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One dimensional self-assembly of mouse embryonic stem cells using an array of hydrogel microstrands

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Abstract

The ability of embryonic stem (ES) cells to self-renew indefinitely and to differentiate into multiple cell lineages hold promise for advances in modeling disease progression, screening drugs and treating diseases. To realize these potentials, it is imperative to study self-assembly in an embryonic microenvironment, as this may increase our understanding of ES cell maintenance and differentiation. In this study, we synthesized an array of one-dimensional alginate gel microstrands and aqueous microstrands through an SU-8 filter device by means of capillary action. Furthermore, we investigated self-assembly behaviors and differentiation potentials of mouse ES cells cultured in microstrands of varying diameters. We found that microstrands with an aqueous interior facilitated high density cell culture and formed compact microtissue structures, while microstrands with gelled interiors promote smaller cell-aggregate structures. In particular, we noticed that ES cells collected from one dimensional aqueous microstrands favored the differentiation towards cell lineages of endoderm and mesoderm, whereas those from gelled microstrands preferred to differentiate into ectoderm and mesoderm lineages. In addition to providing a “liquid-like” tubular microenvironment to understand one dimensional self-assembly process of ES cells, this alginate hydrogel microstrand system also offers an alternative way to manipulate the stem cell fate-decision using bioengineered microenvironments.

Keywords

Stem cell; Alginate; Hydrogel; Self-assembly; Microfiber; Microfluidics

1. Introduction

Embryonic stem (ES) cells hold promise in combating various diseases and have the potential to be exploited in drug discovery and diagnostics due to their capacity to self-renew indefinitely and their ability to differentiate into multiple cell types [1-3]. Living cells organize into functional units through self-assembly [4-8], and understanding cellular self-assembly is of paramount importance for engineering various tissue constructs and understanding life [4, 5]. Although self-assembly at the molecular level has been intensively investigated, the study of self-assembly at the cellular level is somewhat lacking, partly due

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to its complexity [9]. Better understanding of ES cell self-assembly in an embryonic microenvironment will provide insights in tissue morphogenesis/organogenesis and offer strategies for effective expansion and differentiation of ES cells, leading to advanced cell therapy, tissue regeneration, and disease modeling.

According to the concept of semi-solid “soft matter” (the living system possessing complexity and flexibility) [6, 10], and “tissue fluidity” (monodispersed cells reconstructing tissues through morphogenetic movement, differential adhesiveness and cell aggregation) [11-16], embryonic tissues can be considered as liquids [7]. Therefore, it is highly desirable to develop a soft and “liquid-like” system platform to mimic the embryonic microenvironment to study the self-assembly behavior of ES cells.

One commonly used approach to assemble mouse ES cells into embryoid bodies under gravity-driven self-assembly is the “hanging drop” technique, which has been used for self-assembly of neuronal microtissue and other structures as well [17, 18]. However, this technique is tedious, and not suitable for high-throughput and long-term culture. Microfabrication techniques in combination with hydrogels has been used to assemble cells into a variety of structures [19-23], such as spheroids [24], toroids [11, 25], rods [26], and honeycombs [27], with the purpose to investigate dynamic cellular self-assembly [28]. It has been shown that microtissue self-assembly is driven by cell-cell contact and intercellular adhesion, which involves connexins [8], cadherins [29], and actin cytoskeletal tensions [30]. Considering the requirement of “tissue fluidity”, alginate hydrogel has great potential to mimic the embryonic microenvironment due to its gentle gelling behavior [31], reversible cross-linking [32], tunable flexibility,[33] and non-adhesive property [34]. Alginate hydrogel can be fabricated into macrobeads (with a diameter in several mm) [35], microbeads (with a diameter < 1 mm and with a hydrogel core) [36-39], microcapsules (with a diameter < 1 mm and a liquid core enclosed within a spherical hydrogel membrane) [40, 41], microfibers (solid hydrogels) [42, 43] and microtubes (liquid core with hydrogel shell structures) [44-46]. It has been shown that alginate microbeads or microcapsules support the maintenance and/or differentiation of mouse or human ES cells [47-56]. Alginate microcapsules or microtubes with a liquid core can provide a “liquid-like” microenvironment for self-assembly of cells. Small diameter structures ($\leq 200 \mu\text{m}$) are desirable and create a microenvironment with better mass transfer of oxygen, nutrients, and metabolic wastes. In this regard, alginate hydrogels shaped into micro-tubular structures with diameters $\leq 200 \mu\text{m}$ are more readily fabricated, compared to spherical alginate microcapsules with similar diameter.

Here we present an approach to fabricate an array of alginate hydrogel microstrands (long microfibers and microtubes) through a microfabricated SU-8 filter device by means of capillary action. We name long microfibers with a homogenous gel core “alginate gel microstrands”, while long microtubes with a liquid core and an alginate/polylysine (ALG/PLL) shell are referred to as “ALG/PLL aqueous microstrands”. The diameter of these microstrands can be controlled from 30 - 300 μm , while the length is 3 cm or longer. Since the aspect ratio (length/diameter) of the microstrand is larger than 100, we consider them to be one-dimensional microstructures. When the diameter of alginate microstrands are around 30 μm , which is close to the size of ES cells, cells can line-up in the microstrand in a pearl-on-a-string fashion. We investigate the self-assembly behavior of mouse ES cells in one dimensional alginate microstrands with various diameters having either a gel or liquid core. We further examine the potential of these encapsulated ES cells to differentiate into ectodermal, mesodermal, and endodermal cell types.

2. Materials and Methods

Mouse ES cell culture

Mouse CCE ES cells were obtained from StemCell Technologies (Vancouver, Canada) [57, 58]. Mouse ES cells were cultured in gelatin coated flasks in a standard cell culture incubator at 37 °C, 5% CO₂ and maintained in an undifferentiated state in growth medium consisting of Dulbecco's Modified Eagle's Medium (DMEM with 4.5 g/l D-glucose) supplemented with 15% (v/v) fetal bovine serum (FBS), 100 U/ml penicillin, 100 µg/ml streptomycin, 0.1 mM non-essential amino acids, 10 ng/ml murine recombinant leukemia inhibitory factor (LIF; StemCell Technologies, Vancouver, Canada), 0.1 mM monothioglycerol, and 2 mM L-glutamine and 1 mM sodium pyruvate (Sigma Aldrich, St. Louis, MO).

Fabrication of SU-8 filter

The SU-8 filter was fabricated using standard photolithography techniques (Fig. 1). First, a silicon substrate was cleaned using Piranha (3:1 H₂SO₄:H₂O₂), rinsed with DI water and then dried with nitrogen. H₂SO₄ was purchased from Transene Company (Danvers, MA.) and H₂O₂ from Puritian Products (Bethlehem, PA). Then a release layer of omnicoat (Microchem, Boston, MA) was spun on the wafer at 3000 rpm using a spin coater (Brewer Science, Rolla, MO) and baked on a hot plate at 200°C for 1 minute. SU-8 50 (Microchem) was spin-coated to a final thickness of approximately 50 µm and baked at 65°C for 5 minutes, 95°C for 20 minutes, and was then cooled to room temperature. The SU-8 was then exposed to 365 nm light using an EVG 640 Contact Aligner (EV Group, Albany, NY). Two separate UV exposures were used to create the support structure and the open vertical channels. The support structure surrounding the "hole area" was exposed with a dose of 500 mJ/cm², while the area with the through-holes was exposed with a dose of 200 mJ/cm². Lastly, the substrate was baked at 65°C for 1 minute, 95°C for 10 minutes, cooled to room temperature and placed in the PGMEA developer (Microchem) overnight. The SU-8 filter was developed and released from the substrate by immersion in PGMEA. SU-8 filters were removed from the silicon substrate and then sterilized by soaking it in 70% ethanol for 20 min and then washing 3 times with sterile 1X phosphate buffered saline (PBS).

Fabrication of alginate hydrogel microfibers and alginate/PLL aqueous microstrands

Mouse ES cells were added to 1.5% alginate of 250 cps at 25 °C (Sigma Aldrich, St. Louis, MO) to make the ES cell-alginate mixture at 1×10^6 cells/ml. The SU-8 microfilter was placed floating on 100 mM of CaCl₂ solution. 2.5 µl of ES cell-alginate mixture was pipetted on top of the open microchannels/holes in the SU-8 filter and flowed immediately through the microfluidic channels by capillary action. Once the alginate and ES cell blend made contact with the CaCl₂ solution, carboxylate and hydroxyl groups in alginate coordinated to calcium ions and formed the alginate gel microstrands. To produce an aqueous environment, microstrands were soaked in 0.01% poly-L-lysine (PLL) (Molecular weight = 30,000-70,000 kDa; Sigma) for 10 minutes, removed and replaced by 1.6% sodium citrate for 5 minutes, which acts as a chelating agent to liquefy the gel microstrand interior while the PLL coating maintains the cylindrical structure. Mouse ES cells encapsulated in both types of microstrands were then cultured for 5 days in an incubator at 37°C with 5% CO₂. Maintenance medium was changed every other day, and cells morphologies were optically imaged using an inverted Nikon TS100F microscope (Micro Video Instruments, Avon, MA).

Cell viability assay

Cell viability after encapsulation in alginate/PLL microstrands was measured by a live/dead stain made of calcein AM and ethidium homodimer (EthD-1) (Sigma Aldrich, St. Louis, MO). Calcein AM was converted into a strongly green fluorescent dye in live cells via intracellular esterases. Dead cells, on the other hand, had strong red fluorescence because EthD-1 binds to nucleic acids after entering through ruptured cell membranes. The live/dead stain working solution is composed of 2 μ M calcein AM and 4 μ M EthD-1. After formation of alginate microstrands, cells were incubated in the live/dead stain working solution for 30 minutes. Live and dead cells were then observed under the inverted fluorescence Nikon TS100F microscope (Micro Video Instruments).

Recovery of mouse ES cell from alginate microfibers and microstrands

To release cells from alginate gel and ALG/PLL aqueous microstrands, the maintenance medium was replaced on day 5 with 1.6% sodium citrate and incubated at 37°C for 10 minutes and 30 minutes respectively. A higher incubation time was needed to release cells from the aqueous microstrands due to the PLL coating. Cells from each condition were centrifuged to obtain a cell pellet, and then cultivated on glass coverslips for 3 days.

Immunocytochemistry

Standard immunocytochemistry protocol was used to stain cells released from alginate hydrogel microstrands. After being kept in culture for 3 days, the cells on glass coverslips were fixed with 4% paraformaldehyde, followed by permeabilization with 0.2% Triton X-100 and blocked with 5% FBS in PBS. ES cells were subsequently incubated with three primary antibodies, including anti-nestin, anti-Myf-5, and anti-PDX-1 (Santa Cruz Biotechnology, Santa Cruz, CA), followed by the incubation with secondary antibodies (Invitrogen). Antibodies with Alexa Fluor® 594 were used for the detection of nestin, Alexa Fluor® 488 for Myf-5, and Alexa Fluor® 647 for PDX-1. Samples were observed under a Nikon epifluorescence microscope using filter cubes for FITC, Texas Red, and Alexa 647 (Micro Video Instruments).

3. Results

3.1 Synthesis of alginate gel microstrands and ALG/PLL aqueous microstrands

An array of alginate microstrands was fabricated in a straight-forward approach using our microfabricated SU-8 filter device. SU-8 is a non-cytotoxic, epoxy-based, negative photoresist that is commonly used in biological studies [59]. The 4 by 4 cm SU-8 filter, which consists of an array of vertical channels/holes, with diameters ranging from 30 to 300 μ m, was fabricated using standard photolithography techniques (Fig. 1). The SU-8 filter spotting an array of vertical microchannels is shown in Fig. 1b. SEM imaging showed the representative porous structures in the SU-8 filter and the smooth side wall of the vertical channels (Fig. 2a and b).

To create alginate hydrogel microstrands containing mouse ES cells, we first placed the SU-8 filter floating on 100 mM CaCl_2 solution in a Petri dish (Fig. 3a). Next, we pipetted a mixture of 1.5% alginate and mouse ES cells on the top of the filter to cover the microchannels. The ES cell-alginate mixture flowed through these microchannels driven by capillary action and made contact with CaCl_2 solution. Alginate gel microstrands formed simultaneously once it was in contact with CaCl_2 as the negative carboxylate and hydroxyl groups in alginate coordinated with positive calcium ions in CaCl_2 to form complexation bonds (Fig. 3b). After approximately two minutes, continuous, long alginate gel microstrands with entrapped mouse ES cells inside had formed, hanging under the SU-8

filter (Fig. 3c). The diameter of produced microfibers was approximately the same as the size of the pore on the SU-8 microfluidic filter.

To produce an aqueous, “liquid-like” microenvironment, we incubated alginate gel microstrands containing ES cells with 0.01% PLL to form a uniform ALG/PLL coating on the exterior of the microstrands, followed by dissolving the inner calcium alginate hydrogel with 55 mM sodium citrate. The ALG/PLL membrane retained the cylindrical structure of the microstrand when the inner core was liquefied by sodium citrate. Using this approach, we obtained ALG/PLL aqueous microstrands which mimic the “liquid-like” embryonic microenvironment.

To determine whether our encapsulation procedure affect cell viability, we performed live/dead staining immediately after the microstrand formation using calcein AM and EthD-1. The staining revealed that most ES cells remained alive as indicated by green calcein (Fig. 3d).

Compared to spherical microbeads and microcapsules, it is very easy to achieve high cell density in our tubular/cylindrical microstrands. For example, we successfully encapsulated mouse ES cells in alginate gel microstrands with a fiber diameter around 90 μm , at an initial density of 1×10^6 cells/ml, 1×10^7 cells/ml, and up to 1×10^8 cells/ml alginate (Fig. 4a-c), where the last value is on the same order as the cell density in tissue.

3.2 The self-assembly behavior of mouse ES cells in alginate microstrands

Next, we investigated the self-assembly behavior of mouse ES cells encapsulated in ALG/PLL aqueous microstrands with various diameters, and compared to their behavior in alginate gel microstrands. Using an SU-8 filter with arrays of microfluidic channels at 30, 60, 90, 180, and 300 μm , we first fabricated an array of alginate hydrogel microstrands by pipetting 2.5 μl ES cells in alginate solution (1×10^6 cells/ml) onto each set of microchannels floating on CaCl_2 solution. As mentioned previously, ALG/PLL aqueous microstrands were formed by coating with PLL and liquefying with sodium citrate. Mouse ES cells in both microenvironments, i.e. solid, alginate gel microstrands and liquefied, alginate/PLL aqueous microstrands, were cultured in medium in the absence of leukemia inhibitory factor (LIF) for 5 days. As can be seen in Fig. 5, mouse ES cells cultured in these various microstrands, produced dissimilar cell morphologies depending on the size of the fiber diameter. This indicates these microenvironments affect the self-assembly of ES cells. The liquefied ALG/PLL aqueous microstrand environment promoted cellular self-assembly and formed high density, compact cellular structure (Fig. 5a-d). In ALG/PLL aqueous microstrands, monodispersed ES cells could move freely and interact with each other. The morphology of the self-assembled tissue architecture depends on the diameter of the fiber. When the fiber diameter was below 100 μm , ES cells elongated along the ALG/PLL aqueous microstrands and formed a long cylindrical cell colony (Fig. 5a and b). When the fiber diameter was about 180 μm , irregular cellular clusters formed and filled out the entire space of the ALG/PLL aqueous microstrand (Fig. 5c). When the fiber diameter approached 300 μm , cellular elongation occurred along the ALG/PLL aqueous microstrand and was accompanied by projecting extensions radially. In this way, ES cells assembled into a long, continuous, stable microtissue with a well-organized branching structure along the ALG/PLL microstrands (Fig. 5d).

In comparison, ES cells in solid alginate gel microstrands at sizes below 100 μm (Fig. 5e and 5f) appeared to grow at a much slower rate as compared to the gel microstrands with larger fiber diameters (Fig. 5g and 5h). In addition to the growth rate, solid, alginate gel microstrands with larger diameter showed self-assembly of cells into branching structures as opposed to the formation of smaller cell clusters as those seen with smaller fiber diameter.

For example, in alginate gel microstrands, with diameters less than 60 μm , ES cells clustered in very small aggregates (Fig. 5e and f). When the diameter of the gel microstrand was above 100 μm , small ES cell aggregates began to accumulate into larger aggregates and organized into a branched structure (Fig. 5g). When the diameter of the microfiber was over 200 μm , cellular elongation occurred and cells organized into many discontinued, branch-like structures (Fig. 5h). These results demonstrated that compared to gel microstrands, mouse ES cells in one dimensional ALG/PLL aqueous microstrands appeared to i) grow fast, ii) fill up the entire space, and iii) self-assemble into well-organized structures.

3.3 Differentiation potential of self-assembled ES cells in microstrands

We further examined the differentiation potential of self-assembled ES cells encapsulated in ALG/PLL aqueous microstrands and ES cells entrapped in alginate hydrogel microfibers. We performed immunocytochemistry to stain the ES cells with markers for the three germ layers (ectoderm, mesoderm and endoderm). In the absence of LIF, which is a growth factor responsible for maintaining mouse ES cells in an undifferentiated state, ES cells would undergo spontaneous differentiation in ALG/PLL aqueous microstrands or alginate gel microstrands. After culturing in media lacking of LIF for 5 days, mouse ES cells were released from ALG/PLL aqueous microstrands or alginate gel microstrands with a diameter less than 200 μm , and allowed to adhere to glass coverslips. On day 3, ES cells were fixed and stained with antibodies against nestin (neural cell marker), Myf-5 (muscle cell marker), and PDX-1 (pancreatic cell marker) to demonstrate their ability to differentiate into cell types of ectoderm, mesoderm and endoderm, respectively. We found that mouse ES cells collected from ALG/PLL aqueous microstrands favored differentiation into cell types expressing low levels of nestin (Fig. 6a), high levels of Myf5 (Fig. 6b), and in particular, high levels of PDX-1 (Fig. 6c). On the other hand, ES cells released from alginate gel microstrands showed preference towards differentiation into cell types expressing the ectoderm marker, nestin (Fig. 6d) and mesoderm marker, Myf-5 (Fig. 6e). Very few ES cells expressed PDX-1 after culturing in an alginate gel microstrand environment (Fig. 6f). These results demonstrated that cells from ALG/PLL aqueous microstrands preferred to differentiate towards endoderm and mesoderm lineages whereas cells collected from the alginate gel microstrands favored towards ectoderm and mesoderm differentiation.

4. Discussion

We have developed a simple approach to synthesize alginate gel microstrands and ALG/PLL aqueous microstrands using an SU-8 filter device driven by capillary action followed by polyelectrolyte complexation. Alginate gel microfibers can be synthesized using a poly(dimethylsiloxane) (PDMS) microfluidic device in which a capillary glass pipet is embedded vertically and two pumps are used to create a coaxial flow [42], and alginate hollow fibers formed using pre-perforated PDMS with three separated inlets for three-layered coaxial flow [46], a coaxial triple cylinder [45] or spinneret [43], or a microfabricated nozzle [44]. All these fabrication processes either involve the complex design for microfluidic devices or require flow control. Compared to these methods, our approach offers a straight forward method to produce microstrands. Once the SU-8 filter is fabricated, alginate hydrogel microstrands with varied diameters could be formed instantly by placing an alginate solution on the top of the SU-8 filter that is floating on a CaCl_2 solution. Microstrands that provide a liquid microenvironment are formed via polyelectrolyte complexation with PLL and then liquefying with sodium citrate; a similar approach used to form ALG/PLL microcapsules [31, 40] or microtubes [44].

Here we demonstrate for the first time the concept of one dimensional self-assembly and “liquid-like” tubular microenvironments, leading to high density cell culture and organization of ES cells in microstrands. At higher cell densities, the transport of oxygen,

nutrients, and metabolic wastes by diffusion becomes critical because the diffusion limit is around 100 – 200 μm [60, 61]. The limitation in diffusive transport of growth factors and chemical cues to EBs or ES cell clusters needs to be considered when differentiating ES cells [62]. Since the fiber diameter of our microstrands can be less than 200 μm , oxygen and nutrients could reach encapsulated ES cells through simple diffusion. Thus, ES cells encapsulated in ALG/PLL aqueous microstrands grew rapidly, reached high cell density, and self-assembled into compact tissue. ALG/PLL aqueous microstrands offer a “liquid-like” microenvironment for self-assembly of ES cells. In such a “liquid-like” microenvironment, cells moved freely and self-assembled into aggregates, clusters, or branching structures through the coordination of cell movement, orientation, elongation, extension, and contraction [63].

ALG/PLL microcapsules for ES cell culture typically has diameters above 400 μm , with an initial cell density no higher than 1×10^7 cells/ml, where the size of the cell aggregates is smaller than the diameter of the microcapsules. As mentioned previously, with large diameter beads, there are several issues with controlling the microenvironment and reaching high cell densities. In contrast, our ALG/PLL aqueous microstrands have several advantages. First, the diameter of ALG/PLL microstrands could be easily controlled to be smaller than 100 μm , which would avoid the diffusion limitation. Second, cell densities as high as 1×10^8 cells/ml can be supported, allowing cells to self-assemble into more compact tissue. Third, long microstrands could serve as a building block to be arranged into complex tissue structures, while the array format allows easy scale-up. Finally, ALG/PLL aqueous microstrands might provide suitable conditions for directly differentiating ES cells into gland tissue. In addition, a tubular shaped hydrogel is needed for mimicking and recreating various tissue structures in the body, for example, blood vessels, nerve crest, urethra, fallopian tube, digestive tracts and others.

Alginate microspheres (microbeads or microcapsules) has been used to formulate EBs [49] and differentiate ES cells into ectodermal (e.g., neural lineage [64]), mesodermal (e.g., cardiac cells [49, 65], osteogenic lineages [54, 66]), and endodermal lineages (e.g., definitive endoderm cells [52], hepatocytes [50, 67, 68], insulin-producing cells [51, 69]). We found that ALG/PLL aqueous microstrands promoted compact ES cell organization and induced high level of PDX-1 expression. PDX-1 is a transcription factor essential for pancreatic development and insulin-producing β -cell maturation. During embryonic development, PDX-1 expressing progenitor cells in the definitive endoderm give rise to the developing pancreatic buds, leading to the whole pancreas [70]. The pancreas is a gland organ with endocrine and exocrine function. The endocrine part of the pancreas is comprised of a compact collection of cells organized into clusters and cords. Combined with our results, this suggests that ALG/PLL aqueous microstrands may be favorable for the differentiation of ES cells into pancreatic cells and other gland cells. For example, it has been demonstrated that mouse ES cells long-term cultured in ALG/PLL microcapsules can differentiate into insulin-producing cells [51, 69]. We find that ALG/PLL aqueous microstrands presented compact, high density cell aggregates with higher expression of the endodermal marker (PDX-1), whereas alginate gel microstrands exhibited smaller, discrete cell aggregates with higher expression of the ectoderm marker, nestin. These results are consistent with the report that smaller cell aggregates showed higher ectodermal markers compared to the larger aggregates, while larger aggregates showed the increased expression of mesodermal and endodermal markers compared to smaller cell aggregates when growing ES cells in microfabricated wells [71].

All together, the array of alginate microstrands demonstrated here provides a flexible, “liquid-like” microenvironment for exploring one dimensional self-assembly of ES cells into compact tissue. Understanding and manipulating one dimensional self-assembly of ES cells

will provide insights in stem cell-based tissue regeneration, cell therapy, and 3D tissue model for drug discovery.

5. Conclusions

In this study, we have successfully fabricated ALG/PLL aqueous microstrands to mimic the “liquid-like” embryonic microenvironment. Mouse ES cells exhibit self-assembly behaviors in ALG/PLL aqueous microstrands as well as alginate gel microstrands. Aqueous microstrands facilitate high density cell culture and promote compact microtissue formation whereas gelled ones favor the formation of small cell aggregates or clusters. The morphology of self-assembled microtissues depends on the diameter of the microstrands. When the diameter of the microstrand is below 100 μm , mouse ES cells in the alginate/PLL aqueous microstrand grew freely and self-assembled into a tubular structure, while in alginate gel microstrands they present a “pearl-on-a-string” fashion. Mouse ES cells self-assemble into branching structures when the diameter is above 180 μm . As far as the differentiation potential is concerned, compact microtissues in ALG/PLL aqueous microstrands show higher preference toward the endoderm and mesoderm lineages, as compared to a higher inclination toward ectoderm and mesoderm lineages for cells recovered from alginate gel microstrands. This research demonstrates self-assembly of mouse ES cells in one dimensional alginate hydrogel microstrands and provides an innovative stem cell micro-cultivation system as a platform for biological studies and therapeutics development.

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Fig. 1. Schematics of the fabrication of the SU-8 microfluidic filter device using photolithography. (a) Piranha clean of the silica wafer. (b) Omnicore treatment for the release layer. (c) SU-8 50 coating. (d) Exposure. (e) Post-exposure bake. (f) Develop. (g) Schematics of the microchannel array of the SU-8 filter device.

Fig. 2. SEM images of the porous microfluidic SU-8 filter. (a) Top view. (b) Cross-section. Scale bar = 20 μm.

Fig. 3.

Encapsulation of mouse ES cells in alginate hydrogel microstrands. (a) Schematics of the formation of microstrands by adding one drop of cell-alginate solution onto the array of microfluidic SU-8 filter device which is floating on the top of the CaCl_2 solution. (b) Optical image of the array of alginate hydrogel microstrands. Scale bar = 40 μm . (c) Optical image of long, continuous microstrands. (d) Fluorescent image of mouse ES cells in a microstrand stained by calcein AM/EthD-1 (green: live cells; red: dead cells).

Fig. 4. High density of mouse ES cells encapsulated in alginate hydrogel microstrands. (a) Initial cell density at 1×10^6 cells/ml. (b) 1×10^7 cells/ml. (c) 1×10^8 cells/ml.

Fig. 5. Optical images of mouse ES cells encapsulated in alginate/PLL aqueous microstrands (a-d) in comparison with those entrapped in alginate gel microstrands (e-h) after culturing for 5 days. The “liquid-like” microenvironment in alginate/PLL microstrands promotes the formation of compact cellular structure and the morphology of the self-assembled tissue architecture depends on the diameter of the fiber.

Fig. 6.

The differentiation potential of mouse ES cells recovered from alginate/PLL aqueous microstrands (a-c) and alginate gel microstrands (d-f) revealed by the expression of markers of ectoderm – nestin (a and d), mesoderm – Myf-5 (b and e), and endoderm – PDX-1 (c and f). Scale bar = 50 μ m.