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# Flexible and hollow micro-ring-electrode arrays for multi-directional monitoring of 3D neuronal networks

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Three-dimensional (3D) cell culture based *in vitro* models shown great promise and able to replicate partially the development and complexity of *in vivo* nerve tissue<sup>1,2,3</sup>. The functional characterization of 3D cell cultures is challenging. Because, most of the *in vitro* neural interfacing technologies are developed for 2D cell cultures, where it limits the access to the networks within the cultures<sup>4</sup>. Hence, high-performance electrophysiological platforms that allow seamless integration with soft and stable tissue system, and multi-directional bio interfacing capabilities for long term are required.

In this context, we report for the first time the fabrication of ‘Stargate’ look alike flexible and hollow micro-ring electrode array (SG MEA) with hole at the centre (40- $\mu\text{m}$  to 150- $\mu\text{m}$  diameter). The SG MEA was microfabricated on a 20- $\mu\text{m}$  thick layer of flexible and biocompatible parylene C configured with 4 to 6 electrode sites. All gold electrode rings were coated with the conducting polymer poly(3,4-ethylenedioxythio-phenylene):poly(styrene-sulfonate) (PEDOT:PSS) to lower the impedance (5-8 k $\Omega$ ) and obtain a better signal-to-noise ratio during extracellular recordings. The hollow ring shape of SG electrode architectures, with geometrical surface area of 289  $\mu\text{m}^2$  to 1299  $\mu\text{m}^2$  were designed specifically in such a way that it offers more freedom to the internal dynamics of cell cultures and multidirectional sensing of neuron activities within the 3D neuronal network, from both front and back-end of the SG MEA.

To monitor the neuronal networks from within, the SG MEA was incorporated vertically to the cell culture chamber. Next, the neuronal cultures made up of rat embryonic hippocampal cells (E18) were grown in 3D, guided with porous silica beads assembly<sup>5</sup>. Silica beads (40- $\mu\text{m}$  diameter) provide a growth surface to move, transfect and culture highly differentiated neurons

without disrupting their membrane integrity. Consequently, it offers distinct subsets of neurons having constrained connectivity on different beads and consistent mechanical properties to keep them intact for longer periods. Later, the long term, stable and comprehensive 3D neuronal network recording was demonstrated over the course of neuron culture development at single cell resolution. The obtained results shows that our SG MEA combined with 3D monitoring approach holds a great potential in understanding the neuronal circuit formations and dynamics in multiple dimensions.

## References

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