NC STATE Wilson College of Textiles

Improving Facial Skeletal Muscle Regeneration using Surface Modified Collagen-PCL Knitted Textile Scaffolds

Monica V. Deshpande[1], Kun Luan[2], Andre J. West[1], Mahmoud Rouabhia[3], Martin W. King[1,4]

[1]Wilson College of Textiles, North Carolina State University, Raleigh, NC, USA, [2]College of Natural Resources, North Carolina State University, Raleigh, NC, USA, [3]Dental Medicine, Laval University, Quebec, Quebec, Canada, [4]College of Textiles, Donghua University, Shanghai, China

BACKGROUND

✴ ***Hemifacial microsomia:***

- Congenital craniofacial malformation characterized by underdevelopment of head and neck tissue on one side of the face, causing asymmetry and functional loss leading to psychological problems such as an inability to smile.
- Observed in ~600 newborns worldwide [1], and in ~4200 newborns in the USA [2].
- Current treatments:
  Surgical reconstruction in young patients including removal of defective tissue and the realignment and correction of jaw bones, followed by filling the defects with biological tissue grafts to restore normal function.
- Drawbacks: Autografts lead to donor site morbidity and allografts lead to higher rejection risks.

Fig 1: Clinical images showing range of deformities in patients suffering from hemifacial microsomia [3]

✴ ***Factors affecting Functional Skeletal Muscle Engineering:***

- Scaffold design >
  - Physical and mechanical properties: Total porosity, Pore size distribution, Strength and Flexibility.
  - Bioactive surface characteristics: Hydrophilicity for improved cell attachment and proliferation.
- Culture conditions >
  - Dynamic Cell Culture System: Mechanical straining to mimic the native skeletal muscle tissue development environment.
  - Co-culture Systems: Biological cues enhancing cell differentiation and tissue development.

OBJECTIVES

To demonstrate viable textile technologies to fabricate a scaffold which is capable of regenerating soft facial tissue to replace autologous biological grafts, *by ---*

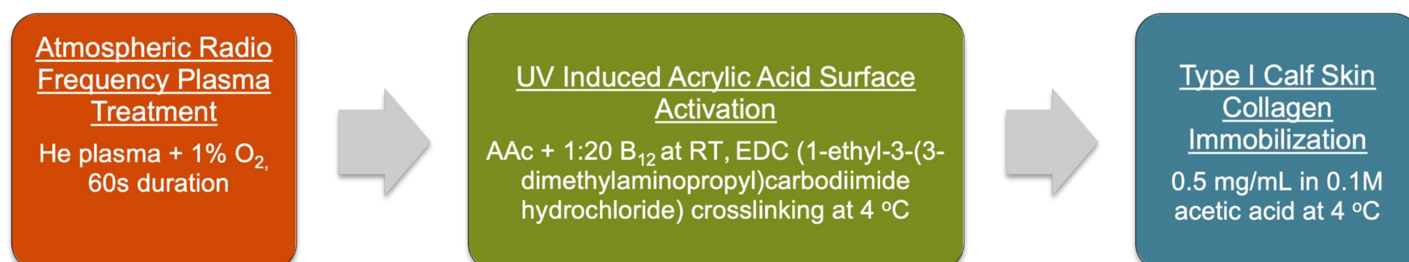
1. Fabricating biodegradable, narrow width knitted scaffolds.
2. Improving hydrophilicity of the scaffold to optimize cell attachment and proliferation.
3. Evaluating the biocompatibility of the scaffolds for skeletal muscle regeneration in monoculture system.

METHODS

➢ ***Scaffold Fabrication:***

- 18-gauge flat knitted 2-ply poly(ε-caprolactone) (PCL) multifilament yarn.
- Auxetic basket and arrowhead designs, control: 1x1 rib stitch design.
- Ultrasonic cleaning with Triton X-100 nonionic detergent at RT
- Heat setting at 45°C.

➢ ***Surface Modification:***



➢ ***Auxetic Design for Tissue Regeneration:***

- **Auxetic knitted textile:** Characterized by negative Poisson's ratio. Self folding structure governed by fabric design geometry and elastomeric component.
- **Dynamic bioreactor:** Unidirectional cyclic loading for tissue maturation. Auxetic scaffold will ensure elimination of lateral compressional forces on cells during cyclic loading.

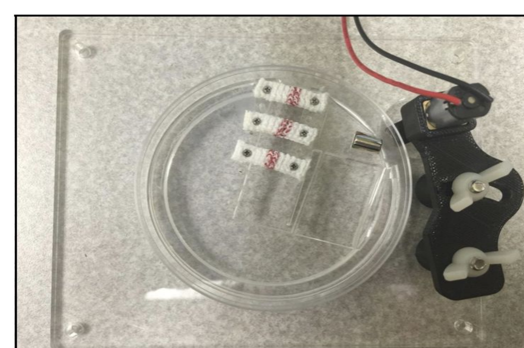


Fig 2: The NCSU MTJ Dynamic Bioreactor [4]

RESULTS

➢ ***Physical & Mechanical Properties:***

Parameters	1x1Rib	Arrowhead	Basket
Fabric width (mm)	10	17	13
Total porosity (%)	74.2	82.4	81.9
Bursting Strength (kPa)	408.89 (±29.97)	423.95 (±38.8)	232.81 (±71.15)
% Elongation at Break	194.77 (±6.96)	179.08 (±6.63)	171.85 (±12.89)

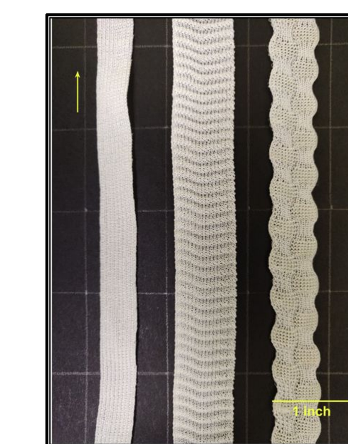


Fig 3: Narrow-width Knitted PCL scaffolds: (from left) a. 1x1Rib b. Auxetic Arrowhead c. Auxetic Basket.

➢ ***Improved Surface Characteristics:***

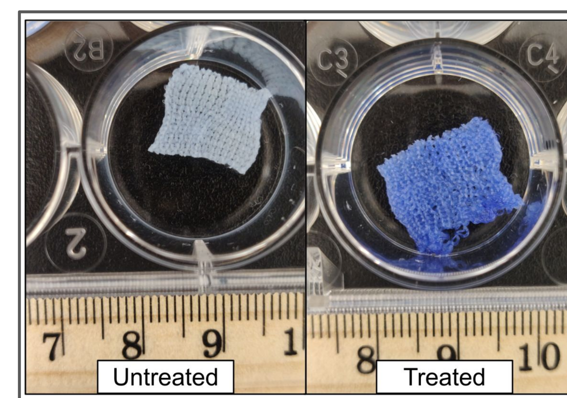


Fig 4: Aniline blue staining test images.

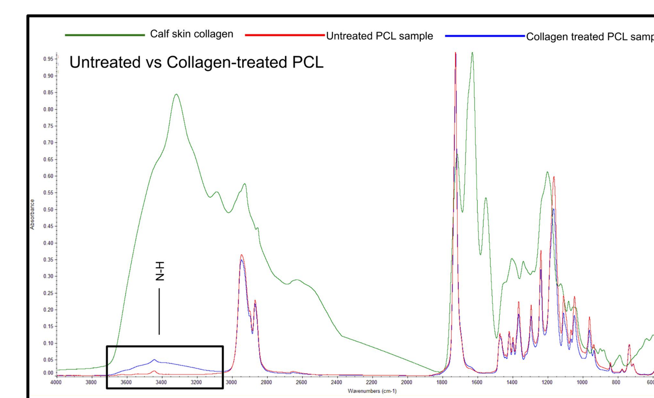


Fig 5: FTIR spectra of collagen treated PCL scaffold.

Elemental composition of the untreated PCL and collagen-treated PCL samples

Elements	Untreated	Collagen-PCL
C	90.9 %	85.8 %
O	9.2 %	11.7 %
N	-	2.2 %
Na	-	0.2 %

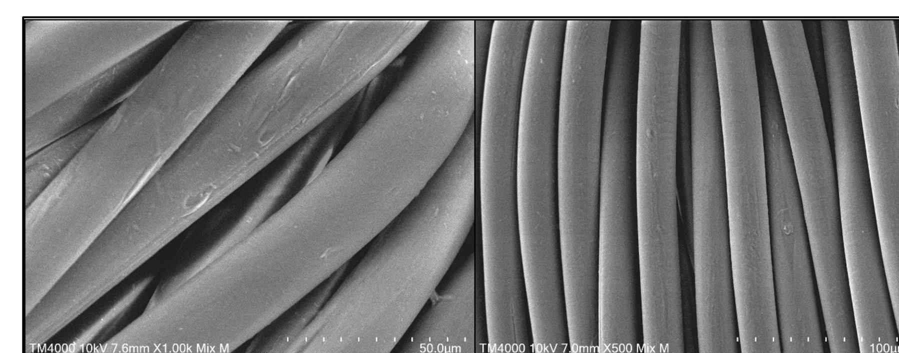


Fig 6: SEM images of collagen-PCL scaffold surface at 500x (left) and 1000x (right) magnification showing superficial collagen immobilization.

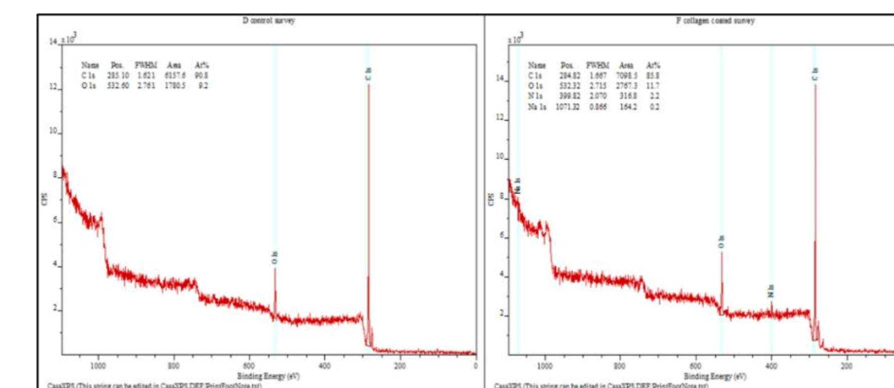


Fig 7: XPS spectra of untreated (left) and collagen-treated (right) PCL scaffolds.

➢ ***Improved Cell Attachment & Proliferation:***

- 10-day *in vitro* cell culture with C2C12 mouse skeletal muscle cells

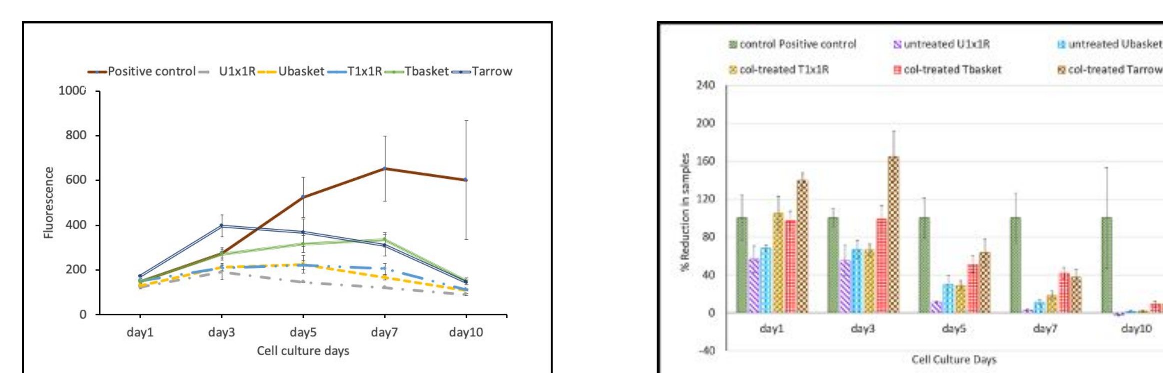


Fig 8: C2C12 static 10-day cell culture cell metabolic activity assay a. alamarBlue™ fluorescence (left) b. %Reduction (right).

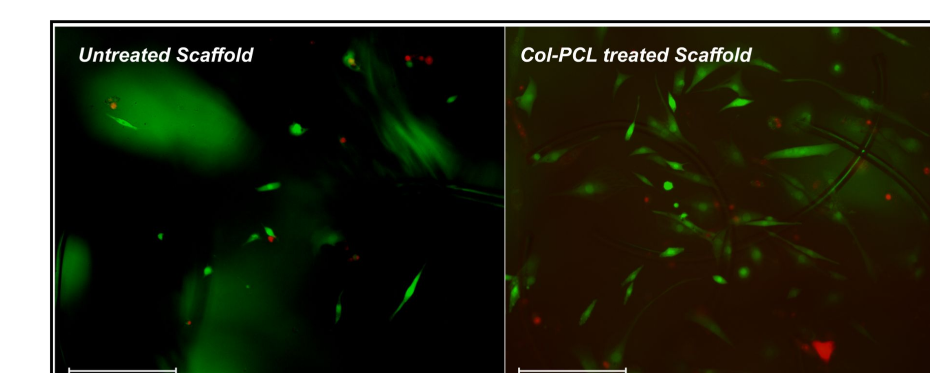


Fig 9: Live/Dead™ cell staining assay images of treated and untreated scaffolds on Day10 of cell culture (C2C12).

- 7-day *in vitro* cell culture with PC-12 rat nerve cells

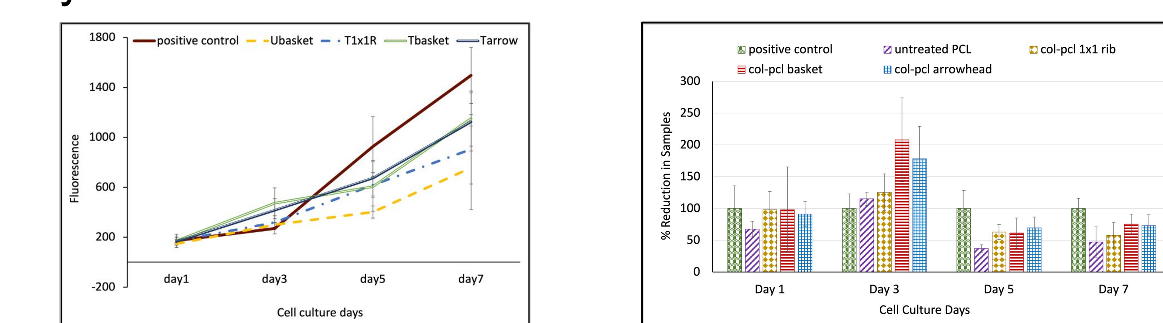


Fig 10: PC-12 static 7-day cell culture cell metabolic activity assay a. alamarBlue™ fluorescence (left) b. %Reduction (right).

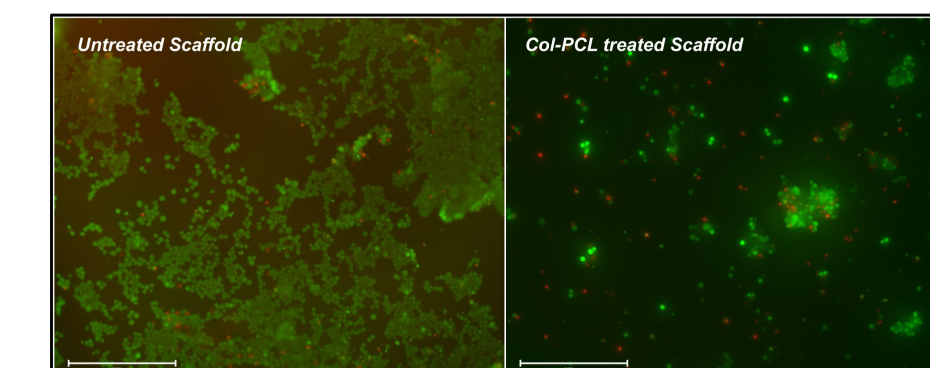


Fig 11: Live/Dead™ cell staining assay images of treated and untreated scaffolds on Day7 of cell culture (PC-12).

FUTURE WORK

- Evaluate surface modified three-dimensional scaffold structures in terms of craniofacial muscle regeneration in static co-culture systems.
- Compare auxetic scaffolds with conventional knitted structures in terms of craniofacial tissue regeneration in separate and co-cultured static and dynamic culture systems.

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CONTACT

- Monica V. Deshpande: mdeshpa3@ncsu.edu Dr. Martin W. King: mwking2@ncsu.edu