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Nanostructured Active Biomaterials for Tissue Engineering Applications

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Abstract: In recent years, considerable effort has been devoted to the design and controlled fabrication of nanostructured materials with functional properties. The layer by layer buildup of the polyelectrolyte multilayered films from oppositely charged polyelectrolytes [1] offers new opportunities for the preparation of functionalized biomaterial coatings. This technique allows the preparation of supramolecular nano-architectures exhibiting specific properties in terms of control of cell activation and may also play a role in the development of local drug or gene delivery systems. In our team, peptides, proteins or DNA, chemically bound to polyelectrolytes, adsorbed on or embedded in such architectures, have been shown to retain their biological activities. The challenge is now to develop a novel generation of multifunctional architectures and to use them for clinical applications.

Keywords: Active biomaterials, nanostructured architectures, multilayered films, bone regeneration, gene delivery, drug delivery, stem cells.

1. ACTIVE BIOMATERIALS FOR LOCAL DRUG OR GENE THERAPIES

We examined the effect of the embedding depth on Protein (PA) activity by comparing TNF-α amounts secreted by THP-1 cells grown on architectures where PA was incorporated under an increasing number (0-30) of poly(L-glutamic acid) (PGA) and poly (L-lysine)(PLL) bilayers. We demonstrated that this protein embedded in a multilayered film keeps its activity. We have demonstrated for the first time that cells can interact with proteins incorporated in polyelectrolyte multilayer films and elucidated the mechanism by which cells come in contact with the active protein in the case of monocytic cells. Our results showed that cells communicate with embedded proteins through local film degradation and pseudopods formation [2].

We described also the biochemical and morphological response of monocytes in contact with surfaces functionalized with a melanocortin derivative (alpha-melanocyte stimulating hormone, alpha-MSH) peptide incorporated in PGA/PLL multilayer assemblies. Here, we coupled the alpha-MSH peptide to the carrier PGA. We have shown that the presence of this peptide in the multilayers confers anti-inflammatory properties to the coating. This suggests that the multilayer film acts as a reservoir in the sense that the number of layers deposited and thus of the quantity of alpha-MSH accessible to the cells controls their response. In addition to the anti-inflammatory effect of films containing alpha-MSH we observed, by in situ atomic force microscopy (AFM), morphological differences induced by the presence of alpha-MSH in the films. Cells in contact with a film containing PGA-alpha-MSH undergo morphological changes that can be interpreted as the development of pseudopods. Number, diameter and fine structure of these rigid "fiber-like" extensions change with time [3].

We have also reported that it is possible to incorporate active drugs into the multilayered films. The Piroxicam (Px) drug was incorporated into the films under the form of complexes with a charged cyclodextrin (cCD). The anti-inflammatory property was evaluated by determining the inhibition of TNF-alpha production by human monocytic cells. We investigated the anti-inflammatory activity and long lasting efficiency of this formulation. These formulation containing CD-Px complexes were shown to act as efficient reservoir devices for Px. Moreover, by adjusting the architecture, one can control the time over which the film is active.

Such modularly functionalized coatings could, in the future, become potent tools for the modification of biomaterial surfaces as applied to implants, prostheses or in tissue engineering [4-6].

Recently, we have demonstrated for the first time the sequential induction of nuclear and /or cytoplasmic

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expression products, mediated by β-cyclodextrin embedded in a PEM film. The results demonstrate that cells can be transfected first with a cytoplasmic response and later on with the nuclear response. These results indicate clearly that we are able for the first time to propose a biomaterial coated with multilayer films that interact with cells by inducing sequential and specific interactions (nuclear or cytoplasmic) depending upon the embedding level of the active vectors. Our results also showed that cyclodextrins can act as promising molecular templates with large potential for development as a new generic series of gene delivery vectors. In this study, we have described the fabrication of substrates containing β-cyclodextrin-DNA complexes embedded in a PEM films in which specific expression of nuclear or cytoplasmic proteins are selectively and sequentially produced. We have shown that films on which cyclodextrin-DNA complexes are adsorbed can act as an efficient gene delivery tool to transfect cells. This novel type of coating combines the simplicity of the construction by adsorption processes and the simultaneous interfacial delivery of different effectors molecules. These results should have a significant impact on the development of new localized gene therapies and open the route to numerous potential applications [7-9].

2. ACTIVE AND STRUCTURED BIOMATERIALS FOR TISSUE ENGINEERING

Recently, tissue engineering has merged with stem cell technology to develop new sources of transplantable material for injury or disease treatment. Eminently interesting are bone and joint injury disorders because of the low self-regenerating capacity of the matrix secreting cells. Recently, we have shown that BMP-2 and TGFβ_1 embedded in a multilayer can drive embryonic stem cells to cartilage and bone differentiation depending on supplementary co-factors. The new project entitled "Preparation of structured cartilageneous biomaterials by controlled cell and matrix spraying" concerns a highly innovating and original way to prepare functionnalized composite biomaterials containing cells, growth factors and various matrix elements distributed within perfectly controlled buildups. The approaches commonly used today are for most of them based on the use of gels and matrices in which only one cell type is included. Moreover, these approaches often not offer any possibility to restore the architecture of the native tissue to be rebuilt. The architecture of cartilage is complex, characterized by multiple cell types interacting within matrixes in three-dimensions. In order to recreate the characteristic functions of the tissue in the artificial construct, our strategy is based on the buildup of a biomaterial in which, during the procedure, the various elements constituting the natural architecture are progressively incorporated. This approach would permit to reproduce the organization of the cartilage and to adjust the thickness of the buildup to the lesion depth. Moreover, the spraying procedure in which the different constituents are progressively brought in contact should increase the cohesion of the whole material, which certainly represents a major weakness in the proposed biphasic materials. The large flexibility in the combination of different biomaterials should open in the future large opportunities in the treatment of articular cartilage lesions of different localization needing often large composition variations and changes in the architectures. Finally, when validated, the concept should open large opportunities in the tissue-engineering domain to repair other tissues.

In summary, we have shown that one can construct by spray deposition alginate gel layers containing cells with adjacent bioactive polyelectrolyte multilayers. The alternation of biofunctionalized multilayers with gel layers containing cell constitutes an essential step towards the buildup of stratified architectures where the cellular activity can be tuned as a function of the position of active molecules (growth factors) in the architecture and the nature of polyelectrolytes multilayers. Spray deposition is a simple and inexpensive technique that is able to create three dimensional cell structures *in vitro* for tissue engineering applications [10].

We also have reported that we are able to induce bone formation by using actives multilayered films and stem cells. Interest in the development of new sources of transplantable materials for the treatment of injury or disease has led to the convergence of tissue engineering with stem cell technology. Bone and joint disorders are expected to benefit from this new technology because of the low self-regenerating capacity of bone matrix secreting cells. In this study, the differentiation of stem cells to bone cells using active multilayered capsules is presented. The capsules are composed of PGA and PLL with active growth factors embedded into the multilayered film. The bone induction from these active capsules incubated with embryonic stem cells was demonstrated *in vitro*. We report here the first demonstration of a multilayered capsule-based delivery system for inducing bone formation *in vivo*. This new strategy is an alternative approach for *in vivo* bone formation. Strategies using simple chemistry to control complex biological processes would be particularly powerful as they make simpler production of more easily controlled therapeutic materials.

This work is also the first demonstration of the use of a multilayered capsule-based delivery system for inducing bone formation *in vivo*. By using this new formulation, we can incorporate different kinds of active molecules aimed at different applications, such as gene therapy, drug therapy or tissue engineering. This innovative approach, should also find applications in other domains of complex tissue restorations [11,12].

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