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Microscopy techniques in muscle tissue engineering

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Tissue engineered muscle constructs have been developed as alternatives to autografts or allografts to be used especially in regeneration of normal anatomy and physiology of the damaged tissue. Components of tissue engineering approach are cells, scaffolds and regulators. Since lots of cells are needed for production of a tissue equivalent, stem cells have drawn attention due to their ability to proliferate easily in *in vitro* and to differentiate into many cell types. The most prominent stem cell candidates that can be safely used in clinical treatments are the mesenchymal stem cells (MSCs).

The tissue engineering approach involves cell propagation *in vitro* and a subsequent cell seeding on 3D biodegradable scaffolds where the cells attach, grow and form the new tissue substitute as they differentiate. It is well known that the cell carriers, with their chemical, biological, architectural and physical properties, have a substantial effect on the behavior of anchorage dependent MSCs. Both skeletal and cardiac muscle fibers are oriented parallel to each other in their native tissue and this structural organization is very important in terms of proper tissue functionality. Fibrous scaffolds that mimic the natural ECM and possess an aligned structure can be used to control cellular orientation.

Both natural and synthetic biodegradable polymers are used to obtain 3D scaffolds for muscle tissue engineering. Besides serving as cell carries, natural polymers can provide biological cues to the cells to control stem cell fate. On the other hand, synthetic polymers can be easily modified to obtain tailor-made scaffolds and may better preserve tissue integrity until the new tissue is fully regenerated. It is very common nowadays to combine the natural and the synthetic polymers to form a hybrid scaffold that possesses the properties of both and thus may have a synergistic effect on the cells. Microscopy is of utmost importance in characterization of both the scaffolds and the final tissue engineered constructs.

The goal in this study was to design polymeric mats with aligned microfibers that can serve as scaffolds in tissue engineering of both skeletal muscle and myocardial tissues. Aligned microfibrous mats were obtained by electrospinning a polyester blend (PHBV (5% HV), P(L-D,L)LA (70:30) and poly(glycerol sebacate) (PGS)) and a blend of hyaluronic acid (HA), collagen (COLL) and PLA/PCL (70:30). Fiber diameter and orientation in the mats were determined via scanning electron microscopy (SEM). The distribution of the biological component in the HA:COLL:PLA/PCL fibers was revealed by bright field microscopy of histochemically stained mats. Human skeletal muscle stem cells (hSkMSCs) were seeded on the PHBV:PLDLLA:PGS mats to align them and promote unidirectional myofiber formation. Simultaneous construct vascularization protocol was developed by coculturing hSkMSCs with human endothelial cells (HUVEC or ECFC). Cell alignment and capillary network formation was assessed with fluorescence microscopy: the endothelial cells formed both unidirectional and transverse capillary networks, and hSkMSCs were able to form aligned myotubes.

The microfibrous PHBV:PLDLLA:PGS mats were seeded with MSCs from human umbilical cord matrix (Wharton's Jelly) to obtain a construct that can serve as a myocardial patch [1]. Cellular alignment and cell penetration within the mats were investigated with confocal microscopy. The 3D myocardial construct design involved two biodegradable macroporous tubes, to allow transport of growth media to the cells within the construct, and cell seeded, aligned fiber mats wrapped around them. SEM was used to visualize the final construct. The 3D construct was cultured in a microbioreactor by perfusing the growth media transiently through the macroporous tubing, sectioned at the end of the culture and examined by fluorescence microscopy: enhanced cell viability, uniform cell distribution and preservation of the cell alignment in the perfused 3D construct were confirmed.

1. Kenar H, Kose GT, Toner M et al. Biomaterials 32 (2011), p.5320.