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Cell patterning without chemical surface modification: Cell–cell interactions between printed bovine aortic endothelial cells (BAEC) on a homogeneous cell-adherent hydrogel

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Abstract

Cell printing offers the unique ability to directly deposit one or multiple cell types directly onto a surface without the need to chemically pre-treat the surface with lithographic methods. We utilize biological laser printing (BioLP™) to form patterns of bovine aortic endothelial cells (BAECs) onto a homogeneous cell adherent hydrogel surface. These normal cells are shown to retain near-100% viability post-printing. In order to determine whether BAECs encountered shear and/or heat stress during printing, immunocytochemical staining experiments were performed to detect potential expression of heat shock proteins (HSP) by the deposited cells. Printed BAECs expressed HSP at levels similar to negative control cells, indicating that the BioLP process does not expose cells to damaging levels of stress. However, HSP expression was slightly higher at the highest laser energy studied, suggesting more stress was present under these extreme conditions. Printed BAECs also showed preferential asymmetric growth and migration towards each other and away from the originally printed pattern, demonstrating a retained ability for the cells to communicate post-printing.
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Keywords: BioLP; Cell patterning; Cell printing; Tissue engineering; BAECs

1. Introduction

Chemical surface modification is often used to form two-dimensional patterns of cells [1–4]. Surface modification techniques, such as photolithography and various stamping or soft lithography approaches, form adjacent cell adhesive and non-adhesive molecular patterns on a surface [5–11]. Cell patterns are then formed via cell–surface interaction by exposing the chemically modified surface to a concentrated cell solution. High definition lines and microarrays of cells with resolution spanning from several cell diameters to a single cell have been formed. Many experiments utilize these surfaces to investigate various cell–surface and cell–cell interactions and to determine the surface's role in cell growth, adhesion, migration, and differentiation [12–15].

With the onset of various methods to print mammalian cells, it is now possible to directly form cell patterns without the aid of chemical surface modification [16–23]. As an added benefit, these printers can often form three-dimensional cell patterns, which is necessary for future tissue engineering applications such as layer-by-layer printing of heterogeneous cell constructs. For example, a modified ink jet apparatus has shown the ability to print large cell aggregates into three-dimensional patterns that then fuse to form a unified shape [22]. Recent studies, however, suggest that up to 10% of the cells may be lysed during ink jet deposition due to heat and/or shear stress present during printing [23]. Other techniques rely upon syringe-like micropens to deposit scaffold/cell suspensions that then solidify into pre-designed shapes after printing [21].

BioLP, or biological laser printing, has demonstrated the ability to directly form cell patterns (single cell to multiple cell/spot resolution) in both two and three dimensions as well as creating heterogeneous, or multi-cell type, patterns [17,18]. However, laser printing studies to date have used carcinoma cell lines. The carcinoma cells used in these studies are all continuous cell lines that have undergone genetic and phenotypic changes, and therefore may not represent the in vivo condition ("The

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QIAGEN Transfection Resource Book", qiagen.com) [24,25]. One example of this is the characteristic ball-shaped aggregate growth of printed carcinoma cells rather than extension, migration, and differentiation often observed with primary cells [16,17]. In addition, carcinoma cell lines are more robust than primary cells, presenting the question as to whether primary cell lines could survive the potentially damaging shear stress that may be present during a BioLP experiment [17].

Studies detailed in this article extend upon previous BioLP experiments by directly forming viable patterns of primary bovine aortic endothelial cells (BAECs) onto homogeneously cell adherent surfaces. For these cell patterns, we find asymmetric growth and migration that acts to linearly join printed spots of cells originally spaced over 100 μm apart. This result indicates that printed BAECs retain their ability to communicate and interact with their neighbors positioned several cell diameters away, a crucial requirement for cells seeded into scaffolds for tissue engineering applications. In order to determine the level of potential heat and/or shear stress encountered by the cells during the printing process, heat shock protein (HSP) expression by the cells is monitored over a wide range of laser energies from threshold (below which no cells are printed) to 10 times this threshold. At all laser energies, HSP expression by printed BAECs is small and similar to that of the negative control cells. However, expression of HSP is slightly higher under the most extreme experimental conditions (10 times energy), indicating that HSP expression may be a good marker to determine the level of stress endured by cells during future printing experiments.

2. Materials and methods

2.1. Cell culture

Normal bovine aortic endothelial cells (lot #1065) from Cell Applications, Incorporated (San Diego, CA) were cultured in BAEC Growth Medium (Catalog number B211-500 Lot 180) mixed with BAEC Supplemental Growth Medium (10% final concentration) in a humidified 37 °C, 5% CO_2 incubator. Media was changed every 2–4 days. Cells were grown to 70% confluence before splitting or harvesting for printing. Cells in passage numbers 2–4 were used for all printing experiments. To prepare cells for printing, centrifuged cells were decanted and re-suspended in an equal volume of 50% BAEC medium, 45% BAEC supplemental growth medium, and 5% glycerol. The final cell concentration used for the printing experiments was $\sim 10^8$ BAECs/mL. The resulting cell solution was homogeneously spread onto the target at 2 μL/cm^2.

2.2. BioLP

As shown in Fig. 1, BioLP uses laser pulses (MPB Technologies PSX-100 Excimer Laser, 248 nm, 2.5 ns FWHM, E_{max} = 5 mJ, rep rate = 0.1–100 Hz) focused onto a support (target) to transfer small aliquots of biological material, in this case a concentrated solution of BAECs, from the target to a receiving substrate. The target is an optically transparent quartz plate coated on one side with a thin (10–100 nm) ultraviolet absorption layer usually formed from a metal or metal oxide. The opposing side of the metal layer is coated with a 10–100 μm thick layer of BAEC cells and media (see cell culture section above for details). When a focused UV laser pulse is directed at the target-absorption layer interface, the metal layer absorbs greater than 99.9% of the laser energy, converting the photon energy into thermal and/or mechanical energy [17]. Previous studies used Comet assays to determine that even when greater than 90% of the incident laser energy penetrates through the target to the cells, the cell–laser interaction does not induce significant single or double strand breaks in the intracellular DNA [19]. In the present studies, the energy imparted to the absorption layer results in a small aliquot of the cell solution to be propelled away from the target towards the receiving substrate. Adjusting the energy and spot diameter of the incident laser pulse controls the diameter (30–300 μm) and volume (hundreds of fL to several nL) of the deposited aliquot. The current rate of transfer is variable up to 100 spots per second, but could be upwards to thousands of spots per second with faster repetition rate lasers. The BioLP resolution is

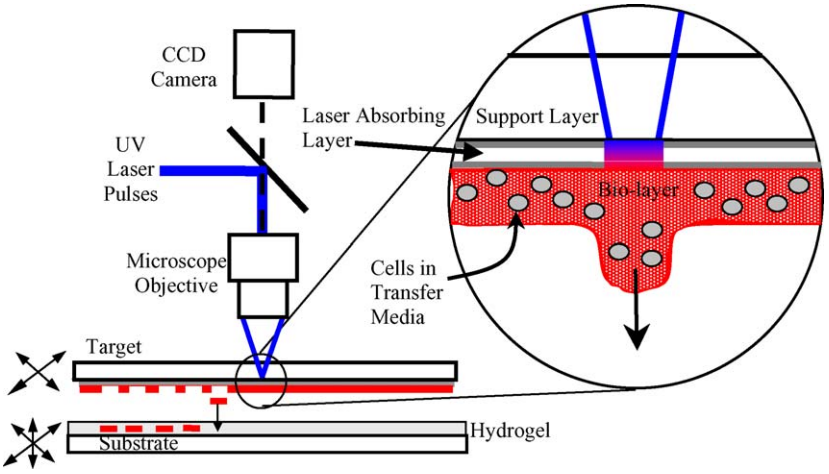

Fig. 1. Schematic of the BioLP apparatus. Details for laser printing mammalian cells to a hydrogel surface.