

Direct-Write Construction of Tissue-Engineered Scaffolds

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ABSTRACT

A computer-controlled *xyz* dispensing system called the Biological Architecture Tool (BAT) has been extensively tested in the creation of multilayered and three-dimensional biological objects: tissue scaffolds and plain and patterned cellular-array slides. The BAT dispensing system has proven its versatility and reliability in tissue engineering and biological experiments. The potential employments of modified versions of the *xyz* dispensers for *in vivo* minimally invasive surgery and other *in vitro* aspects of biological and medical research are discussed.

INTRODUCTION

The modern tissue engineering (TE) community regards biodegradable supporting and shape-forming scaffolds as crucial parts of novel tissue constructs. The community acknowledges that in many cases the human body is unable to directly regenerate removed or lost tissue. Reconstructing the proper three-dimensional (3D) organization of cellular and extracellular elements is a necessity. Otherwise, the removed volume eventually will be filled with nonfunctional scar tissue, at best [1]. To fulfill successfully their structural and functional roles, tissue scaffolds should be highly penetrable to cells and vital fluids. Porous scaffolding constructs become a paradigm, which has stipulated the involvement of leaching and gas expansion techniques in the fabrication of scaffolds [2].

The *in vitro* construction of tissue scaffolds poses comparatively fewer problems. Molding and 3D-printing polymerization techniques allow the fabrication of sophisticated elements [3]. The injection of biodegradable plastics, hydrogels, and cellularized composites in bone reconstruction and orthodontic surgery can be regarded as simplified versions of *in vivo* scaffolding [2, 4]. However, the minimally invasive surgery (MIS) of the near future will inevitably require *in vivo* and *in situ* fabrication of complex, multicellular, spatially organized, and functional elements of tissue and organs [1, 5].

The study objective was to explore the potential of using the Sciperio Biological Architecture Tool (BAT) dispensing technology for future *in vivo* and *in situ* fabrication of tissue scaffolds and cell constructs.

EXPERIMENT

Poly(propylene fumarate) (PPF), poly(propylene fumarate-*co*-ethylene glycol) (PPF-PEG) hydrogel, and bis(2,4,6-trimethylbenzoyl)phenylphosphine oxide (BAPO) photoactivator were obtained from Rice University. Synthesis of these polymers has been described elsewhere [6]. Commercial sucrose sugar, in 200–500- μm and 5–30- μm grain-size brands, was used as leaching filler. A modified TL003 Facial Sunlamp (Saidel Inc., Renton, WA) was used as an ultraviolet

(UV) source for photoactivated crosslinking of PPF and PPF-PEG. The typical polymer paste used for scaffolding construction was composed of PPF or PPF-PEG containing 10 wt % dichloromethane (Aldrich Chemical, Milwaukee, WI) and 0.5 wt % BAPO. In most experiments, this paste was mixed with 50–80 wt % sugar filler. UV curing cycles for the paste layers were 30–60-s exposures under the TL003 lamp, which was held 15 cm above the samples to be cured. The Sciperio BAT *xyz*-dispensing system was supplied with ULTRA™ barrels, adapters, and dispense tips (EFD, East Providence, RI) and a rotational scanning system based on the Keyence LK Laser Displacement Meter (KEYENCE Corp. USA).

Laboratory-safe specimens of *Staphylococcus epidermidis* and *Escherichia coli* bacteria were obtained from Drs. Chang-An Yu and Kenneth Bartels (College of Veterinary Medicine, Oklahoma State University, Stillwater, OK). Human white blood cells (WBC's) supplied to Sciperio by Dana-Farber were drawn from healthy blood donors and fixed by 2% paraformaldehyde in phosphate-buffered saline (PBS). Fixed cells, approximately 10^7 per milliliter, were sedimented by mild spinning (2,500 revolutions per minute for 30 minutes in a benchtop preparative centrifuge), then mixed with aliquots of supernatant to the desired concentration.

RESULTS AND DISCUSSION

Three-dimensional images of parts of the human body, typically obtained by magnetic-resonance imaging (MRI) or X-ray tomography (XT), are ordinarily presented as arrays of “image slices.” In a reciprocal fashion, 3D objects can be recreated in similar layer-by-layer (LBL) mode. The Sciperio syringe-based dispensing system used in these experiments was originally designed for the construction of two-dimensional (2D) objects, whether linearly drawn or raster-filled. It was readily proved, however, that the same system could be successfully used for the LBL reconstruction of 3D objects if each layer was solidified before dispensing the next, as illustrated in Figure 1.

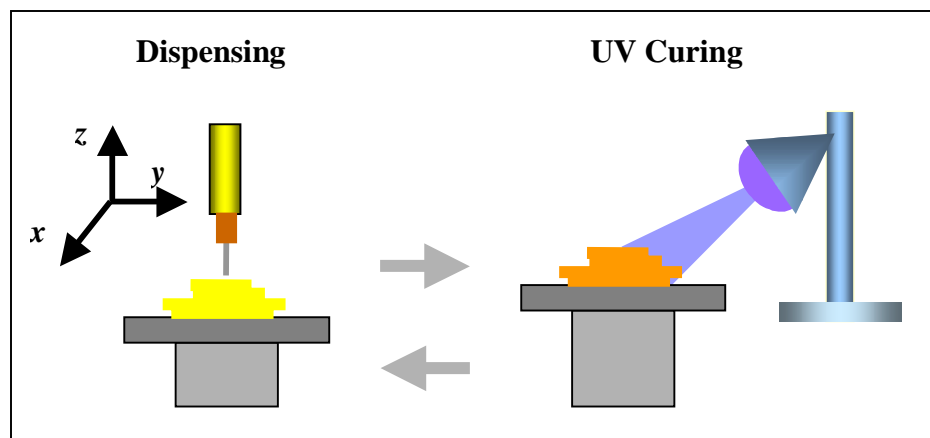


Figure 1: Layer-by-Layer Reconstruction of a 3D Object Using Photocurable Plastics

Physical model of the human external ear

A physical model of the human external ear was selected as tentative object to be reconstructed in LBL mode from biodegradable PPF paste as shown in Figures 2–3. This particular physical

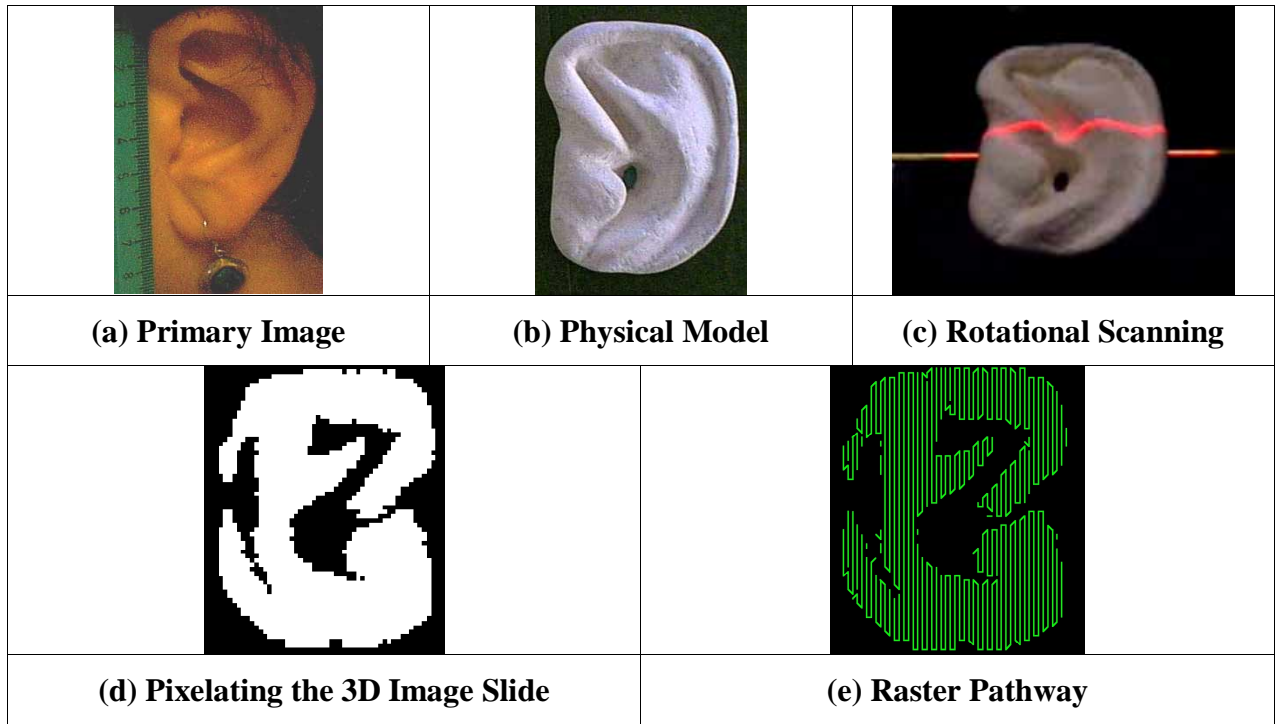


Figure 2: Modeling, Scanning, and Digitizing the Human External Ear

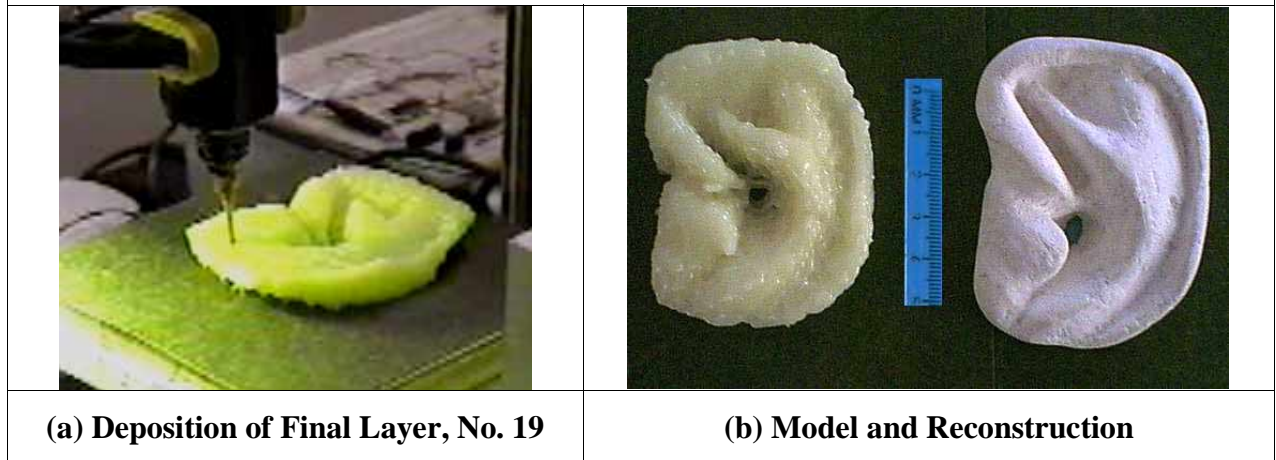









Figure 3: Reconstruction of the Human Ear Model

model was chosen for convenience; since the objective was to explore the recreation process in full, a technique of scanning and digitizing 3D objects was developed for the BAT. To preserve negative-angle elements, in which material overhangs the edge, an alternating raster-filling mode was used, in which the raster direction was turned 90° for every other layer. A total construct of 19 layers, each 0.8 mm thick, was mounted and UV-cured. The dispensing nozzle had a 1.4-mm inner diameter (ID). The paste was 40 wt % photocurable PPF precursor and 60 wt % fine-grained sugar. Although the time consumed by this first full-scale reconstruction experiment was unacceptably long (more than three hours), it still demonstrated ways to achieve faster and more accurate results.

However slow and awkward the LBL raster-filling technique currently appears, it promises to remain a basic feature of future MIS experiments, wherein small, newly formed cavities in the body of a surgical patient should be filled *in situ* with spatially organized, scaffolded tissue constructs. Switching from highly viscous plastic components to less viscous hydrogels can significantly increase deposition speed.

Deposition of bacteria

Experiments with nonhazardous laboratory brands of bacteria were undertaken to check and to prove the capability of the Sciperio xyz system to deposit cellular cultures in fine patterns, with delicacy and without contamination of undesired areas or surfaces. A fine capillary assembly with a 30- μm -ID orifice was used to provide accurate deposition. Mature cellular cultures were used as obtained, without additional manipulation. The company name “SCIPERIO” was used as the deposition pattern. Highly visible patterns were constructed by bacterial growth in a 37- $^{\circ}\text{C}$ incubator, as shown in Figure 4.

			0 hr
			12 hr
			36 hr
(a) Capillary Assembly Poised Over Petri Dish	(b) <i>Staphylococcus epidermidis</i>	(c) <i>Escherichia coli</i>	(d) Incubation Time
Figure 4: Fine-Patterned Deposition of Bacteria			

Deposition of human white blood cells

Experiments with human WBC’s were performed in pursuit of the objective of creating high-cell-density slides for the detection and analysis of rare cancer cells with Dana–Farber’s Rare Event Imaging System (REIS) [7]. For these purposes, it proved critically important to demonstrate the capability of a mechanical xyz deposition system reliably to create highly populated, monodisperse cell slides. The creation of monolayer cell samples with high areal densities is important to the efficiency of rare-event detection. Furthermore, the ability to form patterned slides suitable for further experiments and manipulations represented an autonomous interest.

Two types of experiments were conducted. In the first variant, cells were deposited directly onto plain glass slides (Corning) together with a gelatinizing agent. The cells were not attached to the slide surface, but rather became encapsulated within the gel sheet, which measured 10 mm by 10 mm in area, $\approx 25 \mu\text{m}$ thick, as shown in Figure 5.


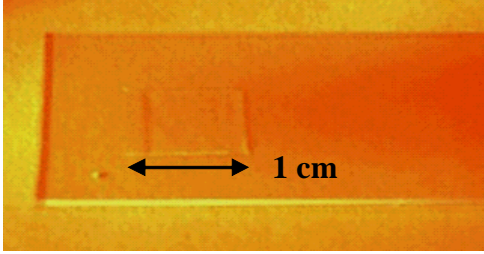
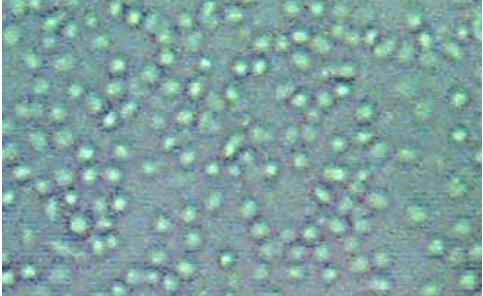
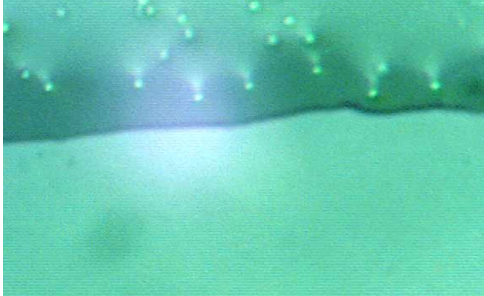
	
<p>(a) Sensoric Dispenser Depositing Encapsulated Cells in a Raster Mode</p>	<p>(b) Standard Gelatinized Slide, 10 mm × 10 mm × 25 μm</p>
	
<p>(c) Encapsulated Cells, $\approx 8 \times 10^5 \text{ cm}^{-2}$, Magnification 500×</p>	<p>(d) Edge of the Slide, Magnification 500×</p>

Figure 5: Direct Deposition of Human White Blood Cells

	
<p>(a) Patterned Slide Immediately After Deposition, Magnification 10×</p>	<p>(b) Same Slide After Six Successive Water Washes, Magnification 10×</p>
	
<p>(c) Selected Area Before Washing, Magnification 500×</p>	<p>(d) Selected Area After Washing, Magnification 500×</p>

Figure 6: Direct Deposition of Human White Blood Cells

In the second procedure, a concentrated suspension of fixed WBC's was deposited onto a specially prepared multilayer slide with a hydrophilic surface. After soft drying, the cells occurred firmly attached to the surface and remained there undisturbed after multiple extensive washings in PBS or water, as shown in Figure 6. The cell areal density was approximately $3 \times 10^6 \text{ cm}^{-2}$.

In the first experiment, the deposited cells formed a consistent monolayer (Figure 5(d)) that can provide reliable focusing and cell counting. In the second experiment, maximal cell density on the slide surface and firm cell attachment to the surface were successfully achieved.

Sensoric dispensers

The cornerstone of the experiments described above on cell deposition was the concept of the sensoric tool. This device is able to fulfill its function while simultaneously using an artificial sense of touch to maintain controllable contact with the surface. The idea was realized in Sciperio's proprietary designs for a sensoric quill-pen and capillary dispenser. These instruments eliminated concerns about roughness and curvature of the slide or scaffold surface, variability of the distance to the surface, the necessity of accurately positioning the flat slides, etc.

CONCLUSIONS

The experiments presented have demonstrated the promising capability of the Sciperio BAT prototype dispensing system in the creation of multilayer and two- or three-dimensional biological objects, including tissue scaffolds and cellular constructs. When modified and reconstructed for specific experiments and tasks, computer-controlled dispensing machines could become an integral part of a medical or biological laboratory.

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