Iron-doped apatite nanoparticle adjuvants for enhanced phage therapy delivered through electrospun fibers

Jessica M. Andriolo
University of Montana, Missoula, MT 59812
JGregory@MTech.edu
John P. Murphy, M. Katie Hailer, Marisa L. Pedulla, Jack L. Skinner
Montana Tech, Butte, MT 59701

When bacteria are exposed to specific iron-doped apatite nanoparticles (IDANPs) prior to the introduction of bacteriophage (phage), they are up to 2 times more susceptible to death by phage.[1-2] For a century, phage therapy has been used to treat bacterial infection in humans,[3] but was largely abandoned upon the discovery of traditional antibiotic therapies.[4] Today, the great challenge of antibiotic-resistant bacterial strains calls for alternatives to the antibiotics we have relied on for decades. IDANPs used in our research are composed of calcium, phosphate, and hydroxyl ions and resemble hydroxyapatite, a mineral known to be biocompatible and analogous to the inorganic constituent of mammalian bone and teeth.[5] The most effective IDANPs at increasing phage infection are synthesized at 25 °C, with 30 % iron, and with 0.5 mM citrate (Fig 1). IDANPs synthesized below or above 25-45 °C, with other amounts of iron, or with altered levels of citrate, do not cause significant increases in phage infections. Reagents of 30 % IDANPs have been tested alone and do not increase phage infection to the extent of the fully-formed IDANP. The specificity of this effect has fueled an ongoing effort in our labs to characterize IDANPs, understand IDANP interaction with bacterial surfaces (Fig 2), minimize IDANP cytotoxicity, and to develop IDANPs for therapeutic purposes. Phage have previously been incorporated into biocompatible polymer fibers using coaxial electrospinning and maintained 100 % viability upon release.[6-7] We aim to create a polymer fiber bandage with controlled release for IDANP-assisted phage therapy treatment of topical wounds. We have previously determined that bacterial exposure to IDANPs for one hour prior to the introduction of phage results in maximum effect. In our labs, we have fabricated IDANP-impregnated poly(ethylene) oxide (PEO) fibers which dissolve rapidly in an aqueous environment to allow IDANPs adequate time to interact with bacteria prior to phage introduction (Fig 3A). We have also incorporated phage into coaxial polycaprolactone (PCL)/phage buffer fibers (Fig 3B). Future work aims to fabricate an electrospun bandage with superficial, rapid-release, IDANP-doped PEO fibers electrospun on top of slower-release, blended PEO/cellulose diacetate (CDA) or PEO/PCL fibers which contain phage. An example of the bandage described can be seen in Fig 3C, where fibers were also aligned (as described previously[8]) for predictable release. The composite bandage created will be tested in liquid bacterial cultures, on biofilms, and phage viability and release kinetics will be evaluated via plaque assay and epifluorescence microscopy.

[1]Andriolo JM, et al., *J. Vac. Sci.Technol. B* 32(6), 2014. [2]Andriolo JM, et al., *IEEE Trans. Nanobioscience* 15, 8 (2016), [3]d'Herelle, *Bull NY Acad. Med.* 7, 5 (1931), [4]Flemming, *Br. J. Exp. Pathol* 10, 3, (1929), [5]Palmer LC, et al., *Chem. Rev.* 108, 11 (2008), [6]Korehei R, et al., Carbohydr. Polym. 100, (2014), [7]Korehei R, et al., *J. Appl. Microbiol.*, 114, 5 (2013), [8]Beisel JD, et al., *J. Vac. Sci. Technol. B* 34, 6 (2016).

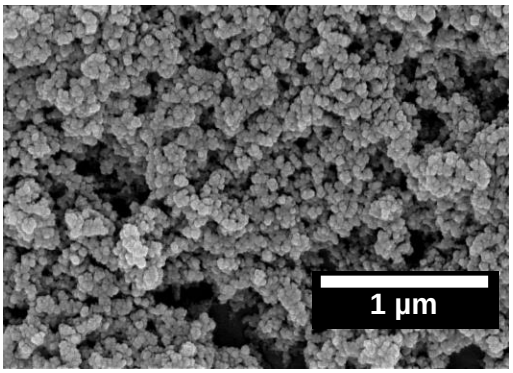

Fig 1. Scanning electron micrograph of IDANPs synthesized at 25 °C, doped with 30 % iron, and citrate functionalized.

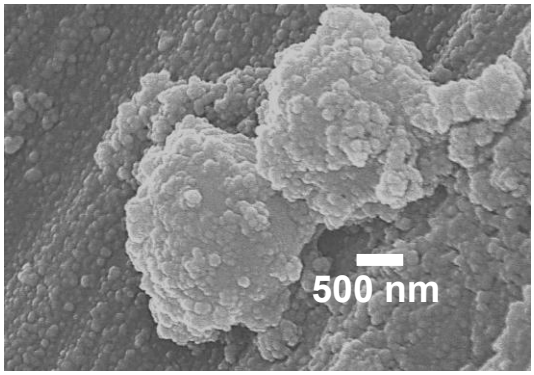

Fig 2. Scanning electron micrograph of *S. aureus* covered in IDANPs seen in Fig 1.

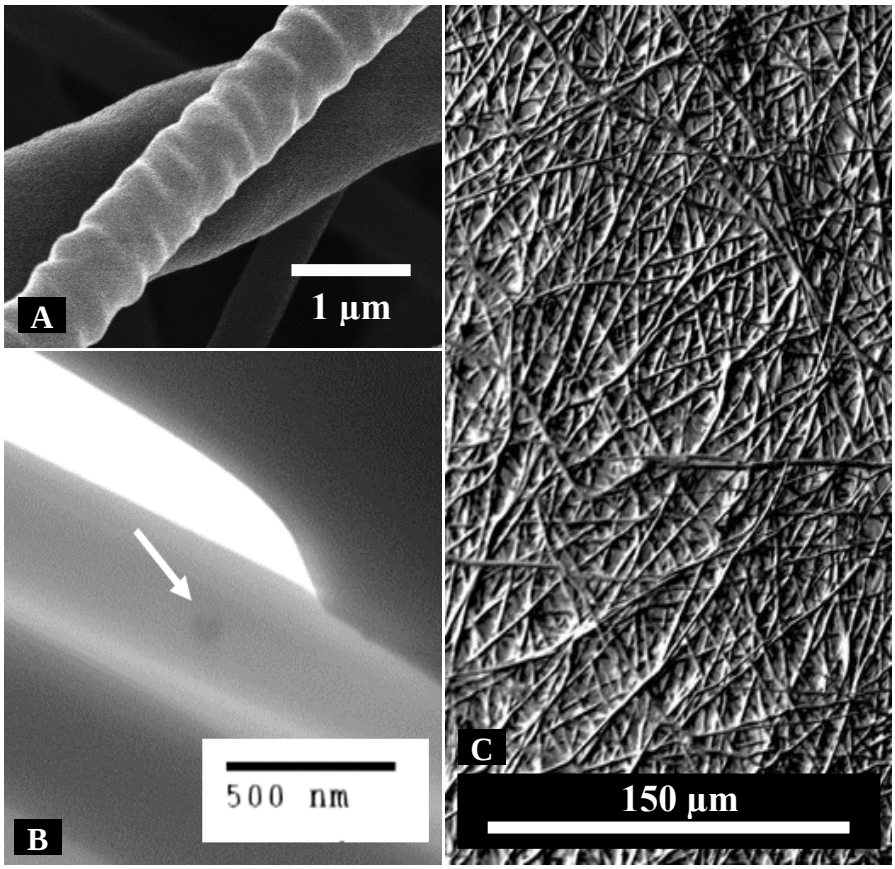

Fig 3A. Scanning electron micrograph of PEO fibers doped with IDANPs seen in Fig 1. **Fig 3B:** Transmission electron micrograph of icosahedral phage heads observed in coaxial PCL fibers. **Fig 3C.** Scanning electron micrograph of a preliminary bandage composed of IDANP-impregnated PEO fibers on top of coaxial PCL fibers containing phage.