

Development of Tunable Surface Coatings via Layer-by-Layer Deposition

T. Croll^{1,2}, A. O'Connor¹, G. Stevens¹, J. Cooper-White^{2,*}

¹ University of Melbourne, Victoria, Australia ² University of Queensland, Queensland, Australia
*Corresponding Author

INTRODUCTION: A promising approach to the surface engineering of biopolymers is the so-called “blank slate” – the creation of a non-interactive surface which can then be functionalised to provide only the desired interactions when placed in a biological environment. One highly versatile avenue towards this goal is layer-by-layer electrostatic deposition of polyelectrolytes (biologically derived or otherwise). The oppositely charged polysaccharides hyaluronic acid (HA) and chitosan in particular appear to be promising candidates for this technique.

METHODS: Numerous surfaces, including a number of alkanethiol monolayers, aminolysed poly(lactic-co-glycolic acid) (PLGA) and tissue culture polystyrene (TCP) were coated layer-by-layer with HA and chitosan in the presence of carbodiimide as a crosslinking agent. Buildup, stability, and protein resistance/binding were followed using a quartz crystal microbalance (QCM). X-ray photoelectron spectroscopy (XPS) was used to follow the build-up on PLGA surfaces. In addition, the *in vitro* cell response to these surfaces were analysed using fluorescence microscopy techniques.

RESULTS: QCM analysis showed very similar build-up behaviour on different surfaces and at 10-fold different polymer concentrations (50 and 500 µg/mL). No change in mass was observed when a multilayer surface was challenged with fibronectin, albumin or collagen IV under physiological conditions. However, collagen IV bound readily in a reduced salt, low-pH buffer, in the presence of carbodiimide. This collagen was further used as a template for the self-assembly of proteins from dilute Matrigel.

When placed in contact with 10% foetal bovine serum in DMEM, mass was lost corresponding closely to the mass of the final HA layer, presumably due to the action of hyaluronidase. However, this loss was prevented by incubating the surface with 10 mM acetate in the presence of carbodiimide – it is hypothesized that the carbodiimide mediated the esterification of the acetate to the HA hydroxyl groups. Interestingly, no mass increase was noted in this case, indicating that the surface retained its non-adhesive properties.

XPS analysis of the carbon 1s spectrum of treated PLGA surfaces showed a progressive change as the number of layers increased, reaching a steady state with no evidence of the characteristic peaks of PLGA after 5 layers.

The *in vitro* response of NIH-3T3 fibroblasts to the treated surfaces strongly reflected the results observed in the QCM. While cells spread and multiplied quickly on untreated and aminolysed PLGA and TCP, cell adhesion was almost entirely removed upon the application of 3 ½ HA/chitosan bilayers, with all observed cells remaining rounded. On collagen IV-coated multilayer surfaces, adhesion, spreading and multiplication were restored to control levels.

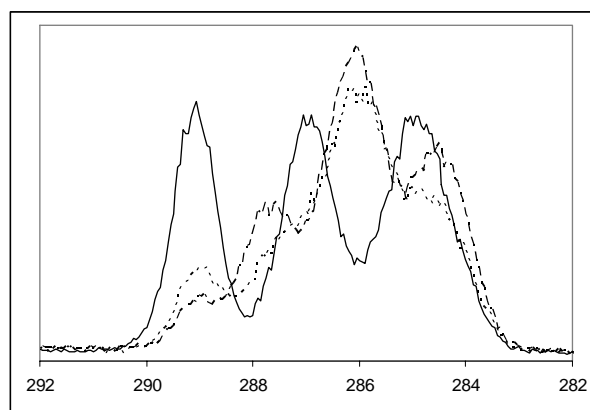


Figure 1. XPS C1s spectrum of aminolysed PLGA (solid line), a 3 ½ bilayer HA/chitosan coating (dotted line) and 3 ½ bilayers + collagen IV (dashed line).

CONCLUSIONS: Layer-by-layer modification provides an extremely facile, versatile method firstly for the production of highly non-interactive surfaces, and the subsequent functionalisation of these surfaces to provide specific interactions with cells. Much of the flexibility inherent in this method remains to be tapped.

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