3D printing of cardiac patches with integrated sensors and actuators to regenerate the infarcted heart

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Myocardial infarction (MI; heart attack) is associated with sudden death as well as significant morbidity and mortality. MI results from blockage of one of the coronary arteries that supply the cardiac tissue, leading to ischemia of a segment of the heart. This process eventually leads to the death of contractile cells and the formation of a scar tissue. Since cardiomyocytes cannot proliferate, the cardiac tissue is unable to regenerate, leading to chronic cardiac dysfunction. Cardiac tissue engineering has evolved as an interdisciplinary field of technology combining principles from the material, engineering and life sciences with the goal of developing functional substitutes for the injured myocardium. Rather than simply introducing cells into the diseased area to repopulate the injured heart and restore function, cardiac tissue engineering involves the seeding of contracting cells in or onto 3-dimensional (3D) biomaterials prior to transplantation. Following implantation and full integration in the host, the scaffold degrades, leaving a functional cardiac patch on the defected organ. However, once the 3D cardiac patches have been engineered, *in vitro* assessment of their quality in terms of electrical activity without affecting their performance is limited. This situation might lead to implantation of cardiac patches with limited or no potential to regenerate the infarcted heart. Therefore, engineering an implantable tissue that can provide information on its own function and actively intervene with the tissue function would contribute immensely to the success of this tissue engineering approach. In this research we focused on the development of a method in which recording and stimulating electrodes were simultaneously 3D printed together with extracellular matrix (ECM)-based hydrogel and cardiac cells to generate a microelectronic cardiac patch (microECP). To this end, we developed a unique formulation of autologous, thermoresponsive ECM-based hydrogels, originated from decellularized omental tissue, which can be easily and safely extracted from the patient. These hydrogels, that self-assemble under physiological temperature, have been found to support cultivation and tissue organization of cardiac cells. The printing process is executed using a multi-nozzle 3D printer that extrudes a unique formulation of conducting materials (based on a mixture of graphite flakes in PDMS) for electrode fabrication, alongside cradiomyocyte-containing ECM hydrogel that serves as “bio-ink”. The electrodes in the hybrid patch have been found to be elastic, mechanically durable and electrically conductive (Figure 1 and 2). Microscopic analysis and biochemical assays revealed that cardiomyocytes maintained good viability and functionality while growing in close proximity to the printed electrodes. We have demonstrated the capacity of the electrode-containing constructs to implement real-time recordings of cardiac extracellular potentials and actively control and interfere

with the patch function by applying acute electrical stimulation at different frequencies. This resulted in activation and synchronization of the contraction of the cells throughout the patch (Figure 3). Future experiments will be conducted to investigate the potential of the printed electronics patch to perform in vivo and to improve heart function following infarction. In conclusion, we have developed and tested precisely-printed electronic cardiac patches that enable monitoring and regulation of the integrated engineered tissue. Such functions would provide control over the in vitro process of cardiac tissue engineering, and guarantee successful (and controlled) regeneration of the diseased heart following implantation. It will provide quality assurance for the engineered tissue prior to implantation, which is extremely important to attain appropriate structural and electrical integration with the healthy part of the heart. The technology has the potential to significantly decrease mortality and improve the quality of life for millions of patients.

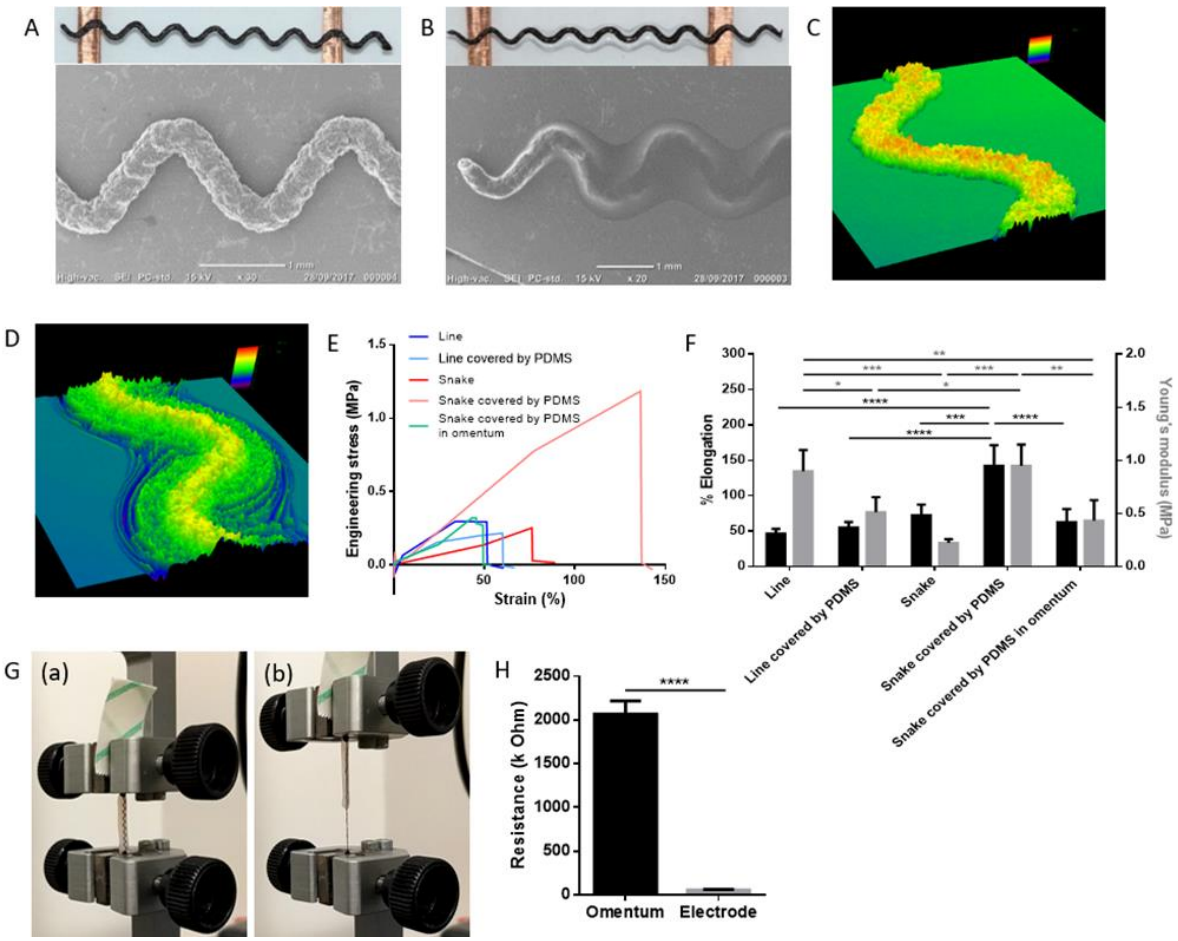

Figure 1. 3D printing of electrodes. Snake-shaped graphite electrode, without (A,C) and with (B,D) PDMS passivation coating in the electrode center. (A-B) Upper panels: photographs of the electrodes, lower panels: SEM images. (C-D) Confocal laser scanning microscope images. (E) Stress vs. strain diagram and (F) the calculated Young's modulus and elongation percentage (n=3) of different electrode variations; graphite straight vs. snake-shaped electrodes and with vs. without passivation coating and ECM hydrogel. (G) Photographs of the tension examination of graphite electrode in omentum hydrogel at the beginning of the exam (a) and before the torn (b). (H) Resistance measurements of the electrode and the surrounding omentum-based hydrogel.

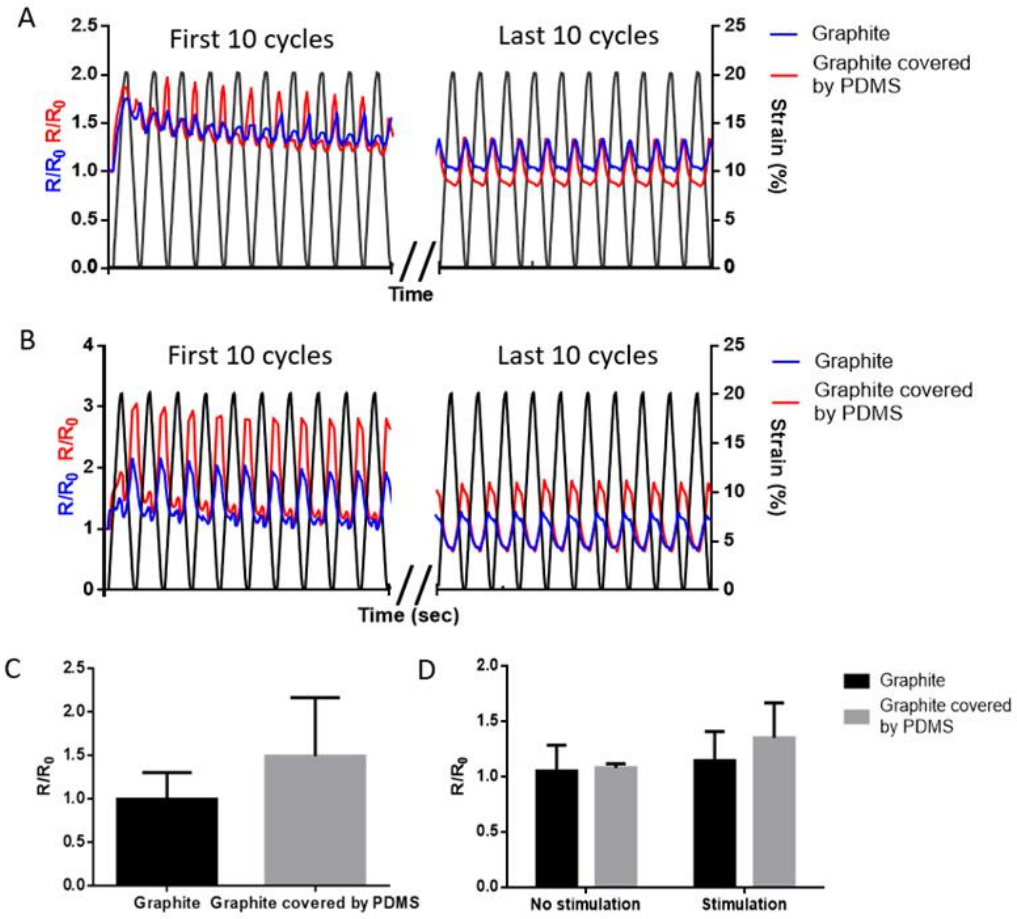

Figure 2. Electrical function of the printed electrodes. Electrical resistance during repetitive bending (A) and tension examinations (B) at the 10 first and last cycles (of 1000 cycles). The test was performed on graphite snake-shaped electrodes w/ and w/o passivation. (C) Resistance after two months at physiological conditions. (D) Resistance after pulsed electrical stimulation of 3V at 1Hz for 30min.

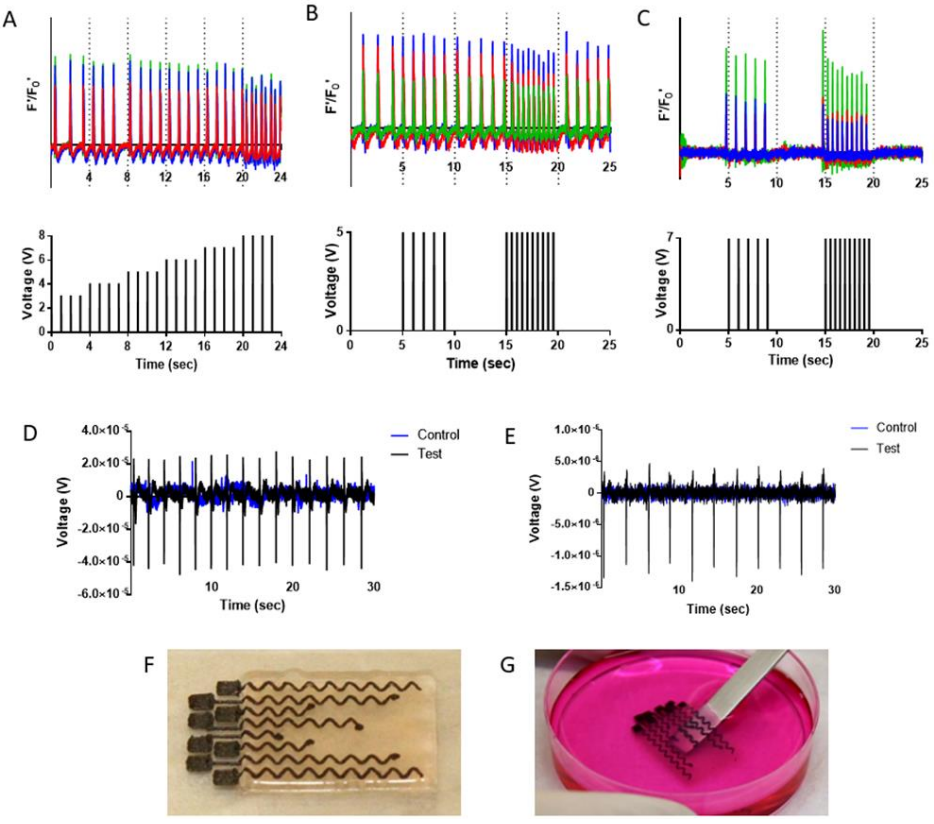

Figure 3. Electrical stimulation and monitoring of neonatal cardiomyocytes using graphite electrodes. (A) Stimulation threshold of 5V at 1 Hz on 2D cardiomyocytes culture and (B) on/off stimulation of 5V at 1 and 2 Hz through printed T-shaped graphite electrodes. (C) On/off stimulation of 7V at 1 and 2 Hz on 3D culture of cardiomyocytes in omentum hydrogel. Upper panel represents first derivative of the normalized Ca2+ signal, the lower panel represents the voltage applied. (D) Monitoring of the electrical activity of cardiomyocytes in 2D cultures and (E) in 3D cultures in omentum hydrogel. Test signal is obtained from contracting cardiomyosytes in warm medium; control signal is obtained from the same culture in cold medium (when contraction is inhibited). (F-G) 3D printed graphite electrodes in omentum hydrogel.