Electrospun Wound Healing Devices Containing Antimicrobial Eutectic Solvents Resist Biofouling by Microbial Pathogens

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Introduction

Wound healing is a complex process consisting of a cascade of events; however, several limitations can hinder this process. Pathogens presenting as a microbial biofilm within wounds can slow the healing process. If the wound is unable to close, this can lead to an increased incidence of medical complications in addition to more extensive therapeutic procedures.

We have previously demonstrated that wound healing devices comprised of electrospun proteins associated with skin have been shown to accelerate the healing process relative to conventional wound dressings. Furthermore, the inclusion of the antibacterial ionic liquid/deep eutectic solvent (IL/DES) choline geranate (CAGE) into these materials afforded the devices an ability to resist microbial corruption yet still serve as an effective platform for cellularization by human dermal fibroblasts. In this work, we have synthesized electrospun scaffolds with the new IL/DES, which has favorable antibacterial properties against pathogens commonly associated with some non-healing wounds. We also report preliminary material and antimicrobial characteristics of these scaffolds.

Materials and Methods

Synthesis of IL/DES

Like CAGE, the new IL/DES is synthesized via salt metathesis and proceeds in quantitative yield. The IL/DES, Compound A, retains antibiofilm activity and contains a molecule which targets various pathogenic fungi (e.g. *Candida albicans*)[3]. Another IL/DES, Compound B, was similarly synthesized salt metathesis reactions where we vary the composition of anions and neutral species.

Figure 1. Synthesis of choline geranate (CAGE).[1,2]

Microbial Corruption Assay

Scaffolds containing various concentrations of Compound A were saturated with an actively proliferating culture of *Candida albicans*. After challenge times of 30 min and 2 hours, the scaffolds were sonicated to dislodge any adherent cells and colony viability determined via dilution and enumeration on solid media.

PrestoBlue and CyQUANT Assays

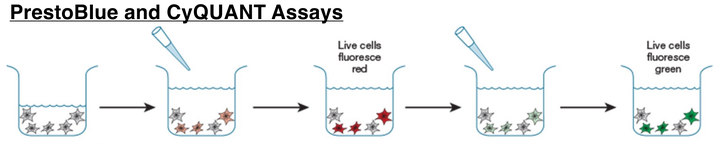

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Figure 2. Cellular Viability of adult human dermal fibroblast (hDF) cells were measure using the PrestoBlue and CyQUANT assays. The PrestoBlue assay used resazurin when uptaked into the living cell, fluoresced red, and detected cell metabolic activity. The CyQUANT assay measured cellular DNA content when the green fluorescent nucleic acid stain was taken up by living cells, and a background dye taken up by dead cells and masking the fluorescence.

Scaffold Construction

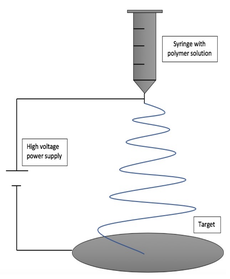

Figure 3. The process of electrospinning. Electrospinning scaffolds involved a high voltage to guide protein fibers from a solution through a charged needle to an oppositely charged target. The solution contained 10% gelatin (GE) protein in a HFIP solvent with various Compound A concentrations (200*µ*l, 100*µ*l, 50*µ*l, 25*µ*l of 100% solution) The flow rate was 1mL/hour at 16KV and 12cm distance. The scaffolds were crosslinked with 50% glutaraldehyde vapor.

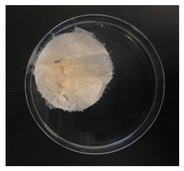

Figure 4. Visual depiction of a representative gelatin scaffold containing 40% Compound A.

Scaffold Characterization

Resistance of Scaffolds to Biofouling

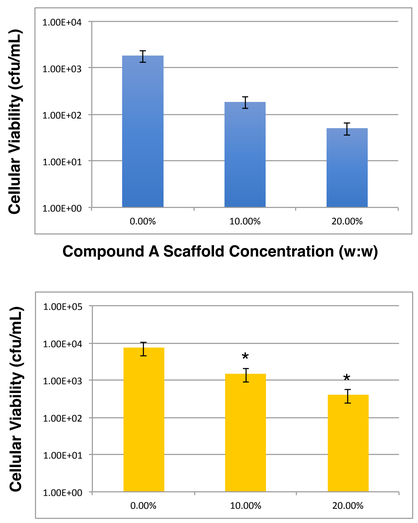

Figure 5. Microbial corruption assay. A solution (20 µL) of actively proliferating *Candida albicans* was introduced to scaffolds containing various concentrations of Compound A and incubated for 30 minutes (blue) or 2 hours (yellow). (* = $p < 0.05$).

SEM Analysis of Scaffolds

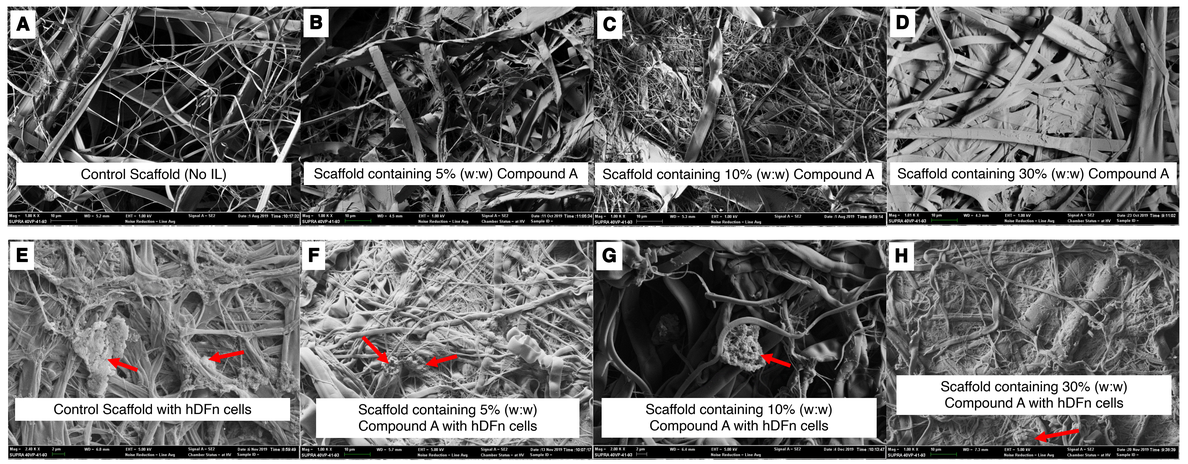

Figure 6. Scanning electron microscope (SEM) images of electrospun gelatin scaffolds. Human dermal fibroblast cells (hDFn) are denoted with red arrows. Images A-D correspond to different concentrations (i.e. 0%, 5%, 10%, and 30%) of Compound A. Respectively, images E-H reflect the concentrations of the images above, except with hDFn cells. SEM images of Compound A containing scaffolds depict the materials becoming less porous as the IL/DES concentration increases. Scaffolds with Compound A were able to grow hDFn cells.

hDF Cellular Viability

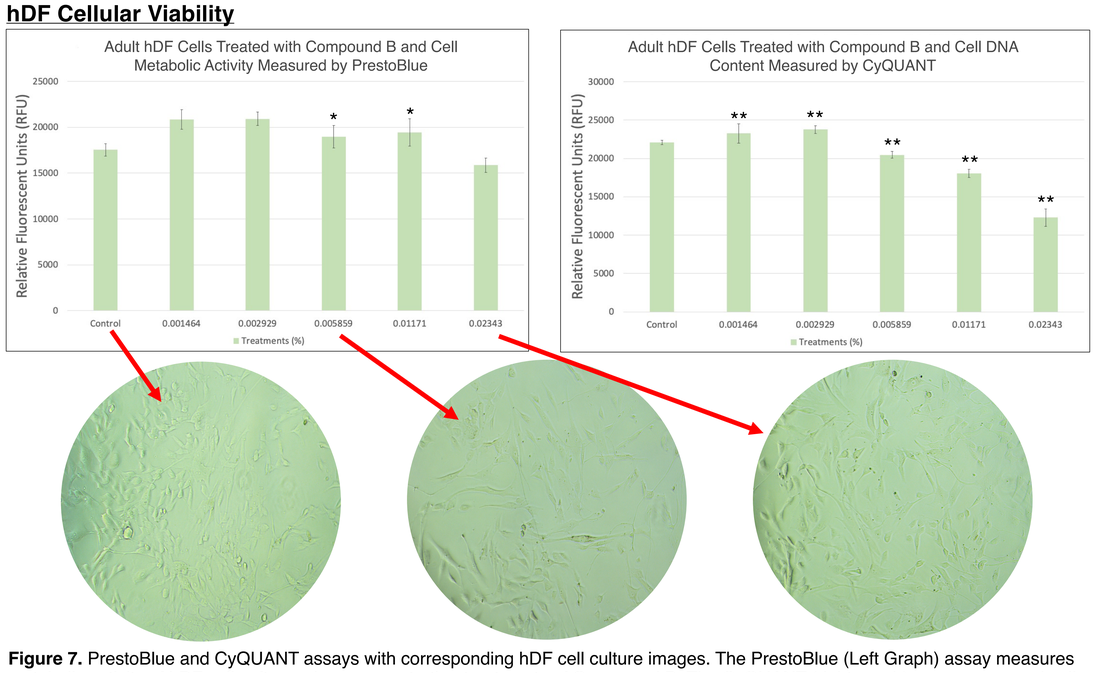

Figure 7. PrestoBlue and CyQUANT assays with corresponding hDF cell culture images. The PrestoBlue (Left Graph) assay measures metabolic activity of hDF cells after various concentration treatments of Compound B. The CyQUANT (right graph) assay measured cell DNA content of hDF cells after various concentration treatments of Compound B. Red arrows correspond concentrations to hDF cells imaged after 24-h treatments. As concentrations increased, hDF cells begin to show signs of growth in unfavorable environments. In the control image, the cells are elongated, fuller, and more concentrated. However, as concentration increases, the cells are shorter, smaller, and less concentrated. (* < p = 0.05, ** < p = 0.01).

Conclusions

The novel IL/DES were characterized to determine cellular compatibility properties, antimicrobial properties, and challenged in applications. The results we observe to date indicate that the IL/DES is versatile for the different applications that can be used in healthcare. In particular:

- The hDF cells do not experience any observable deleterious effects when in the presence of some concentrations of the Compound B.
- Incorporating novel eutectic solvents within scaffolds decreases porosity of of scaffolds, however, allows for growth of hDFn cells.
- Preliminary antimicrobial data suggests the Compound A scaffolds resist biofouling by a common microbial pathogen associated with skin/wound infections.

Future Directions: Novel IL/DES implemented into surface modifications for medical devices.

References

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