PERSPECTIVE

Multilayered Polyelectrolyte Films: From Active Molecular Delivery to Vaccine Therapy

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ABSTRACT A paper in this issue discusses the use of multilayered polyelectrolyte films incorporating a hydrolytically degradable polymer for transcutaneous drug or vaccine delivery. Dried films could be deconstructed rapidly, and a model protein antigen (ovalbumin) and/or immunostimulatory molecules could be released with different kinetics. Both molecules could also be delivered to antigen-presenting cells in the skin (Langerhans cells). The described approach provides a new route for the storage of vaccines. Successful antigen processing and presentation to lymphocytes remain ongoing challenges before the concept can be used in the next generation of vaccines.

Polyelectrolyte multilayer (PEM) films consist of self-assembled layers obtained by the alternated deposition of polyanions and polycations.[1] The approach is very popular because of the ease in constructing such films, by either dipping, spin-coating, or alternated or simultaneous spraying. The interest in the concept lies in the fact that almost any charged molecule that has affinity for another species can be used for the film assembly. Even uncharged molecules can be included in the architectures *via* hydrogen bonding or hydrophobic interactions with other macromolecules.

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Polyelectrolyte multilayer films have been suggested for various applications, including optoelectronics, fuel cells, and biomedicine. In this latter domain, numerous publications have been devoted to biomaterial surface coatings, preparation of micro- or nanoparticles, or free-standing membranes, with the aim to confer to the resulting material specific properties enabling interactions with the surrounding proteins, cells, or tissue.[2]

Properties Favoring Biological Applications. The facility to "biofunctionalize" polyelectrolyte films renders them particularly attractive for biological applications. Biofunctionalization can be easily achieved by adsorbing or including in the architecture any biologically active molecule (*i.e.*, growth factor, drug, peptide, enzyme, DNA, siRNA, *etc.*).[3−6] The active molecules can be delivered to the cells after enzymatic degradation of the architecture induced by the cells. Another interesting concept for drug delivery from PEM films is based on the use of hydrolytically degradable polyions.[7] Generally, the embedded drug molecules, in particular proteins, retain their bioactivity once released from the film onto which they were adsorbed or embedded.

The work by Su *et al.* in the present issue goes a significant step further.[8] These authors show that previously dried films assembled with ovalbumin, a model vaccine antigen, and poly(β-amino esters), a family of hydrolytically degradable polyelectrolytes, are degraded after initial contact with skin. Moreover, the active antigen molecules are taken up by the cells once they are in the presence of an aqueous medium or in the tissue (skin) (Figure 1). These observations are important for clinical or medical use since the dry form allows easier and longer storage possibilities (*e.g.*, in the form of a patch) and embedded samples could be prepared long before use in clinical applications.

An important property of these assemblies is related to the possibility of varying the amount of the embedded drug. It has been shown that embedding the molecules within the PEM architecture can be enhanced considerably by the use of exponentially or supralinearly growing films, instead of linearly growing ones.[9] Indeed,

See the accompanying Article by Su *et al.* on p 3719.

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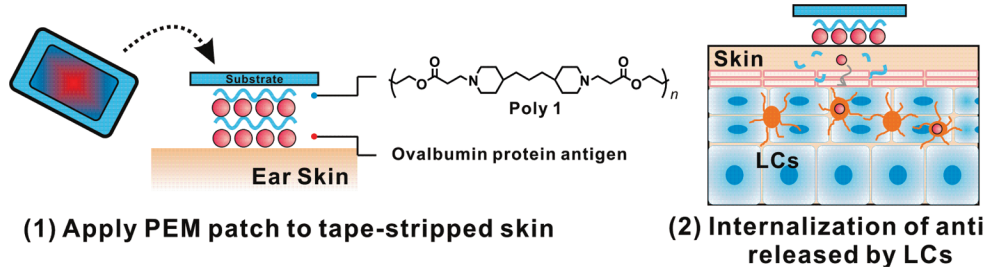

Figure 1. (1) Schematic representation of a PEM patch on a substrate that is applied onto tape-stripped ear skin. (2) Ovalbumin is released from the patch as the multilayer disassembles and penetrates into the basal layer of the epidermis. Reproduced with permission from ref 8. Copyright 2009 American Chemical Society.

supralinear growing films may attain thicknesses in the micrometer range after only a modest number of deposition cycles. The drug or protein brought into contact with an exponentially grown, often strongly hydrated film, diffuses through the whole film, which acts as a reservoir. In this way, more active molecules can be taken up, compared to the case of a linear film, where both the thickness and the compact nature of the film are detrimental to storing molecules. For clinical drug delivery, it is also important to be able to tune the duration of the release. An example of this is shown in Figure 2. Here, a drug (pyroxicam, Px) was complexed with cyclodextrin (CD) and embedded in different poly(glutamic acid)/poly(L-lysine) (PGA/PLL) buildups. When exposed to the leukemia cell line THP-1, the intensity and duration of the amount of secreted tumor necrosis factor-alpha (TNF-α) can be tuned by the number of polyelectrolyte layers deposited above the active molecule.

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Another consideration in fabricating drug delivery constructs is the possibility of inducing a cascade of reactions, which may increase the potency or efficacy of the active drug molecule. For example, by including complexes of plasmid DNA and poly(ethyleneimine) in polyelectrolyte films aimed at cell transfection in addition to a peptide molecule (NDPMSH) bound to poly(L-glutamic acid) as a signal molecule, both active molecules were able to react with melanoma cells independently.[10] This led to cell transfection and cell activation *via* the peptide signaling pathway in a synergistic manner to enhance cell transfection rate.[10] It has also been demonstrated that embedding two types of transfection vectors expressing human transcription factors enabled targeting of either the nucleus or the cytoplasm reaction (Figure 3).[11] Depending on the architecture, the distance from the surface, and the number of polyelectrolyte layers separating the two embedded active constructs, a sequential response was obtained.[11] By reversing the deposition sequence of both vectors in the architecture, the transfection sequence could also be reversed.

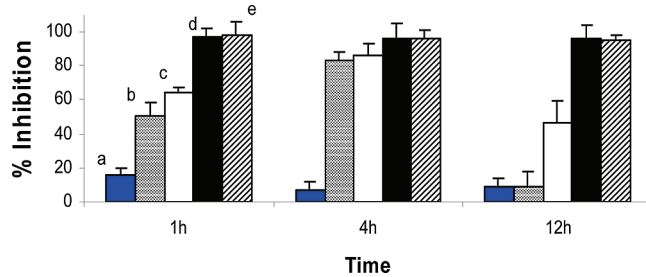

Figure 2. Inhibition of tumor necrosis factor-alpha (TNF-α) secretion by stimulated THP-1 cells grown on multilayered films containing a cyclodextrin–pyroxicam (CDPx) complex. Cells were deposited on multilayered films built with poly(ethyleneimine) (PEI), poly(L-glutamic acid) (PGA), and poly(L-lysine) (PLL). The variation in inhibition indicates that there is an optimal balance between the composition and number of polyelectrolyte layers in the film: (a) PEI-(PGA-PLL)$_6$-Px, (b) PEI-(PGA-PLL)$_6$-CDPx, (c) PEI-(PGA-PLL)$_6$-CDPx-PLL-(PGA-PLL)$_3$, (d) PEI-(PGA-PLL)$_3$-CDPx-PLL-(PGA-PLL)$_3$-CDPx-PLL-(PGA-PLL)$_3$, (e) PEI-PGA-(PLL-CDPx)$_3$. Reproduced with permission from ref 6. Copyright 2004 Wiley-VCH Verlag GmbH & Co. KGaA.

Similarly, the work by Hammond's and Irvine's groups in this issue shows that the release kinetics of the vaccine component (ovalbumin) could be tuned by combining with a short oligonucleotide designed to increase the efficacy of the antigen. The release times in the range of 2–6 days are of particular interest for clinical use, and specifically, distinct delivery schedules of vaccine components may be particularly important in a vaccine strategy.

Biofunctionalized Polyelectrolyte Films *In Vivo*. Safe and effective systemic delivery remains a major challenge still impeding the use of new therapeutics in the clinic. Most of the trials for clinical applications concerning drug delivery *in vivo* were performed *via* multilayered polyelectrolyte capsules.[12] In this respect, targeting polyelectrolyte films in the form of capsules toward specific cells could offer tremendous help because deleterious side effects could be avoided. This approach has been pursued, in particular, in cancer therapy applications. For clinical use, this route was developed generally through intravenous injection. Intravenous administration requires particles with reduced size to allow their passage in small blood capillaries. The charged particles often nonselectively bind to proteins rapidly upon injection into the biological fluid, rendering targeting from the active molecule inefficient. For delivery to be effective, specific capsules have to be transported to a specific tissue. One of the strategies employed has been to insert magnetic nanoparticles in the shell of the capsules and to direct the particle to a specific area by magnetic field.[13] Other possibilities are particle functionalization with targeting ligands or monoclonal antibodies targeting transmembrane glycoproteins expressed by cancerous cells.[14] For these approaches, only very few assays have been performed directly *in vivo* in animal models to date.

The Future of Layer-by-Layer Films as a Tool for Vaccine Delivery. Besides the problems described above for drug delivery, vaccine delivery raises specific difficulties. Among them is the need to target the body's defense system represented by antigen-presenting cells such as dendritic cells (DCs), which are responsible for the appropriate immune responses. Oligopeptides have demonstrated efficient responses once used for the prevention of various diseases. Different delivery strategies have been proposed that are based on the use of liposomes, emulsions, particles, or capsules also prepared by the layer-by-layer (LbL) method. The Caruso group used a cysteine-modified polypeptide bound to poly(methacrylic acid).[15] The films were constructed based on hydrogen bonding with poly(vinylpyrrolidone). The system was found to become active in the presence of a natural reducing agent, glutathione. The work by Irvine's and Hammond's groups in this issue demonstrates for the first time the possibility of using multilayered polyelectrolyte films with embedded active molecules as a vaccine delivery system *in vivo* using a patch model applied to tape-stripped skin (Figure 1).

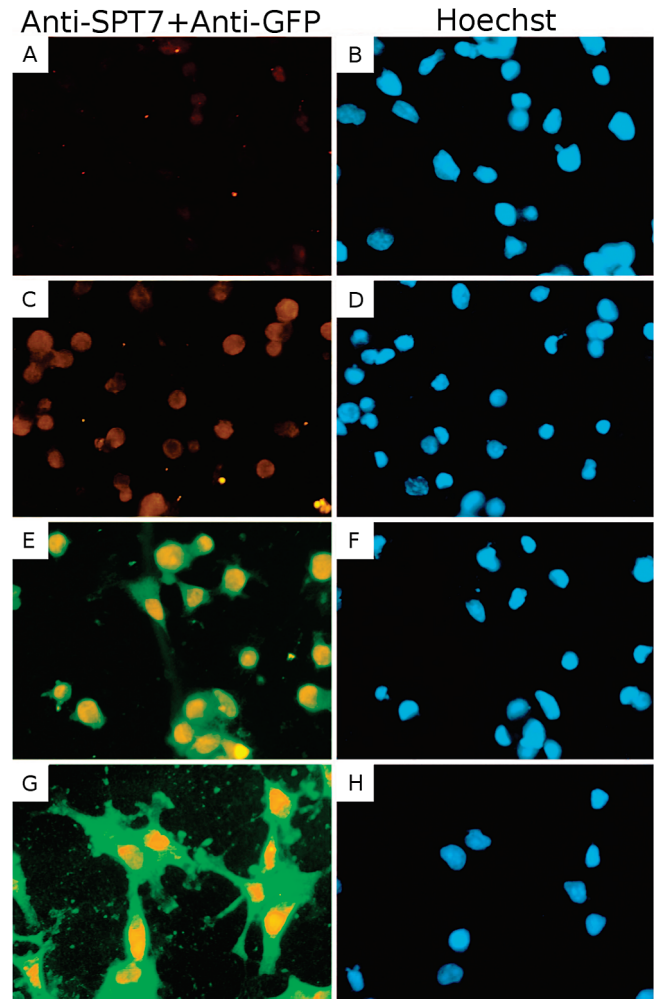

Figure 3. Expression of the nuclear transcription factor SPT7 and enhanced green fluorescent protein (EGFP) in cytoplasm in COS cells grown on the surface of $(PLL\text{-}PGA)_5$ pCD-$(PGA\text{-}PLL)_5$-PGA-PCD-$(PGA\text{-}PLL)_5$ multilayered films (A,B) or $(PLL\text{-}PGA)_5$-pCD-pEGFP-pCD-$(PGA\text{-}PLL)_5$-PGA-pCD-h5PT7pTL-pCD-$(PGA\text{-}PLL)_5$ multilayered films for 2 (C,D), 4 (E,F), and 8 h (G,H). The expression of SPT7 (red) and EGFP (green) was detected by using mouse monoclonal anti-SPT7 and rabbit polyclonal anti-GFP as primary antibodies and Cy3-conjugated goat antimouse and Alexa Fluor 488 goat antirabbit as secondary antibodies (A,C,E,G). Nuclei were visualized by Hoechst 33258 (B,D,F,H). PLL and PGA as in Figure 1, pCD (pyridylamino-β-cyclodextrin). Reproduced from ref 11. Copyright 2006 National Academy of Sciences.

The assembly demonstrated by Su *et al.* enabled the delivery of ovalbumin together with adjuvant oligonucleotide molecules aimed to improve the efficiency of the vaccine model molecule in an *in vivo* murine ear skin model.[8] This opens the route for vaccination, but different hurdles have yet to be surpassed. Antigen delivery to DCs is a complex problem including antigen transport to DCs, antigen uptake by DCs, and relevant antigen processing and presentation, which are dependent on the mode of DC activation and antigen uptake, and finally, on the functional role of the material itself. The strategy reported by Irvine's and Hammond's groups appears promising for achieving targeted and prolonged delivery to DCs. Successful antigen processing and presentation to CD4+ and CD8+ lymphocytes remain the ongoing challenge. Once these challenges are overcome, one could foresee using this concept in the next generation of vaccines.

> Only very few assays have been performed directly *in vivo* in animal models to date.

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