JOURNAL OF Applied Polymer SCIENCE

A Polyurethane-Based Nanocomposite Biocompatible Bone Adhesive

Kevin J. Schreader,[1] Ilker S. Bayer,[2,3] Derek J. Milner,[4] Eric Loth,[3] Iwona Jasiuk[1,4]

[1]Department of Mechanical Science and Engineering, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801
[2]Center for Biomolecular Nanotechnologies@UNILE, Smart Materials Platform, Istituto Italiano di Tecnologia, Arnesano, LE 73010, Italy
[3]Department of Mechanical and Aerospace Engineering, University of Virginia, Charlottesville, Virginia 22904
[4]The Institute for Genomic Biology, Regenerative Biology and Tissue Engineering Theme, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801
Correspondence to: I. Jasiuk (E-mail: ijasiuk@illinois.edu)

ABSTRACT: A novel polyurethane-based foam-like adhesive reinforced with nanosized hydroxyapatite (HA) particles was developed and investigated for bone-to-bone bonding applications in terms of mechanical adhesion and biocompatibility. The adhesive has a hierarchical structure with HA particles at the nanoscale level and pores at the micro-scale level. This adhesive was tested mechanically in the three principal loading modes anticipated: shear, tension, and compression. Standard testing procedures were used when available. Tensile strength of primed adhesive showed a four-fold increase in adhesion on unmodified bone and a nearly two-fold increase in adhesion to primed bone as compared with the conventional bone cement. Biocompatibility was initially assessed *in vitro* using cell culture tests, which showed positive interaction with the adhesive. Then, a second biocompatibility test was performed using *Xenopus laevis* limbs to assess an *in vivo* response. The results indicated that the adhesive material produces a normal response consistent with control specimens. However, long-term observations and tests with additional species are needed to demonstrate full biocompatibility. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

KEYWORDS: adhesion; bone repair; nanocomposite; polyurethane; hydroxyapatite particles

Received 15 February 2012; accepted 17 May 2012; published online
DOI: 10.1002/app.38100

INTRODUCTION

Current methods for fracture stabilization of bone tissue typically require metal hardware to be affixed to the bone resulting in many challenges and limitations in this technology. The use of microsystems is particularly important in trauma surgery such as in fractures of infraorbital area, frontal sinus wall, and reconstruction of the skull.[1] The development of rigid microplates with screws in maxillofacial fractures has revolutionized treatment of related trauma, but yet many improvements are possible.[2,3] Although capable of very high mechanical strength, the use of screws can result in stripping the bone due to potential over-tightening when inserted and loosening over time resulting in dislocation of the fixture and poor anatomical healing.[1] Additional drawbacks for screws include fractures from pilot holes, bone resorption from stress shielding, devascularization from exposure, and growth disturbance.[2–4] The resulting limitation on the tissue size and geometry with the current technology motivates the investigation of alternate techniques for bone fracture stabilization.

An adhesive bone bonding system holds potential advantages that cannot be realized with the use of metal screw systems. Because an adhesive spreads the force over a larger contact area, it can be used in situations where surrounding bone material is weak or even osteoporotic.[5] Utilizing an adhesive allows the force to be transmitted throughout the contact area minimizing possible stress shielding effects that could otherwise occur.[6] An adhesive also reduces concerns that rigid fixation may be responsible for bone atrophy due to the high stiffness of the metal plates.[7]

However, unique challenges are present in the bonding of biological material in the *in vivo* conditions. Primary among these is the interface where hydrophobic polymer and hydrophilic bone come into contact.[8] To overcome the incompatibility between polymers and bone, an amphiphilic primer can be used to modify the surface energy. The primer can decrease the barrier between the lower surface energy of polymer and the higher surface energy of the hydrophilic bone surface resulting in a significantly improved adhesion.[2,3,8] The composition of bone and dentin are similar with both being primarily made of the inorganic hydroxyapatite (HA), organic collagen, and water.[2] Dentin priming agents have already been well developed and thus are natural choices for preliminary

bone bonding studies as it has been shown that they are advantageous in increasing adhesion strength.[2,3]

Despite challenges, there are also new opportunities with the use of an adhesive fixation technique. The ideal adhesion system will provide initial stabilization and then degrade with time to allow gradual load transfer to the bone until it is finally fused. It has already been observed that enzymes appear capable of recognizing and acting on substrates such as a polyurethane contributing to the degradation process.[9] An adhesive system could further work as a targeted drug delivery agent to enhance healing if bioactive compounds are incorporated within the adhesive system to promote bone ingrowth, or antibiotics to prevent infection at the trauma site.[10] It is also necessary that all parts of the adhesive system meet requirements to enable healing and prevent damage. Numerous standards have been set forth for optimum performance including the following: the adhesive and its degradation products should be nontoxic, biocompatible to bone and surrounding tissue, bond in a wet environment, and have practical preparation and application.[6] These potential advantages of an adhesive fixation system make it an attractive option once all such performance requirements can be satisfied.

Several common engineering adhesives: epoxy resins, polyurethanes, and cyanoacrylates have been proposed for biological applications, with mixed outcomes. Epoxy resins exhibited poor bonding in wet conditions, tissue necrosis from polymerization heat, and dubious toxicological properties.[6,11] The use of cyanoacrylates was questionable due to the toxic effects of some monomer types, higher infection rates, and low shear strength.[6] The synthetic adhesive that showed the most overall promise for an adhesive application in biological specimens was polyurethane based. The use of polyurethane polymers has also received a great deal of attention for a wide range of potential *in vivo* applications including scaffolds and hard tissue replacement.[7,9,12–15] This demonstrated ability of polyurethane for use in a biological setting made it a strong candidate for the investigation of bone bonding, and ultimately was the chosen adhesive for this study.

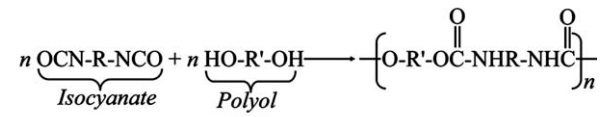
This study seeks to develop the biocompatible polyurethane adhesive reinforced with nanosized HA particles to enhance bonding between the hydrophobic polymer and hydrophilic bone. The particles are incorporated within moisture-curable polyurethane to provide a durable and practical bond. This mixture can have favorable degradation properties since enzymes have been observed to be capable of recognizing and degrading polyurethane components.[9] This attribute is combined with foam-based interconnectivity of the adhesive, which may aid in healing by promoting bone ingrowth. Herein, such an adhesive is developed and tested with respect to bonding strength and biocompatibility. Mechanical characterization of the cured adhesive was conducted in three principal loading modes: shear, compression, and tension. Biocompatibility was assessed with *in vitro* cell culture tests and *in vivo* testing with Xenopus Laevis frog limbs.

MATERIALS AND METHODS

Adhesive Components and Application

The baseline adhesive, used in this study, is cross linked by moisture-curing polyurethane chemistry. Moisture-curing polyurethanes can be designed with a wide range of physical properties, from soft and flexible to hard and rigid.[16,17] The curing of these adhesives is based on the reaction between isocyanates and oligomers with terminal hydroxyl groups (polyols) as shown in Figure 1. During curing, the water isolation of the reaction system is essential, because the free isocyanate groups might undergo reaction with the moisture contained in the air or the substrate (Figure 1). The polyol chemistry and structure determine the final properties of the cured polyurethane as well as its adhesive and bonding properties. The polyurethane adhesive used in the present study is developed jointly by Kaneka and Nippon Polyurethane industry located in Yokohoma, Japan and is composed of methylene diphenyl diisocyanate (MDI, isomers and homologues), polymeric MDI and a biodegradable polycaprolactone-based polyol (44% by weight), the exact chemical structure of which is registered as trade secret.

Scheme 1. Reaction between Isocyanate and Hydroxyl Groups



Scheme 2. Reaction between Isocyanate and Water

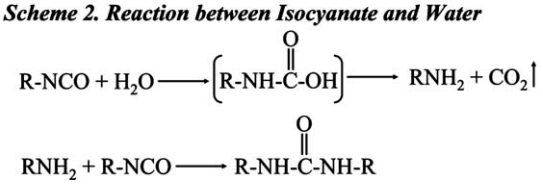


Figure 1. Moisture-based chemical reaction of polyurethane.

Initially a spray system was considered to apply the polyurethane directly. However, a large volume fraction of voids was formed during the polymerization resulting in high porosity foam, which limited its mechanical properties. To address this issue, a small amount of water was added to the mixture to initiate crosslinking in the polyurethane.[14,18] The condensation reaction that occurs with water drives the polyurea reaction and releases carbon dioxide gas (see Figure 1) as a byproduct that produces a foam structure with variable porosity depending on the fraction of water addition.[19] The result can vary from the high-porosity foam with about 80% voids and 2-3 mm pores, to the dense foam with micro porosity and interconnectivity. The latter foam structure has improved mechanical properties and the interconnecting pores can promote ingrowth of cells and tissue, which is preferable for tissue regeneration.[13]

To combine the water with the polyurethane compound, an ultrasound bath was used for 1 min while the bath was periodically agitated to release dissolved gasses. The ultrasound mixing improved polymerization and resulting mechanical properties and dramatically reduced the time to achieve proper consistency for application ranging from 25 min down to 10 min. This is important in a clinical application where the preparation time and curing time should be minimized. After 10 min of sonicated mixing and reaction, the adhesive obtained the consistency of a foamy paste, which proved optimal for brush-on application. FS30 Mechanical Ultrasonic Cleaner (Fisher Scientific, Pittsburg, PA) was used for sonication.

To improve biocompatibility and mechanical performance, and promote osteoconduction, HA nanoparticles of size $\leq$200 nm

were added to water and separately sonicated. This suspension was then combined at 1% concentration by volume with the above polyurethane-based paste and stirred to promote shear mixing of the HA and polymer adhesive. The paste adhesive with and without the nanoparticles was then compared with bone cement as a baseline. Commercially available two-component self-polymerizing acrylic bone cement (methyl metacrylate) was obtained from Heraeus Medical Components, St. Paul, MN, developed for hip, knee and shoulder defects (Palacos R). The cement contained no antibiotics. The physicochemical properties of this and similar commercial cements were reviewed by Lewis[20] and will not be repeated here for brevity.

Shear Strength Testing

Shear strength was measured using shear lap test involving two lap elements bonded together. For these tests, a clear acrylic adherent was chosen because of the difficulty in obtaining bone samples of the appropriate geometry and size. Also, this choice allowed visual observation of the failure behavior and conformed to existing standards to make comparison of results with other adhesives. The acrylic adherent was abraded with 120 grit sandpaper in the adhesive zone and thoroughly cleaned. Once the adhesive achieved paste consistency, it was applied to the acrylic adherent. A variety of adhesives were investigated including: the present polyurethane-based adhesive, the polyurethane-based adhesive reinforced with HA particles, and bone cement. The adhesive extended approximately 6.4 mm beyond the overlap length of 12.7 mm and was held in place with a 3 N clamp force according to recommendations in standard ASTM D 1002. In accordance with the standards a crosshead displacement rate of 1.3 mm/min was chosen. In polyurethane samples, the ratio of polymer to water used to produce the desired porosity was 7 parts polymer to 1 part water. Bonded samples were placed in an oven at 38 degrees C for 90 minutes. The samples were then tested for early properties after the 90 minute curing period and after 20 hours in ambient conditions to evaluate a fully cured state. For each test, early properties and cured state, 5 samples with the polyurethane-based paste, 4 samples with polyurethane reinforced with HA, and 4 samples with bone cement were tested. Testing was carried out on an MTS Insight 2 kN testing machine with Testworks 4 used in processing test data.

Compression Testing

Although a specific standard was not available for compression test of the composite adhesive, general guidelines from the ASTM D 695 were used as a reference for compression testing. To assess load bearing of the composite adhesives, bulk samples were prepared in 4-mL glass vials, which were broken after curing to produce cylindrical load elements. Compression samples were cylindrical in shape with a 2:1 height to diameter ratio. A ratio of seven parts polymer to one part water was used to produce desired low-porosity with pore sizes of 1 mm or less. There were two samples of each group tested (early properties, and fully cured). Compression testing was performed with steel platens at 1.3 mm/min displacement rate to 10% strain using the MTS Insight 2 kN testing machine.

Tensile Strength Testing

Bovine femur of an unknown age was obtained from Animal Sciences Laboratory at the University of Illinois at Urbana-Champaign and kept frozen until processing and use. The femur was sectioned and each piece of solid cortical bone was then abraded on a polisher until the cross sections were roughly rectangular with typical dimensions of $6.5 \times 18 \times 32$ mm^3. A precision saw was used to make a cut transverse to the longitudinal direction of the femur at 16 mm, which generated the surfaces to be bonded. The bone was kept moist with phosphate buffered saline (PBS) solution throughout processing. A liquid dentin bonding primer Clearfil^{TM} SE bond (Kuraray America, Inc., New York, NY) was tested as an amphiphilic agent to promote bonding with the polymer adhesive by application to the surface 10 min before the adhesive. A ratio of seven to one polymer to water was again used in the polyurethane adhesive preparation. A second group was tested using a two-part self-polymerizing poly(methyl methacrylate) bone cement with trade name Palacos R. The testing included three samples of polyurethane groups, two samples of bone cement without surface primer and one sample of bone cement with surface treatment. All samples were bonded under wet conditions, wrapped in PBS soaked gauze, and placed in an oven at 38°C for 2 h and 1 day time periods before testing. Flash was removed from the outside surfaces of the samples. The samples were cooled to room temperature before testing. Testing was performed with scissor grips at 1.3 mm/min displacement rate.

Next, titanium rods grade Ti6Al/4 V obtained courtesy of Nexxt Spine, LLC (Fishers, IN) with diameter 9.52 mm were bonded to the cortical bone on the outer longitudinal bone surface. The bovine cortical bone samples were cut and abraded on a polisher to flat surfaces with typical dimensions $6.5 \times 18 \times 16$ mm^3. The shafts of the rods were wrapped in Teflon tape to isolate the adhesive contact area. The bone surface was treated with dentin primer applied 10 minutes before the adhesive. Each test group consisted of three samples. A ratio of seven to one polymer to water was used in the adhesive preparation. All samples were bonded under wet conditions, covered in PBS soaked gauze, and placed in an oven at 38°C for 2 h and were cooled to room temperature before testing. Testing was performed with scissor grip on the bone and vice grips on the metal rods with a 1.3 mm/min displacement rate.

Cell Culture Assessment

C_2C_{12} myoblast cells were used for i*n vitro* biocompatibility testing. To test the adhesive for effects on cell adhesion, survival and differentiation, glass culture slides were coated with the polyurethane adhesive, soaked in water, then sterilized overnight under UV light to inhibit bacterial or fungal contamination. Slides were then soaked in cell culture medium for 6 h before plating with cells. Slides were placed in 10 cm culture dishes and seeded with 5.0×10^5 myoblasts in growth medium (low glucose Dulbecco's Modified Eagle Medium (DMEM Invitrogen, Carlsbad, CA) supplemented with 20% fetal bovine serum and 1% penicillin-streptomycin). After reaching confluence, myoblast cultures were switched to differentiation medium (low glucose DMEM supplemented with 5% horse serum and 1% penicillin-streptomycin) and allowed to undergo myogenic differentiation for four days. On the fourth day, slides were immunostained for sarcomeric myosin heavy chain expression using the monoclonal antibody MF20 (DSHB, Iowa City, IA) and AF488-conjugated goat anti-mouse IgG (Invitrogen, Carlsbad, CA). Samples were counterstained with blue-fluorescent DNA stain DAPI for visual identification of nuceli. Samples were observed on a Leica DMI4000B microscope (Leica Microsystems Inc, Wetzlar, Germany), and images were captured using a Qimaging Retiga 2000 camera (Qimaging,

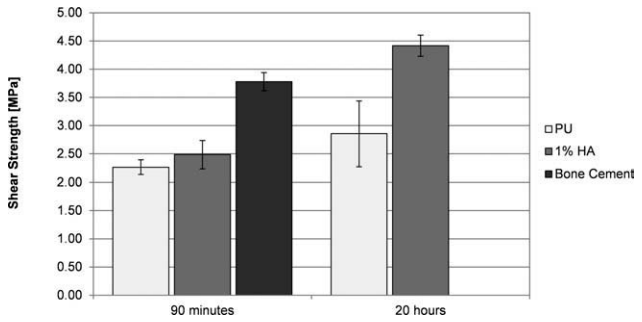


Figure 2. Results of lap shear tests showing ultimate shear strength of polyurethane (PU), polyurethane with 1% HA, and bone cement.

Surrey, BC, Canada) and Image Pro Plus software (Media Cybernetics Inc, Bethesda, MD). To determine if myogenic differentiation is enhanced or inhibited by culture on the adhesive, fusion indicies were calculated. Images from six 20X fields were randomly captured from immunostained samples on three independent adhesive slides and three glass coverslips. Nuclei present in myotubes were counted and presented as a percentage of total nuclei. Data are presented as mean ± SEM.

In Vivo Assessment

Biocompatibility testing was conducted using the hindlimb tarsus bone in adult *Xenopus laevis* as the model because of previous data on repair of long bone critical size defects.[21] Typical outside dimension of the tarsus was 1 mm with bone cross-sectional area of 0.26 mm^2. The size limitations of the species prevented adequate study of the adhesive bonding of bone *in vivo* with available surgical techniques. The polyurethane adhesive was prepared with 1% HA by volume. The procedure included removal of a 1-1.6 mm section of the tarsus bone in the posterior limb. A blunt hypodermic needle ensured placement of 0.2 mL of adhesive into the cut section. This joint section was advantageous due to the opposing bone maintaining the mechanical stability of the limb immediately following the procedure. A total of 6 specimens were used, 2 as a control that had a tarsus section cut, and 4 that received the adhesive in the cut section. At 15 days post surgery the animals were sacrificed and tarsus limb segment samples were fixed overnight in 4% paraformaldehyde and processed for cryosectioning. Sections of thickness 35 μm were obtained and stained with haematoxylin and eosin for routine histological analysis. Images were taken of the sections to determine the local cellular and immunological response to the adhesive. All surgeries and animal care were performed in accordance with the University of Illinois at Urbana-Champaign Institutional Animal Care and Committee (UIUC IACUC) procedures and approved protocols.

RESULTS

Shear Strength Results

Ultimate shear strengths of bone for the polyurethane adhesive, polyurethane adhesive reinforced with HA particles, and bone cement were compared (Figure 2). At the early stage (90 min), the polyurethane samples (unreinforced and reinforced with 1% HA) showed no difference while the bone cement samples showed superior properties. At 20 h, the polyurethane adhesive with 1% HA reinforcement showed significant improvement. The failures were primarily adhesive in nature for all groups (Figure 2). Some tests of the samples of the later abraded polyurethane and bone cement groups failed the adherents. Because of the fast cure rate the bone cement tested at 90 min represents nearly the full strength. Additional testing of sonication time showed a decrease in strength at times exceeding 1 min, but no negative effects at shorter times.

Compression Strength Results

Compression tests yielded the elastic modulus of foam samples with and without HA particles. The compressive strength was measured at 10% strain (Figure 3). The strain for measurement of the compressive strength was chosen based on the material behavior to be within the plateau stress region before the densification region and damage to the foam structure. The pure polymer and HA composite polymer had similar compressive strength. The results were not statistically significantly different at $P < 0.05$. Using the stress strain curves a Young's modulus for both polymer and polymer HA composite were calculated (Figure 4). The testing showed a lower modulus for the HA composite foam.

Porosity of the polyurethane adhesive with and without HA reinforcement was measured using an Archimedes principle. The adhesive foam with 1 wt % HA reinforcement had 46% porosity while the adhesive foam with no HA particles had 40% porosity. Also, the internal microstructures of the polyurethane foam with and without HA inclusions were observed through SEM imaging. The images indicate that the polyurethane samples contain mostly regular spherical cavities of around 200 μm diameter with smaller pores between cells with typical diameter of 3 μm (Figure 5). The polyurethane with HA inclusions contains more irregular voids with a greater range of sizes, averaging around 250 μm in diameter with smaller pores of about 5 μm (Figure 6). Although a good degree of porosity was observed for both samples, the interconnectivity of the pores, desired for cell penetration, appears to be somewhat inefficient. The foams with HA reinforcement contain more interpenetrating pores. Because recent works based on this new class of isocyanate-functional adhesives reported in literature investigate mostly biocompatibility issues,[21] tuning of the morphological characteristics of such new class of adhesives will be considered

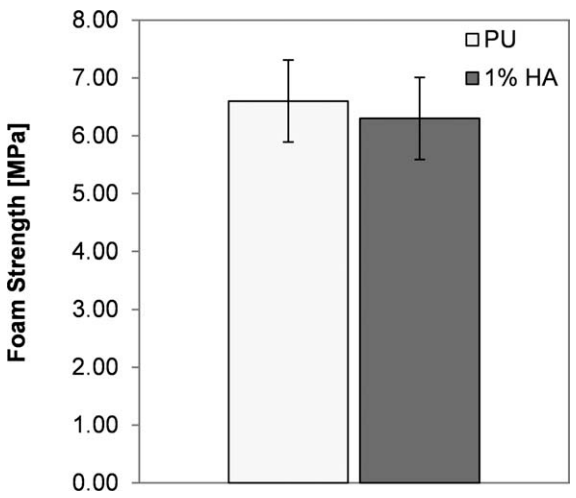


Figure 3. Compressive strength at 10% strain for polyurethane and polyurethane with HA.

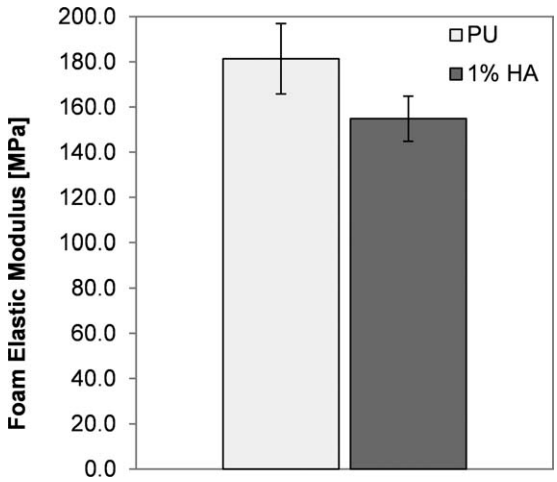


Figure 4. Compressive elastic modulus for polyurethane and polyurethane with HA.

in more detail in a future work. It is expected that by increasing the nanoparticle concentration or including other biomaterials into the adhesive formulation such as collagen, alginic polymers or other calcium containing nanoparticles will allow to further fine tune porosity and its characteristics.[22,23]

Bone Tensile Strength Results

All samples showed an adhesive failure with the bone surface (Figure 7). Also, all unprimed bone tensile samples showed lower strength. The application of the dentin primer before the adhesive was applied demonstrated a significant increase in bond strength for all groups. Bone cement was also tested for comparison and formed weaker bonds than the polyurethane samples.

The debonding failure strength under tension for a titanium rod adhered to the bone surface was tested (Figure 8). The adhesive mixed with HA resulted in a generally stronger bond force, but the degree of variance was also larger in this group.

Cell Culture Results

Cell culture testing was conducted on glass slides coated with polyurethane adhesive. Myoblast cells cultured in the medium on the adhesive samples were easily able to attach to the coated

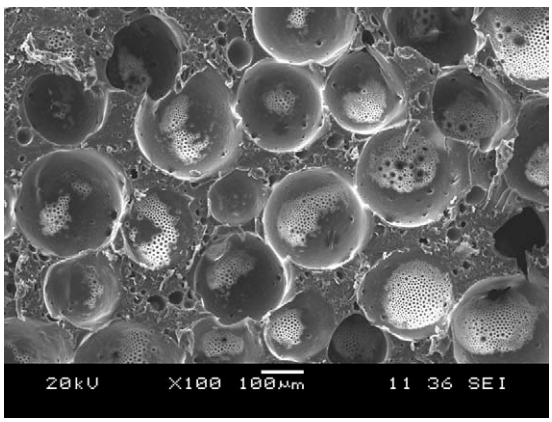


Figure 5. SEM image of polyurethane foam.

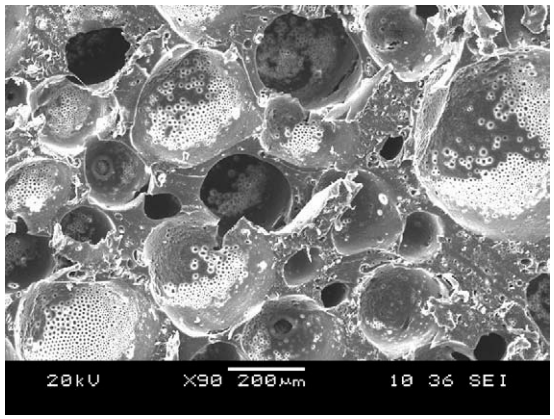


Figure 6. SEM image of polyurethane foam containing 1% HA.

slides. There was no apparent effect of the adhesive on viability or cell proliferation, as dead cells were not observed in the culture medium of dishes containing the adhesive-coated slides. In addition, cultures plated on the slides rapidly reached confluence at the same interval after plating seen on standard tissue-culture dishes (2.0–2.5 days after culture seeding—data not shown).

After reaching confluence, cells were induced to undergo myogenic differentiation by switching to growth-factor reduced medium (DMEM + 5% horse serum). As in control cultures, myoblasts cultured on the adhesive readily differentiated into myotubes, as indicated by their change in morphology and expression of sarcomeric myosin heavy chain. Myogenic differentiation was not inhibited or enhanced by culture on the adhesive, as the myotube fusion index for cultures grown on the adhesive was identical to cells grown on glass coverslips (Figure 9).

In Vivo Results

Samples used for *in vivo* biocompatibility tests showed osteoclasts remodeling the outer damaged bone surface in all samples, and the formation of a significant collar of periosteal cartilage, indicating the onset of the bone repair process. An immune response is visible in the form of clusters of immune cells in both the control specimens [Figure 10(C, D)] and experimental specimens treated with the adhesive [Figure 10(F, G)]. These clusters can be observed near the sutures used to

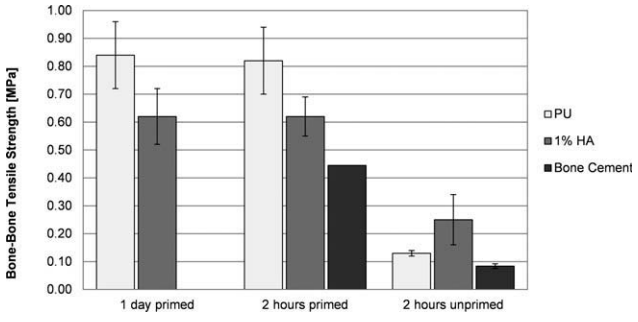


Figure 7. Bone-to-bone tensile bond strength for polyurethane, polyurethane with HA, and bone cement.

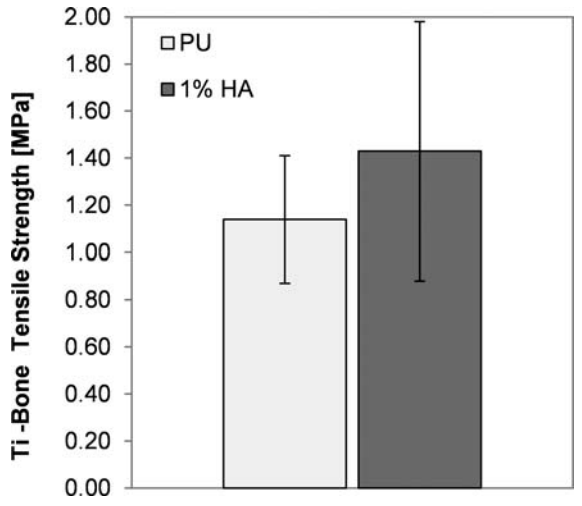


Figure 8. Results of bone to Ti rod bonding tests at 2 hours showing tensile strength of polyurethane and polyurethane with HA.

close the incision, as well as near areas containing the adhesive. Specimens treated with the adhesive showed a somewhat increased immune response compared to the control. However, the immune reactions are localized to the immediate area of the adhesive, and no detrimental effects were observed near the distal or proximal ends of the bone, away from the fracture site. In addition, areas of muscle damaged during surgery can be observed, with necrotic fibers being cleared by macrophages and immune cells, and regenerating myofibers forming. Areas of muscle damage were not enhanced in adhesive-treated samples compared to controls.

DISCUSSION

The shear testing demonstrated the pure adhesive can achieve about 80% of its full strength within 90 min of application. The failures in the polyurethane samples were almost all adhesive in nature. This indicates that the performance was limited by the given time for the adhesive to bond to the acrylic rather than the maximum cohesive strength of the adhesive developed through curing. The bone cement is chemically similar to the adherent and likely contributed to its high bond strength even without abrading the surface. All of the composite samples with HA showed higher strength.

The compressive modulus showed a decrease with HA content. Although HA has a higher modulus than the polymer, it did not effectively transfer the potential reinforcement effect possible for the composite. The observed variations in the pore structure could account for the lower modulus measured on a larger scale sample even if local properties of the material were higher. In addition, these results can be explained by higher porosity of the polymer foam with HA particles (46%) than that of the nonreinforced polymer foam (40%). The overall compressive strength did not significantly decrease for the samples with HA inclusions, thus the HA is still a recommended addition because of the benefits of the larger and more interconnected pores for potential cell infiltration with the foam.

The chosen bovine bone test sections were solid cortical bone with no visible porosity and a flat surface. These sections represent the most challenging scenario for bonding because such smooth surface does not allow for mechanical interlocking with the adherent, but instead requires the intermolecular forces at the interface to bear the load. This makes proper wetting of the surface by the adhesive very important and in this system an amphiphilic primer proved to help overcome the surface energy mismatch with wet bone. In many existing studies on potential bone adhesive agents the bone surface was dry or it was not stated that wet conditions were maintained during the application of the adhesive to replicate reasonable conditions expected *in vivo*.[3,8,22] This mitigates the wetting and surface energy problem at the interface, which leads to higher adhesion strength results than would be achievable with wet conditions.

The surface primer used in this investigation was not optimized for use on bone material or for the adhesive used. However, the nearly two-fold increase in strength that it promoted in our polymer and over fourfold increase with bone cement demonstrate the importance of this component in any adhesive system. Our adhesive showed a fourfold better adhesion on unmodified bone and nearly twofold better adhesion to primed bone compared with bone cement. This favorable result is not unexpected because bone cement is intended to fill space and primarily uses

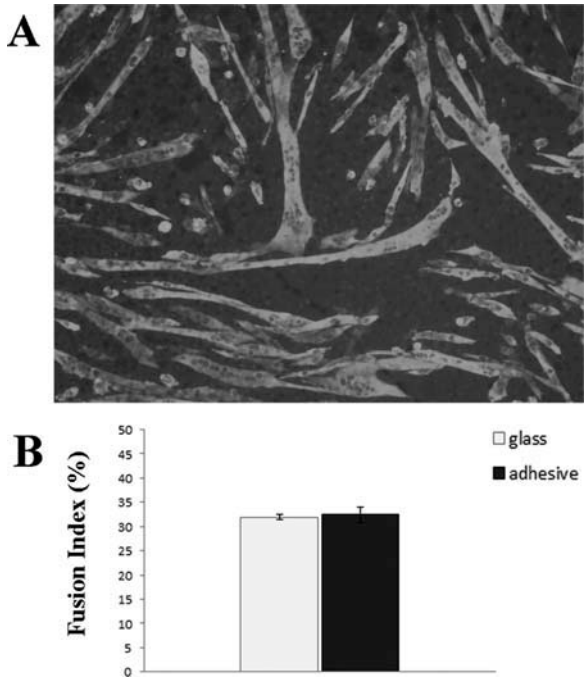


Figure 9. Cell culture testing of polyurethane adhesive. (A) Differentiation of multinucleate myotubes from myoblast cultures is observed on polyurethane coated slides. Myotubes express sarcomeric myosin heavy chain (green). Nuclei are labeled with DAPI (blue). (B) Fusion indices (% nuclei in myotubes) calculated from cultures differentiated on glass or polyurethane adhesive indicate myogenic differentiation is not influenced by culture on the adhesive. Data are mean ± SEM. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

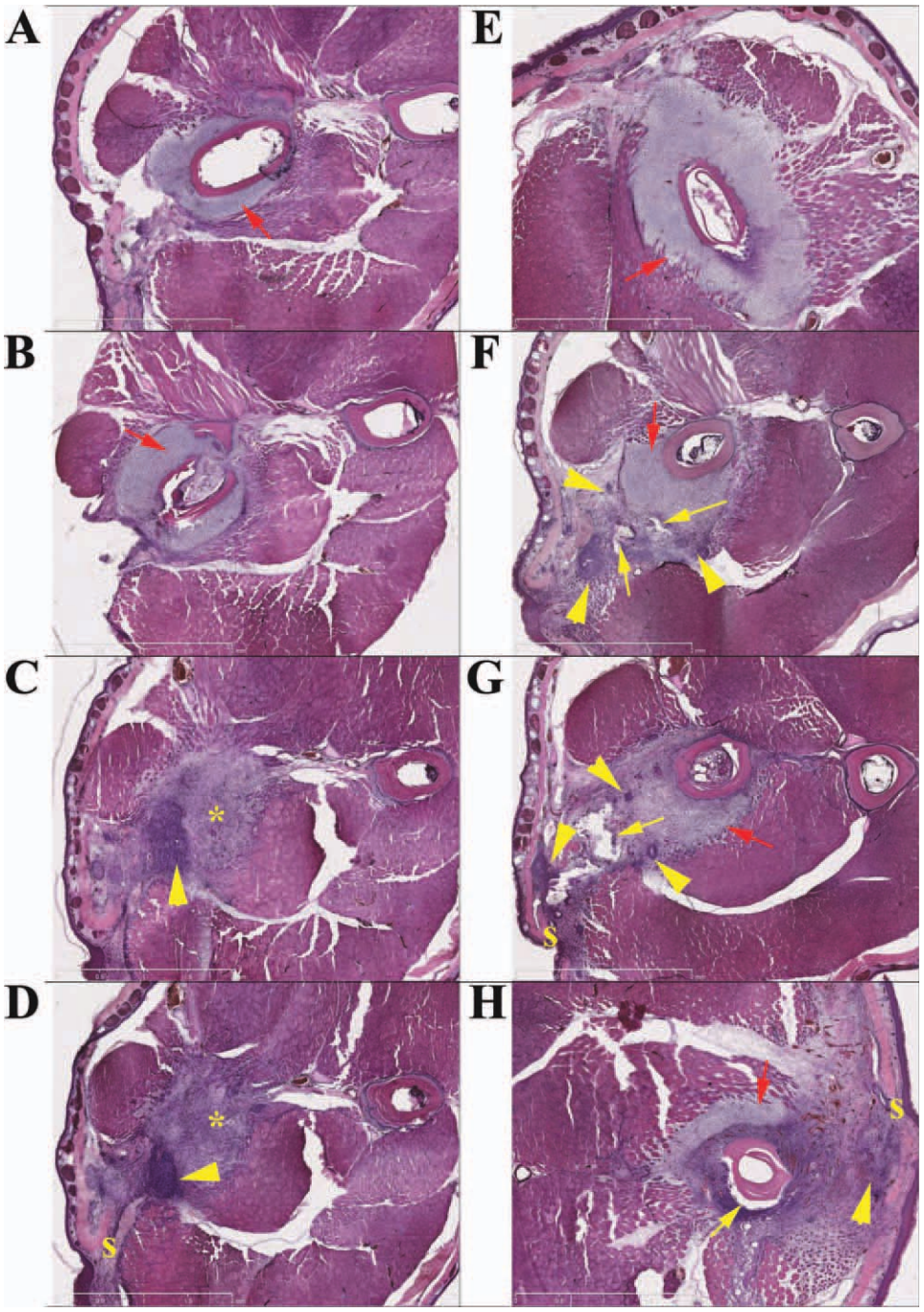


Figure 10. (A–D) Histological cross sections from control animals taken near the bone defect site. (A, B) Sections taken from areas proximal to (A) and at the fracture site (B) show periosteal cartilage formation (red arrow). (C, D) Sections from a different control animal taken from the gap between the fractured ends of the bone show precartilaginous granulation tissue (asterisk) along with a large mass of immune cells (yellow arrowheads). A suture insertion site (s) can be seen in (D). (E–H) Histological sections from near the bone defect site taken from three different animals [(E), (F–G) and (H)] treated with adhesive. (E) shows a section proximal to the bone fracture site demonstrating periosteal cartilage (red arrow) formation with no immune response evident. (F–G) show sections near the fracture site displaying areas of immune cell concentration (yellow arrowheads) and concentrations of adhesive (yellow arrows). A site of suture insertion can be seen in (G). (H) section from another adhesive-treated animal near the fracture site with an area of adhesive near the bone (yellow arrow) and a subdermal area of diffuse immune cell infiltration near a suture insertion site (s). Scale bar for all image = 2 mm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

mechanical interlocking with pores and friction to rigidly hold its placement against bone.[2,8] This improvement is quite encouraging for applications that conventionally use bone cement, and it may also help reduce the use of mechanical fasteners in some specific cases where the stress levels given in Figures 7 and 8 are acceptable.

The bone to metal rod testing had a larger variation compared to the other testing methods. The variation was due in part to the difficulty of the test method. To obtain the tensile strength performance required careful control from the grips to make the bonded surface perpendicular to the applied force. Any slight misalignment resulted in the rod acting as a lever for the moments creating an asymmetric stress distribution across the bonded area and premature failure of the bond. Also, the level of standard deviation can be high when working with biological materials due to the inherent variations in geometry, chemical composition, and microstructure.[2]

Initial biocompatibility testing was performed *in vitro* using mouse myoblastas. Cell tests were conducted on glass slides coated with polyurethane adhesive. Myoblast cells cultured in the medium on the adhesive samples were able to attach to the coated slides, and were able to grow and differentiate normally (Figure 9). For *in vivo* biocompatibility testing, we used a fracture of the hindlimb tarsus bone in adult *Xenopus laevis* as a model system, based on our previous work on repair of long bone critical size defects in this animal.[24] Images of histological sections from the control and adhesive groups were taken from in and around the defect area (Figure 10). Although our observations demonstrated that an immunological response was visible in areas of adhesive deposition in experimental samples, there was no widespread immunological response throughout the tarsus bone segment, nor was there any observation of widespread tissue necrosis in samples treated with the adhesive. Normal histological observations of fracture repair and muscle damage repair were evident in the adhesive-treated samples, similar to the normal histological responses observed in the control specimens.

This study has several limitations. The amount of water used to prepare the adhesive influences polymerization and thus the final microstructure of cured adhesive. The process of preparation of the adhesive, detailed in section 2.1, which was done prior to placing the adhesive in the *in vivo* environment, involved a specific amount of water. This initial amount of water influences the initiation of crosslinking and early curing stage. The excess water *in vivo* in the later stages of curing is expected to have lesser effect on the final foam structure. However, this issue needs to be further investigated and will be the subject of a future study. Second, it is postulated that the calcium phosphate particles can improve osteoconductivity and increase initial spread of serum proteins compared to the polymer surface.[3] The increased interconnectivity of the pores observed in the sample prepared with HA may also be beneficial to cell migration and ingrowth, and this is recommended as an avenue for future study. Furthermore, the addition of bioactive compounds should be investigated as they may potentially deliver bone growth factors to a fracture site. Finally, long-term observations and tests with additional species are needed to demonstrate full biocompatibility.

CONCLUSIONS

An adhesive for bone to bone bonding applications was developed consisting of polyurethane foam matrix and reinforcing HA crystals. Shear and tensile strength results were obtained and compared with bone cement strengths. Compression test was used to measure elastic moduli of adhesives. Microstructures were imaged using scanning electron microscopy. In terms of mechanical testing, under tensile loading our adhesive showed a fourfold better adhesion on unmodified bone and nearly twofold better adhesion to primed bone compared with bone cement. This improvement is quite encouraging for applications that conventionally use bone cement, and also shows promise to reduce mechanical fasteners in certain applications. In terms of biocompatibility, *in vitro* tests showed normal growth and differentiation of cells on the adhesive. *In vivo* histological examination showed elevated immunological response, but limited only to close vicinity of the adhesive.

ACKNOWLEDGMENTS

We gratefully acknowledge the support from the Grainger Foundation and the National Science Foundation (CMMI 09-27909, Dr. Ken Chong). We also thank Mr. Patrick Redwood and Ms. Deepika Chitturi for their assistance in *Xenopus laevis* surgeries and histology.

REFERENCES

1. Schortinghuis, J.; Bos, R. K. M.; Vissink, A. *J Oral Maxillofac Surg* **1999,** *57*, 130.
2. Endres, K.; Marx, R.; Tinschert, J.; Wirtz, D. C.; Stoll, C.; Riediger, D.; Smeets, R. *Biomed. Eng. Online* **2008,** *7*, 16.
3. Maurer, P.; Bekes, K.; Gernhardt, C. R.; Schaller H.; Schubert J. *Int. J. Oral Maxillofac. Surg.* **2004,** *33*, 377.
4. Shermak, M.; Wong, L.; Inoue, N.; Crain, B.; Im, M.; Chao, E. *Plast. Reconstr. Surg.* **1998,** *102*, 309.
5. Grossterlinden, L.; Janssen, A.; Schmitz, N.; Priemel, M.; Pogoda, P.; Amling, M. *Biomaterials* **2006,** *27*, 3379.
6. Heiss, C.; Kraus, R.; Schluckebier, D.; Stiller, A.; Wenisch, S.; Schnettler, R. *Eur. J. Trauma* **2006,** *32*, 141.
7. Mano, J. F.; Sousa, R. A.; Boesel, L. F.; Neves, N. M.; Reis, R. L. *Compos. Sci. Technol.* **2004,** *64*, 789.
8. Smeets, R.; Riediger, D.; Wirtz, D.; Marx, R.; Endres, K., Materialwissenschaft und Werkstofftechnik **2007,** *38*, 178.
9. Santerre, J. P.; Woodhouse, K.; Laroche, G.; Labow, R. S. *Biomaterials* **2005,** *26*, 7457.
10. Hafeman, A. E.; Zienkiewicz, K. J.; Carney, E.; Litzner, B.; Stratton, C.; Wenke, J. C.; Guelcher, S. A. *J. Biomater. Sci. – Polym. Ed.* **2010,** *21*, 95.
11. Lipatova, T. E. In Biopolymers/Non-exclusion HPLC; Advances in Polymer Science 79, Springer-Verlag: Berlin Heidelberg, **1986.**
12. Eglin, D.; Mortisen, D.; Alini, M. *Soft Matter* **2009,** *5*, 938.
13. Guelcher, S. *Tissue Eng. Part B: Rev.* **2008,** *14*, 3.
14. Guelcher, S.; Patel, V.; Gallagher, K.; Connolly, S.; Didier, J.; Doctor, J. *Tissue Eng.* **2006,** *12*, 1247.

15. Liu, H.; Zhang, L.; Zuo, Y.; Wang, L.; Huang, D.; Shen, J.; Shi, P.; Li, Y. J. *Appl. Polym. Sci.* **2009,** *112*, 2968.
16. Harikrishnan, G.; Umasankar Patro, T.; Khakhar, D. V. *Ind. Eng. Chem. Res.* **2006,** *45*, 7126.
17. Guan, J.; Song, Y.; Lin, Y.; Yin, X.; Zuo, M.; Zhao, Y.; Tao, X.; Zheng, Q. *Ind. Eng. Chem. Res.* **2011,** *50*, 6517.
18. Guelcher, S.; Srinivasan, A.; Hafeman, A.; Gallagher, K.; Doctor, J.; Khetan, S. *Tissue Eng.* **2007,** *13*, 2321.
19. Kricheldorf, H. R.; Nuyken, O.; Swift, G. Handbook of Polymer Synthesis; Marcel Dekker: New York, **2005.**
20. Lewis, G. J. *Biomed. Mater. Res.* **1997,** *38*, 155.
21. Feng, L.; Milner, D. J.; Xia, C.; Nye, H. L. D.; Redwood, P.; Cameron, J. A.; Stocum, D. L.; Fang, N.; Jasiuk, I. *Tissue Eng. Part A* **2011,** *17*, 691.
22. Perry, M.; Youngson, C. *Br. J. Oral Maxillofac. Surg.* **1995,** *33*, 224.