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Fabrication of a scaffold from protein-based electrospun yarn for skin tissue regeneration

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Introduction

As the largest organ in the human body, the skin's primary function is to serve as a protective barrier against the invasion of harmful substances from the external environment. Additionally, it participates in metabolic processes, playing a resorptive and thermo-regulatory function, defending against microorganism, and taking parts in the immunological process (Boer et al. 2016). The structure of the skin consists of three layers, the outermost epidermis, inner dermis and subcutaneous hyperdermis composed of fat and connective tissue (Rnjak et al. 2011). Figure 1 illustrates the overall structure of three layers.

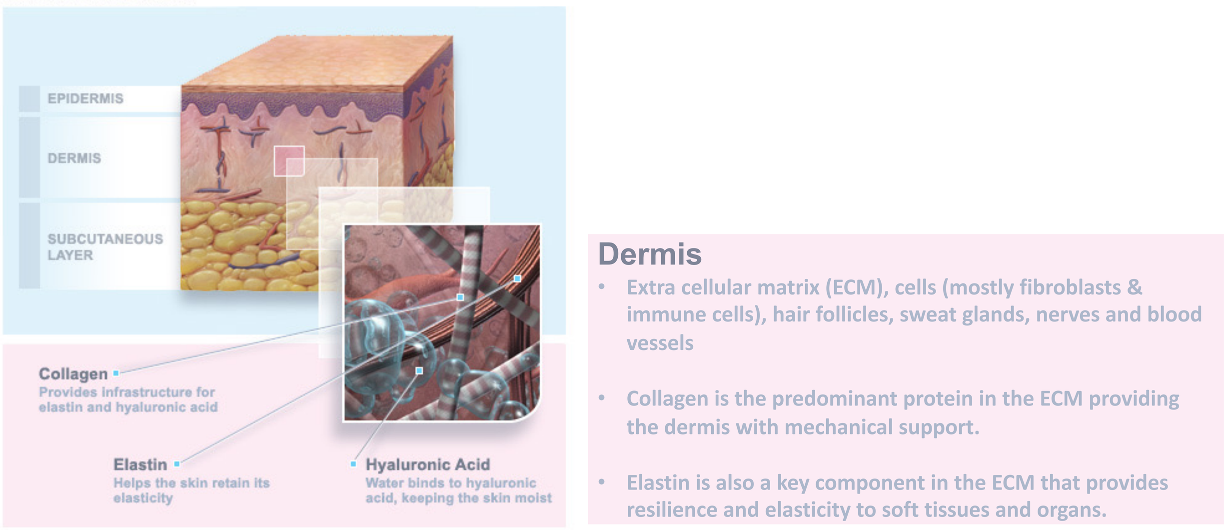


Figure 1: Overall Structure of the skin: hypodermis, dermis, and epidermis.

According to the report from World Health Organization(WHO), burns are a global public health problem, accounting for an estimated 11 million burn injuries annually worldwide. Approximately 180,000 of which are fatal. The treatment of burn injuries comes at a high cost for both health care and society, accounting for about $118 million in direct costs and $172 million in indirect costs, for a total of $290 million annually (Banfield et al, 2015).

The classification of burn injuries depends on their depth and sizes, which is critical in wound management. Determination of the depth is estimated by using commonly observed clinical features. The extent and depth of injury is typically described in turns of first, second, third and fourth degrees. Figure 2 gives a visual illustration of the classification of burn injuries by depth.

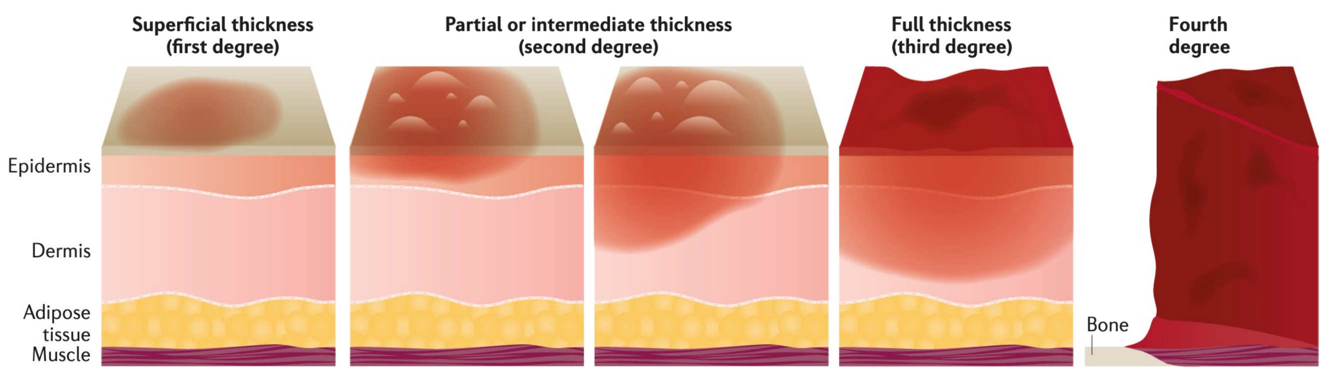


Figure 2: Common clinical patterns resulting from major burn injuries (Jeschke et al.2020)

There are many ways of treating burn injuries like with the use of autografts, allografts, xenografts and synthetic materials. However, the limited availability of these grafts, serious infections and toxicity can severely restrict their clinical use. Therefore, the strategy of tissue engineering is to seed cells on a biodegradable acellular scaffold so as to create cell-sheets directly which can construct a skin graft that is structurally and biochemically similar to skin tissue. This will lead to a more complete and quicker tissue remodeling and reconstruction.

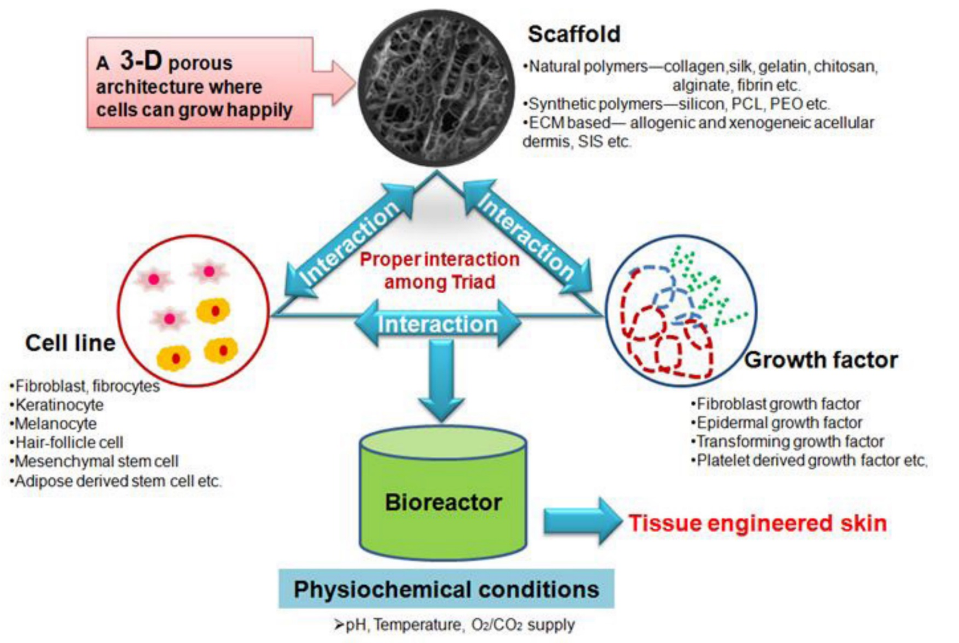


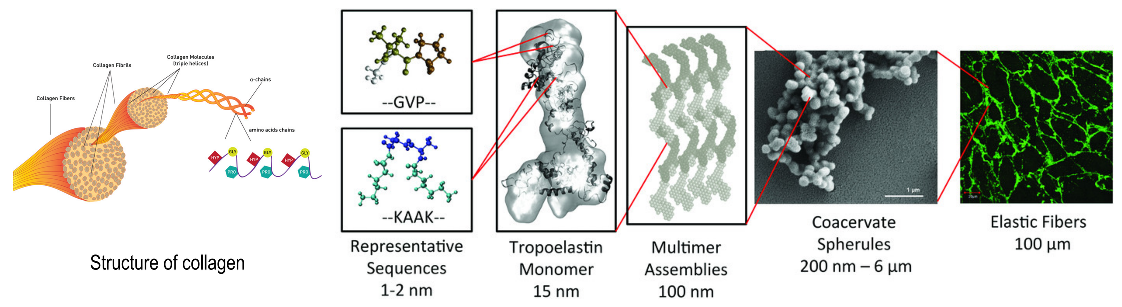
Figure 2: Strategy of tissue engineering(Dhasmana et al.2018)

The design considerations of tissue engineering include providing an ECM equivalent with the necessary functional and structural properties. It should aid in repair and support the proliferation of skin cells. The scaffold can provide a permissive environment for the development of nerves, the immune response and other functions such as the reduction in scarring. The overall structure should provide the necessary mechanical support over a sufficient period of time so as to complete healing.

Material and Methods

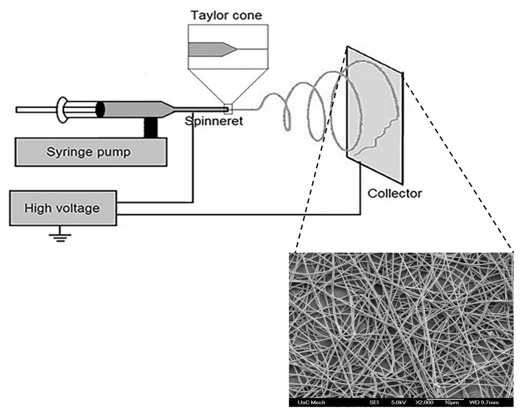
Materials

- Type I collagen from calf skin(MP Biomedicals, Irvine, CA)
- Type A gelatin from porcine skin (Millipore Sigma)
- Polycaprolactone, approx Mw 150,000 (Scientific Polymer Products Inc. ,NY)
- Recombinant human dermal tropoelastin (Axolotl Biologix Inc.,Sedona, AZ).
- 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP, 99.0+%, Fishier Scientific)

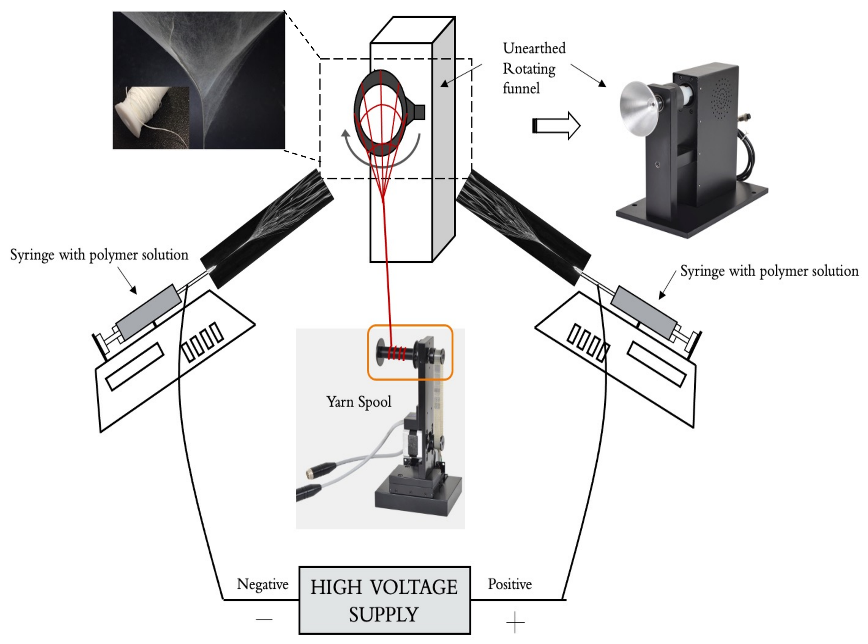


Methods

Trandititional Electrospinning process



Modified Electrospinning set up

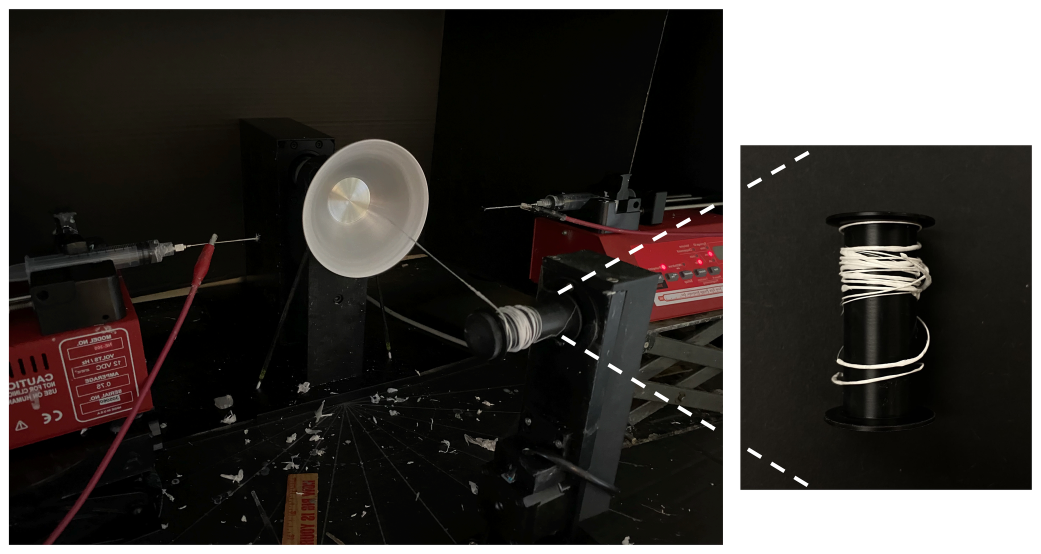


In order to spin the highly twisted continuous electrospun yarns, the mixed protein solutions were pumped through two 18-gauge needles. The two nozzles connect separately to positive and negative charges were placed on either side of the rotating funnel collector. Under high voltage, nanofibers were produced and deposited on the rotating funnel forming a web within the funnel rim. A plastic pipette was used to pull a hollow nanofibrous cone from the center of the rotating funnel. The cone was drawn and wrapped on a revolving winder to produce a continuous yarn of nanofibers.

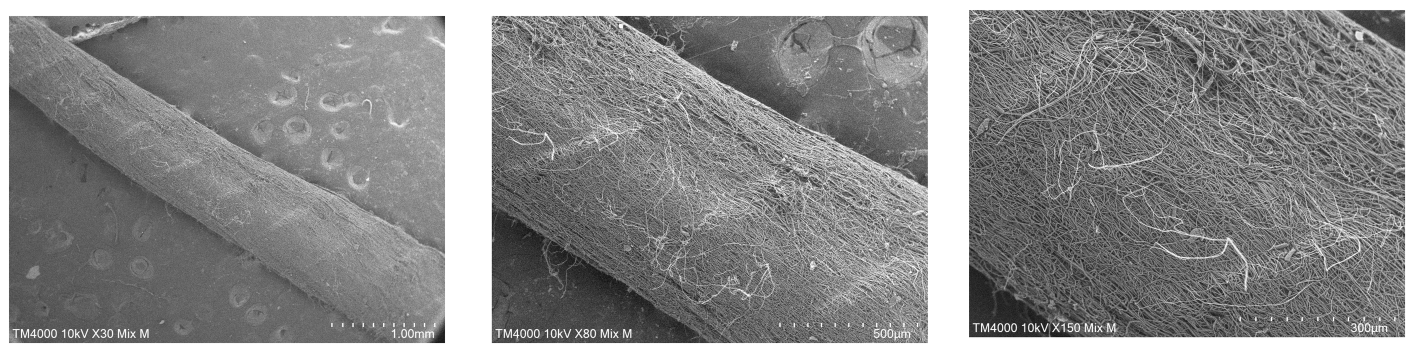
Advantages of using modified electrospinning setup for electrospun yarn

- 2D constructs with denser nanofibrous limiting cell infiltration into the materials
- Relatively high mechanical strength
- Yarns can be post processed into well-defined mats, tubes or other 3D fibrous scaffolds
- High alignment of oriented nanofibers promotes greater actin production and focal adhesion to achieve high cell proliferation

Preliminary studies

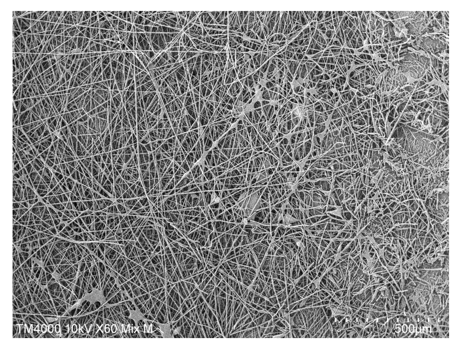


- Materials: 15%w/v PCL:Gelatin at ratio of 2:1 in HFIP
- Setup parameters:
- Funnel speed: 250rpm
- Spool speed: 10rpm
- Voltage: 17Kv
- Flow rate of positive charged syringe pump: 3ml/h
- Angle and distance from positive charged syringe pump to edge of funnel: 30°，6.5cm
- Flow rate of negative charged syringe pump: 1.8ml/h
- Angle and distance from negative charged syringe pump to edge of funnel: 30°，5.5cm
- Distance from funnel to yarn spool: 17cm



SEM Images of electrospun nanofiber yarns

VS



SEM Images of electrospun web

Future plan

- Introduction of collagen and tropoelasin into the scaffolds and evaluate how modifying electrospinning setup variables affects the performance of yarn morphology and its mechanical properties. Analyze material characteristics and examine their biocompatibility.
- Fabricate prototype skin grafts using textile technologies from the optimized novel twisted collagen-tropoelastin electrospun yarns. Evaluate how the incorporation of tropoelastin/collagen blended yarns affects the proposed textiles-based grafts and examine the mechanical properties as well as analyze the cell proliferation performance.
- Developing scaffolds with biomechanical properties, such as cell proliferation, ECM secretion and endothelial network formation, and culturing stem cells on the electrospun tropoelastin-collagen scaffolds by applying pulsatile radial stretching in a dynamic bioreactor.

Reference

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