Bacteriophage Encapsulation in Poly(Ethylene Glycol) Hydrogels Significantly Reduces Bacteria Numbers in an Implant-Associated Infection Model of Bone Repair

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Statement of Purpose: Implant-associated infections account for over 1 million infections per year, typically leading to device failure and removal, causing significant patient morbidity. Bone fractures and non-union defects often require surgical intervention where biomaterials, bone grafting, or protein delivery (BMP-2) are used to repair the injury. In the US, nearly 112,000 orthopedic device infections occur annually[1]. This motivates the development of dual functional materials capable of both healing bone and preventing infection.

Currently, few robust models are available to study how pathogenic bacteria interact with regenerative therapies. Our lab has recently developed a poly(ethylene glycol) (PEG)-based hydrogel to deliver BMP-2 and integrin-specific peptides facilitating bone regeneration in a critical-sized radial segmental defect in a mouse,[2] which has been extended to evaluate antimicrobial properties. Bacteriophages are viruses specific to bacteria causing lysis. The phage infection amplifies and propagates through the pathogen, but is self-limited in that it cannot infect eukaryotic cells, and is cleared when the host bacteria is eliminated. This provides on-demand response to pathogens, while reducing the development of antibiotic resistance. The main objective of this study is to evaluate the feasibility and efficacy of bacteriophage presenting hydrogels in an infection model of bone repair.

Methods: PEG hydrogel synthesis: PEG-maleimide hydrogels[3] were reacted with the collagen mimetic peptide GFOGER and cross-linked with the protease degradable peptide VPM as outlined in Fig. 1.

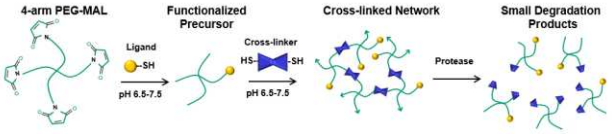

Figure 1: PEG-mal hydrogel synthesis schematic.

Bacteriophage release from PEG hydrogels: Bacteriophage ϕPaer14 was encapsulated in the PEG hydrogel and incubated in DPBS. Released bacteriophage was collected and measured at various time points using the soft agar overlay technique.

Bacteriophage effects on hMSC differentiation: Human mesenchymal stem cells (hMSC) were co-encapsulated with ϕPaer14 and cultured in osteogenic media. Alkaline phosphatase (ALP) was measured after nine days.

Bacteriophage delivery to mouse radial segmental defect: A 2.5mm radial defect male C57/B6 mice was created and hydrogels containing BMP-2, Pseudomonas aeruginosa PsAer-9 pSEVAplaxA, and ϕPaer4 were implanted in the defect (Fig. 3A). At 8 weeks, implants were recovered, degraded, and assayed for viable bacteria.

Results: Encapsulated ϕPaer14 is released linearly (R=0.96) from PEG hydrogels swelled in DPBS. Released phage is lytic against the PsAer-9 as determined by plaque formation (Fig. 2A). The presence of ϕPaer14 does not inhibit the differentiation potential of hMSCs encapsulated in PEG hydrogels cultured in osteogenic media as determined by ALP activity (Fig. 2B). BMP-2 loaded hydrogels containing no bacteria, PsAer-9, or PsAer-9 and ϕPaer4 were implanted into a mouse radial defect model (Fig. 3A). After 8 weeks, viable bacteria was recovered in all PsAer-9 groups demonstrating a well-defined, controlled infection model. Importantly, the ϕPaer4 treated hydrogels significantly reduced the number of viable bacteria recovered at 8 weeks after surgery, $p < 0.05$ (Fig. 3B).

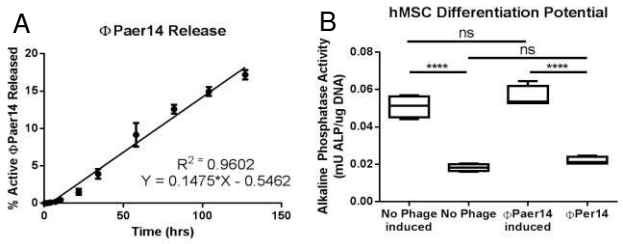

Figure 2: A) Active ϕPaer14 released from hydrogel, **B**) hMSC ALP expression after culture in osteogenic media in the presence of bacteriophage (**** = $p < 0.001$)

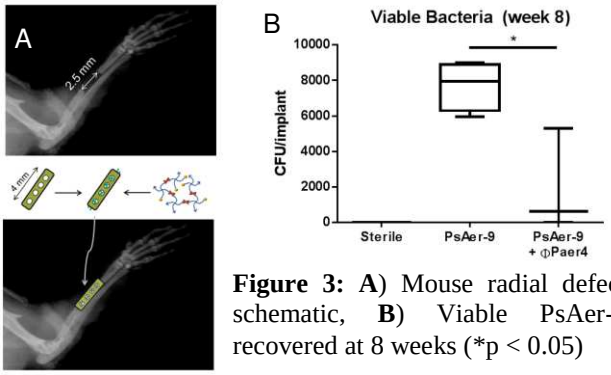

Figure 3: A) Mouse radial defect schematic, **B**) Viable PsAer-9 recovered at 8 weeks (*$p < 0.05$)

Conclusions: We demonstrate bacteriophage can be encapsulated into PEG hydrogels without inhibiting bactericidal ability. The presence of bacteriophage does not alter the differentiation potential of hMSCs suggesting they do not disrupt bone formation. Bacteriophage delivery significantly reduces viable bacteria in a chronic implant infection model, providing proof of concept for their feasibility as an antimicrobial strategy. Further investigation is required to assess the biocompatibility of bacteriophage, as well as the immune response and effects on bone formation of bacteriophage delivery.

References: 1. Nair, MB; Curr opin Biotechnol. 2013, 22, 721-725; 2. Shekaran, A; Biomaterials. 2014, 35, 5453-5461; 3. Phelps, EA; Adv Mater. 2012, 24, 64-70.

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