BIST Ignite Project Progress Report

January 2020

1. Title of the project

Programable 3D printed living biobots with nanoelectronics for rational sensing/local stimulation

2. Acronym

ElectroSensBioBots

3. Names and centres of the PIs

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4. Abstract

The rational integration of living entities in hybrid biorobotic systems provided for advanced bioactuators with unique properties such as adaptability to their immediate environment, their capability to response to external stimuli or self-healing. Indeed, 3D printing technologies have allowed the fabrication of functional and welldefined structures whose cells contractile behavior permitted their use as biohybrid actuators at different scales. Both cardiac and skeletal muscle cells have been explored, but the latter has been preferred for providing better controllability, implementation in 3D environments and presenting force adaptability. One of the main interests on such biohybrid robots relies on developing improved control systems and to better understand how the biological events undergoing can affect the final robot performance. Although the mechanical properties of whole biorobot designs has been controlled by using light/electrical stimuli, and training protocols implemented to obtain distinct force generation by modulating the maturation process, it is crucial to move towards a more controlled and local stimulation approach to achieve more refined and complex motion patterns. The ElectroSensBioBots project main purpose is to achieve local stimulation by the integration of flexible graphene based microelectrodes in 3D printed biohybrid robotic systems, obtaining also (i) a better understanding of their biological behavior by implementing a real-time spatiotemporal

monitoring performed by the same electrodes, allowing in a later stage (ii) biorobots with programmable actuation. The first stage of the ElectroSensBioBots project has been mainly focused on coupling 3D printed skeletal muscle cell laden constructs to flexible graphene-based microelectrode arrays (gMEAs) in order to evaluate local stimulation by applying different protocols. From one side, different fabrication approaches and bioactuators designs were explored while accounting for the adhesion and biocompatibility of the 3D printed constructs on the surface of gMEAs. Additionally, the optimized stimulation protocols were studied in a 3-post force measurement system prepared with (i) C2C12 skeletal muscle cell construct or (ii) human muscle (hSMC) cell construct, and (iii) a biohybrid robot based on C2C12 cells. In order to evaluate the feasibility of applying local stimulation in the biohybrid structure (i.e. 3-post setup, biobot) we integrated it into a home-made modular setup where 3 gMEAs can independently apply local electrical fields. Local stimulation was qualitatively evaluated by using optical data analysis, obtaining successful local stimulation when the distance between the electrode and the 3D muscle cell construct was less than 300 μm and applying a voltage over 400 μA. Pulse training protocols were applied to achieve better force generation, and although no significant improvement was observed, no evidence of muscle fatigue was shown. Local stimulation was also tested on a skeletal muscle based biobot, demonstrating the feasibility of differentially stimulate different edges of the same structure. Therefore, we successfully demonstrated local stimulation in 3D printed living systems, both in a 3-post configuration of great use for quantitative studies to evaluate the effect of training protocols, and in biobots configuration. In this later case, such differential stimulation can lead to controlled guidance and more complex motion patterns when gMEAs will be integrated in the biobot structure, along with the possibility to achieve programmable biobots whose biological performance can be also real-time monitored by the same electrodes that are locally inducing its actuation. Therefore, in the second stage of the project, our two main objectives will rely first (i) on achieve a better understanding of the mechanotransduction mechanisms taking place on the 3D printed biohybrid system when is locally stimulated (i.e. effect of training protocols, drug to modulate contractility) by using the 3-post system, and on (ii) later integrating two flexible gMEAs between the compliant skeleton and the cell construct in each edge of the biobot structure. In this manner, direct contact will be ensured to later induce differential local stimulation and explore complex motion patterns/advanced guidance, as well allowing the first time the on-demand actuation and programming of a 3D printed living biobot.