Direct Plotting of Three-Dimensional Hollow Fiber Scaffolds Based on Concentrated Alginate Pastes for Tissue Engineering

Yongxiang Luo, Anja Lode, and Michael Gelinsky*

Dedicated to Professor Wolfgang Pompe on the occasion of his 70th birthday

Tissue engineering has been developed as a very active field since the definition was promoted. However, vascularization of tissue-engineered constructs remains the key challenge for its clinical application and is still not solved effectively. Several attempts have been made to realize vascularization in vitro and in vivo using cell-based and scaffold-based strategies. Cell-based strategies often try to fabricate vascular structures in vitro through deposition of cells (such as spheroids consisting of endothelial and smooth muscle cells) layer-by-layer. However, utilization of this method is still limited by poor mechanical properties and high complexity. Scaffold-based strategies, which focus on fabricating 3D porous scaffolds or hydrogels including an artificial vascular-like structure and controlled release of growth factors, are promising for promoting vascularization but must be combined with cellular approaches to be successful. Various techniques have been developed for producing such scaffolds and constructs including soft lithography, stereolithography, and direct fugitive ink writing. Almost all of these methods require multi-step processes for achieving 3D structures. Furthermore, fabricating 3D porous scaffolds with controllable macropores and open channel structures under mild conditions as a prerequisite for combination with live cells or biological factors is still a challenge for these techniques.

3D bioplotting, as one of the rapid prototyping techniques suitable for scaffold fabrication, is an attractive method for creating 3D porous constructs with defined properties for tissue engineering. 3D scaffolds consisting of solid strands have been fabricated successfully by this method using a variety of polymers as well as ceramics. In addition, Moroni and co-workers fabricated 3D porous scaffolds with hollow fibers by extruding a mixture of two polymers with different viscosity.

The hollow strands were formed by selectively dissolving the inner polymer of the core-shell structures. However, this method is limited by the requirement of using organic solvents, which prevents plotting with cells or sensitive substances such as growth factors.

Herein, we demonstrate a very simple and direct method to create 3D porous scaffolds with regular macropores and a network of controllable hollow fibers as artificial vasculature-like system by 3D bioplotting under mild conditions. In this approach, a shell/core nozzle tip was constructed by inserting a right angle stainless steel nozzle with smaller diameter (core nozzle) into a conic plastic nozzle with bigger diameter (shell nozzle). The two tips were adjusted on the same level and the connection of the shell/core nozzles was reinforced to make sure that the two tip positions were stable and not contacting each other. Various sizes of shell/core nozzles were obtained by simply altering the combination of the two nozzles.

The materials, selected for our study, were concentrated pastes consisting of alginate and aqueous poly(vinyl alcohol) (PVA) solutions. Alginate is a biocompatible and biodegradable biopolymer, which has been widely used and studied for drug, growth factor and cell delivery and fabrication of scaffolds for tissue engineering. PVA is a water-soluble and biocompatible polymer that can be used to prepare hydrogels. Alginate powder was mixed with aqueous PVA solution (6% w/v) and stirred until concentrated but homogeneous alginate/PVA pastes with alginate concentration of 16.7% w/w were formed. The prepared alginate/PVA pastes were more suitable for plotting than pure aqueous alginate pastes, because PVA improves the viscosity of the paste significantly. In addition, the high alginate concentration of the pastes, not used by other groups for 3D plotting so far, led to more stable and regular 3D structures in comparison to the low alginate concentration (1–4% w/w) which is used normally.

With the shell/core nozzles and the concentrated alginate/PVA pastes, meter-long hollow fibers were easy to prepare even without the requirement of a 3D plotting device. The extruded hollow alginate/PVA fibers of different diameters showed uniform wall thicknesses and we demonstrate that different sizes of the hollow channels could be prepared. The openness and tightness of the hollow alginate fibers was further confirmed by a dye injection test. A liquid red-dye was running through the whole hollow strand from one end of the fiber under a given light pressure, and little drops leaked from the other end. After 90 min of incubation...
in CaCl₂ solution, the red-dye liquid almost vanished from the tube. These results indicated that the hollow fibers are open at both ends and continuous. Furthermore, the data suggest that the hollow alginate fibers have a good permeability and transport capability, which is important concerning the function as artificial blood vessels to transport and distribute oxygen and

Figure 1. Microscopic images of plotted hollow fibers with different sizes of shell/core nozzles (inner diameters; μm): a) 400/100; b) 610/200; c) 840/250; and d) 1190/450. Images were taken from the middle of fibers. e) Pictures of the hollow alginate fiber before (left) and after injection (middle) of red-dye liquid through one end of the fiber and the fiber after being soaked in CaCl₂ solution for 90 min (right). f) Relationship of fiber size with different sizes of shell/core nozzles and g) the relationship of fiber size with plotting speed and dosing pressure with shell/core nozzles of 400/100 μm. Scale bar is 200 μm for (a–d) and 5 mm for (e).
nutrients. The diameters of the hollow fibers were controlled by adjusting the diameters of shell/core nozzles (Figure 1f). The size of the lumen was mainly related with the core nozzle size. However, the outer diameter of the hollow fiber was dependent on the shell nozzle size, as well as on the plotting speed and dosing pressure (Figure 1g). The plotting speed had to be adjusted to the extrusion speed of the materials to obtain uniform and regular fibers and scaffolds.

Our method for preparing hollow alginate fibers is much simpler compared to that using a special microfluidic chip. With this chip, hollow alginate fibers with meter length were also achievable. However, due to the low alginate concentration...
(2% w/w) used with this technique they are fragile and difficult to handle and tend to deform during drying. Furthermore, with this method it is not possible to create uniform 3D scaffolds. Our hollow fibers prepared of concentrated alginate pastes were stable concerning handling after crosslinking in CaCl₂ solution and underwent little deformation during drying although about 30% shrinkage occurred. The ultimate strength of these hollow fibers was significantly higher than that of fibers prepared from low concentrated alginate with the microfluidic chip. Furthermore, our method is very simple and suitable to the fabrication of 3D scaffolds with controllable inner and outer morphology by means of 3D plotting.

Combining the shell/core nozzles with a 3D plotting system, 3D porous scaffolds with defined structure and orientation of hollow channels were easy to be obtained by a one-step process. Figure 2a shows the overview of a plotted alginate/PVA scaffold consisting of 16 layers of hollow strands. The scaffold has a regular shape and open channels at the ends of the fibers. After plotting, the scaffolds were transferred to a 500 mM CaCl₂ solution for crosslinking of alginate and dissolving of PVA. Interestingly, the hollow fiber scaffold was floating on top of the CaCl₂ solution before vacuum was applied and sank to the bottom after evacuation (Figure 2b). Before vacuum was drawn, the hollow fibers were air-filled and the scaffold therefore lighter than the CaCl₂ solution, thus the scaffold floated on top of the solution. This indicated that the hollow strands of the scaffold were open, continuous and empty. However, the scaffolds consisting of non-hollow alginate/PVA strands, produced as control, always stayed at the bottom of the CaCl₂ solution. Scanning electron microscopy (SEM) (Figure 2c,d,f,g) and optical microscopy images further confirmed that the regular hollow fiber scaffolds not only had interconnected macropores (the

Figure 3. a) Ultimate strength of hollow fibers prepared with different sizes of shell/core nozzles. b,c) Compressive strength and d,e) Young’s modulus of 3D hollow fiber scaffolds in dry (b,d) and wet (c,e) states (n = 5) (*p < 0.05).
pores between the strands), but also an open hollow channel structure. High magnification SEM microscopy images revealed that the surface of the hollow strands was smooth and dense, whereas the tube walls were porous with a pore size of ca. 6 μm. These micropores were produced by the dissolution of PVA in CaCl₂ solution and phase separation of the calcium alginate gel during drying. Fourier transform infrared (FTIR) analyses were performed to confirm that PVA was completely removed by dissolution from the alginate/PVA scaffolds during crosslinking (Supporting Information, Figure S3j).

The plotted 3D constructs consisting of hollow strands are suggested to be useful as scaffolds for tissue engineering, either for providing a matrix for in vitro vascularization or for the generation of complex tissues such as liver or kidney. The macropores of the scaffolds could support, e.g., bone tissue formation, while simultaneously the hollow strands can be used to create blood-vessel-like channels. In addition, such matrices might be very helpful for 3D cancer tissue models.

The mechanical properties of the material were considered as an important factor, especially for application as bone tissue implants. Therefore, the compressive strength and Young’s modulus of the alginate hollow fiber scaffolds were evaluated in dry and wet state (soaking in SBF solution for 2 h at 37°C). Our data (Figure 3) revealed that the porous alginate scaffolds with hollow fiber structure had a significantly higher compressive strength and modulus than those prepared by conventional methods and with a low concentration of alginate,[29,31–33] particularly in the dry state. Two distinct advantages of our scaffolds are obvious. One is the uniform structure and regular orientation of the fibers of the plotted scaffolds, both of which contribute to the improvement of the mechanical properties.[38] The other is the development of high concentrated alginate pastes, which makes the alginate fibers suffer no critical deformation during plotting and drying. The mechanical properties of the hollow fiber alginate scaffolds were adaptable by the porosity of the scaffolds and the tube wall thickness of the hollow fibers, which was controlled by the size of shell/core nozzles and the plotting parameters (plotting speed and dosing pressure).

Human-bone-marrow-derived mesenchymal stem cells (hBMSC) were seeded and cultivated on the plotted hollow fiber scaffolds to evaluate their cytocompatibility. After 7 days and 14 days of culture, viable cells were visualized by SEM and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) staining, respectively. Microscopic images shown in Figure 4 illustrate good attachment and spreading of the hBMSC, not only on the outer surface of the hollow fibers (Figure 4a,c), but also to the inner walls of the tubes (Figure 4b,d). These observations indicate that the plotted hollow fiber scaffolds based on concentrated alginate/PVA pastes support cell adhesion and viability.

In conclusion, we have described a very simple and gentle method to create hollow alginate fibers and 3D alginate scaffolds with designed macropores and controllable hollow channels based on concentrated alginate/PVA pastes and adapted shell/core nozzles. Hollow fibers with sizes ranging from 200 μm to the millimeter-scale were achievable. The produced hollow fibers and 3D scaffolds possess mechanical strength and modulus comparable to that of normal, filled strands and scaffolds made thereof and support hBMSC attachment and spreading. This novel method has the potential to support the design of

![Figure 4. a,b) hBMSC seeded on the scaffolds and observed by SEM on day 7 and c,d) stained with MTT using an optical microscope on day 14, respectively. Cells were attached to the surface (a,c) and to the inner lumen (b,d) of the hollow alginate scaffolds.](image-url)
prevascular structures for tissue engineering and regenerative therapies and the creation of complex tissues models. Furthermore, the direct creation of hollow alginate fiber scaffolds was performed under mild and sterile conditions, at room temperature, physiological pH, and without utilization of any organic solvent, and in a highly effective manner (plotting a small scaffold only needs a few minutes). This makes it possible to include different types of living cells and/or growth factors in the plotting process and to use this novel technology for engineering of complex tissue constructs and artificial organs.

**Experimental Section**

*Preparation of Alginate/PVA Pastes and Construction of Shell/Core Nozzles:* The plotting pastes were prepared by mixing sodium alginate powder (Manugel; ISP Alginites Ltd. Waterfield) with PVA (Sigma-Aldrich, Mw = 130 000) as a 6%w/v aqueous solution in a mass ratio of 1 to 5 and stirred until homogeneous pastes were achieved. The pastes were then transferred into plotting cartridges (Globaco GmbH). For preparing hollow fibers and hollow fiber scaffolds, shell/core nozzles were assembled with a very simple method. A stainless steel nozzle with a bent cannula at a 90° angle (Techcon systems GmbH) with smaller diameter were inserted into a conic plastic nozzle with larger diameter in a manner that the tips of the two nozzles were on the same level and did not touch each other (see Supporting Information). Several sizes of shell/core nozzles were made and used in this work, including those with a size of (inner diameter; jm): 400/100, 610/150, 610/200, 840/200, 840/250, and 1190/450.

*Preparation of Hollow Fibers and 3D Hollow Fiber Scaffolds:* The hollow fibers and 3D hollow fiber scaffolds were fabricated by extruding the prepared alginate/PVA pastes through the shell/core nozzles on a receiver (plastic film or cell culture dish) by 3D plotting. The 3D plotting system was developed by Fraunhofer IWS (Dresden, Germany) based on the Nano-Plotter device from GeSiM (Grosserkmannsdorf, Germany) and introduced on the market as a “bio-plotting printer.”[10] The present configuration of the system allows the manufacturing of three different pastes in one scaffold, 3D scaffolds, with structures created by computer assisted design (CAD), were produced by 3D plotting through layer-by-layer deposition under the control of computer-assisted manufacturing (CAM). The plotting speed was 3 mm/s and dosing air pressure ranged from 600 kPa to 800 kPa according to size of the shell/core nozzles. After the 3D hollow fiber scaffolds were composed, they were transferred into 500 μl CaCl2 solution for crosslinking of alginate for 3–5 h. Optionally, vacuum was applied to force the CaCl2 solution to penetrate the lumen of the hollow fibers. Afterwards, the scaffolds were washed with deionized water three times and dried at air and room temperature.

*Characterization of Hollow Fibers and 3D Hollow Fiber Scaffolds:* The single hollow fibers were characterized by reflected-light microscopy (AxioLab A, Carl Zeiss, Germany) and the diameter of the fibers were measured with the help of a software (Axiovision 4.7.2, Carl Zeiss Microimaging GmbH, Germany). For investigating the patency of the single hollow fibers a red food coloring (Wusitta, Germany) was used. The 3D hollow fiber scaffolds were characterized by optical (Stemi 2000-C, Zeiss, Germany) and scanning electron microscopy (Philips XL 30/ESFM, equipped with a field emission gun). For SEM analysis, dried scaffolds were cut with a razor blade and coated with gold. Scaffolds of alginate/PVA and pure alginate without PVA after crosslinking and drying were analyzed by FTIR spectroscopy in transmission mode (Spectrum 2000, Perkin Elmer, USA). The samples were prepared by grinding particles of the scaffolds with KBr.

*Mechanical Testing of the 3D Hollow Fibers and Hollow Fiber Scaffolds:* The ultimate strength of hollow fibers and compressive strength modulus of 3D hollow fiber scaffolds (7 mm × 7 mm × 7 mm) were tested on dried and wet samples (immersed in simulated body fluid (SBF)) for 2 h at 37°C by a mechanical testing machine (INSTRON 5566, equipped with a 10 kN load cell; Wolpert, Germany). The tests were performed by compressing five samples of each type at a constant compression rate of 1 mm min⁻¹.

**In Vitro Cell Culture Experiment:** Fully characterized hBMSC, isolated from bone marrow of healthy young donors after obtaining informed consent, were kindly provided by the Medical Clinic I, Dresden University Hospital “Carl Gustav Carus” (Prof. Bornhäuser and co-workers). The ethics commission of Technische Universität Dresden approved the application of hMSCs for in vitro experiments. Cells in passage 5 were seeded on the plotted hollow fiber scaffolds (6 × 10⁵ cells on each scaffold) and the resulting cell-matrix-constructs were cultured in Dulbecco’s modified Eagle’s medium low glucose (DMEM) containing 9% fetal calf serum, 10 U mL⁻¹ penicillin and 100 μg mL⁻¹ streptomycin (all components purchased from Biochrom, Germany) for 14 days at 37 °C and 8% CO₂. For MTT staining, the cell culture medium was supplemented with 1.2 μM 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma-Aldrich, Germany) and the cell-seeded scaffolds were further incubated for 4 h followed by microscopic analysis (LEICA DFC295, Germany). For preparing SEM samples, scaffolds seeded with cells were washed with PBS twice and fixed with 3.7% formaldehyde in 100 mM CaCl₂ solution for 30 min, washed with distilled water, and dehydrated using a gradiation series of ethanol/distilled water mixtures. Critical point drying was performed with a CPD 030 apparatus (BALT-EC AG, Liechtenstein). Dried samples were coated with gold, and observed using a Philips XL 30/ESFM, equipped with a field emission gun and operated in SEM mode.

**Supporting Information**

Supporting Information is available from the Wiley Online Library or from the author.

**Acknowledgements**

This study was partly funded by the German Federal Ministry for Economics and Technology (BMWi) via AiF and supported by a seed grant of the DFG Center for Regenerative Therapies Dresden (CRTD). The authors thank the China Scholarship Council (CSC) for supporting YL.

Received: August 22, 2012

Published online: November 26, 2012

---