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 up to 8 weeks. Three formulations with either a burst release or extended release in different doses were tested in a rabbit mandibular defect inoculated with *Acinetobacter baumannii* (2×10^7 colony forming units/mL). 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 by culture of the defect, saliva, or blood. Defects with high-dose, extended-release implants had greater soft tissue healing compared to defects with burst release implants, with 8 out of 10 animals showing healed mucosae compared to 2 out of 10 with healed mucosae, respectively. Extended release of locally delivered colistin via a PLGA microparticle carrier improved soft tissue healing over the implants compared to burst release of colistin from a gelatin carrier.

Keywords: Space maintainer, craniofacial reconstruction, antibiotic delivery, infected defect, composite tissue defect

1.0 Introduction

Non-porous space maintainers have been previously 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 an infection due to the presence of oral flora and colonization of the material. Some clinical products, such as polymethylmethacrylate (PMMA)-based bone cements, have incorporated 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 from 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 by using a carboxymethylcellulose or gelatin hydrogel as a porogen, and the resulting porosity allowed for 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 commonly multi-drug resistant bacterial strain that has been observed to have increased incidence of infection in traumatic combat wounds [11-15]. Additionally, because colistin is infrequently used systemically due to 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.

2.0 Materials and Methods

2.1 Materials

Poly(DL-lactic-co-glycolic acid) (PLGA) was obtained from Lakeshore Biomaterials (Birmingham, AL) and had a copolymer 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] 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. The oil phase comprised PLGA in methylene chloride at a concentration of 50 mg/mL. The oil phase was added to the internal phase in 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, in a ratio of 10:1 external phase:oil. The solvent was allowed to evaporate for 4 hours, and the particles were washed, lyophilized, and stored at -20°C. Blank microparticles were fabricated with an internal phase of 0.4 wt% of 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 for all samples in a ratio of 2.11:1 of powder phase to monomer phase as supplied by the manufacturer. To fabricate the space maintainers, the powder phase of the bone cement was first dispersed into 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 colistin in ddH_2O in 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 6h was approximately equivalent to the total cumulative release of the PLGA High group over the 84d 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 intraoperative fabrication and cured *in situ*.

2.4 Colistin Release

The release kinetics of each group containing colistin were determined by high performance liquid chromatogray (HPLC) as previously reported.[7, 8] Each of three space maintainers for each group was placed in 5 mL 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 hrs 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 was 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 (250mm × 4.6mm, Waters) at 45 °C. The elution was performed with 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

with the two components of colistin, colistin A and colistin B, eluted at approximately 16.2 min and 16.9 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 area of colistin A and colistin B versus the colistin concentration. The cumulative release (%) was 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 hours and 39 days were tested by sterile filtering the supernatant and using the solution as a stock solution in the microdilution minimum inhibitory concentration (MIC) protocol according to ISO 20776. Briefly, the stock solution was serially diluted with sterile Mueller Hinton broth (MHB) to 50 μL aliquots with concentrations from 0 mg/L to 32 mg/L. A 0.5 MacFarland standard of *A. baumannii* cultured in MHB was diluted 1:100 in sterile MHB, and 50 μL of the inoculum was added to each well. The experiment was performed in triplicate, and the lowest concentration well without growth after 18 hr of culture at 37°C was denoted the MIC.

For the *in vivo* inoculum, *A. baumannii* was cultured in tryptic soy broth (TSB) (BD, Franklin Lakes, NJ). Colony forming unit (CFU) concentration for inoculation was determined by absorption the morning of each surgery, diluted by sterile TSB to 2×10^7 CFU/mL, and stored on ice until inoculation. Uninoculated defects were inoculated with the sterile TSB.

2.6 Surgical Procedure

All procedures followed protocols approved by the Rice University and University of Texas Health Science Center at Houston Institutional Animal Care and Use Committees. Adult male New Zealand white rabbits weighting 4.3-4.5 kg were used for this study (Myrtle's Rabbitry, Thompsons Station, TN). Four groups were selected for evaluation in an infected composite tissue defect model with 10 implants per group. *A. baumannii* was inoculated into defects filled with three different formulations: Gelatin, PLGA Low and PLGA High. The fourth group served as a material control that was uninfected and the implanted construct had the same formulation as PLGA High. The surgical procedure was completed as previously described.[9, 10, 17] Briefly, a midline incision extending posteriorly from the mentum was used to expose the inferior border of the right hemimandible. The soft tissue and periosteum were lifted from the body of the mandible and a 10 mm diameter bicortical defect was made in the body of the mandible with a dental trephine (Ace Surgical, Brockton, MA) powered by a micromotor handpiece (NSK, Kanuma, Japan) with copious irrigation. A crosscut bur (Stryker, Kalamazoo, MI) was used to cut a 2-3 mm notch in the superior aspect of the defect. The overlying crown was removed, creating an oral mucosal defect with intraoral communication. The defect was inoculated with 100 μL of the bacterial suspension, amounting to 2×10^7 CFU/mL of bacteria, or sterile broth by pipette on the defect walls. The implant was fabricated by first mixing the powder phase into the swollen gelatin until evenly mixed. The monomer phase was added and mixed until doughy, then shaped by hand and packed into the defect. The time from inoculation to packing was maintained at 7 min. A titanium plate was placed over the defect to prevent iatrogenic

fracture. The defect was closed in layers with 4-0 Vicryl suture (Ethicon, Somerville, NJ) in running and subcuticular stitch patterns for the muscle/fascia and skin, respectively. Each rabbit received a fentanyl patch and carprofen subcutaneously for pain management and inflammation, respectively, for 48 hours postoperatively.

2.7 Kidney Function

The kidney function of the rabbits was analyzed at 0, 1 and 5 weeks by measuring plasma creatinine and blood urea nitrogen from blood drawn from the ear vein. The plasma concentrations were measured using an IDEXX Vet Test 8008 (IDEXX Laboratories, Westbrook, ME).

2.8 Sample Culture

At 12 weeks postoperatively, the rabbits were euthanized by barbiturate overdose. A blood sample was taken sterilely from cardiac puncture with 3.8% sodium citrate. The anterior edge of the defect was exposed by sterile dissection. Sterile cotton swabs were used to collect samples from saliva, the anterior edge of the defect, and any abscesses in the craniofacial region. The swabs were cultured at 37°C on tryptic soy agar (BD, Franklin Lakes, NJ) and tryptic soy agar with 5% sheep blood (BD, Franklin Lakes, NJ) for blood samples. Individual colonies were tested for oxidase activity by oxidase reagent (PML Microbiologicals, Wilsonville, OR). The same colonies were smeared on glass slides and stained with Gram's stain. All Gram-negative oxidase-negative colonies were further identified by the API 20 NE kit (bioMerieux, Marcy l'Etoile, France).

2.9 Gross Observation

At the time of euthanasia, the right hemimandible was explanted. The mucosa over the defect was classified as healed or non-healed and the other oral mucosa was classified as dehisced or non-dehisced. The mandibles were then placed in 10% neutral buffered formalin for fixation for 72 hours. After 72 hours, the mandibles were transferred to 70% ethanol.

2.10 Histology

The mandibles were dehydrated in serial solutions of ethanol (70-100%) and embedded in methylmethacrylate. After complete polymerization, 10 μm sections were cut coronally using an inner circle diamond microtome (Leica, Wetzlar, Germany) and stained with methylene blue and basic fuchsin. Three blinded reviewers scored each specimen according to the scoring system shown in Table 2 to assess the tissue response around the implant and within the pores of the implant.[18] Images were obtained using an AxioImager Z.2 microscope (Zeiss, Oberkochen, Germany).

2.11 Statistical Analysis

The colistin released at each timepoint, the MIC of each group, and the creatinine and blood urea nitrogen values were compared using ANOVA with post-hoc analysis by Tukey's honestly significant difference test. The classifications of oral mucosae were compared using the Fisher-Freeman-Halton test with post-hoc analysis by Fisher's exact test. The histological scores of the space maintainers were compared using the Kruskal-

Wallis test with post-hoc analysis by the Mann-Whitney U test. An *a priori* level of significance was set at α=0.05. All analyses were performed using MATLAB (Version R2011B, Natick, MA).

3.0 Results

3.1 Colistin Release

Colistin was released for up to 8 weeks from the space maintainers *in vitro*. Figure 1 shows the release of colistin from each of the groups. Figure 1A shows cumulative release of colistin over 12 weeks. Cumulative release of colistin from each group was significantly different from the other groups at every timepoint ($p<0.05$). Figure 1B shows percent cumulative release over 12 weeks. At 6h and 12h, percent cumulative release of colistin from PLGA High and PLGA Low groups were not significantly different from each other, but Gelatin released significantly more of its loaded antibiotic than either of the other groups. Between 12h and 39d, all groups were significantly different from each other at every timepoint ($p<0.05$). At 42d, PLGA High was different from both PLGA Low and Gelatin, and at 46d, PLGA High was different from only PLGA Low. From 49d until 84d, there was no significant difference between any group ($p>0.05$).

3.2 Bacterial Susceptibility

The susceptibility of *A. baumannii* to colistin, colistin released from the Gelatin, PLGA Low and PLGA High groups was measured as the MIC according to ISO 20776 using the microdilution method. Colistin standard had an MIC of 4.00 ± 0.00 μg/mL. At

the 6 hour timepoint, colistin from the Gelatin group had an MIC of 1.67 ± 0.58 μg/mL, from the PLGA Low group had an MIC of 4.00 ± 0.00 μg/mL, and from the PLGA High group had an MIC of 3.33 ± 1.15 μg/mL. At the 39 day timepoint, colistin from the Gelatin group did not inhibit growth as there was no detectable colistin (i.e. no MIC determined), from the PLGA Low group had an MIC of 3.33 ± 1.15 μg/mL, and from the PLGA High group had an MIC of 4.00 ± 0.00 μg/mL. The MICs of each group are not statistically significant from each other ($p>0.05$).

3.3 Animal Care

Surgery was performed on 45 rabbits, with 5 prematurely euthanized animals that were not included in the analysis. All prematurely euthanized animals had complications unrelated to the treatment but rather due to general problems including unresolved postoperative diarrhea, neurological deficits due to spinal fracture, and unresolved foot wounds. The remaining 40 animals were healthy and were euthanized at 12 weeks postoperatively.

3.4 Kidney Function

Mean values for creatinine and BUN levels were within normal range (creatinine: 0.8-1.8 mg/dL; BUN: 10-24 mg/dL) for all animals, and there were no significant differences between groups at each time point (Figure 2). Creatinine and BUN remained below the threshold that would indicate acute tubular necrosis due to nephrotoxic systemic levels of colistin.

3.5 Gross Observation

In addition to healed (Fig. 3A) and non-healed (Fig. 3B) mucosal defects, some rabbits exhibited a separate medial mucosal dehiscence as seen in Figure 3C. The black arrows in Figures 3B and 3C indicate exposed implant through non-healed and dehisced mucosae, respectively. Figure 4A shows the number of healed mucosae in each group and Figure 4B shows the number of dehisced mucosae in each group. There were a significantly greater number of healed mucosae in the PLGA High group compared to the Gelatin group ($p<0.05$). There were no significant differences between groups for the dehiscences.

3.6 Sample Culture

All saliva swabs and 3 defect swabs grew bacteria; however, none of these bacteria proved to be *A. baumannii*. Colonies grown from saliva swabs were excluded based on Gram stain, oxidase presence, or with the API 20NE kit. No blood swabs grew bacteria. Two defects had abscesses; however, only one grew bacteria, which were gram positive.

3.7 Histology

Histological assessment of the space maintainers explanted from mandibles confirmed the presence of healed (Figures 5A and 5E) and non-healed (Figure 5C) oral mucosae in various samples. Histologic scoring of the tissue-implant interface revealed that the PLGA High group had significantly higher histological scores than the Uninfected group ($p<0.05$). As seen in Figure 6A, this is shown by all PLGA High

samples receiving a score of 2, representative of a highly organized fibrous capsule (Figure 5F). The Uninfected group had scores of 0, 1, and 2, indicating an abundance of inflammatory cells (Figure 5B), a poorly organized fibrous capsule, and a highly organized fibrous capsule, respectively. Although there were instances of direct bone implant contact (Figure 5D), this did not comprise the majority of the interface and thus could not receive a score of 3 or 4. No other significant differences were found for the tissue-implant interface. None of the histological scores for the pore tissue were significantly different ($p>0.05$), including scores of 0, 1, 2, and 3, representing exclusively inflammatory cells, a majority of inflammatory cells, immature fibrous tissue, and fibrous tissue and bone, respectively, as indicated in Table 2.

4.0 Discussion

This study evaluated porous space maintainers fabricated from clinically available materials *in vitro* and *in vivo* in an infected rabbit composite tissue defect for their effect on wound healing, kidney function, presence of *A. baumannii*, and tissue response to the construct.

Colistin was released *in vitro* over 12 weeks, and the total amount of colistin released at each timepoint as well as percentage of total colistin released was determined (Figure 1A and 1B). Gelatin was shown to have a high burst release, as evidenced by higher percentage of colistin released at early timepoints compared to PLGA High and PLGA Low groups (Figure 1B). By the end of the 12 weeks, there was no significant difference between the percentage cumulative release of the three groups (Figure 1B), although these values did not reach 100%. The most likely explanation for this is the

existence of non-interconnected pores in the construct, which sequester either gelatin or PLGA microspheres and prevent the release of some antibiotic. The colistin released from the constructs was tested against *A. baumannii in vitro* and showed effectiveness to *A. baumannii* similar to that of non-loaded colistin. This result verifies that the use of these fabrication and delivery methods for the colistin do not significantly alter the bioactivity of the antibiotic.

In the infected composite tissue defect, PLGA High group implants, which released colistin over an extended period of time *in vitro*, showed increased soft tissue healing compared to implants in the Gelatin group, which released colistin as a burst release *in vitro*. While this is an indirect measurement of infection clearance, previous studies have shown decreased healing in the presence of infection.[19, 20] Previous studies in non-porous PMMA based materials showed similar results in that the duration of release impacted reduction in bacterial load.[2, 5, 21] This may be due to some bacteria entering a sessile state as biofilm, thereby increasing the effective concentration necessary for clearance of the infection. These bacteria may then proliferate and colonize the wound after the short duration of antibiotic delivery, whereas they remain sessile with continued delivery of antibiotics in the extended release groups.[22]

For the first time using this model, a dehiscence separate from the intentional mucosal defect was observed. As seen in Figure 3C, these dehiscences were located on the lingual aspect of the mucosa overlying the implant. Because dehiscences were seen in the Uninfected group of this study, these dehiscences were likely not due to the presence of the inoculated bacteria. The primary differences in formulation between the samples in this study and the samples used in previous studies are the use of gelatin as a porogen and

the delivery of antibiotics.[9,10] These dehiscences could therefore be due to the presence of gelatin, colistin, or their combination. Although gelatin cannot be excluded as a cause of dehiscence, it is perhaps a less likely culprit as previous studies have not shown dehiscence when gelatin is implanted in the oral and maxillofacial region, though these studies are not extensive.[23,24] Previous studies have shown that antibiotics, while intended to target bacteria, can have detrimental effects on viability and function of host cells *in vitro*.[25,26] This toxic effect seen *in vitro* could lead to breakdown of the soft tissue, fenestration, and dehiscence *in vivo*. It is also possible that the combination of gelatin and colistin may lead to increased local toxicity if the gelatin causes increased tissue exposure to colistin by retaining released colistin in the area.

In addition to efficacy in infection clearance, safety was measured through the analysis of kidney function by plasma concentration of BUN and creatinine. The early discovery of nephrotoxicity with colistin has led to increased research into local delivery of the drug for increased local concentration with decreased systemic exposure.[16,27-29] This study showed that with high local delivery to the wound site, the negative systemic effects of colistin administration were avoided. Local delivery strategies may allow for increased application of powerful antibiotics that were previously underused clinically due to severe systemic adverse effects.

Clearance of the infection was also evaluated by cultures of the saliva, blood, and defect at 12 weeks. While three of the defects and all of the saliva swabs grew bacteria, none were identified to be *A. baumannii*. In the normal rabbit, saliva swabs would culture many different bacteria due to physiologic mucosal colonization. In addition, these bacteria could inoculate and colonize the porosity of the implant through the

mucosal defect. This is corroborated by the few defect swabs that did culture bacteria that were not identified as *A. baumannii*. This finding is consistent with previous studies where increased inflammation in highly porous space maintainers was attributed to colonization of the implant by oral flora.[9] Additionally, a recent study showed that 37% of traumatic injuries resulted in polymicrobial infections.[30] Thus, while the delivery of a single antibiotic is likely inadequate alone to treat such polymicrobial wound infections, the animal model used in this study presents a complex environment very similar to the targeted problem area.

Although there were significant differences in gross wound healing between the PLGA High and Gelatin groups, no significant difference was observed between the two groups in the histological response of the tissue-implant interface and the tissue within the pores. As seen in Figure 6A, many of the samples received scores of 2 for a mature fibrous capsule; however, for several of these samples, there was a layer of inflammatory cells between the fibrous capsule and the implant as shown in Figure 5B. This has been previously demonstrated to be due to continued release of proinflammatory molecules being released from the implant resulting in the invasion by inflammatory cells.[31] Inflammatory molecules from bacterial colonization or the degradation products of PLGA or gelatin may be released from the implant at late time points, resulting in the recruitment of inflammatory cells. These differences are reflected in the organization of the fibrous capsule for implants from the current study. Finally, there are several instances of direct bone-implant contact either at the interface or in the pores of the implant, as shown in Figure 5D, illustrating that after 12 weeks, there is some growth of bone around and in the implant.

This study would be strengthened by the use of infected untreated controls to verify that an initial infection with *A. baumannii* was achieved. However, an untreated infection in the craniofacial region presents animal welfare concerns, including pain and discomfort, and was accordingly not included in the study. There are several important strengths to this study. First, the formulations used in this study allow for comparison of the effects of extended release and burst release of colistin on gross and microscopic tissue healing. Second, dose effects of the extended release formulation were also investigated by using a high and low dose loading of colistin-loaded PLGA microparticles into the constructs. Last, the measurement of BUN and creatinine as well as soft tissue healing allowed for safety and efficacy measures in the same model.

Considering the results, there are a few areas from this study that could receive further investigation. While this study elucidated the role of colistin release from the space maintainer against *A. baumannii* in this defect, the expansion of this study to investigate various antibiotics, bacterial strains and combinations thereof is important to address the clinical nature of many traumatic facial injuries. Additionally, further study into the mechanisms and relationships of the tissue regeneration with respect to infection clearance and delivery of the antibiotic is important.

Conclusions

This study analyzed the *in vitro* release characteristics of colistin from porous PMMA space maintainers and evaluated the safety and efficacy of these antibiotic-containing constructs *in vivo* in an infected rabbit mandibular defect. Extended, high-dose antibiotic delivery via PLGA microparticles appeared to improve gross soft tissue healing

compared to burst release via a gelatin carrier in an infected composite tissue defect model over a period of 12 weeks. These results indicate that duration of delivery affects soft tissue healing. Local delivery did not result in systemic side effects, specifically nephrotoxicity. Prolonged delivery of antibiotics may mitigate failure of surgical implants placed in contaminated wounds, obviating the need for systemic treatment and reducing systemic side effects, while simultaneously improving healing.

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Figure Legends

Figure 1. *In vitro* release of colistin from constructs presented as mean + standard deviation (n = 3). (A) Cumulative release of colistin from each group. Every group is significantly different from all other groups at all timepoints ($p < 0.05$). (B) Percentage cumulative release of colistin from each group. The Gelatin group releases significantly more of its loaded drug at 6h compared to PLGA High and PLGA Low. All groups are significantly different at 12h through 39d. At 42d, PLGA High is significantly different from both PLGA Low and Gelatin, and at 46d, only PLGA High and PLGA Low are different from each other ($p<0.05$). There is no significant difference between groups from 49d until 84d.

Figure 2. Plasma concentrations of (A) BUN and (B) creatinine evaluated preoperatively (week 0) and at 1 and 5 weeks postoperatively to assess kidney function. Data are presented as means ± the standard deviation (n = 10). Normal ranges: BUN: 10-24 mg/dL and creatinine: 0.8-1.8 mg/dL. There were no significant differences between groups for BUN or creatinine plasma concentrations.

Figure 3. Gross photographs of oral mucosae 12 weeks postoperatively. Images show (A) well-healed, (B) non-healed, and (C) medially dehisced mucosae. Black arrows in (B) and (C) indicate exposed implant through non-healed and dehisced mucosae, respectively.

Figure 4. (A) Number of healed versus non-healed mucosal defects as well as (B) dehisced and non-dehisced mucosae for each group. * indicates a significant difference between the PLGA High and the Gelatin groups ($p < 0.05$).

Figure 5. Representative low (2X) (A, C, E) and high (10X) (B, D, F) magnification histological images stained with methylene blue and basic fuchsin (A, B: Uninfected; C, D: Gelatin; E, F: PLGA High). Titanium plates are indicated with a (p) and the implant with an (i). Healed mucosa over the implants in (A) and (E) are indicated with black arrows. Implant exposure in a non-healed defect (C) is indicated with a black arrowhead. High magnification images (B), (D), and (F) were taken from low magnification images (A), (C), and (E), respectively, at a location indicated with a rectangle to display tissue-implant interface features. (B) shows an abundance of inflammatory cells (ic) inside a thick fibrous capsule (c) at the tissue implant interface. In (D), bone (b) is seen in direct contact with the implant (i). (F) is a high magnification image of a highly organized fibrous capsule (c).

Figure 6. Distribution of histological scores of the (A) tissue-implant interface and the (B) tissue within the pores of the implants using the scoring system described in Table 2. * indicates a significant difference between the Uninfected and the PLGA High groups for the tissue-implant interface score ($p < 0.05$).

Tables

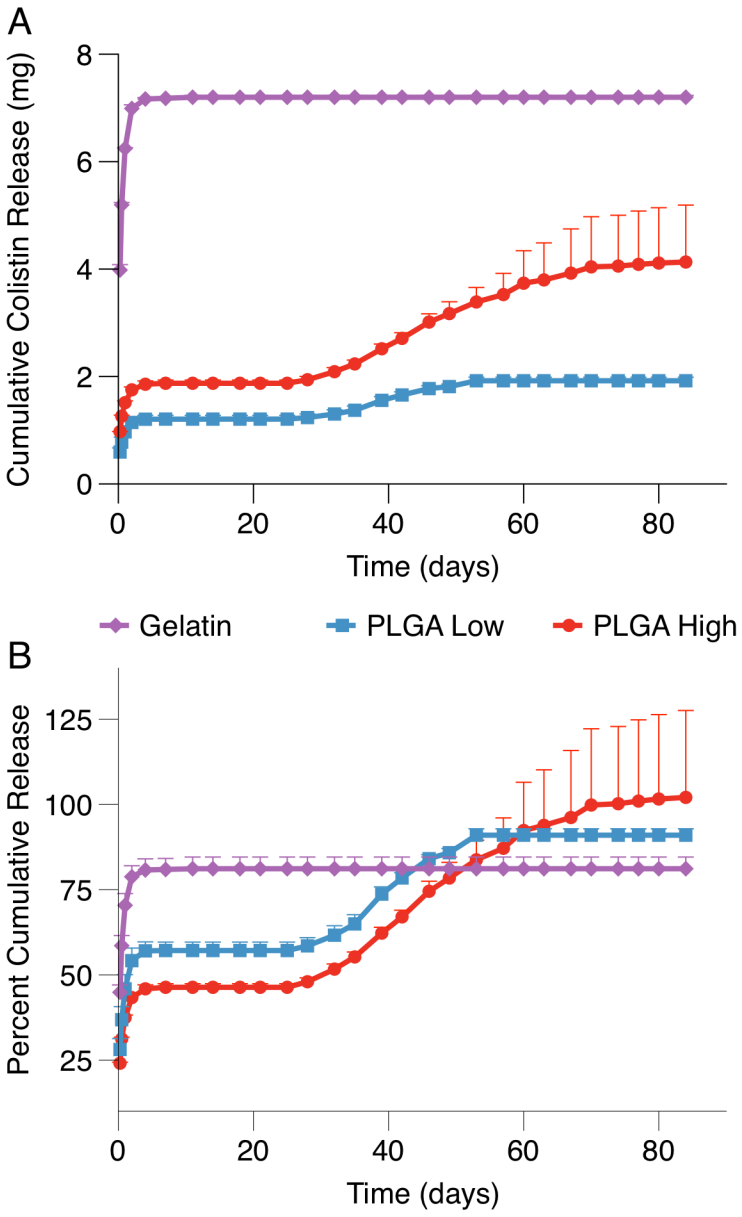
Table 1. The composition of PMMA/PLGA/gelatin/colistin constructs examined.

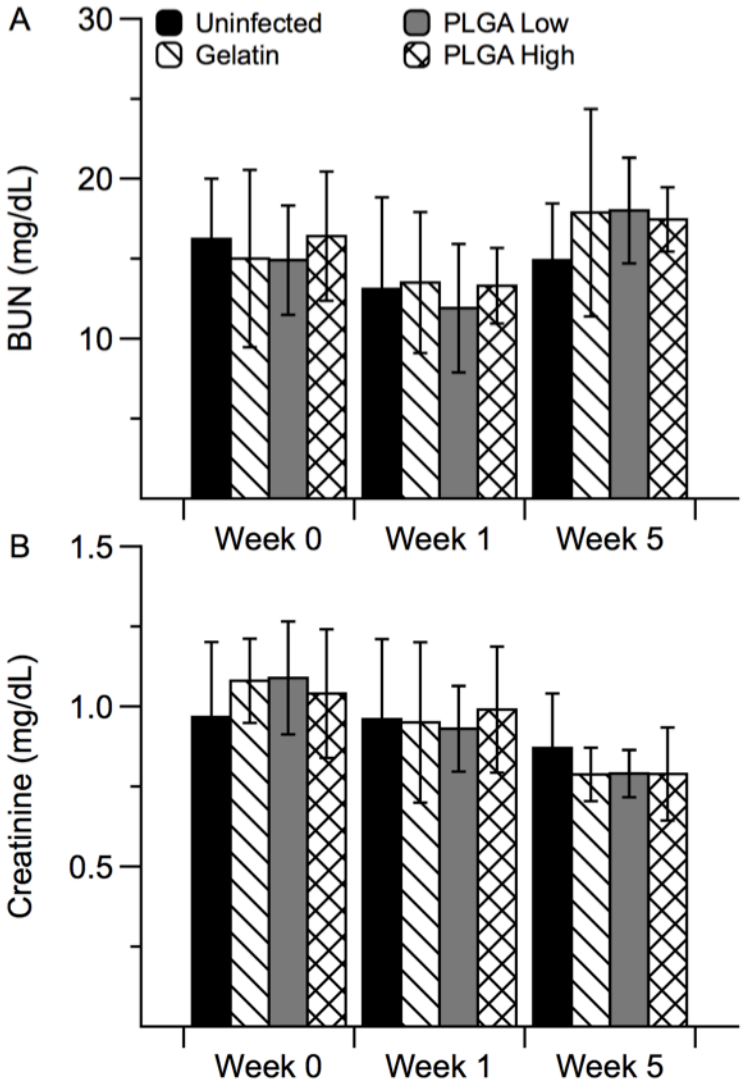
	Gelatin matrix parameters		Implant composition				
Group	Swelling Ratio	Drug in gelatin matrix (wt%)	Gelatin matrix (wt%)	Powder phase of bone cement (wt%)	Monomer phase of bone cement (wt%)	Colistin-loaded PLGA (wt%)	Calculated drug content (wt%)
Uninfected	1:1.9	0	26.7	42.3	20.0	11.0	0.67
Gelatin	1:1.9	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

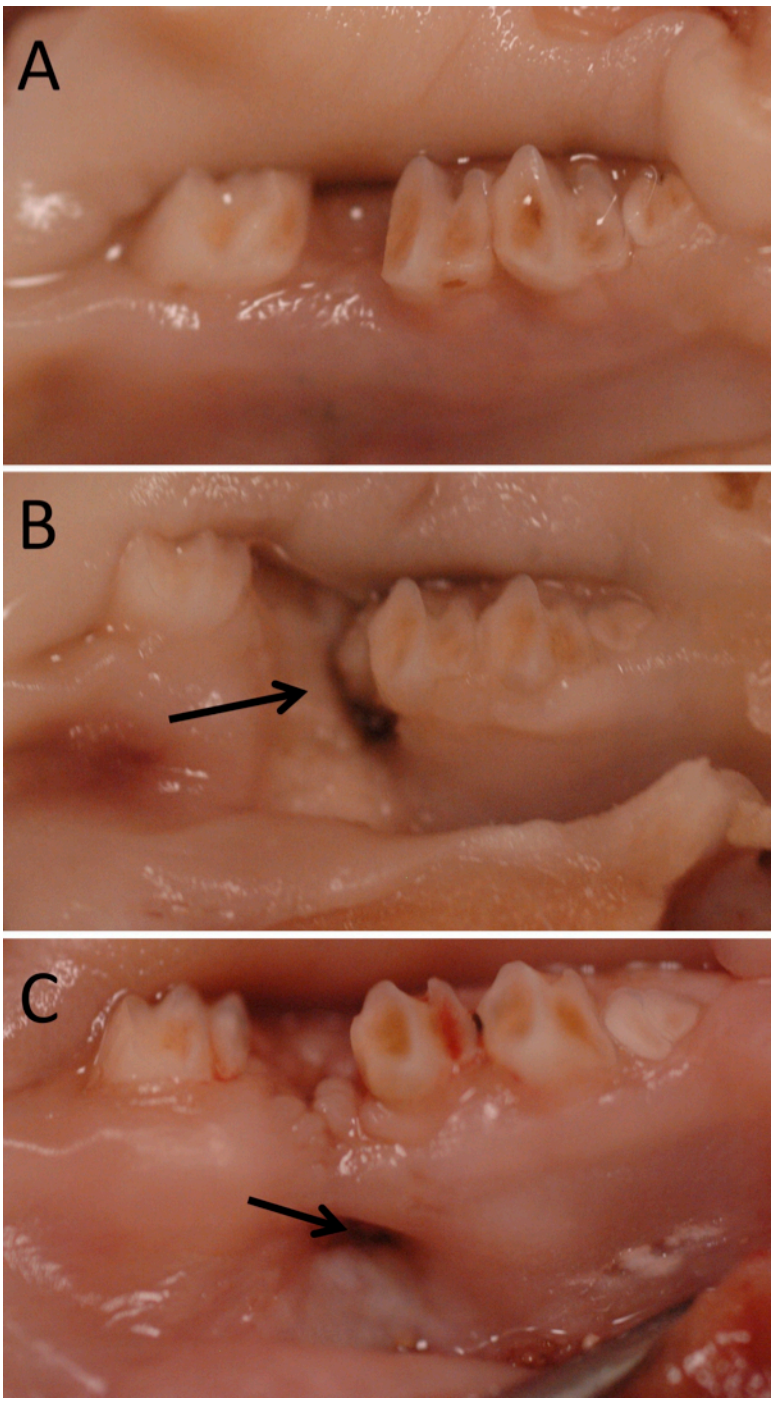
* in the PLGA Low group there is an additional 5.5 wt% of blank PLGA microparticles.

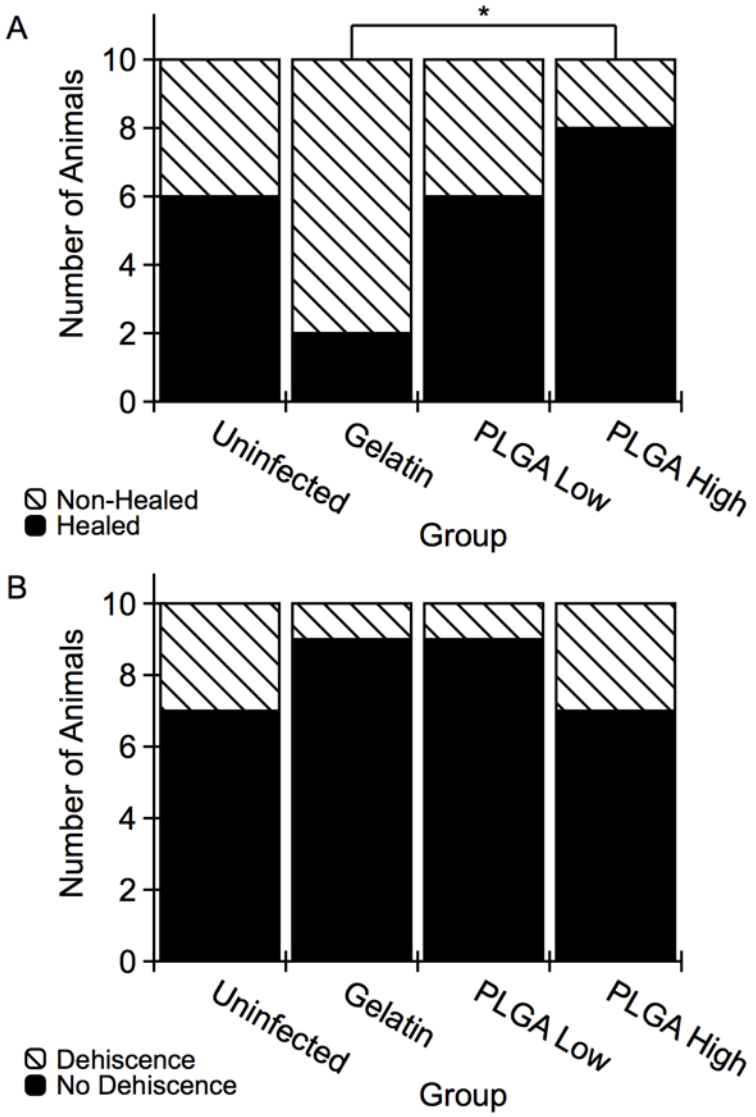
Table 2. Histological scoring system for the implant tissue interface and the tissue in the pores of the implant.[18]

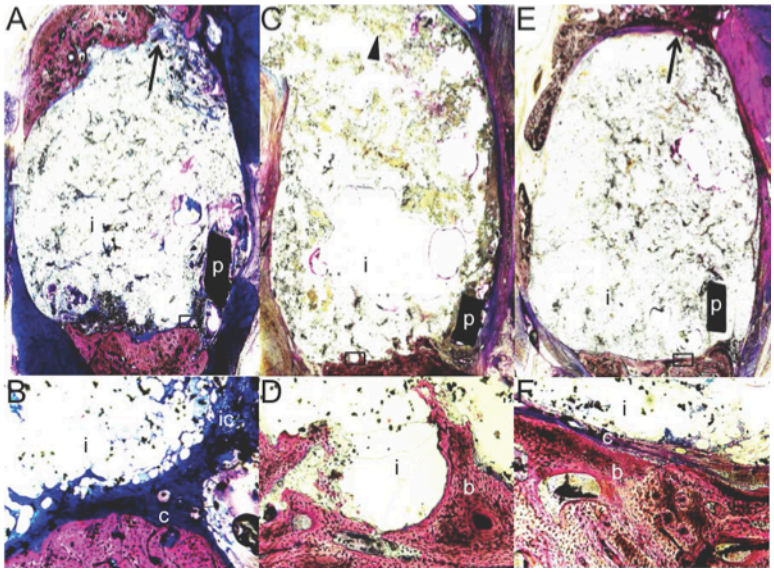
Hard tissue response at the implant-bone interface	Score
Direct bone-to-implant contact without soft interlayer	4
Remodeling lacuna with osteoblasts and/or osteoclasts at surface	3
Majority of implant is surrounded by fibrous tissue capsule	2
Unorganized fibrous tissue (majority of tissue is not arranged as capsule)	1
Inflammation marked by an abundance of inflammatory cells and poorly organized tissue	0
Hard tissue response within the pores of the scaffold	
Tissue in pores is mostly bone	4
Tissue in pores consists of some bone within mature, dense fibrous tissue and/or a few inflammatory response elements	3
Tissue in pores is mostly immature fibrous tissue (with or without bone) with blood vessels and young fibroblasts invading the space with few macrophages present	2
Tissue in pores consists mostly of inflammatory cells and connective tissue components in between (with or without bone) or the majority of the pores are empty or filled with fluid	1
Tissue in pores is dense and exclusively of inflammatory type (no bone present)	0

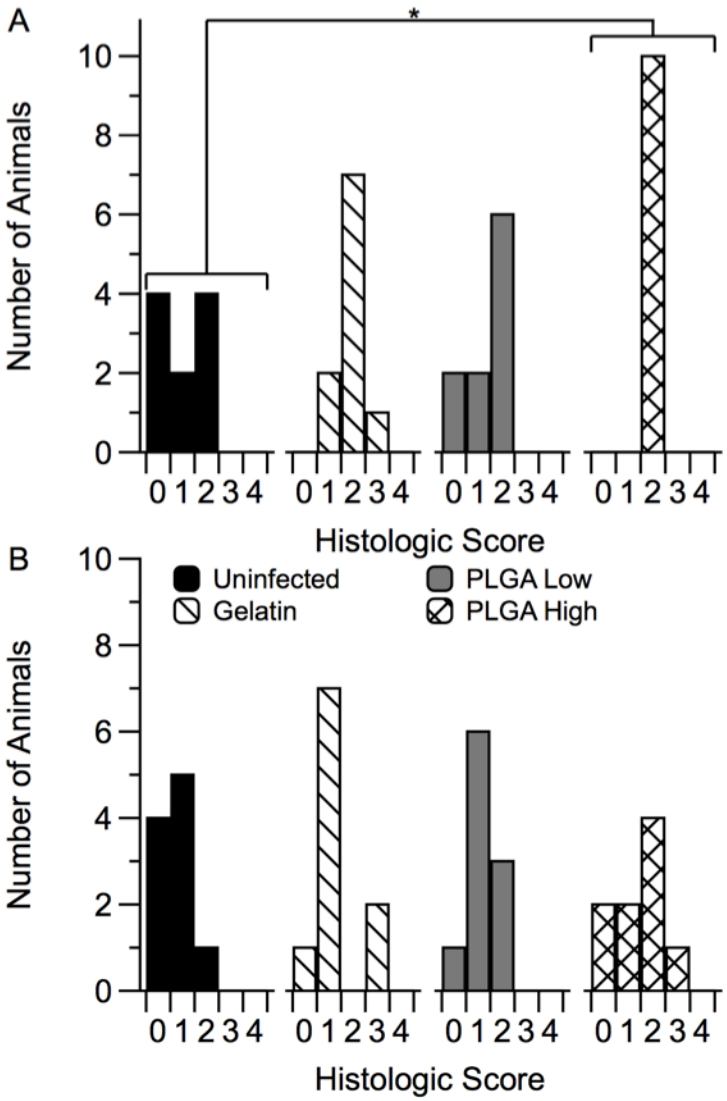

A
Cumulative Colistin Release (mg)
8
6
4
2
0
0
20
40
60
80
Time (days)
Gelatin
PLGA Low
PLGA High
B
Percent Cumulative Release
125
100
75
50
25
0
20
40
60
80
Time (days)


A
30
20
10
BUN (mg/dL)
Uninfected
Gelatin
PLGA Low
PLGA High
Week 0
Week 1
Week 5
B
1.5
1.0
0.5
Creatinine (mg/dL)
Week 0
Week 1
Week 5


A
B
C


A
*
Number of Animals
10
8
6
4
2
0
Uninfected
Gelatin
PLGA Low
PLGA High
Non-Healed
Healed
Group
B
Number of Animals
10
8
6
4
2
0
Uninfected
Gelatin
PLGA Low
PLGA High
Dehiscence
No Dehiscence
Group


A
C
E
i
p
i
p
i
p
B
D
F
ic
i
c
i
b
i
c
b


A
*
Number of Animals
10
8
6
4
2
0
0 1 2 3 4
0 1 2 3 4
0 1 2 3 4
0 1 2 3 4
Histologic Score
B
Uninfected
Gelatin
PLGA Low
PLGA High
Number of Animals
10
8
6
4
2
0
0 1 2 3 4
0 1 2 3 4
0 1 2 3 4
0 1 2 3 4
Histologic Score