Osteomyelitis is one of the most challenging complications after joint replacement, orthopedic reconstructive surgery, and fracture repair. Infection remains the most common complication associated with limb-sparing procedures in small animals and can reportedly affect up to 49% of patients. In fact, osteomyelitis was reported as the single most common complication in a retrospective study of 253 tibial plateau leveling osteotomies.

Local antimicrobial treatment has become crucial in the elimination of orthopedic infections in humans and equids and is gaining popularity for use in small animals. This approach relies on local delivery of antimicrobials to achieve higher tissue concentrations while avoiding systemic toxic effects. Antimicrobial-loaded PMMA pellets are the most commonly used carriers for local delivery of antimicrobials in veterinary surgery and are used as a control method for evaluation of new carriers. However, PMMA inhibits bone formation, releases toxic substances during curing, provides a surface for bacterial colonization, and is not resorbable, thereby requiring surgical removal. Therefore, research interests in procedures.

Effects of porcine small intestinal submucosa on elution characteristics of gentamicin-impregnated plaster of Paris

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Objective—To evaluate effects of small intestinal submucosa (SIS) on elution properties of plaster of Paris (POP).

Sample Population—27 POP cylinders, 27 POP spheres, and 9 polymethylmethacrylate (PMMA) spheres.

Procedures—Pellets were loaded with gentamicin (50 mg/g) and divided into 7 groups of 9 beads each: PMMA spheres; POP cylinders coated with 0, 4, or 8 layers of SIS; and POP spheres coated with 0, 4, or 8 layers of SIS. Gentamicin concentration was measured 6, 12, 18, 24, 32, and 48 hours and 3, 4, 5, 7, 14, 21, 28, 35, and 42 days after wrapping. Porosity was evaluated via scanning electron microscopy. Curvature factor of elution curves, total amount of drug released (TDR), time required to reach 50% of total release (TDR_{t50}), and number of days with concentrations ≥1 µg/mL were compared among groups.

Results—SIS decreased the curvature factor and increased the TDR_{t50} and TDR of POP spheres and cylinders. Curvature factor of the PMMA-release curve remained lower than that for any POP group, but all POP groups wrapped in SIS released more gentamicin than PMMA spheres. Gentamicin concentrations remained ≥1 µg/mL in SIS-wrapped POP and PMMA groups throughout the study. Wrapping POP in SIS minimized the increase in porosity of pellets.

Conclusions and Clinical Relevance—Wrapping POP with SIS slows the release and increases the amount of gentamicin leaching from spheres and cylinders. All groups wrapped in SIS maintained antimicrobial concentrations greater than the minimum inhibitory concentration of most pathogens. (Am J Vet Res 2007;68:171–177)

Abbreviations

PMMA Polymethylmethacrylate
POP Plaster of Paris
SIS Small intestinal submucosa
MIC Minimum inhibitory concentration
TDR Total amount of drug released at infinity
TDR_{t50} Time needed for the cumulative drug release to reach 50% of TDR
\( \gamma \) Curvature factor of the cumulative release-versus-time curves
human orthopedics have focused on the development of resorbable systems for local delivery. Among these, POP has been used as a biodegradable, osteoconductive filler that is readily available and releases most of its antimicrobial content.\textsuperscript{12,13} Medical-grade calcium sulfate is available as pellets formulated to slow its otherwise extremely rapid rate of resorption. These pellets have been used to treat osteomyelitis in humans but are cost prohibitive for use in small animals.\textsuperscript{13} From a practical standpoint, there is currently no resorbable carrier that provides local delivery of antimicrobials for use in veterinary medicine.

Our long-term goal is to design a biodegradable carrier that can be used by practitioners to provide localized delivery of antimicrobials while stimulating bone healing (osteochonduction) in patients with osteomyelitis. Wrapping POP in porcine SIS is a novel approach. Small intestinal submucosa is available on the veterinary market as a biological wound dressing.\textsuperscript{12,13} Although most clinical applications have focused on reconstruction of soft tissues, the properties of porcine SIS are relevant to orthopedics. Indeed, this product primarily contains collagen I along with some osteoinductive growth factors, such as transforming growth factor-β and fibroblast growth factor.\textsuperscript{14} Collagen I forms 95% of the organic phase of bone and is used as an osteoconductive carrier for growth factors.\textsuperscript{15} In addition, porcine SIS has antibacterial properties,\textsuperscript{16–18} presumably attributable to cefropins, a family of proteins that lyse bacterial cell membranes. Combined, these properties make porcine SIS an attractive candidate to adjust the elution behavior of POP and enhance bone formation in infected tissues.

The objective of the study reported here was to evaluate the effects of SIS on the elution characteristics of gentamicin from POP pellets. We hypothesized that SIS would slow the release of antimicrobials from POP cylinders and spheres and maintain concentrations greater than the MIC for most pathogens throughout the study.

**Materials and Methods**

**Preparation of pellets**—Sixty-three pellets (27 POP cylinders, 27 POP spheres, and 9 PMMA spheres) impregnated with gentamicin (50 mg/g) were prepared\textsuperscript{19,20} for use in the study. Gentamicin was selected because it is the antimicrobial used most commonly to impregnate PMMA for treatment or prevention of osteomyelitis in veterinary orthopedics.\textsuperscript{5–7}

The control compound was PMMA\textsuperscript{a} spheres prepared as described elsewhere.\textsuperscript{1} Briefly, 10 g of PMMA powder, 5 mL of liquid monomer, and 5 mL (500 mg) of gentamicin sulfate solution\textsuperscript{b} were thoroughly mixed for 2 minutes by use of a sterile spatula. Each batch was poured into a silicon mold\textsuperscript{c} to make spheres (5 mm in diameter).

Plaster of Paris powder\textsuperscript{d} was sterilized by heating for 4 hours at 100°C.\textsuperscript{19} Five milliliters (500 mg) of gentamicin sulfate was added to each 10-g portion of POP powder. Four milliliters of sterile water was added to the mixture and evenly blended. A 3-mL syringe was used to inject the paste into spherical or cylindrical molds. The POP spheres were prepared in the same silicon mold\textsuperscript{c} that was used to prepare the PMMA spheres. Rubber tubes\textsuperscript{e} were used as molds to prepare POP cylinders (20 mm in height and 2 mm in diameter). Thirty minutes after POP and PMMA pellets were cured, they were randomly allocated into sets (3 pellets/set) and weighed.

**Elution study**—Seven groups (9 pellets/group) were prepared for the study. The groups comprised PMMA spheres; POP cylinders wrapped in 0, 4, or 8 layers of SIS; and POP spheres wrapped in 0, 4, or 8 layers of SIS. Pellets assigned to SIS groups were wrapped in porcine SIS.\textsuperscript{1} Four or 8 layers of SIS (each layer was 150 µm thick) were sutured in position with a simple interrupted pattern by use of 4-0 polydioxanone suture material (Figure 1). This bioresorbable monofilament material was selected because it would allow degradation of pellets after implantation and minimize the risk of nidus formation.

Within each treatment group, 3 pellets were placed in each of 3 sterile, 17 × 100-mm polystyrene culture tubes.\textsuperscript{5} Each tube contained 3 mL of normal canine serum.\textsuperscript{5} Therefore, elution experiments were repeated 3 times for each group, as recommended for in vitro tests.\textsuperscript{22–25} Initiation of the elution period was designated as time 0 on day 0.

Canine serum was selected for use as the elution fluid, and pellets were agitated at a setting of 50 rounds/ min at 37°C on a shaking incubator to best simulate in vivo conditions.\textsuperscript{23,24–25} The volume of elution fluid was set at 1 mL/pellet to approximate the volume of fluid around a pellet implanted in tissue.\textsuperscript{23,30} Serum (3 mL) was collected 6, 12, 18, 24, 32, 40, and 48 hours and 3, 4, 5, 7, 14, 21, 28, 35, and 42 days after start of elution and replaced with 3 mL of fresh serum at each evaluation time, as described in other elution tests.\textsuperscript{30,31,22,23,33} Collected serum samples were stored at –20°C until assayed.\textsuperscript{34}

**Measurement of gentamicin concentration**—Gentamicin concentration in each elution sample was determined with a particle-enhanced turbidimetric inhibition immunoassay\textsuperscript{30,33} by use of a commercially available system.\textsuperscript{5} Particle-enhanced turbidimetric inhibition immunoassay is sensitive, easy to perform, and a more cost-effective method than high-performance liquid chromatography. The immunoassay was validated for use on canine serum by measuring quadruple samples of canine serum that contained 6 known concentrations of gentamicin (100, 50, 10, 1, 0.5, and 0 µg/mL). The minimum detectable concentration of gentamicin in our study was 0.5 µg/mL, which is consistent with results of another study.\textsuperscript{33} The calculated interassay coefficient of variation was 6.18%. Regression coefficient of the concentration–measurement curve was 99%.

**Scanning electron microscopy**—Morphologic changes of the pellets and SIS wrapping were evaluated on days 0 and 42 by use of scanning electron microscopy of 3 samples from each group. Samples were fixed as described elsewhere\textsuperscript{36} before they were examined on a scanning electron microscope\textsuperscript{5} at 20 KV. Digital images were analyzed by use of computer software.\textsuperscript{5} This software automatically measures black areas in the 2 × 2-mm referent field, which correspond to voids within the material. Porosity was then expressed as the percentage of the surface that consisted of pores.
Data analysis—Pellets were weighed, and the initial gentamicin content of each pellet was calculated on the basis of the loading concentration (50 mg/g) and the weight of each pellet. Concentrations of gentamicin subsequently measured in the elution medium were adjusted on the basis of the initial gentamicin content and compared among groups. The amount of gentamicin released during the first 48 hours of elution was expressed as a percentage of the initial antimicrobial content and compared among groups. Kinetic curves were established over 42 days of elution and fitted to a cumulative release model by use of pharmacokinetic software. Cumulative drug release was calculated by use of the following equation:

\[
\text{Cumulative drug release} = \frac{(\text{TDR} \times t^\gamma)}{(t + \text{TDR} t_{50}^\gamma)}
\]

where \(t\) is the time of sample collection and \(\gamma\) is a factor that averages instant slopes at sample collection time points.

Variables estimated by use of this pharmacokinetic model included the amount of antimicrobial released (represented by TDR) and speed of release (represented by \(\gamma\) and TDR\(_{t_{50}}\)). The curvature factor of the curves (ie, \(\gamma\)) represents the overall speed of release throughout the entire study, whereas TDR\(_{t_{50}}\) is more representative of the initial speed of release. A shorter TDR\(_{t_{50}}\) and greater \(\gamma\) correlate with faster release.

Adequate concentrations of antimicrobial were defined as concentrations higher than the established MIC of gentamicin (1 \(\mu\)g/mL) for pathogens most commonly isolated from small animals clinically affected with osteomyelitis.\(^22,30,35\) The number of days on which the released concentrations for each group equaled or were higher than 1 \(\mu\)g/mL was then calculated.

Statistical analysis—Parametric data were compared among groups by use of a repeated-measures ANOVA. When indicated, a post hoc test was conducted by use of the Fisher least significant difference test with adjusted \(P\) values. Values of \(P \leq 0.05\) were considered significant.

Results

All groups released measurable amounts of gentamicin during the 42 days of the study. Elution curves had a bimodal pattern, with an initial release of high concentrations followed by a slower sustained release (Figure 2). The percentage of the initial gentamicin content released after 48 hours of elution in each group was calculated (Table 1). The POP cylinders that were not wrapped in SIS released less antimicrobial during the first 48 hours of the study, compared with release for POP cylinders wrapped in 4 or 8 layers of SIS. Similar results were obtained with spheres wrapped in 4 layers of SIS. Mean \(\pm\) SEM initial release of gentamicin from PMMA spheres (12 \(\pm\) 1.7%) did not differ from that of POP cylinders that were not wrapped in SIS but was less than that of POP spheres that were not wrapped in SIS (29 \(\pm\) 3.5%).

Mean \(\pm\) SEM TDR ranged from 12.6 \(\pm\) 0.9% of the initial content for POP cylinders not wrapped in SIS to 61.7 \(\pm\) 0.05% for POP cylinders wrapped in 8 layers of SIS (Table 1). Regardless of shape, TDR increased when POP pellets were wrapped in SIS (Table 1). The TDR\(_{t_{50}}\) was greatest for POP spheres and cylinders wrapped in 8 layers of SIS, whereas POP spheres and cylinders not wrapped in SIS, POP spheres and cylinders wrapped in 4 layers of SIS, and PMMA spheres had the shortest TDR\(_{t_{50}}\) (Table 1). Regardless of shape, TDR\(_{t_{50}}\) increased when the number of SIS layers increased from 4 to 8.
Values for \( \gamma \) were consistent with the other results (Table 1). Within each shape, wrapping pellets in SIS decreased the curvature of cumulative release curves (0 vs 4 or 8 layers). Although the lowest \( \gamma \) value in all biodegradable groups was achieved for POP spheres wrapped with 8 layers of SIS, it was still greater than that for PMMA spheres.

The PMMA spheres maintained a concentration \( \geq 1 \mu g/mL \) for a longer period than did POP spheres not wrapped in SIS. All groups except POP spheres not wrapped in SIS released gentamicin concentrations \( \geq 1 \mu g/mL \) for 42 days. Wrapping POP spheres in SIS increased the number of days with concentrations \( \geq 1 \mu g/mL \) (Figure 2).

The SIS remained grossly intact at the end of the study in all groups. Morphologic changes evident during scanning electron microscopy appeared especially obvious in POP pellets wrapped in 4 layers of SIS or POP pellets that were not wrapped in SIS (Figure 3).

Table 1—Mean \( \pm \) SD values for 7 groups (9 pellets/group) of PMMA spheres or POP spheres or cylinders that contained gentamicin (50 mg/g) and were used to evaluate release of gentamicin during a 42-day period.

<table>
<thead>
<tr>
<th>Variable</th>
<th>PMMA</th>
<th>POP cylinders*</th>
<th>POP spheres*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Weight (mg)</td>
<td>680.3 ( \pm ) 42.5</td>
<td>642.7 ( \pm ) 15.4</td>
<td>624.3 ( \pm ) 22.5</td>
</tr>
<tr>
<td>Initial drug release (%)</td>
<td>12.3 ( \pm ) 1.7 ( \text{a} )</td>
<td>12.6 ( \pm ) 1.7 ( \text{a} )</td>
<td>54.0 ( \pm ) 5.3 ( \text{b} )</td>
</tr>
<tr>
<td>TDR (mg/mL/( % ))</td>
<td>1.7 ( \pm ) 0.2 ( \text{a} )</td>
<td>1.4 ( \pm ) 0.1 ( \text{a} )</td>
<td>5.7 ( \pm ) 0.3 ( \text{b} )</td>
</tr>
<tr>
<td>TDR(_{50}) (d)</td>
<td>0.30 ( \pm ) 0.01 ( \text{a} )</td>
<td>0.20 ( \pm ) 0.01 ( \text{a} )</td>
<td>0.30 ( \pm ) 0.00 ( \text{b} )</td>
</tr>
<tr>
<td>( \gamma )</td>
<td>1.0 ( \pm ) 0.0 ( \text{a} )</td>
<td>0.3 ( \pm ) 0.3 ( \text{a} )</td>
<td>2.4 ( \pm ) 0.2 ( \text{c} )</td>
</tr>
</tbody>
</table>

*The POP cylinders and spheres were wrapped in 0, 4, or 8 layers of porcine SIS and placed in canine serum to determine elution characteristics. †Values reported are mean \( \pm \) SEM. ‡Represents the percentage of drug released within 48 hours after onset of the elution study. §Represents the cumulative concentration released from each pellet. ¶Represents cumulative release percentage of the initial gentamicin content of each pellet.

Values for \( \gamma \) were consistent with the other results (Table 1). Within each shape, wrapping pellets in SIS decreased the curvature of cumulative release curves (0 vs 4 or 8 layers). Although the lowest \( \gamma \) value in all biodegradable groups was achieved for POP spheres wrapped with 8 layers of SIS, it was still greater than that for PMMA spheres.

The PMMA spheres maintained a concentration \( \geq 1 \mu g/mL \) for a longer period than did POP spheres not wrapped in SIS. All groups except POP spheres not wrapped in SIS released gentamicin concentrations \( \geq 1 \mu g/mL \) for 42 days. Wrapping POP spheres in SIS increased the number of days with concentrations \( \geq 1 \mu g/mL \) (Figure 2).

The SIS remained grossly intact at the end of the study in all groups. Morphologic changes evident during scanning electron microscopy appeared especially obvious in POP pellets wrapped in 4 layers of SIS or POP pellets that were not wrapped in SIS (Figure 3).

The greatest porosity was measured in POP pellets not wrapped in SIS, whereas the lowest porosity was obtained when POP pellets were wrapped in 8 layers of SIS (Figure 4).

**Discussion**

The main findings of the study reported here were that wrapping POP spheres and cylinders in SIS slowed the release of antimicrobial (decrease in \( \gamma \) and increase in TDR\(_{50}\)); wrapping POP spheres and cylinders in SIS decreased the curvature of cumulative release curves (0 vs 4 or 8 layers). Although the lowest \( \gamma \) value in all biodegradable groups was achieved for POP spheres wrapped with 8 layers of SIS, it was still greater than that for PMMA spheres.

The PMMA spheres maintained a concentration \( \geq 1 \mu g/mL \) for a longer period than did POP spheres not wrapped in SIS. All groups except POP spheres not wrapped in SIS released gentamicin concentrations \( \geq 1 \mu g/mL \) for 42 days. Wrapping POP spheres in SIS increased the number of days with concentrations \( \geq 1 \mu g/mL \) (Figure 2).

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The greatest porosity was measured in POP pellets not wrapped in SIS, whereas the lowest porosity was obtained when POP pellets were wrapped in 8 layers of SIS (Figure 4).
cylinders in SIS increased TDR, with all POP pellets wrapped in 4 or 8 layers of SIS releasing more antimicrobial than was released from PMMA; antimicrobial concentrations remained ≥ 1 µg/mL throughout the study for POP pellets wrapped in SIS and PMMA spheres, and wrapping POP spheres and cylinders in SIS minimized the increase in porosity detected during scanning electron microscopy.

Wrapping POP spheres or cylinders in 8 layers of SIS slowed the rate of antimicrobial release (decrease in γ and increase in TDR γ50), compared with results for POP pellets of similar shape. Increasing the thickness of wrapping from 4 to 8 layers further increased TDR γ50 for both spheres and cylinders. Although the TDR γ50 nearly doubled when spheres and cylinders were wrapped with 8 layers of SIS, the overall magnitude of this increase (approx 7 hours) may not be clinically important. Nonetheless, γ reflected the speed of release throughout the study, and γ decreased when pellets were wrapped in 4 or 8 layers of SIS. These findings most likely resulted from the physical barrier created by layers of SIS, which slowed diffusion of antimicrobial and the dissolution of POP. Other investigators have reported similar results and were able to extend the release of vancomycin to > 5 weeks by coating POP pellets with 6 layers of polylactide-co-glycolide polymer. Although this approach may be valid, polymers are not widely accessible to veterinarians and do not contain growth factors.

Wrapping POP in SIS increased the total amount of antimicrobial released by spheres and cylinders. This finding may have resulted from a release of gentamicin naturally contained in SIS, interference of SIS with the technique used to measure gentamicin concentrations, or an increase in the release of antimicrobial by SIS-wrapped POP. Porcine SIS may have inherent antibacterial properties but is devoid of intrinsic gentamicin.29 In a preliminary study, samples of SIS were incubated in serum to verify a lack of interference with the immunoassay. Therefore, it is more likely that the greater amounts (TDR) of antimicrobial measured in SIS-wrapped POP groups resulted from enhanced gentamicin release during 1 or both phases of the study. Indeed, the elution curves of POP pellets in the study had a bimodal pattern, with a burst of release during the first 48 hours of the study that was followed by a sustained release; this bimodal pattern of release has been reported in other studies.5,10,19,24,37 The initial rapid release of gentamicin from POP and PMMA has been attributed to diffusion of antimicrobial from the exposed surface of the pellets into the surrounding medium.20,17,38 Release of antimicrobial from POP then results from diffusion along the concentration gradient between the center toward the periphery of the pellet. The amount of antimicrobial released is proportional to the dissolution of POP and continues until dissolution is complete.29,37

The increase in porosity measured during scanning electron microscopy would be consistent with dissolution of the pellets, but it was inversely correlated with the number of layers of SIS. In other words, SIS wrapping appeared to protect POP pellets from structural changes and dissolution. These results reflected the barrier effect of SIS but do not support dissolution of POP as a major mechanism for increasing antimicrobial release from wrapped pellets. Instead, SIS appears to facilitate the initial leaching of antimicrobial from the surface of POP cylinders and spheres. This theory is especially supported by the amount of antimicrobial released from cylinders at 48 hours. Additional studies are warranted to explore the mechanism behind this increase in antimicrobial release.

Although wrapping POP pellets in SIS slowed release of antimicrobial, γ for all POP pellets (including those that were not wrapped in SIS) remained greater than that for PMMA spheres. This would suggest that PMMA released gentamicin slower than any of the POP groups. Although the mechanism responsible for elution of drugs from PMMA after the initial leaching phase remains controversial, diffusion of antimicrobial via interconnected cracks and pores is considered to be the main mechanism for the slower release observed in the later phase of elution from PMMA spheres.23,38 Therefore, the lack of dissolution of PMMA explains the slower elution properties, compared with those for a biodegradable carrier such as POP. This was confirmed by use of scanning electron microscopy in the study reported here, whereby the number of pores on the surface of PMMA spheres at the end of the study was less than in POP cylinders or spheres. However, the other index of speed of release (ie, TDR γ50) did not differ between PMMA spheres and POP pellets not wrapped in SIS and was lower in PMMA spheres than for POP pellets wrapped in 8 layers of SIS.

The discrepancy between γ and TDR γ50 can be explained by the impact of TDR on TDR γ50. Wrapping POP with SIS increased TDR, thereby increasing TDR γ50. The value for TDR γ50 ranged from 6 to 13 hours and was therefore largely influenced by the kinetics of release during the first 48 hours of the study, whereas γ is equally affected by all phases of the elution curves. 

Poly(methylmethacrylate) released less antimicrobial than any of the SIS-wrapped POP groups, which confirmed results of other studies.24,39 Incomplete release of antimicrobial from PMMA pellets has been established,23,36,40 with cumulative release ranging from 2% to 25% of the initial drug content. Slower release and lack of biodegradability are reasons that PMMA spheres maintained gentamicin concentrations > 1 µg/mL for a longer period than POP spheres not wrapped in SIS, despite incomplete release from the PMMA spheres.

Wrapping POP in SIS improved elution characteristics by slowing the release and increasing the amount of gentamicin leaching from spheres and cylinders. Increasing the amount of SIS from 4 to 8 layers further slowed this release, presumably because of a barrier effect. All SIS-wrapped groups and PMMA maintained antimicrobial concentrations sufficient to treat or prevent bone infections caused by bacteria with an MIC ≤ 1 µg/mL. However, the mechanism by which these concentrations were achieved varied among groups. Whereas PMMA spheres had a slower and incomplete release, POP pellets wrapped in SIS had a faster and more complete release of gentamicin. The lower amount of antimicrobial released from PMMA spheres, compared with results for the POP pellets, resulted from lack of biodegradability of PMMA.
Although microscopic evidence of POP dissolution was provided during scanning electron microscopy at the end of the study reported here, all POP spheres and cylinders maintained their physical structure without gross evidence of disintegration after 42 days of elution. This finding contrasts with in vivo studies in which calcium sulfate pellets were resorbed within 4 to 8 weeks after implantation. This discrepancy illustrates the limitations inherent to in vitro elution studies and the inability to reproduce all in vivo mechanisms, such as cellular degradation. Nonetheless, elution studies remain a prerequisite for the selection of potential systems for delivery of drugs.

The POP spheres and cylinders wrapped in 8 layers of SIS had elution properties closest to those of PMMA. Additional investigation is warranted for use in small animals to treat or prevent osteomyelitis.

References


5. Santschi EM, McGarvey L. In vitro elution of gentamicin from SIS had elution properties closest to those of PMMA. Additional investigation is warranted for use in small animals to treat or prevent osteomyelitis.