coating options such as HA, PMMA, PLA, PGA, and others. The elimination of an initial nidus of infection with the use of orthopaedic metallic devices in contaminated wounds by using a coating was sought as the primary objective. Chitosan seems to be a reasonable option and presents some potential advantages from these preliminary investigations.

Other surface coatings such as HA and PMMA have been investigated as potential coatings for internal fixation devices [5, 16–19, 21]. Although these potential coating materials are good candidates, they each have limitations. Hydroxyapatite and PMMA are susceptible to bacterial adherence and the resultant bacterial resistance [9]. Therefore, these materials do not have the inherent bacteriostatic properties. Chitosan is an intriguing coating possibility as a result of its reported bacteriostatic characteristics [4, 8]. In this particular study, unloaded (no gentamicin) chitosan had a small zone of inhibition present, confirming this reported bacteriostatic characteristic.

Hydroxyapatite and other similar coatings also have limitations because of their brittle structure, leading to eventual fracture of the coating [4, 5]. In functional testing, chitosan compares favorably with these types of coatings with only minimal delamination occurring [16–18]. After standard functional testing coatings of HA and other brittle materials are retained to a substantial degree [16–18]. This preliminary testing showed a 90% coating retention with chitosan using a more applicable testing model (irregular surface of a screw).

Other degradable polymeric coatings such as PLA and PGA also have been investigated for use as coating materials on orthopaedic devices. Although these coatings are good candidates for coating devices, they also have reported biocompatibility issues. Poly (lactic acid) and PGA have degradation products with high acidity [12, 14, 23, 24]. These degradation products can cause local inflammation on degradation [12, 14, 23, 24]. Chitosan has no such detrimental effect in vitro on degradation and has a more tolerant pH than these other polymeric coatings [4, 8]. The initial cytotoxicity testing of chitosan validated the reported biocompatibility in the presence of fibroblast and osteoblast precursor cells.

This preliminary investigation showed the capacity of chitosan coatings to be sufficiently bonded to stainless steel medical devices and to contain therapeutic agents (antibiotics). Biocompatibility, zone of inhibition, and elution testing show chitosan has the potential to be used as a coating for orthopaedic devices, whereas functional simulated bone testing suggests the coating strength is sufficient to be used in these applications. The limitations of this study are the small sample size, only in vitro evaluations, limited antibiotic loading levels, and the use of only one antibiotic. Therefore, the use of an antibiotic-loaded chitosan coating on stainless steel bone screws as internal fixation devices for contaminated bone fracture fixation may be considered after an optimization of antibiotic loading, additional evaluation of other antibiotics, and expanded in vitro and in vivo investigations.

Acknowledgments We thank Wright Medical Technology (stainless steel bone screws), Vanson (chitosan), and Pacific Research Laboratories (sawbone samples).

References

1. Aimin C, Chunlin H, Juliang B, Tinyin Z, Zhichao D. Antibiotic loaded chitosan bar: an in vitro, in vivo study of a possible treatment for osteomyelitis. *Clin Orthop Relat Res*. 1999;366:239–247.
2. Bloom BS, Esterhai JLJ. Musculoskeletal infection: impact, morbidity, cost to society, medicine, and government. In: Esterhai JLJ, Gristina AG, Poss R, eds. *Musculoskeletal Infection*. Park Ridge IL: American Academy of Orthopaedic Surgeons; 1992:5–11.
3. Bumgardner JD, Wiser R, Elder SH, Jouett R, Yang Y, Ong JL. Contact angle, protein adsorption and osteoblast precursor cell attachment to chitosan coatings bonded to titanium. *J Biomater Sci Polym Ed*. 2003;14:1401–1409.
4. Bumgardner JD, Wiser R, Gerard PD, Bergin P, Chesnutt B, Marin M, Ramsey V, Elder SH, Gilbert JA. Chitosan: potential use as a bioactive coating for orthopaedic and craniofacial/dental implants. *J Biomater Sci Polym Ed.* 2003;14:423–438.
5. Campbell AA, Song L, Li XS, Nelson BJ, Bottoni C, Brooks DE, DeJong ES. Development, characterization, and anti-microbial efficacy of hydroxyapatite-chlorhexidine coatings produced by surface-induced mineralization. *J Biomed Mater Res*. 2000;53:400–407.
6. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing—Eighteenth Informational Summit. CLSI Document M100–317*. Villanova, PA: Clinical and Laboratory Standards Institute; 2008.
7. Correlo VM, Boesel LF, Bhattacharya M, Mano JF, Neves NM, Reis RL. Hydroxyapatite reinforced chitosan and polyester blends for biomedical applications. *Macromol Mater Eng*. 2005;290:1157–1165.
8. Di Martino A, Sittinger M, Risbud MV. Chitosan: a versatile biopolymer for orthopaedic tissue-engineering. *Biomaterials*. 2005;26:5983–5990.
9. Gristina AG, Naylor PT, Myrvik QN. Molecular mechanisms of musculoskeletal sepsis. In: Esterhai JLJ, Gristina AG, Poss R, eds. *Musculoskeletal Infection*. Park Ridge, IL: American Academy of Orthopaedic Surgeons; 1992:21–25.
10. Hamilton V, Yuan Y, Rigney DA, Chesnutt BM, Puckett AD, Ong JL, Yang Y, Haggard WO, Elder SH, Bumgardner JD. Bone cell attachment and growth on well-characterized chitosan films. *Polymer International*. 2007;56:641–647.
11. Harris LG, Mead L, Muller-Oberlander E, Richards RG. Bacteria and cell cytocompatibility studies on coated medical grade titanium surfaces. *J Biomed Mater Res A*. 2006;78:50–58.
12. Huang C, Li J, Zhu J, Li P, Xie G, Gong Y. [A comparative study on two different absorbable intramedullary nails in treating metacarpal and phalanx fractures][in Chinese]. *Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi*. 2004;18:360–363.
13. Khor E, Lim LY. Implantable applications of chitin and chitosan. *Biomaterials*. 2003;24:2339–2349.
14. Li H, Chang J. Preparation and characterization of bioactive and biodegradable wollastonite/poly(D,L-lactic acid) composite scaffolds. *J Mater Sci Mater Med*. 2004;15:1089–1095.
15. Mabry RL, Holcomb JB, Baker AM, Cloonan CC, Uhorchak JM, Perkins DE, Canfield AJ, Hagmann JH. United States Army Rangers in Somalia: an analysis of combat casualties on an urban battlefield. *J Trauma*. 2000;49:515–528; discussion 528–529.
16. Moroni A, Faldini C, Marchetti S, Manca M, Consoli V, Giannini S. Improvement of the bone-pin interface strength in osteoporotic bone with use of hydroxyapatite-coated tapered external-fixation pins: a prospective, randomized clinical study of wrist fractures. *J Bone Joint Surg Am*. 2001;83:717–721.
17. Moroni A, Faldini C, Pegreffi F, Giannini S. Fixation strength of tapered versus bicylindrical hydroxyapatite-coated external fixation pins: an animal study. *J Biomed Mater Res*. 2002; 63:61–64.
18. Moroni A, Heikkila J, Magyar G, Toksvig-Larsen S, Giannini S. Fixation strength and pin tract infection of hydroxyapatite-coated tapered pins. *Clin Orthop Relat Res*. 2001;388:209–217.
19. Moroni A, Vannini F, Mosca M, Giannini S. State of the art review: techniques to avoid pin loosening and infection in external fixation. *J Orthop Trauma*. 2002;16:189–195.
20. Patzakis MJ. Microorganisms in nature, disease: the surgeons perspective. In: Esterhai JLJ, Gristina AG, Poss R, eds. *Musculoskeletal Infection*. Park Ridge IL: American Academy of Orthopaedic Surgeons; 1992:31–32.
21. Piza G, Caja VL, Gonzalez-Viejo MA, Navarro A. Hydroxyapatite-coated external-fixation pins: the effect on pin loosening and pin-track infection in leg lengthening for short stature. *J Bone Joint Surg Br*. 2004;86:892–897.
22. Prasitsilp M, Jenwithisuk R, Kongsuwan K, Damrongchai N, Watts P. Cellular responses to chitosan in vitro: the importance of deacetylation. *J Mater Sci Mater Med*. 2000;11:773–778.
23. Safinia L, Datan N, Hohse M, Mantalaris A, Bismarck A. Towards a methodology for the effective surface modification of porous polymer scaffolds. *Biomaterials*. 2005;26:7537–7547.
24. Schachter DM, Kohn J. A synthetic polymer matrix for the delayed or pulsatile release of water-soluble peptides. *J Control Release*. 2002;78:143–153.
25. Schmidmaier G, Lucke M, Wildemann B, Haas NP, Raschke M. Prophylaxis and treatment of implant-related infections by antibiotic-coated implants: a review. *Injury*. 2006;37(suppl 2): S105–112.
26. Zalavras CG, Patzakis MJ, Holtom PD, Sherman R. Management of open fractures. *Infect Dis Clin North Am*. 2005;19:915–929.