Nanometer-Thick Collagen/Calcium Phosphate Coatings with Improved Mechanical and Biological Performance

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Statement of Purpose: Although the biological efficacy of calcium phosphate (CaP) coatings for bone implantology has been confirmed in several clinical follow-up studies[1], the current generation of CaP coatings is associated with problems related to poor mechanical properties and limited osteoconductivity. Since their purely ceramic composition does not reflect the composite nature of native bone tissue, this study has adopted a bioinspired strategy towards improvement of mechanical and biological performance involving electrospray co-deposition of CaP-collagen composite coatings onto titanium (Ti) implants. Moreover, the coating thickness of these composite coatings was reduced from the micron- to nanometer-scale in an attempt to optimize their mechanical behavior even further. Since it is unknown to what extent the thickness of CaP-based coatings can be reduced without compromising their osteogenic potential, the relationship between coating thickness and biological performance of CaP and CaP-collagen coatings was evaluated by means of an in vitro cell culture experiment.

Methods: CaP-collagen composite coatings were deposited onto machined Ti discs using a commercially available electrospray deposition device (AST, Bleiswijk, The Netherlands). The electrospray deposition technique was selected since this technique allows simultaneous deposition of both biomolecules and CaP at a temperature of 25 °C and a relative humidity of 15%[2]. Commercially available ethanol-based CaP suspensions (Berkeley Advanced Biomaterials Inc.) were diluted with 0.01M acetic acid to a final concentration of 600 µg/ml CaP. For the deposition of collagen-CaP composite coatings, rat tail collagen type I (BD Biosciences) was dissolved in the above-mentioned CaP suspensions at a collagen concentration of 400 µg/ml yielding a final CaP/collagen precursor ratio of 6:4. All precursor solutions were electrosprayed at a flow rate of 0.05 ml/h, a high voltage of about 10-11.5 kV and a nozzle-to-substrate distance of 40 mm. Deposited coatings were characterized i) physicochemically (SEM, AFM, ATR-FTIR), ii) mechanically (standardized tape tests according to ASTM D-3359 and quantification of coating retention by measuring the Ca amount before and after tape testing) and iii) biologically by culturing primary rat bone marrow cells for several time periods up to 4 weeks.

Results: The electrosprayed nano-sized CaP particles formed a densely packed, uniform coating on the Ti discs, whereas CaP particles were entangled within a fibrous morphology for the CaP-collagen composite coatings (Figure 1). Electrospray deposition times of 15 and 60 minutes yielded coating thicknesses (as measured by AFM) of 55 and 255 nm for CaP coatings and 90 and 290 nm for CaP-collagen coatings, respectively. A decrease in CaP coating thickness resulted into a considerable decrease in coating delamination from the implant surfaces for all experimental groups. Co-deposition of collagen significantly improved coating adhesive and cohesive strength, resulting in a remarkably high coating retention of up to 97% for coating thicknesses below 100 nm. The mechanical properties were improved by the entanglement of the CaP within the collagen fiber-like network, whereas the additional organic collagen linkage to the Ti surface contributed to the increased interfacial bonding between coating and Ti implant surface.

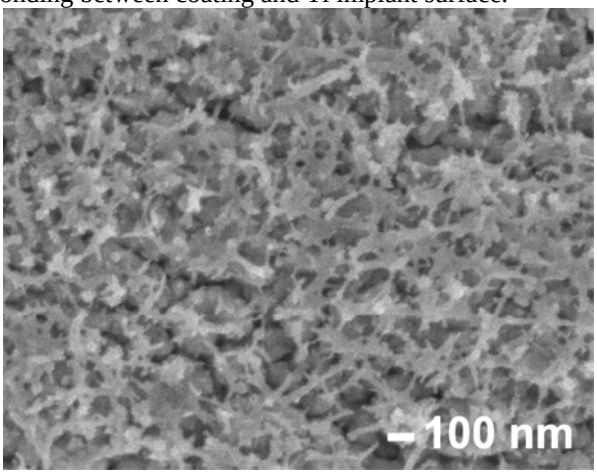

Figure 1. Typical morphology of CaP-collagen coatings

Generally, cell culture experiments showed that all CaP and CaP-collagen composite coatings supported proliferation and differentiation of osteoblast-like cells, whereas collagen-containing coatings enhanced osteoblast differentiation compared to pure CaP coatings, as indicated by reduced proliferation and accelerated mineral deposition after 8, 12, and 16 days of cell culture. Collagen-containing coatings were able to exert an osteogenic effect even at the minimum coating thickness of 90 nm. To the best of our knowledge, this is the first time that CaP-based coatings of less than 100 nm are reported to possess the capacity to induce osteogenic effects in vitro.

Conclusions: Nanometer-thick CaP and CaP-collagen composite coatings were successfully fabricated using the electrospray deposition technique. Co-deposition of collagen significantly improved coating adhesive and cohesive strength. Cell culture experiments showed that CaP or CaP-collagen coatings of thickness below 100 nm were able to induce osteogenic effects in vitro. These results indicate that a strong synergy exists between collagen and CaP that can be exploited to fabricate nanometer-thick coatings with superior mechanical and biological properties. In vivo experiments need to be carried out in order to prove if these nanometer-thick coatings are able to outperform traditional micron-thick bioceramic coatings for bone implantology.

References:

1: Epinette JA. Hip Int. 2008;18:69-74.
2: De Jonge LT. Acta Biomater 2009;5:2773-82.