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A Microfluidic Platform based on LIG Electrodes and Machine learning for Real-Time Skeletal Muscle Analysis

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Summary:

Lab-on-chip technologies offer powerful tools for studying biological processes in physiologically relevant microenvironments. We present a microfluidic platform for real-time analysis of muscle contraction, uniquely integrating Laser-Induced Graphene electrodes fabricated via direct CO_2 laser engraving on polyimide tape adhered to polystyrene. This rapid, low-cost method enables precise electrical stimulation of muscle tissues. Time-lapse microscopy captured the resulting contractions, which were quantitatively analysed using machine learning algorithms to extract contractile features.

Keywords: Laser Induced Graphene, Time-lapse Microscopy, Lan-On-Chip, Skeletal Muscle Contraction.

Background, Motivation and Objective

Lab-on-chip (LoC) platforms are increasingly adopted for in vitro modeling of physiological processes, offering fine control over microenvironments and enabling high-throughput analysis. In particular, LoC systems are well-suited for studying muscle tissue contraction dynamics, a process that requires precise stimulation and real-time monitoring [1]. Electrical stimulation is commonly used in microfluidic devices to induce action potentials in excitable cells, typically implemented via integrated electrodes. These electrodes are often fabricated from metals such as gold or platinum, which require complex microfabrication steps and may pose biocompatibility challenges [2]. Laser-induced graphene (LIG) has emerged as a promising alternative for bioelectronic interfaces due to its excellent electrical conductivity, cytocompatibility, and ease of fabrication [3]. Our approach integrates LIG electrodes directly onto the LoC polystyrene substrate. Starting from commercial polyimide tape (PI), we pattern LIG via laser engraving and peel off the residuals. This technique avoids chemical etching or metal deposition steps and allows the electrodes to be easily and precisely positioned only at the extremities of the microfluidic chamber. This fabrication method ensures compatibility with cell cultures and offers a straightforward path to customization and miniaturization. As a result, the central culture area remains untouched and fully transparent, enabling high-quality optical monitoring. Unlike approaches where the LIG remains on the polyimide substrate, resulting in an opaque surface [4], our transfer technique preserves transparency in the culture zone, which is crucial for real-time optical inspection and imaging. Indeed, we integrate time-lapse microscopy to capture contraction events at high temporal resolution, allowing for direct, video-based quantification of cell activity. To extract meaningful metrics from the time-lapse videos, we employ machine learning techniques to perform motion quantification [5]. This automated image processing pipeline enhances the precision and throughput of contraction analysis.

Methods description

The lab-on-chip device was fabricated using laser cutting techniques on sheets of Polystyrene (PS) from Petri dishes, Polymethyl Methacrylate (PMMA) and Cyclic Olefin Polymer (COP). LIG was synthesized on commercial PI tape, which was directly bonded onto PS substrates. After the laser processing, the non-functionalized residual portions of the tape were carefully peeled off, leaving only the patterned conductive regions adhered to the substrate, as in Fig.1. Electrodes were characterized both electrically with four probe measurements on 8x8 mm^2 squares and morphologically at Scanning Electron Microscopy (SEM), evaluating the effects of laser processing parameters such as power and speed. C2C12 murine myoblasts were cultured and differentiated on the chip for approximately 20 days, with daily medium refill. At the end of the differentiation period, mature myotubes were obtained, forming skeletal muscle fibers capable of responding to electrical stimulation. Stimulation was delivered using an arbitrary waveform generator, applying square wave signals at a frequency of 0.5 Hz with varying duty cycles. Contractions were recorded via time-lapse video microscopy through an inverted optical microscope at a temporal resolution of ~10 frames per second. The videos were analyzed using machine learning algorithms to identify contraction centers and quantify displacement and contraction velocity.

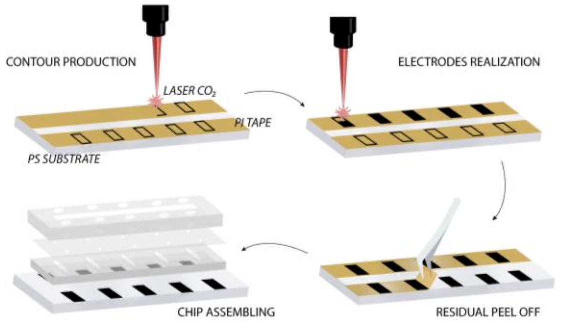

Fig. 1. Scheme of the LIG on PI tape production process and subsequent chip assembling.

Results

The electrical characterization of the LIG electrodes enabled the optimization of the fabrication parameters. Both electrical and morphological analyses revealed directional structure. SEM imaging highlights how the directionality of the laser pattern applied to the polyimide is imprinted onto the plastic substrate, resulting in a well-defined microstructure morphology (Fig. 2). This structural anisotropy is also reflected in the electrical measurements, as resistance values differ significantly depending on the direction of interrogation. Once assembled, the chip supports the replication of skeletal muscle cells, which, upon maturation, form elongated fibers, myotubes, as shown in Fig. 2. Electrical stimulation, optimized at 0.5 Hz with a 100 ms pulse duration, induced muscle contractions that were frequency-aligned. The contraction velocity quantified through an image velocimetry analysis is also reported in Fig. 2.

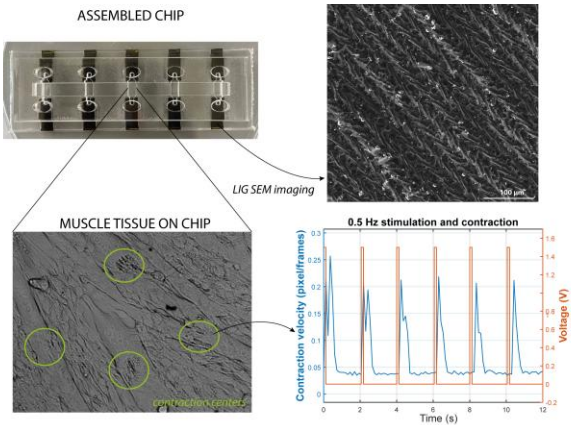

Fig. 2. Assembled chip, SEM images (scale bar 100 µm), muscle tissue in lab-on-chip chamber, stimulation and relative contraction velocity trends.

The resulting device with the integration of LIG electrodes allows for efficient electrical stimulation and real-time monitoring of muscle contraction dynamics, highlighting the platform's potential for customizable bioelectronic assays.

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