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Evaluation of antibiotic releasing porous polymethylmethacrylate space maintainers in an infected composite tissue defect model

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ABSTRACT

This study evaluated the in vitro and in vivo performance of antibiotic-releasing porous polymethylmethacrylate (PMMA)-based space maintainers comprising a gelatin hydrogel porogen and a poly(DL-lactic-co-glycolic acid) (PLGA) particulate carrier for antibiotic delivery. Colistin was released in vitro from either gelatin or PLGA microparticle loaded PMMA constructs, with gelatin-loaded constructs releasing colistin over approximately 7 days and PLGA microparticle-loaded constructs releasing colistin for up to 8 weeks. Three formulations with either burst release or extended release at different doses were tested in a rabbit mandibular defect inoculated with *Acinetobacter baumannii* (2×10^7 colony forming units ml^{-1}). In addition, one material control that released antibiotic but was not inoculated with *A. baumannii* was tested. *A. baumannii* was not detectable in any animal after 12 weeks on culture of the defect, saliva, or blood. Defects with high dose extended release implants had greater soft tissue healing compared with defects with burst release implants, with 8 of 10 animals showing healed mucosae compared with 2 of 10 respectively. Extended release of locally delivered colistin via a PLGA microparticle carrier improved soft tissue healing compared with implants with burst release of colistin from a gelatin carrier.

1. Introduction

Non-porous space maintainers have previously been shown to enhance craniofacial reconstruction by maintaining the bony structure and soft tissue envelope for later bone regeneration [1]. However, implantation of a foreign body into an area where bone has been resected due to trauma or pathology could precipitate infection due to the presence of oral flora and colonization of the material. Some clinical products, such as polymethylmethacrylate (PMMA)-based bone cements, incorporate antibiotics for local control of infection [2–6]. The release of these antibiotics is rapid, and the majority of the loaded antibiotic remains in the cement, rendering them ineffective at long-term infection control [2].

Previous work in our laboratory has utilized particulate delivery systems, such as gelatin and poly(DL-lactic-co-glycolic acid) (PLGA) microparticles, to control the release of antibiotics from porous PMMA-based constructs [7,8]. The porosity of these constructs was generated using a carboxymethylcellulose or gelatin hydrogel as the porogen, and the resulting porosity allowed greater cumulative release of antibiotic [7–10]. In these studies colistin, a polypeptide antibiotic, was incorporated into either gelatin or PLGA microparticles. Colistin was selected due to its efficacy against *Acinetobacter baumannii*, a common multidrug-resistant bacterial strain that is showing an increased incidence of infection in traumatic combat wounds [11–15]. Additionally, because colistin is infrequently used systemically due to its nephrotoxicity, it is an ideal choice for local delivery due to both decreased systemic concentrations and increased local concentrations [16].

This study investigated the use of porous PMMA/PLGA/gelatin/colistin constructs as antibiotic-releasing space maintainers by characterizing their release kinetics in vitro and evaluating their efficacy in vivo in a rabbit infected composite tissue defect model. The goal of the study was to assess the effects of antibiotic dose and release kinetics on wound healing, infection clearance, kidney function, and tissue response to the construct.

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2. Materials and methods

2.1. Materials

PLGA with a co-polymer ratio of 50:50, a weight average molecular weight of 61.1 kDa, and a number average molecular weight of 37.3 kDa, as measured by gel permeation chromatography [7], was obtained from Lakeshore Biomaterials (Birmingham, AL). Colistin sulfate salt was purchased from Sigma–Aldrich (St Louis, MO). Poly(vinyl alcohol) (PVA) was 88% hydrolyzed with a nominal molecular weight of 22 kDA and was purchased from Acros Organics (Geel, Belgium). Surgiflo Hemostatic Matrix (Ethicon, Somerville, NJ) was used as a source of gelatin. Bone cement was obtained from Depuy Orthopaedics (Smartset HV, Warsaw, IN).

2.2. Microparticle fabrication

PLGA microparticles containing colistin were fabricated as previously described [7]. Briefly, a water in oil in water double emulsion solvent extraction technique was used. The internal phases consisted of colistin dissolved in a solution of 0.4 wt.% PVA at a concentration of 325 mg ml^{-1}. The oil phase comprised PLGA in methylene chloride at a concentration of 50 mg ml^{-1}. The oil phase was added to the internal phase at a ratio of 20:1 oil:internal phase and homogenized. The water/oil emulsion was added to the external phase, a solution of 0.4 wt.% PVA with 0.5 M NaCl, at a ratio of 10:1 external phase:oil. The solvent was allowed to evaporate for 4 h, and the particles were washed, lyophilized, and stored at −20 °C. Blank microparticles were fabricated with an internal phase of 0.4 wt.% PVA without antibiotic. The entrapment of colistin-loaded microparticles was determined as previously described [7].

2.3. Space maintainer fabrication

Gelatin matrix was swollen in a 1:1.9 ratio of solution weight to gelatin weight. The swollen gelatin matrix comprised 30 wt.% of the total space maintainer mass. Bone cement was used at a ratio of 2.11:1 of powder phase to monomer phase for all samples, as supplied by the manufacturer. To fabricate the space maintainers the powder phase of the bone cement was first dispersed in the gelatin matrix. The monomer phase of the bone cement was added and mixed, and the space maintainer was molded and allowed to cure.

Colistin was loaded into the constructs using either the gelatin matrix or PLGA microparticles. For groups with colistin loaded into the gelatin matrix the gelatin matrix was swollen with 150 mg ml^{-1} colistin in double distilled H_2O at a ratio of 1:1.9 solution weight to gelatin weight. For groups with colistin loaded into the PLGA microparticles, PLGA microparticles comprised 11 wt.% of the total mass and were added to the powder phase of the bone cement. For the PLGA High group this 11% consisted entirely of colistin-loaded microparticles, while for the PLGA Low group approximately half of the colistin-loaded microspheres were replaced with blank PLGA microparticles. Table 1 summarizes the groups used for all analyses, including the calculated drug content based on the entrapment efficiency for the PLGA groups and the concentration for the Gelatin group. The Gelatin group was formulated such that the burst release of colistin at 6 h was approximately equivalent to the total cumulative release of the PLGA High group over the 84 day study. Of note, although the weight percentages of each component between groups may differ, each construct contained the same absolute amount of gelatin, PMMA powder, and liquid monomer. Each sample was sterilely aliquoted in sterile containers for intra-operative fabrication and cured in situ.

2.4. Colistin release

The release kinetics of each group containing colistin were determined by high performance liquid chromatography (HPLC) as previously reported [7,8]. Each of three space maintainers for each group was placed in 5 ml of phosphate-buffered saline (PBS) (pH 7.4) at 37 °C under mild agitation. The supernatant from each sample was completely removed and replaced with fresh PBS at 6 and 12 h and at 1, 2, 4, 7, 11, 14, 18, 21, 25, 28, 32, 35, 39, 42, 46, 49, 53, 56, 60, 63, 67, 70, 74, 77, 81 and 84 days. The supernatant was filtered with a 0.2 μm filter and the colistin concentration determined using a HPLC system. The HPLC system comprised a Waters 2695 separation module and a 2996 photodiode array detector (Waters, Milford, MA) with an XTerra RP 18 column (250 × 4.6 mm, Waters) at 45 °C. Elution was performed at a flow rate of 0.5 ml min^{-1} in a mobile phase consisting of acetonitrile (HPLC grade) 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 λ = 214 nm, with the two components of colistin, colistin A and colistin B, eluting at approximately 14 and 14.7 min, respectively. Standard solutions of colistin in PBS (pH 7.4) were tested in the range 5–1000 μg ml^{-1}. Calibration curves were obtained using the combined peak area of colistin A and colistin B versus the colistin concentration. The cumulative release (%) is expressed as the percent of total colistin released over time.

2.5. Bacterial culture and susceptibility

Acinetobacter baumannii (isolate 170) was obtained from Brooke Army Medical Center as a cultured specimen from a deep wound of a soldier returning from Operation Iraqi Freedom. Antibiotics released from the Gelatin, PLGA Low, and PLGA High groups at 6 h and 39 days were tested by sterile filtering the supernatant and using the solution as a stock solution in the microdilution

Table 1
Composition of the PMMA/PLGA/gelatin/colistin constructs examined.

Group	Gelatin matrix parameters		Implant composition				Calculated drug content (wt.%)
	Swelling ratio	Drug content (wt.%)	Gelatin matrix (wt.%)	Bone cement		Colistin-loaded PLGA (wt.%)	
				Powder phase (wt.%)	Monomer phase (wt.%)		
Uninfected	1:1.9	0	26.7	42.3	20.0	11.0	0.67
Gelatin	1:1.9	4.9	30	47.5	22.5	0.0	1.55
PLGA Low	1:1.9	0	26.7	42.3	20.0	5.5*	0.36
PLGA High	1:1.9	0	26.7	42.3	20.0	11.0	0.67

* In the PLGA Low group there is an additional 5.5 wt.% blank PLGA microparticles.

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