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MULTI-STRUCTURAL COLLAGEN SCAFFOLDS TO RECONSTITUTE COMPLEX TISSUE ARCHITECTURES

MOTIVATION

Mimicking the natural structure of 3D-tissues is an essential step to enhance the quality of in vitro cell culturing. 3D scaffolds with a porous structure or a membrane-like shape are available from many suppliers. However, those cell matrices are limited in dimensions, functionality and complexity and they are made of different material compositions. Products combining different structures in one scaffold are not available. The aim of this project is to design 3D scaffolds with different tissue architectures including pores, barriers and channels by using only insoluble collagen as biomaterial.

METHODS

FABRICATION OF INSOLUBLE NATIVE COLLAGEN DISPERSIONS

The scaffold structures were constructed from porcine collagen dispersions. To obtain these dispersions, the skins were decellularized using different cleaning steps to eliminate non-collageneous proteins and to reduce the DNA content to < 50 ng/mg dry mass. The clean skins were grounded with a mincer, the granule was swollen in hydrochloric acid solution and dispersed in a dispersion plant to receive a homogeneous slurry. This slurry was used to construct different collagen structures including membranes, channels and porous scaffolds.

Figure 1\The final slurry: a smooth and finely dispersed dispersion of native collagen fibers

SCAFFOLD STRUCTURING PROCESSES

The multiple structures of the scaffolds were realized by combining different drying techniques, like lyophilization and convective drying, and extrusion processes. Pores were generated by lyophilization using a dry mass content between 1-3 %. Changing the pH of the slurry before freeze drying resulted in a closed surface on top of the scaffold, serving as a barrier or outer layer for epithelial cell adhesion.

PORE, MEMBRANE AND CHANNEL DESIGN PROCESSES

The collagen slurry´s pH was increased up to 5.5 and the slurry was filled in multi-well plates. Increasing the pH > 4.5 leads to a separation of water and protein. After a short settling time the samples were lyophilized with a defined freeze and dry regime as shown in figure 2. Membranes were prepared by convective drying of the slurry in a petri dish.

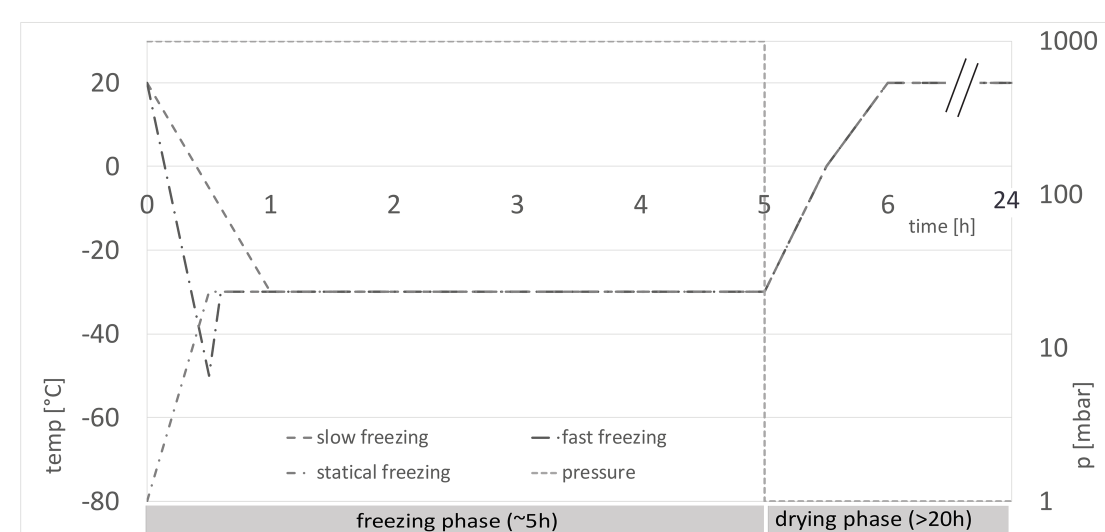

Figure 2 \Lyophilization program for pore designing

- Dispersion is cooled down slowly (0.9 K/min), fast (2.3 K/min) and static (-80 °C, shock freezed).
- Drying is performed at 20 °C under vaccum conditions up to 20 h.

Hollow fibers were prepared by extrusion of a highly concentrated slurry (> 4 %) using a special nozzle. The strand was extruded directly into a solvent bath to drain the fibers. To integrate the hollow fibers into the scaffolds, dry strands were embedded with collagen slurry and lyophilized. As an alternative way, canules were used as placeholders to generate perfusable channels. All materials were cross-linked, dried and sterilized.

RESULTS

PORE SIZE

The pore structure is mainly influenced by the dry mass content and the pH of the collagen slurry. Additionally, the lyophilization regime forms the pores. Deep and static temperatures (<-30 °C) result in very small pores, which are not usable for cell culturing.

Freezing the slurry with a gradient of 0.9 K/min led to pore sizes of about ~150 µm and an open pored scaffold promoting cell migration and supply of nutrients.

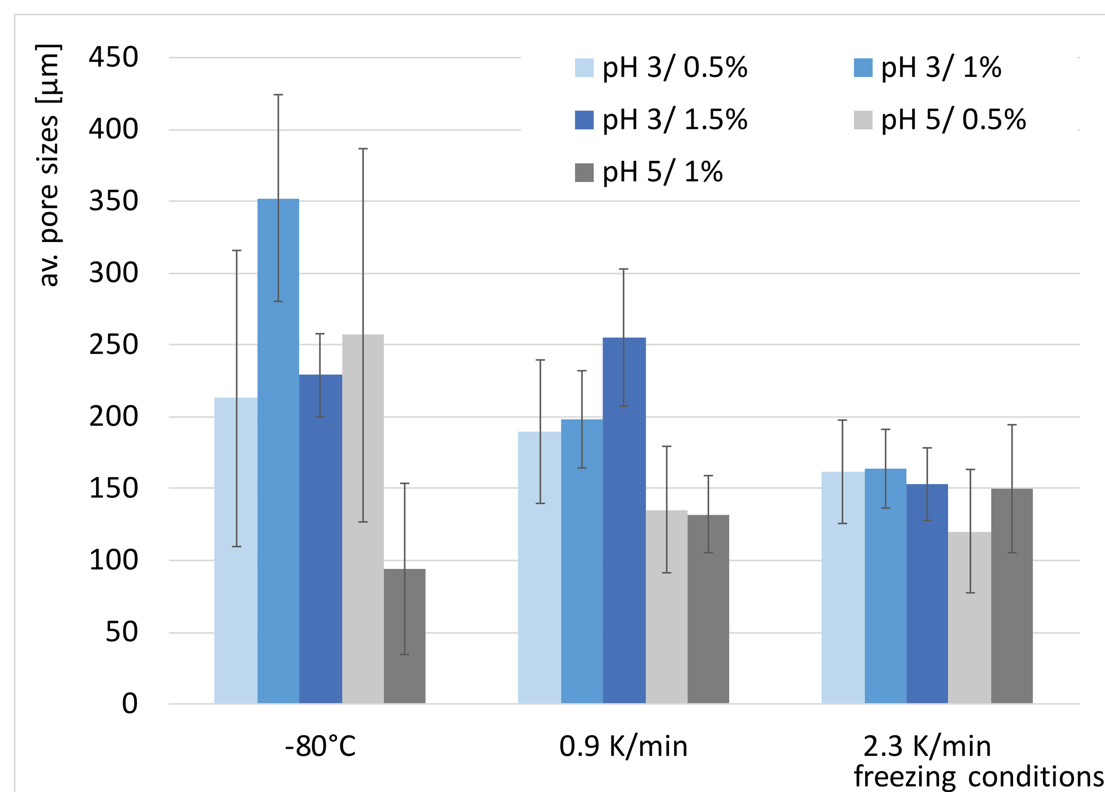

Figure 3\Pore Sizes in Scaffolds made of collagen slurry disolved in acetic acid

MEMBRANES

The closed top layer of the scaffold showed a relatively smooth surface and enabled the growth of endothelial cells. Further cell culture experiments confirmed the suitability of the multi-structural scaffold.

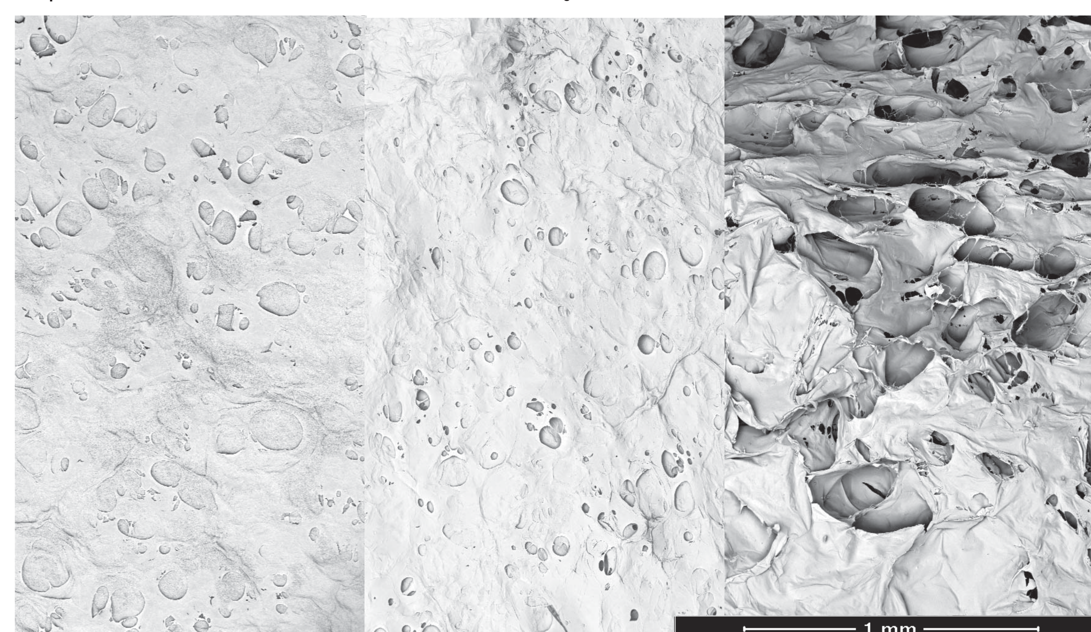

Figure 4\Scaffold surfaces depending on pH of the collagen slurry, soluted in acetic acid; neutralizing the slurry up to pH 7 results in closer surfaces on the top of the scaffolds

CHANNELS

Embedding the crosslinked hollow fibres resulted in perfusable channels, the oxygen perfusion is shown in figure 5.

The scaffold was cultivated with 2 cell types succesfully, endothelial cells inside the channel and fibroblasts in the pores (fig. 6).

Surrounding canules with a collagen slurry (pH > 5.5) led to opened (metallic canules) or almost closed (plastic canules) channels inside a scaffold.

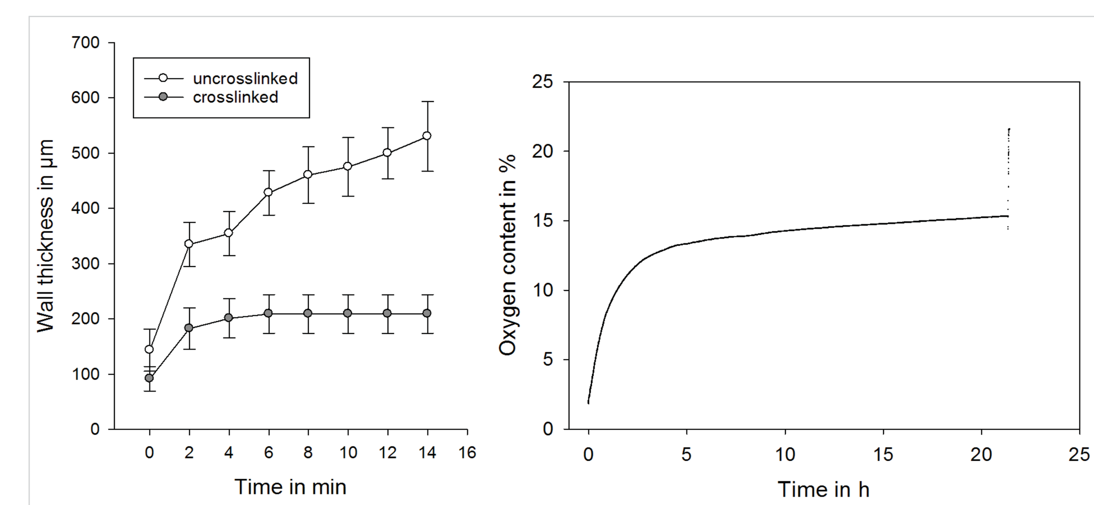

Figure 5\ Wall thickness of collagen channels and oxygen content in medium perfused through a crosslinked channel. After ~20 min an equilibrium is reached in the culture medium.

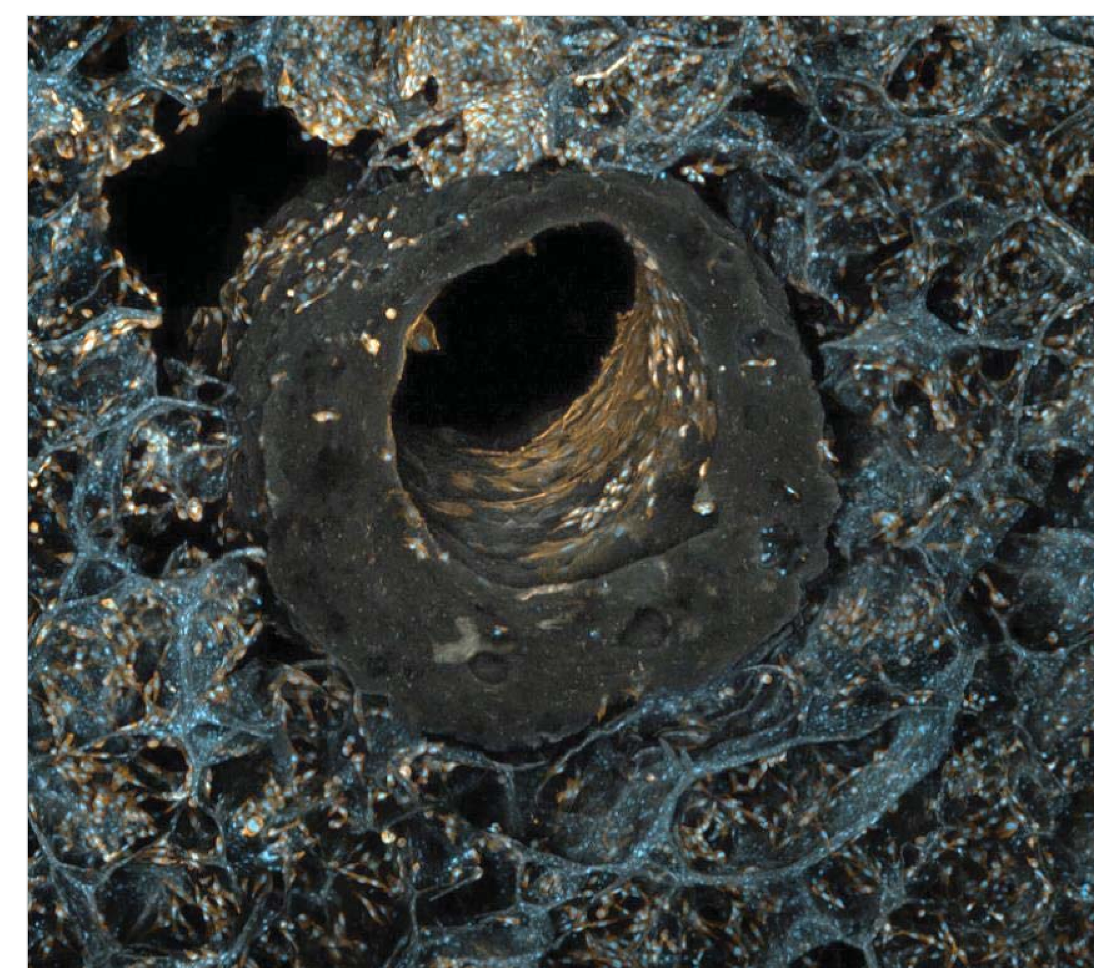

Figure 6\Cells inside a hybrid scaffold system, perfused within a lab-on-achip system (fluorescence microscopy; blue: nucleus (DAPI) and collagen; yellow: cytoskeleton (phalloidin))

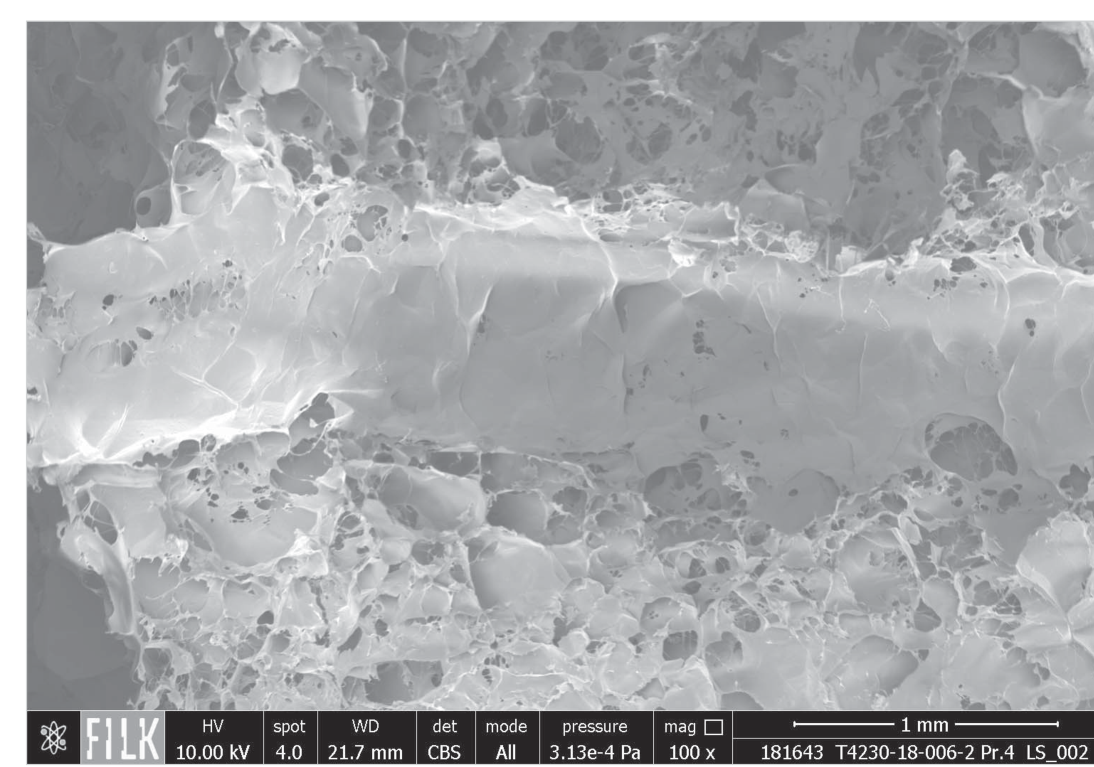

Figure 7\Channel with a closed lumen surface inside a scaffold generated by surrounding a plastic canule with collagen slurry

CONCLUSIONS

Combining special material parameters such as the pH and dry mass of the collagen slurry and using special support, it was possible to generate a perfusable scaffold system with pore structures, channels and a closed top layer made in a one step process. The support contains 2 channels equipped with a syringe for cell inoculation. The same ports were further used for the perfusion with the culture media. In co-culture experiments it was shown that two different cell types are able to grow within a multi-structural scaffold containing channels, a porous matrix and a closed top layer.

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ACKNOWLEDGEMENT
The research project MF150076 was partly funded in the framework of the funding program "INNO-KOM-OST" of the Federal Ministry of Economics and Energy based on a resolution of the German Bundes-tag. We would like to thank for the support granted.