Craniofacial Skeletal Muscle Regeneration with Auxetic Designed Collagen-PCL Knitted Scaffolds in Alternative Cell Culture Conditions

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Statement of Purpose: Hemifacial microsomia is characterized by underdevelopment of head and neck tissues on one side of the skull causing facial asymmetry [1]. It results in several functional and psychological difficulties, such as problems in chewing, breathing, hearing, and smiling [2]. Current surgical treatment, mostly carried out in infants and children, requires use of autografts, increasing donor site morbidity, or allografts, associated with a higher risk of rejection [3,4]. The proposed study provides an alternative approach by developing synthetic, biocompatible and biodegradable textile scaffolds with the ability for *in vitro* regeneration of facial skeletal muscle using mature autologous precursor cells.

The study includes fabrication of novel auxetic designed weft knitted scaffolds using elastomeric biodegradable poly-ε-caprolactone (PCL) multifilament yarn, and their surface modification using plasma induced surface activation and type I collagen immobilization. To enhance the regeneration of functional skeletal muscle on the scaffold, cyclic uniaxial mechanical stimulation in a dynamic bioreactor and co-culture with nerve cells is proposed. These two cell culture conditions will mimic the conditions of native skeletal muscle, simultaneously allowing better functional skeletal muscle regeneration.

Methods: To fabricate an auxetic and highly porous biodegradable scaffold, we used elastomeric PCL multifilament yarn and weft knitting technology with two auxetic designs. To improve the cell attachment, the scaffold's surface was modified with radiofrequency plasma activation and type I bovine dermal collagen immobilization. Biocompatibility in terms of the extent of cell proliferation and metabolic activity was evaluated in *in vitro* cell culture with C2C12 mouse myoblasts and PC-12 rat nerve cells.

The ability to regenerate a functional skeletal muscle tissue is to be determined through a series of mono- and co-culture studies involving the above cell lines and a dynamic bioreactor assembly. Two types of coculture with and without cell culture inserts over the scaffolds were compared. The extent of tissue regeneration is to be evaluated with the help of cell metabolic activity, cell viability and migration assays and quantitative PCR for expression of muscle tissue specific genes.

Results: Figure 1 shows alamarBlue™ assay results comparing the cell metabolic activity of C2C12 cells over 10 days of cell culture between single cell line monoculture and coculture with PC-12 cells. The higher fluorescence in the scaffolds in coculture indicates high cell metabolic activity during coculture with nerve cells compared to monoculture conditions. The cell metabolic activity between the untreated and treated PCL scaffolds is also compared. Figure 2 shows comparison of confocal images of scaffolds in monoculture and coculture on Day 10 of the cell culture which shows a higher number of viable cells and a higher live to dead cell ratio in coculture conditions compared to monoculture. Two types of coculture with and without a cell culture insert were also compared. A high standard deviation obtained from that containing the cell culture insert shows that the 3D scaffolds better supported regeneration of a 3D viable tissue.

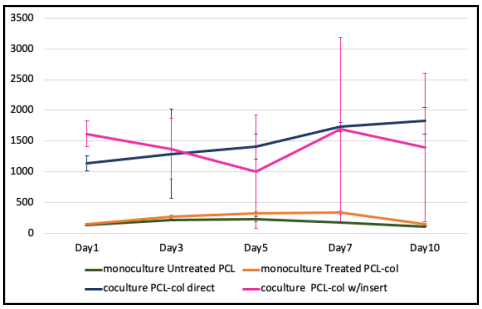

Figure 2. Cell metabolic activity of C2C12 cells in monoculture & co-culture obtained from alamarBlue™ fluorescence assay.

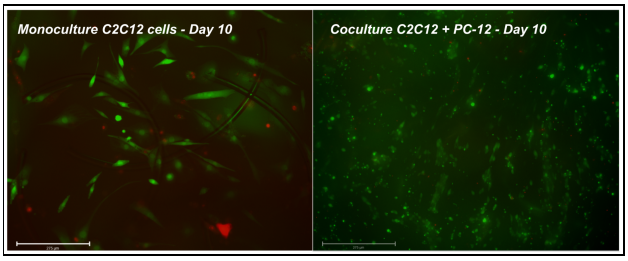

Figure 3. Live/Dead™ staining images on Day10 of cell culture of monoculture (left) and coculture (right) on collagen-PCL scaffolds.

Conclusions: This study has demonstrated the potential in providing an alternative approach to the use of biological grafts for facial reconstruction by developing a synthetic, biodegradable textile scaffold which has the ability to regenerate facial skeletal muscle tissue in vitro using autologous primary cells. The surface modification as well as the coculture conditions have shown significant improvement in cell attachment and metabolic activity during *in vitro* cell culture.

References:

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