Video Article

Gradient Strain Chip for Stimulating Cellular Behaviors in Cell-laden Hydrogel

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Abstract

Artificial guidance for cellular alignment is a hot topic in the field of tissue engineering. Most of the previous research has investigated single strain-induced cellular alignment on a cell-laden hydrogel by using complex experimental processes and mass controlling systems, which are usually associated with contamination issues. Thus, in this article, we propose a simple approach to building a gradient static strain using a fluidic chip with a plastic PDMS cover and a UV transparent glass substrate for the stimulation of cellular behavior in a 3D hydrogel. Overloading photo-patternable cell prepolymer in the fluidic chamber can generate a convex curved PDMS membrane on the cover. After UV crosslinking, through a concentric circular micropattern under the curved PDMS membrane, and buffer washing, a microenvironment for investigating cell behaviors under a variety of gradient strains is self-established in a single fluidic chip, without external instruments. NIH3T3 cells were demonstrated after observing the change in the cellular alignment trend under geometry guidance, in cooperation with strain stimulation, which varied from 15 - 65% on hydrogels. After a 3-day incubation, the hydrogel geometry dominated the cell alignment under low compressive strain, where cells aligned along the hydrogel elongation direction under high compressive strain. Between these, the cells showed random alignment due to the dissipation of the radical guidance of hydrogel elongation and the geometry guidance of the patterned hydrogel.

Video Link

The video component of this article can be found at <https://www.jove.com/video/53715/>

Introduction

Serving as a block material that mimics a native microenvironment, a hydrogel containing extracellular matrix (ECM) can re-build biomimetic tissue scaffolds to support cell growth. To possess the functions of a tissue, organized cell alignment is an essential requirement. Various 2D (*i.e.*, cells cultured on a surface) and 3D (*i.e.*, cells encapsulated in a hydrogel) cell alignments have been achieved by culturing or encapsulating cells in or on flexible substrates with micro- or nano-patterns¹. 3D cell alignment in microarchitecture is more attractive, as the microenvironment is closer to the native tissue construct^{2,3,4}. One common approach for 3D cell alignment is the geometric cue of hydrogel shape^{2,3}. Because of the restricted space for cell proliferation in the short-axis direction, cells aim to align along the long-axis direction in a micro-patterned hydrogel. Another approach is to apply tensile stretch to the hydrogels to achieve cell alignment parallel to the stretch direction^{4,5}.

Biophysical stimulation on ECM hydrogels, such as compressive strain or an electrical field, can regulate cell functions for proper tissue integration, proliferation, and differentiation^{1,2,3}. Much research has been done to investigate cellular behavior by applying one strain condition at a time using multiple mechanical control units^{4,6,7,8,9}. For example, the use of mechanical step motors squeezed or stretched on a 3D cell-encapsulated collagen hydrogel has been a common approach^{7,10}. However, such controlling equipment requires extra space and faces the issue of contamination in the incubator^{7,9,11,12}. In addition, the large instrument cannot give a precise control environment to provide high reproducibility¹³.

Considering that cell-laden hydrogels are usually employed on the micro-scale for biomedical applications, it is advantageous to combine MEMS techniques to generate a range of strain/stretch stimulation to simultaneously investigate cell behaviors in 3D biomimetic constructs *in vitro*^{2,14,15,16,17,18}. For example, using gas pressure to deform the PDMS membrane in microfluidic chips can give rise to various strains, driving cell differentiation to different lineages^{9,16}. However, there are many technical challenges, such as complicated chip fabrication processes in a clean room and the software control integration of motors, pumps, valves, and compressed gases.

In this work, we demonstrate a simple approach to obtain a self-sustaining gradient static-strain microfluidic chip by employing a concentric circular hydrogel pattern and a flexible PDMS membrane. Unlike most of the existing approaches, our platform is a portable and disposable miniature device that can be fabricated outside a yellow room and that possesses self-generating gradient strains on concentric cell-encapsulated hydrogels, without external mechanical equipment during the incubation. 3T3 fibroblast cell behaviors influenced by a combination of hydrogel shape and a variety of tensile stretch guidance cues were demonstrated during the observation of cell alignment within 3D ECM-mimetic environments in the gradient strain chip for 3 days.

Protocol

1. GelMA Synthesis

1. Weigh 10 g of gelatin powder and add it to a glass flask with 100 mL of Dulbecco's phosphate-buffered saline (DPBS). Put a magnetic stir bar into the flask and place the flask on a stirring hot plate.
2. Cover the flask with aluminum foil to avoid water evaporation. Set the hot plate temperature to 50-60 °C and the stirrer at 100 rpm for 1 h to dissolve the gelatin powder well.
3. After the gelatin has dissolved, add 8 mL of methacrylic anhydride very slowly (one drop per second) using a pipette. Let it react at 60 °C for 3 h.
4. Add pre-warmed DPBS (40 °C) to the flask to a final volume of 500 mL and allow this mix well for 15 min to stop the reaction.
5. Meanwhile, cut a dialysis membrane (14 kDa cut-off molecular weight) into several 25 cm-long tubes. Immerse them in deionized (DI) water for 15 min and make a knot to close one end of the dialysis tubes.
6. Load the appropriate amount (30 - 60 mL) of the polymer solution into the dialysis tubes and close the other end. Place them in a 5-L plastic beaker with DI water for a week. Renew the DI water twice a day and maintain the solution at 40 - 50 °C during the dialysis process.
7. Collect the solution from the tube in a 500-mL glass bottle. Pour ~450 - 500 mL of solution in a 500-mL filter cup (pore size of 0.22 µm) and apply a vacuum to the filter cup to force the solution to pass through the filter membrane for sterilization.
8. Transfer the sterilized polymer into several 50-mL sterilized tubes and store them in a -80 °C freezer for 3 - 5 days.
9. Freeze-dry the -80 °C polymer for 1 week using a freeze dryer to form GelMA. Store the GelMA in a -80 °C freezer.

2. 3-(Trimethoxysilyl)propyl Methacrylate (TMSPMA) Modification

1. Cut commercial glass slides into two small pieces (25 mm x 37.5 mm) and immerse them in 0.5 M NaOH solution for 4 h. Wash the slides with a large amount of DI water.
2. Place the slide on a rack inside a glass container with 95% ethanol and clean using an ultrasonicator at 43 kHz for 15 min. Air dry the glass slide.
3. Immerse the glass slides in 5% TMSPMA in 99.5% ethanol for 1 h.
4. Wash the slides in 95% ethanol, air dry the slides, and anneal the TMSPMA coating in an oven at 80 °C for 2 h.

3. Chip Fabrication

1. Take one 2 mm- and one 0.3 mm-thick polymethylmethacrylate (PMMA) plate, apply double-sided tape to one side of the PMMA surface, and release the liner on one side. Leave two 2 mm-thick PMMA plates and one 1 mm-thick plate without double-sided tape.
2. Laser-cut a 2 mm-thick PMMA plate without double-sided tape to 42 mm x 30 mm to make the bottom plate. Cut a 2 mm-thick PMMA plate with double-sided tape to make the boundary frame, with outer dimensions of 42 mm x 30 mm and inner dimensions of 37.5 mm x 25 mm.
3. Laser-cut the 0.3 mm-thick PMMA plate with double-sided tape into a 12-mm center circle with two 2 mm-wide and 8 mm-long flow channels on opposite sides of the circle (**Figure 1a**).
4. To prepare the PMMA mold for casting the PDMS cover, assemble the three pieces of PMMA components from steps 3.2-3.3 (**Figure 1b**) using double-sided tape.
5. Laser-cut a 2 mm-thick PMMA plate with double-sided tape into a 5 cm x 5 cm piece with a 3 x 3 array of 8.5 mm x 8.5 mm hollow rectangles. Cut another 5 cm x 5 cm piece with a 3 x 3 array of 4-mm hollow circles. Laser-cut a 1 mm-thick PMMA plate without double-sided tape into a 5 cm x 5 cm PMMA bottom plate.
6. Prepare the PMMA mold for casting the PDMS plug by assembling the three pieces of PMMA components from step 3.5 (**Figure 1c**) using double-sided tape.
7. Prepare the PDMS cover and PDMS plug by properly mixing 30 g of PDMS elastomer and 3 g of PDMS curing agent; degas the mixture under a vacuum chamber for 1 h.
8. Cast 1.8-2.0 g of the mixture into the PMMA mold for the PDMS cover and use the appropriate amount to fill each cavity of the PMMA mold for the PDMS plug. Cast 10 g of uncured PDMS mixture in a blank 10-cm plastic plate. Put these molds in a vacuum chamber to degas for 30 min.
9. Cure the PDMS for 2 h at 80 °C. After cooling down the cured PDMS on the mold, detach the PDMS covers and the PDMS plugs from the PMMA molds.
10. Punch two holes with diameters of about 3 mm at the ends of flow channels of the PDMS covers.
11. Cut the PDMS sheet molded from the 10-cm plastic plate into many 1 cm x 1 cm cubes and punch a 3-mm hole in each PDMS cube. Glue two uncured 1 cm x 1 cm PDMS cubes onto the openings of the PDMS cover (to serve as medium reservoirs and to aid in the curing process of the gradient circular hydrogel patterns) and cure for 1 h at 80 °C.
12. Bond the PDMS covers with the two PDMS reservoirs onto a TMSPMA-coated slide by pre-treating the bonding side of the PDMS cover and the TMSPMA-coated slide under an oxygen plasma machine (30 W RF power (high mode) and 600 mTorr compressed air) or a high-frequency electronic corona generator (115 V, 50/60 Hz, 0.35 A) for 90 s of O₂ plasma treatment.
13. Contact the plasma-treated surface of the PDMS covers and the TMSPMA slide and press them closely for permanent bonding through the formation of an Si-O-Si bond.
NOTE: Placing the chip in an oven at 80 °C for 1 h can further enhance the bonding strength.
14. After cooling, immerse the chips in 95% ethanol for 15 min and air dry. Then, sterilize the chips under UV irradiation for 1 h and store them in a box wrapped in aluminum foil in the laminar hood.

4. Static Gradient Strain on the Cell-laden Hydrogel

1. Print and cut a piece of photomask, 25 mm x 37.5 mm in size, by printing the layout in **Figure 2b** on a transparent film. Adjust the printed size of **Figure 2b** to match the dimension in **Figure 2a**.
 2. Prepare 100 mL of DMEM medium with 10% FBS, 1% Pen-Strep, and 250 mL of DPBS in a 37 °C water bath to use as the cell culture medium.
 3. Weigh 25 mg of freeze-dried GelMA into 0.3 mL of prewarmed (37 °C) cell culture medium in a 1.5-mL black microcentrifuge tube. Put the microcentrifuge tube on a laboratory stirrer/hot plate until the GelMA dissolves in the medium.
 4. Weigh 50 mg of photoinitiator into 1 mL of DPBS in a microcentrifuge tube and place it in an 80 °C oven for 15 min or until the photoinitiator has dissolved.
 5. Take 25 μ L of the 10% photoinitiator from step 4.4 and add it to the microcentrifuge tube from step 4.3. Pipette several times to mix well.
 6. Count 3×10^6 NIH 3T3 cells using an automated cell counter. Centrifuge the suspension at 200 x g for 5 min, discard the supernatant, and re-suspend the cells in 175 μ L of cell culture medium.
 7. Add the cell solution from step 4.6 to the microcentrifuge tube from step 4.5 to get a prepolymer cell solution of 5% GelMA, 0.5% photoinitiator, and $\sim 6 \times 10^6$ 3T3 cells/mL. After mixing, load 100 μ L of cell prepolymer in a 100- μ L micro syringe.
 8. Manually align (see **Figure 3a**) a piece of the photomask onto the bottom slide of the sterilized gradient strain chip and simply fix the position using a small drop of DI water in between. Connect the 100- μ L micro-syringe loaded with prepolymer cell solution to the inlet of the chip.
 9. Place (see **Figure 3b**) 50 μ L of prepolymer cell solution in the flow channel using the micro-syringe and then plug the outlet using a PDMS plug. Inject an extra 40 μ L of solution to create a convex bulge in the circular PDMS membrane.
 10. Move the chip with the photomask (bottom), micro-syringe (inlet), and PDMS plug (outlet) from step 4.9 under a UV lamp (365 nm, 9 mW/cm²) and expose it for 30 - 45 s to crosslink the concentric circular hydrogel in the fluidic chamber.
 11. Remove the PDMS plug and the micro-syringe to release the liquid pressure (see **Figure 3c**). Use a 1-mL syringe loaded with prewarmed DPBS to wash out uncrosslinked resins 3 times by flushing from the inlet to the outlet.
 12. Fill the flow channel with about 100 μ L of cell culture medium.
 13. Place the chip in a sterilized culture dish and culture in a 5% CO₂ atmosphere at 37 °C for a week. Refresh the medium every day.
 14. Take images of three chips on day 0 after 4 h of incubation, as a control group, and three chips on day 3, as the experimental set, using a microscope with a 20X objective. Measure the line width of each hydrogel from line 1 to line 12 using software (e.g., ImageJ) to calculate the compress strains (**Figure 4**).
- NOTE: The elongation percentage is calculated by dividing the value of the line width difference between 40 μ L and 0 μ L by the line width at 40 μ L.

5. Cell Staining for Alignment Analysis

1. Use a syringe to inject 4% paraformaldehyde (PFA) in DPBS at RT into the flow chip for 15 min for the fixation of the cell-laden hydrogel.
NOTE: Caution. PFA is toxic and should be handled with care.
2. Replace the solution with 0.5% cell membrane permeating solution in DPBS for 10 min to permeabilize the cell membrane at RT.
3. PBS wash the samples 3 times with 5- to 10-min interval between washes (using a loaded syringe, as in step 5.1).
4. Load 1% BSA solution into the fluidic channel for 45-60 min at RT (using a loaded syringe through the inlet port).
5. Add 1.5 mL of methanol into the vial with Alexa Fluor 488 phalloidin to yield a final concentration of 6.6 μ M stock solution.
6. Take 5 μ L of Alexa Fluor 488 phalloidin from step 5.5 and dilute it in 200 μ L of DPBS with 0.1% BSA to form a final concentration of 0.165 μ M Alexa Fluor 488 phalloidin.
7. Add 200 μ L of the mixture solution to the fluidic channel using a micropipette and incubate the chip at 37 °C for 45 - 60 min for actin staining. PBS wash (as above) the samples 3 times.
8. Prepare 1 μ g/mL DAPI in PBS, flow it through the chip, and stain cell nuclei at 37 °C for 5 min.
9. Pipette DPBS into the fluidic channel to wash out the staining solution and refill the fluidic chamber with PBS solution to take images with a fluorescence microscope.
10. Capture the fluorescent images of the 3T3 cells in the hydrogels using an inverted fluorescence microscope under 40X magnification with a CCD detector and filter sets of ex/em at 488/520 nm and 358/461 nm for Alexa Fluor 488 phalloidin (actin) and DAPI (nucleus), respectively.

Representative Results

To compare the mechanical variations between each circular hydrogel in the completed gradient strain stimulation chip, we measured the line widths of each circular hydrogel in two of the same chips, with injection volumes of 0 μ L (**Figure 4a**) and 40 μ L (**Figure 4b**), respectively. The percent elongations at each circle were calculated by dividing the elongations in the 40 μ L-injected chip by the line widths of the corresponding hydrogels in the 0 μ L-injected chip (**Figure 4c**). The hydrogel is an incompressible material, so the vertical contracting strain will be equivalent to the lateral elongation strain. Therefore, the percent elongations in the chip with the 40- μ L injection volume show 15 - 65% elongation, which can be converted to the compressive strain (see **Supplementary File 1**).

Fluorescent staining of the cell nuclei and F-actin with DAPI and phalloidin, respectively, was done to analyze the cellular alignment. The DAPI staining provided data on nuclear orientation, and the phalloidin staining was applied to assess cell spreading. **Figure 5a-c** shows the cell alignment in the gradient strain chip. In line 1, the 3T3 cells aligned along the radial direction. In hydrogel 7, the cells aligned randomly, and cells aligned along the circular direction in line 12. In accordance with the fluorescent staining images, a 90° shift from the angle of cell alignment of the 3T3 cells in line 1 (maximum hydrogel elongation in the radial direction) to the alignment angle of the cells with long-axis alignment in line 12 (lowest hydrogel elongation in the radial direction) was discovered.

In previous research^{2,9}, cells in 200- μm line-patterned hydrogels aim to align along the long-axis direction of the hydrogel. However, in this study, we observed that the elongated stretch on the 200- μm hydrogels in the short-axis direction provided another factor to affect and dominate the cellular alignment by controlling the percentage of the strain on the hydrogel. For the 65% strain on line 1, the radial alignment proved that the elongation stretch of the hydrogel dominates the cell alignment. For the 15% strain on line 12, the circular alignment proved that the long-axis effect dominated the cell alignment. For the 40% strain on hydrogel 7, the cells aligned randomly due to the neutralization of the geometry guidance and strain effect.

Figure 1. PMMA Mother Mold for PDMS Sheet and Plug Fabrication. (a) The separated components of the PMMA mold, including a bottom plate, boundary frame, and flow channel. After assembly with double-sided tape, (b) the PMMA mother mold for the flow sheet is formed. (c) Another PMMA mold is assembled for the PDMS plug. The red number represents the depth. (unit: mm) [Please click here to view a larger version of this figure.](#)

Figure 2. Plastic Photomask Design for the Cell-laden Hydrogel Micropattern. There are two openings with triangle shapes (2 mm at the bottom line and 6.5 mm in height) connecting to the flow channels to supply fresh cell culture medium. (a) The plastic photomask is labeled with the dimension. The period of the concentric circle is 400 nm, and the duty cycle is 50%. The diameter of the center circle is 2 mm. (b) The photomask layout, without labels for laser printing on a plastic transparent film. [Please click here to view a larger version of this figure.](#)

Figure 3. Fabrication Processes of the Gradient Strain Cell-laden Hydrogel Along the Radial Direction in a PDMS Fluidic Chip. (a) A plastic photomask is aligned and stuck under the chip with a TMSPMA-coated fluidic channel. A micro-syringe with prepolymer cell solution is inserted into the inlet of the chip and used to inject about 50 µL to fill the flow channel. (b) The outlet of the flow channel is closed with the PDMS plug and an additional 40 µL of prepolymer cell solution is injected. The glass bottom is UV-patterned for 30 s to fabricate the concentric circular hydrogel in the flow chip. (c) The liquid pressure is released in the flow channel by unplugging the outlet and the un-crosslinking mixture is washed out with DPBS. (d) A chip with static gradient strain applies concentric cell-laden hydrogels that are ready for cell culturing. During the UV crosslinking process, (e) a non-continuous gradient height of hydrogel along the radius is formed. (f) After unplugging the outlet, the PDMS membrane becomes flat and applies gradient stress on the cell-encapsulated hydrogels. [Please click here to view a larger version of this figure.](#)

Figure 4. The Pure Hydrogel Elongation Gradient. The elongation line width and percentage of pure hydrogel without cell encapsulation in the gradient strain chip on day 3 with the (a) 0-µL (control group) and (b) 40-µL injection volumes. (c) The elongation percentage is calculated by dividing the value of the line width difference between 40 µL and 0 µL by the line width of 40 µL. [Please click here to view a larger version of this figure.](#)

Figure 5. Fluorescent Actin-nucleus Stain images of the 3T3 Cells Encapsulated in the Gradient Chips on Day 3. The cell alignment direction in (a-c) line 1, (d-f) line 7, and (g-i) line 12 reveal radial alignment, random alignment, and circular alignment, respectively. The green and blue colors show the actin and nucleus stains, respectively. The dotted white line represents the boundary of the hydrogel. Scale bar: 200 μ m. [Please click here to view a larger version of this figure.](#)

Supplementary Figure 1. Calculation of the Dome-shaped PDMS Curvature. $H(x)$: the convex PDMS curve; H_0 : the maximum height difference between the PDMS dome before and after deformation; r : the radius of the dome; V : the over-injection volume of the blue region, which causes the PDMS deformation as a dome. See **Supplementary File 1** for details. [Please click here to download this figure.](#)

Supplementary File 1. [Please click here to download this file.](#)

Discussion

In this paper, we report on a simple approach to compare cell alignment behavior after hydrogel shape guidance and tensile stretch. A flexible PDMS membrane creates a dome-shaped curvature for generating different heights of concentric circular hydrogels. After releasing the pressure, the PDMS membrane automatically applies force to the micro-patterned hydrogels to form gradient strain/elongation, with a maximum at the center and a minimum at the outer boundary. As the formation of the gradient strain is designed by the flexible PDMS membrane and the handling of the fluidic chip, there are several important parameters that should be attended to: (i) Precise control of the thickness of the PDMS membrane is crucial to adjust the gradient strain value. If the membrane is too thick, even the maximum injection volume of cell prepolymer will not be able to generate a proper convex curve in the PDMS membrane for crosslinking a gradient height of cell-laden hydrogels. In contrast, a too-thin PDMS membrane cannot apply sufficient force to the hydrogels. Please check that the weight of uncured PDMS in the PDMS cover mold is around 1.6-2.0 g per chip. (ii) Contamination prevention is very important during the crosslink handling of the cell-laden hydrogel in the fluidic chip. Before cell culturing in the incubator, thoroughly washing with sterilized PBS in the fluidic channel and using 75% ethanol to wipe the surface of the chip can help to avoid the contamination issue. (iii) The concentration of photoinitiator and the dosage of UV exposure should be carefully controlled and in the range of ~0.1% - 2% (0.5% is recommended). Over-crosslinking the hydrogel and overdosing the UV irradiation will result in low cell viability. (iv) The line-width of the patterned hydrogel should not be too large. Otherwise, the nutrient replacement rate in the thick hydrogels will not be able to support cell proliferation. Usually, less than 300 μ m is recommended. The spacing between two hydrogel circles can be varied, and a 50% duty cycle is recommended. (v) While washing or refilling the flow channel with solution, the formation of bubbles should be avoided. Gently pipetting the solution in the chip can help to remove bubbles.

The concept of gradient strain generated by PDMS-deformed curvature can be further upgraded to applying dynamic gradient strains and can be integrated with biochemical stimulation, which can benefit many studies on functional tissue regeneration. The simple fluidic injection module with a PDMS plug can be replaced by any advanced fluidic system for extended experimental control. The PMMA mold can also be replaced by a microfabricated SU-8 mold or a bulk-etched silicon mold.

This gradient strain chip with a circular cell-laden hydrogel can generate static compressive force on the 3D hydrogel without external mechanical or electrical machines. Therefore, it provides a fast screening platform for investigating cell behaviors in a series of strain conditions,

without the risk of contamination issues caused by the operation of external machines. However, time-controlled strain stimulation is not achievable because the PDMS membrane generates strains until the degradation of the hydrogel.

Disclosures

The authors have nothing to disclose.

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References

1. Simmons, C.S., Petzold, B.C., Pruitt, B.L. Microsystems for biomimetic stimulation of cardiac cells. *Lab Chip*. **12** (18), 3235-3248 (2012).
2. Aubin, H. *et al.* Directed 3D cell alignment and elongation in microengineered hydrogels. *Biomaterials*. **31** (27), 6941-6951 (2010).
3. Guan, J. *et al.* The stimulation of the cardiac differentiation of mesenchymal stem cells in tissue constructs that mimic myocardium structure and biomechanics. *Biomaterials*. **32** (24), 5568-5580 (2011).
4. Wan, C.R., Chung, S., Kamm, R.D. Differentiation of embryonic stem cells into cardiomyocytes in a compliant microfluidic system. *Ann Biomed Eng*. **39** (6), 1840-1847 (2011).
5. Huh, D. *et al.* Reconstituting organ-level lung functions on a chip. *Science*. **328** (5986), 1662-1668 (2010).
6. Li, X., Chu, J.S., Yang, L., Li, S. Anisotropic effects of mechanical strain on neural crest stem cells. *Ann. Biomed. Eng.* **40** (3), 598-605 (2012).
7. Butcher, J.T., Barrett, B.C., Nerem, R.M. Equibiaxial strain stimulates fibroblastic phenotype shift in smooth muscle cells in an engineered tissue model of the aortic wall. *Biomaterials*. **27** (30), 5252-5258 (2006).
8. Ramon-Azcon, J. *et al.* Gelatin methacrylate as a promising hydrogel for 3D microscale organization and proliferation of dielectrophoretically patterned cells. *Lab Chip*. **12** (16), 2959-2969 (2012).
9. Park, S.H., Sim, W.Y., Min, B.H., Yang, S.S., Khademhosseini, A., Kaplan, D.L. Chip-Based Comparison of the Osteogenesis of Human Bone Marrow- and Adipose Tissue-Derived Mesenchymal Stem Cells under Mechanical Stimulation. *PLoS One*. **7** (9), e46689 (2012).
10. Gould, R.A. *et al.* Cyclic Strain Anisotropy Regulates Valvular Interstitial Cell Phenotype and Tissue Remodeling in 3D Culture. *Acta Biomater.* **8** (5), 1710-1719 (2012).
11. Kurpinski, K., Chu, J., Hashi, C., Li, S. Proc. Anisotropic mechanosensing by mesenchymal stem cells. *Natl Acad Sci USA*. **103** (44), 16095-16100 (2006).
12. Sim, W.Y., Park, S.W., Park, S.H., Min, B.H., Park, S.R., Yang, S.S. A pneumatic micro cell chip for the differentiation of human mesenchymal stem cells under mechanical stimulation. *Lab Chip*. **7** (12), 1775-1782 (2007).
13. Vader, D., Kabla, A., Weitz, D., Mahadevan, L. Strain-Induced Alignment in Collagen Gels. *PLoS One*. **4** (6), e5902 (2009).
14. Aguado, B.A., Mulyasmita, W., Su, J., Lampe, K.J., Heilshorn, S.C. Improving viability of stem cells during syringe needle flow through the design of hydrogel cell carriers. *Tissue Eng Part A*. **18** (7-8), 806-815 (2012).
15. Wan, J. Microfluidic-Based Synthesis of Hydrogel Particles for Cell Microencapsulation and Cell-Based Drug Delivery. *Polymers*. **4** (2), 1084-1108 (2012).
16. Moraes, C., Wang, G., Sun, Y., Simmons, C.A. A microfabricated platform for high-throughput unconfined compression of micropatterned biomaterial arrays. *Biomaterials*. **31** (3), 577-584 (2010).
17. Keung, A. J., Kumar, S., Schaffer, D. V. Presentation Counts: Microenvironmental Regulation of Stem Cells by Biophysical and Material. Cues. *Annu Rev Cell Dev Biol*. **26**, 533-556 (2010).
18. Segers, V.F., Lee, R.T. Stem-cell therapy for cardiac disease. *Nature*. **451** (7181), 937-942 (2008).
19. Hsieh, H.Y. *et al.* Gradient static-strain stimulation in a microfluidic chip for 3D cellular alignment. *Lab Chip*. **14** (3), 482-493 (2014).