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3D Printed Bioelectronic Platform with Embedded Electronics

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Abstract

Silver nanoparticle based microelectrodes embedded between layers of hydrogel material were successfully fabricated. 3D bioprinting is employed to print the entire bioelectronics platform comprising of conducting silver ink and Gelatin methacryloyl (GelMA) hydrogel. The additive manufacturing technique of bioprinting gives design freedom for the circuit, saves material and shortens the time to fabricate the bioelectronics platform. The silver platform shows excellent electrical conductivity, structural flexibility and stability in wet environment. It is tested for biocompatibility using C2C12 murine myoblasts cell line. The work demonstrates the potential of the fabricated platform for the realization of practical bioelectronic devices.

INTRODUCTION

Flexible and embedded electronics is of huge interest for biomedical applications. Bioelectronic devices have shown promise to provide novel healthcare solutions. However, for the new-age electronics to work for biomedical field, it needs to be biocompatible. Hence, it is imperative to move away from hard and inflexible substrates such as silicon and glass. There are only few reports that have experimented with biocompatible substrates for electronics. He *et al.* laid down nanoparticles over textured hydrogels for large-scale micropatterning [1]. The group demonstrated that assembled particles could be transferred and integrated into alternative templates while retaining their properties for bioassays. In another effort, researchers integrated a hydrogel based microfluidic chip with Electric Cell-substrate Impedance Sensing (ECIS) technique to apply to a high-throughput, real-time cell viability assay and drug screening [2]. A passive wireless sensor was combined with a variety of hydrogel materials for biomedical and chemical sensing applications by Sridhar *et al*. [3]. Ahn et al. created silver nanowire based microelectrodes on a soft and biocompatible hydrogel [4]. However they used a lengthy and tedious transfer process to shift the silver nanowires from glass to hydrogel substrate.

As the world moves towards more complex, multi-functional and flexible biomedical devices, there arises a need to explore new ways to fabricate them. Additive manufacturing (AM) commonly referred to as 3D printing, is novel manufacturing technique that helps to build a prototype by adding layer-upon-layer of material [5-7]. AM is a disruptive technology that promises to build complex geometries in short time and is able to customize the end product according to the end-user. Bioprinting is a type of AM technique that has attracted tremendous attention recently, as it offers automated and advanced manufacturing platform to construct complex bioengineered prototypes [8,9].

In this work, we employ bioprinting to print different bio- and electronic inks to form a multilevel platform. We successfully fabricated a highly conductive silver nanoparticle based hydrogel platform. Printed hydrogel material forms the base of the platform, upon which electronic circuit is printed. The printed circuit is then sandwiched between another layer of printed hydrogel. The printed bioelectronics platform is functional as soon as it is printed, needs no post-processing and stable under wet environment.

Experimental

Preparation of Inks

GelMA was prepared by reacting Gelatin Type A (Porcine skin, Sigma-Aldrich, G2500) with Methacrylate Anhydride (Sigma-Aldrich, 276685). 1X Phosphate Buffered Saline (PBS) was diluted from 10X PBS (pH 7.2, Vivantis, PB0342) using deionized (DI) water. Gelatin powder (10% w/v) was dissolved in 1X PBS Methacrylate Anhydride was added at 1.4% v/v dropwise into the solution and the reaction is continued for 2 h at 50 °C. The reaction is quenched by adding pre-warmed 1X PBS at 40 °C. The mixture was transferred into dialysis tubing (Sigma-Aldrich, D0530) for dialysis in DI water for 4 days at 40 °C. Finally, the solution was lyophilized for 7 days to obtain pure GelMA, and was stored at −20 °C until further use. GelMA, varying concentration of sodium chloride (NaCl) and a photoinitiator are mixed together homogeneously to form the bio ink. NaCl solution at 0M, 0.25 M, 0.5 M, 0.75 M and 1 M is prepared and added to GelMA. Silver ink (20 wt%) in DI water was synthesized according to the reported literature [10] and used without modification.

Printing the Platform

Biofactory® (RegenHu, Germany) with a microvalve setting was used to print the bioelectronics platform. Printing toolpaths were prepared using the in-built CAD software. Bioink was printed using a 30 G needle at 20 kPa with a writing speed of 2000 mm/min on a clean glass slide. Bioink was crosslinked through exposure of UV for 120 s. Silver ink was printed using same print head with a nozzle diameter of 100 μm.

Characterization

The investigation on morphology of the printed materials was carried out using scanning electron microscope (SEM), JOEL JSM-5600LV model. Parametric analyzer (Keithley 4200) and probe station were used to measure the sheet resistance of conducting tracks. Keithley 2400 SourceMeter® SMU Instruments was employed to supply the current to the heating coil. The temperature of the printed coil was measured and imaged using a forward looking infrared (FLIR) E4 thermal camera. C2C12 murine

myoblasts were used for the biocompatibility testing of the printed platform. C2C12 cells were cultured till 70–90% confluency using growth media consisting of DMEM/High Glucose (Hyclone™, GE Life Science) supplemented with 15% Fetal Bovine Serum (FBS, Gibco®, Life Technologies) and 1% 100X Antibiotic- Antimycotic (Hyclone™, GE Life Science) and trypsinized prior to experiments. 100,000 C2C12 were manually seeded onto the platform and cultured for a period of 7 days.

Results and Discussion

Bioprinting is a computer-aided technology that is able to build biological constructs, organs and scaffolds through 'layer-by-layer' precision placement of materials. Bioprinting imparts added spatial control and functionality to biofabrication methodology. Schematic of fig 1 depicts step-by-step process of fabricating the bioelectronics platform through microvalve-based bioprinting method. A layer of Gelatin methacryloyl (GelMA) hydrogel is laid down on glass substrate. GelMA is used as it possesses tunable properties while containing cell-binding sites, deeming the material to be biocompatible [11]. This is followed by printing of the desired electrical circuit on top using silver nanoparticle ink. Another layer of hydrogel is finally printed on top to form a sandwich structure. No post processing is required and the platform is functional as soon as it is printed.

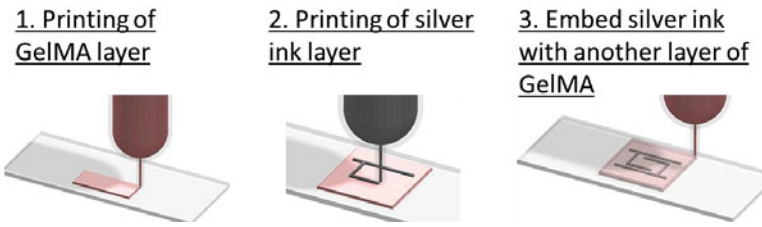

Figure 1. Schematic of the bioprinting process using microvalve for fabricating the bioelectronics platform with electronic circuit sandwiched between hydrogel layers.

The bioink is made from solid GelMA by dissolving it in NaCl. Silver nanoparticles are dispersed in DI water to make the electronic ink. Both inks are well dispersed and do not precipitate on standing (fig 2a). As-printed platform with electronic ink sandwiched between wet hydrogel layers is shown in fig 2b. The freshly prepared platform is swollen and has high water content. However, there is no spreading or leaking of silver nanoparticle ink in the wet environment, and the entire platform is stable, due to the high viscosity of the gelatin ink and due to rapid sintering of the particles upon contact with NaCl. Stability of the bioelectronics platform in wet environment is an important consideration for in-vivo applications. Upon drying the overall thickness of the platform is reduced (fig 2c). Drying of the hydrogel layers does not cause any collapse, crumble or crack the platform. The side view of the dried platform clearly shows that the platform is still intact and does not peel off from the glass substrate (fig 2d).

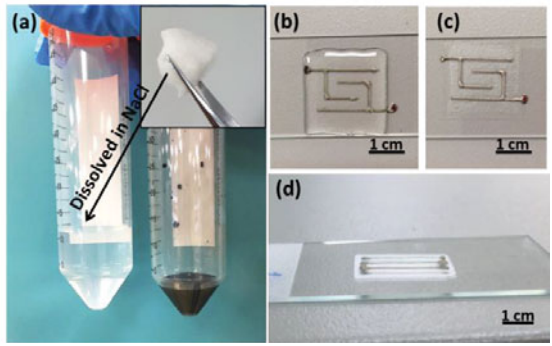

Figure 2. (a) bio- and electronic- ink used for printing the platform. Inset shows solid GelMA that is dissolved in NaCl to form the bio ink. (b) As-printed, (c) dried and (d) side view of the fabricated bioelectronics platform using GelMA hydrogel and silver nanoparticle materials.

The fabricated bioelectronics platform is peeled-off from the glass substrate. The resulting platform is free-standing and highly flexible. It provides conformal coverage, as can be seen in fig 3a, where it is supported on a glass rod. The GelMA material renders the platform transparent, except at the places where silver ink is printed (fig 3b). The platform is easy to handle and does break (fig 3c). The free-standing platform can be folded and bend without breaking the encapsulated circuit. 3D bioprinting has the unique capability to design the platform in any shape and size, and thus to customize it based on end-user requirements. Apart from giving design freedom, bioprinting also enables laying down different circuit designs at required spatial coordinates. Fig 3d shows an electrical heater design printed instead of the interdigitated electrodes. All the printed circuits are functional upon applying the external bias. Fig 3e depicts the current flow through the interdigitated circuit to light up a light emitting diode (LED). Similarly on powering the electrical micro-heater, temperature can be raised up to 40 °C as shown in the forward looking infrared (FLIR) image (fig 3f).

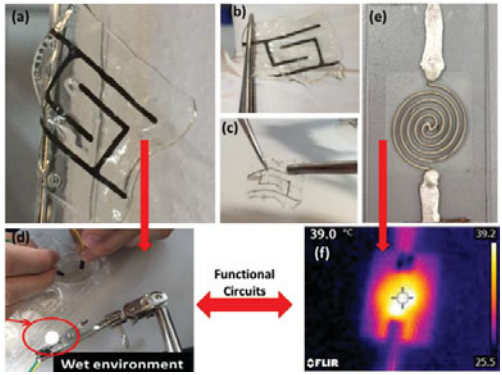

Figure 3 (a, b, c) depict the printed bioelectronics platform with sandwiched interdigitated electrodes. (d) Image shows the working of the platform through lighted LED. (e) Printed micro-heater coil design on the platform with (f) its equivalent heat signature.

SEM characterization was carried out to investigate the morphology of the printed layers. The SEM micrograph of the printed silver nanoparticle ink on GelMA layer is shown in fig 4a. The printed line width is approximately 200 μm, and is crack-free and continuous. A magnified image of the printed tracks reveals closely packed silver nanoparticles (fig 4b). The silver nanoparticles form a crack-free and homogenous film. A uniform coverage is important to have good electrical conduction in the printed layers. It is to be noted that only one print pass is used for fabricating the platform for all inks. Cross-section of the printed platform reveals thickness of ~ 100 μm and ~ 60 μm for GelMA and silver layers respectively (fig 4c). The sandwich structure of silver nanoparticles between GelMA layers can be easily observed in SEM (fig 4d). The multi-material platform is stable due to good interfacial bonding between the layers.

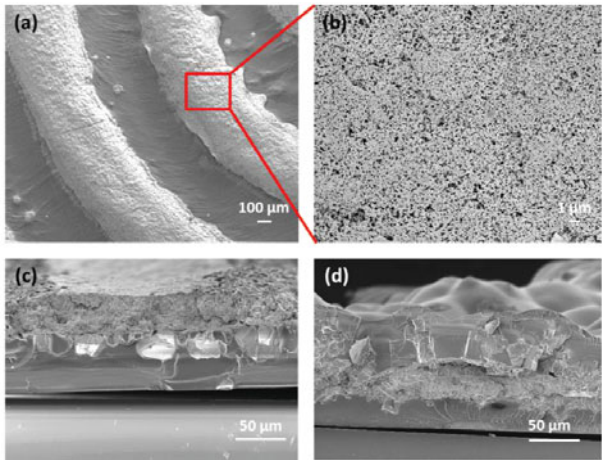

Figure 4. SEM micrographs of (a) printed silver coil design on GelMA and (b) magnified image of printed silver nanoparticle ink. Cross-sectional image of (c) silver printed over GelMA and (d) silver sandwiched between GelMA layers.

The unique property of silver ink to be functional without any post-processing comes from the self-sintering nature of silver nanoparticles. As published by Magdassi *et al*. [10], chloride ions are required to sinter the silver nanoparticles at room temperature. When the silver nanoparticles come in contact with the chloride ions, the nanoparticles are no longer stable and undergo coalescence process, eventually leading to sintering at the room temperature. Thus NaCl is added to the bio ink of GelMA before printing silver. The concentration of NaCl in GelMA is varied from 0.25 to 1 M to obtain the optimized ink. Fig 5a shows the computed sheet resistance of the printed silver tracks on GelMA layer for various NaCl concentrations using four-point probe measurements. Sheet resistance values of 0.032, 0.010, 0.014 and 0.04 Ω are obtained for 0.25, 0.5, 0.75 and 1 M NaCl samples respectively. The optimum value for electrical conduction in printed silver ink tracks is achieved for 0.5 M NaCl in GelMA. This is probably due to better fixation of the silver nanoparticles upon contact with the GelMA material. Hence 0.5 M NaCl is used in GelMA to fabricate the bioelectronics platform.

The biocompatibility of the printed platform was tested using C2C12 murine myoblasts cell line. Quantitative assay using PrestoBlue™ shows that C2C12 cultured on the fabricated bioelectronic platform gradually increases throughout different time point. Figure 5b showed that high percentage of C2C12 cells were alive (green) as compared to the number of dead cells (red). A large number of green cells indicate high cell viability. The platform is resilient to any breakage or degradation even after 7 days of culture. The proliferation of C2C12 cells across the platform was uniform, thus indicating that the platform can support cell growth.

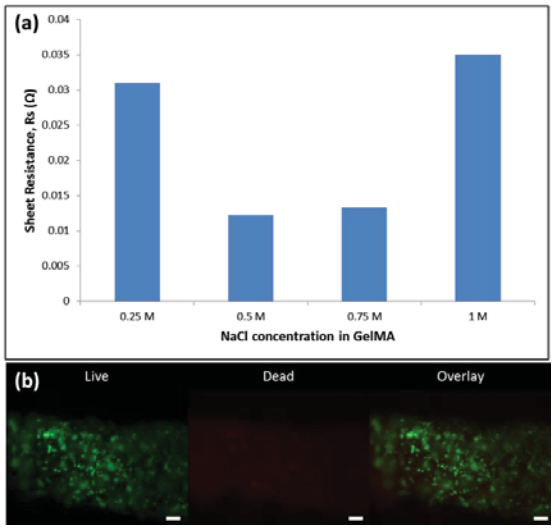

Figure 5. (a) Bar graph depicting the sheet resistance values of printed silver ink tracks on GelMA layer with varying concentration of NaCl. (b) Fluorescence microscopy of C2C12 stained for Live cells (Green), Dead cells (red) and overlay, 7 days after culturing (Scale bar = 50 μm).

Conclusion

In conclusion, the work demonstrates the efficacy of bioprinting technique for fabricating bioelectronics platforms with sandwiched electrical circuits. The encapsulation of the electrical circuitry not only protects it from wear and tear, but also shields the human body from the direct contact of the metal tracks. Silver nanoparticle ink is used to print the electronic circuit design, while GelMA hydrogel is used as the biomaterial for the top and bottom layer. Design freedom of laying down different electrical circuits through bioprinting is also shown. Finally, the platform is tested for biocompatibility to access its suitability in biomedical applications.

Acknowledgment

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