Nanomechanical Properties of Hydroxyapatite (HA) with DAB Dendrimers (poly-propylene imine) Coatings onto Titanium Surfaces

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Bone is a highly specialised composite consisting of hydroxyapatite (HAP) nanoplates [1], collagen and other proteins, a structure allowing synergistic structure and function during bone mineralization [2]. Implantable biomaterials for bone-grafting must possess characteristics mimicking those of natural bone in order to be successful in their application. As a consequence, the research efforts are now being directed towards biomimetic approaches for the synthesis of HAP crystals of various morphologies, employing polymers such as monosaccharides [3] and related polymers [4] or more recently dendrimers [5-7] to modulate crystal nucleation and growth. It is also known that the use of biomolecules containing specific amino acid residues, such as arginine, lysine or specific peptide sequences, e.g. the RGD sequence, encourages cell attachment, proliferation and differentiation on HA surfaces [8].

In this respect, through the presented work we provide a new route for the formation of functional coatings of HA onto titanium implant surfaces employing a cationic fourth generation diaminobutane poly(propylene imine) dendrimer (DAB) bearing 32 amine end groups for the synthesis of HAP nanorods to increase the coating strength and adhesion and to potentially induce osteogenic cell activity.

Dendrimers exhibit a highly precise architecture [9], as they are highly branched macromolecules composed of a core molecule, a large number of branches regularly extending from the core, and terminal (or surface) groups suitable for further functionalization. They have a definite molecular weight and size, in contrast to the usually broad molecular weight distribution of linear polymers, a near spherical shape for generation numbers larger than 4 and are able to encapsulate metal ions, or organic molecules (e.g. drugs) [10,11]. However, up to now, dendrimer mediated coatings of HAP onto Ti surfaces have not been studied yet.

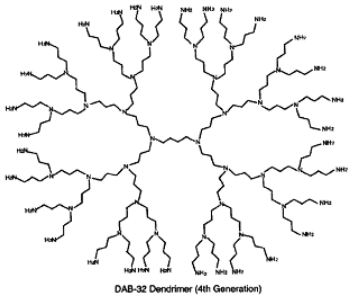


Figure 1: Schematic representation of DAB dedrimer.

For HAP synthesis, aqueous solutions of $CaCl_2$ (0.1 M; Sigma-Aldrich), Na_2HPO_4 (0.06 M; >99%; Fluka, Buchs, Switzerland) and 1,4-diaminobutane poly(propylene imine) dotriacontaamine dendrimer, DAB (0.2 M; DSM Fine Chemicals,) were prepared with three different calcium:dendrimer molar ratios, viz. 2:1, 1:1, 1:2. The resulting HAP suspensions were either used without any further treatment or hydrothermally treated at 80°C for 16h, or at 130°C for 6h. Then the suspensions after several centrifugation and washing steps were employed to coat cpTi coupons 1x1cm (3mm thickness, Aldrich) which had been previously polished with SiC paper for evaluating the nanomechanical properties.

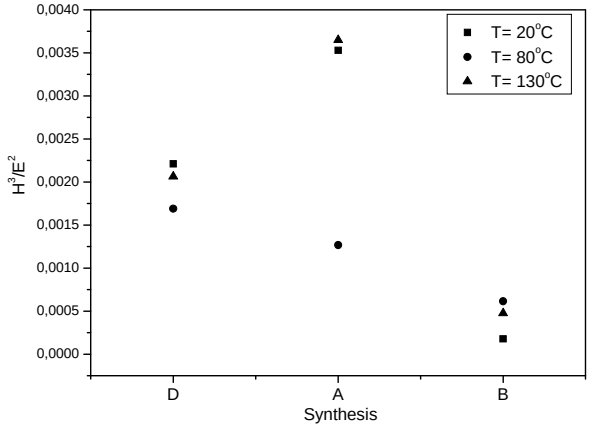
The indentation analysis was performed using a Hysitron TriboLab® Nanomechanical Test Instrument that is capable of performing indentations. The equipment allows the application of loads from 1 to 10.000μN and the recording of penetration depths as a function of applied loads with a high load resolution (1 nN) and a high displacement resolution (0.04 nm). A Berkovich diamond indenter was used with a maximum load of 1000μN at room temperature. Since nanoindentation test results are very sensitive to the quality of the surface of the specimens, samples were first polished before indentation testing.

Coatings of all the three different calcium:dendrimer molar ratios (D, A &B, Table I) synthesized at room temperature or after hydrothermal treatment at 80 or 130 ^{o}C were tested at load 1000μN. As a result of the synthesis conditions the grain size distribution of the coatings as well as their DAB content are different resulting in different H & E values measured. Prior to testing all the different coatings developed, indents were performed at the coating of synthesis D at 130^{o}C on Ti substrates polished with different SiC papers in the range 200-1000. Coatings on Ti substrates ground at 400 grit led to higher H & E values and higher H/E and H3/E2 ratios which measure the coatings resistance in plastic deformation. Thus, polishing at 400 has better adhesion with the coating, since resistance to plastic deformation of the coating has the higher values.

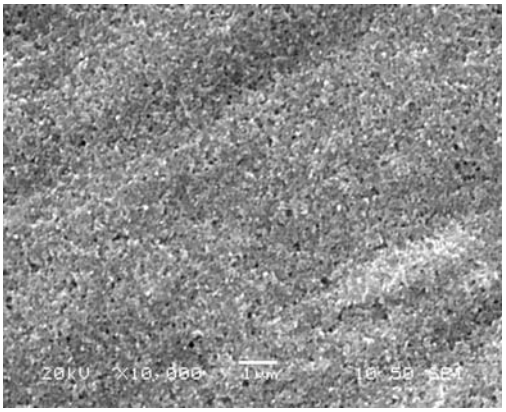
Table I: Concentration and H&E values of three syntheses at each temperature.

		T= 20^{o}C		**T= 80^{o}C**		**T= 130^{o}C**	
Synthesis	**Ratio of Ca/dendrimer**	**H(GPa)**	**E(GPa)**	**H(GPa)**	**E(GPa)**	**H(GPa)**	**E(GPa)**
D	2:1	3,15	118,89	2,76	111,53	2,78	102,03
A	1:1	4,26	147,98	2,38	103,17	3,9	127,5
B	1:2	1,36	91,55	0,992	39,86	1,57	90,15

Nanoindention analysis of all coatings (A, B & D) revealed a plastic behavior. It is observed (Table 1) that at temperatures 20 and 130^{o}C higher H and E values appear for coating of synthesis A. At temperature 80^{o}C coating of synthesis D appear higher H and E values. It was revealed that higher values of resistance in plastic deformation (H3/E2) had synthesis A at temperatures 20 and 130^{o}C. For temperature 80^{o}C synthesis D had higher value of the ratio (Fig.2a). SEM analysis revealed that all coatings have porosity, with pores almost the size of grains of each coating. At Fig.2b is shown a typical example of a SEM image for synthesis D at 130^{o}C.



(a)



(b)

Figure 2: (a) Resistance to plastic deformation vs three different synthesis coatings at different temperatures and (b) SEM image of coating with synthesis D at 130^{o}C.

Concluding with the study, it was shown that a better adhesion property of coatings on Ti substrates has smoothing 400. All coatings have a plastic behavior but higher H & E values have synthesis A, which has for all temperatures the smallest grain size, a medium percentage of polymer mass encapsulated in grain size and smaller pores than coating of synthesis D.

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