Biosensors and Bioelectronics 102 (2018) 365–371

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

A novel 3D bioprinted flexible and biocompatible hydrogel bioelectronic platform

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ARTICLE INFO

Keywords:
Bioelectronic
Bioprinting
Bioink
Hydrogel
Electrode
Flexible
Biocompatible
Heating coil

ABSTRACT

Bioelectronics platforms are gaining widespread attention as they provide a template to study the interactions between biological species and electronics. Decoding the effect of the electrical signals on the cells and tissues holds the promise for treating the malignant tissue growth, regenerating organs and engineering new-age medical devices. This work is a step forward in this direction, where bio- and electronic materials co-exist on one platform without any need for post processing. We fabricate a freestanding and flexible hydrogel based platform using 3D bioprinting. The fabrication process is simple, easy and provides a flexible route to print materials with preferred shapes, size and spatial orientation. Through the design of interdigitated electrodes and heating coil, the platform can be tailored to print various circuits for different functionalities. The biocompatibility of the printed platform is tested using C2C12 murine myoblasts cell line. Furthermore, normal human dermal fibroblasts (primary cells) are also seeded on the platform to ascertain the compatibility.

1. Introduction

Last decade has seen emergence of high-performance, multi-functional, flexible and printed electronic devices that have contributed to the development of the new age, conformal and non-invasive biomedical devices. There is huge interest in developing materials and electronics further that can provide new capabilities for organ-electronics interfaces and implantable devices. Rogers *et al.* pioneered the field of epidermal electronics where electronic devices were printed on the elastomeric substrates and directly applied to the skin for health and wellness monitoring (Yeo et al., 2013). In another profound work, his team developed ultra-thin silicon wafers to fabricate bioresorbable flexible electronic systems for clinical applications (Yu et al., 2016). Xie et al. also demonstrated advanced nanoelectronic brain probe to overcome the mechanical mismatch between soft tissues and hard electronics (Xie et al., 2015).

The advances in electronics are also paving way for another similar field, the bioelectronics. Bioelectronic platforms bring bio-species and electronics onto a single stage, and thus provide tools to monitor the activity of the cells and tissues. The knowledge extracted from the measurements using the platform allows understanding how the tissues behave and grow in a certain environment. A recent surge of funding and interest in bioelectronics by companies like Galvani Bioelectronics, GlaxoSmithKline and Alphabet has assured the future of the field. An attempt towards the integration of biomaterials and electronics was carried out by Ahn et al. by fabricating silver nanowire based microelectrodes and transferring them on a biocompatible hydrogel (Ahn et al., 2014). In another similar work an poly (3,4-ethylenedioxythiophene) (PEDOT) and polyurethane (PU) blend was used as the conducting material for fabricating electronics on DN hydrogel films for advanced tissue engineering(Sasaki et al., 2014). Tal Dvir's group took the field a step further by engineering a flexible cardiac patch integrating cells with electronics in a nanofiber network (Feiner et al., 2016). The patch helped the living cells to interact with electronics and thus improve the tissue function.

Although all the above work contributed significantly in advancing the field, there are few limitations that need to be addressed. All the devices were fabricated using conventional microfabrication techniques, and peeling/transfer processes were used to move the system to other substrates. The fabrication procedure puts limitations on the materials to be explored, repeatability and scalability. This work addresses some of the issues and adds to the existing knowledge by harnessing the advanced manufacturing process of 3D bioprinting (Khoo et al., 2015; Lee and Yeong, 2016; Ng et al., 2016a, 2016b; Wang et al.,

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https://doi.org/10.1016/j.bios.2017.11.039
Received 4 September 2017; Received in revised form 30 October 2017; Accepted 10 November 2017
Available online 16 November 2017
0956-5663/

2015) to design and fabricate free-form bioelectronic platforms, where electronic and biomaterials co-exist in synergy. One of the attractive features of 3D printing, also called additive manufacturing, is the variety of materials that can be processed in various shapes and sizes (Lee et al., 2017; Murphy and Atala, 2014; Saengchairat et al., 2017).

We have developed and optimized the bioprinting process, to facilitate the deposition of bio- and electronic- materials on a single platform with full spatial control. The platform is fabricated via extrusion-based printing of Gelatin methacryloyl (GelMA) hydrogel (Tan and Yeong, 2015) and drop-on-demand microvalve-based printing of silver nanoparticle ink (Ng et al., 2017a; Ng et al., 2017b). Bioprinting enables fast and simple fabrication and the platform is ready for use as soon as it is printed, thus requiring no post processing. The fabrication procedure is done using one-equipment and is completed in three easy steps making it cost-effective, quick and easy to scale-up. Obtaining the conductivity in one simple step without the need for heating is derived from the unique property of the silver nanoparticles to undergo a chemical sintering process at room temperature, in presence of chloride ions. Microelectrodes and heating coil are printed and embedded in the platform to provide electrical stimulation and heating, respectively, which can then be exploited for various applications. This work demonstrates an important technological goal of printing multi-materials in different layouts, and possible spanning in three dimensions; to fabricate flexible and biocompatible microelectrodes. The bioelectronics platform provides a good conformal coverage, works in wet environment and can be used as regenerative template.

2. Materials and methods

2.1. Synthesis of GelMA

GelMA was synthesized 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 using a magnetic stirrer at 600 rpm keeping the temperature at 60 °C. 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 (MWCO: 12400, 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.

2.2. Preparation of Bioink and Silver ink

The hydrogel bioink is composed of GelMA, sodium chloride and a photointiator. Sodium chloride (Sigma Aldrich) solution with various concentrations of 0 M, 0.25 M, 0.5 M, 0.75 M and 1 M were prepared, followed by adding GelMA until it fully dissolves. 10% w/v 2-Hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone (Sigma Aldrich) was dissolved in ethanol as the photoinitiator for the photopolymerization reaction. 0.02% v/v (i.e. 20 µl in 1 mL of GelMA solution) of photoinitiator was added to the bioink prior to loading of the print cartridge. Silver ink (20 wt%) in DI water was synthesized according to the reported literature (Magdassi et al., 2010) and used without modification.

2.3. Printing of the bioelectronic platform

Biofactory® (RegenHu, Germany), comprising of a pressure-based extrusion system, a microvalve setting and a UV curing pen, was used as the printing platform for this study. Printing toolpaths were prepared using the in-built CAD software, BioCAD. Bioink of 5% w/v GelMA dissolved in 0.5 M NaCl was loaded in the printing cartridge. The bioink was extruded via a 30 G needle at 20 kPa with a writing speed of 2000 mm/min to form a square platform (20 mm × 20 mm). The

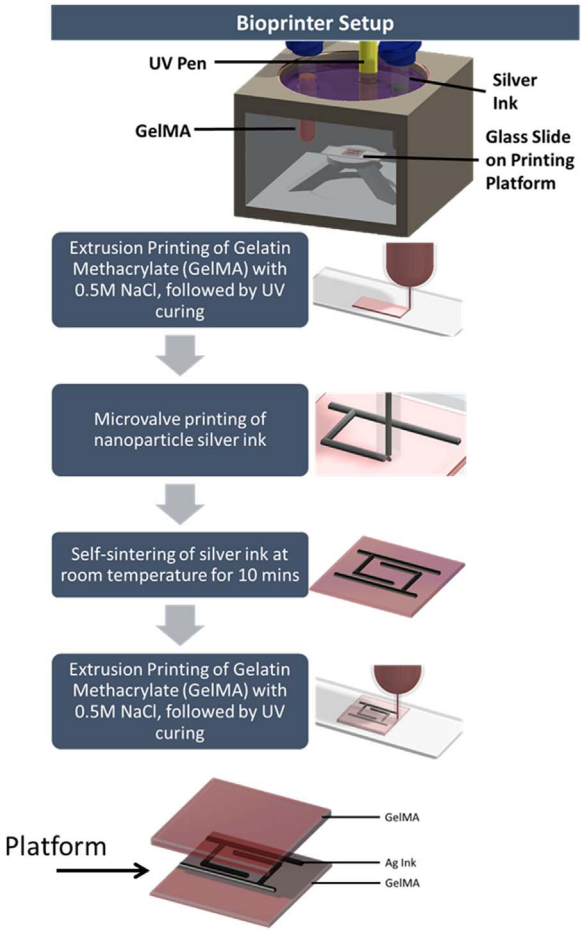

Fig. 1. Schematic depicting the complete 3D bioprinting process to fabricate the bioelectronics platform.

bioink was crosslinked through exposure of UV for 120 s at the intensity of 360 mJ/cm. Silver ink was printed using a microvalve-based print head with a nozzle diameter of 100 µm. A printing pressure of 0.25 bars and feed rate of 200 mm/min were used for drop-on-demand printing of silver droplets to create the pre-defined electrode structures. Two designs are used in this work to print the electronic circuit- an interdigitated electrode and a circular heating element. Finally, bioink was added on top of the silver ink using the same setting as previously mentioned, thus forming a sandwiched structure. A step by step procedure of printing the bioelectronics platform is depicted in schematic of Fig. 1.

2.4. Rheology of GelMA

Different types of GelMA (with varying concentration of NaCl) were prepared to investigate the effect of NaCl concentration on the printability of PVP-based bioinks. The rheological properties of different GelMA samples were characterized using a Discovery hybrid rheometer (TA instruments, USA). All the measurements were conducted within the linear viscoelastic region and the viscosities of different GelMA samples were analyzed for shear rates ranging from 0.1 to 1000 /s at a constant temperature of 27 °C. All measurements were performed in triplicate.

2.5. Cell culture

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