Chemically Defined Plant Leaf-derived Biomaterials for Muscle Tissue Engineering

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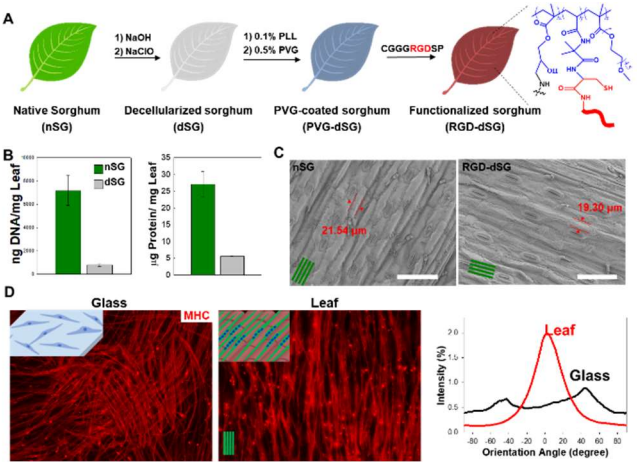
Statement of Purpose: Muscle tissue can recover by themselves upon injuries, however, in severe scale of muscle injury called volumetric muscle loss, muscle tissues lose their regenerative capability. To regenerate functional muscle tissues, muscle cells need to be aligned parallelly and form hierarchical structures. Recently, decellularized plant-derived scaffolds have been studied as a new natural material.[1,2] The plant-derived materials are sustainable and biocompatible and contain pre-vascularized and highly porous structures. Especially, leaves of monocot species such as sorghum have parallel surface topography which can promote muscle cell alignment and formation of functional muscle tissue. However, the plant materials need additional treatment due to the lack of cell adhesive properties. Previously, we developed a polymer which can form a stable and thin layer of coating with chemically defined surface to a variety of platforms.[3-5] This polymer coating is also applicable on the plant materials to support cell adhesion. In this study, we cultured human embryonic stem cell (hESC) derived myogenic progenitor cells on the functionalized plant leaf-derived materials to study muscle cell behaviors. We assume that the plant materials can be used as a noble platform for cell culture and their structural features can promote cellular alignment, differentiation, and muscle contraction.

Methods: Sorghum leaves (80-day-old) were collected from Wisconsin Crop Innovation Center in Madison, Wisconsin. The leaves were treated by NaOH (5%) for 24 hours and 50 % bleach solution until fully discolorized (30–60 min) both at room temperature. The decellularized leaves were rinsed with DI water multiple times and stored in ethanol at -20 ºC until usage, and DNA and protein contents were assessed by CyQuant® and Bradford assay. Poly(PEGMEMA-*r*-VDM-*r*-GMA) (PVG) polymer was synthesized by RAFT polymerization[3] and characterized by GPC and NMR. The leaves were coated sequentially with poly-L-lysine (PLL) (70–150 kDa) for 1 hour and PVG copolymer (50–70 kDa) for overnight both at room temperature, and the coating was evaluated by FTIR. The polymer coated leaf was reacted with RGD peptide. The leaf topography was analyzed by SEM. hESC (H9)-derived myogenic progenitor cells were cultured and expanded by the spherical culture.[6] The cells were seeded on the functionalized leaf scaffold and cultured in differentiation medium for three weeks. Cellular alignment, myogenic differentiation, and myotube contraction were observed by immunocytochemistry (MF-20) and calcein-AM staining.

Results: During the decellularization process, the sorghum leaves were discolorized until became translucent and white color, and their DNA and protein contents reduced significantly. The PLL and PVG polymer were coated on



(Figure 1. (A) Scheme of leaf scaffold preparation. (B) Leaf materials decellularization result. (C) SEM images of the leaf materials. (D) Cellular orientation of hESC-derived myogenic cells after two weeks.)

the leaf scaffold uniformly and the azlactone ring signal at 1818 cm^{-1} was detected by FTIR. Cells were cultured for 1 day and rinsed out to test adhesion. The cells did not adhere to the PVG coated substrates but adhered to the RGD functionalized substrates. Cellular alignment and differentiation were monitored for three weeks. In the first week, both leaf and glass showed slight myogenic differentiation of cells, but only the leaf showed parallelly aligned cells to the topography. During the second week, cells on both materials presented significant elongation, multinucleation, and MHC expression. However, the glass showed only local cell alignments in random direction, while the leaf showed highly aligned cell morphologies and narrow orientation angle distribution. In the last week of culture, cells migrated closely and formed thick myotube bundles. The cells on the leaf showed huge contraction at the beginning and periodic contraction in parallel direction under stimulation. But cells on the glass contracted at first in random direction and lost contractibility.

Conclusion: In this study, we demonstrated that the decellularized plant leaf materials and polymer coating can offer a platform for muscle tissue engineering. The unique properties of plant materials give great results of cell viability, alignment, differentiation, and formation of myotube bundles. Moreover, diversity of plant materials may extend their applications in different types of tissues.

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