Journal of the Mechanical Behavior of Biomedical Materials 82 (2018) 329–337

Contents lists available at ScienceDirect

Journal of the Mechanical Behavior of Biomedical Materials

journal homepage: www.elsevier.com/locate/jmbbm

Acute exposure of white-tailed deer cortical bone to *Staphylococcus aureus* did not result in reduced bone stiffness

Ariel Nicole Kunde, Victoria Jane Frost, Meir Max Barak*

Department of Biology, Winthrop University, Rock Hill, SC 29733 USA

ARTICLE INFO

Keywords:
Cortical bone
Stiffness
Osteomyelitis
Staphylococcus aureus

ABSTRACT

Staphylococcus aureus (*S. aureus*) is the main source of osteomyelitis in adults. The end-result of untreated osteomyelitis is bone necrosis and distraction of bone structure. While bone tissue can heal and remodel its structure to ameliorate its mechanical properties, so far no study has tested the mechanical properties of cortical bone tissue exposed to *S. aureus*. With the increase usage of bone banks as a source of bone graft supply, it is important to screen for any possible pathology that may affect the bone graft success to function normally in the receiving patient. This study tested the effect of acute exposure to *S. aureus* on cortical bone stiffness. We have postulated that the incubation of cortical bone with *S. aureus* for 48 h will result in a significant decrease in bone stiffness. Sixty-five bone cubes (2 × 2 × 2 mm) were prepared from the cranial and caudal aspects of four white-tailed deer mid-diaphysis humeri. First, all bone samples were tested to determine their stiffness in the three principle orientations (axial, radial and transverse). Next, bone samples were incubated for 48 h with *S. aureus* (32 cubes, experimental group) or with sterile distilled water (33 cubes, control group). Finally, all cubes were mechanically tested again and each stiffness value was compared to the original value obtained from the same cube. Our results revealed that overall, acute exposure to *S. aureus* did not significantly decrease bone stiffness and thus our working hypothesis could not be supported. Therefore, our findings support the current tissue collection screening methods employed by bone-graft banks.

1. Introduction

Osteomyelitis is bone inflammation caused by bacterial infection that can be classified based on the duration of infection (chronic or acute), etiology (Waldvogel classification system), or based on anatomic and physiologic characteristics (Cierny-Mader staging system). Acute osteomyelitis is typically thought of as progressing over several days or weeks (Lew and Waldvogel, 2004) and occurring before osteonecrosis (Chihara and Segreti, 2010). Chronic osteomyelitis on the other hand, evolves over months or years, typically recurs in the same area and is accompanied by bone destruction (Lew and Waldvogel, 2004).

Although polymicrobial presence is usually seen at infection sites of osteomyelitis, *Staphylococcus aureus* (*S. aureus*) is the most commonly isolated bacteria (Chihara and Segreti, 2010). *S. aureus* causes 60–90% of bone infections in children and more than 95% of infections in adults (Rosa et al., 2015). *S. aureus* contain several virulence factors such as adhesins, toxins, and other protein-degrading enzymes that enhance its invasiveness and pathogenic ability (Cassat et al., 2013; Cunningham et al., 1996; Dinges et al., 2000). Rydén et al. (1997) proposed that *S. aureus* have developed cell receptors that recognize and bind to amino acid sequences in extracellular matrix proteins of bones as an evolutionary mechanism to increase virulence (Rydén et al., 1997). Adhesins, for example, are cell surface receptors that allow the pathogen to gain access to the host by binding to laminin, fibrin, collagen, and other components of bone matrix (Foster, 1996; Tong et al., 2015).

The end-result of untreated osteomyelitis is bone necrosis and distraction of bone structure, which leads to a decrease in the ability of bone to support physiological loads. While bone tissue can heal and remodel its structure to ameliorate its mechanical properties (such as stiffness and strength), so far no study has tested the mechanical properties of cortical bone tissue exposed to *S. aureus*. Several researchers have developed experimental models for creating bone infection in rabbits (Del Pozo et al., 2009; Gaudin et al., 2011), rats (Rissing et al., 1985), dogs (Fitzgerald, 1983), mice (Li et al., 2008), and sheep (Kaarsemaker et al., 1997), however these works focused on the pathogenesis and treatment of osteomyelitis in vivo and did not address the possible effect of *S. aureus* infection on bone quality. Currently, according to the Food and Drug Administration's guidance for human cell, tissue, and cellular and tissue based product donors (HCT/

* Corresponding author.
E-mail addresses: kundea2@mailbox.winthrop.edu (A.N. Kunde), frostv@winthrop.edu (V.J. Frost), barakm@winthrop.edu (M.M. Barak).

https://doi.org/10.1016/j.jmbbm.2018.04.003
Received 22 July 2017; Received in revised form 15 March 2018; Accepted 3 April 2018
Available online 04 April 2018
1751-6161/

Ps), eligible donors must not have had a diagnosis of sepsis "immediately preceding death," but there is no mention of previous osteomyelitis diagnosis as a donor restriction (USDHHS, 2007). Furthermore, the American Association of Tissue Banks requires that bone-graft banks currently subject donors to an extensive physical and medical history review for risk of complications with transplantation before accepting a tissue, however the donor suitability screening only restricts tissue that has or is being treated for bacterial infection at time of examination (AATB, 2016). More research which will elucidate effects of *S. aureus* exposure and infection on the biomechanical properties of bone may lead to tighter guidelines for screening in relation to osteomyelitis history. With increased usage of bone banks as a source of bone graft supply, it is important to screen for any possible pathology that may affect bone quality and the bone graft success to function normally in the receiving patient.

Our study examines the effect that *S. aureus* inoculation has on cortical bone stiffness. As cortical bone mechanical properties depend on the quality and spatial arrangement of its collagen and mineral components (Augat and Schorlemmer, 2006; Sharir et al., 2008), we hypothesize that inoculation of cortical bone cubes with *S. aureus* for a period of 48 h (i.e. mimicking the onset of acute infection), will result in decreased bone stiffness. To test our hypothesis, we subjected cortical bone cube samples from the cranial and caudal aspects of the mid diaphysis of white-tailed deer humeri to compressional stress before and after they were inoculated with *S. aureus* for 48 h and measured their tissue stiffness (Young's modulus). A second set of cubes (control group) was subjected to the same protocol and timeline except for the *S. aureus* inoculation. This group served to verify that any stiffness decrease in the inoculated cubes (if existed) was not due to the time that had passed between the first and second compression tests or to structural damage caused by the first mechanical testing. The Young's moduli of the bone cubes within each group (control and experimental) were ultimately compared between the first and second mechanical testing to determine if bone stiffness was compromised after acute exposure to *S. aureus*.

2. Materials and methods

2.1. Bone sample preparation

Four white-tailed deer humeri were obtained from a local processing factory right after they were captured and killed. All humeri were intact with no sign of any pathology. Sex and age were unknown, but all bones had active growth plates, indicating these were juvenile individuals still in their growing phase. Sixty-five $2 \times 2 \times 2$ mm bone samples were cut using a low speed water-cooled diamond saw (TechCut 4 precision low speed saw, Allied Technologies). Thirty-two samples were cut from the cranial aspect and thirty-three from the caudal aspect of the bone. All cubes were cut parallel to the orthogonal axes of the bone (Fig. 1). Samples were stored frozen at −20 °C in individual 1.5 mL Eppendorf tubes containing a water soaked paper towel and were thawed in about 1 mL of water at 4 °C for 24 h before mechanical testing.

2.2. Inoculum preparation

S. aureus (ATCC-12600) were obtained from the American Type Culture Collection (Manassas, VA, USA). Upon arrival, the bacterial sample was immediately inoculated in nutrient broth (Difco™Nutrient Broth, BD) and incubated at 37 °C for 48 h. This initial inoculum was stored as 1 mL stock cultures in 40% glycerol at −80 °C. For each batch of cubes tested (4–6 bone cubes), 1 mL stock culture was thawed and added to 10 mL nutrient broth and then incubated at 37 °C for 48 h. Subsequently, 1 mL of the infected broth was transferred to a 10 mL new sterile nutrient broth that was incubated for another 48 h at 37 °C to be used as the test suspension for each inoculated group.

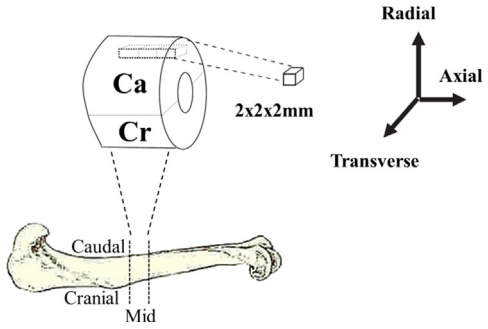

Fig. 1. A schematic illustration of the white-tailed deer humerus and the location from which the bone cubes were cut. The cubes (measured $2 \times 2 \times 2$ mm) were prepared from the humerus mid-caudal and mid-cranial regions, parallel to the three primary axes of the bone (axial, radial, and transverse).

2.3. Experimental timeline

Each experimental trial consisted of 4–6 bone cubes and lasted 6 days from time of thawing. Day one consisted of thawing the bone samples for 24 h. During day two, *S. aureus* was transferred to a new broth to grow for 48 h. Concurrently, each bone cube was loaded in compression for the first time. At the end of compression testing, each cube was set to sterilize in ethanol for 24 h. The cubes were removed from ethanol and washed with sterile distilled water (dH_2O) on day three. On day four, bone samples were rinsed once more with sterile dH_2O, surfaces swabbed and plated to verify sterility. All cubes from the group except one were inoculated with *S. aureus* for 48 h at 37 °C (experimental group). The last cube was kept in sterile dH_2O and served as a control (control group). On day six, all cubes were decontaminated with chlorhexidine gluconate and rinsed in dH_2O. One cube from the experimental group was crushed, made into a liquid suspension with dH_2O, and plated on nutrient agar to test for bacterial growth. The surfaces of the other cubes were swabbed and plated on nutrient agar. Finally, all the remaining experimental and control bone samples were tested in compression. To keep the control and experimental groups at similar sizes, several cycles of the above protocol were run with just control cubes. Overall, 32 cubes were included in the experimental group and 33 cubes were included in the control group.

2.4. Ethanol testing

All bone cubes required sterilization before beginning the inoculation process. The most common methods of bone sterilization include a variety of chemical agents and irradiation. Irradiation as a sterilization technique, however, has been shown to have a degrading effect on bone that significantly alters its biomechanical properties (Haimi et al., 2008). The ideal sterilization candidate for bone tissues undergoing mechanical testing is one that has a minimal effect on its mechanical properties. Evidence from previous studies suggests that bone preservation for short periods in ethanol contributes only a small change in pre-yield mechanical properties (i.e. bone stiffness) if the bone is washed with sterilized water before the experiment (Beaupied et al., 2006; Linde and Sørensen, 1993; Mick et al., 2015; Turner and Burr, 1993; Wieding et al., 2015). To verify that ethanol would not significantly affect our cortical bone samples stiffness, we have designed a preliminary experiment. Eight cortical bone samples (same dimensions and from the same bones used for our experiment) were loaded in compression both before and after 24 h in 91% ethanol and washing with sterile dH_2O. The results were analyzed with a non-parametric Wilcoxon signed-rank test to determine differences between pre and post-treatment stiffness in all three orientations. No significant difference was observed in the axial, radial or transverse orientations ($P > 0.05$).