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Econazole-releasing porous space maintainers for fungal periprosthetic joint infection

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Abstract

While antibiotic-eluting polymethylmethacrylate space maintainers have shown efficacy in the treatment of bacterial periprosthetic joint infection and osteomyelitis, antifungal-eluting space maintainers are associated with greater limitations for treatment of fungal musculoskeletal infections including limited elution concentration and duration. In this study, we have designed a porous econazole-eluting space maintainer capable of greater inhibition of fungal growth than traditional solid space maintainers. The eluted econazole demonstrated bioactivity in a concentration-dependent manner against the most common species responsible for fungal periprosthetic joint infection as well as staphylococci. Lastly, these porous space maintainers retain compressive mechanical properties appropriate to maintain space before definitive repair of the joint or bony defect.

Graphical Abstract

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Electronic supplementary material

The online version of this article (<https://doi.org/10.1007/s10856-018-6073-1>) contains supplementary material, which is available to authorized users.

Modifying Bone Cement for Antifungal Therapy

Composition	Structure	Bioactivity
Polymethylmethacrylate
Polymethylmethacrylate + Econazole
Polymethylmethacrylate + Econazole + Carboxymethylcellulose

1 Introduction

In order to facilitate musculoskeletal reconstruction, space maintainers have been leveraged to preserve anatomy while also functioning as local drug depots to treat local infection. Space maintainers have been used in a variety of anatomical defects, including cranial, mandibular, and joint spaces [1–3]. These devices have been loaded with a variety of antimicrobials for the local treatment of osteomyelitis as well as periprosthetic joint infections [4].

While relatively rare, fungal periprosthetic joint infection (FPJI) is a clinically challenging condition that often results in significant patient morbidity, mortality, and economic burden. Resection arthroplasty, a major surgical procedure involving the removal of the prosthesis, is required in up to 94% of cases [5]. Persistent infection, disease progression leading to amputation, and mortality have been reported in up to 32, 13–16, and 3–5% of cases, respectively [2, 5].

Candida species are by far the most commonly reported in FPJI, accounting for 80–90% of infections [5–7] with *C. albicans* as most prevalent [5–7]. *Aspergillus* species are the next most commonly reported species with *A. fumigatus* as most prevalent [5]. In addition to FPJI, *Candida* and *Aspergillus* are also the two most common species associated with fungal osteomyelitis [8]. Another major complication arising in FPJI is co-infection with bacterial species (occurring in as many as 16–55% of cases [5, 9]). *Staphylococci* are the most common co-infecting bacterial species, reported in 34–80% of patients with bacterial co-infection [5, 9].

The current state of the art for the treatment of FPJI is two stage resection arthroplasty [8]. In the first stage, the infection is treated with extended systemic antifungals, surgically removing the infected prosthesis, debriding the area of pathogens and biofilm, and in some cases, implanting a bone space maintainer in the joint space (in addition to treatment with systemic antifungal therapy). The second stage is definitive prosthesis re-implantation and follows after infection is cleared (typically 6–24 weeks after the first stage [6]). The space maintainer is made of bone cement, polymethylmethacrylate (PMMA) and may be loaded with antimicrobials for local drug release. Antibiotic-loaded PMMA space maintainers have

been shown to be effective in the clinical treatment of bacterial periprosthetic joint infections [4]. However, there have been several key disadvantages in the use of antifungal-loaded PMMA space maintainers.

Many typical antifungals show very low elution from PMMA [10, 11]. For example, in a study of amphotericin B release from PMMA, only 0.03% of loaded drug was observed to be released and no drug was detectable after 1 week of elution [12]. Minimal antifungal elution from PMMA is most likely due to a combination of factors: (1) As PMMA is solid and non-biodegradable, release is limited to drug bound on its surface while the majority of drug remains encapsulated and unavailable for release; (2) heat generated from the exothermic reaction of PMMA polymerization may degrade or otherwise deactivate some antifungals; and (3) certain chemical groups within anti-fungals, such as the unsaturated carbon bonds within amphotericin B, may be disrupted during PMMA polymerization, resulting in limited bioactivity. Furthermore, space maintainers releasing traditional antifungals are at risk for bacterial co-infection [2, 5, 9].

In order to address these shortcomings, we have designed porous space maintainers capable of eluting antimicrobial agents at greater concentration and for longer duration than current solid space maintainers [13, 14]. Carboxymethylcellulose (CMC), also known as cellulose gum, is an inexpensive carbohydrate-based powder that can be reconstituted in water to form a biocompatible gel. CMC has been a component of implantable Food and Drug Administration-regulated devices for over two decades [15, 16]. CMC gel can be added to PMMA before polymerization to create a space maintainer with an embedded sacrificial aqueous gel; once the space maintainer is placed in an aqueous environment, the gel diffuses out of the space maintainer, leaving behind a network of interconnected pores within the PMMA [17]. These pores increase the available surface area for the elution of encapsulated drugs [13, 14]. Additional benefits to the use of CMC include its ability to act as a heat sink during PMMA polymerization, resulting in a lower reaction temperature [17]. Lastly, the roughness introduced to the space maintainer surface has been shown in a rabbit mucosal defect to promote healthy soft tissue interactions compared to traditional solid space maintainers [18].

In this study, porous space maintainers were loaded with econazole, an antifungal agent that has activity against *Candida*, *Aspergillus*, and *Staphylococcus* [19–21]. Potential chemical interactions between econazole and PMMA were characterized by proton nuclear magnetic resonance spectroscopy (^1H -NMR) and gel permeation chromatography (GPC). Compared to solid space maintainers, porous space maintainers were evaluated for radiographic features, antimicrobial bioactivity, and mechanical properties.

2 Materials and methods

2.1 Space maintainer fabrication

PMMA econazole-loaded space maintainers (porous and non-porous) at three different loading concentrations (Table 1) were prepared by established methods [17]. All constructs were prepared using a 2:1 powder phase bone cement: liquid phase bone cement (Great Lakes Orthodontics Limited, NY) wt/v ratio. As a ratio of 1:40 g antibiotic: bone cement

(2.5 wt%) has been defined as “low-dose” and ratio of 2:40 g antibiotic: bone cement (5 wt%) has been defined as “high-dose” for antibiotic-loaded space maintainers [22], 2.5 and 5 wt% were chosen as the loading concentrations in this study.

Briefly, econazole (Econazole Nitrate, Sigma-Aldrich, St. Louis, MO) was stirred in powder bone cement in a 5 wt% (“High”) or 2.5 wt% (“Low”) ratio. No econazole was added to “Blank” groups. For porous groups, a 9 wt% aqueous carboxymethylcellulose (CMC) gel was created by dissolving sodium carboxymethylcellulose (Spectrum Chemical, New Brunswick, NJ) in di-ionized water. CMC gel was mixed into porous groups in a 30 wt/wt% CMC/PMMA concentration [17]. After mixing, liquid phase bone cement was added and stirred vigorously. Upon doughing, the mixture was added to polytetrafluoroethylene molds and allowed to cure for 24 h. For elution and bioactivity assays, space maintainer discs of 6 mm in diameter and 1 mm height were fabricated. For mechanical compressive evaluation, cylinders of 6 mm in diameter and 12 mm in height were fabricated (per the standard ISO 5833 [23]). All constructs were conditioned at room temperature for at least 24 h before any testing was performed. Constructs subjected to microbiological tests underwent sterilization by ethylene oxide [14].

2.2 Polymer characterization

To better elucidate any potential interactions between loaded drug (econazole) and PMMA during polymerization, proton nuclear magnetic resonance spectroscopy was performed to determine any changes in chemical groups and gel permeation chromatography was performed to determine any changes in chain length. Given that potential interactions between CMC and PMMA have already been extensively evaluated in previous work [17, 24], solid space maintainers with a high loading dose of econazole (5 wt%) and with no econazole (blank) were evaluated.

2.2.1 ¹H-NMR spectroscopy—Ten milligram of fabricated solid blank space maintainer, solid high-dose space maintainer, and econazole alone were dissolved in 1 mL of deuterated chloroform (Sigma-Aldrich, St. Louis, MO) each. These samples were subjected to ¹H-NMR spectroscopy using a 400 MHz spectrometer (Bruker, Billerica, MA) and analyzed with TOPSPIN 3.0 software (Bruker).

2.2.2 Gel permeation chromatography—Specimens were dissolved in chloroform (Sigma-Aldrich, St. Louis, MO) at a concentration of 20 mg/mL under mild agitation at room temperature and filtered by injection through a 0.45 µm filter (Whatman, Kent, UK). The resulting solution was assayed for molecular weight by gel permeation chromatography (GPC) based on previously reported methods [17, 24]. The GPC system (Waters, Milford, MA) consisted of a pump (Waters Model # 155), injection module (Waters # 717), and refractive index detector (Waters Model # 410). GPC was performed ($n = 3$) at a flow rate of 1 mL/min and temperature of 30 °C using a Phenogel column (300 × 7.80 mm, 5 µm particle size, Waters) and calibrated using linear PMMA standards with molecular weights ranging from 2.58 to 981 kDa (Waters, Milford, MA). Number average molecular weight (M_n), weight average molecular weight (M_w), and polydispersity index (PI) were measured and reported.

2.3 Radiographic characterization

MicroCT was performed on discs (6 mm in diameter and 1 mm in height) from each group in Table 1 ($n = 3$ per group) with an x-ray tube voltage of 80 kV and current of 124 μ A with no filter and at a voxel size of 17.1 μ m. The images were reconstructed for analysis using the software NRecon (version 1.6.9.18, SkyScan) and CTAn (version 1.15.4.0, SkyScan) with a 15% beam-hardening correction and an 85–255 binary threshold. Total porosity and percentage open pores were calculated in CTAn.

2.4 Antimicrobial characterization

2.4.1 Zone of inhibition—Zone of inhibition analysis was performed on clinical isolates of fungi (*Candida albicans* Y4215 and *Aspergillus fumigatus* Af293) and on a commercially-available methicillin-susceptible *Staphylococcus aureus* (*S. aureus* ATCC29213). Discs (6 mm in diameter $\times$ 1 mm in height) from all groups in Table 1 ($n = 3$ per group per organism) were evaluated. Discs were tested based on standardized methods as defined by the Clinical and Laboratory Standards Institute [25] (Standard M44-A for fungal testing and Standard M02-A12 for *S. aureus*). Briefly, discs were placed on plates seeded with $1\text{--}2 \times 10^8$ CFU/mL of each organism (one type of organism per plate). These plates were incubated at either 35 °C (*C. albicans*) or 37°C (*A. fumigatus* and *S. aureus*). After either 18 h (*S. aureus*) or 24 h (*C. albicans* and *A. fumigatus*) of incubation, plates were examined for zone of inhibition. The diameter of the zone of inhibition was measured and rounded to the nearest millimeter.

2.4.2 Duration of bioactive elution—Discs (6 mm in diameter $\times$ 1 mm in height) were incubated in 2 mL sterile phosphate buffered saline (PBS) at 37°C under mild agitation ($n = 3$ per group in Table 1). At day 0.5, 1, 2, 3, and every other day afterwards, supernatant was collected, frozen, and replaced with fresh PBS. The ability of the supernatant to inhibit *C. albicans* Y4215 over time was evaluated. 100 μ L of supernatant from each disc at each time point was added to 100 μ L of 5×10^3 *C. albicans* (effectively diluting the supernatant at 1:1 ratio) and incubated for 48 h at 35 °C based on the minimum inhibitory concentration guidelines for *Candida* species (Standard M44-A for fungal testing). Wells consisting of fresh econazole (25 μ g/mL), fresh PBS, and fresh PBS that were not inoculated served as controls. After 48 h of incubation, wells were read and reported as having *C. albicans* growth or no growth.

2.5 Mechanical characterization

Cylinders from all 6 groups in Table 1 ($n = 5$ per group) were placed in 1 L di-ionized water at room temperature under mild agitation for 48 h to remove soluble CMC and then dried by lyophilization [17, 24]. Compressive and bending testing was then performed as specified by ISO 5833 [23]. Briefly, a Mini Bionix 858 Mechanical Testing System (MTS Systems Corporation, Eden Prairie, Minnesota) with a 10 kN load cell was utilized for both compression and bending assays. For compression testing, a compressive force at a cross-head speed of 20 mm/min was applied until either fracture or passing of the upper yield point ($n = 5$ per group in Table 1). The compressive strength (determined by 2% offset yield) and compressive modulus was measured using TestWorks 4 (MTS Systems Corporation).

Bending modulus and strength was measured in accordance to ISO 5833 [23] with a modification in the calculation of modulus as explained below. The distance between the inner and outer loading points was 20 mm and the distance between the two outer loading points was 60 mm. Force was applied at a cross-head speed of 5 mm/min until specimens failed ($n = 4$ per group in Table 1). Data were collected using the software TestWare-SX as provided by MTS Systems Corporation. Bending modulus, as calculated from ISO 5833, requires the slope of force/displacement data between a force of 15 and 50 N. However, as porous PMMA had an ultimate bending force of less than 50 N, the slope of force/displacement from tare force to yield force was used to calculate modulus. Otherwise, bending strength and modulus were calculated as described in ISO 5833.

2.6 Statistical analysis

Total porosity, percentage open porosity, diameter of zones of inhibition, compressive offset yield strength, and compressive modulus were compared by a one-way analysis of variance with post hoc analysis by Tukey's honestly significant difference ($\alpha = 0.05$) and molecular weights (number average, weight average, polydispersity index) were compared by a two-tailed T -test ($\alpha = 0.05$). All statistics were performed using JMP Pro 11.0 (SAS, Cary, NC).

3 Results

3.1 Space maintainer fabrication

Space maintainers were successfully fabricated as both discs (6 mm diameter $\times$ 1 mm height) and cylinders (6 mm diameter $\times$ 12 mm height). On gross inspection, solid PMMA space maintainers were smoother in surface texture (Supplemental Fig. 1). Increased econazole loading resulted in greater space maintainer opacity.

3.2 Polymer characterization

3.2.1 ^1H -NMR spectroscopy—Upon analysis of PMMA polymerized in the presence and absence of econazole, no changes in chemical structure of either the polymer or the antimicrobial were noted. Representative sections of scans are shown in Supplemental Fig. 2.

3.2.2 Gel permeation chromatography—The M_n , M_w , and PI of space maintainers made from PMMA polymerized in the presence or absence of econazole (5 wt%) can be found in Table 2. There were no statistically significant differences in any molecular weight values between PMMA polymerized in the presence or absence of econazole.

3.3 Radiographic characterization

All groups in Table 1 ($n = 3$ per group) were radiographically scanned and reconstructed by microCT (Fig. 1). Reconstructed constructs were analyzed to determine total porosity as well as the proportion of open to closed pores (Fig. 2). Non-porous space maintainer groups had total porosity (Fig. 2a) ranging from 0.3 to 0.6% regardless of econazole loading (no significant differences amongst non-porous groups). Porous groups had a total porosity ranging from 19.3 to 26.4% regardless of econazole loading (no significant difference

amongst porous groups). At each level of econazole loading, all porous groups had significantly greater total porosity than their non-porous counterparts.

Open pores are those that communicate with other pores and the outside environment through an interconnected network. Closed pores are void spaces that do not communicate. Open pores (%) is a measurement of the relative amount of open pores out of all total available pores (Fig. 2b). The open pores of non-porous groups ranged from 10.3 to 56.3%; the high loading group had significantly greater open pores than the blank and low loading group. The open pores of the porous groups ranged from 96.8 to 99.1% regardless of econazole loading (no significant difference amongst porous groups). At each level of econazole loading, all porous groups had significantly greater open porosity than their non-porous counterparts.

3.4 Antimicrobial characterization

Antimicrobial bioactivity was assayed by disc diffusion assay against the two most common FPII species (*C. albicans* and *A. fumigatus*) as well as the most common bacterial species associated with superinfection (*S. aureus*). The zone of inhibition produced by the discs was measured and compared across groups to determine the effect of porosity and econazole loading on antimicrobial activity.

Inhibition ranged from 0 to 32 mm, based on species, porosity, and loading dosage (Fig. 3). Regardless of porosity, no unloaded space maintainers (blank groups) demonstrated any inhibition against the three pathogen species. For all species, there were no significant differences between porous and non-porous groups at the low loading dose. For all groups, high loading groups demonstrated significantly greater inhibition than low loading groups. For all groups, the porous high loading group demonstrated significantly greater inhibition than the non-porous high loading group.

In corroboration with the disc diffusion study, unloaded space maintainers were not capable of inhibiting the growth of *C. albicans* and porous groups had greater inhibition than non-porous groups at the highest loading dose (67 vs. 33% inhibition at 48 h) as shown in Table 3. High loading groups also had more sustained inhibition over the 48 h compared to low loading groups, which only suppressed *Candida* growth in the first 12 h. Of note, the non-porous group had greater inhibition at 12 h than the porous group. Control wells showed no growth in wells with fresh econazole (25 µg/mL), growth in wells with inoculated fresh PBS, and no growth in wells with fresh PBS that were not inoculated.

3.5 Mechanical characterization

Cylinders (6 mm in diameter, 12 mm in height) underwent compressive and bending testing per the standard ISO 5833 (Fig. 4). Compressive yield strength ranged from 95.1 to 99.0 MPa for non-porous groups and from 29.5 to 30.8 for porous groups (Fig. 4a). Compressive modulus ranged from 2077.0 to 2329.6 MPa for non-porous groups and from 703.9 to 780.7 MPa for porous groups (Fig. 4b). Porous groups had significantly decreased strength and modulus compared to non-porous groups with no significant effects based on econazole loading at either low or high dosage. The addition of econazole at either loading dose did not significantly affect either of the mechanical properties. This held true for both non-porous

and porous groups. Similarly, porous groups had significantly decreased bending strength (Fig. 4c) and modulus (Fig. 4d) compared to non-porous groups under the same econazole loading conditions. Bending yield strength ranged from 69.7 to 89.6 MPa for non-porous groups and from 27.4 to 33.8 MPa for porous groups. Bending modulus ranged from 5682.4 to 7557.9 MPa for non-porous groups and from 2734.7 to 4268.6 MPa for porous groups.

4 Discussion

In this study, the effect of porosity and drug loading on antifungal-eluting space maintainers was studied by chemical, radiographic, microbial, and biomechanical means. Imidazole-based compounds have been utilized as chemical initiators for free radical polymerization of PMMA and other polymer systems [26]. Therefore, the first objective of this study was to polymerize PMMA in the presence of econazole to determine if there are any interactions between the compounds at a molecular and macromolecular level. By $^1\text{H-NMR}$ spectroscopy and gel permeation chromatography, polymerizing PMMA in the presence of econazole (5 wt%) did not affect the function either the antifungal drug or the polymer (Table 2).

While the effects of introducing CMC as a leachable porogen to PMMA are well-described [17, 24, 27], it was important to determine if the introduction of econazole had any effect on the architecture and topography of the final porous space maintainer. It was determined that econazole had no effect on the total porosity of these constructs (Fig. 2a), and that the total porosity was similar to that found in other studies using the same amount of CMC [24]. Introduction of CMC greatly increased the total porosity of constructs from less than 1% to greater than 20%. However, total porosity is not the only important metric when evaluating the additional surface area created for additional elution of drug. A greater percentage of open pores suggests formation of an interconnected network with communication to the environment. Porous groups had much greater open porosity than non-porous groups (Fig. 2b). While econazole loading had no statistically significant effect in porous groups on open porosity, the highest loading dose resulted in significantly greater open porosity among non-porous groups. It is possible that the addition of greater amounts of econazole may alter the mixing and/or packing of PMMA during polymerization, resulting in a greater degree of open pores (total porosity did not change).

As an antimicrobial device, econazole-loaded space maintainers were effective in inhibiting the growth of the most common fungal species associated with FJIPs and fungal osteomyelitis [8] as well as the most common bacterial species associated with superinfection [5, 9]. At the high loading dose, porous space maintainers were superior to non-porous space maintainers in microbial inhibition (Fig. 3). In addition, this inhibition was sustained for at least 12 h and for as long as 48 h in the high loading groups (Table 3). While not commonly used since the advent of more effective antifungal drugs, econazole has well-characterized activity against *Candida* and *Aspergillus* [19–21]. Against fungal pathogens, econazole prevents the synthesis of ergosterol (through 14α -demethylase inhibition), a critical component of the fungal membrane [21]. However, sterols are not thought to have significant roles in the bacterial structure and are therefore not considered antibacterial agents [28]. Despite this, we observed a zone of inhibition observed against *S. aureus* (17

mm in the porous high dose group) (Fig. 3c). As blank groups demonstrated no inhibition, this effect cannot be attributed to PMMA or CMC. It has previously been demonstrated that econazole nitrate has *in vitro* activity against *S. aureus*; in one study, econazole nitrate was found to be effective against several clinical isolates of *S. aureus* (as well as other common gram positive species, such as streptococci) with minimum inhibitory concentrations as low as 0.03 µg/mL [29]. However, the drug was not effective against gram negative species, including *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Escherichia coli*, and *Klebsiella pneumoniae*, suggesting that the mechanism of action of econazole against bacteria may be related to components of the bacterial cell wall. Other speculated mechanisms of action include increased membrane permeability to potassium ions and inhibition of nitric oxide dioxygenase [28, 30]. Further studies must be performed to elucidate the mechanism of econazole against *S. aureus*. In this study, it was found that econazole is an attractive therapeutic to elute from the porous space maintainer given its activity against the most common fungal species and most common bacterial species responsible for superinfection in FPJI and fungal osteomyelitis.

The release study chosen was stringent as the minimum inhibitory concentration for the given clinical *C. albicans* isolate was relatively high (4 µg/mL), the large volume of supernatant relative to the small space maintainer (2 mL), and the need for further dilution of the release supernatant with the *C. albicans* concentrate during assay (1:1 dilution). In total, in order to inhibit *Candida* in this assay, space maintainers would need to release at least 16 µg per time point. In a different collection of 420 clinical isolates of *C. albicans*, 99% of isolates had a minimum inhibitory concentration of 0.06 µg/mL or less [19]. The clinical isolate used in this study was particularly robust with inhibition requiring ~67× greater concentration of econazole than other isolates. Despite this challenge, at the high loading dose, the release products of space maintainer discs were capable of inhibiting all growth for at least 12 h and porous groups inhibited growth in two out of three specimens for at least 48 h (Table 3). As it is believed that most periprosthetic joint infections are due to inoculation at the time of surgery rather than hematogenous dissemination at a later time, providing local high-concentration therapy in the immediate post-operative time is critical; therefore, 12 h of inhibition may be sufficient to clear and/or prevent infection [9, 31].

Lastly, porous econazole-loaded space maintainers were tested under compressive and bending forces per ISO 5833 (“Implants for surgery-Acrylic resin cements”) [23]. As tension is not considered a major element of the typical biomechanical stresses on space maintainers, it is not included in ISO 5833 and thus was not examined in this study. Even at the highest loading dose (5 wt%), there were no significant differences between groups based on econazole loading (Fig. 4). In studies using solid PMMA, it has previously been noted that antibiotics loaded up to 5 wt% often result in negligible changes in space maintainer mechanical properties [4]. As has been found in previous studies involving porous space maintainers [17, 24], introducing porosity significantly decreased space maintainer mechanical properties (Fig. 4). However, even with decreased mechanical properties, these space maintainers may still be sufficient for their intended application. Given the temporary nature of space maintainers, it has been argued that diminished mechanical properties are acceptable in light of increased antimicrobial activity [32]. Furthermore, space maintainers are often mechanically reinforced when implanted. In the

majority of clinical studies of antibiotic-loaded PMMA for use in hip arthroplasty, space maintainers had additional mechanical support from surgical hardware [4]. In instances in which CMC-based porous PMMA bone space maintainers were implanted in preclinical animal models [13, 14, 18] and human patients [1], there were no reports of mechanical failure. Therefore, despite diminished mechanical properties, econazole-loaded porous space maintainers may still be appropriate for temporary space maintenance in an infected tissue defect.

A strength of this space maintainer-based strategy is the combination of clinically-available materials to create a new device. CMC is a substance “generally regarded as safe” by the Food and Drug Administration (FDA) [33] and PMMA has been regulated by the FDA for orthopaedic applications as early as 1976 [34]. Porous PMMA (by mixing with CMC) has been evaluated for biocompatibility in a number of animal models [13, 14, 18] as well as used to improve surgical outcomes in human patients without any signs of toxicity [1]. Similarly, econazole has been marketed as a regulated therapeutic in the US since 1982 [35]. Econazole is regarded as non-cytotoxic and some advocate for its use replacing other antifungals in cell culture systems due to its superior safety profile [20]. Local delivery of econazole through drug delivery vehicles enables high concentrations at infected sites without systemic side effects or local toxicity as reviewed recently [36]. Given that this approach uses components that are regulated in other existing products by the FDA and have a long-standing history of biocompatibility, the translation of this technology from benchtop to clinical practice may be more rapid than the use of entirely novel components.

5 Conclusions

In this study, we have developed econazole-releasing porous space maintainers that are capable of inhibiting the growth of *C. albicans*, *A. fumigatus*, and *S. aureus*. Econazole, with activity against fungi and gram positive bacteria, is a desirable choice for the treatment of fungal periprosthetic joint infection and fungal osteomyelitis. Compared to traditional solid space maintainer devices, we found that porous space maintainers were capable of greater pathogen inhibition. This may be due to increased surface area available for drug elution, as both total porosity and percentage open pores increased with incorporation of a leachable porogen into the space maintainer. While decreased, the mechanical properties of the econazole-releasing porous space maintainers are still sufficient for their intended biological function.

Fungal infection of musculoskeletal tissues, including the joint space and bone, carry tremendous clinical consequence. As the materials for these econazole-loaded space maintainers (PMMA, CMC, and econazole) are relatively inexpensive and widely available, this is an attractive strategy that is capable of rapid translation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Henslee AM, Spicer PS, Shah SR, Tatara AM, Kasper FK, Mikos AG, et al. Use of porous space maintainers in staged mandibular reconstruction. *Oral Maxillofac Surg Clin North Am.* 2014; 26:143–9. [PubMed: 24794263]
2. Jakobs O, Schoof B, Klatte TO, Schmidl S, Fensky F, Guenther D, et al. Fungal periprosthetic joint infection in total knee arthroplasty: a systematic review. *Orthop Rev.* 2015; 7:5623. <https://doi.org/10.4081/or.2015.5623>.
3. Giannoudis PV, Faour O, Goff T, Kanakaris N, Dimitriou R. Masquelet technique for the treatment of bone defects: tips-tricks and future directions. *Injury.* 2011; 42:591–8. <https://doi.org/10.1016/j.injury.2011.03.036>. [PubMed: 21543068]
4. Anagnostakos K, Furst O, Kelm J. Antibiotic-impregnated PMMA hip spacers: current status. *Acta Orthop.* 2006; 77:628–37. <https://doi.org/10.1080/17453670610012719>. [PubMed: 16929441]
5. Azzam K, Parvizi J, Jungkind D, Hanssen A, Fehring T, Springer B, et al. Microbiological, clinical, and surgical features of fungal prosthetic joint infections: a multi-institutional experience. *J Bone Jt Surg Am.* 2009; 91:142–9. <https://doi.org/10.2106/JBJS.I.00574>.
6. Hwang BH, Yoon JY, Nam CH, Jung KA, Lee SC, Han CD, et al. Fungal peri-prosthetic joint infection after primary total knee replacement. *J Bone Jt Surg Br.* 2012; 94:656–9. <https://doi.org/10.1302/0301-620X.94B5.28125>.
7. Anagnostakos K, Kelm J, Schmitt E, Jung J. Fungal periprosthetic hip and knee joint infections clinical experience with a 2-stage treatment protocol. *J Arthroplast.* 2012; 27:293–8. <https://doi.org/10.1016/j.arth.2011.04.044>.
8. Bariteau JT, Waryasz GR, McDonnell M, Fischer SA, Hayda RA, Born CT. Fungal osteomyelitis and septic arthritis. *J Am Acad Orthop Surg.* 2014; 22:390–401. <https://doi.org/10.5435/JAAOS-22-06-390>. [PubMed: 24860135]
9. Miller AO, Gamaletsou MN, Henry MW, Al-Hafez L, Hussain K, Sipsas NV, et al. Successful treatment of Candida osteoarticular infections with limited duration of antifungal therapy and orthopedic surgical intervention. *Infect Dis.* 2015; 47:144–9. <https://doi.org/10.3109/00365548.2014.974207>.
10. Sealy PI, Nguyen C, Tucci M, Benghuzzi H, Cleary JD. Delivery of antifungal agents using bioactive and nonbioactive bone cements. *Ann Pharmacother.* 2009; 43:1606–15. <https://doi.org/10.1345/aph.1M143>. [PubMed: 19755620]
11. Rouse MS, Heijink A, Steckelberg JM, Patel R. Are anidulafungin or voriconazole released from polymethylmethacrylate in vitro? *Clin Orthop Relat Res.* 2011; 469:1466–9. <https://doi.org/10.1007/s11999-010-1643-7>. [PubMed: 20963525]
12. Goss B, Lutton C, Weinrauch P, Jabur M, Gillett G, Crawford R. Elution and mechanical properties of antifungal bone cement. *J Arthroplast.* 2007; 22:902–8. <https://doi.org/10.1016/j.arth.2006.09.013>.
13. Spicer PP, Shah SR, Henslee AM, Watson BM, Kinard LA, Kretlow JD, et al. Evaluation of antibiotic releasing porous polymethylmethacrylate space maintainers in an infected composite tissue defect model. *Acta Biomater.* 2013; 9:8832–9. <https://doi.org/10.1016/j.actbio.2013.07.018>. [PubMed: 23891810]
14. Shah SR, Tatara AM, Lam J, Lu S, Scott DW, Bennett GN, et al. Polymer-based local antibiotic delivery for prevention of poly-microbial infection in contaminated mandibular implants. *ACS Biomater Sci Eng.* 2016; 2:558–66.

15. Kusunoki M, Ikeuchi H, Yanagi H, Noda M, Tonouchi H, Mohri Y, et al. Bioresorbable hyaluronate-carboxymethylcellulose membrane (Seprafilm) in surgery for rectal carcinoma: a prospective randomized clinical trial. *Surg Today*. 2005; 35:940–5. <https://doi.org/10.1007/s00595-005-3061-0>. [PubMed: 16249848]
16. Zavanah PN, Putnam D. Materials in surgery: a review of bio-materials in postsurgical tissue adhesion and seroma prevention. *Tissue Eng Part B Rev*. 2008; 14:377–91. <https://doi.org/10.1089/ten.teb.2008.0226>. [PubMed: 18816187]
17. Wang L, Yoon DM, Spicer PP, Henslee AM, Scott DW, Wong ME, et al. Characterization of porous polymethylmethacrylate space maintainers for craniofacial reconstruction. *J Biomed Mater Res B Appl Biomater*. 2013; 101:813–25. <https://doi.org/10.1002/jbm.b.32885>. [PubMed: 23359449]
18. Kretlow JD, Shi M, Young S, Spicer PP, Demian N, Jansen JA, et al. Evaluation of soft tissue coverage over porous polymethylmethacrylate space maintainers within nonhealing alveolar bone defects. *Tissue Eng Part C Methods*. 2010; 16:1427–38. <https://doi.org/10.1089/ten.tec.2010.0046>. [PubMed: 20524844]
19. Richter SS, Galask RP, Messer SA, Hollis RJ, Diekema DJ, Pfaller MA. Antifungal susceptibilities of *Candida* species causing vulvovaginitis and epidemiology of recurrent cases. *J Clin Microbiol*. 2005; 43:2155–62. <https://doi.org/10.1128/JCM.43.5.2155-2162.2005>. [PubMed: 15872235]
20. Wyler R, Murbach A, Mohl H. An imidazole derivative (Econazole) as an antifungal agent in cell culture systems. *Vitro*. 1979; 15:745–50.
21. Heel RC, Brogden RN, Speight TM, Avery GS. Econazole: a review of its antifungal activity and therapeutic efficacy. *Drugs*. 1978; 16:177–201. [PubMed: 98315]
22. Jiranek WA, Hanssen AD, Greenwald AS. Antibiotic-loaded bone cement for infection prophylaxis in total joint replacement. *J Bone Jt Surg Am*. 2006; 88:2487–500. <https://doi.org/10.2106/JBJS.E.01126>.
23. ISO 5833: Implants for surgery—Acrylic resin cements [database on the Internet]. International Organization for Standardization; 2002. [Accessed 15 Dec 2017]
24. Tatara AM, Shah SR, Sotoudeh M, Henslee AM, Wong ME, Ratcliffe A, et al. Effects of electron beam sterilization on mechanical properties of a porous polymethylmethacrylate space maintenance device. *J Med Devices*. 2015; 9:024501.
25. Watts, JL. Clinical Institute LS. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals: approved standard. National Committee for Clinical Laboratory Standards; 2008.
26. Morell M, Ramis X, Ferrando F, Serra À. New improved thermosets obtained from diglycidylether of bisphenol A and a multiarm star copolymer based on hyperbranched poly (glycidol) core and poly (methyl methacrylate) arms. *Macromol Chem Phys*. 2012; 213:335–43.
27. Tatara AM, Wong ME, Mikos AG. In vivo bioreactors for mandibular reconstruction. *J Dent Res*. 2014; 93:1196–202. <https://doi.org/10.1177/0022034514547763>. [PubMed: 25139360]
28. Sud IJ, Feingold DS. Action of antifungal imidazoles on *Staphylococcus aureus*. *Antimicrob Agents Chemother*. 1982; 22:470–4. [PubMed: 6291453]
29. Kokjohn K, Bradley M, Griffiths B, Ghannoum M. Evaluation of in vitro activity of ciclopirox olamine, butenafine HCl and econazole nitrate against dermatophytes, yeasts and bacteria. *Int J Dermatol*. 2003; 42:11–7. [PubMed: 12895182]
30. Helmick RA, Fletcher AE, Gardner AM, Gessner CR, Hvitved AN, Gustin MC, et al. Imidazole antibiotics inhibit the nitric oxide dioxygenase function of microbial flavohemoglobin. *Antimicrob Agents Chemother*. 2005; 49:1837–43. <https://doi.org/10.1128/AAC.49.5.1837-1843.2005>. [PubMed: 15855504]
31. Darouiche RO, Hamill RJ, Musher DM, Young EJ, Harris RL. Periprosthetic candidal infections following arthroplasty. *Rev Infect Dis*. 1989; 11:89–96. [PubMed: 2916098]
32. Seldes RM, Winiarsky R, Jordan LC, Baldini T, Brause B, Zodda F, et al. Liquid gentamicin in bone cement: a laboratory study of a potentially more cost-effective cement spacer. *J Bone Jt Surg Am*. 2005; 87:268–72. <https://doi.org/10.2106/JBJS.C.00728>.
33. Majewicz, TG., Erazo3Majewicz, PE., Podlas, TJ. Encyclopedia of polymer science and technology. Wiley-Interscience; New York: 2003. Cellulose ethers.

34. Bauer TW, Smith ST. Bioactive materials in orthopaedic surgery: overview and regulatory considerations. *Clin Orthop Relat Res.* 2002; 395:11–22.
35. Kaitin KI, Mattison N, Northington FK, Lasagna L. The drug lag: an update of new drug introductions in the United States and in the United Kingdom, 1977 through 1987. *Clin Pharmacol Ther.* 1989; 46:121–38. [PubMed: 2758722]
36. Firooz A, Nafisi S, Maibach HI. Novel drug delivery strategies for improving econazole antifungal action. *Int J Pharm.* 2015; 495:599–607. <https://doi.org/10.1016/j.ijpharm.2015.09.015>. [PubMed: 26383840]

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Fig. 1. Representative microcomputed tomography reconstructions of non-porous (top) and porous (bottom) space maintainers, loaded with 0, 2.5, or 5.0 wt% econazole (blank, low, and high, respectively). Error bars represent standard deviation. Scale bar = 2 mm

Fig. 2. Microcomputed tomography-generated radiographic data. **a** Total porosity (%). **b** Proportion of open pores (%). Error bars represent standard deviation for $n = 3$. Those not sharing the same letter are significantly different ($p < 0.05$)

Fig. 3.

Zone of inhibition data for **a** *C. albicans*, **b** *A. fumigatus*, and **c** *S. aureus*. No zone of inhibition was observed for any of the blank groups. Error bars represent standard deviation for $n = 3$. Those not sharing the same letter are significantly different ($p < 0.05$)

Fig. 4. Compressive yield strength **a** and modulus **b** and bending yield strength **c** and modulus **d** of porous and non-porous econazole-loaded space maintainers. Error bars represent standard deviation for $n = 5$ (compression) and $n = 4$ (bending). Those not sharing the same letter are significantly different ($p < 0.05$)

Table 1

Factors (porosity and econazole loading concentration) investigated, resulting in a total of six different groups

Factors	Porosity	Loading dose
# Levels	2	3
Levels	Solid	Blank
	Porous	Low
		High

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Table 2

Molecular weight (number average, weight average, and polydispersity index) of polymer chains in space maintainers

Group	M_n (kDa)	M_w (kDa)	PI
Polymerized without econazole	157.3 ± 24.3	588.7 ± 91.3	3.23 ± 0.10
Polymerized with econazole	185.1 ± 27.8	638.6 ± 118.0	3.44 ± 0.13
<i>p</i> value	0.89	0.59	0.08

Table 3

Release assay results of supernatant from space maintainers tested against *Candida albicans* after 12, 24, and 48 h of incubation G = growth and NG = no growth

Group	12 h	24 h	48 h
Non-porous; 0 wt%	G, G, G	G, G, G	G, G, G
Non-porous; 2.5 wt%	NG, NG, NG	G, G, G	G, G, G
Non-porous; 5 wt%	NG, NG, NG	NG, NG, G	NG, G, G
Porous; 0 wt%	G, G, G	G, G, G	G, G, G
Porous; 2.5 wt%	NG, G, G	G, G, G	G, G, G
Porous; 5 wt%	NG, NG, NG	NG, NG, G	NG, NG, G