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Fabrication of hASCs-laden structures using extrusion-based cell printing supplemented with an electric field

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ABSTRACT

In this study, we proposed a hybrid cell-printing technique that combines a conventional extrusion-based cell-printing process with an electrohydrodynamic jet. The electric field stabilized the extruded struts of cell-embedding-hydrogel and reduced the damage to dispensed cells caused by the high wall shear stress in the dispensing nozzle. The new cell-printing process was optimized in terms of various processing parameters, applied electric field strength, nozzle movement speed, and distance between the nozzle tip and working stage. Using the optimal cell-embedding hydrogel composition (1×10^6 cells mL^{-1} in 4 wt% alginate) and cell-printing process parameters (applied voltage, 1 kV; nozzle movement speed, 12 mm s^{-1}; distance, 0.7 mm; current, 10.67 ± 1.1 nA), we achieved rapid and stable fabrication of a cell-laden structure without loss of cell viability or proliferation, the values of which were similar to those of the process without an electric field. Furthermore, by applying the same pneumatic pressure to fabricate cell-laden structures, considerably higher volume flow rate and cell viability at the same volume flow rate were achieved by the modified process compared with conventional extrusion-based cell-printing processes. To assess the feasibility of the method, the hydrogel containing human adipose stem cells (hASCs) and alginate (4 wt%) was fabricated into a cell-laden porous structure in a layer-by-layer manner. The cell-laden structure exhibited reasonable initial hASC viability (87%), which was similar to that prior to processing of the cell-embedding-hydrogel.

Statement of Significance

The extrusion-based cell-printing process has shortcomings, such as unstable flow and potential loss of cell viability. The unsteady flow can occur due to the high cell concentration, viscosity, and surface tension of bioinks. Also, cell viability post extrusion can be significantly reduced by damage of the cells due to the high wall shear stress in the extrusion nozzle. To overcome these limitations, we suggested an innovative cell-printing process that combines a conventional extrusion-based cellprinting process with an electric field. The electric field in the cell-printing process stabilized the extruded struts of bioink and dramatically reduced the damage to dispensed cells caused by the high wall shear stress in the dispensing nozzle.

1. Introduction

Tissue engineering has developed rapidly during the last decade. The technique facilitates regeneration of a variety of damaged tissues and organs. To successfully regenerate various tissues, polymeric scaffolds have been widely used. These scaffolds should generate nontoxic byproducts during degradation and have good biocompatibility and appropriate mechanical properties [1]. In addition, highly porous structures provide paths for the transport of nutrients and metabolic waste and induce vascularization *in vivo* [2]. Using scaffold-based tissue engineering, damaged tissues and organs, including bladder [3], myocardium [4], skin [5], cartilage [6,7], and bone [8], have been treated effectively. However, despite the advantages of scaffold-based tissue engineering, several issues remain, including low cell-seeding efficiency, inhomogeneous cell growth after prolonged culture, and difficulty embedding cells in the desired region of the scaffold [9,10].

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One potential solution to these problems is a cell-printing process, which offers the advantages of flexible cell retention, homogeneous distribution of cells at the required locations, and efficient delivery of growth factors [10–14]. Several cell-printing techniques are available, including extrusion [15], laser [16], microvalve [17], inkjet [18], and heterogeneous cell-laden processes [19]. For example, Koch et al. successfully fabricated a 20-layer fibroblast- and keratinocyte-laden collagen structure mimicking natural skin using a laser-based bioprinting process [20]. Also, Xu et al. demonstrated a new technique to manipulate cell-encapsulating microscale hydrogels using acoustic waves, which can be utilized in regenerative medicine and cell/drug delivery [21]. In the study by Fuh [22], L929 rat fibroblasts were successfully delivered through a nozzle (36 μm in diameter) using an inkjet technique. Kolesky et al. [19] fabricated a heterogeneous 10T1/2 fibroblast-laden structure with vasculature using a cell carrier, gelatin methacrylate, and a three-dimensional (3D) co-printing method [19]. The results suggested a new approach to the design of 3D cell-laden structures with a micro-engineered environment, which could be useful in drug screening, angiogenesis, and stem cell studies [19].

Of these cell-printing techniques, the extrusion method is considered the simplest process for fabricating 3D scaffolds in which cells can be encapsulated stably. Recently, our research group has proposed several extrusion methods supplemented with aerosol cross-linking [23], a low-temperature processing working stage [24], and a core-shell nozzle [25], to obtain precisely designed macroscale cell-laden structures with micropores and reasonable cell viability.

However, the drawbacks of the extrusion-based cell printing method are unstable flow and potential loss of cell viability. Unsteady flow from a pressurized microscale nozzle can occur due to the high cell concentration in the cell-embedding-hydrogel and its viscosity and surface tension (*i.e.*, Rayleigh–Plateau instability) [26–28]. Also, cell viability can be reduced by damage of the cells [20], the plasma membrane [29], and protein structure [30], due to the wall shear stress in the extrusion nozzle [31] and the prolonged period required to fabricate a highly porous complex structure [32].

Here, to overcome these limitations, we combined extrusion-based cell printing with an electrohydrodynamic jet (EJ) process. The EJ method, originally used in commercial jet printing [33], has been applied to produce liquid alginate beads containing cells [34,35]. L. Gasperini et al. fabricated hollow cylindrical structure containing 3T3 mouse fibroblast cells using an EJ method [34]. However, the proposed EJ technique has several limitations for fabricating precisely designed cell-laden pore structures with high cell viability to replace complex 3D anatomic defects, because the process requires a high electric field strength (12–20 kV) and showed little capability with regard to 3D shape fabrication [34]. Also, cell-electrospinning has been used to obtain a fibrous cell-laden structure [36]. However, the system to obtain the fibrous structure should be required in high electric field strength over 7 kV. Furthermore, the electrospun cell-laden hydrogel fibers can be extremely difficult to fabricate three-dimensionally designed porous structure because the electrospun fibers can be easily merged in each other and the fibers were deposited with the random two-dimensional structure.

In this work, to obviate the need for high electric field strength and overcome the low shapeability, we combined the EJ technique and an extrusion-based cell-printing method. The cell-laden solution was extruded using pressurized air; to reduce the shear stress in the nozzle wall and obtain stable flow of the cell-laden hydrogel, we used an electrostatic force directed to a grounded working stage, similar to the EJ process. The electric field strength applied during the process was 1–3 kV. To prevent high current flow between the nozzle tip and grounded stage, we attached a glass substrate to the working stage. The optimum electric field strength for fabrication of homogeneous cell-laden struts that resulted in high initial cell viability was first determined. After fabrication, cells were cultured in the porous structure for various periods, and the effect of the electric field on their activities was investigated. Based on the work, we can confirm that the pressure-assisted cell dispensing supplemented with an electric field can enable to fabricate much more stable cell-laden struts due to the electrostatic forces between the nozzle and grounded working plate and also provide significantly higher cell viability of the printed struts due to the lowered wall shear stress within the microsize-nozzle compared to the normal pressure-assisted cell dispending method.

2. Experimental section

2.1. Preparation of cell-laden hydrogel

Alginate hydrogels were prepared by the following method, described in our previous work [37]. A mixture of alginate (FMC BioPolymer, Drammen, Norway) and phosphate-buffered saline (PBS) was prepared to obtain a 4 wt% alginate solution. To increase the viscosity of the solution, 0.5 wt% $CaCl_2$ (Sigma–Aldrich, St. Louis, MO, USA) was added at an alginate:$CaCl_2$ ratio of 7:3. Osteoblast-like cells (MG63, CRL-1427; ATCC, Manassas, VA, USA) and human adipose stem cells (hASCs, Anterogen Corporation, South Korea) were added to the solution to a density of 1×10^6 mL^{-1} using a three-way stopcock.

2.2. Single-line test of cell-laden hydrogel

To perform a single-line test of cell-laden struts under various electric field conditions, we used a computer-controlled three-axis robot system (DTR2-2210T, Dongbu Robot, Bucheon, South Korea) equipped with a dispensing system (Fig. 1(a, b)). A glass substrate (thickness: 1 mm; microscope glass; Marienfeld, Germany) was used to block the current flow from the nozzle tip (nozzle diameter: 150 μm) to the working stage. A power supply (HVDC, SHV300RD-50K; ConverTech, South Korea) was used to apply the electric field. Because application of high pneumatic pressure to the cell-embedding-hydrogel within the nozzle may reduce cell viability, we applied a moderate pneumatic pressure, 70 ± 10 kPa. The nozzle movement speeds were 6 and 12 mm s^{-1}. The cell-laden alginate solution was loaded into a syringe barrel. An aerosol process using a $CaCl_2$ solution (aerosol flow rate of $CaCl_2$ (5% (w/v) in TBS (Tris-buffered saline), 1.45 ± 0.2 mL min^{-1}) was used to tentatively crosslink the surface of the extruded struts [38] for stable cylindrical strut fabrication (Fig. 1(c)). After fabrication of a single layer, the cell-laden struts were further cross-linked with $CaCl_2$ solution (2% (w/v)) for 2 min (Fig. 1(d)) and rinsed in pure TBS to remove residual calcium ions (Fig. 1(e)).

2.3. Characterization of dispensed cell-laden struts

The dispensed struts were visualized by optical microscopy (BX FM-32; Olympus, Tokyo, Japan) and scanning electron microscopy (SEM, SNE-3000M; SEC Inc., Suwon, South Korea).

2.4. Culture of cell-laden alginate structures

The four-layered cell-laden structures (16 mm × 16 mm × 1.8 mm) were cultured and maintained in minimal essential medium (MEM; Life Science, St. Petersburg, FL, USA) containing ascorbic acid (50 μg/mL), 10-mM β-glycerophosphate, 10% fetal bovine

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