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Antibiotic-releasing porous polymethylmethacrylate constructs for osseous space maintenance and infection control

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ABSTRACT

The use of a strategy involving space maintenance as the initial step of a two-stage regenerative medicine approach toward reconstructing significant bony or composite tissue defects in the craniofacial area, preserves the void volume of bony defects and could promote soft tissue healing prior to the subsequent definitive repair. One of the complications with a biomaterial-based space maintenance approach is local infection, which requires early, effective eradication, ideally through local antibiotic delivery. The purpose of this study is to develop a dual function implant material for maintaining osseous space and releasing an antibiotic to eliminate local infection in bony defects. Colistin, a polymyxin antibiotic, was chosen specifically to address infections with *Acinetobacter* species, the most common pathogen associated with combat-related traumatic craniofacial injuries. Porous polymethylmethacrylate (PMMA) constructs incorporating poly(lactic-*co*-glycolic acid) (PLGA) microspheres were fabricated by mixing a clinically used bone cement formulation of PMMA powder and methylmethacrylate liquid with a carboxymethylcellulose (CMC) hydrogel (40 or 50 wt%) to impart porosity and PLGA microspheres (10 or 15 wt%) loaded with colistin to control drug release. The PMMA/CMC/PLGA construct featured mild setting temperature, controllable surface/bulk porosity by incorporation of the CMC hydrogel, reasonably strong compressive properties, and continuous drug release over a period of 5 weeks with total drug release of 68.1–88.3%, depending on the weight percentage of CMC and PLGA incorporation. The concentration of released colistin was well above its reported minimum inhibitory concentration against susceptible species for 5 weeks. This study provides information on the composition parameters that enable viable porosity characteristics/drug release kinetics of the PMMA/CMC/PLGA construct for the initial space maintenance as part of a two-stage regenerative medicine approach.

1. Introduction

Craniofacial trauma is among the most debilitating forms of injury facing civilian and military populations due to the important aesthetic and functional role of the craniofacial complex [1,2]. Blast injuries and injuries from high velocity projectiles (e.g., those encountered on the battlefield) often require a staged repair where the surgical revision, however, is sometimes complicated by distortion of surgical landmarks, diminished volume of the defect space, fibrosis of the tissue bed and/or local contamination [3–8]. Over the course of a staged reconstruction, the placement of a temporary, alloplastic implant may eliminate many of the aforementioned complications [9,10]. Toward treating traumatic craniofacial injuries with significant bone/tissue loss, our laboratory is developing a two-stage regenerative medicine approach consisting of a first stage using temporary space maintenance to not only maintain the void space but also to prime the wound site for later definitive reconstruction [10]. The initial space maintenance using a non-biodegradable implant material (i.e., a space maintainer)

Abbreviations: ANOVA, analysis of variance; CMC, carboxymethylcellulose; FDA, Food and Drug Administration; HPLC, high-performance liquid chromatography; ISO, International Organization for Standardization; MDR, multidrug-resistant; MIC, minimum inhibitory concentration; microCT, microcomputed tomography; MMA, methylmethacrylate; PBS, phosphate buffered saline; PLGA, poly(lactic-*co*-glycolic acid); PMMA, polymethylmethacrylate; PVA, poly(vinyl alcohol); SEM, scanning electron microscopy.

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preserves the original dimensions of the bony defects and prevents soft tissue ingrowth while, importantly, allowing for wound/tissue healing over the material. Successful space maintenance creates a soft tissue envelope with definitely preserved volume and well-healed surrounding tissues, ideal for the placement of a tissue engineering construct designed for bone regeneration during the subsequent reconstruction stage [9–12]. In order to advance the development of this two-stage regenerative medicine approach, we herein designed a polymethylmethacrylate (PMMA)-based space maintainer featuring a porous structure to promote wound/tissue healing over the material and implant retention at the host site [10]. In addition, an antibiotic delivery system is incorporated to mitigate/prevent local infections, thereby reducing the potential for any infection-related complication associated with space maintenance.

Self-hardening PMMA cement has been successfully used in a variety of orthopaedic conditions because of its appealing physical and chemical characteristics [13,14]. The non-degradability allows PMMA constructs to maintain sufficient mechanical support over an extended time period. It can also be molded intraoperatively to fill complex defects, making PMMA implants particularly suited for osseous space maintenance in oral and craniofacial reconstructions [9,15–17]. While conventional PMMA cement has been previously used in space maintenance applications, problems with implant extrusion or wound dehiscence have been reported [10,11,18]. It has been demonstrated that the porous structure of an implant material plays an essential role in anchoring the material to the host by allowing for rapid ingrowth of fibrovascular and soft tissue into the pores to promote wound healing and the formation of a stable interface [10,18–22]. A porous PMMA structure thus becomes the gold standard for designing an effective space maintainer in the envisioned two-stage approach.

Infections following traumatic injuries, including combat wound infections and osteomyelitis, are a common occurrence [7,8,23–25]. Latent or active posttraumatic and postsurgical infections may potentially hinder wound healing and tissue regeneration, underscoring the importance of effective, early eradication/prevention by antibiotic drugs. The multidrug-resistant (MDR) *Acinetobacter baumannii* species has been demonstrated to be the predominant organism recovered in trauma-related infections sustained by US soldiers in Iraq and Afghanistan [24–26]. Colistin, one of the last-resort antibiotics for this species, holds promise as an effective antimicrobial agent [27–30]. The parenteral administration of colistin requires a long course of therapy due to the poor penetration of the antibiotic into bone. Long-term parenteral colistin administration is associated with a high incidence of nephrotoxicity and neurotoxicity [28,30], whereas a local delivery of antibiotic drugs promises to achieve local therapeutic drug levels over an extended duration while eliminating systemic exposure to potentially toxic drug concentrations [31–33]. Consequently, the development of a local antibiotic delivery system delivered through an implant becomes a potentially important strategy in space maintenance.

Building upon the advantages of a porous implant structure to improve incorporation into the surrounding tissue bed through the incorporation of controlled drug delivery to address local infections, a space maintainer was designed as a poly(lactic-*co*-glycolic acid) (PLGA) microsphere-incorporating porous PMMA construct. The antibiotic drug colistin was first loaded into biodegradable PLGA microspheres, and the PLGA microspheres were then mixed with the PMMA cement, while a carboxymethylcellulose (CMC) hydrogel component was co-incorporated to impart porosity throughout the constructs [10,21,34]. All components in these PMMA/CMC/PLGA constructs are regulated by the United States Food and Drug Administration (FDA) for orthopaedic and/or craniofacial applications. A construct successfully developed from this combination has the potential to transition readily from experimental research into clinical use.

The aim of the present *in vitro* study was to elucidate the influence of material composition of PMMA/CMC/PLGA constructs on their physical properties and provide predictive insight into the expected space maintenance and drug delivery capability of the space maintainer over time *in vivo*. Specifically, it was hypothesized that the overall porosity would be tailored by the incorporation of CMC hydrogel and would not be significantly changed with the addition of PLGA microspheres. The incorporation of PLGA microspheres in porous PMMA constructs was hypothesized to allow for a sustained, high concentration colistin release over weeks. To test these hypotheses, four formulations of PMMA/CMC/PLGA constructs with 40–50 wt% CMC and 10–15 wt% PLGA microsphere incorporation were investigated for surface and bulk morphology, porosity, pore interconnectivity and compressive mechanical properties initially and throughout a degradation process of 12 weeks. *In vitro* drug release kinetics were also examined over a period of 5 weeks.

2. Materials and methods

2.1. Materials

Poly(lactic-*co*-glycolic acid) (PLGA) (copolymer ratio of 50:50, weight average molecular weight of 61.1 kDa and number average molecular weight of 37.3 kDa as determined by gel permeation chromatography based on polystyrene standards) was purchased from Lakeshore Biomaterials (Birmingham, AL). Colistin sulfate salt was purchased from Sigma-Aldrich (St. Louis, MO). PMMA cement (SmartSet®, High Viscosity) was from DePuy Orthopaedics Inc. (Warsaw, IN). Carboxymethylcellulose sodium was purchased from Spectrum® chemical MFG Corp. (Gardena, CA). Poly(vinyl alcohol) (PVA) (88% hydrolyzed, nominal molecular weight 22 kDa) was from Acros Organics (Geel, Belgium). All other reagents were purchased from Sigma-Aldrich (St. Louis, MO) and used as received.

2.2. Preparation of colistin-loaded PLGA microspheres

Colistin-loaded PLGA microspheres were fabricated by a water-in-oil-in-water (W/O/W) double emulsion solvent evaporation technique [35]. Briefly, PLGA polymer (2.0 g) was dissolved in methylene chloride at a concentration of 50 mg/mL as an oil phase, and colistin (650 mg) was dissolved in 2 mL distilled water (internal aqueous phase) containing 0.4 wt% PVA. The colistin solution was dispersed in the polymer solution by a homogenizer (PRO250, Pro Scientific Inc., Monroe, CT) at 26,000 rpm for 30 s. This stable W/O emulsion was slowly added into an aqueous solution (external aqueous phase, 400 mL) containing 0.4 wt% PVA and 0.5 M NaCl under stirring at 500 rpm and the solution was stirred at 500 rpm for 30 min. Solvent removal and microsphere hardening was achieved by stirring at 300 rpm for another 3.5 h. The microspheres were isolated by centrifugation, washed with distilled water three times, and then vacuum-dried for 24 h.

The drug content in the PLGA microspheres was determined by first dissolving microspheres in methylene chloride and then extracting colistin with phosphate buffered saline (PBS) (pH 7.4). Briefly, 20 mg colistin-loaded microspheres were dissolved in 1 mL methylene chloride and then 20 mL PBS was added to the solution. The solution was vigorously stirred for 2 h allowing for the extraction of colistin by the aqueous phase and evaporation of the organic solution.

The colistin concentration in the PBS buffer was analyzed by high-performance liquid chromatography (HPLC) (Waters®, Milford, MA) [36]. The HPLC system consisted of a Waters 2695 separation module and a 2996 photodiode array (PDA) detector. The separation was performed using an XTerra® RP 18 column (250 cm × 4.6 μm, Waters®) at a column temperature of 45 °C and a flow rate of 0.5 mL/min in a mobile phase consisting of acetonitrile (HPLC grade with 0.1 vol% trifluoroacetic acid) and water (HPLC grade with 0.1 vol% trifluoroacetic acid). Peaks were eluted with a linear gradient of 10–65% acetonitrile in water over 20 min. Absorbance was monitored at $\lambda = 214$ nm. The two main components colistin A and colistin B were eluted at approximately 14.9 min and 15.7 min, respectively. Standard solutions with colistin in PBS buffer (pH 7.4) were tested in the range of 5–1000 μg/mL. Calibration curves were obtained using the combined peak areas of colistin A and colistin B versus the colistin concentration.

2.3. Preparation of microsphere-incorporating porous PMMA constructs

Antibiotic-releasing porous PMMA constructs were fabricated by mixing a clinical grade bone cement formulation of PMMA powder and MMA liquid with a CMC