Communication

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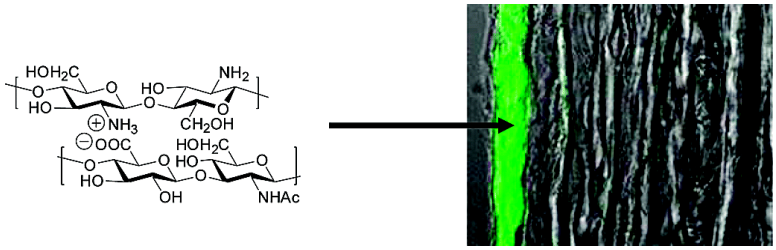

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Nanocoatings onto Arteries via Layer-by-Layer Deposition: Toward the in Vivo Repair of Damaged Blood Vessels

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The layer-by-layer self-assembly of polyelectrolytes has emerged as a powerful and versatile, yet simple, strategy to engineer surfaces with specific properties.[1] Applications in the biomedical field are scarce, but they hold great promise, as the method permits the construction of thin films containing macromolecules, such as proteins, enzymes, or nucleic acids, with targeted properties onto a variety of substrates.[2] In this Communication, we describe the deposition of self-assembled nanocoatings onto arteries, as a means not only to protect an artery damaged during the revascularization procedure against blood coagulation, but also to control the healing processes by incorporating bioactive molecules within the multilayer.

Indeed, revascularization procedures are often plagued with complications related to the reobstruction of the treated artery, that is, restenosis, resulting from injuries induced during the procedure to the vascular wall. These injuries denude the protective endothelial cell lining (endothelium) and initiate excessive vascular cell proliferation within the artery.[3] Systemic and local drug delivery via catheters or nanoparticles has been attempted, but such treatments have been largely unsuccessful in alleviating restenosis, due to inefficient targeting of the drug to the site of injury.[4] Ideal strategies against restenosis should prevent the growth of blood thrombi on damaged arteries, enhance healing, and prevent the proliferation of vascular cells. Interest in local delivery has, however, been recently diverted by the success of drug eluting stents.[5] In this Communication, we demonstrate that it is possible to build on damaged arteries a nanoscale self-assembled multilayer obtained by alternating depositions of two polysaccharides, hyaluronan (HA), a polyanion, and chitosan (CH), a polycation. The two polysaccharides (Figure 1) have been chosen in view of their biocompatibility, healing capabilities, and antiinflammatory properties.[6]

First, we characterized in detail the self-assembly of the CH/HA polyelectrolytes on a model surface, collagen-coated glass slides, using an ^{111}In-radio-labeled HA.[7] Multilayer growth was a linear function of the number of layers[8] (slope = 5.1 μg ^{111}In−HA/cm^2 per layer deposited; $r^2 = 0.99$, [NaCl] = 0.14 M) (Figure 2). The electrostatically driven adsorption of the polymers occurred within a few seconds. Increasing the ionic strength of the polymer solutions (from 0.14 to 1.0 M NaCl) resulted in a decrease of the amount of deposited polymer. Quartz microbalance (QCM) studies indicated a hydrated thickness of about 70 nm for 5 bilayers. Using radio-labeled HA instead of HA resulted in an increase of the multilayer thickness (~30%). The stability of the multilayer was monitored by incubation of a (CH/^{111}In−HA)$_5$ self-assembly in phosphate buffer saline (PBS) at 40 °C. No loss of radioactivity was detected after 1 week.

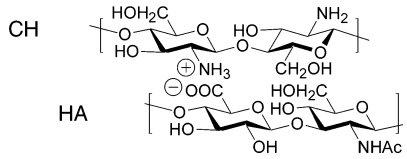

Figure 1. Chemical structure of chitosan (CH) and hyaluronan (HA).

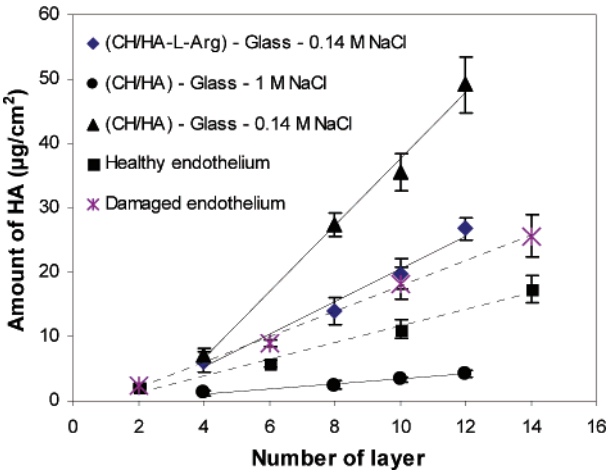

Figure 2. Amount of ^{111}In−HA vs layers. Solid lines: CH/HA (moderate and high ionic strength) and CH/HA−L-Arg on collagen-coated glass. Dashed lines: CH/HA on damaged or healthy endothelium. Linear regressions are presented excluding the first bilayer due to the substrate effect.

The multilayer was then constructed on damaged and healthy aortic porcine arteries, using a perfusion chamber matching closely the conditions achievable in vivo. Strong adhesion of the coating on the artery was secured by depositing first a layer of chitosan, a polycation exhibiting excellent bioadhesive properties toward negatively charged surfaces such as those presented by damaged arteries. Linear build up profiles were obtained (Figure 2) with lower amounts of polymers being deposited on healthy endothelium, as compared to damaged arteries.

The successful deposition of the multilayer and its retention on the arterial wall was monitored by confocal microscopy detection of CH/FITC−HA (fluorescein isothiocyanate) multilayers. A top view of the coated artery indicated that the multilayer uniformly covered the damaged artery. The multilayer-coated artery was subsequently subjected to physiological shear by perfusion in PBS. Imaging of transversal sections showed that the multilayer was retained on the vascular wall after a 24 h perfusion (Figure 3). Remarkably, the multilayer, or at least the fluorescently labeled HA, was detectable within the arterial wall, an indication of the polymer diffusion. Note that an assembly of 5 bilayers allowed the incorporation within the vascular wall of about 20 times more HA (18.2 μg/cm^2 vs 0.86 μg/cm^2) than passive infusion of a HA

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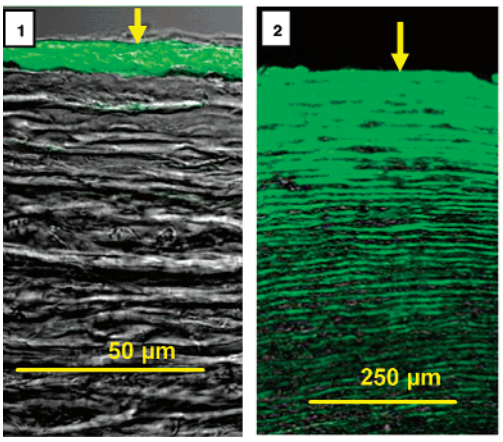

Figure 3. Confocal microscopy imaging of a section of a $(CH/HA\text{-}FITC)_5$-coated damaged artery showing the transmural disposition of the fluorescently labeled polymer after (1) 0 h and (2) 24 h perfusion with PBS. The arrow indicates the coated surface exposed to the PBS flow.

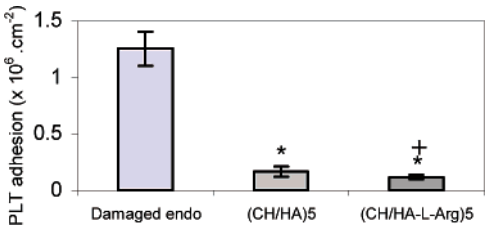

Figure 4. Platelet adhesion ($n \geq 4$) on damaged endothelium (damaged endo), $(CH/HA)_5$-, and $(CH/HA-L\text{-}Arg)_5$-coated damaged endothelium ((CH/HA)5 and (CH/HA-L-Arg)5). $*$ represents $p < 0.05$ vs damaged endo; $+p < 0.05$ vs (CH/HA)5 by the paired student *t*-test.

solution (1 mg/mL HA in 0.14 M NaCl, 15 min). HA, together with other anionic polysaccharides, such as heparin or fucoidans, is known to inhibit vascular cell proliferation.[6a,9] Because the biological response of the damaged artery in vivo has been correlated to tissue concentration rather than to the administrated dose of active polysaccharides,[10] the CH/HA nanocoatings may act as highly effective inhibitors of restenosis.

Next, CH/HA multilayer-coated arteries were placed in contact with blood to assess, via an in vitro platelet adhesion assay, the protective effect of the nanocoating against platelet adhesion onto damaged arteries. The growth of thrombus on damaged arterial surfaces was significantly inhibited by the CH/HA multilayers (Figure 4; 87% reduction in platelet adhesion, $p < 0.05$). This observation corroborates a previous report that polylysine/alginate self-assembly can act as a barrier material against cell attachment when deposited in vitro onto extracellular matrix-coated surfaces.[11] Insulation of the vascular wall from blood components, such as platelets and growing thrombi, results in reduced activation of vascular cells.[12] By cutting off this physiological response, one can expect to block the restenosis pathway early on.

Finally, we set out to evaluate whether CH/HA multilayers can act as localized drug-release systems and promote the artery healing process. Thus, we deposited onto arteries a multilayered assembly containing L-arginine. This low molecular weight cationic peptide has been reported to reduce restenosis.[13] It is the precursor of nitric oxide (NO), which is known to affect vascular tone and wall dynamics, to inhibit monocyte and platelet adhesion, and to prevent vascular cell proliferation. Multilayers were constructed by sequential deposition of a CH solution and a solution of a HA/L-arginine complex (L-arginine/disaccharide molar ratio: 1/5).[14] Under these conditions, it was possible to incorporate L-arginine (213 ng/cm^2 in 5 bilayers) within the multilayer, but the amount of HA per layer was significantly lower than that in the case of CH/HA multilayers of identical layer number (Figure 2). The small amount of drug loaded within the multilayer was a result of its extraction during the adsorption of the chitosan layers. The release profile of [^{3}H] L-arginine from a multilayer placed in PBS featured an initial burst (~80% release) followed by a slow linear regime.[15]

Incorporation of L-arginine in the CH/HA nanocoating further improved its protective effect against platelet adhesion, as demonstrated by a reduction of 30% in the amount of platelet adhesion after 90 min ($p < 0.05$), as compared to arteries protected by a nanocoating devoid of L-arginine. Note that, as compared to unprotected damaged arteries, L-arginine loaded CH/HA self-assemblies reduced platelet adhesion by 91% ($p < 0.05$). Conjugation of biologically active components, such as antiproliferative drugs or hormones to the polymers, is under investigation. This approach is expected to significantly enhance the amount of drug incorporated in a multilayer and to allow its sustained presentation or its gradual release within the arterial wall.

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Supporting Information Available: Experimental details, QCM experiments, and L-arginine release profile (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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