

Material Surface Modification with Organized Multilayer Assemblies of Biological Macromolecules

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INTRODUCTION: Artificial polymer scaffolds are used by tissue engineering to accommodate cells and guide their growth. The cell interactions with scaffolds which themselves do not specifically promote the growth of cells but satisfy the other necessary criteria, such as, mechanical properties, shape, morphology, biodegradability, etc., can be specifically affected by modifications of the scaffold surface. The controlled successive immobilization of biological and synthetic macromolecules ¹ has provided a technology for creating functional interfaces between artificial materials and biological media, e.g. blood compatible coatings ² or biorecognition layers on optical immunosensors ³. The technique makes possible to set up assemblies composed of a selected number of molecular layers of various macromolecules arranged in a designed order.

METHODS: The multilayer assemblies were prepared on polystyrene surfaces (PS) by successive deposition of molecular layers of laminin (LA), collagen IV (C), gelatin (G), polylysine (PL), poly(ethyleneimine) (PI), dextran sulfate (D), and bovine serum albumin (A). The first monolayer was immobilized by hydrophobic adsorption of a protein on PS. The second and third monolayers were adsorbed utilizing electrostatic interactions between molecules in solution and oppositely charged surface monolayer, i.e. interaction of LA⁻ negatively charged above isoelectric point (pI) or polyanion D⁻ with G⁺ or C⁺ positively charged below their pI and interaction of polycations PI⁺ or LA⁺ with A⁻ or LA⁻ above pI. G, C, and D were adsorbed from citrate buffer pH 4, LA, A, PI, and PL were adsorbed from phosphate buffered saline pH 7.4, (PBS). The deposition procedure was observed in real time and stability of the assemblies in PBS was tested using surface plasmon resonance (SPR) ³ on gold chips coated with a PS film. The morphology of the surfaces was observed in PBS using atomic force microscopy (AFM). The adhesion and growth of mouse embryonic stem cells line D3 was tested in PS culture dishes coated with the assemblies.

RESULTS: The molecular assemblies remained immobilized on PS without observable changes after 2-days incubation in PBS.

Table 1. Mass of differentiated (d) cells grown for 48 hours on coated PS surfaces

Arrangement of molecular layers immobilized on PS surface								
<u>PS</u>	<u>PS</u>	<u>PS</u>	<u>PS</u>	<u>PS</u>	<u>PS</u>	<u>PS</u>	<u>PS</u>	<u>PS</u>
LA	C	C	C	G	G	A	A	A
		D	LA	D	LA	PI	PL	PL
		C		G			A	LA
							PL	
Cell culture (cell mass related to that on uncoated PS=1)								
1.5	2.3	3.6	3.0	4.5	4.2	0.9	0.8	1.6

Mass values similar to those in Table 1 were observed if the differentiation of cultivated cells was inhibited. The mass of non-differentiated (nd) cells on PI containing surface increased considerably (nd/d=14) when they were seeded in serum-free medium.

DISCUSSION & CONCLUSIONS: The stability of the coatings during the cell cultivation might be different from that in PBS. However, the results indicate, that assemblies containing extracellular matrix proteins (C, G, LA) were able to promote significantly the cell growth. Potentially, the adsorption technique allows us to prepare assemblies tailored for individual cells on scaffolds of any shape and morphology fabricated from any material on which the first protein layer can be immobilized.

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ACKNOWLEDGEMENTS: This research was supported by the Grant Agency of the Czech Republic (203/02/1326) and by the Ministry of Education, Youth, and Sports, CR (LN 00A065)