Sustained Release of Functional Antibiotics From a Keratin Hydrogel

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Statement of Purpose: Keratins are naturally-derived proteins whose formulation flexibility makes them advantageous as a biomaterial platform. These materials have broad application in rengenerative medicine due to their ability to support cell attachment, proliferation, and migration (Hill, 2010). Their utility in delivering therapuetic agents (Ciprofloxin and rhBMP-2) has also been demonstrated using a crude extract of keratin (Saul 2011; deGauzman, 2013). Here we describe the release of Streptomycin, Neomycin, Ciprofloxin, and Cephazolin from a highly purified keratin biomaterial. The antiobiotics remain active against targeted bacteria and show no signs of toxicity using *in vitro* assays.

Methods: Keratin was purified by KeraNetics by using a patented process in a 21CFR820 validated facility. The lyophilized extract was weighed into 30 mL conical tubes to the desired weight to volume percentage and hydrated with an aqueous solution containing the antibiotics and allowed spontaneously form hydrogels overnight at 37 °C. Release studies were performed by placing 100 μL aliquots of the keratin hydrogels in microcentrifuge tubes and overlaying them with 100 μL DPBS. DPBS was then exchanged and analyzed at regular intervals. Total protein was determined by Lowry assay and antibiotics were quantified by fluorescence (Ciprofloxin), light absorption (Cephazolin), size exclusion chromatography followed by UV absorption (Streptomycin), ELISA (Neomycin, Europromixa, Netherlands). Antibiotic activity was tested in 3 bacterial strains *S. aureus* (Gram-positive), *P. aeroginsa* (Gram-negative), and *F. magnus* (Gram-positive, anaerobic) by a broth inhibition assay. One mL of keratin hydrogel was placed in 10 mL of Mueller-Hinton broth (*S. aureus* and *P. aeroginsa*) or Clostridial media (*F. magnus*) containing 10^5 colony-forming units (cfu) per mL as determined by McFarland standard. The broth was sampled and exchanged daily for 7 days, serially diluted at 1:10 to dilutions of 10^9 to allow determination of the number of cfu present in the broth. After a week, the samples were diluted to 10^4 cfu/mL to determine bacterial inhibition time course. Toxicity of the formulations was determined by culturing primary fibroblast and keratinocytes (Lonza, Walkersville, MD) in normal growth media (per manufacturer's instructions) containing the antibiotic loaded gels. Proliferation was measured by CellTiter 96 Aqueous Assay (Promega, Madison , WI)

Results: A 15% (w/v) hydrogel with the highly purified keratin led to release profiles for Ciprofloxin similar to those previously reported (Saul, 2011) with a less purified keratin extract. Release profiles of various concentrations of Neomycin, Streptomycin and Cephazolin were also determined. The percent release of each antibiotic strongly correlated to the total protein release from the keratin hydrogles. To determine if the minimum inhibitor concentration (MIC) was achieved, concentrations from the release assay were used to calculate a theoretical concentration in the bacterial inhibition assay to choose the appropriate concentration of antibiotic (20 mg/mL for each antibiotic except Ciprofloxin, for which 2mg/mL was used). Ciprofloxin, the broad spectrum antibiotic, was effective against all strains of bacteria tested and inhibited growth for 14-19 days. Cephazolin, primarily active against Gram-positive bacteria, showed inhibition for 14 days against *S. aureus* and *F. magnus* and was ineffective against *P. aeroginsa*. Streptomycin and Neomycin are typically associated with efficacy against Gram-negative bacteria (*P. aeruginosa*), and in our studies, they achieved inhibition for 4 days and 8 days, respectively. However, they also achieved a long-term effect against both Gram-positive strains including up to 31 days against *S. aureus*. These formulations were further tested in culture with mammalian cells without bacterial spike to assess biocompatibility. The proliferation of keratinocytes was improved on day 2 in culture with the keratin hydrogels both with and without antibiotics. By day 7 there were no significant differences suggesting that the keratin antibiotic mixture did not lead to any quantifiable reduction in cell viability under these culture conditions. The proliferation of fibroblasts in the presence of antibiotic-loaded keratin hydrogels was not statistically different than the no antibiotic condition or the no treatment control.

Conclusions: Keratin hydrogels were able to deliver bioactive Neomycin, Streptomycin, Cephazolin, and Ciprofloxin for over a week. This finding is clinically relevant to the treatment of skin wounds and burns. Wound and burn treatments require cleaning and replacement of dressings at least every 3 days. Our results suggest that use of keratin in wound dressings could maintain antibiotics levels above the MIC, reducing the number of dressing changes for burn and wound care patients and prevent infection during the entire wound contact periods.

References:

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