Novel Scaffold Design that Adds a Third Dimension to Engineering Complex Tissues

Ting He[1], Katharina Sippel[1, 3], Alfred O. Inman[2], Nancy Monteiro-Riviere [2], Sang Jin Lee[4], Martin W. King[1,5],
[1]College of Textiles, [2]Center for Chemical Toxicology Research & Pharmacokinetics, North Carolina State University, Raleigh, NC, USA, [3] ITA, RWTH Aachen University, Aachen, Germany, [4] Wake Forest Institute of Regenerative Medicine, Winston-Salem, NC, USA. [5]College of Textiles, Donghua University, Shanghai, China

Introduction and Objectives: The healthcare crisis in the USA is driving growing interest in regenerative medicine and tissue engineering research. Despite significant successes in developing a single tissue type on a thin scaffold, there is a need for techniques that can develop composite tissues [1]. Most of the current techniques, such as the phase separation and emulsion freezing, cannot create large samples with interconnected open pores. Other fabrication methods, such as electro-spinning, are limited to a small scale laboratory operation producing scaffolds with limited thickness and poor reproducibility [2]. In response to these challenges we propose a 3D textile scaffold with variable design in thickness, total porosity, distribution of pore sizes, and mechanical properties. We have harnessed the latest warp knitting technology to obtain a reliable prototype which has commercial potential. Our goals are 1) to explore the design perspectives of different composite structures and mechanical properties 2) to fabricate 3D scaffolds and determine whether they will be mechanically functional, biocompatible and able to support the adhesion and proliferation of various cell lines.

Materials and Methods: The design requirements for the various tissue engineering scaffolds have not been defined precisely, but estimated properties can be inferred by reference to published literature.

Tissue Application	Cell Lines
urethra, bladder	epithelium, smooth muscle cells
vascular	endothelium, smooth muscle cells
skin, wound care	epithelium, keratinocytes, fibroblasts
oral mucosa	epithelium, smooth muscle cells
muscle-tendon junction	fibroblast, smooth muscle cells
pancreas, liver	islets, hepatocytes
cartilage	chondrocytes

Table 1. Current clinical collaborators evaluating our textile tissue engineered scaffolds

The choice of degradable polymers is determined by the designed rate of degradation and the mechanical requirements of the targeted application. We chose warp knitting technology because of its productivity and versatility. It works well with a large range of options of FDA proved materials such as polylactic acid (PLA) and polycaprolactone (PCL). In order to study the influence of the thickness and the pore size distribution on cell behavior, an initial permanent prototype was knitted from non-degradable polyethylene terephthalate (polyester) yarns. Based on our preliminary simulation in ProCad WarpKnit 3D software, we designed a 3D sandwich scaffold using a spacer fabric structure which consisted of small pores (30-40 µm) on both outer surfaces to help cell attachment, and large pores (200-500µm) in the middle layer to encourage nutrient and oxygen transport and to promote angiogenesis. The pore size along the thickness can be controlled by the knitting parameters and the different pore sizes along the width can be achieved by using yarns with different diameters. The 3D spacer fabric prototype 24G4GBXII was fabricated from textured 150 denier polyester yarn with 12.5µm filament diameter and 48 filaments (Unifi, Inc., Greensboro, NC) (Figure 1.C-F). The physical characteristics, mechanical and biological properties of the prototype were tested by standard methods.

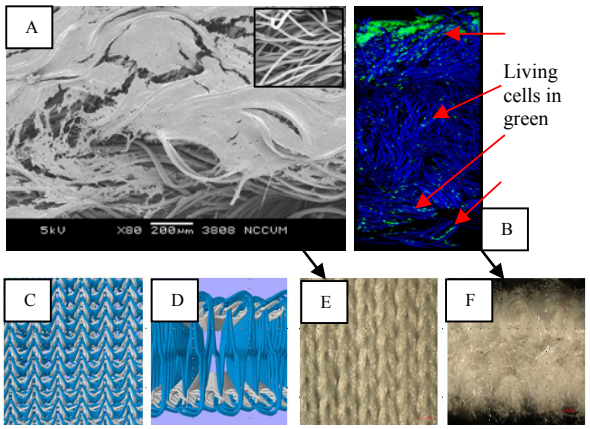

Figure 1. SEM image of the scaffold surface with HDF cells (A); LSCM image of the side view with HDF cells (B); face and side views of simulated 3D spacer fabric (C and D) and knitted spacer fabric (E and F).

Results: The prototype has a thickness of 28.32 mm and total porosity as 85. 9 %. An MTT assay was used to compare the cell proliferation of human dermal fibroblasts (HDF) over 15 days. Scanning electron microscopy (SEM) of the surface layer (Figure 1.A) and laser scanning confocal microscopy (LSCM) confirmed that the 3D scaffold successfully promoted faster cell the thickness of the knitted spacer fabrics (Figure 1.B). The mechanical properties have been evaluated in terms of the tensile strength, Young's modulus, ultimate elongation, compression rate, extent of recovery (78.6%), bursting strength and stiffness.

Conclusions and Future Work: We successfully fabricated a novel 3D scaffold with desired characteristics. A new prototype using degradable polymers such as PLA and PCL will be fabricated and evaluated by the same methods. The tissue ingrowth and the change of scaffold structures during the degrading process will be examined. The following *in vitro* study will include co-culturing different cell lines such as human dermal fibroblasts (HDF) and normal human epidermal keratinocytes (NHEK).

References: [1] Mikos AG, Herring SW, Ochareon P, et al. 2006. Engineering complex tissues. Tissue Eng 12(12): 3307.
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