**Synthesis and collection of collagen nanotubes for biomaterials application.**

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**Statement of Purpose:**
The extracellular matrix (ECM), which regulates cell behaviour, presents a complex structure on the micro- and nanoscales. The creation of environments mimicking the ECM is an important challenge in the field of biomaterials science and tissue engineering. Layer-by-layer (LbL) assembly is an attractive method to incorporate ECM proteins into nano-objects. However, while the LbL assembly of synthetic polyelectrolytes is well known, the build-up of multilayers using proteins is not trivial due to their intramolecular organisation which influence charge distribution of protein molecules. The aim of this study is to use collagen, the most abundant ECM protein, (i) to synthesize collagen-containing nanotubes using LbL assembly within the pores of polycarbonate membrane, (ii) to collect these nanotubes by electrophoretic deposition (EPD) on flat surfaces and (iii) to investigate the effect of resultant surfaces for cell culture applications.

**Materials and methods:**
LbL assembly was performed by alternate adsorption steps of collagen (COL) and poly(styrene sulfonate) (PSS) using acetate buffer of pH 4.7. Assembly was first investigated on flat silicon substrates using quartz crystal microbalance with dissipation monitoring (QCM-D). The nanotubes were synthesized into pores of polycarbonate track-etched membrane with pore diameter of about 500 nm. They were then liberated by the template dissolution in DMF and further collected using EPD. Indium tin oxide (ITO)-coated conducting glass slides (8-12 Ohms resistivity) were used as both working and counter electrode, whereas platinum wire was used as a pseudo reference electrode. Distance between electrodes was maintained at 5 mm. The effect of applied voltage and solution concentration on deposition of nanotubes was investigated. The characterization of surfaces modified by nanotube deposition was carried out by using optical and scanning (SEM) electron microscopes. Fluorescently labeled nanotubes were also synthesized by incorporating an inner layer of fluorescent poly(allyl amine) hydrochloride (Flu-PAH) during LbL assembly along with PSS, while always maintaining collagen in the outer layer of the nanotubes.

**Results:**
Collagen was successfully incorporated into LbL assemblies, allowing size-controlled collagen-containing nanotubes to be obtained by the template method. In situ QCM-D monitoring of LbL build-up showed a significant decrease of the resonant frequency ($\Delta f$) as a function of the alternate deposition of PSS and COL, demonstrating that COL can be used as a polycation for LbL assembly [1]. COL nanotube deposition by EPD was shown to increase with applied potential and with concentration in solution. SEM and fluorescence microscopic analyses showed that nanotubes synthesized and collected by these methods have controlled dimensions as shown in figure 1. The use for cell culture of these surfaces modified by collagen-based nanotubes is currently under investigation.

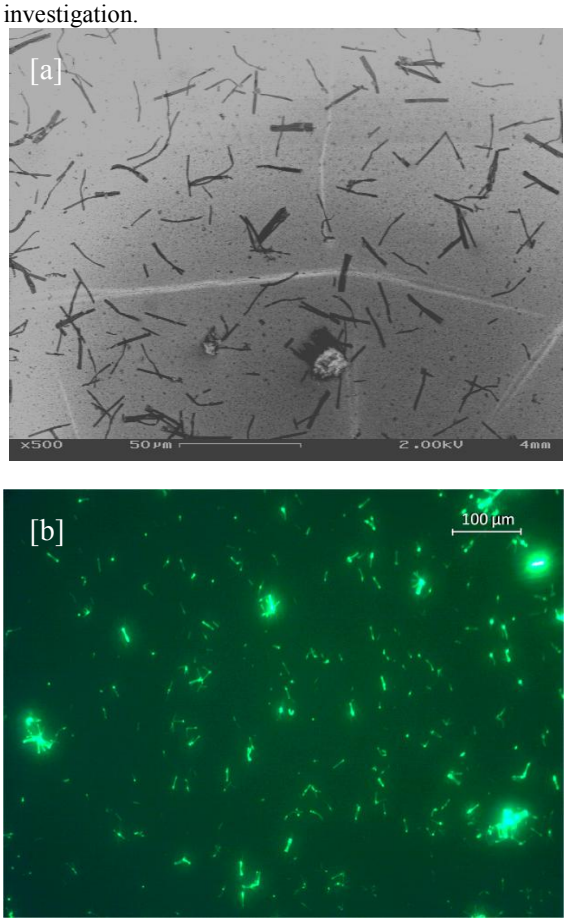


Figure 1. [a] SEM and [b] fluorescence image of electrophoretically deposited $(COL/PSS)_3$-(Flu-PAH/PSS)$_3$ nanotubes of 500nm diameter.

**Conclusions and perspectives:**
Collagen was successfully incorporated into LbL assemblies, allowing size-controlled collagen-containing nanotubes to be obtained by the template method. EPD technique was successfully applied for collection of these protein nanotubes, and the surface density of nanotubes could be adjusted. These nanotube-coated ITO surfaces are currently being investigated for cell culture applications.

**References: [1]** J. Landoulsi, C.J. Roy, C.C. Dupont-Gillain, S. Demoustier-Champagne. Biomacromolecules. 2009 **10**:1021-4.

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