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FABRICATION OF HYBRID CELL-MICROBEAD CONSTRUCTS USING LASER DIRECT-WRITE OF ALGINATE MICROBEADS AND ADHERENT BREAST CANCER CELLS

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INTRODUCTION

Microbeads are becoming popular tools in tissue engineering as 3D microstructure hydrogels. The gel nature of microbeads enables them to sequester soluble factors and mammalian cells, and their high surface area-to-volume ratio allows diffusion between the bead and the environment [1,2]. Microbeads are thus good systems for drug delivery and can serve as 3D microenvironments for cells. To fully maximize their potential as delivery systems and microenvironments, it is highly desirable to create spatially-precise hybrid cultures of microbeads and mammalian cells. Precise placement of microbeads in proximity to patterned cells will allow the study of spatial cellular interactions, paracrine signaling, and drug delivery.

One of the most widely used materials for microbead fabrication is alginate, because it is biocompatible and degradable, and both the mechanical properties and permeability can be tuned [3]. Alginate microbeads can encapsulate cells or drugs [1,2], and the permeability of the bead allows drug to slowly diffuse out, and nutrients to diffuse in, supporting encapsulated cells.

Alginate bead fabrication typically involves alginate dropped or moved into a solution of calcium chloride [4,5]. Immobilization of the bead can be accomplished in a separate step, but this step is difficult and cannot precisely immobilize single beads. To date, microbeads have not been immobilized in a controlled pattern on the same planar substrate as viable, adherent cells.

To address these limitations, the aim of this study was to precisely place both microbeads and cells on the same planar substrate using laser direct-write (LDW). LDW has previously been utilized to pattern cells [6]. We hypothesized that the addition of calcium chloride on the printed substrate would enable the serial printing of cells with laser direct-write, followed by the placement of microbeads by calcium crosslinking of LDW-printed alginate on the same planar substrate. This method would ultimately lead to the creation of hybrid cell-microbead constructs.

METHODS

Cell maintenance:

GFP-labeled MDA-MB-231-gfp (M231) human breast cancer cells (ATCC, Manassas, VA) were grown in Dulbecco's modification of Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum and 2 mM L-glutamine. Cells were maintained in a cell culture incubator at 37 °C, 95% humidity, and 5% carbon dioxide.

Laser direct-write:

A gelatin-based laser direct-write (LDW) technique [6] was adapted to deposit cells and alginate microbeads on polystyrene Petri dishes (**Figure 1**). Briefly, a PLL-coated Petri dish was spin-coated with 10% gelatin/DMEM to serve as the receiving substrate for LDW. After refrigeration at 4 °C to allow the gelatin to harden, the dish was washed with 1% calcium chloride/DMEM and placed in a cell culture incubator.

M231 cells were trypsinized and resuspended in media for cell printing, or 2% alginate for microbead fabrication. To prepare "print ribbons", UV-transparent quartz disks (Edmund Optics, Barrington, NJ), were spin-coated with 2% alginate/10% gelatin/cell culture-grade water for microbeads and 20% gelatin/DMEM for cells. Cell suspensions were placed on the respective ribbons, and excess media or alginate was removed. Cells and beads were printed serially, with each ribbon placed in the path of a 193 nm excimer laser (Teosys, Crofton, MD) pulse. The ribbons were placed 600 μm from the receiving substrates, and computer aided design/computer aided manufacturing (CAD/CAM) capabilities of the system allowed programmed movements of the stages for precise printing. After

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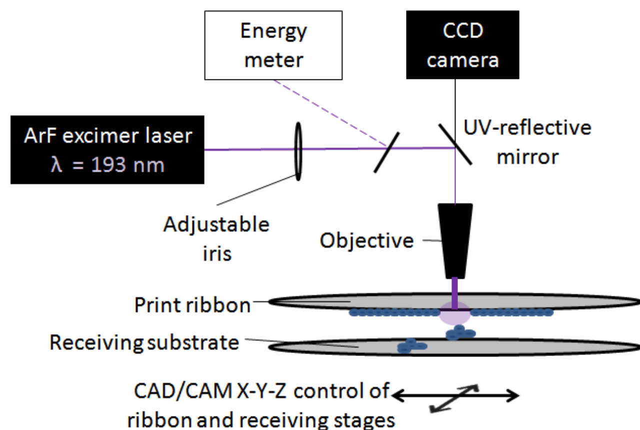

Figure 1. LDW schematic for cell deposition and bead fabrication. To use these techniques serially, the print ribbon was replaced with one loaded with a new material.

printing and a 15 minute incubation at 37 °C, media was added to the printed cell/bead system for cell survival.

Image acquisition:

Images of cell and microbead patterns were captured using a Zeiss Z1 microscope with Axiovision software (Carl Zeiss, Thornwood, NY).

RESULTS

We have demonstrated that alginate microbeads can be laser-fabricated and placed in the same controlled pattern as viable mammalian cells patterned by LDW. After LDW of viable M231 cells, alginate was printed to form microbeads in a single step on the same substrate. Cells and beads were printed by LDW in a "checkerboard" pattern, with each printed spot of cells adjacent to a printed bead (**Figure 2**). Microbeads were consistently formed with diameters of 100 µm, and remain visible in the pattern after three days. Over time, cells attached and spread out, and they continue to express GFP (**Figure 2c**). Beads remained immobilized in the printed pattern.

DISCUSSION

The survival of printed cells and formation of alginate microbeads on the same substrate is an important advancement over LDW of cells alone, allowing many new studies based on cellular patterning. Previously, signaling to cells patterned by LDW was limited to paracrine signaling from other cells, chemical signals homogeneously distributed in the media, and mechanical signals from the substrate.

The ability to control the placement of microbeads in a precise location relative to printed cells allows microbeads to be used as point chemical sources. Because discrete chemical sources could not previously be placed with respect to the printed pattern, it was not possible to investigate the effect of point sources on migration, differentiation, or cellular protein expression in LDW patterns. With this technique, delivery of growth factor to only a certain subset of a cell population is possible. Microbeads placed within a cell pattern can release molecules, and this release profile is typically one of exponential decay. Encapsulation of cells that secrete a desired factor allows steady-state molecule release, provided long-term survival of encapsulated cells. Thus, placement of microbeads and cells on the same substrates allows many more parameters of cell signaling to be studied.

With this method, microbead fabrication and placement occurs in a single step due to the alginate immediately cross-linking with

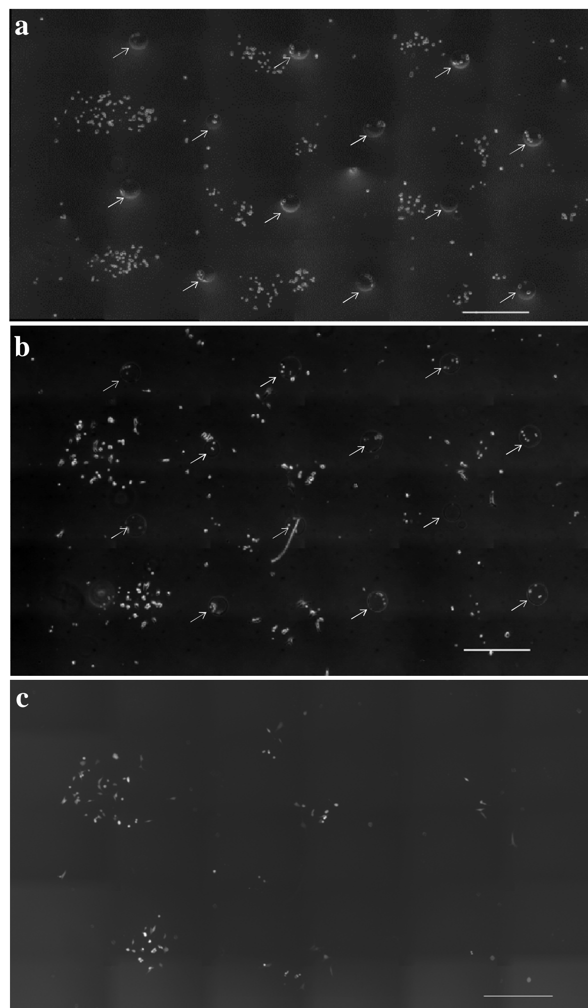

Figure 2: Pattern of cells and beads in a "checkerboard" pattern (a) immediately after printing and (b-c) 3 days after printing. Beads are marked (c) Cells continue to express GFP after 3 days, indicating viability. Scale bar is 500 µm.

calcium chloride on the substrate upon printing. We were able to combine bead formation with LDW of cells by washing the receiving substrate with 1% calcium chloride solution. This proved gentle enough for cell survival, yet sufficiently concentrated to enable bead formation. While cells were encapsulated in microbeads for this study, this technique is easily adapted to also fabricate microbeads with drugs or molecules sequestered in the alginate, allowing a wide-range of applications in tissue engineering and regenerative medicine.

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