Comparison of Bio-Inks for Free-Hand 3D Bioprinting Directly Onto Moving Human Anatomy

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INTRODUCTION

Advances in bioprinting have enabled synthetic tissue, organ, and skin construction via additive manufacturing techniques [1]. The most common bioprinting approach involves depositing hydrogel solutions embedded with bio-inks via pressure driven syringes [2] or via inkjetting [3]. Inkjet approaches are a viable alternative since they do not damage the cell yet permit high-speed control. Prior art has demonstrated the benefits of bioprinting for tissue engineering [4]. However, prior art has emphasized open loop deposition on planar, stationary surfaces. This is sufficient for laboratory settings where culture dishes are utilized or subjects can be sedated and the anatomy fixed. As envisioned in [5], some clinical settings may benefit from depositing bio-inks onto moving anatomy such as an unfixtured hand of a burn patient that must move during therapy to maintain range of motion for skin grafts. Alternatively, a hand-held precision bioprinting tool (Fig. 1 Top) may move relative to patient anatomy or be scaled down for use in laparoscopic surgery (Fig. 1 Middle) and contend with unpredictable anatomical motion.

The gap in prior art has been the demonstration of an additive manufacturing technique capable of depositing and adhering viable biomaterials directly onto unconstrained, non-planar, moving anatomy. The objective of this paper is to demonstrate the feasibility of robotically depositing and adhering bioprinting-compatible materials. We evaluated two bio-inks for their accuracy and adhesion during bioprinting directly onto moving human anatomy.

MATERIALS AND METHODS

As in [5], the Leap Motion (Leap Inc, Mountain View CA) was used to track the human hand at 120Hz and a Nordson EFD PICO Pμlse piezo jetting system was used to propel the fluid onto the hand when it was in the correct position. Here, the system ran on a Linux PC using the Robot Operating System (ROS), with a graphical display of the current position of the hand relative to the remaining pixels in the pattern, a suggested target highlighted (Fig. 2), and a height bar showing the acceptable distance from the hand to the jet (~1cm). The system allowed either the user to move their hand relative to the system, or an operator to move the system relative to the user's hand.

Two hydrogels were used. These hydrogels are biocompatible and are used as scaffolds for bioprinting [6]. The first consisted of Sodium Alginate that was deposited onto the hand, followed by an aqueous Calcium Chloride solution airbrushed onto the hand to crosslink the Alginate (as in [5]).

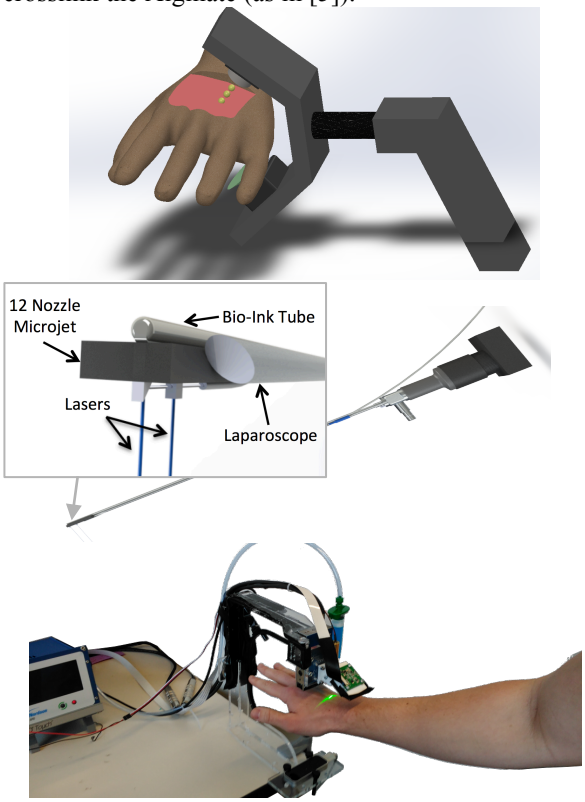

Fig. 1 Conceptual design of additive manufacturing directly onto moving human anatomy (Top). Conceptual printing device for laparoscopic surgery (Middle). Experimental setup, showing user's hand below the PICO Pμlse and above the Leap Motion (Bottom).

A second hydrogel was synthesized with deionized water containing 10% GelMA (gelatin methacrylate) and 0.5% LAP (lithium phenyl-2,4,6-trimethylbenzoyl-phosphinate) as a photoinitiator to allow use of a 405nm flashlight to crosslink between layers. This natural bio-ink is also proven to be compatible with a variety of cell types [7].

Blue food dye was used to color the hydrogels to allow for a computer vision-based evaluation of the 2D accuracy from scans of the finished gels on a flatbed scanner at 600 DPI (Fig. 4). The scans were then compared to the target template (Fig. 3) to determine

True Positive (TP), False Negative (FN) and False Positive (FP) areas. These values were then used to determine True Positive Rate (TPR), False Negative Rate (FNR) and False Discovery Rate (FDR) to provide metrics that were independent of the template image.

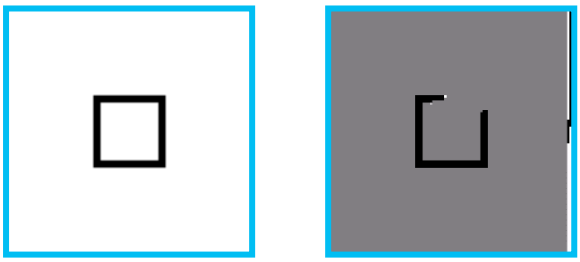

Fig. 2 The target template (Left) and an in-progress user view (Right). 1 pixel = 1mm width.

RESULTS

The robotic system was able to detect and track the subject's hands with sufficient speed. The user was able to ink at 1.8 pixels per second, leading to an average layer time of 182 seconds.

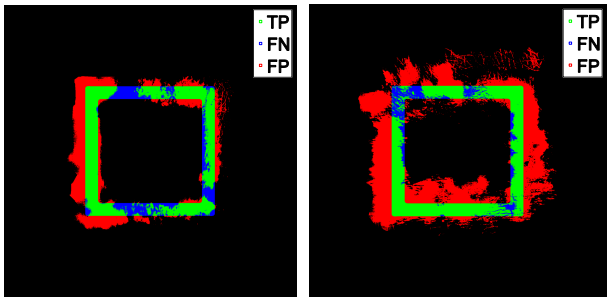

Fig. 3 True Positive (TP), False Negative (FN) and False Positive (FP) areas for the Calcium Alginate Hydrogel (Left) and the GelMA Hydrogel (Right)

The Calcium Alginate provided moderate layer to layer adhesion and poor adhesion to the skin of the hand, being easily peeled away by even stretching the underlying skin. The GelMA hydrogel provided good layer to layer adhesion, as well as good adhesion to the underlying skin. Table 1 shows True Positive Rate (TPR) and False Discovery Rate (FDR) for both gels.

	Bio Com-patibility	Cross Link	Adhesion	TPR	FDR
Alginate	Good	Slow	Poor	75%	46%
GelMA	Good	Fast	Good	82%	65%

Tab. 1 Comparison of Calcium Alginate to GelMA Hydrogel

DISCUSSION

Both proposed hydrogels show promise as bio-inks for additive manufacturing on moving anatomy, however both need further development. The Calcium Alginate's requirement of the aqueous Calcium Chloride caused the gel to run, and the crosslinking was incomplete leading to poor layer adhesion. The GelMA hydrogel became too warm in the PICO Pµlse thus becoming too liquid, causing the gel to run and not hold shape, leading to a large False Positive area. If this were adequately addressed (eg by controlling viscosity via temperature or an additive like glycerol) GelMA would prove superior to Alginate for this application.

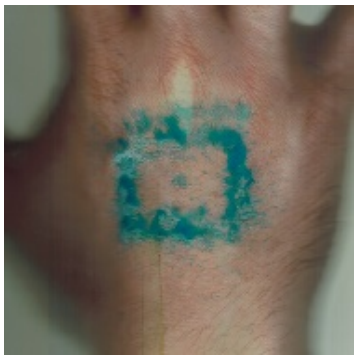

Fig. 4 Raw scan of hydrogel on hand.

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REFERENCES

[1] Murphy, Sean V., and Anthony Atala. "3D bioprinting of tissues and organs." Nature biotechnology 32, no. 8 (2014): 773-785.

[2] Lee, Vivian, Gurtej Singh, John P. Trasatti, Chris Bjornsson, Xiawei Xu, Thanh Nga Tran, Seung-Schik Yoo, Guohao Dai, and Pankaj Karande. "Design and fabrication of human skin by three-dimensional bioprinting." Tissue Engineering Part C: Methods 20, no. 6 (2013): 473-484.

[3] Nakamura, Makoto, Akiko Kobayashi, Fumio Takagi, Akihiko Watanabe, Yuko Hiruma, Katsuhiro Ohuchi, Yasuhiko Iwasaki, Mikio Horie, Ikuo Morita, and Setsuo Takatani. "Biocompatible inkjet printing technique for designed seeding of individual living cells." Tissue engineering 11, no. 11-12 (2005): 1658-1666.

[4] Skardal, Aleksander, David Mack, Edi Kapetanovic, Anthony Atala, John D. Jackson, James Yoo, and Shay Soker. "Bioprinted Amniotic Fluid□Derived Stem Cells Accelerate Healing of Large Skin Wounds." Stem cells translational medicine 1, no. 11 (2012): 792-802.

[5] J. J. O'Neill, R. A. Johnson, R. L. Dockter and T. M. Kowalewski, "3D bioprinting directly onto moving human anatomy," 2017 IEEE/RSJ International Conference on Intelligent Robots and Systems (IROS), Vancouver, BC, 2017, pp. 934-940. https://www.youtube.com/watch?v=6VrozlyELdw&t=3s

[6] K. Y. Lee and D. J. Mooney, "Alginate: properties and biomedical applications," *Progress in polymer science*, vol. 37, no. 1, pp. 106– 126, 2012.

[7] Yue, Kan, Grissel Trujillo-de Santiago, Mario Moisés Alvarez, Ali Tamayol, Nasim Annabi, and Ali Khademhosseini. "Synthesis, properties, and biomedical applications of gelatin methacryloyl (GelMA) hydrogels." Biomaterials 73 (2015): 254-271.