U937 cells readily undergo differentiation into macrophages. U937 cells were induced to differentiate with the addition of 100 nM PMA in the culture medium for 48 hours. Prior to differentiation, U937 cells have rounded morphology and are non-adherent (Panel A). Fully differentiated cells are larger in size and adhere to the culture dish (Panel B).

**Results**

U937 cells can successfully differentiate into the macrophage lineage upon the addition of PMA. U937 cells can be laser manipulated into desired positions in hydrogel. Reporter systems easily monitor differentiation in real-time in hydrogel. Cells in 3.4 kDa and 20 kDa hydrogels show similar viability. Methodologies for placement of MSCs into a 3D scaffold are being established.

**References**


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**Abstract**

Mesenchymal stem cells (MSCs) have been targeted for use in cell-based therapies, regenerative medicine, and tissue engineering. For MSCs to be utilized in these applications, further elucidation of environmental and biochemical stimuli directing differentiation is needed. A limitation to studying directed MSC differentiation is that traditional culture systems represent a heterogeneous population. The objective of this study is to understand specific environmental cues and signals directing differentiation. To achieve this goal, we created a three-dimensional poly (ethylene glycol) diacrylate (PEDGA) based microenvironment that supports cell survival and signal transduction. PEDGA environments allow transport of small molecules, demonstrate biocompatibility, and are easily modified. Laser tweezers manipulate cells and place them in any desired location. Coupled with photopolymerizable hydrogels, they provide control of the environment by specific placement of cells and signals. U937 cells were used to develop hydrogel microenvironments since they are easily differentiated into macrophages by small molecules (phorbol 12-myristate 13-acetate; PMA). Visual monitoring systems utilizing fluorescent reporter vectors were created to analyze cellular activity by transfecting a GFP reporter gene controlled by a CMV promoter. Differentiation was monitored by transfecting a DrRed gene driven by PMA induced TNF-α and osteopontin promoters. RT-PCR analysis shows that 50 nM PMA stimulation results in upregulation of TNF-α and osteopontin expression within 2 and 4 hours, rapidly detecting differentiation. Optimal microenvironments have adequate PEDGA pore size, molecule diffusion, and minimal cell death from UV exposure, photoinitiation, or laser manipulation. Microenvironments were optimized for placing U937 cells in 5%-10% of 400 mW or 3.4 kDa hydrogel with 0.1%-0.2% photoinitiator and polymerized with UV light for 10-15 seconds. Fluorescence microscopy analysis of GFP expression and Molecular Probes Live/Dead kit indicate that cellular metabolic activity was optimized with 5%-3.4 kDa PEDGA, 0.2% photoinitiator and 15 seconds UV exposure. Lower PEDGA concentrations (5%-3.4 kDa) displayed higher metabolic activity (45%) compared to 6-10% PEDGA concentrations (30%) 48 hours after trapping in hydrogel. These data demonstrate that U937 cells remain metabolically active in hydrogel for sufficient time to monitor differentiation. After development of optimal microenvironments, cells were manipulated with laser tweezers into 3x3 arrays and monitored for cellular activity. One hour after trapping, all cells remained metabolically active. After trapping, cells remained 55% metabolically active after 48 hours, reflecting similar metabolic activity to non-arrayed cells in hydrogel (45%) and reflecting that laser manipulation does not affect cellular activity. Lastly, differentiation in hydrogel was demonstrated by adding 50 nM PMA to the culture medium and monitored by visualization of DrRed fluorescence. After complete optimization of microenvironments using U937 cells, MSCs will be encapsulated in hydrogel with lineage-specific differentiation signals. In conclusion, we have created a cellular microenvironment system where the use of laser trapping in conjunction with a PEDGA microenvironment provides a platform for studying directed differentiation of MSCs.

**Introduction**

Multipotent adult mesenchymal stem cells (MSCs) have a potential of treating several diseases via cell based therapies. A rate limiting step to obtaining full clinical potential of MSCs is obtaining a homogeneous population of differentiated stem cells to inject in the patient. Stem cell differentiation has been well established in human culture, however cell signals in this environment are heterogeneous. Signals from surrounding cells, other cell types, and other unknown factors released into the culture medium can affect differentiation. To study cellular differentiation on a single-cell basis, we have created a three dimensional cell culture system where cells and signals can be placed in desired locations with sub micron precision via laser manipulation. Laser trapping and hydrogel environments for cell culture and differentiation are being optimized with the human lymphocytic cell line U937. We chose U937 cells because they can endure severe culture conditions, and are easily and visibly differentiated into the macrophage lineage. Hydrogel microenvironments are composed of poly (ethylene glycol) diacrylate (PEDGA). Signals to induce differentiation can be presented to the cells in several manners. Bathing the hydrogels in media containing differentiation signals, placing signals in microparticles that can be manipulated to release signals at desired times, and placing cells secreting signals into the environment. Laser and culture systems have been optimized using U937 cells, and we are now further investigating U937 cell signaling and modifying parameters for porcine MSCs. At the conclusion of this study we will establish methodologies for trapping and differentiating cells in a 3D scaffold for investigating cellular differentiation.

**Materials and Methods**

**Vector Construction and Cell Transfection**

- The pEGFP-N1 plasmid was transfected into cells as described.
- TNF-α and OPN-DrRed constructs were created by excising the CMV promoter from the plasmid.
- The pEGFP-N1 plasmid, co-transfected with plasmids expressing GFP and the transfection mixture was amplified by PCR with primers containing restriction sites for cloning into the pEGFP vector.
- Plasmids were transfected into U937 cells with the Amaxis nucleofection kit V.

**Laser Trapping**

- U937 and MSCs were trapped in a 3x3 array using a ZeissNeo-Fluor 100X, 1.25NA objective at 1X900 with a time-averaged power per trap at the sample of 8mW/trap.

**Hydrogel Formation**

Cells were trapped in 5-10% 400mW, 3.4kDa or 20 kDa poly (ethylene glycol) diacrylate solution with 0.1%-0.2% 2-hydroxy 2-methylpropiophenone as a photoinitiator. Hydrogels were crosslinked by exposing solution to a focused UV beam at 6mw/cm2 for 5-20 seconds.

**U937 Differentiation**

Cells were washed in PBS and reseeded in RPMI 1640 supplemented with 10% FBS at a concentration of 1X10^6 cells/ml.