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Cell-encapsulating alginate microsized beads using an air-assisted atomization process to obtain a cell-laden hybrid scaffold

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Abstract

The air-assisted atomization process is a method for straightforwardly spraying liquid to obtain microsized capsules. In this study, we used the atomization process to obtain homogeneous cell-laden alginate-based microbeads for tissue regeneration. To achieve appropriate processing conditions, various weight fractions of alginate, distances between the spraying nozzle and collector, and applied air pressures were applied. Through the selected conditions (3 wt% of alginate, 7 cm distance between the nozzle and collector, and 90 kPa air pressure), we prepared homogeneous microsized cell-laden beads (average diameter = 15.5 ± 6.1 μm) with reasonable cell viability (> 90%). The feasibility of the sprayed microbeads was evaluated in terms of *in vitro* cellular activities, cell proliferation, and the morphology of the proliferating cells. In particular, the cell-sprayed micro-beads showed 1.74-fold greater cell proliferation rate compared to the macro-sized cell-laden beads. Furthermore, the sprayed cell-laden alginate beads, prepared with the selected processing conditions, were combined with melt-plotted polycaprolactone struts to obtain a new hybrid scaffold. The cells in the hybrid scaffold were viable and proliferated well over several culture periods.

Introduction

Biomedical scaffolds used for tissue regenerative material have evolved rapidly because recent computer-aided manufacturing methods have been applied to obtain various natural and synthetic biomaterials containing manipulated micro-internal pore structures.[1,2] Although these fabrication methods have provided many advantages, including precise micro-internal pore architecture and controllable mechanical properties of the scaffolds, nonhomogeneous cell distribution and growth within the scaffolds after long culture periods have been identified as shortcomings of the scaffolds.[3,4] For example, for cells seeded on top of rapid-prototyped thick scaffolds, the cell seeding efficiency was low and even the cells attached on the scaffold may reside in the bottom layer.[5] Additionally, with an electrospun fibrous mat as a scaffold, the cells can easily attach on top of the mat, but low migration into the mat has been reported due to insufficient pore size generated by the nanoscale fibers.[6] In efforts to overcome these deficiencies of conventional scaffolds, cell-laden or cell-encapsulated hydrogels fabricated using cell printing[7-10] or cell electrospinning[11-14] have been used as a cell-releasing reservoir, which is inter-located between the pre-designed synthetic scaffolds, within the scaffolds. Combinatorial methods include the following: a cell-printing/melt-plotting method to fabricate interdigitated cell-laden hydrogel struts between micro-sized poly(ε-caprolactone) (PCL) struts,[15] cell injecting into a thermoplastic mesh-mold,[16] and a cell-electrospinning/melt-plotting method to obtain hybrid scaffolds consisting of cell-laden fibers and melt-dispensed perpendicular PCL struts.[17] Regarding the homogeneous cell distribution using cell-laden hydrogels and pre-defined pore architecture/mechanical support of the synthetic materials, these combinatorial techniques use advanced manufacturing tools to prepare efficient biomedical scaffolds.

Here, we suggest another new strategy for a combinatorial technique to obtain homogeneous cell distribution/growth within synthetic biomedical scaffolds using an air-assisted cell-

spraying method onto pre-designed synthetic microsized struts. To achieve this goal, appropriate processing conditions for the air-assisted spraying technique should be selected. The air-assisted atomization process (AAAP) uses an aerodynamic force to overcome the combined influences of viscosity and surface tension of the polymeric solution.[18] Various studies on the disintegration processes of various solution jets under high-speed gas-streaming conditions have been reported.[19]

Recently, AAAP was used to directly prepare cell-encapsulating beads consisting of living cells and medical-grade polydimethylsiloxane (PDMS).[20] Achieving a significantly uniform cell distribution and reasonable cell viability in microsized beads could provide an innovative cell-laden material for tissue engineering.

To obtain cell-laden microbeads, bio-electrospraying technique has been used, and the method is the exposure of an electric field between the charged nozzle (needle) and the grounded electrode to draw a solution jet which generates cell-laden fibers or droplets. In the method, the cells are directly exposed to an electric field which negatively influences the viability of the cells.[11,12] As another method, a microfluidic device is used to fabricate cell encapsulated microsized hydrogel particles by manipulating emulsion droplets inside microchannels. Besides its clear advantages, microfluidic method requires too much solution to fill up the microfluidic device and due to the dead volume of the solution in the device and the pump, some samples may be lost.[21, 22] In addition, the method requires intricate fabricating protocols for designing valves and difficult manipulation of the laden-cells in the microfluidic domain.[23]

To overcome the limitations, here, we proposed air-pressure to generate cell-laden microbeads and this technique was combined with 3D printing method to obtain 3D hybrid structure. Unlike electrospraying method, we achieved a cell viability of >92% by simply controlling the process conditions of air-assisted cell-spraying method. This result can be

because of the use of air instead of an electric field. Furthermore, compared to microfluidic method, the cell-spraying technique provides a short process time and a more efficient combination with the 3D printing for the fabrication of hybrid scaffolds.

In this study, to achieve homogeneous microsized beads and reasonable cell viability of the sprayed alginate-based cell-laden microcapsules, various concentrations (1, 2, and 3 wt%) of alginate solution, as the cell-delivering vehicle, and the two major parameters of AAAP processing conditions, the distance (3, 5, 7, and 10 cm) between the nozzle and collector and various applied air pressures (0-350 kPa), were investigated. Through various biological characterizations including cell viability, using a live/dead assay, and cell proliferation, determined using the MTT assay, the microcapsulated osteoblast-like-cells (MG63) fabricated using the selected processing conditions were viable and proliferated significantly, as compared with a control in which air pressure was not applied. Furthermore, to show the feasibility for fabricating a hybrid cell-laden scaffold, the cell-laden alginate beads were sprayed onto the surface of a porous microsized PCL mesh structure in a layer-by-layer manner, and the hybrid structure was cultured for several periods.

Experimental

Preparation of cell-laden alginate solutions

High-G-content LF10/60 alginate (FMC BioPolymer, Drammen, Norway) was mixed with phosphate buffered saline (PBS) to obtain three alginate solutions (1, 2, and 3 wt%). The alginate solutions were cross-linked with 0.5 wt% $CaCl_2$ solution (Sigma-Aldrich, USA) to increase their viscosities. The mixture ratio of the alginate and the calcium chloride solution was 7 : 3. After slight cross-linking the solutions, the osteoblast-like-cells (MG-63, CRL-1427; ATCC, Manassas, VA, USA) and human adipose-derived stem cells (hASCs, Anterogen corporation, South Korea) were added to the solutions using a three-way stopcock

at a density of (1.0 to 1.6) × 10^6 mL^{-1}.

Production of cell-laden microbeads

Cell-laden alginate solutions were injected into a syringe pump and the syringe was connected to a core-shell nozzle (inner diameter of the core nozzle = 250 μm; diameter of the shell nozzle = 800 μm). In the core region, the alginate solution (flow rate: 0.5 mL min^{-1}) was set; for the shell region, compressed air was injected at various pressures. Next, the alginate solutions were sprayed onto a poly(ethylene terephthalate) film. The pressurized air, which plays a major role in the atomization process, can force the alginate solution into microsized droplets through the orifice of the core/shell nozzle. During the spraying process, an aerosol of 10 wt% of $CaCl_2$ solution (flow rate: 25 ± 2.5 μL min^{-1}), obtained with a humidifier (Tess-7400; Paju, South Korea), was used simultaneously to tentatively cross-link the sprayed particles during the process, resulting in the formation of cell-laden microsized beads. A detailed scheme of the atomization process is shown in Figure 1. The size and distribution of the sprayed microbeads were visualized and measured by optical microscopy (BX FM-32; Olympus, Tokyo, Japan).

Fabrication of a cell-laden hybrid scaffold

To obtain a hybrid scaffold consisting of cell-laden alginate microsized beads and a microsized synthetic mesh structure, we used poly(ε-caprolactone) (PCL, M_n = 70,000-90,000 Da, T_m = 60°C, Sigma-Aldrich, USA). Using a three-axis robot system (DTR3-2210-T-SG; DASA Robot, South Korea) connected to the melt-dispensing system, we can achieve a mesh structure of PCL in a layer-by-layer manner to obtain a three-dimensional (3D) structure having a homogenous rectangular pore size. The detailed processing conditions were as follows: temperature = 100°C, applied pneumatic pressure = 260 ± 5 kPa, nozzle

moving velocity = 10 mm s^{-1}. The cell-laden hybrid structure was obtained in the next procedure. First, the melted PCL was drawn by the controlled process and the cell-laden alginate beads were sprayed on top of the perpendicular PCL struts. The spraying time was set at 10 s. The procedure was repeated several times until the designed 3D structure was obtained. After fabricating the 3D structure, the final hybrid architecture was completely cross-linked through a second cross-linking process (2 wt% $CaCl_2$ for 1 min). Thus, the hybrid structure consisted of PCL strut layers and cell-laden alginate beads on the PCL struts.

In vitro *cell culture*

After fabricating sprayed cell-laden micro-sized beads, those were cultured and maintained in minimal essential medium (MEM; Life Science, St. Petersburg, FL) containing 10% fetal bovine serum (Gemini Bio- Products, Sacramento, CA) and 1% antibody (Antimycotic; Cellgro, Manassas, VA). The samples were incubated in an atmosphere of 5% CO_2 at 37 ^oC and the medium was changed every second day.

Initial live and dead cells

After spraying 4 h, to obtain images of live/dead cells, the cell-laden beads were exposed to 0.15 mM calcein AM and 2 mM ethidium homodimer-1 for 45 min in an incubator. Stained cells were analyzed by fluorescence microscopy. Stained images were captured in which green color indicated live cells and red color indicated dead cells. The ratio of the number of live cells to the total number of cells (including live and dead cells) was calculated using ImageJ software, and the value was normalized to the initial cell viability (the value before cell-alginate extrusion). The initial cell viability was determined using Trypan blue (Mediatech, Herndon, VA).

MTT assay and DAPI/Phalloidin

We used the MTT assay to analyze the cell proliferation (Cell Proliferation Kit I; Boehringer Mannheim, Mannheim, Germany) for 1, 3, and 7 day cell-culture periods. This assay is based on the cleavage of the yellow tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) by mitochondrial dehydrogenases in viable cells to produce purple formazan crystals. Cells on the surface were incubated with 0.5 mg mL^{-1} MTT for 4 h at 37 ^{o}C. Absorbance was measured at 570 nm using a microplate reader (EL800; Bio-Tek Instruments, Winooski, VT, USA). Five samples from each incubation period were tested, and each test was performed in triplicate.

After 3 and 7 days of cell culture, the microbeads and cell-laden hybrid scaffold were analyzed via diamidino-2-phenylindole (DAPI) staining. Fluorescence staining was performed to characterize the nuclei of the cells in the specimen (Invitrogen, Carlsbad, CA). Phalloidin was used to visualize the actin cytoskeletons of cells in the specimen by fluorescence microscopy (CKX41; Olympus).

Statistical analysis

All data are presented as means ± SD. Statistical analyses were performed using the SPSS software (SPSS, Chicago, IL). Statistical analyses consisted of single-factor analyses of variance (ANOVAs). In all analyses, $p < 0.05$ was taken to indicate statistical significance.

Results and Discussion

Effects of alginate weight fraction

The air-assisted jet process involved a three-axis robot to move the spraying tool, an air-spraying system connected with the core-shell nozzle, and various air filters (Fig. 1). In the core region, the cell-laden alginate was injected at a flow rate of 0.5 mL min^{-1}, and in the

shell region, various air pressures were applied to fabricate microsized cell-laden beads.

To achieve proper conditions for the air-spraying process, we observed the effects of weight fractions (1, 2, and 3 wt%) of alginate solution on the formation of microsized cell-laden beads and cell viability of the sprayed beads. During the process, the applied air pressure in the shell nozzle was fixed at 90 kPa, the distance between the nozzle tip and collector was 7 cm, and spraying time was set at 10 s. To efficiently form sprayed cell-laden alginate beads, aerosols of $CaCl_2$ solution (10 wt% of $CaCl_2$ and flow rate = 25 μL/min) were spread over the collecting stage during the process. The aerosol of the $CaCl_2$ solution tentatively cross-links the spraying alginate beads.[10] Figure 2(a) shows optical images of cell-laden alginate beads air-sprayed with and without the aerosol cross-linking process using the $CaCl_2$ solution. In the test, the weight fraction of alginate was set at 3 wt%. As shown in the optical images, the formation of cell-encapsulated alginate beads was successfully achieved with the process using the aerosol of $CaCl_2$ solution versus the process without it. Based on this, we adopted the aerosol tentative cross-linking process in the microsized cell-laden bead formation.

Figure 2(b, c) shows the optical and fluorescence images of cell-sprayed beads for three different weight fractions of alginate, which were mixed with osteoblast-like MG63 cells and hASCs at a density of $1.0\text{-}1.6\times10^5$ cells mL^{-1}. As shown in the optical images of MG63-sprayed cell-beads, as the weight fraction of alginate increased, the average diameter of the sprayed beads increased (Fig. 2(d)). For 1, 2, and 3 wt% alginate, the average diameters of the cell-laden beads were 9.2 ± 5.6 μm, 10.6 ± 4.9 μm, and 15.5 ± 6.1 μm, respectively. This was because the alginate solution with higher viscoelastic properties can be stretched more by the air flow in the shell region of the nozzle tip, and when the stretched threads break up, larger droplets were obtained due to the recoiling phenomenon of the stretched threads.[24] Thus, a solution having higher normal stress can cause larger bead sizes. However, although the average diameter was smaller in the low weight fraction (1 wt%) of alginate, it did not

provide uniformly dispersed cell-laden beads, whereas for the 2 wt% and 3 wt% alginate, the beads were homogeneously distributed. This may be because the cells in the low weight fraction of alginate can aggregate after the cell-spraying process due to the low viscosity and low cross-linking concentration of the alginate, resulting in aggregated cell beads. In addition, when comparing the cell beads fabricated using 2 wt% and 3 wt% of alginate, the beads using 2 wt% alginate did not fully encapsulate the cells due to the low viscosity of the cell-encapsulating matrix compared with those with 3 wt% of alginate, although the fabricated beads were stably formed.

Cell viability of the air-sprayed cell beads was examined using fluorescence imaging 4 h after the spraying process. Figure 2(e) shows the cell viability for the sprayed cell-laden microbeads (MG63 and hASCs). The viability of both cells encapsulated in the three different alginate concentrations for all sprayed cell beads was above 92%.

Effects of distance

To assess the effect of spraying distance between the spraying nozzle and collector on bead formation and cell viability, we used four distances (3, 5, 7, and 10 cm) under conditions of 3 wt% of alginate, 90 kPa of air pressure in the shell region, and spraying time = 10 s. Figure 3(a,b) shows fluorescence and optical images describing alginate bead formation and live/dead cells for various spraying distances. While cells were sprayed on the collector, in terms of bead formation, the distances of 3 and 5 cm were too short to form spherical beads because the scattering window was too narrow, resulting in convergence of the sprayed beads (aggregated beads). However, at a distance greater than 5 cm, the beads were well-developed. Using the sprayed beads, we measured cell viability for the four different distances (Fig. 3(c)). Except for 10 cm, cell viability was over 90%. The low cell viability for the 10 cm distance may be because the cells sprayed by the air pressure in the shell nozzle collided more

severely with the collector, thus damaging the cells, although the alginate does shield the encapsulated cells somewhat.

Effects of air pressure

The mixture of 3 wt% alginate and osteoblast-like MG63 cells at a density of 1.0-1.6×10^5 cells mL^{-1} in the core region was successfully sprayed at a distance of 7 cm at various air pressures (0, 50, 90, 150, 250, and 350 kPa) in the shell nozzle. Figure 4(a) shows live/dead images of the sprayed microsized beads obtained with various air pressures in the shell region. As shown, when air was not applied in the shell nozzle, the bead size was approximately 500-650 μm and cell viability at 4 h was 91.5±2.3%. As the pressure (P) in the shell region was increased to 350 kPa, the microsized beads were well-developed and the size of the beads decreased because of the shear and normal stress due to air pressure. The air pressure effect for droplet size was described in reference.[25] Additionally, with regards to the 50 kPa air pressure, the encapsulated cells were grouped in big macro-beads (diameter = 100-120 μm), while at pressures above 90 kPa, one cell per one bead was achieved. In terms of cell viability, the viability of the fabricated beads (0, 50, 90, and 150 kPa) was above 90% and the values were similar to that with no applied pressure in the shell nozzle (Fig. 4(b)). However, for 250 and 350 kPa, cell viability was reduced significantly to approximately 72% and 54%, respectively. This was because the high pressure in the shell nozzle can cause too high shear and normal stresses, damaging the sprayed cells and causing a stronger collision on the target plate, although the alginate hydrogel shields the embedded cells to some degree.

The density of the sprayed cells on the collector can be an important parameter too, because a wide range of scattered cell-laden beads can cause cell loss. Figure 4(c) shows the cell-sprayed density (%) for the applied air pressures. The cell density (cell number/mm^2) was measured and normalized to the value of the cell-laden beads fabricated with no air pressure.

As expected, the cell density was reduced significantly with increased applied pressure. With respect to air pressure above 150 kPa, the relative cell density was below 20% versus that with no applied air pressure.

In vitro *cell culture*

To examine cell proliferation of the cell-laden beads, we used the following processing conditions: spraying distance = 7 cm, 3 wt% of alginate mixed with the cells, and spraying time = 10 s with various pressures (0, 50, 90, and 150 kPa). Because too low initial viability of the cell-laden beads was observed at a pressure greater than 150 kPa, we selected the range below 150 kPa. Figure 5(a) shows the proliferation of viable cells determined using the MTT assay. The optical density (OD) in the control (cell-laden beads fabricated with no pressure) was significantly higher than that of the other sprayed cells for 1 day. This was due to the different cell densities (cell number/mm^2). However, with increasing culture periods, the cells sprayed with 90 kPa air pressure showed the highest proliferation. To observe the increasing rate of viable cell number, we used the slope of the OD and the time period. The increasing rate of the cells is displayed in Figure 5(b); the cells sprayed with 90 kPa air pressure showed the highest proliferation rate. We believe this was because of the efficiently thin thickness of the cell-encapsulating alginate. For the macrosized cell-laden beads fabricated with no pressure, the cell density was highest but the embedded cells did not fully proliferate because of the strongly bonded alginate matrix. However, the homogeneously microsized cell-laden beads at pressures of 90 and 150 kPa were covered with a thin alginate layer, so that the cells proliferated more readily due to the rapid degradation of the thin alginate layer, although the density of spraying cells was relatively low. Also, the differences in cell proliferation between the cell-laden beads fabricated at pressures of 90 and 150 kPa may be due to the different cell-spraying densities.

Figure 5(c) shows live/dead cells of the sprayed cell-laden microsized beads at 3 days. As seen in the images, most cells laden in alginate beads sprayed with an air pressure of 90 kPa and with no pressure (control) were alive. These results indicate that the spraying process is safe, both with and without air pressure. It is known that the stained cytoskeleton shows the morphology of proliferated cells. The sprayed MG63 cells were cultured for 3 days and subsequently stained with DAPI/phalloidin (Fig. 5(d)). As shown in the images, in the cell-laden alginate sprayed with 90 kPa, the cells showed polygonal spindle-like structures and were distributed homogeneously, while the control (no air spraying)shows that cells embedded in the alginate did not proliferate due to the thick encapsulation layer of alginate.

Fabrication and in vitro *cell culture of the cell-laden hybrid scaffold*

From previous results, we determined optimal settings for the cell-spraying process: 3wt% of alginate, a distance of 7 cm, and an air pressure of 90 kPa. Using these selected conditions, we aimed to fabricate a cell-laden hybrid scaffold. First, a PCL mesh structure (strut diameter = 432 ± 47 μm and pore size = 554 ± 32 μm) was plotted with the processing conditions described in Table 1. After plotting the PCL structure, the cell-laden alginate beads were sprayed onto the PCL mesh structure in a layer-by-layer manner. The fabricating procedure is described in Figure 6(a). Figure 6(b) shows optical images of the cell-sprayed structure. Figure 6(c) demonstrates the live/dead cells on the surface and cross-section of the PCL struts of the cell-sprayed structure after 7 days. The cells in the mesh structure were alive, and viability was around 90%. To examine the cellular activities of the sprayed cells, fluorescence images (DAPI (blue)/phalloidin (red)) of the surface of the PCL strut after 7 days of culture were obtained (Fig. 6(d)). The cells on the surface of the PCL mesh structure proliferated well, demonstrating that the sprayed cells survived and underwent cytoskeleton reorganization. Based on these results for the hybrid scaffold, we conclude that

the air-spraying process using cell-laden alginate may be a useful tool to fabricate various hybrid scaffolds.

Conclusions

Here, cell-laden alginate beads fabricated using an aerodynamic spraying process were successfully prepared. By manipulating various processing parameters, such as the weight fraction of alginate, the distance between the spraying nozzle and collector, and the air pressure applied in the shell nozzle, optimum processing parameters were determined to prepare homogeneous microsized cell-laden alginate beads with reasonable cell viability. To evaluate their feasibility as a tissue regenerative material, *in vitro* cellular activities, cell proliferation, and morphology of proliferated cells for the cell-laden microbeads were examined. The optimally sprayed cell-laden alginate beads were viable during several culture periods and proliferation was significantly higher than the control (no air pressure). By combining the cell-laden alginate beads and melt-plotted PCL struts, we successfully fabricated a new cell-laden hybrid scaffold. The hybrid scaffold was cultured for several periods and the cell-laden alginate beads attached on the PCL struts were viable and proliferated well. Based on these results, the cell-laden alginate beads manufactured using the air-spraying process may serve as a supplementary tool to obtain various cell-laden hybrid scaffolds for tissue engineering applications.

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Table 1. Processing parameters of melt-plotted PCL and cell-laden alginate beads for the hybrid scaffold.

Cell-laden hybrid scaffold	PCL plotting	Cell-alginate spraying
Nozzle moving velocity (mm s^{-1})	10	1.75
Processing temperature (℃)	100	36.7
Pneumatic pressure (kPa)	260 ± 5	90 ± 1
Core nozzle size (μm)	ID - 350	ID - 250, OD - 510
Shell nozzle size (μm)		ID - 800, OD - 1020
Strut size (μm)	432 ± 47	-
Pore size (μm)	554 ± 32	-
Flow rate of calcium chloride (μl min^{-1})	-	25
Laden cells (cells ml^{-1})	-	1.0 x 10^6

Figures

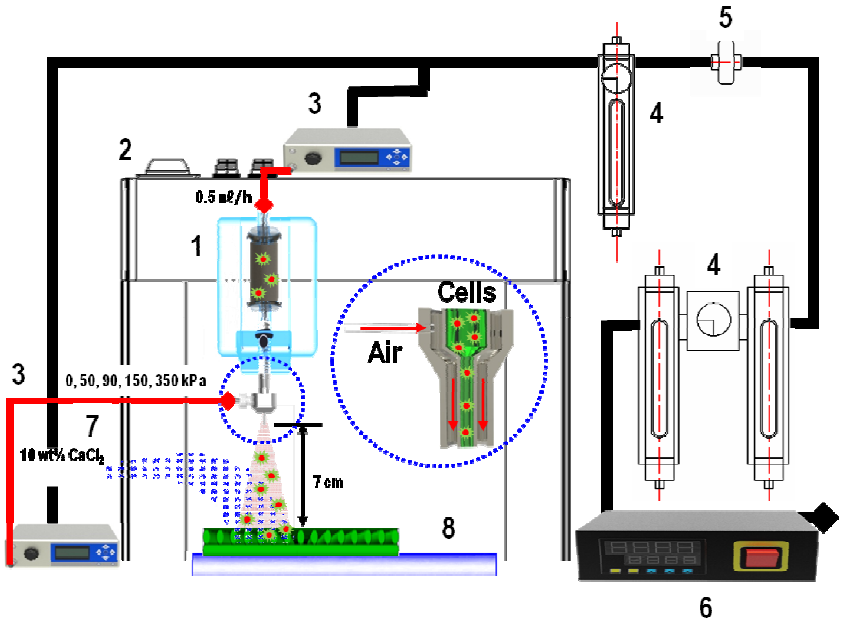


Figure 1. Scheme of the cell-spraying device: (1) a cell-laden alginate spraying system using a core-shell nozzle, (2) three-axis robot system, (3) a controller system for dispensing air, (4, 5, 6) filtering systems for air, (7) an aerosol system for $CaCl_2$solution, and (8) a working stage.

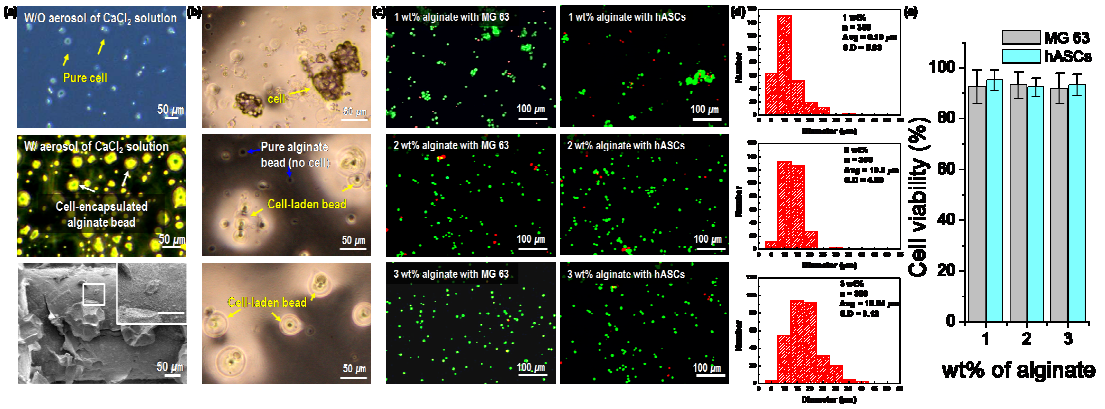


Figure 2. (a) Optical and SEM images of aerodynamically sprayed cell-laden alginate beads with and without an aerosol of $CaCl_2$ solution during the process. (b) Optical and (c) fluorescence (live = green, dead = red) images of cell-laden alginate beads sprayed with three different alginate concentrations (1, 2, 3 wt%). (d) Diameter distribution of cell-laden alginate beads. (e) Cell viability of the cell-laden beads with three different alginate concentrations.

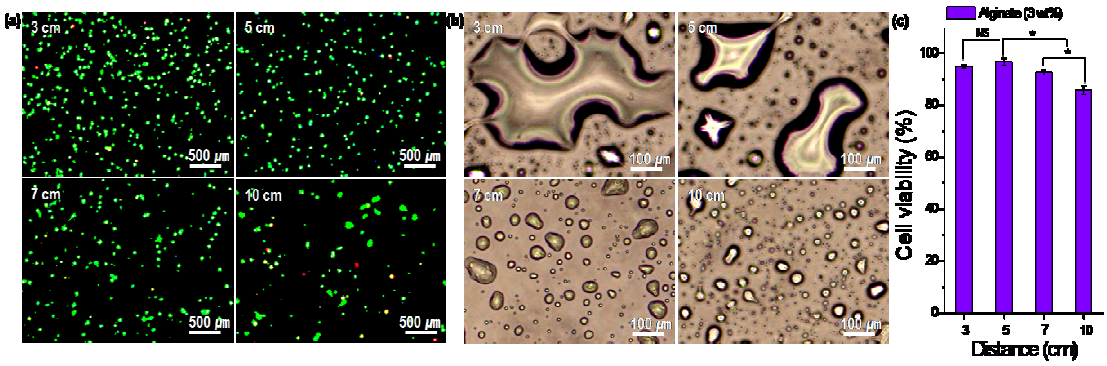


Figure 3. (a) Fluorescence (live = green, dead = red) images and (b) optical images of cell-laden alginate beads sprayed at four different distances (3 cm, 5 cm, 7 cm, and 10 cm) between the spraying nozzle and collector. (c) Cell viability of cell-laden beads fabricated at the various distances.

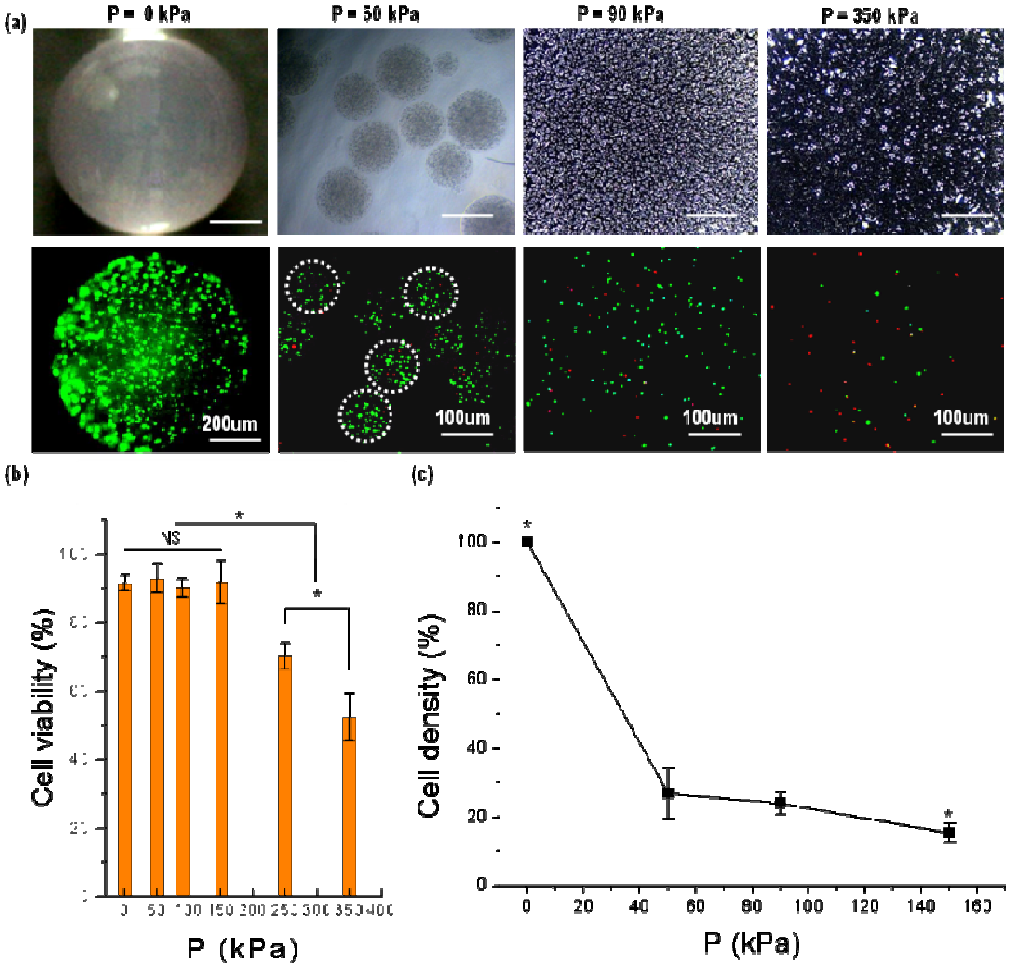


Figure 4. (a) Optical and fluorescence (live = green, dead = red) images of cell-laden alginate beads sprayed with various air pressures (0, 50, 90, and 350 kPa) in the shell nozzle. (b) Cell viability and (c) the relative density (%) of sprayed cell-laden alginate beads per the same collecting area.

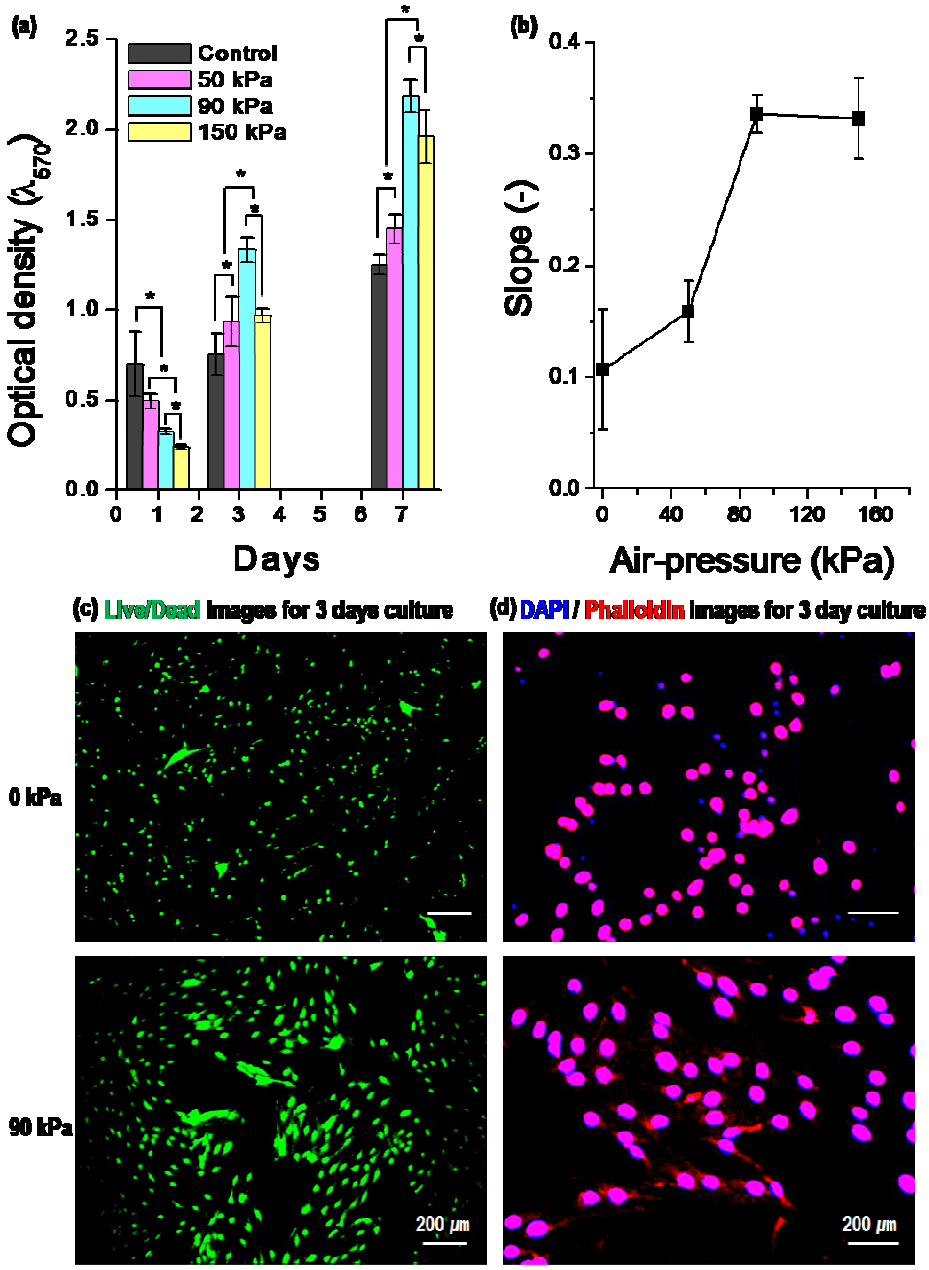


Figure 5. (a) Results of the MTT assay for the cell-laden alginate beads fabricated with several air pressures (0 [control], 50, 90, and 150 kPa) and (b) the slope of the MTT result describing the rate of cell proliferation. (c) Live/dead and (d) DAPI/phalloidin images of the cell-laden beads fabricated using 0 pressure (control) and 90 kPa.

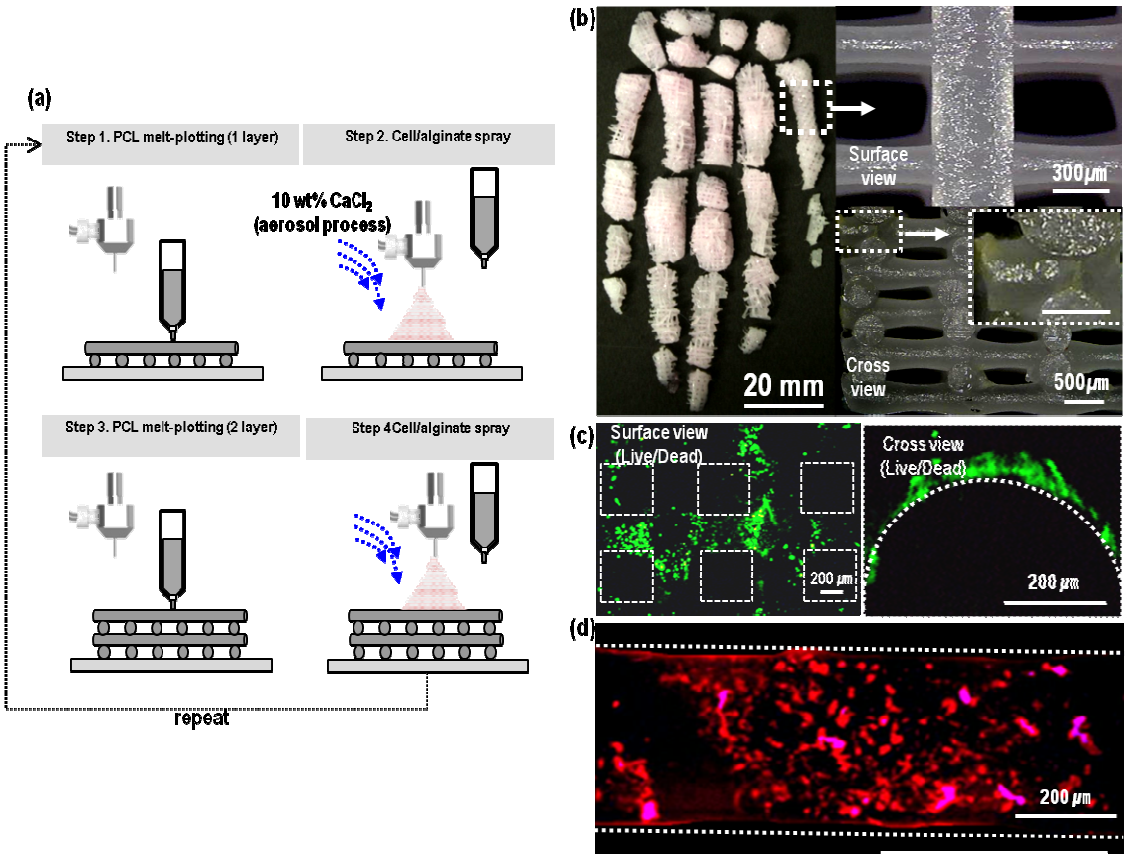
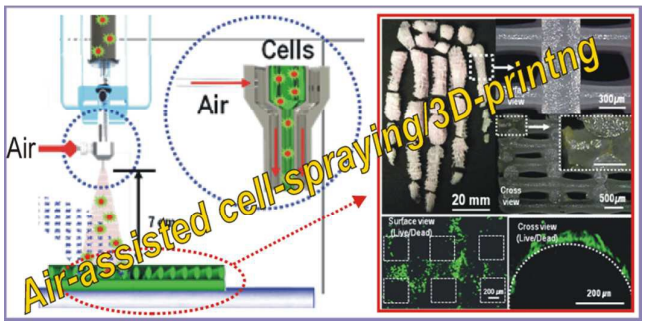


Figure 6. (a) Schematic description of hybrid scaffold fabrication. (b) An optical image showing the cell-laden hybrid scaffold. (c,d) Live and DAPI/phalloidin images of the hybrid scaffold after a 7-day culture period.

Graphical abstract



Here, the atomization process to obtain homogeneous cell-laden microbeads was proposed, and they were sprayed simultaneously onto the surface of a PCL mesh structure in a layer-by-layer manner to obtain the cell-laden hybrid structure.