# Functionalized polyethylene glycol hydrogels optimized for 3D neurite outgrowth of cortical and hippocampal neurons

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## I. Introduction and prior art

POLYETHYLENE glycol (PEG) hydrogels were optimized to promote neurite outgrowth of encapsulated dissociated cortical and hippocampal neurons. Being very weakly interactive with biological systems, PEG hydrogels represent an ideal "blank" 3D matrix in which the bioactivity of molecules can be studied. This weak interactivity also means PEG hydrogels are non-immunogenic, and therefore promising for various *in vivo* cell and drug delivery applications, as well as to enhance defect bridging or neural interfaces. They were shown to be biocompatible with primate brain upon implantation [1], and to support the 3D outgrowth of neurites from robust peripheral neuron clusters [2]. But gels that were optimized for 2D culture of neural progenitor cells were found to be non-permissive to 3D growth [3], and in PEG gels that undergo hydrolysis over time, neurites were found to extend through the gel only from neural clusters and at a very late stage of degradation [4]. As a result, PEG hydrogels which meet the stringent requirements of 3D cultures from dissociated central neurons are lacking. We generated functionalized PEG gels that fill this gap.

## II. Materials and methods

4-arm-PEG-vinyl sulfone (PEG-VS4) was prepared by reaction of 4-arm-PEG-thiol (Laysan Bio, 20kDa) with a 50 fold excess of divinyl sulfone (Sigma) in triethanolamine (TEOA) buffer pH8.0 for two hours and purified by dialysis (DS~90% by NMR). The medium for the E17 rat embryo primary hippocampal and cortical neurons consisted of neurobasal+1xB27+1xGlutamax (Gibco). For cell encapsulation, concentrated solutions of PEG-VS4 in PBS (pH 7.4, Gibco), adhesion peptide CSRARKQAAS**IKVAV**SADR-NH2 (IKVAV, Anaspec) in NaCl 0.9%w/v, enzymatically cleavable peptide Ac-GCRDGPQGIWGQDRCG-NH2 (ECP [5]) in PBS, HEPES 300mM pH7.4 and of cells in medium supplemented with 0.75%w/v hyaluronan (HA, Sigma, ~1.6MDa) were combined to get final concentrations of 1.3%w/v PEG-VS4, stoichiometric amount of enzymatically cleavable peptide, 100uM IKVAV, ~15%v/v HEPES buffer, 1e7cells/ml and 0.375%w/v HA. The mixture was left to gel for one hour before adding medium, half of it renewed every 3-4 days.

## III. Results

Optimization of mechanical properties, gelling conditions, attachment cues and degradability yielded gels enabling very fast and early neurite outgrowth of dissociated central neurons. A strong requirement was the very low macromer content, to form soft gels which can be easily disrupted and have larger pores. Among the three laminin-mimetic peptides RGD, YIGSR and IKVAV, only the latter was found to be effective to promote attachment and growth of neurons in a preliminary trial. The convenient pH8.0 usually used [5] being cytotoxic to neurons, we prepared the gels at pH7.4, stabilized with HEPES. Gelling in these conditions takes ~25min, which prompted the addition of a small amount of high viscosity HA to keep the neurons immobile during gelling. We expect these hydrogels to find applications in *in vitro* neurology and in neural engineering.

## References

[1] K. B. Bjugstad, D. E. Redmond, K. J. Lampe, D. S. Kern, J. R. Sladek, and M. J. Mahoney, "Biocompatibility of PEG-based hydrogels in primate brain.," *Cell transplantation*, vol. 17, no. 4, pp. 409–15, Jan. 2008.

[2] L. Marquardt and R. K. Willits, "Neurite growth in PEG gels: Effect of mechanical stiffness and laminin concentration," *Journal of biomedical materials research. Part A*, vol. 98, no. 1, pp. 1–6, Jul. 2011.

[3] K. J. Lampe, R. G. Mooney, K. B. Bjugstad, and M. J. Mahoney, "Effect of macromer weight percent on neural cell growth in 2D and 3D nondegradable PEG hydrogel culture.," *Journal of biomedical materials research. Part A*, vol. 94, no. 4, pp. 1162–71, Sep. 2010.

[4] M. J. Mahoney and K. S. Anseth, "Three-dimensional growth and function of neural tissue in degradable polyethylene glycol hydrogels.," *Biomaterials*, vol. 27, no. 10, pp. 2265–74, Apr. 2006.

[5] G. P. Raeber, M. P. Lutolf, and J. a Hubbell, "Molecularly engineered PEG hydrogels: a novel model system for proteolytically mediated cell migration.," *Biophysical journal*, vol. 89, no. 2, pp. 1374–88, Aug. 2005.

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