# Polyelectrolyte Multilayer Coatings for Implant Osseointegration

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***Abstract*—The number of arthroplasties is rapidly increasing, however most materials used for such applications lack in osseointegration. The improvement of the bone/implant interface has received great attention for many years, with special reference to titanium-based implants. The interface between bone and implant has been considered both by physical approaches focused on surface topography and by chemical/biochemical surface modification by incorporation of organic molecules. The work described here is focused on the fabrication of implant coatings by layer by layer self-assembly of Collagen I (COL) and Hyaluronic acid (HA). The multilayer structure has been characterized by SEM and AFM, and the Titanium substrates coated with this multilayers have been tested with 3T3 cells seeded on Titanium supports. The results show that these coatings are promising for the improvement of implant osseointegration. This fabrication method is easily reproducible, versatile and economic.**

## I. Introduction

CONSIDERING that in the last decade the incidence of arthroplasty has increased not only in elder population but also in younger patients [1-3], stable integration of orthopedic implants with host bone is crucial to avoid short term revisions [4-7]. In order to improve implant performances, a new and effective approach consists in applying bio-inspired coatings on bone-implant interface to enhance bone tissue direct apposition rather than fibrous encapsulation [8, 9]. Bioinspired nanocoatings at the interface between bone and implant have been found to improve implant performances. Collagen and hyaluronan have been found to be very promising for the development of engineered tissues and biomaterials for tissue engineering and regeneration [10-12]. Collagen I is the principal structural protein of the organic bone matrix and together with growth factors and adhesion proteins it affects cell-matrix interactions. Hyaluronic acid (HA), another integral part of ECM, is a biocompatible and biodegradable linear polysaccharide with bacterial inhibitory effect [13-15]. The approach described here fabricates implant coatings based on collagen and on hyaluronan in order to improve cell adhesion and growth around the implant, while decreasing bacterial adhesion.

Polyelectrolytes multilayer coatings have the potential to ensure high longevity and excellent biocompatibility, guiding osteoblast adhesion, proliferation and differentiation at the implant-bone interface [16-19] and reducing the risks of bacterial infection, one of the primary cause of mechanical aseptic loosening in situ. The fabrication method used is based on sequential addition of oppositely charged polyelectrolytes onto a template of any shape. This process is called Layer by Layer assembly [20-22]. This technique is highly reproducible, economic and the architecture can be controlled at nanometer scale by parameters such as pH, concentration of polyions solution and ionic strength. This technique has been applied to the fabrication of ultra-thin films on surfaces of any size and shape, using a wide variety of polyelectrolytes [23-32].

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## II. Materials and Methods

### A. Materials

Cationic type I collagen from calf skin (COL, Sigma product number C8919), anionic Hyaluronic acid sodium salt from rooster comb (HA, Sigma product number H5388) and cationic poly (ethyleneimine) (PEI, average Mw_25.000, Aldrich product number 40,872-7) were used for the ultrathin film formation. PEI solution was prepared in pure water at a concentration of 5 mg/ml. COL was diluted in 0.1M acetic acid solution at a concentration of 1mg/mL, stirred for three hours in a becker using a magnetic bar, then diluted again in purified Milli-Q water at a final concentration of 0.2 mg/mL. HA was used as received and diluted in purified Milli-Q water at a final concentration of 0.5 mg/mL. All solutions were adjusted to a value of pH equal to 4 using HCl 0.1M. Water, used in the experiments for the solutions preparation and washing, was purified by Milli-Q system and had a resistance of 18.2 MΩcm.

### B. Ultrathin films preparation

Study of the process of ultrathin films fabrication was performed on planar surfaces using a quartz crystal microbalance instrument working in liquid environment (QCM-Z500, KSV Instruments, Helsinki, Finland). The QCM-Z500 instrument measuring principle is based on the analysis of the quartz crystal impedance at multiple overtones. The obtained parameters are used to calculate the properties of adsorbed layers such as mass, density and thickness. PEI/(HA/COL)3 multilayers were deposited on gold-coated 5 MHz AT-cut quartz crystals. Before adsorption, the quartz crystals were cleaned with H2SO4 at 150°C for 20 min followed by washing in purified Milli-Q water. After each use the quartz crystals were renewed. Considering that the quartz crystal surface is negatively charged, due to partial oxidation in air, a first layer of positive PEI was deposited to make easier the following

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deposition of the structure (HA/COL)n. Specific solutions were alternatively introduced into the measurement chamber and were left in contact with the quartz crystal for 5 min for Milli-Q water (first calibration step), for 10 min for PEI adsorption, 20 min for HA deposition and 20 min for COL deposition. After each adsorption step, water at pH 4 was purred into the chamber and left in contact with the crystal for 5 min in order to remove the unabsorbed molecules. The data analysis and calculation of thickness were performed using the QCM Impedance Analysis software (KSV Instruments, version 3.11).

### *C. Characterization*

A Scanning Electronic Microscope (SEM, Hitachi S-2500) at an acceleration voltage of 10kV observed the surface morphology of titanium substrates. Being the titanium samples conductive, there was no need to perform a substrate metallization.

Silicon samples were air dried at room temperature, and then analyzed at the AFM by using a custom build set-up driven by R9 advanced controller (RHK technology) in air at room temperature. Data acquisitions were carried out in tapping mode at scan rates between 0.4 and 0.7 Hz, using rectangular Si cantilevers (NCHR, Park Systems) having the radius of curvature less than 10 nm and with the nominal resonance frequency and force constant of 330 kHz and 42 N/m, respectively.

### *D. Cell Cultures*

3T3 mouse fibroblastic cell line was used instead of human cell line to carry out preliminary cell culture studies on the titanium samples. An amount of 50.000 cells/100 µL medium was homogeneously seeded onto the samples. Adhesion and proliferation of cells was evaluated after 1 and 3 days by using a cell viability test (Presto Blue test by Invitrogen). Using a spectrophotometric test at 570 and 600 nm was measured the absorbance of the supernatant. The degree of cell proliferation was expressed as fold difference from cells cultured on non functionalized titanium substrates. To assess cellular adhesion, morphology and distribution on the different samples we used SEM imaging.

## III. Results and Discussion

The assembly of the multilayer coating has been firstly realized on planar substrates of quartz with the architecture $PEI(HA/COL)_6$. The constructive protocol was characterized by QCM in order to be optimized. The frequency shift due to the adsorption of charged polyelectrolyte onto the quartz crystal resonator shows a gradual growth of the film (Fig. 1.). The frequency gap of each assembly step is used to quantify the mass adsorption and thickness for each layer thanks to Kanazawa-Gordon equation [33].

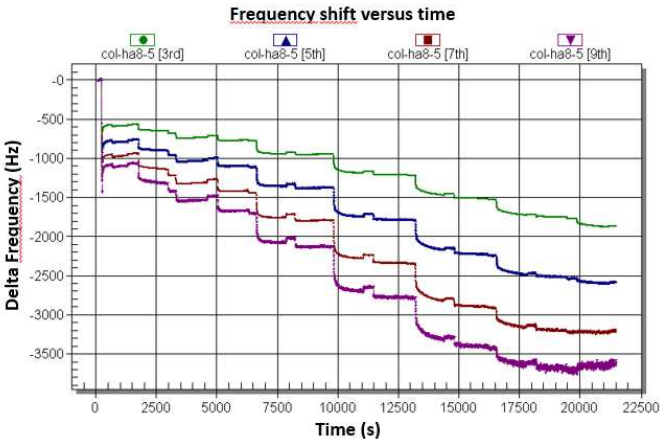


Fig. 1. Frequency gap for each fabrication step in time

The graphic plotted to describe the increasing thickness for each bilayer (HA/COL) deposited reveals a mean growth of 30-40 nm (Fig. 2.). This increase can be considered linear.

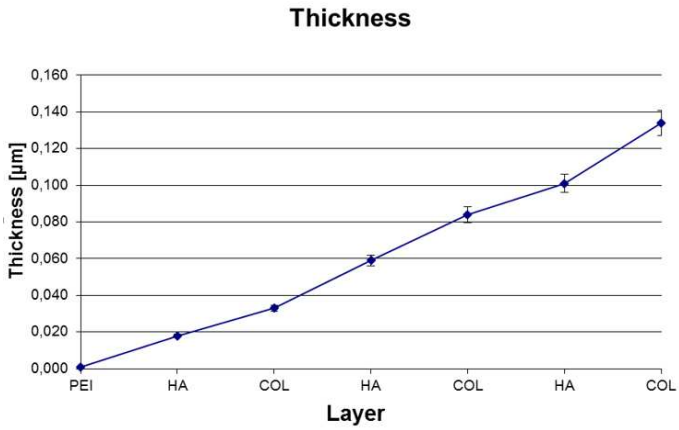


Fig. 2. Growth of thickness in µm for a three bilayer structure

Bioinspired coating obtained with the adsorption of bioactive macromolecules are capable to enhance osseointegration, improving cells adhesion, spreading and proliferation on implant surfaces [34]. In this work, we used porous titanium and smooth titanium substrates (Fig. 3.).

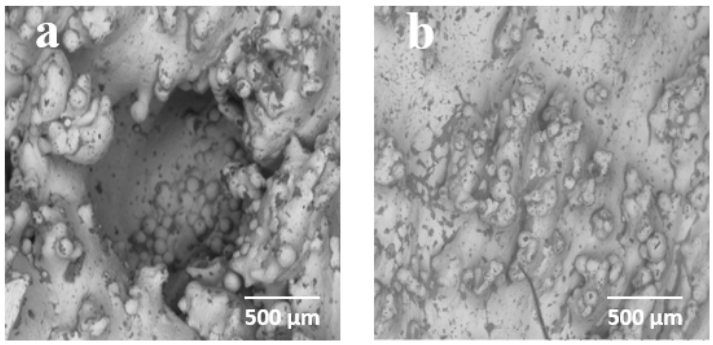


Fig. 3. SEM image of a Ti porous sample covered with 8 bilayers of HA/COL (panel a); SEM image of a Ti smooth sample with 8 bilayers

Confirmation about the success of the deposition protocol of PEI(HA/COL)$_8$ is shown in Fig. 4. This image compares non-treated titanium substrate (panel a) and functionalized titanium substrate (panel b). The coating levelled the nanometric features of the titanium dioxide interface, making it smoother in respect to rough pristine titanium.

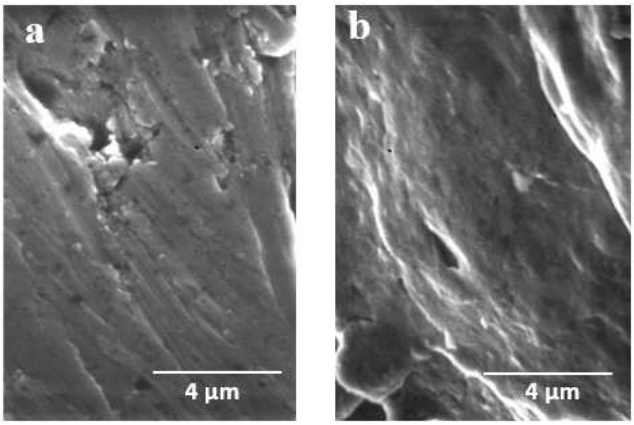


Fig. 4. SEM image comparison between pristine titanium (panel a) and functionalized titanium (panel b)

The same deposition protocol was performed on planar silicon to assess if the adsorbed fibers of collagen assumed a preferential orientation. PEI/(HA/COL) architecture was deposited on the silicon samples, then characterized via AFM. The superior spatial resolution of the AFM image (Fig. 4) reveals the presence of fibrillar aggregates and a random disposition of collagen fibers. LbL technique cannot guide the assembly of fibers in ordered unit, but it is useful to functionalize the interface topography of biomaterials improving osteoconductive performances of the implants.

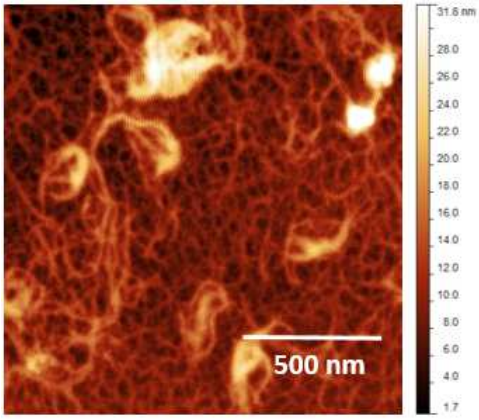


Fig. 4. AFM characterization of silicon substrate functionalized with PEI/(HA/COL)

In vitro growth of cells, evaluated via PrestoBlue assay, shows the greater biocompatibility of PEI(HA/COL)$_8$ on porous titanium substrates than other functionalized samples. All samples present the same adhesion potential at day one, but proliferation tests conducted after 3 days of culture show that eight bilayer porous titanium is significantly better than other samples.

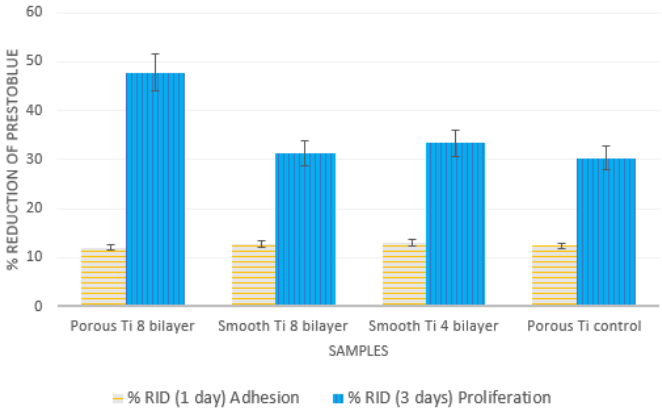


Fig. 5. Prestoblue reduction on different samples with different coatings

The 8 bilayers structure has been SEM characterized. Results are shown in Fig. 6.

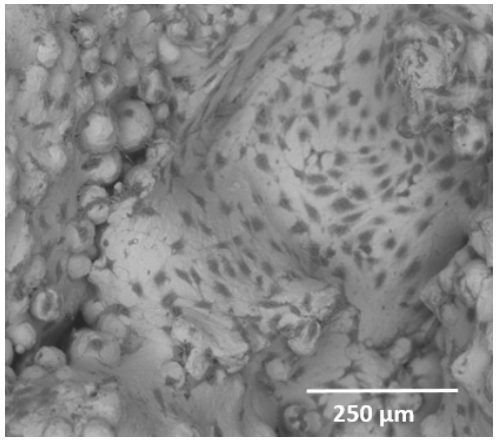


Fig. 6. SEM image of a porous titanium sample functionalized by PEI(HA/COL)8 with seeded cells

## IV. Conclusion

Future biomaterials need to be designed considering every cascade of biological event that takes place when a biomedical implant is inserted in the human tissue. Implant covered with multilayer of bioactive macromolecules can avoid main limitations of arthroplasties as micromotion at the interface and peri-implant infections. These biocoatings can enhance functionality and biological efficacy of biomaterial, avoiding bacterial adhesion and maximizing osteoconductive processes.

The development of these polyelectrolyte multilayer coatings can be regarded as a first step to the realization of a smart coating capable of enhance osteoconductivity and reduce bacterial infections in biomaterials.

## ACKNOWLEDGMENT

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