3D Printing of Biohybrid Electrical Stimulation Platform to Promote Insulin Secretion of Pancreatic β Cell

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1. Introduction

Islet transplantation, a promising treatment for diabetes mellitus, has not yet been a golden standard due to the lack of donor islets and their low therapeutic efficacy. The electrophysiological activities of the β cells in pancreatic islets are crucial for appropriate insulin secretion and blood glucose level control. In recent studies, electrical stimulation (E-stim) is applied to induce the calcium ion influx triggering the insulin granule exocytosis. However, the exact physiological process and cellular behaviors are yet to be studied. 3D cell culture platform provides high versatility to replicate the microenvironment of the tissue and modify the culturing conditions. In this work, an electrode-integrated biohybrid platform is printed for pancreatic tissue. For E-stim without damage to tissue, conductive and biocompatible composite ink was fabricated by integrating carbon nanomaterials into biocompatible polymers. Moreover, to recapitulate the electrolytes around the islets, the β cells are embedded in pancreas decellularized ECM (pdECM) ink. The intracellular calcium concentration with E-stim can be directly evaluated on the platform. The 3d printed biohybrid platform applies E-stim to the tissue and potentiates the direct and controlled analysis of cellular behavior.

Keywords : Electrical stimulation; Pancreatic β cell, 3D printing

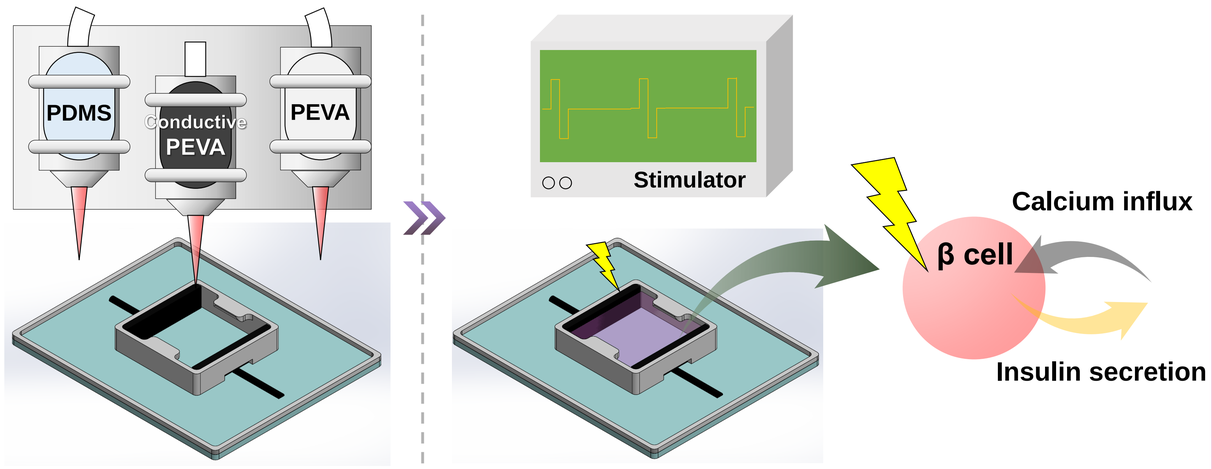
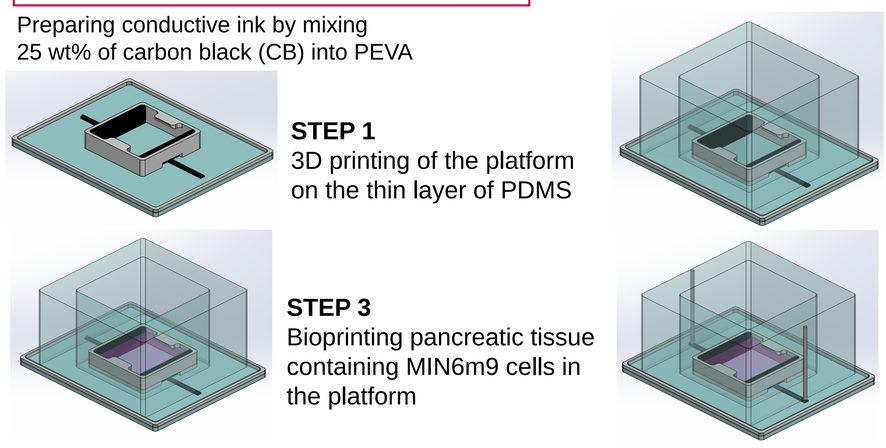


Figure 1. Schematics of the biohybrid system and electrical stimulation.

2. Materials & Methods

Fabrication process of a biohybrid system

Preparing conductive ink by mixing 25 wt% of carbon black (CB) into PEVA



STEP 1
3D printing of the platform on the thin layer of PDMS

STEP 2
Attaching PDMS well for containing culture medium

STEP 3
Bioprinting pancreatic tissue containing MIN6m9 cells in the platform

STEP 4
Wiring to stimulation device with stainless needle

Figure 2. Schematics of fabrication process of a biohybrid system.

Circuit diagram for biphasic pulse stimulation and E-stim characterization

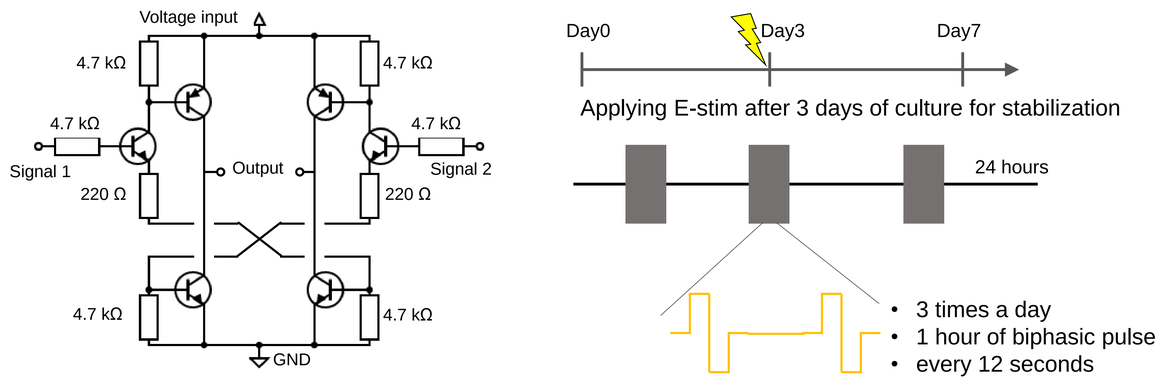


Figure 3. Description of experimental conditions for electrical stimulation.

3. Results

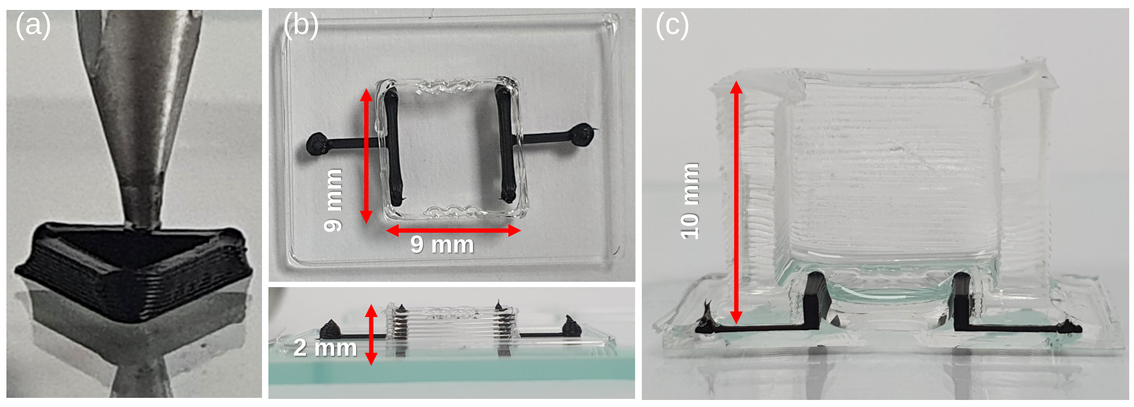


Figure 4. 3D printing of CB-PEVA ink and characterization biohybrid platform. (a) printing fidelity of CB-PEVA ink (layer height: 0.1mm). (b) The dimension of biohybrid platform (c) Cross section of final biohybrid platform including PDMS well.

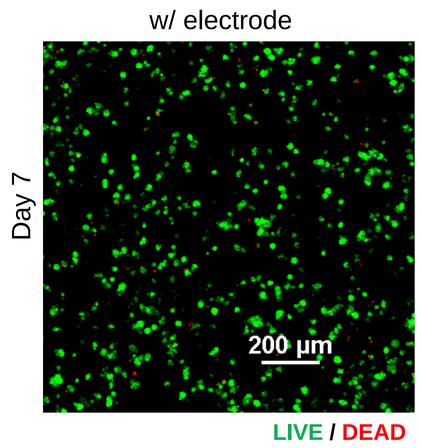


Figure 5. Viability test of the CB/PEVA electrode. Both platforms with and without the electrode showed high viability (> 90%).

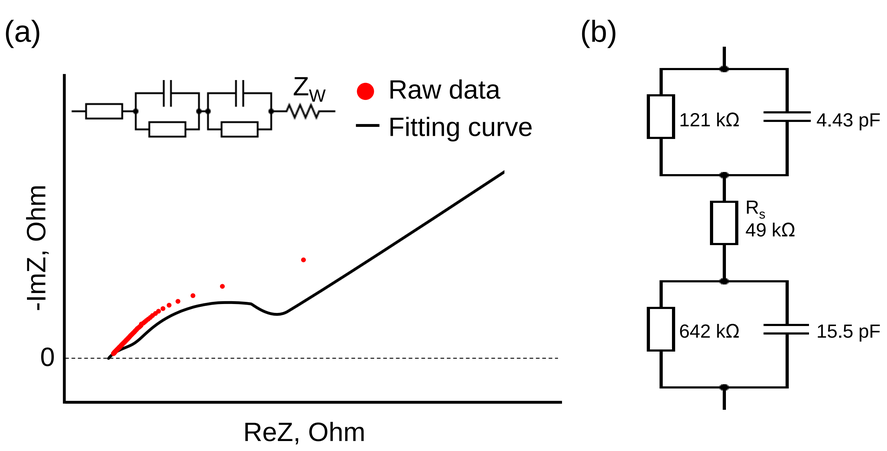


Figure 6. Electrochemical impedance spectroscopy (EIS) analysis of biohybrid platform. (a) fitting of EIS result and (b) equivalent circuit generated with the result.

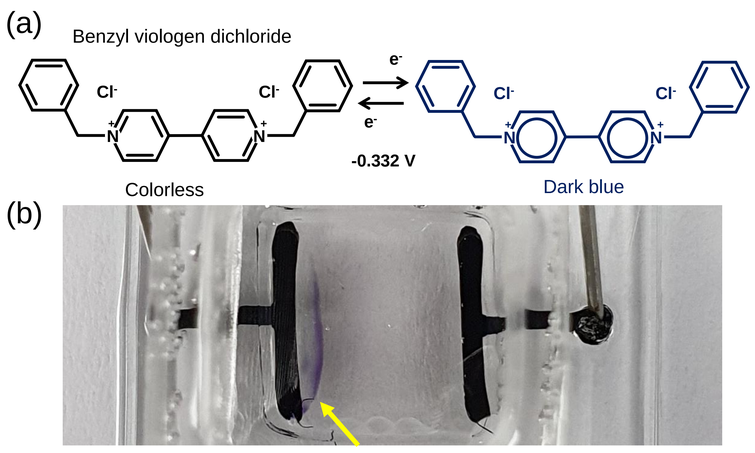


Figure 7. E-stim from the electrode was observed using benzyl viologen dichloride (BVD). (a) BVD shows electrochromic behavior and turns dark blue at -0.332V. (b) With monophasic E-stim, dark blue color was distinguished at the anode electrode (yellow arrow).

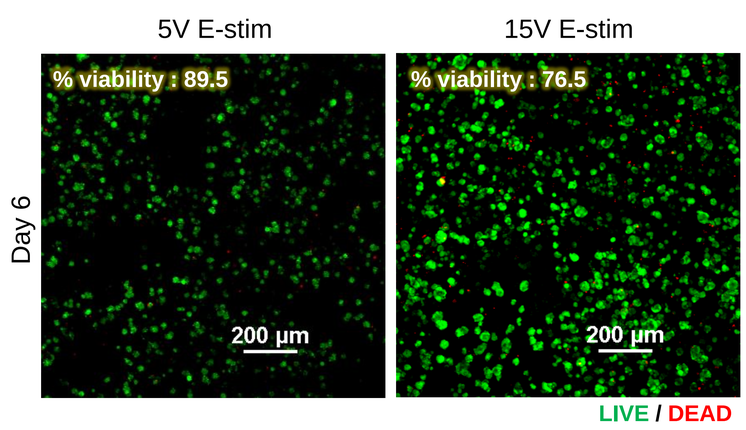


Figure 8. Viability test with 5 V, 15 V of E-stim. (b) After 3 days of culture with E-stim, tissue with 5 V stimulation maintained high viability but tissue with 15 V stimulation showed low viability of around 75 %.

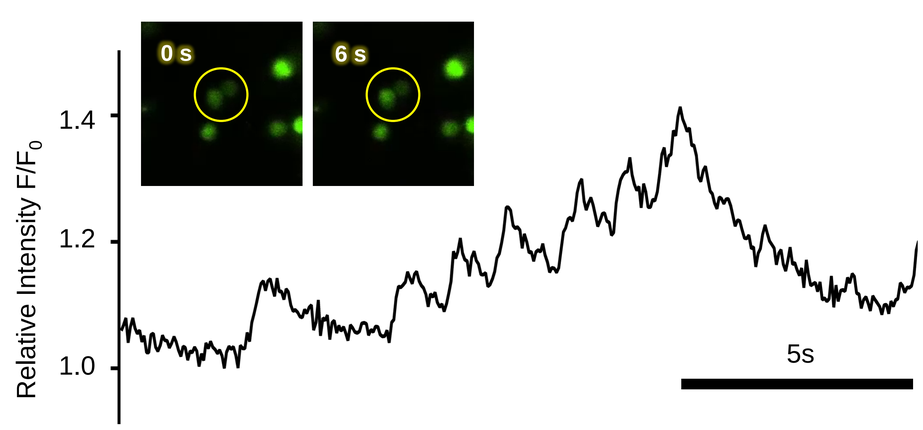


Figure 9. The effect of E-stim was observed with calcium fluorescence. Increased intracellular calcium ion concentration with E-stim can be seen (yellow circle). Relative intensity shows step-wise increase of intracellular [Ca^{2+}] with E-stim of 1 Hz.

4. Conclusion

The ability to integrate E-stim to the culture system was demonstrated using 3D printed biohybrid system. The biocompatible conductive ink compromised with carbon black and PEVA showed high printability and fidelity, and the platform with the desired structure was constructed (**Fig. 4 and 5**). The electrical properties of the CB-PEVA ink were evaluated with EIS, and the equivalent circuit was simulated to calculate the electrical field applied to the tissue (**Fig. 6**). The electrochromic property of BVD was used to test the E-stim (**Fig. 7**). Although the dark blue color was observed, the system needed high voltage (< 20 V) agreeing with the high capacitance observed with EIS and indicates low faradaic reaction related to tissue damage. The increase of intracellular calcium level indicated the modified intracellular behavior through ion channels (**Fig. 9**).This work demonstrated the potential of E-stim integrated into 3D tissue culture platform with biocompatible conductive ink. The biohybrid system promotes the *in vitro* investigation of the effect of E-stim on cellular behavior and also in vivo implantation device for higher therapeutic efficacy.

5. Acknowledgement

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