Materials Science and Engineering C 42 (2014) 168–176

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

The influence of low concentrations of a water soluble poragen on the material properties, antibiotic release, and biofilm inhibition of an acrylic bone cement

Josh A. Slane [a,b,*], Juan F. Vivanco [b,e], Warren E. Rose [c], Matthew W. Squire [d], Heidi-Lynn Ploeg [a,b]

[a] Materials Science Program, University of Wisconsin-Madison, Madison, WI, USA
[b] Department of Mechanical Engineering, University of Wisconsin-Madison, Madison, WI, USA
[c] Pharmacy Practice Division, School of Pharmacy, University of Wisconsin-Madison, Madison, WI, USA
[d] Department of Orthopedics and Rehabilitation, University of Wisconsin-Madison, Madison, WI, USA
[e] Facultad de Ingeniería y Ciencias, Universidad Adolfo Ibañez, Viña del Mar, Chile

ARTICLE INFO

Article history:
Received 23 January 2014
Received in revised form 26 March 2014
Accepted 6 May 2014
Available online 22 May 2014

Keywords:
Bone cement
Infection
Drug release
Mechanical properties
Biofilm

ABSTRACT

Soluble particulate fillers can be incorporated into antibiotic-loaded acrylic bone cement in an effort to enhance antibiotic elution. Xylitol is a material that shows potential for use as a filler due to its high solubility and potential to inhibit biofilm formation. The objective of this work, therefore, was to investigate the usage of low concentrations of xylitol in a gentamicin-loaded cement. Five different cements were prepared with various xylitol loadings (0, 1, 2.5, 5 or 10 g) per cement unit, and the resulting impact on the mechanical properties, cumulative antibiotic release, biofilm inhibition, and thermal characteristics were quantified. Xylitol significantly increased cement porosity and a sustained increase in gentamicin elution was observed in all samples containing xylitol with a maximum cumulative release of 41.3%. Xylitol had no significant inhibitory effect on biofilm formation. All measured mechanical properties tended to decrease with increasing xylitol concentration; however, these effects were not always significant. Polymerization characteristics were consistent among all groups with no significant differences found. The results from this study indicate that xylitol-modified bone cement may not be appropriate for implant fixation but could be used in instances where sustained, increased antibiotic elution is warranted, such as in cement spacers or beads.

1. Introduction

Prosthetic joint infection is one of the most devastating complications that can occur following total joint replacement with a prevalence rate of approximately 2.2% for both hip and knee implants [1]. Often times, these infections are attributable to bacterial colonization through biofilm formation on the implant's surface, which makes treatment with traditional systemic antibiotics exceedingly difficult [2]. As a prophylactic measure against infection, antibiotics are often incorporated into bone cement in order to provide local drug administration at the implant site and avoid systemic toxicity concerns. Despite some positive outcomes with this technique [3], the relative hydrophobic nature of bone cement limits the amount of antibiotic that can be released and typically only ~10% of the total incorporated drug is eluted from the cement [4]. Moreover, the antibiotic release profile normally observed is characterized by a high initial burst release followed by a low, non-therapeutically effective phase [5] wherein biofilm formation may still persist.

In an effort to enhance antibiotic elution, inert soluble poragens can be added to bone cement. As the poragen dissolves in vivo, an interconnected porous network is left behind which increases fluid infiltration into the cement, theoretically leading to increased, prolonged antibiotic diffusion [4,6]. A wide variety of materials have been studied including soluble sugars [7–11], chitosan [12,13], tricalcium phosphate [14], and glycine [15]. These materials are typically employed in high poragen/cement loading ratios (e.g. 22 g xylitol per 40 g cement [16]) which have a significant adverse impact on the cement's mechanical properties rendering them unsuitable for prosthesis fixation in primary arthroplasty. Therefore, there is a necessity to investigate alternative cement formulations that utilize lower poragen concentrations in hopes of achieving high antibiotic release levels without inducing deleterious effects on the cement's mechanical properties.

Xylitol is a material that shows high potential as a filler within bone cement. It is a highly water-soluble crystalline powder that is non-immunogenic, inexpensive and widely available. Xylitol has demonstrated in animal models to affect bone metabolism and lead to increased bone density [17] and improved structural/mechanical properties [18].

* Corresponding author at: Materials Science Program, University of Wisconsin-Madison, 1513 University Avenue, Room 3046, Madison, WI 53706, USA. Tel.: +1 608 263 6692; fax: +1 608 265 2316.
E-mail address: jaslane@wisc.edu (J.A. Slane).

http://dx.doi.org/10.1016/j.msec.2014.05.026
0928-4931/

More importantly, it has been shown to have an inhibitory effect on bacterial adhesion against both *Staphylococcus aureus* [19,20] and *Pseudomonas aeruginosa* [21], two bacterial strains that are commonly isolated in cases of prosthetic joint infection. The action of xylitol does not prevent bacterial growth rather it can stop planktonic bacteria from adhering to a surface, which is the first step in biofilm formation [22]. Despite these potential benefits, the biofilm inhibition properties of a bone cement modified with xylitol have not been studied.

In order to extend the use of a xylitol-modified bone cement for load bearing applications, such as in primary arthroplasties or in articulating cement spacers used in revision procedures, a thorough understanding of the resulting impact on the cement's properties is needed. Therefore, the purpose of this study was to quantify the static mechanical properties, cumulative antibiotic release, biofilm inhibition properties and thermal characteristics of a commercially available antibiotic-loaded bone cement modified with various concentrations of xylitol.

2. Materials and methods

2.1. Bone cement preparation

Palacos R+G bone cement (Heraeus Medical GmbH, Wehrheim, Germany) was used as received for all testing. Each Palacos unit contained a 40.8 g powder sachet (with 0.5 g gentamicin pre-mixed) and a 20 mL methyl methacrylate monomer ampoule. Five experimental conditions were examined: standard Palacos R+G and the addition of 1, 2.5, 5 or 10 g of xylitol to a single cement unit. Prior to use, xylitol (≥99%, Sigma-Aldrich, St. Louis, MO) was crushed with a ceramic mortar and pestle and sieved to a particle size of 95–125 μm. The xylitol was combined with the powder component of the cement using a tumbler mixer operating at 2 Hz for approximately 2 min. The powder component of the control samples (plain Palacos R+G) was also mixed in the same manner to ensure consistency across all groups. Cement mixing was then performed by hand at atmospheric conditions according to the manufacturer's instructions. Once the dough phase of the cement was reached, the cement was transferred into custom aluminum molds and cured for 1 h. Following removal from the molds, samples to be used for mechanical testing were wet ground with 400 grit silicon carbide paper and cured using either a dry or wet method. In the former, samples were placed in atmospheric conditions for 24 ± 2 h prior to testing. For the wet method, samples were submerged in 15 mL of phosphate buffered saline (PBS) and placed in an incubator operating at 37 °C for 21 days. Samples used for gentamicin elution and biofilm inhibition testing were cured using the dry method only. It is important to note that xylitol is highly soluble in water [6] (0.66 g/mL) yet relatively insoluble in methyl methacrylate.

2.2. Mechanical testing

Mechanical testing was performed in air at ambient conditions using an electromechanical materials testing frame (Criterion C43.104, MTS Systems, Eden Prairie, MN) with force and displacement data recorded at 100 Hz. Static compression and four-point flexural testing were conducted in accordance with ISO 5833 [23], with the only modification being the addition of the wet curing process. These wet samples were kept hydrated at 37 °C until immediately prior to testing to mitigate the effect of sample drying and temperature change. A minimum of 7 and 10 samples were used for flexural (75 × 10 × 3.4 mm) and compressive (12 × 6 mm) testing, respectively. The ultimate compressive strength was calculated using the 2% offset method, as described in ISO 5833, and the compressive modulus was taken as the slope of the linear portion of the stress–strain curve. The flexural strength, σ_F, was calculated from

$$\sigma_F = \frac{3Fa}{bh^2} \tag{1}$$

where F is the applied load at failure, a is the distance between inner and outer supports (20 mm), b is the sample width and h is the sample thickness. The flexural modulus, E, was calculated using [24]:

$$E = \frac{a\left(3Lx - 3x^2 - a^2\right)}{12I}\,\frac{\Delta F}{\Delta d} \tag{2}$$

where L is the distance between outer supports (60 mm), x is the position at which deflection is measured, I is the area moment of inertia and $\Delta F/\Delta d$ is the slope of the linear portion of the force–displacement curve. In this study, displacement was measured at the position of load application; therefore, $x = a$.

Fracture toughness testing was conducted on a minimum of five wet cured samples (44 × 10 × 3 mm) using the single-edge notched beam method at a loading rate of 10 mm/min. A slow-speed diamond blade band saw operating under water irrigation was used to create a notch in each sample that was then sharpened with a fresh razor blade. The final crack length was between 0.45 and 0.55 of the sample thickness, as measured using a stereomicroscope. The mode I plain strain fracture toughness, K_{Ic}, was calculated from [25]:

$$K_{I_c} = \frac{3PL}{2bw^{3/2}}\,f(x) \tag{3}$$

where P is the maximum applied load, L is the lower span length (40 mm), b is the sample thickness and w is the sample width. The function $f(x)$ is a calibration factor that is dependent on the sample's crack length, a, and width:

$$f(x) = 1.93\left(\frac{a}{w}\right)^{1/2} - 3.07\left(\frac{a}{w}\right)^{3/2} + 14.53\left(\frac{a}{w}\right)^{5/2} - 25.11\left(\frac{a}{w}\right)^{7/2} + 25.8\left(\frac{a}{w}\right)^{9/2} \tag{4}$$

The microstructural morphology of the failure surface of wet cured four-point bending samples was investigated with scanning electron microscopy (SEM). Representative samples from each cement group were mounted on aluminum stubs covered with carbon tape and then sputter coated with gold for 30 s at 45 mA. Images were then obtained with a LEO DSM 1530 field emission SEM (Zeiss-LEO, Oberkochen, Germany) using an acceleration voltage of 5 kV and a working distance of 6–8 mm.

2.3. Porosity and gentamicin release

The porosity percentage of the cements were determined using Archimedes' principle [26]. Cylindrical samples (6 × 4 mm) were submerged in 10 mL of deionized water and the mass of the samples was monitored until there was no further change. Upon full hydration, the wet mass and submerged mass of the samples were determined with a density determination kit (A&D Weighing, Adelaide, Australia) and the porosity was calculated. All testing was performed in triplicate.

Five cylindrical samples (6 × 4 mm) from each experimental group were submerged in 5 mL of sterile PBS and placed in an incubator shaker operating at 37 °C and 60 rpm. At predetermined time intervals (1, 2, 4, 8, 10, 15, 25 and 45 days), 3 mL of the PBS was aspirated off and the sample was placed into fresh PBS. The aspirated fluid was then stored in cryotubes at −20 °C until time of analysis. The amount of gentamicin present in the collected PBS was determined through an indirect spectrophotometric method [27]. Briefly, a derivatizing solution was made by adding 2.5 g of *o*-phthaldialdehyde, 62.5 mL methanol and 3 mL 2-mercaptoethanol to 560 mL of a 0.04 M sodium borate solution. Equal portions of the collected PBS solution, *o*-phthaldialdehyde reagent and isopropanol (to prevent precipitates) were combined with a vortex mixer and allowed to react for 30 min at room temperature. The absorbance was then measured at 332 nm using a spectrophotometer (Varian Cary 300 UV-Vis, Agilent Technologies, Santa Clara, CA). A linear