Assessment of Bacteriophage Activity in Poly(Ethylene Glycol) Hydrogels

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Statement of Purpose: In cases of severe bone fracture, biomaterial implants may be used to stabilize the bone and promote healing. However, surgical implants account for roughly one million hospital-related infections annually in the US[1], making the prevention of implant-associated infection a significant clinical problem. A potential solution involves engineering biomaterial scaffolds to resist infection through the incorporation of antimicrobial agents such as bacteriophages. Bacteriophages (or phages) are viruses that can only infect and replicate in the presence of bacterial cells, killing their hosts and providing an on-demand response to infection. We have recently established a model for bone repair based on a critical-sized segmental defect in the murine radius that can be healed by implanting poly(ethylene glycol) (PEG) hydrogels that deliver low doses of BMP-2[3]. The purpose of this study is to engineer these PEG hydrogel implants to resist infection against an engineered luminescent strain of Pseudomonas aeruginosa, PsAer-9 pSEVAplaxA, through the incorporation of active bacteriophage.

Methods: Evaluation of bacteriophage efficacy against PsAer-9: PsAer-9 was incubated at 37˚C in synthetic interstitial fluid with proportional numbers bacteriophage (ϕPaer4, ϕPaer14 or both) and optical densities (OD) at 590nm were recorded at 24hr.

Bacteriophage encapsulation in PEG hydrogels: PEG hydrogels functionalized with the collagen mimetic peptide GFOGER were cross-linked with a protease-degradable peptide to encapsulate $5x10^7$ plaque forming units (PFU) of bacteriophage (ϕPaer14, ϕM4, or neither). Hydrogels were placed in 25% tryptic soy broth inoculated with $5x10^6$ colony forming units (CFU) of PsAer-9 and cultured at 37°C and 180rpm for 32 hr. At various time points, OD at 550nm and viable bacteria of the culture were measured.

In vivo delivery of encapsulated bacteriophage: PEG hydrogels were functionalized with GFOGER and BMP-2 and cross-linked to encapsulate either PsAer-9 ($3x10^5$ CFU/gel) or both PsAer-9 and ϕPaer4 ($6x10^6$ PFU/gel). A non-healing critically sized 2.5 mm radial defect was created in C57/B6 mice and the hydrogel was implanted. At day 7, implants were removed and analyzed for viable bacteria and active bacteriophage.

Results: At high initial bacterial concentrations, all three phage treatments showed significant reduction in OD when compared to the bacteria only control (Fig. 1A). ϕPaer14 showed a greater reduction in OD as compared to ϕPaer4, which led to its selection for the in vivo study. The combined treatment showed a significantly greater reduction than ϕPaer4 or ϕPaer14 individually, demonstrating a synergistic interaction between the two in controlling for bacterial growth (Fig. 1A). In assessing the efficacy of bacteriophage incorporating hydrogels in vitro, we showed a significant decrease in viable bacteria due to the ϕPaer14 hydrogel treatment as compared to the no phage and inactive phage controls. Additionally, there were no significant differences between the free and hydrogel incorporated bacteriophages, suggesting that bacteriophage encapsulation has no significant decrease in phage activity as measured (Fig. 1B). Although our ϕPaer14 presenting hydrogels did not significantly reduce bacterial recovery from our in vivo study, active bacteriophages were successfully recovered from all phage presenting implants, suggesting in vivo persistence of ϕPaer14 a week after implantation (Figure 1C).

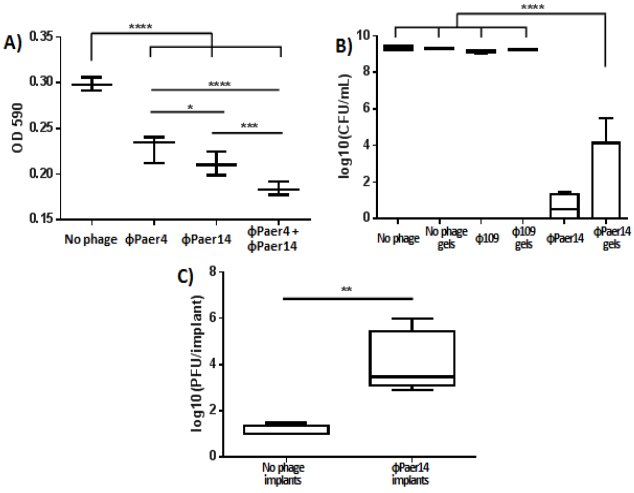

Figure 1: A) Optical density of suspension after 24 hr phage treatment. **B)** Viable bacterial counts after 32 hr incubation with encapsulated bacteriophage. **C)** Bacteriophage recovery 1week after in vivo implantation (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

Conclusions: We showed a synergistic interaction between ϕPaer4 and ϕPaer14 in suspension, continued efficacy of ϕPaer14 against planktonic bacteria in vitro after hydrogel incorporation and in vivo persistence of ϕPaer14 in our hydrogel scaffold one week after implantation. Altogether, these results suggest that bacteriophage incorporation in hydrogel scaffolds may be an effective way of controlling for infection. Future work includes evaluation of how modifying certain parameters in our hydrogel formulation, such as altering bacteriophage concentration and combining multiple bacteriophages, affects the efficacy of our treatment both in vitro and in vivo.

References: [1] Darouiche et al. N Engl J Med, 2004, 350, 1422-1429. [2] Widmer et al. Clin. Infect. Dis. 2001, 33, S94-S106. [3] Shekaran et al. Biomaterials. 2014, 35, 5453-5461.

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