Electronic Supplementary Information

Bio inspired titanium coatings: Self-assembly of collagen- alginate films for enhanced osseointegration

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\textbf{Figure S1. XPS survey of APTES on TiO}_2
Appearance of the NH\textsubscript{2} peak in the N 1s region after APTES functionalisation (in red).
Figure S2. Increase in Rhodamine B fluorescence intensity upon deposition of alginate layers. Intensities correspond to the maxima of the wavelengths in Figure 2b, at ~585 nm.

Figure S3. AFM imaging of a) height of the 9 layer film is calculated to be on average 30 nm and b) phase image of the same film, showing an example of fiber (in blue) extending to ~1.8 µm in length connecting to other fibers.
Atomic force spectroscopy (AFS)

Film elasticity was determined from nanoindentation experiments preformed on a NanoWizard II AFM (JPK, Berlin, Germany) acquiring force-distance (f-d) curves in liquid medium. Measurements were performed with a 2 μm borosilicate colloidal probe attached to the cantilever (Novascan Technologies, USA). The spring constant of the cantilever was calibrated through the thermal noise method in the medium where measurements were preformed (Na acetate and PBS buffers). The spring constant had a value of 0.065 N/m. 200 f-d curves were acquired with set point forces of 0.5, 0.1 and 0.05 nN over a sample area of 10 μm × 10 μm. For each film, a total of five different sample areas were probed and the resulting data screened and processed using the JPKSPM Data Processing software. The Young’s elastic modulus (E) of each film was obtained by fitting the force data in the entire compressive part (curve) of the indentation cycle to the Hertz model (Equation 1), assuming a Poisson ration of 0.40. Further statistical analysis of resulting E values was performed with OriginPro 2015 software.

Experiments were conducted with a colloidal probe and the resulting F-d curves were fitted with the Hertz model (Equation 1) that best describes our system, considering non-adhesive elastic contact between a stiff sphere and an elastic surface. We consider that our evaluation of the elastic modulus is a rational approximation concerning numerous assumptions and variables introduced in the experimental setup and analysis. These variables are for example the size and shape of the probe, loading force and further analytical variables such as fixing contact point and baseline or Poisson ratio used to fit the curves to the model. However, under the same experimental parameters and equal approach for curve fitting for the two samples, we can compare their E values and estimate their relative differences in elastic properties. In order to decrease the variability coming from uneven topography and to improve the quality of the results, force curves were collected with a rather large statistical population of at least 500 curves across different sample areas. Representative f-d curve for experiments conducted on these films are shown in Figure S4. Figure S5 shows apparent E values for two sets of films; 5 layer films where alginate is deposited as the last layer (Figure S5 a) and 6 layers with collagen as the last layer in Figure S5 b. Mean E values between these 2 sets are quite similar resulting in apparent E values of ~ 35 kPa for LbL deposited films and ~ 10 kPa for crosslinked with a higher error for non-crosslinked (LbL) films. E values themselves are in a nice agreement with the literature for collagen fibers11 as well with the elasticity of soft bone tissue of 25-40 kPa.10 From the results shown here, we can drive two conclusions. Crosslinked films, though visualized as a more compact structure, have apparently a lower Elastic modulus than the ones assembled by LbL in acid. Since the calculation of the elastic moduli of thin films based on AFS is typically affected by the characteristics of the substrate on top of which the film is assembled, the so called “substrate effect” our results are unexpected. As titania has a Young’s modulus of 200-300 GPa, one would expect that given the decrease in height of the crosslinked film the apparent E would be much more significantly affected by the substrate underneath. Nevertheless, even though the thinner, crosslinked films showed lower apparent elastic moduli. This can be considered as an indication that
crosslinking significantly altered the mechanical properties of the multilayers. Furthermore, we observe this behavior in both 5- and 6-layer films. Since crosslinking will lead to increased coupling between polymer fibers, we can expect that upon indentation a significant fraction of the energy dissipation will be converted into tensile load across the fibers (and associated elastic response) rather than in dislodging them from their resting position. We can expect the inverse tendency in the case of LBL films, which can make them more susceptible to the “substrate effect”, leading to an increase in the apparent elastic modulus. The decrease in the apparent E for the crosslinked film can be explained by a tighter mechanical coupling between individual fibers. Another conclusion that we can draw is that the film is highly homogeneous. This is manifested in the standard deviation of the mean E value (distribution curves in Figure 4 c and 4 d). Wider distribution of apparent Young’s modulus in the case of the LbL films indicates less homogeneous coating due to variability in the indentation curves across the sample while crosslinked films seems to be more homogeneous regarding their elastic properties.

Figure S4. AFS typical f-d curve, raw data fit. Bright red is the approach curve and dark red is the retract curve in the cycle.
Figure S5. Young's modulus (E) of biofilms before and after crosslinking. Mean E for a) films consisted of 5 layers and for b) 6 layer films, measured with a loading force 0.5 nN. Error bars are representing standard error of the mean value. In a) the E values correspond 5 layer films with alginate as a last layer and in b) to 6 layer films where collagen is deposited as a last layer. Distribution of Young’s modulus for c) films with alginate deposited as a last layer and d) collagen deposited as a last layer. Distribution curves are Lognormal fit to the histograms of apparent Young’s modulus.
Figure S6. Statistical analysis of mean values for ALP stained area by tukey comparison with with $P > 6.12476E-4$. 
Figure S7. Average osteoblast cell number after 24 hours of incubation in osteogenic medium. Average number is calculated from three samples and cell counts are expressed as cell per area of 318x318 μm