The Role of Keratin in Cell Adhesion to Complex Geometries

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INTRODUCTION: Osseointegration offers an innovative solution for regaining functionality following limb loss through direct integration of prosthetic devices with the skeletal system. This direct attachment to the hard tissue provides enhanced stability, improved load distribution, and better sensory feedback compared to traditional socket-based prostheses. However, long-term dermal adhesion remains a significant issue for transcutaneous devices, leading to complications such as infection, soft tissue breakdown, and implant loosening. Achieving permanent implant/dermal interface adhesion has the potential to significantly improve patient outcomes, where the incorporation of keratin in tissue scaffolds has demonstrated enhanced cellular adhesion and proliferation. Specifically, keratin is a collection of fibrous proteins found in many tissues throughout the body. It has been shown to promote cell attachment in simple *in vitro* and *in vivo* environments, improving stability and nutrient exchange for tissue formation. To expand the application of keratin to osseointegrated environments, multiple tissue scaffold fabrication methods should be combined to create clinically relevant scaffold geometries. For this study, the combination construct being tested is composed of electrospun fibers (ESF) and 3-dimensionally printed (3DP) frameworks. ESF have been implemented to quickly create tailorable, polymer meshes to facilitate tissue ingrowth. However, the thin and flexible nature of ESF matrices lacks structural support and mechanical stability. In contrast, 3DP offers a cost-effective, precise, and relatively efficient manufacturing solution for producing stable frameworks. However, common 3DP technology is unable to print the necessary microstructure required for appropriate tissue regeneration. Through the combination of these two methods, a modular scaffold can be fabricated that supports cellular adhesion/ingrowth while maintaining 3-dimensional complexity. We hypothesize that incorporating mechanically stable, 3DP structures with keratin-doped ESF scaffolds will exhibit improved cellular adhesion when cultured at complex angles, with translational applications in improved dermal sealing for transcutaneous prosthetics.

METHODS: This research seeks to evaluate the role of keratin (KA) in promoting cellular adhesion in constructs simulating clinically relevant geometries. The combined constructs themselves are fabricated by fixation of keratin-doped electrospun polycaprolactone (PCL) fibers in 3DP polylactic acid (PLA) scaffolds. Microporous electrospun meshes were created from 5% combined (wt/v) PCL and KA dissolved in 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP). For the fibroblast study, 1%:4% and 3%:2% KA:PCL concentrations were used. 5% PCL ESF scaffolds without keratin served as a control. Fibers were affixed in a collection of hexagonal PLA frames. The frames consisted of an outer hexagonal ridge across which the fibers were stretched, and an inner hexagonal frame which was inserted into the outer ridge, mechanically securing the ESFs. The modular frames were printed with platform supports (Figure 1) to alter the angle of the scaffold during cell culture with the purpose of observing fibroblast adhesion at various angles. Frame supports tiled the ESFs at 0, 30, 60, and 90 degrees from the well plate surface. Human dermal fibroblasts (hDFs; 50,000 cells/construct; ATCC) were then seeded onto these combined scaffolds, and cell adhesion/morphology was assessed over two weeks. Scanning electron microscopy (SEM) images of the scaffolds were obtained to assess fiber diameter and pore size. Confocal microscopy was also used to monitor cell adhesion, spreading, and extracellular matrix (ECM) deposition through actin filament staining. After SEM imaging, scaffold analyses were run on imageJ and Python. Resulting ESF pore areas and fiber diameters were evaluated using two-way ANOVA. Ongoing work is focused on evaluating adhesion using an adhesion assay in which cells are counted before and after a phosphate-buffered saline (PBS) wash. Adhesion counts are then analyzed for significance using two-way ANOVA to assess adhesion retention at the varied angles.

RESULTS: This study aims to provide insights into the combined effects of keratin additives and 3DP scaffolds on transcutaneous engineering applications. SEM imaging was used to assess pore size and fiber diameter (Figure 2). It was shown that the increased KA composition significantly decreased the average pore area of the meshes ($P<0.05$). Without the presence of keratin, 5% PCL pore sizes averaged 1.024 μm^2, but significantly decreased to 0.426 μm^2 in the 1% keratin, 4% PCL group. Pore areas significantly decreased again to 0.286 μm^2 and 0.287 μm^2 in the 2% keratin/3% PCL and 3% keratin/2% PCL groups respectively, though there was no confirmed significance between the 2% and 3% pore area averages ($P<0.05$). Fiber diameters exhibited a similar trend, thinning from 258 nm in the 0% keratin, 5% PCL group to 208, 183, and 136 nm in the 1, 2, and 3% keratin concentration groups ($P<0.5$). By combining 3DP foundations and KA-doped ESF, this study demonstrated the potential for modular, 3D fastening of fibers via mechanical clasps. The ability of the cells to proliferate and adhere to complex surface angles supports the potential for scaffolds with clinically relevant geometry. The findings of this study support the potential improvement of osseointegration via utilization of KA-doped, 3DP scaffolds to seal the implant-disrupted dermal barrier.

DISCUSSION: This research provides novel insights into the effects of scaffold geometries, the bioactivity of KA, and the cellular behavior response of fibroblasts to a combination construct. This *in vitro* study provides valuable insights into keratin scaffold modifications and their effects on fibroblast behavior. The shrinking ESF features noted within the data can be mitigated with adjusted spinning and solution parameters, however the small pore sizes are expected to have limited effect on cell adhesion and are more relevant for infiltration. Once fibroblast adhesion is confirmed on the angled surfaces, the modular construct can be assembled for full thickness tissue sample evaluation as it conforms and adheres to the polyhedral geometry. For this preliminary study, there are some limitations that must be considered. Firstly, the complexity of the cellular responses within 3-dimensional scaffolds cannot be fully induced in vitro. The interactions between the fibroblasts and KA are also limited due to the use of a cell line which limits the primary cell behavior. The potential variations in KA distribution within the ESF also pose challenges in achieving uniform cell adhesion and infiltration. Additionally, long-term effects on cell behavior and tissue maturation are not included in this study. Further work would be needed to assess long-term adhesion using non-biodegradable materials. Despite these challenges, this research paves the way for innovative strategies to enhance tissue adhesion. By understanding the interplay between KA additives, 3-dimensional geometries, and cellular behavior, this work supports future advancements in tissue engineering and regenerative medicine, facilitating the development of more effective clinical solutions for dermal adhesion.

SIGNIFICANCE: Limb loss affects millions with increasing rates expected by 2050. Osseointegrated prostheses offer improved stability through direct bone integration; however, with persisting risks of infection, there remains a need for improved implant-dermal interface solutions.

IMAGES:

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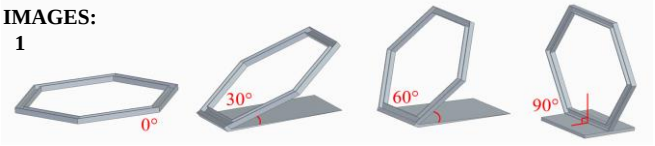

Figure 1: The 3DP mechanical fasteners used to suspend the keratin-doped ESFs for assessment of the force direction's effect on fibroblast adhesion.

Figure 2: Pore sizes of ESFs with increasing keratin concentration solutions displaying significant decreases in pore area with concentrations up to 2%.

Figure 3: ESF diameters with increasing keratin concentration solutions displaying significant decreases in fiber size between the 0 and 1, and 2 and 3% KA groups.

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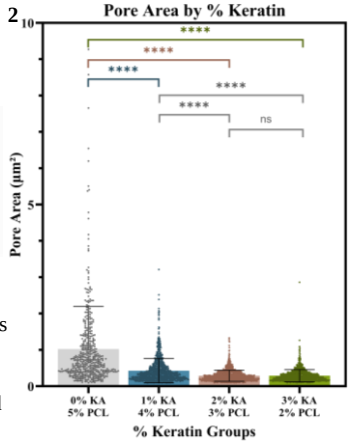

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