Keratin Biomaterials Promote Sustained Release and Bioactivity of Therapeutic Agents

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Statement of Purpose:
Biomaterial constructs can act as a physical guide for regenerating cells, a matrix for stem cells, and a reservoir for growth factor release, making their use pre-eminent in the field of tissue engineering. We are exploring keratin-based biomaterials derived from human hair for a number of applications including wound healing, nerve bridging, and segmental bone defects. Injuries resulting from trauma and particularly battlefield injuries that require biomaterials and/or tissue engineering approaches are highly prone to acute bacterial infection from environmental opportunistic pathogens. Therefore, we have explored the potential to incorporate antibiotics into the design of our keratin systems to limit acute bacterial infections. We hypothesized that the physical and chemical nature of keratin proteins would allow for the sustained release and bioactivity of antibiotics for inhibition of bacterial proliferation in acute models of infection.

Methods:
Keratin proteins were extracted from human hair by an oxidative process described previously (1). The extracted keratin proteins were formed into hydrogels at 20% w/v with or without ciprofloxacin-HCl. For release studies, keratin gels were covered in phosphate-buffered saline (PBS). At specified timepoints, PBS was removed and the amount of ciprofloxacin released from the gels to the PBS was quantified by fluorescence (excitation/emission = 360nm/460nm).

Bioactivity of the ciprofloxacin was determined by an *in vitro* broth inhibition assay with a *Staphylococcus aureus* cell line (29213, American Type Culture Collection, MRSA negative). Keratin hydrogels with or without ciprofloxacin-HCl were inoculated daily with 10^8 c.f.u. of *S. aureus* and incubated for 24 hours. The resulting suspensions were plated on sheep blood agar and the total number of colony forming units determined by counting. Agarose hydrogels were used as a control for comparison to a diffusion-mediated hydrogel system.

The *in vivo* efficacy of the system was determined in a subcutaneous mouse infection model. All animal experiments were conducted under a protocol approved by the Wake Forest University Health Sciences Animal Care and Use Committee (ACUC). A small incision was made in the backs of mice and the subcutaneous space opened by blunt dissection. 10µL of 10^{10} c.f.u./mL of *S. aureus* 29213 was injected followed by keratose hydrogels with or without ciprofloxacin-HCl. At 1 or 2 weeks, animals were humanely euthanized. The site of infection was swabbed and plated onto sheep blood agar to quantify the number of colony forming units.

Error bars indicate standard error of the mean. Data were collected in triplicate. Student t-tests were used to compare groups.

Results:
Ciprofloxacin release from the keratin hydrogels was detectable for 21 days and at levels above the minimum inhibitory concentration (MIC) of *S. aureus* 29213 for 15 days. Agarose controls showed detectable antibiotic release for only 9 days and above the MIC for only 5 days (Figure 1).

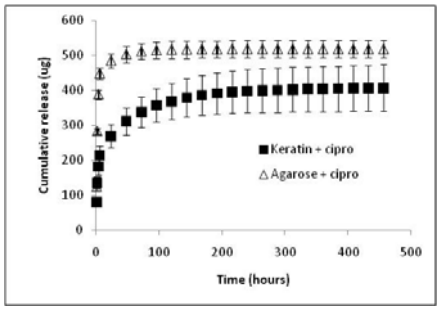

Figure 1. Ciprofloxacin release from keratin hydrogels and agarose hydrogel controls.

In vitro broth inhibition of S. aureus reflected results of the release studies (Figure 2). Keratin hydrogels achieved inhibition of bacterial growth for 14 days compared to agarose hydrogels, which inhibited growth for 4 days. The decrease in bacterial load with keratin gels was significantly less than with agarose gels over the two week duration of the experiment ($P < 0.05$).

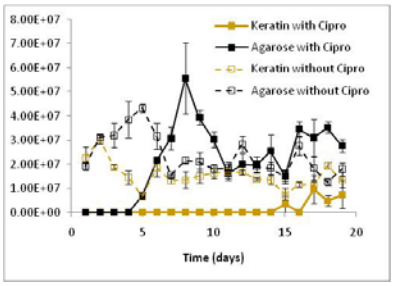

Figure 2. *S. aureus* inhibition with keratin hydrogels with or without ciprofloxacin and agarose hydrogel controls.

Subcutaneous in vivo studies indicate that keratin-released antibiotics significantly inhibit bacterial growth over the course of two weeks. We are currently applying this system to a rat femur defect model to assess the bacterial inhibition in a more clinically-relevant system.

Conclusions:
We demonstrate that keratin biomaterials exhibit unique antibiotic release profiles and inhibit bacterial growth *in vitro* and *in vivo* for two weeks. These materials provide a mechanism to curb bacterial infection following traumatic and battlefield injuries in which tissue engineering approaches are used to achieve regeneration.

References:
1. Sierpinski P, et al. Biomaterials 2008, **29,** 118-128.