Optimizing Mesenchymal Stem Cell Wound Healing Properties on Electrospun Silk with Selenium Nanoparticles

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Statement of Purpose:

Current treatments for skin wound healing provide limited healing and do not promote skin repair. Therefore, an alternative approach that actively participates in the endogenous wound healing process would significantly improve wound healing and expand the scope of skin treatment to more serious conditions, such as severe burns and epidermal replacement.

In addition to their stemness properties, mesenchymal stem cells (MSCs) have a number of cell signaling properties that are very beneficial to wound healing. Human MSCs (hMSCs) act as pericytes[1], cells that wrap around a blood vessel and participate in blood vessel formation and maintenance. hMSCs have also been shown to promote endogenous wound healing processes and demonstrate improvements to many skin healing endpoints[2,3]. In addition, cells around skin are especially responsive to physical cues and nanotopographies, and hMSC differentiation activity is significantly affected by nanotopography[4]. Thus, a nanofeatured surface that upregulates wound healing activity of hMSCs would have tremendous potential for wound healing and fundamentally alter the paradigm of skin regeneration. The objective of this study is to electrospin silk scaffolds, a proven platform for skin, embedded with selenium nanoparticles, a novel bactericidal agent[5], to enhance the wound healing properties of mesenchymal stem cells, especially antibacterial properties. Selenium is a natural part of our diet and may have stem cell differentiation properties.

Methods:

Silk purification-Silk from *Bombyx mori* silkworm was purified by the following protocol[6]: 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[5]-3 ml of 25 mM Na_2SeO_3 (Alfa Aesar) were reduced by adding 3 ml of 100 mM GSH (TCI America) for 0 and 60s (t_1). After mixing the reactant solutions, 2M NaOH was used to bring the pH of the solution to the alkaline regime for 75 and 15 s (t_2).
Electrospinning-Aqueous solutions containing 8% silk/formic acid were used to create silk scaffolds. Selenium nanoparticles were synthesized at t_1 and t_2 respectively of 60/15 and 0/75 to determine the optimal concentrations of selenium nanoparticle deposition to decrease bacteria growth. Electrospinning conditions were at 24 kV, 7 cm to collector, and 35% relative humidity at room temperature.
Materials characterization-After making the proposed electrospun scaffolds, a series of experiments were conducted to characterization the scaffolds. 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-Human mesenchymal stem cells (Lonza) 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 a duration of 2 weeks after plating cells on these samples.
Bacterial co-culture experiments-Bacterial strains *Staphylococcus aureus, Staphylococcus epidermis*, and *Pseudomonas aeruginosa* (all from ATCC) were cultured with the same 4 sample groups as in the mammalian cell culture experiments. Bacteria were grown in 0.3% tryptic soy broth (Sigma-Aldridch) 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 nanoparticles significantly reduced bacteria growth at concentrations as low as 7.8 μg/mL (as an example, *Staph. aureus* is shown in Figure 1)[5].

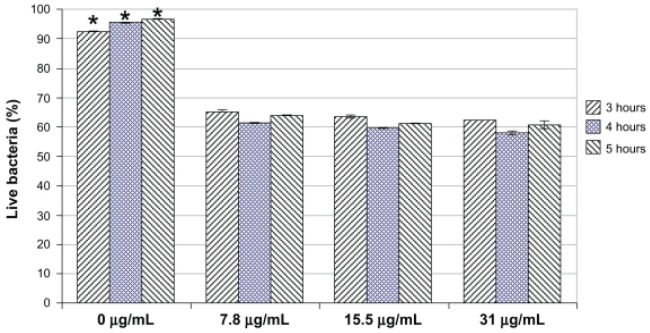

Figure 1. The antibacterial effects of selenium nanoparticles at 7.8, 15.5, and 31 μg/mL. Data = mean +/- SEM; N = 3; * $p<0.01$ compared to selenium treatments at respective time periods.

Additional characterization and effects on skin cells and stem cells will be presented.

Conclusions:

Current skin treatments do not actually promote skin healing and thus cannot address serious skin conditions, such as severe burns. A cell-based therapy utilizing the ability of hMSCs to heal wounds may address this

shortcoming. An electrospun silk scaffold, shown to have beneficial skin properties, embedded with selenium nanoparticles, a novel antibacterial agent and possible stem cell differentiation factor, is thought to provide synergistic wound healing in combination with hMSCs. Selenium is a naturally occurring element present in the body. Antibacterial concentrations of selenium (7.8 μg/ml) are 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.

Acknowledgement: The authors would like to thank Northeastern for funding. Disclosure: Senstruct, a start-up company based on this SeNP work.

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