Aminopropyl silica-based hybrid films: an approach to improve neuronal cell adhesion and differentiation

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Statement of Purpose: The sol-gel process is a very flexible and biologically benign synthesis method. Consequently, it has many advantages to produce solid phase biointerface and culture surface to cells[1]. Also, flexibility of the sol-gel process allows easy adjustments of the synthesis conditions to tune the substrate for a more synergist effect. Silica based materials have been successfully employed as cell culture substrates and neural microelectrode coating[2,3]. It was shown that 100 nm-thick silica coating adhered to neural electrodes successfully without adverse effects on electrical properties at physiological frequency (i.e. 1 kHz).[2] However, the silica coating used was not conducive to neuron adhesion. Considering the function of neural electrodes, it is highly desirable to use coatings supportive of neuron adhesion for further neural integration with the implants. Neuronal adhesion and differentiation can be influenced by chemical, compliance and topography characteristics of a substrate. For example, substrates containing amino groups have been shown to improve cell attachment as a result of favorable proteins adsorption. In addition, a combination of these key factors can lead to improved conditions for neuronal growth[4]. Thus, in this study we investigate a hybrid silica film containing aminopropyl groups as a potential coating for neuronal adhesion and differentiation.

Methods: Silica-aminopropyl films were prepared by sol-gel process from Tetraethoxysilane (TEOS) and Aminopropyltriethoxysilane (APTES) (5:1) using nitric acid as catalyst and ethanol as solvent. The resulting sol after 12 h was deposited on piranha treated glass using spin-coating technique. Poly-L-lysine and bare silica films were used as controls. Samples were sterilized using 70% ethanol for 30 min and rinsed 5 times for 5 min each with sterile water. Substrates were characterized by AFM and water contact angle (WCA) measurements. For adhesion assays, PC12 cells, a rat adrenal pheochromocytoma cell line, and primary cortical neurons obtained from 7-day-old chicken embryos were used. The cells were seeded at 10,000 and 50,000 cells/cm^2, respectively. PC12 cells were cultured in F12-K supplemented with 1% horse serum, 0.2% fetal bovine serum, and 1% penicillin/streptomycin. Primary cortical cells were cultured in DMEM supplemented with 10% FBS, 1% penicillin/streptomycin, and 1% L-glutamine. The cultures were carried out at 37 °C in a 5% CO_2 atmosphere. Adhesion assay investigated the number of adhered cells after 24 h using the sum of ten 10x field images in triplicate. Cell morphology after 72 h was also examined via fluorescent staining of actin filaments (Alexa Fluor 488 phalloidin).

Results: The hybrid films obtained using sol-gel process presented low roughness as shown by AFM image in a z scale of 8 nm (Fig.1). The wettability determined by WCA for the hybrid materials was 84 ± 4 °. Support of neural adhesion by the hybrid material was observed for both PC12 cells and primary cortical cells (Fig.1), in comparison to PLL control. Bare silica showed no adhesion. In addition, both types of cells presented neurite development evidenced by the fluorescent staining (Fig.2) after 72h of culture. It suggests that the hybrid silica substrate can also support neuron differentiation.

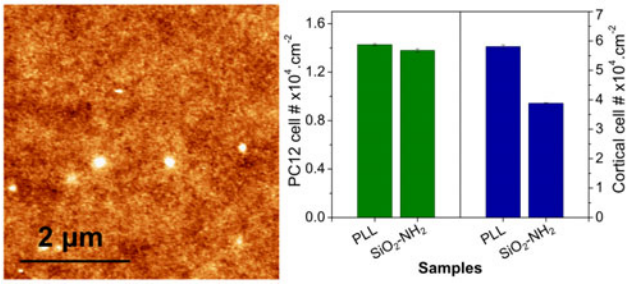

Fig. 1. (Left) AFM image of SiO_2-NH_2 sample (z scale 8 nm). (Right) Neuronal adhesion after 24 h (*p<0.01).

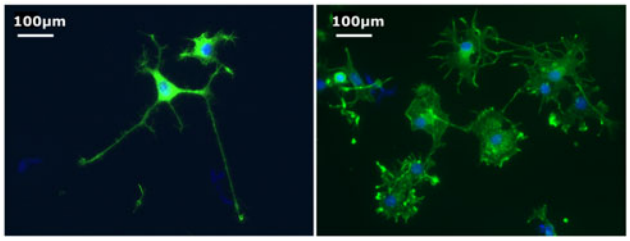

Fig. 2. Fluorescent staining showing morphology of (left) PC12 cells and (right) primary cortical neurons after 72 h culture on SiO_2-NH_2 substrates.

Conclusions: Hybrid silica materials containing aminopropyl groups were shown to support neural attachment and differentiation. This behavior could be attributed mainly to the presence of the amino groups. We hypothesize that further tuning the aminopropyl group concentration in the film could lead to additional improvements in neuron attachment and differentiation. A detailed quantification of neurite extension and branching are currently underway.

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References: **[1]** Jedlika S.S., Rickus J.L., J Mat Chem. 2006 (16:3221-3230). [2] Piece A.L., Otto K.J., J Neurosci Meth. 2009 (280:1061-110). [3] Hickman G.J., Perry C.C., J Mater Chem. 2012 (22:12141-12148) **[4]** Roach P., Alexander M.R., Surf Sci Rep. 2010 (65:145-173).