Communication

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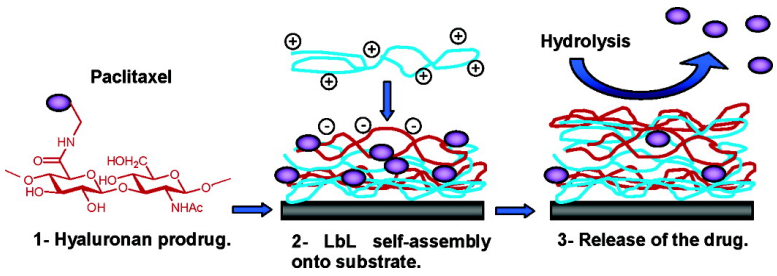

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Delivery Platform for Hydrophobic Drugs: Prodrug Approach Combined with Self-Assembled Multilayers

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Over the past decades, a number of approaches have been proposed to achieve site-specific and time-controlled delivery of therapeutics, thus alleviating undesired side effects and enhancing the efficacy of a treatment.[1] The so-called "prodrug approach", whereby a drug is linked to a biocompatible polymer via a hydrolyzable bond, has emerged as one powerful method for controlling the release of a variety of compounds to a targeted site. This methodology has advantages in solubilization of the drug, especially in the case of hydrophobic drugs and tunability of the drug pharmacokinetics.[2] Paclitaxel is one of the most potent chemotherapeutic agents, particularly in the treatment of breast and ovarian cancers.[3] Its clinical use has remained rather limited due to difficulties in effectively formulating this poorly water soluble drug. Nonetheless, recently there have been notable achievements in the implementation of paclitaxel-eluting-endovascular implants in interventional cardiology, which led to a drastic reduction of complications associated with coronary revascularization procedures.[3b,4] Also, several paclitaxel prodrugs have been synthesized and are currently being evaluated in antitumor therapies.[5]

Another class of polymeric biomaterials that shows great promise in various therapies includes the polyelectrolyte multilayers (PEMs) constructed by the layer-by-layer (LbL) technique.[6] The ability to incorporate bioactive molecules in PEMs has generated considerable interest in the field of drug delivery. Strategies explored to date involve, for example, the release of DNA from degradable PEMs, the use of core−shell structures loaded with various compounds, or the pH-mediated delivery of charged low molecular weight drugs via electrostatic interactions or hydrogen bond formation.[7] Application of these strategies remains rather ineffective in view of the creation of efficient controlled delivery systems. In this communication, we report for the first time the amalgamation of the prodrug approach and the LbL technique, using a hyaluronan (HA) ester prodrug of paclitaxel as the polyanion to construct PEMs with chitosan (CH), a polyamine. Multilayers formed by HA and CH have previously been shown to restore blood compatibility of injured arteries, alleviating the risks of post-angioplasty restenosis of blood vessels.[8]

The prodrug HA-Pac (Figure 1) was prepared by linking paclitaxel to HA via a labile succinate ester linkage. A 2′ hemisuccinate derivative of paclitaxel was prepared first, as previously reported,[9] activated to the corresponding *N*-hydroxysuccinimide (NHS) ester and linked to an amine-modified HA. The successful formation of the paclitaxel NHS ester was confirmed by ^{1}H NMR analysis, in particular by the appearance of a signal at 2.6 ppm, attributed to the succinimide methylene protons and the downfield shifts of the signals due to the protons 2′-H and 3′-H,

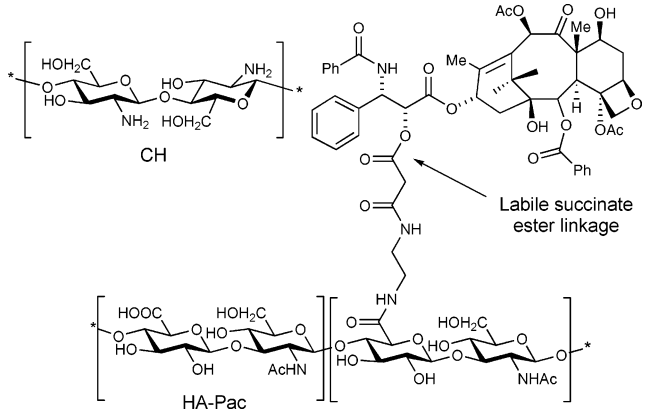

Figure 1. Structure of the HA prodrug of paclitaxel (HA-Pac) and CH.

from 4.70 to 5.49 ppm and 5.79 to 5.97 ppm, respectively.[10] The level of paclitaxel incorporation onto HA had to be kept low, to preserve the water-solubility of the prodrug. Two HA-Pac samples, with paclitaxel loading of 3 and 6 mol % (vs HA disaccharide units, as determined by UV absorbance, $\lambda_{max} = 228$ nm) were obtained, but only HA-Pac containing 3 mol % exhibited sufficient solubility in aqueous NaCl for use under standard LbL build-up conditions (0.5 mg/mL).

A cell viability assay was conducted, subjecting murine macrophages J774 to increasing doses of HA-Pac to ascertain that paclitaxel retained its activity when linked to HA. The percentages of cell survival were 108 ± 39, 37 ± 7, and 26.7 ± 6 for macrophages treated with equivalent paclitaxel amounts of 0.1, 1, and 10 μg ($p < 0.05$ for doses of 1 μg and 10 μg in comparison to control cells by ANOVA-Dunnett). Unmodified HA has no cytotoxic effect.

Next, we set up to construct multilayers of CH and HA-Pac, the latter playing the dual role of structural element of the PEM and of the macromolecular carrier of the drug. The PEM buildup was monitored in situ in a dissipation enhanced quartz crystal microbalance (QCM). A continuous decrease of the QCM frequency shift (Δf), which is proportional to the mass deposited on the sensor surface, as a function of layer number confirmed the occurrence of build-up (Figure 2). The shifts observed for CH/HA-Pac multilayers were more pronounced compared to those for the CH/HA coating. This trend may indicate that a larger amount of HA-Pac (vs HA) is deposited in each step. It may also reflect the higher viscoelasticity of CH/HA-Pac multilayers, compared to that of CH/HA multilayers. Both factors contribute to the frequency shift observed.

The CH/HA-Pac build up was observed by tapping mode atomic force microscopy (AFM). AFM images of multilayers present features characteristic of CH/HA PEM, with the appearance of small

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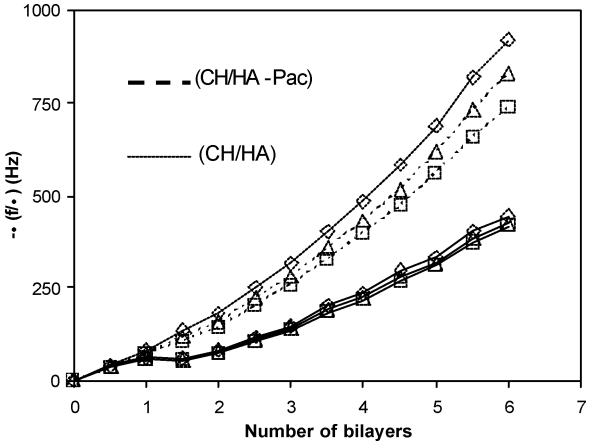

Figure 2. QCM resonance frequency shifts as a function of the number of layers for the first three harmonics; □: 5 MHz, △: 15 MHz, ◇: 25 MHz.

islets that grow in size (50 to ∼200 nm) as a function of bilayer number and eventually coalesce into larger domains (see Supporting Information).[11] Significant islet coalescence takes place after deposition of HA-Pac(CH/HA-Pac)$_2$, whereas unmodified multilayers HA(CH/HA)$_2$ still feature distinct small islets. These observations, together with the QCM results concur, indicating that the presence of the hydrophobic paclitaxel moieties does not prohibit the construction of multilayers, although the growth mechanism may be affected.

The release of paclitaxel from a HA-Pac(CH/HA-Pac)$_9$ nanocoating placed in contact with water was monitored as a function of time by measuring the UV absorbance of released paclitaxel (see Supporting Information). Under these conditions the half-life of paclitaxel was ∼3 h. Multilayers constructed under identical conditions but with rhodamine-labeled chitosan were subjected to the same release test. UV spectra of water placed in contact with these multilayers presented a significant absorbance at 240 nm, due to paclitaxel, but negligible at 550 nm, the wavelength of rhodamine absorbance, vouching for the stability of the multilayers throughout the release experiment. The total amount of paclitaxel released was 1.8 μg/cm^2 which is in agreement with the expected total amount loaded into the PEM extrapolated from our previous study with radiolabeled HA.[7c]

The efficacy of the paclitaxel-loaded PEMs was investigated in a cell viability assay (Figure 3). Twenty-four-well plates were coated with HA-Pac(CH/HA-Pac)$_9$ or HA(CH/HA)$_9$ PEMs and UV sterilized. J774 macrophages were plated onto the coated surfaces and cultured for 4 days in RPMI media. Cells cultured onto paclitaxel-loaded PEMs displayed a 95% reduction in viability ($p < 0.05$ by ANOVA-Dunnett) while cells cultured onto HA(CH/HA)$_9$ were healthy (100.2% cell survival).

In summary, we have demonstrated the possibility of self-assembling paclitaxel-loaded PEMs using a hyaluronan ester prodrug of paclitaxel. This prodrug approach could be applied to other therapeutic molecules of interest. The numerous available strategies for such a macromolecular conjugation offer convenient ways to achieve better control of the pharmacokinetics. One could easily envisage with this strategy to design PEMs with multiple drugs and/or with complex release behaviors based on appropriate chemical design of the prodrugs. Combined to the compatibility of

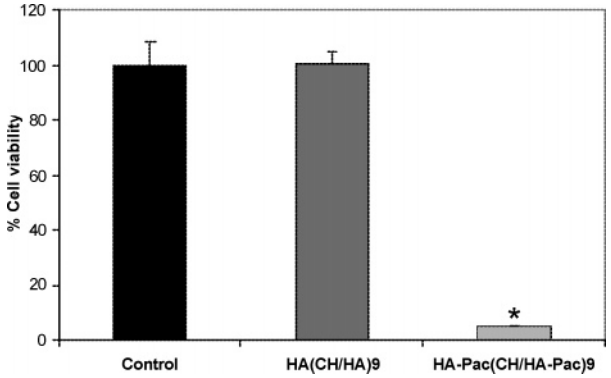

Figure 3. Cell viability assay (MTT): J774 macrophages were cultured onto HA(CH/HA)$_9$ and HA-Pac(CH/HA-Pac)$_9$ multilayers for 4 days. (*) $p < 0.05$ vs control by ANOVA-Dunnett test.

the LbL methodology with complex geometries, nano/microparticules or living tissues, this approach could be of significant interest in the design of new therapeutic drug delivery strategies.

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Supporting Information Available: Experimental details, NMR and AFM data, release profiles, and cell viability assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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