Tissue Engineering for Palate Regeneration using Nanofiber Array-based Cell Construct

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Statement of Purpose: Cleft palates represent the most prevalent congenital craniofacial birth defect in humans [1]. Current treatments for cleft palate have relied on surgery and autografts, which are associated with donor site morbidity and chronic pain. Tissue engineering offers a new avenue for palate repair. However, the high strength of natural palate relative to its dimension has posed major challenges to the design of a tissue-engineered construct. The natural palate possesses a unique alternating compact/spongy bone organization rather than an ensheathing compact/spongy configuration as seen in long bone. This structure imparts high mechanical strength to the palate. To mimic the structure of the natural palate, we have recently developed a nanofiber array-based scaffold. Arrays of aligned fibers with individual fiber diameters down to the nanoscale would mimic the organization of natural ECM in bone and provide substrates that allow cell attachment, alignment, and 3-D organization into bone-like structure. Sheets of unidirectionally aligned nanofiber arrays were stacked layer-by-layer in a plied fashion to offer high strength to the scaffold. Dental pulp stem cells (DPSCs), which are readily available in patient's own tooth pulp and have osteogenic potential, were seeded between adjacent layers of nanofiber arrays to constitute a sandwich architecture. To further enhance the strength of the scaffold and facilitate osteogenesis of DPSCs for palate regeneration, the nanofiber array-based scaffold was biomineralized before cell seeding. Using this construct, we have demonstrated robust DPSC attachment, alignment, proliferation, 3-D organization, and osteogenic differentiation into palate-like tissue. Ongoing studies are focused on mechanical testing of the DPSC-scaffold compound in comparison to that of natural palate, and in vivo grafting of the tissue-engineered construct into animal model of cleft palate. Our approach holds promise as an alternative solution for the repair of cleft injuries and malformations through tissue engineering, which eliminates the needs for artificial devices or a second surgery.

Methods: Highly aligned nanofiber arrays were produced using an advanced electrospinning technology developed in our lab. Biomineralization on fiber surfaces was performed by submersing the fibers in a simulated body fluid (SBF) for 28 days. DPSCs were then seeded onto the biomineralized nanofiber arrays and allowed to align along the fibers. The sheets of nanofiber array-DPSC constructs were then stacked at different angles to form a "plied structure" to mimic bone-like assembly. Tensile strength of the construct was determined by a dynamic mechanical analyzer. The stacking angle that leads to the highest mechanical strength of the cell-scaffold construct was determined.

Results: Biomineralization of the nanofiber scaffold in SBF resulted in the formation of a ultra-thin layer of hydroxyapatite (HAp) coating on fiber surfaces, as evidenced by the detection of calcium and phosphate by EDX, X-ray mapping, and by measurements of the reduction in calcium concentration in SBF after biomineralization using a calcium electrode. SEM examination indicated that the HAp coating reproduced the highly aligned morphology of nanofiber arrays. Among the polymers being screened for the fabrication of nanofiber arrays, including Polycaprolactone (PCL), PCL/Chitosan, PCL/Heparin, and PCL/Chitosan/Heparin, PCL/Chitosan/Heparin demonstrated the greatest ability to be biomineralized in vitro, perhaps due to the presence of functional groups on the Heparin molecules that attracted the calcium and phosphate ions. The HAp coating also significantly increased the mechanical strength of the nanofiber arrays. DPSCs that were seeded onto the biomineralized nanofiber scaffold displayed highly aligned morphology (Fig. 1). By stacking sheets of DPSC-nanofiber compounds, 3-D bone-like structure was achieved. Further studies have demonstrated that the seeded DPSCs have high viability, are actively proliferating, and can be induced to differentiate into osteoblasts to form palate-like tissue.

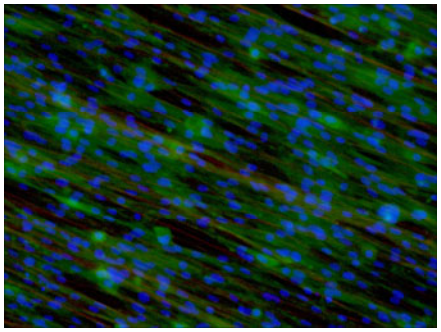

Figure 1: DPSCs aligned along biomineralized nanofiber arrays. Red is the DiI-impregnated nanofibers, green is the actin staining of the DPSCs, and blue is the DAPI staining for cell nuclei.

Conclusions: A tissue engineering paradigm for palate regeneration can be developed based upon the combination of biomineralized nanofiber array scaffolds and osteogenic DPSCs. The biomineralized nanofiber array scaffolds are able to support the viability, attachment, alignment, proliferation, 3-D organization, and osteogenic differentiation of DPSCs into palate-like tissue. When sheets of nanofiber array-DPSC compound were stacked in a layer-by-layer configuration, 3-D palate-like tissue structures with enhanced mechanical strength can be achieved. Ongoing studies in our lab are focused on in vivo grafting of the tissue-engineered construct for palate repair.

References: 1. Derijcke, A. et al. Br J Oral Maxillofac Surg. 1996; 34:488-94.