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ACHIEVING HIGH POROSITY IN SCAFFOLD BUILDING USING ELECTROHYDRODYNAMIC JETTING3D PRINTING

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ABSTRACT: Electrohydrodynamic Jetting (E-Jetting) printing is a promising microfabrication technology for its unique capability of generating and controlling fine fibers at nanometer/micrometer level. Its capability of building highly oriented scaffold makes it superior for some bio-medical applications. The porosity of E-jetted scaffold is essential in order to provide an environment mimicking the functional cells living environment. This paper analyzes fiber diameter and fiber adjacent gap, and proposes an ideal process to build scaffolds with controllable porosity and precisely oriented fibers.

INTRODUCTION

E-Jetting 3D Printing technology is an attractive near field electrohydrodynamic (EHD) printing method for various applications (Zhenget al., 2010). Due to its unique capability of generating fine fibers at nanometer/micrometer level, and direct depositing fibers with precisely controlled pattern and orientation, E-jetting shows an advantage over tradition electrospinning method in building highly oriented scaffold to restore the unique anisotropic mechanical property in some special human tissues, such as articular cartilage, ligament and tendon tissues (Li et al., 2013).

To provide a mimicking living environment for the functional cells, the proper fiber gap is a key property of a scaffold to ensure sufficient supply of fresh culture medium and nutrient, remove metabolites and facilitate cell infiltration and proliferation. From Chang's study, the preferred porosity of scaffolds for cell penetration is in the range of 60%-90%. The pore size of at least 300µm is required in fibrocartilaginous tissues scaffold for minimum pore size and porosity consideration (Chang and Wang, 2011). Building scaffold with desirable pore size and porosity is essential to cell survival in the cell seeding stage.

However, to achieve precisely aligned fibrous scaffold with ultrathin fibers and large fiber gap above 300µm, maintaining consistent interlayer pore size becomes the major challenge. The problem of inconsistency of interlayers pore is shown in Figure 1. The fibers as shown in Figure 1(c) are incapable of supporting itself over the inter-fiber gap of 300µm, thus the gaps (serving as pores) between layers are blocked. This is mainly due to two reasons. Firstly, the insufficient solidification of the ink solution as it jets out of the nozzle tip. When the fiber is deposited on the substrate, the liquid-stage ink solution easily deforms into a thin film by gravitational force and the electric field force. The second reason lies in the fiber gap, so decreasing the gap size would abate the slacking effect of the fiber as indicated in Figure 1: the fiber gap on the right is twice as long as the fiber gap on the left.

To address the two key factors affecting the porosity of E-jetted scaffold, the solidification rate of ink solution and the gap size between adjacent fibers are analyzed in this paper. By optimizing the two key factors, a self-developed E-jetting system is optimized for building a precisely aligned scaffold with a high porosity above 90%.

Figure 1. (a) and (c), schematic View of straight fiber and ‘slacking fiber’. (b). SEM photo for scaffold built with a different fiber gap.

METHODOLOGY

Method of Scaffold Building

Biocompatible and biodegradable polymers such as polylactide (PLA), polyglycolide (PGA), and polycaprolactone (PCL) are commonly used in building scaffolds. In this paper, PCL-acidic solution is chosen as ink material (V. Yogeshwar Chakrapani et al., 2012).

PCL-acidic solution is drawn into thin fibers with solely the electrical force (potential difference in ~2kV). Thin fibers are stacked into a scaffold structure. The operation process is shown in Figure 2. The XYZ- precision stage first moves in horizontal zigzag directions for first layer printing, followed by moving in vertical zigzag directions to print a layer by the E-jetting process directly on top of the first layer, as shown in Figure 2 (a). By repeating this process, a multi-layers scaffold is built as shown in Figure 2(b) and (c).

Figure 2. (a) Schematic showing the printing methodology of fiber pattern (b) Photo of 20 layer structure built with PCL fibers and the microscopic view of a part of it. (c) 3D schematic illustrates isometric view of scaffold.

Calculation of Porosity

The porosity of multi-layer scaffold was calculated using equation (1) (Moroni L et al., 2007).

$$P = 1 - \frac{V_{Scaffold}}{V_{Cube}} = 1 - \frac{\pi d_1^2}{4 d_2 d_3} \quad (1)$$

where P is the scaffold porosity, d_1 is the fiber diameter, d_2 is the fiber gap (gap between adjacent fibers), and d_3 is the layer thickness (fiber gap between adjacent layers) as indicated in Figure 3(b). The shaded square in Figure 3(b) is a repeating unit in the whole structure, since the scaffold is composed of the same fiber pattern in each layer. By calculating the area percentage of empty space of the shaded square, the porosity of the whole structure can be estimated. d_3 is not necessary equal to the fiber diameter because of the fiber slacking effect which will be introduced in the following section.

Figure 3. The schematic of square-mesh scaffold: (a) Isometric View and (b) Cross-sectional View.

RESULTS AND DISCUSSIONS

Effects of concentration and fiber gap

The goal is to achieve desired sub-micron fiber diameter while attaining more consistent solidification speed. The lower concentration of ink material directly affects the solidification speed because the larger proportion of acid solvent takes longer time for evaporation. Therefore, the first stage of the experiments was to find the suitable solution concentration, which gave a good balance between fiber diameter and solidification speed.

In previous research, the concentration ranges from 60wt%-100wt% concentration demonstrates good continuity of fiber property (Padmanabhan et al., 2011). However, for concentration below 60wt%, no interlayer pore can be observed from the cross section view, as shown in Figure 4. Therefore the workable range for solution concentration is around 70wt%-100wt%.

Figure 4. SEM photo of scaffold with 60wt% PCL solution. (a). Top view (b). Cross-section view.

Building Scaffold with Workable Fiber Gap

The optimal fiber gap for cartilage cell growth ranges from 150-300 μm (Chang and Wang, 2011). To verify the fiber gap effect on the interlayer thickness, scaffolds were built for each concentration (from 70wt% to 100wt%) with increased fiber gap from 100 μm to 500 μm with an increment of 100 μm .

At small fiber gap (100 μm), fibers of the next layer are better supported and the pores between layers were maintained, but the small gap decreases the porosity of scaffold. At large fiber gap, the porosity should be higher in theory, but the thickness of the scaffold would be reduced due to fibers' stacking on each other. Thus, an optimum combination of concentration and fiber gap needs to be found in order to achieve a larger porosity.

Evaluation of optimal concentration and fiber gap

Scanning Electron Microscopy (SEM) operating at 15 kV was used for scaffold imaging. The scaffold fiber gap (d_2) was determined by measuring three random pores from SEM image (top view) as illustrated in Figure 5(a). The fiber diameters (d_1) and layer thickness (d_3) were obtained by measuring 3 random fibers and 3 random inter-layer distances from SEM image (cross section view) as illustrated in Figure 5 (b). An example of the measuring method using 70wt% solution, 300 μm designed fiber gap is illustrated in Figure 5. The data using different concentrations and the designed fiber gaps are recorded in Table 1.

Figure 5. SEM photo of scaffold made of 70wt% PCL solution, 300 μm designed fiber gap: (a) Top View (b) Cross-sectional view.

Table 1. Porosity of scaffold made of different solution concentration and designed fiber gap.

Concentration (wt%)	Designed fiber gap (μm)	Diameter d_1 (μm)	Fiber gap d_2 (μm)	Layer thickness d_3 (μm)	Porosity P (%)
70wt%	200	22	186.7	26.7	92.4%
	300	18.7	268.3	18.7	94.5%
	400	18.3	419.5	14.5	95.7%
	500	21.3	470.5	4	81.1%
80wt%	200	31.3	216.5	28.7	87.6%
	300	21.7	293.3	20.7	93.9%
	400	28	410	12.7	88.2%
	500	19.3	480.5	10.5	94.2%

100wt%	200	41.3	226.5	23.3	74.6%
	300	28.7	296.7	22	90.1%
	400	26.7	400	12	88.3%
	500	25.3	516.7	10.7	90.9%

The designed fiber gap in Table 1 refers to the gap distance between two fibers programmed for XYZ-precision stage in code. As observed from Table 1, fiber gap measured is generally smaller than the designed fiber gap. This is probably because the *Taylor cone* at the nozzle tip also has a length of several micrometers. The pattern printed could be slightly different from the designed pattern due to the offset distance at nozzle tip.

Figure 6(a) shows the relationship between porosity and designed fiber gap at different concentration. The results agree with the assumption made at the beginning that both fiber gap and layer thickness would have an effect on the porosity of the scaffold. At small fiber gap of 200 μm , 70wt% solution has been found to give the highest porosity, because its layer thickness is the smallest. At large pore size of 500 μm , 70wt% solution has very low porosity since the fiber is too thin to support itself over a 500 μm span, which has resulted in very small single layer thickness (d_3). Though 80wt% and 100wt% solution has a larger fiber diameter, the single layer thickness is maintained even at large pore size of 500 μm . The trend is not obvious at fiber gap of 300 μm and 400 μm , but the same principle can be applied: high porosity is achieved with a good balance of fiber thickness (d_1) and pore size (determined by both fiber gap d_2 and layer thickness d_3). The fiber gap should be maximized as long as the fibers support its own weight and maintain the inter-layer gap. From Figure 6(b), the highest porosity is 95.7% of scaffold with fiber gap of 400 μm and made of 70wt% solution. However, 400 μm exceed the optimal pore size for cell growth. The second largest porosity 94.5wt% occurred at fiber gap of 300 μm scaffold made of 70wt% solution, and the scaffold with the same fiber gap that is made of 80wt% solution has a similar porosity of 94.2%. 70wt% solution has two major advantages over 80wt% solution. Firstly, fiber diameter of 70wt% solution is generally smaller than that of 80wt% solution. Secondly, solution of high concentration requires higher electric field strength to generate fibers, but high electric field strength easily results in sparking between nozzle and substrate. 70% is preferred.

Figure 6. (a) Fiber Diameter *vs.* Designed Fiber Gap at different Concentration. (b) Porosity *vs.* Designed Fiber Gap at different Concentrations.

CONCLUSIONS

In conclusion, the capability of the self-developed E-jetting system of building high porosity, precisely patterned scaffold is demonstrated. By directly controlling the fiber direction and pattern by the E-jetting system, scaffolds can be potentially built that better recapitulate the original microstructure of cartilage tissue. After analyzing the structure properties of scaffold, the scaffold built with fiber gap of 300 μ m and 70wt% PCL solution has been found to have the porosity of 94.5% that makes it more suitable for cell seeding.

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