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Polyelectrolyte multilayers functionalized by a synthetic analogue of an anti-inflammatory peptide, α-MSH, for coating a tracheal prosthesis

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Abstract

Polyelectrolyte multilayer films made of poly (L-lysine) (PLL) and poly (L-glutamic acid) (PGA) have been functionalized by covalent binding of a synthetic analogue of the anti-inflammatory peptide, α-melanocyte-stimulating hormone (α-MSH) to PGA to create biologically active coatings for tracheal prostheses. The morphology and in vivo stability of the films were investigated by atomic force microscopy and confocal laser scanning microscopy, respectively. For the in vivo evaluation, 87 rats were implanted and examined for a period superior to 3 months. Histological analysis, performed 1 month after implantation, showed a fibroblast colonization of the periprosthetic side and a respiratory epithelium type on the endoluminal side of the implant for all the polyelectrolyte coatings tested. However, for prostheses modified by PGA ending multilayer films, a more regular and less obstructive cell layer was observed on the endoluminal side compared to those modified by PLL ending films. Systemic anti-inflammatory IL-10 production was only detected in rats implanted with prostheses functionalized by α-MSH, demonstrating, in vivo, the anti-inflammatory activity of the embedded peptide into multilayer architectures.

Keywords: Tracheal prosthesis; Polyelectrolyte multilayers; Anti-inflammatory activities

1. Introduction

Total laryngectomy is the surgical procedure used to treat patients with advanced-stage cancer of the larynx [1]. One major consequence of the treatment is a permanent loss of voice [2]. Furthermore, respiration is definitively separated from deglutition, necessitating a permanent breathing opening in the neck. To date, artificial larynx reconstruction faces difficulties to comply simultaneously with the combined constraints of biocompatibility and restoration of the function [3].

After implantation, biomaterials are spontaneously covered by a layer of host proteins followed by inflammatory cell attraction which may lead to degradative activities on the implant surfaces [4], resulting in complications, ultimately leading to the rejection of the prosthesis [5]. To improve the biocompatibility of implanted prostheses, one approach consists in the development of bioinert materials and, through surface modifications, create a bioactive interface that could regulate biological responses in a controlled way using specific cell signaling molecules or adhesion ligands [6].

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Recently, a new approach of tunable surfaces had been proposed to prepare biologically active surfaces. It consists in the alternate layer-by-layer deposition of polycations and polyanions for the build-up of multilayered polyelectrolyte films [7]. The method is versatile, yet simple and applicable for materials of any type, size, or shape (including implants with complex geometries and textures, e.g., stents and crimped blood vessel prostheses [8]).

The build-up of polyelectrolyte multilayer films may be the result of different mechanisms, in particular, linear and exponential growth regime [9,10], leading to more or less rough film topographies and more or less stiff architectures. The physico-chemical properties of multilayer architectures can be largely modified by varying the nature of the polyelectrolytes, the number of deposited layers, pH and ionic strength of the solutions [11,12]. For example, by changing the deposition conditions (pH or ionic strength), which strongly dictate the architecture of the films, it is possible to either prepare cytophilic or cytophobic film [13]. Also the cell behaviour, in term of viability, adhesion, or cytoskeletal organization, may be dependent on the nature of the film constituents [14–18]. For instance, the biocompatibility of poly (L-glutamic acid) (PGA) and poly (L-lysine) (PLL) endings films for SaOS-2 osteoblast-like cells and of PGA ending films for human peridontal ligament cells has been evaluated [14]. In addition, the determinant effect of the outer polyelectrolyte layer on actin and vinculin organization has also been described [15]. Of particular interest was the functionalization of the polyelectrolyte multilayer film with bioactive molecules, such as drugs, enzymes, DNA, or proteins [19–25]. For example, melanoma cells specifically respond to α-melanocyte-stimulating hormone (α-MSH) covalently coupled to PLL and incorporated at different depths in the polyelectrolyte multilayer [23].

Applications in the biomedical field are still scarce but they are very promising. The deposition of self-assembled nanocoatings on to arteries has been described as a means to protect a damaged artery and to control the healing process by incorporating bioactive molecules within the multilayer [8]. Multilayer self-assembly of two polysaccharides, hyaluronan (HA) and chitosan (CH), was also employed to engineer bioactive coatings for endovascular stents [26].

Here, we developed a new material in substitution of tracheal or laryngeal cartilages, made of titanium beads [27]. This material was designed to provide a support for a laryngeal prosthesis. Recently, adhesion of chondrosarcoma cells on these titanium beads modified by PLL, PGA or poly(sodium 4-styrenesulfonate) (PSS) ending multilayers was investigated. 3D titanium surface covered by films terminating with negatively charged PGA or PSS amplified the occurrence and length of cell protrusions, whereas positively PLL charged surface down-regulate both β-tubulin and phosphorylated p44/42 MAPK/ERK expressions. These preliminary data showed the potentiality of polyelectrolyte multilayer implant coatings to modify contractile and protrusive contact-based chondrocyte adhesion [28]. In the present work, prostheses made of titanium beads were coated with bioactive nanocoatings based on the layer-by-layer self assembly of two synthetic polypeptides, PLL and PGA, functionalized by a synthetic analogue of the α-MSH peptide. This peptide was selected for its anti-inflammatory properties [29]. After characterization of the morphology and in vivo stability of the films using atomic force microscopy (AFM) and confocal laser scanning microscopy (CLSM), we carried out an animal study to evaluate the concept in vivo. Eighty-seven rats were implanted and examined for more than 3 months. Histological analyses were performed 1 month after implantation and the inflammatory response was followed by measuring the systemic TNF-α and IL-10 amounts.

2. Experimental

2.1. Preparation of the prostheses

The prostheses, manufactured in collaboration with ONERA (Office National d'Etudes et de Recherches Aérospatial), were made of spherical titanium beads of 400–500 μm diameter. Titanium used for these surgical implants was in conformity with the Association Franç aise de NORmalization standards. The beads were placed into a mold and fused by condensed electrical discharges. The porous space between contiguous beads was about 150 μm. The prostheses sizes were adjusted on the mean values of trachea diameter and length previously determined from identical rats in age and weight to those used for in vivo experimentation. The prostheses consisted of cylindrical tubes of 10 mm length corresponding to six tracheal rings with an external diameter of 5 mm and an internal diameter of 3 mm. On the prosthesis, a slot of 0.8 mm was incised into the tubes and a hole of 1 mm diameter was created at each extremity (Fig. 1a). The pieces obtained were tested for mechanical shock resistance. Before implantation, titanium prostheses were sterilized under ultraviolet light irradiation (254 nm) for 1 h.

2.2. Polyelectrolytes and solutions

PLL (MW 23.4×10^3, Sigma, St. Louis, MO), PLL^{FITC} (MW 50.2×10^3 Sigma, St. Louis, MO) and PGA (MW 54.8×10^3, Sigma, St. Louis, MO) were used without any further purification. PLL, PLL^{FITC} and PGA solutions were prepared at 1 mg/ml in 0.15 M