Electrospinning Silk with Selenium for Antibacterial Skin Applications

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Statement of Purpose: Skin is the largest organ in the body and the primary defense against microbes, viruses, and other interactions with the environment. Silk has been studied for skin regeneration and has been widely used as an additive in cosmetics.[1] Silk promotes collagen synthesis, re-epithelialization, wound healing, atopic dermatitis alleviation, and scar reduction. However, pure silk shows negligible or even negative antibacterial properties.[2] Here, we propose for the first time, an electrospun silk scaffold doped with selenium to address this issue. Electrospun silk scaffolds have smaller interstices and higher surface areas, allowing for more efficient nutrient transfer to skin.[3] In addition, selenium nanoparticles have been shown to have excellent antibacterial properties.[4] By incorporating selenium nanoparticles into silk, we expect to retain silk's beneficial skin healing properties while increasing its antibacterial ability.

Methods:

Silk purification-Silk from *Bombyx mori* silkworm was purified by the following protocol[5]: cocoons were boiled for 30 min in 0.02M Na_2CO_3 then rinsed with water to extract silk. Extracted silk was then dissolved in 9.3M LiBr at 60C before undergoing dialysis in water using a Slide-a-Lyzer cassette (Pierce, MWCO 3500).

Selenium nanoparticle synthesis-3 ml of 25 mM Na_2SeO_3 (Alfa Aesar) were reduced by adding 3 ml of 100 mM GSH (TCI America) and were stabilized by adding 0.15 g bovine serum albumin (Sigma-Aldrich) to 9 mL of distilled water. After mixing the reactant solutions, 1M NaOH was used to bring the pH of the solution to the alkaline regime. Selenium nanoparticles were then collected by centrifugation at 13,000 rpm, sterilized by UV light exposure, and resuspended in sterile distilled water before being used in experiments.

Electrospinning-Aqueous solutions containing a range of silk concentrations were made. A range of selenium nanoparticle concentrations were added to the aqueous solutions of silk to determine the optimal concentrations of selenium nanoparticle deposition to decrease bacteria growth and increase skin growth. Electrospinning conditions such as voltage, distance to collector, and collector type were all varied in order to generate scaffolds of specific silk fiber diameters and geometry.

Materials characterization-After making the proposed electrospun scaffolds, a series of experiments were conducted to characterization the scaffolds. Atomic force microscopy (AFM) was used to characterize surface roughness. X-Ray photoelectron spectroscopy (XPS) gave information about surface chemistry. Water contact angles assessed the hydrophobicity of the substrate. Lastly, scanning electron microscopy (SEM) provided detailed images of the surfaces and information about fiber diameters as well as selenium nanoparticle size.

Cell culture experiments-Skin fibroblasts, CCL-110 (ATCC), and keratinocytes, C0015C (Invitrogen), were cultured on 4 groups of samples: cell culture plates, cell culture plates with selenium alone, cell culture plates with electrospun silk alone, and cell culture plates with electrospun silk and selenium. Cell counts and viability tests were conducted on a daily basis for duration of 2 weeks after plating cells on these samples.

Bacterial experiments-Bacterial strains *Staphylococcus aureus, Staphylococcus epidermis*, and *Pseudomonas aeruginosa* (all from ATCC) were cultured on the same 4 sample groups as the mammalian cell culture experiments. Bacteria were grown in 0.3% tryptic soy broth (Sigma-Aldrich) and plated on TSB-Agar plates. One day after inoculation, samples were counted to check bacterial growth.

All experiments were conducted in triplicate and repeated at least three times.

Results:

Initial results demonstrated that selenium nanoparticle significantly reduced bacteria growth at concentrations as low as 7.8 μg/mL (as an example, *Staph. aureus* is shown in Figure 1)[4].

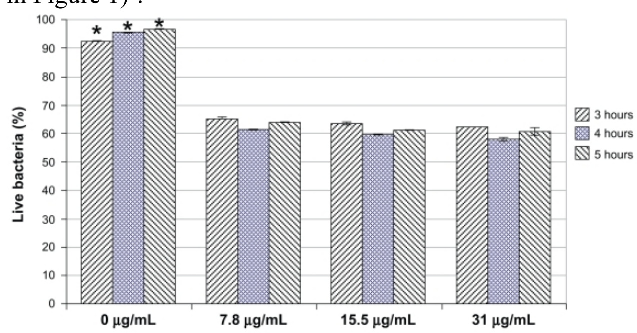

Figure 1. The antibacterial effects of selenium nanoparticles at 7.8, 15.5, and 31 μg/mL. Data = mean +/- SEM; N = 3; * p<0.01 compared to 0 μg/ml at respective time period.

Additional characterization and effects on skin cells will be presented.

Conclusions:

Although silk is already widely used for skin applications, it lacks antibacterial properties, a significant drawback for numerous wound healing applications. Selenium is a naturally occurring element present in the body. Antibacterial concentrations of selenium (7.8 μg/ml) are still far below the selenium toxicity threshold for humans Incorporation of selenium into silk can overcome a significant barrier for the use of silk in skin regeneration applications.

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