Alginate sulfate provides molecular and physical cues for the induction of a 3D neuronal network

G. Palazzolo, N. Broguiere, H. Dermutz, J. Becher, M. Schnabelrauch, and M. Zenobi-Wong

I. Introduction

HEPARAN sulfate proteoglycans are key regulators in brain development, promoting neuronal extension through interaction with cell membrane receptors [1]. Moreover, sulfation is essential for neural differentiation [2]. Here we report that sulfation of the natural polysaccharide alginate can exert effects similar to heparan sulfate, promoting neuronal growth and activity in a three-dimensional environment.

II. Materials and methods

Briefly, alginate sulfate with degree of sulfation of 0.8-1 was synthesized by reaction of alginate (LVG, Novamatrix) with SO_3/Pyridine in dimethylformamide. Cortical neurons were obtained from E17 rat embryos and were encapsulated at a density of 10 million cells/ml in either unmodified or sulfated alginate (2% w/v) which was gelled by 102 mM $CaCl_2$. 3D cultures were maintained for 1 day in DMEM supplemented with 10% FCS and 1.75mM $CaCl_2$, after which they were grown in Neurobasal medium supplemented with B27 and 1.75 mM $CaCl_2$ up to 21 days. As control, cells were cultured in 2D culture chambered glass coated with poly-D-lysine and laminin. Cell viability was assessed through a Live/Dead assay (Life Technologies Ltd). The neuronal network was assessed by immuno-staining of beta III tubulin and microtubule associated protein-2 (MAP-2). Spontaneous activity of neurons was shown by live calcium imaging, where labeled calcium indicators (Oregon Green® 488 BAPTA-1 cell permeant, Life Technologies Ltd) exhibit an increase in fluorescence upon Ca^{2+} binding. Rheological measurements were conducted on an Anton Paar MCR301 rheometer using a 10mm diameter parallel plate geometry to assess the stiffness of the alginates.

III. Results and Discussion

The present work introduces for the first time the biomaterial alginate sulfate for neural tissue engineering. Primary neurons encapsulated in 3D alginate sulfate hydrogels started to extend neurites after only 1 day in culture and underwent extensive neurite formation over 21 days. The neural network stained positively for both the axonal marker βIII tubulin and the dendritic marker MAP-2. Spontaneous activity of the 3D culture could have been shown using a calcium sensitive dye. Furthermore we investigated the stiffness of the material and found that it had a very low shear storage modulus (<100 Pa at frequencies lower than 1Hz). Comparing the rheological properties of unmodified and sulfated alginate, we found that sulfation reduced the stiffness of the material, probably due to a reduction in the groups available for the inter-molecular ionic cross-linking. Based on our results, we believe that alginate sulfate has both physical and biological properties which promote neuronal growth and activity. The relative importance of these two factors will be investigated by interfering with the sulfate/cell receptor interaction and by assessing changes in structure/porosity of the hydrogel due to sulfation. In the future, we aim to use alginate sulfate in combination with neural-repulsive materials to produce 3D patterned hydrogels which can spatially guide neuronal growth. These scaffolds could be potentially applied at the interface between neuro-prostheses and neural tissues, thus improving the function of the implanted devices.

References

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This research is supported by the 3D NeuroN project in the European Union's Seventh Framework Programme, Future and Emerging Technologies (grant agreement n° 296590) and by the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement n° NMP4-SL-2009-229292 (Find&Bind). Authors thank Prof. Jean-Marc Fritschy from University of Zurich for providing the rat embryo cortex.

G. Palazzolo, N. Broguiere and M. Zenobi-Wong are with the Department of Health Sciences and Technology, ETH Zürich, Schafmattstrasse 22, 8093 Zürich, Switzerland (Corresponding author: G. Palazzolo. Phone: +41 44 633 06 13. Mail: gemma.palazzolo@hest.ethz.ch).

H. Dermutz is with the Department of Information Technology and Electrical Engineering, ETH Zurich, Gloriastrasse 35, 8092 Zurich, Switzerland.

J. Becher and M. Schnabelrauch are with Innovent e.V, Department of Biomaterials, Prüssingstraße 27 B, D-07745 Jena, Germany