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Mechanical, material, and antimicrobial properties of acrylic bone cement impregnated with silver nanoparticles

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ABSTRACT

Prosthetic joint infection is one of the most serious complications that can lead to failure of a total joint replacement. Recently, the rise of multidrug resistant bacteria has substantially reduced the efficacy of antibiotics that are typically incorporated into acrylic bone cement. Silver nanoparticles (AgNPs) are an attractive alternative to traditional antibiotics resulting from their broad-spectrum antimicrobial activity and low bacterial resistance. The purpose of this study, therefore, was to incorporate metallic silver nanoparticles into acrylic bone cement and quantify the effects on the cement's mechanical, material and antimicrobial properties. AgNPs at three loading ratios (0.25, 0.5, and 1.0% wt/wt) were incorporated into a commercial bone cement using a probe sonication technique. The resulting cements demonstrated mechanical and material properties that were not substantially different from the standard cement. Testing against *Staphylococcus aureus* and *Staphylococcus epidermidis* using Kirby-Bauer and time-kill assays demonstrated no antimicrobial activity against planktonic bacteria. In contrast, cements modified with AgNPs significantly reduced biofilm formation on the surface of the cement. These results indicate that AgNP-loaded cement is of high potential for use in primary arthroplasty where prevention of bacterial surface colonization is vital.

1. Introduction

The development of prosthetic joint infection (PJI) is one of the most devastating complications that can arise after total joint arthroplasty. Although the current incidence rates are 2.0 and 2.4% for hip and knee replacement procedures, these values are projected to steadily increase [1]. The development of PJI can cause severe physical and emotional pain to a patient while simultaneously placing a significant burden on the healthcare system in terms of cost and resource allocation. Often times, PJI is attributable to bacterial colonization through biofilm formation on the implant's surface, which makes treatment with traditional systemic antibiotics exceedingly difficult [2]. In response, one of the most common prophylactic techniques against PJI is to incorporate antibiotics into acrylic (PMMA) bone cement to prevent bacterial colonization and proliferation by providing local antibiotic delivery directly at the implant site [3].

The recent rise and spread of multidrug resistant (MDR) microorganisms has become a problem of significant importance worldwide. The widespread use of antibiotics over the past several decades has resulted in the development of genetic and biochemical mechanisms that allow bacteria to survive in antibiotic environments [4]. There has been significant concern over the efficacy of commonly used antibiotics within bone cement, particularly gentamicin, due to the aforementioned rise in MDR microorganisms [5,6]. For example, Hellmark et al. [7] obtained 33 clinical isolates of *Staphylococcus epidermidis* during PJI revision procedures and found a 79% resistance to gentamicin. Similar results were confirmed by Thornes et al. [8] who noted an increased resistance to gentamicin-loaded Palacos bone cement in a rat model. It is generally accepted that while the use of antibiotic-loaded bone cement reduces the possibility of PJI, there is an increase in the possibility of bacterial resistance development [9]. Thus, the problem of reduced antibiotic efficacy has created the need to investigate the potential of incorporating new antimicrobials into bone cement [5].

The use of metallic silver as an antimicrobial agent dates back to antiquity where it was commonly utilized to preserve drinking water and wine [10], however, the development of more potent antibiotics eventually displaced the utility of silver in the clinical setting. The availability of silver nanoparticles (AgNPs) has reopened the use of silver in medical applications since the high surface to volume ratio of nanoparticles imparts unique chemical and physical properties which greatly enhance the antimicrobial effects of silver [11]. Within recent decades, AgNPs have been incorporated into a wide array of consumer and medical products such as fabrics, textiles, plastics, cosmetics, catheters, stents, and wound dressings [12]. Despite this wide usage, the exact mechanism behind the antimicrobial properties of silver is still debated.

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Several mechanisms have been proposed including generation of reactive oxidative species, cationic damage to the bacterial cell membrane, silver–amino acid interaction, and silver–DNA interaction [13]. Regardless of which is correct, one key aspect that makes AgNPs an attractive antimicrobial agent is their well-documented activity against a broad spectrum of microorganisms.

Several previous studies have examined the feasibility of modifying acrylic cements with silver particles for dental and orthopedic applications. For example, Fan et al. incorporated silver benzoate into dental resins and found that the resin exhibited an inhibitory effect against *Streptococcus mutans* [14], however, they noted that silver benzoate concentrations above 0.2% wt/wt inhibited the resin from curing properly. Bone cement modified with silver–tiopronin nanoparticles displayed high antimicrobial efficacy against methicillin resistant *Staphylococcus aureus* [15]. Similar findings were observed by Alt et al. using bone cement loaded with silver nanoparticles against strains of methicillin-resistant *S. epidermidis and S. aureus* [16]. Contrasting results have also been found, most notably by Moojen et al. who reported that metallic silver in bone cement did not prevent methicillin-sensitive Staphylococcal infections in a rabbit model [17]. It is important to note that their study did not examine the cement's ability to prevent bacterial colonization, rather only the antimicrobial effect against planktonic bacteria distant from the embedded metallic silver. They speculated that since metallic silver nanoparticles must be ionized before possessing activity, the antimicrobial effect is delayed and primarily a localized effect that protects the cement surface from bacterial colonization [17]. This is an important characteristic of bone cement modified with silver nanoparticles since PMMA is very susceptible to bacterial adhesion and colonization [18], which is the first stage in the biofilm formation.

While previous studies have investigated various acrylics modified with silver nanoparticles, much of the previous work is limited in scope since many important mechanical and material properties have not been examined. Additionally, many of the techniques used to incorporate silver nanoparticles into acrylic cement utilize techniques that would be difficult to implement in a clinical setting, thus limiting their usefulness. Therefore, the purpose of this study was to modify a commercially available acrylic bone cement with various loadings of silver nanoparticles using a simplistic probe sonication technique. The resulting impact on the cement's mechanical properties, material properties, antimicrobial efficacy, biofilm inhibition and biocompatibility was quantified.

2. Materials and methods

2.1. Cement preparation

A commercially available poly(methyl methacrylate) bone cement, Palacos R (Heraeus Medical GmbH, Wehrheim, Germany), was used as received for all testing. A single cement unit contained a 40 g powder sachet and a 20 mL monomer ampoule. Silver nanoparticles (US Research Nanomaterials, Houston, TX, USA) had a primary particle diameter of 30–50 nm and were surface functionalized with polyvinylpyrrolidone (0.2% wt/wt). This functionalization was used to aid in particle dispersion and minimize agglomeration. Three loading ratios were examined: 0.25, 0.5 and 1.0% wt/wt (relative to the cement powder). The particles were incorporated into the liquid monomer using an ultrasonic homogenizer equipped with a solid titanium tip (150VT, Biologics Inc., Manassas, VA, USA). The monomer/AgNP mixture was sonicated for 12 min within a jacketed reaction vessel. To mitigate heating, cold water was continuously pumped through the vessel and the homogenizer was operated in a pulsed power mode. Standard Palacos (no AgNPs) was also prepared in the same fashion, to ensure consistency among all groups. The monomer/AgNP mixture and powder were then combined by hand in a polymer mixing bowl for 30 s, spaulated into aluminum molds, and allowed to cure for 30 min.

2.2. Mechanical characterization

2.2.1. Static mechanical testing

Four-point bending, compression and single-edge notched beam fracture toughness testing were conducted using methods we previously described [19]. Following cement polymerization, samples were wet ground to the proper dimensions using 400 grit silicon carbide paper. Samples were then allowed to cure for 48 ± 2 h in laboratory conditions (21 °C, 22% humidity) prior to testing. A minimum of eight, ten, and six samples were used for each testing method, respectively. All testing was conducted with an electromechanical materials testing frame with force and displacement data collected at 100 Hz (Criterion C43.104, MTS Systems, Eden Prairie, MN, USA).

2.2.2. Dynamic mechanical analysis

The viscoelastic properties (storage/loss modulus, tan delta) of the cements were assessed using dynamic mechanical analysis (RSA III, TA Instruments, New Castle, DE, USA). Flat beam samples (3 mm thickness, 9.91 mm width) were subjected to dynamic strain sweeps from 0.0005% to 0.08% at 37 °C using a three-point bending configuration with a 40 mm span. Strain was increased logarithmically with 30 points measured per decade. These strains are within the range found within the cement mantle surrounding a femoral prosthesis during the single stance phase of gait [20]. Loading frequencies of 1 and 10 Hz were used and a constant static force of 0.1 N was applied to ensure continuous contact with the bending fixture throughout testing.

2.3. Material characterization

2.3.1. Morphology

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

2.3.2. Chemical analysis

Compositional changes in the cement caused by the inclusion of AgNPs were monitored with Fourier transform infrared spectroscopy (Equinox 55, Bruker, Billerica, MA, USA) between 4000 and 750 cm^{-1} at a resolution of 2 cm^{-1}. Cement cross sections were scanned in attenuated total reflectance mode at three random sections on each sample, with 32 scans taken at each location. All collected data were averaged to obtain one spectra per cement.

2.3.3. Thermal characteristics

The glass transition temperatures, T_g, of the cements were determined with differential scanning calorimetry (Q100, TA Instruments, New Castle, DE, USA). Samples (5–7 mg) were sealed in aluminum pans and subjected to heating/cooling/heating cycles between 10–160 °C at 20 °C/min. The T_g was determined from the second heating cycle using the method described in ASTM D3418 [21].

The thermal degradation properties of the cement composites were investigated with thermogravimetric analysis (Q500, TA Instruments). Thermograms were obtained from 30–600 °C in a nitrogen environment at a linear heating rate of 20 °C/min. The initial and midpoint decomposition temperatures were determined, which are the points where 10% and 50% of the material had decomposed, respectively.

2.3.4. Elemental composition

The presence of AgNPs in the cements was confirmed using X-ray photoelectron spectroscopy (K-Alpha XPS, Thermo Scientific, Waltham, MA, USA). Thin cross-sections (~2 mm) were taken from cement beams with a water-irrigated slow speed diamond saw. Initial survey spectra