Antimicrobial Effects of Drug-Containing Electrospun Matrices on Osteomyelitis-Associated Pathogens

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Purpose: To synthesize polydioxanone (PDS)-based drug delivery systems (hereafter referred to as "matrices") containing vancomycin (VANC) and/or rifampicin (RIF) and investigate their effect on the inhibition of biofilm growth containing osteomyelitis (OM)-associated pathogens.

Materials and Methods: PDS matrices were prepared by electrostatic spinning, and the drugs were incorporated as follows: group (G)1, 5wt%VANC; G2, 10wt%VANC; G3, 5wt%RIF; G4, 10wt%RIF; G5, 5wt%VANC+RIF; and G6, 10wt%VANC+RIF. A control group of pure PDS was also electrospun (G7). Biofilms formed by *Staphylococcus aureus* and *S. epidermidis* were grown on the electrospun matrices for 24 hours. The counts of viable cells were assessed after biofilm formation. The fiber morphology and biofilms were imaged using a scanning electron microscope.

Results: G5 and G6 and pure PDS (G7) had the lowest and highest mean number of viable cell counts, respectively ($P < .05$). Small and isolated clusters of bacteria with no mature biofilm present were found on G6.

Conclusions: The results of the present study have provided evidence for the potential use of PDS-based matrices as an effective drug delivery system that could inhibit biofilm formation from OM-associated pathogens.

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Staphylococcus aureus was suspected in an estimated 14.2 million outpatient healthcare visits in the United States during 2005, and approximately 478,000 hospitalizations had a confirmed diagnosis.[1,2] *S. aureus* has been consistently shown through nasal swabs of the anterior nares to colonize 25 to 30% of the human population and is responsible for a number of ailments, including bone and joint infections.[3-5] *S. aureus* is the most common pathogen causing osteomyelitis (OM). Additionally, *S. epidermidis* has been implicated as an opportunistic microorganism of biomaterial-related bone infection and a common skin inhabitant.[6]

OM is an infection of the bone that usually presents with fever, local pain, and tenderness and that, when left untreated, can result in bone necrosis and death. An open wound over a bone and compound fractures have been the major culprits of bone infections owing to the exposure of the bone to skin and environmental bacteria. The commonly implemented treatments for

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OM have involved intravenous (IV) antibiotic therapy, often after drainage of pus and surgical debridement of the infected tissues.[7] Consensus has been lacking on which antimicrobial agent should be selected based on proven efficacy and issues related to the treatment duration and route of administration best for each type of OM.[8,9] However, the typical antimicrobial treatments of OM in adults involving an intense systemic IV approach for 4 to 6 weeks have continued to have significant relapse rates.[10]

The considerable probability of recurring infection has encouraged the development of new therapeutic methods to combat OM and the drawbacks of systemic antibiotics. One disadvantage of IV antibiotics has stemmed from the weakened vascularity regularly associated with surgical debridement and irrigation of the infected area. The resulting decreased bioavailability lowers the chance that significant amounts of the antibiotics will be delivered to the infected areas. Also, systemic antibiotics can cause serious side-effects, including toxicity to uninfected tissues and organs that can result in renal damage.[11] Furthermore, an extended use of systemic antimicrobial agents can produce optimal growth conditions for opportunistic and resistant bacteria, creating additional risk of disease.[12] Thus, local antibiotic delivery could be a safer and more effective therapy for OM. It has been suggested that localized and sustained release of antibiotic-containing biomaterials will maintain the concentration required to eliminate the infection by placing it directly at the infected tissue.[13] In addition, localized treatment will decrease the effect on normal microbial flora.[14]

A number of studies using vancomycin (VANC) incorporated into biodegradable matrices or bioceramics as a local drug delivery system have shown effectiveness in the treatment of staphylococcal infections.[13,15-17] Hirose et al[16] fabricated a biodegradable poly-L-lactide-co-caprolactone sheet loaded with VANC that significantly reduced the viability of *S. aureus* biofilms over time. Another frequently used antibiotic in the development of drug delivery systems to treat staphylococcal infections has been rifampicin (RIF). It has been incorporated into silicone, gelatin-coated polymer meshes, grafts, and a variety of biodegradable polymers.[18-22] Despite some drawbacks as a monotherapy, RIF has demonstrated significant effectiveness in decreasing staphylococcal colonization and biofilm depth in both in vitro and in vivo studies.[18,23,24] Ruckh et al[18] found that RIF-containing poly(caprolactone) electrospun matrices significantly reduced *S. epidermidis* viability and prevented biofilm formation. Individually, VANC and RIF will become less effective against staphylococcal infections over time. This has been consistently shown to be in large part owing to the rapid development in resistance toward RIF and the inability of VANC to penetrate mature biofilms.[25-27] The structure and metabolic activity of *Staphylococcus* biofilms require treatments providing high bioavailability and tissue diffusion. Thus, the antimicrobial effects will be greatly improved with the combination of VANC and RIF in the treatment of *Staphylococcus* biofilms.[19,23,24,26,27] Therefore, the aim of the present study was to synthesize biodegradable polydioxanone (PDS)-based drug delivery systems containing VANC and RIF, at clinically relevant levels using electrospinning, and to investigate their effect on the inhibition of biofilm formation from OM-associated pathogens.

Materials and Methods

MATERIALS

The bacteria used in the present study, *S. aureus* (ATCC 25923) and *S. epidermidis* (ATCC 155), were purchased from American Type Culture Collection (ATCC, Manassas, VA). Violet polydioxanone (PDS II, Ethicon, Somerville, NJ) monofilament system surgical sutures were dissolved using methylene chloride and 1,1,1,3,3,3-hexafluoro-2-propanol (HFP, Sigma-Aldrich, St. Louis, MO) to prepare the PDS for electrospinning. Hexamethyldisilazane (HMDS) and the therapeutic agents of VANC (molecular weight [Mw] 1,485.71 g/mol) and RIF (Mw 822.94 g/mol) were also acquired from Sigma-Aldrich. Tryptic soy broth (TSB) culture media was obtained from Difco (Sparks, MD). Glucose, phosphate-buffered saline, sodium chloride, ethanol, paraformaldehyde, and Columbia blood agar (CBA) supplemented with 5% sheep blood were obtained from Thermo Fisher Scientific (Fair Lawn, NJ). No additional purification was performed on the materials and chemicals used in the present study, and they were used as received.

ELECTROSPINNING OF NANOFIBROUS DRUG-CONTAINING MATRICES

The PDS sutures were initially prepared for electrospinning by removing the attached needles. Next, the violet dye was removed by placing 3-cm lengths of the suture filaments in methylene chloride. The resulting clear PDS filaments were dissolved at 100 mg/mL in HFP and stirred overnight. Solutions containing 5% and 10% of the antibiotic were prepared according to the weight of the PDS by vigorously stirring 1 or both antibiotics directly into the polymer solution overnight. The drug-containing formulations were grouped as follows: group (G)1, PDS+5wt%VANC; G2, PDS+10wt%VANC, G3, PDS+5wt%RIF; G4, PDS+10wt%RIF, G5, PDS+5wt%VANC+RIF; and G6, PDS+10wt%VANC+RIF. A control group of pure PDS was also electrospun (G7).