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Antibiotic-releasing microspheres prevent mesh infection *in vivo*

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ABSTRACT

Background: Infection remains a dreaded complication after implantation of surgical prosthetics, particularly after hernia repair with synthetic mesh. We previously demonstrated the ability of a newly developed polymer to provide controlled release of an antibiotic in a linear fashion over 45 d. We subsequently showed that coating mesh with the drug-releasing polymer prevented a *Staphylococcus aureus* (SA) infection *in vivo*. To broaden the applicability of this technology, the polymer was synthesized as isolated "microspheres" and loaded with vancomycin (VM) before conducting a noninferiority analysis.

Materials and methods: Seventy-three mice underwent creation of a dorsal subcutaneous pocket that was inoculated with 10^4 colony forming units (CFU) of green fluorescent protein (GFP)-labeled SA (10^5 CFU/mL). Multifilament polyester mesh (7 × 7 mm) was placed into the pocket, and the skin was closed. Mesh was either placed alone ($n = 16$), coated with VM-loaded polymer ($n = 20$), placed next to VM-loaded microspheres ($n = 20$) or unloaded microspheres ($n = 10$), or flushed with VM solution ($n = 7$). Quantitative tissue/mesh cultures were performed at 2 and 4 week. Mice with open wounds and explanted mesh were excluded.

Results: Twenty-two of 23 (96%) tissue-mesh samples from mesh alone or empty microspheres were positive for GFP-labeled SA at 2 and 4 wk. Six of seven (86%) samples from the VM flush group were positive for GFP SA at 4 wk. Thirty-eight of 38 (100%) VM-loaded crosslinked cyclodextrin polymers–coated mesh or VM-loaded microspheres were negative for GFP SA at 2 and 4 wk.

Conclusions: Slow affinity-based drug-releasing polymers in the form of microspheres are able to adequately clear a bacterial burden of SA and prevent mesh infection.

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Introduction

The use of synthetic mesh in ventral hernia repair allows the surgeon to achieve a more durable repair that is associated with a lower recurrence rate compared to traditional autotissue repair.[1] However, mesh-related complications such as acute or delayed prosthetic infection have become a concerning clinical problem. Current prevention and nonsurgical treatments for mesh infection are limited, and infection of the mesh often necessitates surgical removal to avoid serious complications, such as enteric fistula.[2] A multisite cohort study of patients undergoing incisional hernia repair with permanent mesh prosthesis at 16 Veterans Affairs hospitals from 1998 to 2002 showed that 5.1% of the 1071 mesh repairs were complicated by subsequent mesh explantation at a median of 7.3 mo (interquartile range, 1.4-22.2).[3] Infection was the most common reason for mesh explantation (69%).[3]

Mesh infection can arise weeks after implantation, but the main prophylactic options available to prevent mesh infection, including pulse lavage, perioperative systemic antibiotics, and antibiotic impregnated mesh and sutures, are all short acting. Additionally, pulse lavage with or without antibiotics lacks evidence to support its efficacy[4,5] and perioperative systemic antibiotics can produce serious side effects, especially with prolonged administration,[6] limiting their use in long-term prophylaxis.

We previously demonstrated the ability of a newly developed polymer to provide controlled release of antibiotic in a linear fashion over 45 d.[7,8] The von Recum laboratory at Case Western Reserve University found that crosslinked cyclodextrin polymers (pCD) loaded with antibiotic release drug in a longer, more linear fashion than chemically similar dextran gels.[7,8] Cyclodextrin (CD) is a ring of 6, 7, or 8 glucose molecules that forms a pocket where drugs can bind and is commonly used in pharmaceutics to increase solubility of poorly soluble drugs. The molecular interaction between the CD pocket and drug is the rate-limiting step in release that determines the amount of free drug able to diffuse out of the polymer. Once a drug is out of the CD pocket it is able to diffuse freely throughout the material and may either diffuse out of the polymer or stick into another CD pocket. This type of release mechanism has been termed affinity-based release.[9] The pCD is capable of extended antimicrobial activity for more than 7 mo even after commercial sterilization.[10] Given the extended time window after surgery during which mesh infections can arise, we believe the extended antibiotic delivery demonstrated by this pCD platform represents a novel approach to prevent mesh infections.

We previously demonstrated that pCD-coated polyester mesh loaded with vancomycin (VM) prevented subcutaneous *Staphylococcus aureus* (SA) infection *in vivo*.[11] Despite the great potential of this polymer, its clinical applicability is limited by the fact that each different polymer-coated product requires individual Food and Drug Administration (FDA) approval.[12] Therefore, a drug-delivery mechanism independent of the prosthesis would be preferable and more broadly applicable to prevent infection of any device. We reformulated the polymer as microspheres that could be administered independently from the prosthetic device, loaded the microspheres with VM and conducted a noninferiority analysis. We designed this study to determine if microsphere polymer complexes are technically practical and efficacious for use against prosthetic infection and to compare antibiotic-loaded microspheres against antibiotic-loaded, polymer-coated mesh with respect to their ability to prevent prosthetic infection.

Materials and methods

Polymer materials

b-Cyclodextrin prepolymer (CD) was purchased from Cyclo-Lab, Budapest, Hungary. Ethylene glycol diglycidyl ether (EGDGE, $M_n = 174$) was purchased from Polysciences, Inc, Warrington, PA. All other reagents were purchased from Fisher Scientific (Pittsburgh, PA) at reagent grade.

Mesh and polymer coating

A 25% w/w CD solution in dimethylformamide was prepared under stirring. Hexamethylene diisocyanate (HDI) was added to the above solution to become glucose:HDI molar ratio of 1:0.32. Known volumes of pCD-HDI solution as prepared above were uniformly coated onto standard pieces (0.7×0.7 cm) of a three-dimensional market approved polyester mesh (Parietex TET; Covidien, Mansfield, MA) in a glass dish, and crosslinked at room temperature 3 days. The polymer-coated samples, hereafter called pCD-coated mesh, were carefully removed from the glass and were immersed in solvent, solvent/water 50/50, followed by Milli-Q water for 1 day each. The samples were then dried at room temperature in a dust free environment. pCD-coated meshes used for drug loading, and animal studies were prepared using this methodology.

Microspheres

The pCD microspheres were synthesized via single water-in-oil emulsification. Briefly, epichlorohydrin crosslinked βCD prepolymer (CycloLab R&D; Hungary) was dried overnight at ~70°C before solubilization in 0.2-M KOH to make a 25% w/v solution by vortexing. After the polymer was fully dissolved, EGDGE (M_n ~174) was added dropwise and vortexed on high for 2 min. The CD/EGDGE mixture was added to 50-mL light paraffin oil (Fisher Scientific) and homogenized for 5 min at 13,000 RPM (Brinkman Kinematica Polytron PT3000 homogenizer equipped with a PT-DA 3012/2 TS blade). The polymer emulsion was then added to 100-mL light paraffin oil stirred at 400 RPM by an overhead stirrer (IKA-Werke Eurostar Euro-St PCV P4S1) to allow crosslinking.

After 48 h, the stirrer was stopped, and the oil was filtered off using a coarse frit glass filter. The crosslinked pCD microspheres were washed successively with excess hexanes, excess acetone, and finished with excess Milli-Q grade water. Microspheres were allowed to dry at room temperature for 2 d.