Dimension-Based Design of Melt Electrowritten Scaffolds

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The electrohydrodynamic stabilization of direct-written fluid jets is explored to design and manufacture tissue engineering scaffolds based on their desired fiber dimensions. It is demonstrated that melt electrowriting can fabricate a full spectrum of various fibers with discrete diameters (2–50 µm) using a single nozzle. This change in fiber diameter is digitally controlled by combining the mass flow rate to the nozzle with collector speed variations without changing the applied voltage. The greatest spectrum of fiber diameters was achieved by the simultaneous alteration of those parameters during printing. The highest placement accuracy could be achieved when maintaining the collector speed slightly above the critical translation speed. This permits the fabrication of medical-grade poly(ε-caprolactone) into complex multimodal and multiphasic scaffolds, using a single nozzle in a single print. This ability to control fiber diameter during printing opens new design opportunities for accurate scaffold fabrication for biomedical applications.

Additive manufacturing (AM) principles have been applied to the fabrication of 3D scaffolds for tissue engineering (TE), including for bone, cartilage, and skin regeneration. AM is particularly pertinent for delivering personalized medicine and one aspect of this is the generation of multiphasic scaffolds, to recapitulate the regional differences in tissue ultrastructure. In this context, multiphasic scaffolds are defined as having regional morphologies that are distinct from each other. Combined with an approach to closely mimic the natural tissue ultrastructure, multiphasic scaffolds have broad applicability within TE.

In addition, bimodal/multimodal fibrous scaffolds provide a breadth of size features within each zone and typically contain small diameter fibers to improve cell adhesion while larger fibers maintain the structural integrity of the overall construct. Most commonly, the small diameter fraction is fabricated by solution electrospinning while the larger diameter fibers are made by various approaches including melt spinning, fused deposition modeling (FDM), or a different electrospinning configuration. Melt processing is used in this study due to a more direct regulatory route to clinical translation, however, similar-scale structures have been direct-written with strongly shear-thinning fluids such as colloidal inks and hydrogel precursors.

The AM technology used here is termed melt electrowriting (MEW) and the configuration of a printer is shown in Figure 1A, with a static syringe heater positioned over a translating stage. Using air pressure, the melt is delivered to a charged nozzle where it is direct-written onto a build plate as a molten fluid column, using an applied voltage to prevent Raleigh–Plateau instabilities. This permits large diameter nozzles to be used, for the production of fibers as small as 820 nm in diameter and as large as 140 µm in planar, or cylindrical rotating collector configurations out of various thermoplastic polymers. The direct-writing of straight fibers is achieved when the speed is higher than a critical translation speed (CTS), which corresponds to the velocity of both the polymer jet and the collector (Figure 1B). This continuous jetting state is influenced by both polymeric and processing parameters. Currently, MEW scaffolds have been fabricated with a constant fiber diameter throughout, with an example shown in Figure 1C. The ability to combine fibers with different diameters is, however, attractive for many TE applications and expands the design perspective for the scaffold.

Here, MEW is developed so that it can be used to fabricate fibers with diameters ranging from 2.0 ± 0.6 to 49.9 ± 2.6 µm into a single printed construct using the same 22G nozzle (406 µm inner diameter), without changing the applied voltage, collector distance, or temperature. The larger fiber diameters, which are comparable in size to the smallest ones achievable with conventional FDM approaches, correlated
with higher air pressure and slow collector speed, while the smallest diameter fibers are manufactured with lower air pressure and rapid collector movement.

All direct-writing experiments produced continuous filaments that did not break or rupture over all speeds and pressures measured, during the print or off-sample. The fiber diameter was optimized using both polymer flow rate (regulated by feed air pressure) and the fiber drawing rate (controlled by the collector speed). For a prediction of the relative diameter change the following relations were applied

\[ D_2 = D_1 \cdot \sqrt{\frac{V_1}{V_2}} \] (1)

and

\[ D_2 = D_1 \cdot \sqrt{\frac{P_1}{P_2}} \] (2)

where \( P_1, V_1 \) and \( P_2, V_2 \) are the pressure and speed values before and after the change respectively. \( D_2 \) is the intended diameter, while \( D_1 \) is the existing diameter. For Equation (1) \( D_1 \) was the diameter of the fibers printed at CTS whereas for \( V_1 \), CTS values were taken (Table 1), while the fiber diameter prediction is only valid above the CTS. In the Equation (2) \( D_1 \) values were preliminarily measured for the lowest pressure used. Although these ratios assume a noncompressible Newtonian fluid only, they were accurate (±10%) for diameter change estimation in the investigated parameter range for the poly(ε-caprolactone) (PCL) melt.

Below the CTS, a fiber is not deposited as a straight line, but as a periodic or sinusoidal pattern (Figure 1B) as previously described. This behavior is similar to falling viscous liquids or elastic rods. Below the CTS, the fiber diameter is constant for the sub-CTS conditions measured here (Figure 1D).

Table 1. The effect of air pressure on the range of fiber diameters achievable with the corresponding CTS.

| Air Pressure [bar] | Collector Speed [100 mm min⁻¹] | Collector Speed [10 000 mm min⁻¹] |
|-------------------|---------------------------------|-----------------------------------|
|                   | CTS [mm min⁻¹] | Fiber diameter [µm] | Coefficient of variation | Fiber diameter [µm] | Coefficient of variation |
| 0.5               | 750 ± 20       | 11.18 ± 0.40        | 3.6%                      | 2.02 ± 0.57         | 28.1%                  |
| 2.0               | 420 ± 22       | 30.51 ± 2.42        | 7.9%                      | 4.29 ± 0.54         | 12.6%                  |
| 4.0               | 300 ± 7        | 49.93 ± 2.62        | 5.3%                      | 6.77 ± 0.93         | 13.7%                  |
With microextrusion direct-writing (e.g., FDM), rapid collector movement can somewhat reduce the fiber diameter,[33] and this effect is demonstrated here to a far greater extent. Mechanical stretching of the MEW fiber commences when the collector speed is greater than the jet speed at the contact point (i.e., above the CTS). Above the CTS, the maximum diameter reduction using the collector speed is between five- and eightfold for the three pressures measured (Table 1 and Figure 1D). As shown in Figure 1E, there is good agreement ($R^2 = 0.974$) between the experimental values and the model equation $D = 26.67 \sqrt[4]{20/V}$ in the range from CTS to $13 \times$ CTS, generated from Equation (1).

One advantage of collector speed-driven diameter control is that it can be performed almost immediately during a print. However, the acceleration length (Figure S1, Supporting Information) is largely defined by the stage specifications and the viscoelastic properties of the jet. As the collector speed increases, the lag between the nozzle and the jet contact point (Figure 1B) is growing and scaffold fidelity decreases.[29,34] Unexpectedly, occasional regions are found where nonlinear fibers existed at high collector speeds ($10000 \text{ mm min}^{-1}$; Figure S2E, Supporting Information), well above the CTS. Controlling the fiber diameter by speed only, therefore, provides limitations on desired diameter and placement.

Varying the mass flow through the nozzle can aid in both extending the range (Figure 1D) and improving precision. When the collector speed was fixed at $1000 \text{ mm min}^{-1}$, increasing the pressure from 0.5 to 4.0 bar increased the diameter almost threefold. For pressures below 0.5 bar under these conditions, the jet became unstable and fiber pulsing resulted.[29] This limited pressure range can be shifted using other processing parameters such as nozzle size, voltage, and the collector distance and material (Figure S3, Supporting Information).[29] These measures could be able to shift and/or extend the diameter window. Similar to the collector speed, a model, $D = 1000 - \sqrt{P/0.5}$, based on Equation (2) provides a good fit ($R^2 = 0.990$) to the experimental data (Figure 1F). When combined, pressure and speed variation allowed sequential printing of the fibers which could have up to a 19-fold increase in diameter (Figure S4, Supporting Information). Fiber diameter in this case can be predicted by the combination of Equations (1) and (2) into one; however, the accuracy of the prediction drops due to error summation.

The change in diameter due to pressure control, however, is not as rapid as when changing the collector speed. The transition between different pressures required a stabilization period, during which a sham construct was printed off-sample at $4 \times$ CTS (Figure 2A). For a period of time, the fiber diameter oscillates until a stable new diameter results, defined here as when the fluctuation is ±10% of new diameter. Based on one diameter measurement every 20 s, the stabilization time exceeded 4 min for the largest pressure changes (Figure 2B). For a more precise determination of the stabilization time and a mathematical description of the process, higher sampling rates (above 1 Hz) might be required (Figure S5A, Supporting Information). Faster collector speeds during the sham print reduced the stabilization time (Figure S5B,C, Supporting Information). It should be noted, however, that the accurate placement of the fiber when the printing direction is changed is affected by both the collector speed and the applied air pressure. The best accuracy for MEW occurs just above the CTS (Figure 2C).

Using the two (electronically controlled) MEW processing parameters (collector speed, pressure), the fiber diameter

![Figure 2. Control of fiber diameter and placement using pressure-speed pairs. A) Schematic of a MEW head and off-sample printing during jet stabilization. B) Fiber diameter measurements for off-sample printing during the stabilization, with $t = 0$ representing the air pressure change. C) Photograph of six direct-written fibers (black lines) and a programmed collector path (dashed red line) approaching and then turning 90° (blue arrows indicate direction of motion). D) SEM image of direct-written fibers, increasing from 5 to 30 µm in 5 µm increments. E) False-colored SEM images showing the stacking of three different diameter fibers and F) three different diameter fibers stacked upon each other three times.](image-url)
can be altered to design multimodal scaffolds based on their desired dimensions. After calibration and fitting of a model curve as per Figure 1E, defined fibers can be printed, as shown in Figure 2D with diameters 5–30 ± 1 µm rotated relative to each other by 30°. Such different diameter fibers can be vertically stacked upon each other (Figure 2E,F) and combined into scaffolds with tailored geometry.

Using this approach, a multiphasic MEW scaffold with smaller pores (scaffold 1; 10 µm diameter fibers; 125 µm spacing) was designed to be connected with a large pore MEW scaffold (scaffold 3; 25 µm diameter fibers; 250 µm spacing) and a membrane between them (scaffold 2). The entire construct was fabricated with a single nozzle, as a single print. Figure 3A is a rendering of three different scaffold morphologies, combined together to form a layered construct. Figure 3B,C shows a scanning electron microscope (SEM) image of such a printed structure, with different-diameter scaffolds on different sides of a membrane. The small diameter fibers (Figure 3A; scaffold 2) are designed to improve cell seeding efficiency and are manufactured utilizing the collector speed method of fiber diameter reduction, as this does not require stabilization time. For the collector speed approach, the necessity to provide a sufficient acceleration path results in a substantial part of a scaffold (Figure 3D) that is not useable, and requires laser cutting to remove the excess material from the central layer. The scaffolds supported the proliferation of human mesenchymal stromal cells (hMSCs) and penetration of the multiphasic scaffold throughout, as shown up to 21 d in vitro (Figure S6, Supporting Information). Figure 3E shows both sides of the multiphasic scaffold after top seeding with hMSCs, while Figure 3F is a magnified image after 7 d in vitro of a large pore where cells proliferate in a concentric manner, while the middle layer (scaffold 2) fibers remain visible (green arrows).

A second example of how multimodal scaffolds can be designed with predetermined fiber diameters is shown in Figure 4. In this instance, both the air pressure and the collector speed were changed to attain the desired dimensions, and an MEW scaffold was fabricated with incrementally smaller fibers. Starting with large diameter fibers (40 µm) at the bottom, the fibers are then graduated in the z-direction down in diameter to 4 µm at the highest point (Figure 4A; Figure S7, Supporting Information). Included within are two small diameter “catching fibers” (7.5 µm) between the two large diameter fibers (Figure 4B–D). When used for the culture of human adipose-derived stem cell (hASC) spheroids, these catching fibers are effective in preventing spheroids from falling through the scaffold (Figure 4E,F). These spheroids can be further cultured within the scaffold, which allows the entire construct to be further manipulated and transferred to other sites.

In conclusion, using changes in both air pressure and collector speed, the PCL fiber diameter is finely controlled “on the fly” and therefore expands the complexity and morphology of the resulting scaffolds. Fiber diameters can be changed over one magnitude and placed accurately using a combination of air pressure and collector speed as controlling parameters. Interestingly, these changes in fiber diameters are valid without any changes in applied voltage and the resulting diameter is remarkably constant. Simple diameter prediction models for both collector speed and air pressure showed good agreement with the experimental values. To maximize positioning accuracy, and achieve the greatest diameter spectrum and for rapid diameter reduction, the collector speed can be used in unison.

**Figure 3.** Example of a multiphasic scaffold. A) A schematic of a dual-sized scaffold design, with a middle layer of small diameter fibers (4 µm) to improve cell seeding efficiency. Image of such a trilayered scaffold visualizing B) scaffold 1 (10 µm diameter fibers; 125 µm spacing) and C) scaffold 3 (25 µm diameter fibers; 250 µm spacing) from above. D) Image of entire printed sample, with an outer, excess part required to accelerate the jet sufficiently for the small diameters of scaffold 2 within the sample (connecting fibers are indicated with blue arrows). E) Top and bottom view of a multiphasic scaffold 21 d after top-seeding of hMSCs. Such cells adhere to, proliferate, and penetrate throughout the scaffold. F) Close up of a pore from scaffold 3, after 7 d in vitro, with fibers from scaffold 2 visible and marked with green arrows.
with air pressure to manufacture a range of multimodal and multiphasic scaffolds, based on their intended dimensions.

Experimental Section

Materials: Medical-grade PCL (Corbion Inc, Netherlands, PURASORB PC 12, Lot# 1412000249, 03/2015) was used as received. Storage/handling of this polymer, for these experiments, is described elsewhere.[29]

MEW Device and Processing Parameters: All samples were fabricated with a previously described custom-built MEW printer. [29] Pressure was regulated from 0.1 to 5 bar in 0.1 bar steps. A 12.5 mm long, 2G nozzle (Nordson Deutschland GmbH, Germany) was used throughout, protruding 0.5 mm beyond the electrode while stainless steel plates were used as a collector. All printing was performed at 21 ± 2 °C ambient temperature, 73 ± 1 °C PCL melt temperature, and a relative humidity of 40 ± 10%. Positive 7.0 kV and negative 1.5 kV voltages were applied to the nozzle and collector respectively. The distance between the nozzle tip and the collector surface (collector distance) was maintained at 6 mm throughout.

Direct-Writing of Fibers: The CTS was determined for the 0.5, 2.0, and 4.0 bar pressures as follows. A series of parallel lines was printed (Figure 2B) with the collector speed decreasing by 10 mm min⁻¹ every four lines. The lowest speed, at which all four lines were visually straight was noted as the CTS.

The fiber diameters after 15 min of stabilization were measured for all combinations of three pressures and five collector speed values: 1) 100, 2) respective CTS, 3) 1000, 4) 3000, and 5) 10 000 mm min⁻¹. Two fibers at least 50 mm in length were printed and six points arbitrarily chosen for measuring in the central area of the sample (Figure S1A, Supporting Information). For the comparison of the experimental data to a mathematical model conditions with 2.0 bar air pressure and 1000 mm min⁻¹ collector speed were investigated in a greater detail. The speed was varied between CTS and 13×CTS with 2×CTS steps, whereas the pressure between 0.5 and 4.0 bar with 0.5 bar steps.

The fiber diameter variation during the stabilization was evaluated for six pressure–speed pair combinations of 0.5, 2.0, and 4.0 bar air pressures (Figure S8, Supporting Information). Two time periods were analyzed for all pressure combinations; 15 and 5 min. After a pressure change every 60 or 20 s respectively a straight segment was printed, in the middle of which fiber diameter was measured. The collector speed for the measured segments was set to 115% of the previously measured CTS. In between each measurement, a jet stabilization was performed off-sample at a constant speed equal to 4×CTS. This test with 5 min duration was also repeated with 1.15×CTS speed during stabilization.

Visualization of Fibers and Statistical Analysis: The CTS was determined with a stereomicroscope (Discovery V20, Carl Zeiss Microscopy GmbH, Germany). All further measurements as well as sample imaging were performed with an SEM (Crossbeam 340, Carl Zeiss Microscopy GmbH, Germany). SEM samples were sputtered with 2 nm platinum before the processing and measurements presented as mean ± standard deviation, (n = 9 samples for all experiments).

In Vitro Culture and Analytics: Human MSCs were obtained from a patient undergoing total hip replacement surgery. Cells were expanded and 1.5 × 10⁶ hMSCs in passage 4 were seeded on top of multiphasic scaffolds with the dimensions of 12 mm x 12 mm. The constructs were cultured for up to 21 d. Cells on scaffolds, after fixation, were visualized using SEM.

Human ASCs were isolated from abdominal lipoaspirates. Cells were expanded and used in passage 3–4.[35,36] Utilizing a liquid overlay technique, 3D spheroids were generated from 5000 hASCs each in agarose-coated 96-well plates. Gradient scaffolds were loaded with spheroids and cultured for 24 h in PBM-2 medium. Cell viability was assessed using the Live/Dead Cell Staining Kit II (Promokine, Germany) and a fluorescence microscope (BX51, Olympus, Germany).

Supporting Information

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

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Conflict of Interest
Coauthor Jodie N. Haigh now works for WILEY-VCH Verlag GmbH & Co. KGaA.

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