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Solving Biocompatibility Layer by Layer: Designing Scaffolds for Tissues

Zernetsch H., Repanas A., Gryshkov A., AL Halabi F., Rittinghaus T., Wienecke S., Müller M., Glasmacher B.[1]

Institute for Multiphase Processes, Leibniz Universität Hannover, Germany

glasmacher@imp.uni-hannover.de

Abstract: *New opportunities for the design of artificial tissue structures via ice templating and electrospinning are described. Exemplarily, developments of vascular grafts, heart valves and nerve guides will be presented.*

Keywords: Functional tissue engineering, scaffold, ice templating, electrospinning, mimicking ECM, fibers

Introduction

Tissue engineering as a field of regenerative medicine is an interdisciplinary field that combines the principles of engineering and life sciences with the goal of restoration, repair or replacement of tissues and their functions. Its underlying principle is the combination of cells and scaffolds, a transitory extracellular matrix, to produce a new functional tissue. Bridging the gap between isolated cells and functioning tissue, the scaffolds become an instructive extracellular microenvironment that actively guides cells both locally and in time towards tissue formation and regeneration. This is achieved by the presentation of specific insoluble biochemical and structural cues to constituent cells in combination with the controlled release of soluble factors with time. In tissue engineering the combination of appropriate cells on a suitable biomaterial as a carrying structure poses a particular challenge. Thus, the cell type and source, the controlled cell seeding of the scaffolds and bioreactor systems for further cultivation and (stem cell) differentiation address further important challenges. Electrospinning and ice templating are versatile techniques in tissue engineering for the production of such scaffolds mimicking the extracellular matrix using different kinds of permanent or resorbable biomaterials with possible incorporation of specific drugs and different structural features.

Methods

Electrospinning is a technique for the production of ultrafine polymer fibers from polymer melts or polymer solutions through electrostatic interaction. The original technique of electrospinning was patented in the first half of the twentieth century by Anton Formhals and Richard Schreiber-Gastell. The filed assemblies and methods aimed at the production of silk-like threads from cellulose acetate. They used cellulose acetate solutions and their filed inventions already featured wheels, mandrels or a metal band to coil up and align the fibres. Formhals also proposed the use of precipitating baths and the possibility of needleless spinning assemblies [1]. Electrospinning as a technology for the production of micro- and nanostructured scaffold materials has gained widespread acceptance in the medical research community over the last decade. The process generates a non-woven fiber mat consisting of one continuous filament with diameters ranging from the micron to nanometer range. It is most often used as scaffold materials in tissue engineering applications due to its similarity to the filamentous microenvironment in native tissues. This similarity often promotes a more positive cell response to the generated fibers than to the bulk material alone. However, the reproducibility of the scaffold structure is often limited by the used electrospinning set-ups that so far do not fully utilize the available potential of the process technology. Here, we describe techniques for the production of aligned fiber structures, multilayered, multiscaled and multifiber scaffolds, fiber modification und functionalization and useful advances in process control.

Ice templating. This technique enables to produce scaffolds with directional porous structures made out of suspensions such as collagen, chitosan, PEO (with alumina particles), fibrin. Examples are shown below in figure 1.

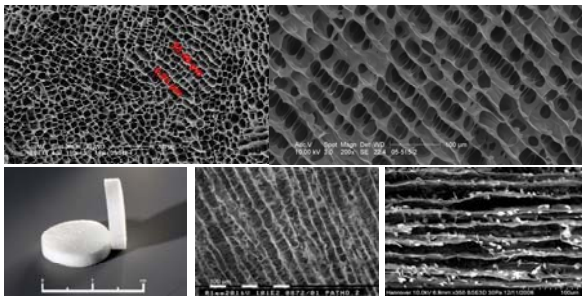

Figure 1: Pictures of structures made by ice templating.

Vascular tissue engineering. In the field of cardiovascular implant technology, synthetic implants, biological grafts and biohybrids are used for valves and vascular grafts. There is a great need for vascular grafts with small inner diameters and heart valve prostheses with growing potential for children. Since the implants that are available today still lack of some biocompatibility features, there is a need for improvement. Functional bioartificial constructs at the time of implantation remain a driving factor for further development. This is due to the active role of a functional endothelial cell layer in mediating blood compatibility and preventing adverse reactions. Thus designing a scaffold to present the correct mechanical and biochemical clues to cells and mechanically stimulating the construct after seeding are vital factors to support physiological tissue formation and cell activity.

Electrospinning offers a unique opportunity to process both synthetic and biological materials, either in pure form or as a combination, to generate nanofibrous mats as tissue engineering scaffolds. The generated fibrous microstructure closely resembles structures in the native extracellular matrix and can be oriented to provide tactile clues to influence cell migration and behaviour. Furthermore, processing different materials in parallel to finetune the internal microstructure allows a wide range in which to modulate mechanical and biochemical properties.
To support and guide neotissue formation, mechanical stimulation of the seeded construct up to the point of implantation is required. This has been recognized by the advent of specialized bioreactors to reproduce the mechanical stimuli in the cardiovascular environment. However, the effects of individual factors (e.g. shear rate and oxygenation level) are still not fully understood especially with regard to their temporal kinetics. Real time microscopic imaging of cells and their response to changes in their micromechanical stimulation may help to better define parameters for stimulation of tissue engineering constructs.

Methods

Developing a synthetic vascular graft with a small inner diameter is one of the great challenges in biomedical engineering. Current prosthesis with an inner diameter less than 6 mm tend to occlude within the first months [2]. Tissue engineering, using cell-seeded scaffolds prepared by methods like salt leaching, directional solidification or electrospinning (fig. 2), is a promising way to overcome this limitation [3]. In order to screen such scaffolds for vascular tissue engineering, standard test methods were successfully adapted to highly porous structures. Tests on suture retention strength, longitudinal and circumferential stiffness, and compliance are performed at 37°C submerged in PBS solution. Hemocompatibility of the scaffold material is evaluated in a dynamic in vitro test setup with anticoagulated porcine whole blood. In a preliminary test run, electrospun PCL/PLA-Scaffolds (i.d. 4 mm) were shown to have excellent suture retention strength and tensile strength. Hemocompatibility testing resulted in a decrease in thrombocyte count of about 20 % over 1 h, which is comparable to the change caused by bare metal stents in a previous study. The set of test methods proved to be suitable for testing highly porous materials and will be used for screening of structures made from various materials produced by electrospinning or ice templating. [4]

Discussion and Conclusions

Scaffold design via electrospinng and ice templating resulted in versatile scaffolds made out of various synthetic respectively biological polymers. The scaffolds exhibited different macroscopic and microscopic structures, various pore sizes and porosities. Scaffolds reinforced with ceramic nanoparticles showed hard-elastic properties for bone replacement, aligned fibres for tendon and fiber mats for heart valve and small vascular graft replacement. Using biochemical cues, nerve guidance is under development. Kinetic drug release studies have been performed to assess the functionality of the scaffold design. Different cell types have been used. Collagen scaffolds made via ice templating were used in stem cell studies from the common marmoset monkey *(Callithrix jacchus)* to its genetic and physiological similarities to humans. Stem cells were used in cell seeding studies applying laser induced cell transfer to locally deposit the cells.

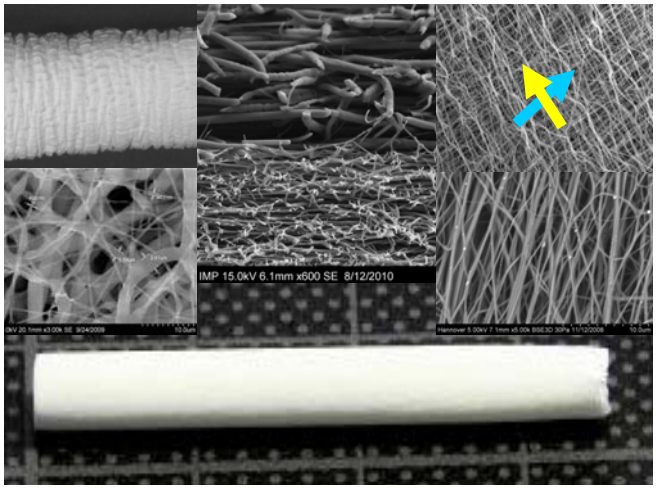

Figure 2: Electrospun 2D and 3D structures.

To guide tissue development, scaffolds must provide specific biochemical, structural and mechanical cues to cells and deliver them in a controlled manner over time. Electrospinning and ice templating have shown to be versatile techniques in tissue engineering for the production of scaffolds. Freeze-dried and electrospun scaffolds showed to address both controlled release and structural cues with adequate pore sizes and resulting porosity enabling cell proliferation and ingrowth.

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