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Studies on the cytocompatibility, mechanical and antimicrobial properties of 3D printed poly(methyl methacrylate) beads

David K. Mills [a, b, *], Uday Jammalamadaka [a], Karthik Tappa [a], Jeffery Weisman [a]

[a] Center for Biomedical Engineering and Rehabilitation Science, USA
[b] The School of Biological Sciences, Louisiana Tech University, Ruston, LA 71272, USA

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ABSTRACT

Osteomyelitis is typically a bacterial infection (usually from Staphylococcus) or, more rarely, a fungal infection of the bone. It can occur in any bone in the body, but it most often affects the long bones (leg and arm), vertebral (spine), and bones of the foot. Microbial success in osteomyelitis is due to their ability to form biofilms which inhibit the wound healing process and increases resistance to anti-infective agents. Also, biofilms do not allow easy penetration of antibiotics into their matrix making clinical treatment a challenge. The development of local antibiotic delivery systems that deliver high concentrations of antibiotics to the affected site is an emerging area of research with great potential. Standard treatment includes antibiotic therapy, either locally or systemically and refractory cases of osteomyelitis may lead to surgical intervention and a prolonged course of antibiotic treatment involving placement of antibiotic-doped beads or spacers within the wound site. There are disadvantages with this treatment modality including insufficient mixing of the antibiotic, lack of uniform bead size, resulting in lower antibiotic availability, and limitations on the antibiotics employed. Thus, a method is needed to address biofilm formations in the wound and on the surface of the surgical implants to prevent osteomyelitis. In this study, we show that all antibiotics studied were successfully doped into PMMA and antibiotic-doped 3D printed beads, disks, and filaments were easily printed. The growth inhibition capacity of the antibiotic-loaded PMMA 3D printed constructs was also demonstrated.

1. Introduction

Osteomyelitis is a difficult-to-treat infectious disease that affects the young and the elderly [1,2]. Treatment of osteomyelitis has improved substantially over the years. However response to treatments vary considerably depending upon the mechanism of infection, its virulence, patient response to treatment, and the nature of the microbial pathogen causing the infection [3–5]. Chronic osteomyelitis requires surgical debridement and high dose antibiotic treatment, given either locally or systemically, for up to six weeks in cases of chronic osteomyelitis [1,4–6]. The selection of an antibiotic regimen in a patient is dependent on many different factors including the causative organism, co-morbidities, severity of the disease, pharmacological cost and concern over antibiotic resistance [1,6,7].

With systemic administration, penetration of the antibiotic into the affected bone is uneven and in some cases, is not effective [7], and the use of high dose concentration can cause other complications [2,6]. Systemic delivery of antibiotics affects the intended site as well as unaffected tissues, and for some patients, raises the risks of cytotoxicity, nephrotoxicity and the potential for an increase in antibiotic resistance [6]. It is clinically advantageous to have treatment without unnecessary and harmful side effects. Local delivery of antibiotics in the management of chronic osteomyelitis has the advantage of delivering high antibiotic concentrations at the site of infection without the systemic toxicity associated with the parenteral route [6,8–10]. The most commonly used non-biodegradable carrier material has been antibiotic-loaded bone cement poly(-methyl methacrylate) (PMMA) in the form of antibiotic-doped beads, nails, spacers or bone cement [11–14]. The constructs can provide high concentrations of broad-spectrum antibiotics to the most critical sites of infection; areas that cannot be reached by

* Corresponding author. Center for Biomedical Engineering and Rehabilitation Science, Louisiana Tech University, Ruston, LA 71272, USA.
E-mail address: dkmills@latech.edu (D.K. Mills).
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systemically delivered antibiotics [8,15]. There are, however, some disadvantages or concerns about the use of antibiotic-loaded PMMA including the high exothermic temperatures generated in PMMA polymerization, limitations on the types of antibiotics that may be employed, uneven antibiotic release rates, and the need for a second surgery to remove the PMMA [15,16].

In response, the use of bioabsorbable or biodegradable material has been developed in recent years that combine the local delivery of antibiotics in biodegradable and osteogenic materials [8,15,16]. These systems have been shown to be promising alternatives for the treatment of osteomyelitis because they can produce bactericidal concentrations for extended periods of time and without the toxicity associated with other delivery systems. Silicate-based systems [16] and bioglasses [17] have been studied for the treatment of chronic osteomyelitis. The local delivery systems that have shown the most promise include osteoconductive bioceramics (calcium sulfate, tricalcium phosphate or hydroxyapatite) or bioceramic composites infused with antimicrobial agents [18–21]. The key advantage of these systems is they bond strongly with bone, act as a void filler, and will convert to a type of hydroxyapatite and promote osteogenesis at the defect site [22,23]. Despite the accepted use of local antibiotic therapy, robust clinical data regarding Indications for use, optimum dosages, types of antibiotics, elution properties and pharmacokinetics are still poorly defined [16]. However, there is a consensus view that a therapy that provides high local drug concentrations for a sustained period is highly desired [6,8,15].

The key features of 3D printing (accuracy, speed, tunability) offer the potential for on-demand, customized, and patient-specific antibiotic treatments, and possibly a technology that can address the disadvantages with current antibiotic carrier systems. It has the potential to provide high concentrations of antibiotics delivered locally and without the harmful side effects seen with the systemic treatments. In our previous work, we have shown the ability to extrude plastic filaments with additives, including metal acetates, ceramic, and drugs (gentamicin, methotrexate) with dopant percentages of up to 25% by weight [26]. This method enables complete customization of the dopants added without inhibiting extruder or 3D printer functionality.[26,27]

In the present work, antibacterial 3D printing beads, disks, and filaments were developed using Fused Deposition Modeling (FDM) and our method for creating bioactive filaments.3D printed PMMA doped with gentamicin sulfate, tobramycin, and nitrofurantoin. It was found that through the manufacturing process, these compounds retained their anti-bacterial growth inhibition properties. The results suggest that 3D printed medical devices can be used as a reservoir for localized delivery of a single drug or a suite of drugs and offer the means to provide local therapeutic levels while avoiding systemic toxicity.

2. Materials and methods

2.1. Materials

All plasticware, such as 2 ml Eppendorf tubes, 96 well plates, and pipettes, were purchased from Mid Scientific, St. Louis, MO. For bacterial culture, 100 mm Mueller Hinton agar plates were purchased from Fischer Scientific (Hampton, NH) and *Escherichia coli* ATCC 11775 Vitroids 1000 CFU were from Sigma-Aldrich (St. Louis, MO). *Staphylococcus aureus* was a gift from Dr. Rebecca Girono, Louisiana Tech University. Gentamicin Sulfate (GS), tobramycin and nitrofurantoin were ordered from Sigma-Aldrich (St. Louis, MO). PLA pellets used for extruding filaments were obtained from Push Plastic (Springdale, AR), KJLC 705 silicone oil used for coating pellets was purchased from Kurt J. Lesker Company (Jefferson Hills, PA). Orthoset 3 Radiopaque PMMA bone cement from Wright Medical (Warsaw, IN) was used for 3D printing PMMA bone cements. ExtrusionBot filament extruder was bought from ExtrusionBot, LLC (Phoenix, AZ). MakerBot Replicator 3D printers were purchased form MakerBot (Brooklyn, NY), Nanodrop used for spectrophotometry was from Thermo Scientific (Wilmington, DE), Vulcan A550 Series Benchtop Muffle Furnaces from Thomas Scientific (Swedesboro, NJ) was used for heating biomaterials. OPTA reagent, isopropyl alcohol, and sodium tetraborate were ordered from Sigma-Aldrich (St. Louis, MO). For modeling 3D constructs, Solidworks 2015 was used. For 3D scanning of objects, A Roland Corporation LP-250 desktop 3D scanner (Osaka, Japan) was used.

2.2. Methods

It is critical to understand how 3D printed bioactive constructs compare to current antibacterial implants and in determining the value of this approach. PMMA bone cement is the current gold standard in implant material for the local delivery of antibiotics. All extruded filaments and constructs were tested against comparable commercial grade PMMA materials (used as controls).

2.3. Coating pellets

An oil coating method was used to enable an even dispersion of drugs on the surface of the PLA and PMMA pellets prior to extrusion. KJL 705 silicone oil was chosen to surface coat pellets because of its thermal stability at extrusion temperatures between 170 and 180 °C. The method required a 20 gm batch of pellets, to which was added 15 μL of silicone oil, and pellets were then vortexed to facilitate a uniform coating. These pellets were transferred to another container to avoid loss of drug powder due to adherence of residual oil on the surface of the mixing container. After switching containers, a calculated amount of a drug in powdered form (gentamicin sulfate, nitrofurantoin tobramycin), was added and the pellets were vortexed again.

2.4. Filament extruder

For extruding filaments from coated PLA pellets and PMMA, a first generation ExtrusionBot filament extruder was used. This device operates using a piston-based auger mechanism. Plastic pellets were fed into the hopper at the top of the device. The piston pushes the pellets down to, and through, a metallic die through a heated element. Sensors arranged around this heating element regulate the extrusion temperature. Dies of different diameter at the bottom of the device can be used to customize the thickness of the desired extruded filament.

2.5. Filament extrusion

The MakerBot 1st generation 3D printer used requires a filament diameter of 1.75 mm to print, accordingly, we used a metal die of the same diameter to extrude all filaments. Each 20-gm batch of GS-coated PLA pellets were extruded at 170 °C maintaining the extruded filament diameter at 1.75 mm. We also tried extruding below 170 °C, but that slowed down the extrusion speed and resulted in thicker filaments which were not suitable for 3D printing. At higher temperatures, PLA melted down completely and flowed through the metal die resulting in filaments of uneven diameter.

2.6. Optimization of 3D printing parameters

Preliminary trials revealed that the print-head temperature and filament feed rate have a direct influence on the material flow in