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Relative impact of uniaxial alignment vs. form-induced stress on differentiation of human adipose derived stem cells

Xin Qu [a, 1], Wei Zhu [a, 1], Samuel Huang [b], Yi-Shuan Li [b], Shu Chien [b], Kang Zhang [c], Shaochen Chen [a, *]

[a] Department of NanoEngineering, University of California, San Diego, La Jolla, CA 92093, USA
[b] Department of Bioengineering, University of California, San Diego, La Jolla, CA 92093, USA
[c] Shiley Eye Center and Institute for Genomic Medicine, University of California, San Diego, La Jolla, CA 92093, USA

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ABSTRACT

ADSCs are a great cell source for tissue engineering and regenerative medicine. However, the development of methods to appropriately manipulate these cells *in vitro* remains a challenge. Here the proliferation and differentiation of ADSCs on microfabricated surfaces with varying geometries were investigated. To create the patterned substrates, a maskless biofabrication method was developed based on dynamic optical projection stereolithography. Proliferation and early differentiation of ADSCs were compared across three distinct multicellular patterns, namely stripes (ST), symmetric fork (SF), and asymmetric fork (AF). The ST pattern was designed for uniaxial cell alignment while the SF and AF pattern were designed with altered cell directionality to different extents. The SF and AF patterns generated similar levels of regional peak stress, which were both significantly higher than those within the ST pattern. No significant difference in ADSC proliferation was observed among the three patterns. In comparison to the ST pattern, higher peak stress levels of the SF and AF patterns were associated with up-regulation of the chondrogenic and osteogenic markers SOX9 and RUNX2. Interestingly, uniaxial cell alignment in the ST pattern seemed to increase the expression of SM22α and smooth muscle α-actin, suggesting an early smooth muscle lineage progression. These results indicate that geometric cues that promote uniaxial alignment might be more potent for myogenesis than those with increased peak stress. Overall, the use of these patterned geometric cues for modulating cell alignment and form-induced stress can serve as a powerful and versatile technique towards controlling differentiation in ADSCs.

1. Introduction

Over the past decades, significant advances in tissue engineering have led to numerous promising approaches and potential therapies for regenerative medicine [1]. The use of stem cells has become a cornerstone in myriad tissue engineering applications, and the rapid development of methods for sourcing stem cells has largely aided this progress. In particular, adipose-derived stem cells (ADSCs), due to their differentiation potential and the relative ease and abundance with which they can be sourced, have attracted much attention within the field. ADSCs can differentiate into a variety of cell lineages, such as smooth muscle cells, chondrocytes, osteoblasts, and neurons [2–9]. Given the powerful utility of ADSCs, the development of methods for appropriately manipulating these cells *in vitro* – and eventually *in vivo* – is pivotal to their adoption in tissue engineering applications. In this work, we studied the differentiation of ADSCs on microfabricated surfaces that feature various geometric patterns to modulate cell alignment and thereby induce different cellular stresses.

Most studies investigating the cellular response of ADSCs to external cues have focused on the use of biochemical signals. However, mechanotransduction events involving cell–cell and cell–material interactions can play a significant role in directing cell activity and fate [10,11]. For instance, Engler et al. used polyacrylamide gels to show that variations in substrate stiffness can induce MSC differentiation towards corresponding cell lineages [12]. The C. S. Chen group demonstrated that endothelial cells can form proliferation patterns that correspond to the internal stresses provided by various geometries designed to direct cell adhesion

* Corresponding author. Department of NanoEngineering, SME Building, MC-0448, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0448, USA.
E-mail address: shc064@eng.ucsd.edu (S. Chen).
[1] These authors contributed equally to this work and should be considered co-first authors.

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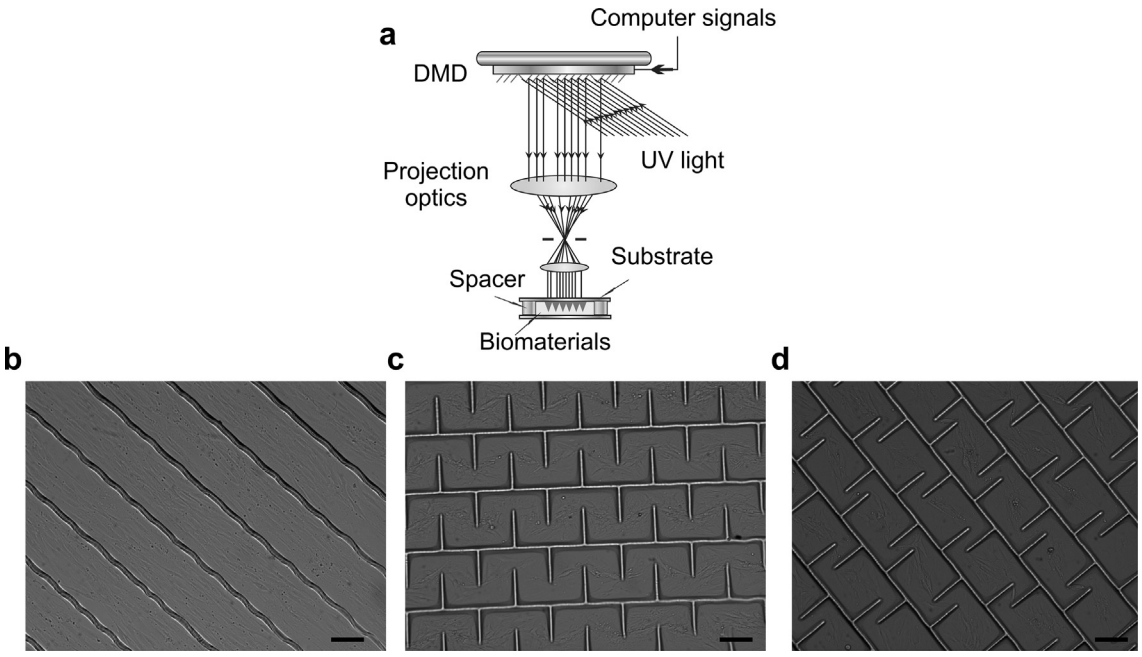

Fig. 1. Schematic view of the DOPsL system and fabricated structures. a) Schematic view of the DOPsL fabrication platform. b–d) DIC images of ADSCs seeded on patterned structures with 20% PEGDA. b) Stripes pattern (ST), c) symmetric fork pattern (SF), d) asymmetric fork pattern (AF). Scale bars are 100 μm.

[13]. They also showed that – in comparison to MSCs that exhibit spheroid morphology – MSCs with a confined cell growth area up-regulate the expression of chondrogenic markers, whereas myogenesis was enhanced with normally spread morphology [14]. Furthermore, independent of soluble factors, osteogenesis was promoted in MSCs cultured on substrates with geometric cues designed to enhance cellular contractility [15]. Others have demonstrated that the expression of muscle-specific genes can be induced in stem cells by multicellular forms that promote uniaxial cell alignment [16]. Interestingly, a recent study by Munoz-Pinto et al. suggested that uniaxial alignment can induce more myogenesis in multipotent mouse stem cells than those with increased cell-substrate stress [17]. However, despite the demonstrated influence that mechanical and geometric cues can have on directing cell fate, to the best of our knowledge the number of studies that have translated these findings to ADSCs has been limited.

Recently, our group developed a mask-free microfabrication method based on dynamic optical projection stereolithography (DOPsL) [18]. Compared to other widely used patterning methods, such as micro-contact printing and conventional lithographic approaches, our system provides greater flexibility in direct printing 3-diemnsional (3D) structures and better biocompatibility for biological materials [18–20]. In this work, we utilized this DOPsL platform to create three designed multicellular forms and further studied the early stage differentiation of ADSCs on microfabricated surfaces to determine the relative impact of uniaxial cell alignment versus form-induced stress.

2. Material and methods

2.1. Scaffold fabrication

Poly(ethylene glycol) diacrylate (PEGDA, Mn = 700), 2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO, free-radical quencher) and 2-Hydroxy-4-methoxy-benzophenon-5-sulfonic acid (HMBS) were purchased from Sigma–Aldrich. Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) photoinitiator was prepared as previously described [21]. Pre-polymer solution was prepared as follows. PEGDA (20 wt% Mn 700, Sigma–Aldrich) was mixed with PBS, LAP (1 wt%) and Tempo (0.01 wt%). The mixture was vortexed (5 min) and sterilized by using a 0.22-μm pore size syringe filter. The PEGDA solution was sandwiched between a methacrylated coverslip and a glass slide holder with a PDMS spacer (50 μm height). Samples were fabricated by 4 s of UV-light (365 nm) exposure using the DOPsL bio-fabrication system (Fig. 1a) [18].

2.2. Cell cultures

ADSCs were purchased from Lonza and cultured according to the protocol provided by the vendor using ADSC™ Growth Media BulletKit™ (Lonza). All experiments were carried out using ADSCs from passages 2 to 5. The cells were harvested and counted based on the general protocol and then seeded onto the scaffold with growth media. Media were initially changed one day after seeding and then refreshed every other day. To assess cell differentiation, the cultures were maintained for 7 days in a 37 °C incubator with 5% CO_2.

2.3. Immunofluorescent staining

Cells were fixed after 7 days of culture and stained for differentiation markers and nuclei. 4% Paraformaldehyde (Electron Microscopy Sciences) was used to fix the cells on the scaffold for 30 min at room temperature, and followed by permeabilization with 0.1% Triton X-100 (Sigma–Aldrich) in PBS with 2% bovine serum album (BSA, Fisher Scientific) for 60 min. The cells were then exposed to 1:100 diluted primary antibodies (smooth muscle a-actin, Novus Bio; SM22α, Santa Cruz; Runx2, Abnova, Sox9, Sigma; PPARγ, Cell Signaling) at 4 °C overnight. Secondary antibodies with fluorescent labels (goat anti mouse-DyLight 488, JacksonImmuno; goat anti rabbit IgG-CF594, Biotium) were applied to samples for 1 h at room temperature. Nuclei were counterstained with Horchest 33258 DNA dye (Invitrogen). Fluorescent images were taken on Leica DMI 6000-B Microscope.

2.4. Finite element modeling of form-induced stress

The cell-substrate stress distributions induced by the three different patterns were modeled using COMSOL Multiphysics according to previously reported model [13,17]. In brief, a three-dimensional finite-element model of the cell-substrate interaction was constructed with a contractile layer and a passive layer using previously reported physical parameters. The cell layer was represented by the contractile layer with a height of 20 μm, a Young's modulus of 500 Pa, a Poisson's ratio of 0.499, a thermal conductivity of 10 Wm^{-1} K^{-1} and a coefficient of expansion of 0.05 K^{-1}. The passive layer was intended to represent the pendant protein chains adsorbed onto the substrate with a height of 4 μm, a Young's modulus of 100 Pa, and a Poisson's ratio of 0.499 [13,17]. Since the surface of the substrate is very rigid, the bottom of the passive layer was treated as fixed constraint. In all simulations, a mesh of free tetrahedral was built with element sizes of 2–5 μm. To simulate the monolayer cell contraction, a thermal strain was induced by a temperature drop of 5°K. The maximum principal stress at the fixed bottom surface was determined.