Summer Scholar Report

An Instrument for Dispensing and Patterning Single Microbeads

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Abstract

We present an instrument for the placement and patterning of single microbeads by dispensing. We dispense these beads using a borosilicate glass micropipette, fabricated using a customized micropipette puller, which is visualized in a custom-fabricated upright microscope. This microscope has a motorized sample stage, which enables reproducible patterning in two dimensions. Our instrument has the potential to array microscale constructs at a resolution not attainable by commercial microarraying equipment, and has substantial implications for biological studies and tissue engineering.

Introduction

Since the invention of microarrays in the 1980s, microscale deposition of biological material on solid substrates has enabled myriad studies of human health. In studies of single cells, arrays are typically created by controlling surface chemistry (e.g., microcontact printing of cell-sized adhesive zones on a culture material[1]) or surface topography (e.g., fabrication of microwells for single-cell capture[2]). These approaches effectively capture cells for analysis, but do not allow for real-time control during the patterning process. Array geometry is determined prior to patterning, as these passive approaches rely on the probability of specific cell-substrate interactions, rather than active positional control of individual cells during the patterning process.

Active control of cellular position in real-time can be achieved by using an additive manufacturing approach. One type of additive manufacturing, 3D printing, has become widely accessible to hobbyists, educators, and scientists in recent years. Material extrusion is not only useful for the rapid fabrication of plastic parts, but for soft, biological objects as well. In bioprinting, a cell-laden ink or hydrogel is dispensed from a needle to produce biocompatible scaffolds. This approach can be used to create two and three-dimensional, hollow and solid products including whole organ replacements and mini-tissues, which are small functional tissue units that can self-assemble into a larger construct.[3] The biocompatibility of these components allows for successful integration into the body, as well as participation in natural biological processes (e.g., angiogenesis).[4] At present, applications of bioprinting range from hollow vasculature to whole kidney prototypes,[5] and this approach is only beginning to be explored.

While the extrusion of cell-laden inks holds great promise for many biological and health-related problems, the field of bioprinting is currently limited by the availability of materials that are printable, as well as the resolution at which these materials can be printed. To facilitate successful bioprinting, the selected material must have specific rheological and crosslinking properties, while also being sufficiently biocompatible.

For these reasons, bioprinting inks are typically limited to collagen, hyaluronic acid, alginate, modified copolymers, photocured acrylates,[5] and ECM (extracellular matrix) mimics such as Matrigel.[6] Depending on the cost, technical features, and type of bioprinting system used, the resolution at which these materials are printed can range from microns to millimeters. In order for the capabilities and clinical relevance of bio-printing to advance, limitations on both size resolution and material selection must be lifted.

One potential solution to these problems is to eliminate the need for carrier inks, and print wholly cellular microstructures one cell at a time. Single-cell resolution can be enabled by micropipettes, which have previously been used to isolate single cells from culture for subsequent analysis. These separations, as well as studies of single-cell mechanical properties by micropipette aspiration, are both enabled by fine control of fluid flow through the micropipette. A handful of commercially available systems[7] possess the machinery to perform single-cell arraying by dispensing, but are not conventionally used to do so. These instruments are very specifically designed to transfer single cells from one container (e.g., a culture dish) to another (e.g., a 96-well plate) for single-cell analysis. In addition to being highly specialized, these systems are also expensive, and often not available to academic laboratories.

To enable ink-free bioprinting with single-cell resolution, we have developed an instrument that allows for real-time visualization of a cell-dispensing glass micropipette positioned just above a culture surface. We use 10 μm polystyrene beads (Polysciences) as model cells to demonstrate the performance of the first iteration of our system. It is our belief that this instrument lays the foundation for high-resolution, ink-free bioprinting of 2D cell patterns and micro-tissues. In the future, a more refined version of this preliminary tool could be used to answer fundamental questions of tissue engineering and biology, specifically those related to cell communication and tissue formation.

Results and Discussion

We constructed an upright microscope to allow for visualization of bead dispensing during experiments (**Figure 1**). Using a milling machine, we fabricated a custom aluminum plate to connect a large optical post to an existing microscope base (Leica). This base contained a brightfield illumination source, and also held the motorized XY

stage (Leica) used for manipulation of the culture surface. Next, we connected a microscope carrier with manual focus controls (Olympus) to the optical post, and used a custom 3D-printed (MakerBot) adapter to attach a DIN microscope tube (Edmund Optics) to the carrier. This tube enabled mounting of the objective lenses (Olympus, Edmund Optics) and camera (Motic).

After development of the imaging system, we added micropipette equipment to enable dispensing experiments. We attached an aluminum collar to the optical post, and used it to connect a 3D-printed arm used to hold the micropipette holder (Warner Instruments) in place. The printed arm held the micropipette holder at a 30° angle relative to the sample stage, which allowed for adequate visualization of the pipette tip inside the sample container, which is a standard Petri dish. This micropipette holder was connected to a syringe using plastic tubing (Nalgene) and luer lock fittings (McMaster-Carr). A syringe pump (Chemyx) was used to control fluid flow through the micropipette. After completion of the instrument, we used custom glass micropipettes to demonstrate the capabilities of our tool.

To fabricate our micropipettes, we used a custom-modified micropipette puller. Micropipettes are created by con- trolled heating and pulling of hollow glass capillaries. The heating element is usually made of platinum, and pullers are offered in vertical and horizontal configurations. In vertical pullers such as ours (Narishige), which can produce a wide variety of tip geometries, pulling force is controlled by adjusting the pull distance and pull mass. Commercial vertical pullers come with a set of masses that are connected to the bottom of the glass capillary in different configurations to achieve different tip geometries. These masses are heavy and only come in two sizes (i.e., 23 or 92 g), precluding fine control over pulling force.

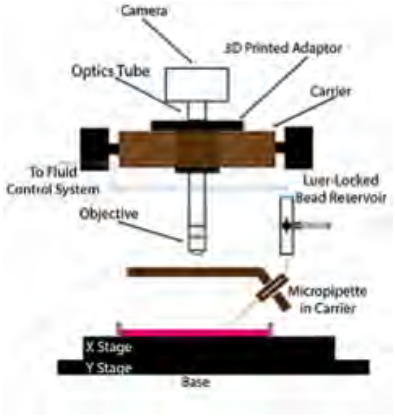

Figure 1*: Schematic of the microbead-dispensing instrument. An upright microscope is used to visualize the bead-filled glass micropipette, which is driven by a syringe pump. A motorized XY stage is used to manipulate the sample container, facilitating reproducible bead patterning.*

For dispensing experiments using 10 μm polystyrene beads, we sought to produce pipettes with a 10-30 μm inner diameter. We attempted to fabricate these pipettes by a two-step pull, in which the capillary is first allowed to fall a set distance (Distance 1) at one heater setting (Heat 1). Next, the heating element moves (by Distance 2) to the center of the hourglass shape formed by the first pull, and the filament is heated to a second setting (Heat 2). On the second pull, the pipette falls the full range of the puller, breaking the capillary at the center of the pulled, microscale section. To fabricate pipettes by the standard approach, we used the following settings—Distance 1: 1 mm, Distance 2: 2 mm, Heat 1: 70 V, Heat 2: 60 V—and a total pulling mass of 117 g.

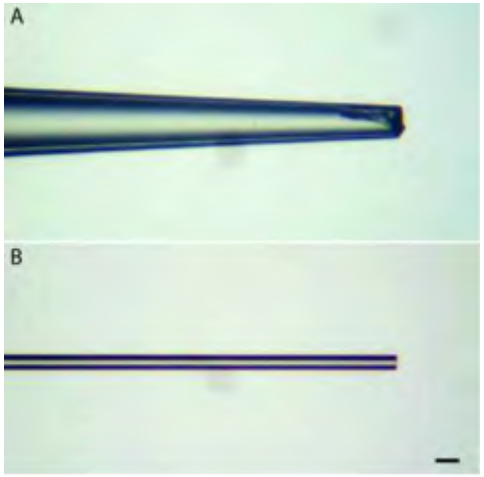

Figure 2: *Micropipettes produced by standard protocol (A) and with custom weights (B). Scale bar is 50 μm.*

Using the standard weights of the device, we could not produce our desired geometries using the puller only. Pipettes pulled using the standard approach (**Figure 2A)** had ~ 50 μm diameters and required a secondary fabrication step known as microforging, in which the pipette tip is brought within proximity of a heated filament to melt and smooth imperfections that are a result of pulling. We used a microforge (Technical Products International) to fire-polish these pipettes and reduce their diameter to an acceptable size, but difficulties associated with microforging (e.g., tip distortion due to melting) led to irreproducible pipette geometries. To circumvent the issues associated with this time-intensive protocol, we fabricated custom weights to enable microforge-free production of 10- 30 μm micropipettes.

To facilitate the attachment of desired masses to the puller, we designed an aluminum piece that had the same mounting features as the manufacturer's weights, and also had a threaded hole (8-32) to which we could attach a rod. After fabrication of the aluminum piece, we attached 3D-printed weights, designed using commercial (SolidWorks) and

open-source (OpenSCAD) programs, to the adapter rod. We controlled the masses of 3D-printed pieces using different design volumes and infill densities. The threaded rod attached to the puller also facilitated the attachment of metal hardware (e.g., washers, nuts, standoffs) that could be used to adjust pull force with greater resolution than the manufacturer's weights. The final protocol for production of our pipettes was a two-step pull, which required both 3D-printed components and off-the shelf metal standoffs. For these pipettes, we used the following settings—Distance 1: 0.5 mm, Distance 2: 1 mm, Heat 1: 78-82 V, Heat 2: 68-72 V—and a total pulling mass of 350 g. By design, the pulled capillary was not separated into two pipettes by the puller. After the second pull step, we pulled the bottom half of the capillary downward by hand, breaking it away from the remainder of the capillary. The bottom pipette was saved for use, and the remainder of the capillary was discarded. Pipettes pulled by this approach (**Figure 2B**) produced 23 µm diameter pipettes (n=39), reducing the range in pipette diameter by 55 µm, from 73 to 18 µm, when compared to the conventional method (**Figure 3**). These micropipettes had flat tips, perpendicular to their parallel walls.

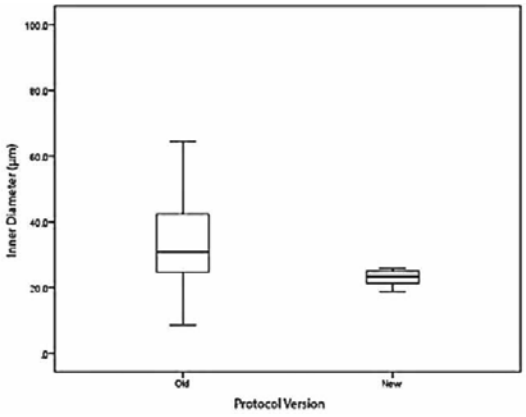

Figure 3: Box plot comparing micropipette diameters resulting from pulls performed using standard and custom equipment.

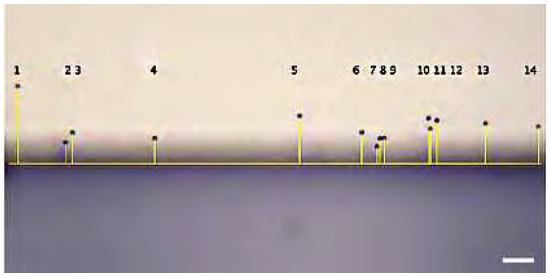

Figure 4: *Accuracy of bead placement was quantified by measuring the distance between dispensed beads and the intended patterning target. Scale bar is 100 µm. To acquire this image, a human hair was taped to the bottom of the sample container and used as a linear target.*

To demonstrate the capabilities of our instrument, we used our glass micropipettes to dispense 10 μm polystyrene beads onto a model culture surface. We designed and purchased a Mylar lithography mask (Advanced Reproduction Corp.) patterned with simple shapes (e.g., lines, circles, squares) to serve as a target for our dispensing experiments. The lines on this target were 25, 50, and 75 μm thick to allow for visualization in the microscope. We loaded a 10^5 bead/mL bead solution into the micropipette before mounting it to the holder, and used a flow rate of 1 μL/minute applied by a 3 mL syringe to drive beads out of the micropipette and onto the tar- get surface. Bead dispensing accuracy was quantified by image analysis (**Figure 4**). In our preliminary experiments, beads landed an average of 90 ± 10 μm from the intended culture surface feature, a line, and the total range of these distances was 156 μm.

Conclusions

In conclusion, we present an approach for high-resolution additive manufacturing of simple cellular microstructures. By developing a new bead dispensing instrument and method for the reproducible manufacture of glass micropipettes, we demonstrate substantial progress toward this goal. While this tool does not yet possess the accuracy or reproducibility necessary for ink-free bioprinting of cellular micro-tissues, future design iterations—including automation of the motorized stage and more precise control of bead flow through the micropipette—could hold great promise for fundamental studies in tissue engineering, cell signaling, and developmental biology.

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