Topography-mediated myofiber formation and endothelial cell sprouting

Almonacid Suarez, A M

DOI:
10.33612/diss.127414004

Summary

Muscle loss or impairment may result from trauma or conditions such as facial palsy. Solutions available to compensate this loss of tissue include revision surgery, which is autografting of a patient's own muscle (flap transplant). However, an ideal solution is to provide a newly formed functional muscle, thereby omitting the need to take healthy tissue from the patient. The technology that allows the construction of this newly formed engineered muscle is called tissue engineering. Tissue engineering consists of providing a scaffold for cells to build a new tissue. Scaffolds provide structural support as well as architecture for the tissue generation. The (bio)chemical composition and physical nature of scaffolds dictate cell behavior. A major part of their physical nature comprises the surface characteristics which include topography, which is investigated in this thesis. Scaffolds are made using different materials – natural, synthetic, or a combination of these. Ideally, using the patient's own cells will avoid immunological rejection of the implanted tissue construct.

Engineering a specific tissue requires mimicking the appropriate morphology of the targeted body part to establish the correct properties and function related to this tissue. In the case of skeletal muscle tissue, it is necessary to acknowledge its shape/geometry and components. Skeletal muscle consists of parallelly (linearly) aligned myofibers surrounded by vessels and held together by the architecture and topography of the extracellular matrix (ECM) The ECM is a supportive scaffold for cells because it regulates cellular proliferation and growth, differentiation, migration, maturation, and homeostasis. Surrounding the cells is the thin basal membrane, composed of collagen IV, laminin, and proteoglycans. Beyond the basal membrane is the interstitial matrix comprising fibrillar proteins such as collagen type I and III that yield strength, and which are embedded in a water-retaining gel of negatively charged polysaccharides (glycosaminoglycans and proteoglycans). This gel also binds regulatory growth factors essential for muscle homeostasis and function.

Alignment of cells is possible using linearly aligned topography. Cells sense topography and guide their cytoskeleton to follow the shape on the substrate. Sinusoidal topographical substrates can be generated using shielded surface oxidation with air plasma. The shielding surface oxidation allows the creation of topography gradients with variability of wavelength and amplitude within the same substrate. Our group has been using this topographical system and has shown that directional gradients are versatile screening platforms that can be used to determine the alignment response of different cell types. In this thesis we describe the implementation of this topographical system to engineer skeletal muscle.

In chapter 2 we investigated the optimum alignment of human myoblasts (muscle stem cells) and hypothesized that myoblasts have a preferred directional topography to proliferate, fuse, and mature to myotubes. We used a directional topographical gradient using polydimethylsiloxane-based (PDMS) substrate containing an aligned topography gradient with sinusoidal features ranging from wavelength (λ) = 1520 nm and amplitude (A)

= 176 nm to λ = 9934 nm and A = 2168 nm. As a result of the alignment, the human myoblast differentiated and contracted spontaneously irrespective of topography size. Besides, the myotubes formed resembled the native muscle by organizing their nuclei to the periphery and having an average diameter of 66 ± 59 µm. In addition, we observed that the differentiation process was inhibited or delayed by the smallest topography in our gradient or by flat surfaces. Thus, choosing the optimal surface topography proved essential for efficient alignment and differentiation of muscle stem cells. This chapter revealed the importance of alignment for myotube functionality.

Skeletal muscle tissue has surrounding vessels. In fact, the microvascular network is extensively branched to support every myotube with no less than four capillaries for efficient exchange of gases, nutrients, and waste products. Therefore, it was necessary to evaluate endothelial cell alignment and sprouting in our topographical system to pursue the vascularization of our aligned myotubes. In Chapter 3, the endothelial cell alignment and sprouting response to different topographical features is described. We speculated that a variety of topographies influence sprouting network formation and alignment of endothelial cells and we found that indeed topography is a major trigger for this type of cell. Endothelial cells aligned on micron-sized topography (wavelengths ranging from 4.8 µm to 9.9 µm and amplitudes ranging from 1015 nm to 2169 nm) while nano-sized topography and flat PDMS surfaces caused endothelial cells to create sprouting networks that formed aggregates. We observed that these aggregates were able to migrate and disintegrate into single cells upon contact with the larger directional topography. In addition, endothelial cell sprouting networks were stabilized on an instructive adipose tissue-derived stromal cell (ASC) monolayer. In earlier research, we showed that ASC monolayers act to support vascular networks.

After determining the influence of the topographical features in both cell types – myoblasts and endothelial cells – it was pertinent to combine both cell types in our system to target pre-vascularized skeletal muscle tissue. Thus, in chapter 4 we evaluated the sprouting capabilities of endothelial cells on top of aligned myotubes in the topography. As a result, we demonstrated that the aligned myotubes produced a network of collagen fibers and laminin that supported the early stages of endothelial sprouting.

Chapter 5 provides a discussion about our findings: that human myoblast proliferation, fusion, and differentiation take place within the topographic parameters we evaluated irrespective of their size. However, there is a preference for larger features because the small ones caused detachment. Additionally, we showed that topography and topography-aligned differentiated myoblasts – myotubes – can trigger capillary network formation but require accessory cells such as pericytes (ASC) to complete the vascularization process *in vitro* for muscle engineering. In conclusion, our results explain how topography plays a significant role in the development of *in vitro* tissues. Topography overruled chemical

triggers by leading the response of endothelial cells and myoblasts in our system. However, further research on these responses is needed, as they vary between cell types and origin.