Supporting information

Surface zwitterionization of customized 3D Ti6Al4V scaffolds: promising alternative to eradicate bone infection

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Apatite coating on Ti6Al4V EBM implants

To optimize the quality of the coatings in terms to obtained highly homogenous and pure nanocrystalline apatite without secondary phases, different ageing times in the sol precursor have been evaluated. The ageing times studied was from 6 to 30 h, which were monitored by determination of pH in the sol precursor. After the aging time, the 3D scaffolds were immersed in the precursor solution and extracted at a constant speed in a dip-coated machine to obtain the coating. Then, the coated-specimens were dried at room temperature during 3 days and subsequently calcined at 550 °C for 10 min.

XRD patterns corresponding to calcined coatings after 6, 8, 24 and 30 h of aging are shown in Figure 1S. XRD patterns corresponding to 6 and 8 h of ageing exhibit small amount of crystalline apatite, being majority the presence of impurities such as CaO and $CaCO_3$ (calcite). By increasing of aging time (24 and 30 h), the XRD diagrams show a gradual improvement in crystallinity of apatite phase (in terms of resolution and intensity of diffraction maxima), while secondary phases gradually decrease until their disappearance. Note the absence of nitrates in all samples, demonstrating that have been entirely removed in the calcination process (550 ° C / 10min). FTIR studies are collected in Figure 1S. In the early times (6 to 8 h), FTIR spectra show mainly bands corresponding to the carbonate group (CO_3^2-) at 870 to 1407 cm^{-1} and bands corresponding to a Ca-P intermediate phase (still immature phase), in agreement to XRD results. However, by increasing the time of aging (24 and 30 h), FTIR spectra show a clear evolution towards the formation of a apatite phase, exhibiting bands at 3570 and 631 cm^{-1} assignable to vibrational modes and voltage of the hydroxyl groups of the nanocrystalline apatite, a very intense band at 1089, 1054, and 961 cm^{-1}, corresponding to the vibration modes link voltage P-O, and a doublet at 603 and 567 cm-1 assignable to the bending mode of the link. Moreover, the observation of bands at 870 to 1407 cm^{-1} corresponding CO_3^{2-} groups is only assignable to their incorporation to the apatite structure and not the presence of calcite, according to XRD studies. Figure S2 provides a comparison by SEM-EDS of the different coated surfaces as function of ageing time. SEM image after 6 h shows a rough and heterogeneous surface formed by spherical particles, which can attribute to the presence of nodes calcite and CaO in agreement with FTIR and XRD results. By increasing of aging time, the obtained coatings evolve towards less rough and smoother surfaces, which is agree with the disappearance of such phases (CaO and calcite) and the majority presence of apatite.

Table S1 shows the EDS analysis corresponding to the chemical composition of the different coatings as function of ageing time. In agreement with the other techniques, for short aging times (6 and 8 h) the Ca/P molar ratios are very high, which is due to the presence of other phase. However, after 24 and 30 days the Ca/P molar ratio is getting closer to a 1.67 value which is characteristic of apatite phase.

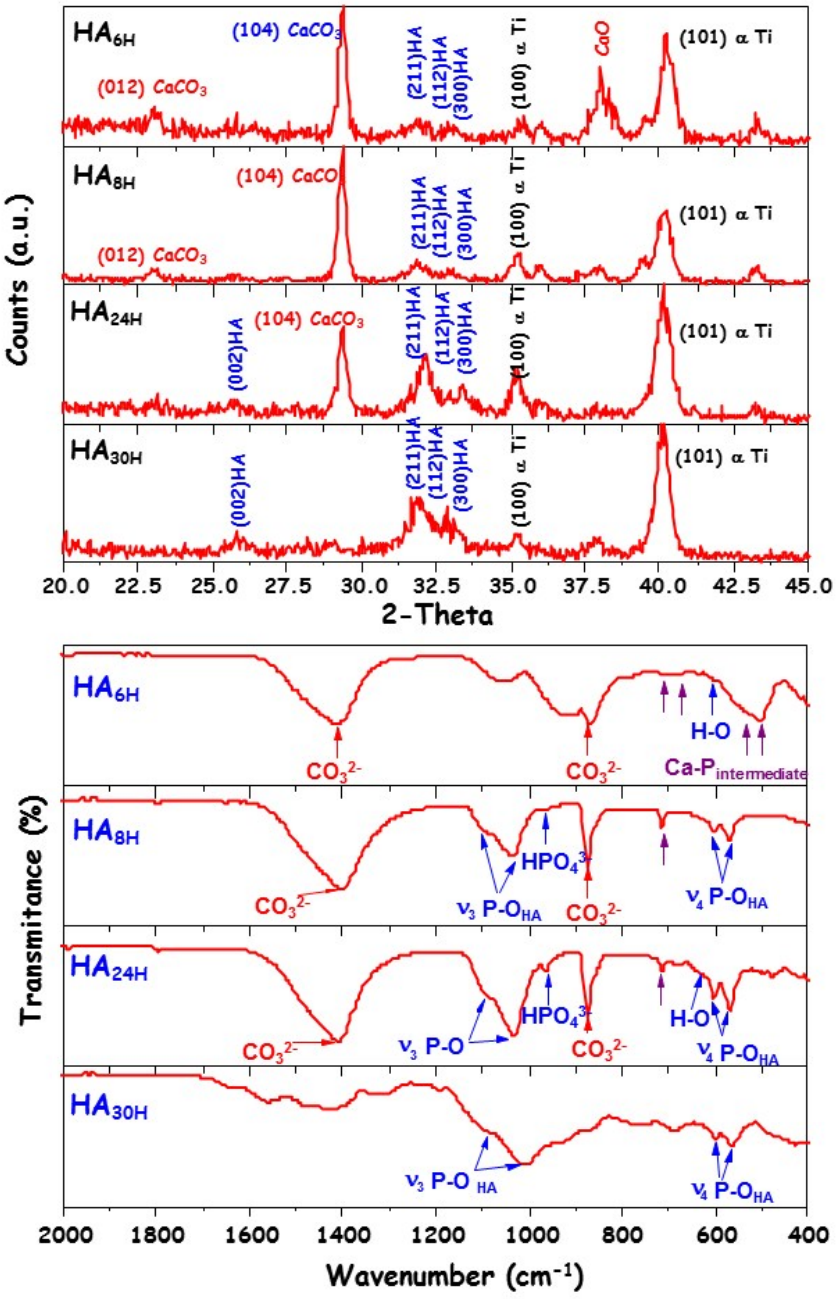

Figure S1: Optimization of apatite coating formation as function of aging time: XRD and FTIR studies of the coatings onto 3D Ti6Al4V scaffolds by using different sols ageing during different times.

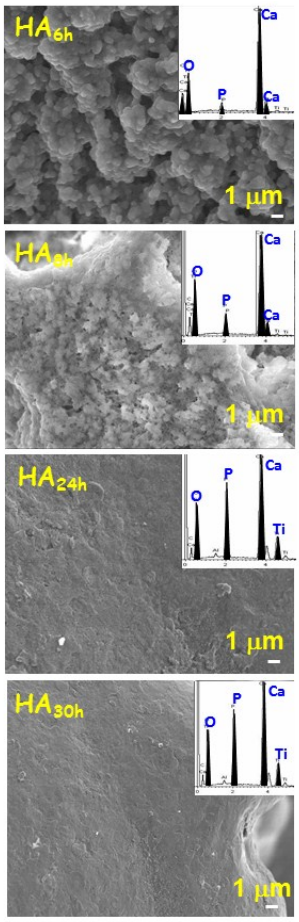

Figure S2: Optimization of apatite coating formation as function of aging time: SEM and EDS studies of the coatings onto 3D Ti6Al4V scaffolds by using different sols ageing during different times.

Table S1: Chemical composition (in atomic percent) and Ca/P molar ratio determined by EDS corresponding to the obtained coatings as function of the ageing time.

Sample	%Ca	%P	Ca/P
HA_{6h}	93.9±1.5	6.2±0.7	15.3
HA_{8h}	85.7±2.5	14.4±1.0	6.0
HA_{24h}	65.0±1.0	34.9±1.0	1.9
HA_{30h}	63.7±1.3	36.3±0.9	1.7

***In vitro* Bacteria assays: Biofilm formation after 48 hours**

Figure S3 shows confocal images on the different samples after being incuabated during 48 h in the *S. aureus* bacteria solution. Similar to 24 h, both Ti and HA-Ti surfaces show biofilm formation, which is evidenced by bacterial conglomerates (green) embedded in the extracellular matrix (blue). In this case staining increases due to increased time with respect to Fig. 6. However, no biofilms are observed in the Zwitter-HATi sample, which displays a few live individual bacterial cells (green) without extracellular formation (blue).

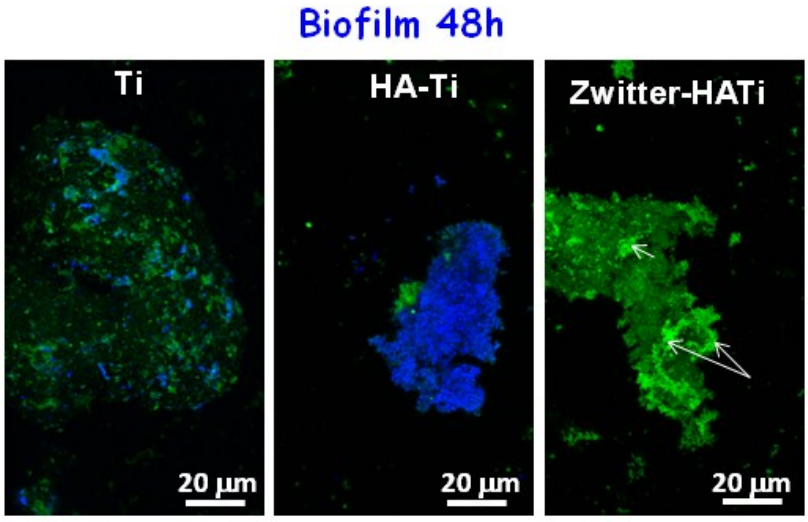

Figure S3: Biofilm formation test after 48 h: Confocal images on pristine (Ti), apatite-coated (HATi) and Zwitter-HATi surfaces incubated during 48 h in *S. aureus* solution. Both Ti and HA-Ti surfaces show biofilm formation which is evidenced by bacterial conglomerates (green) embedded in the extracellular matrix (blue). Note higher blue staining on HA-Ti sample. On the contrary no biofilms are observed in the Zwitter-HATi sample, which displays a few live individual bacterial cells (green) without extracellular formation (blue). It is important to remark that the samples adsorb Syto stain and they are colored by green only the brightest green points correspond to bacteria (see arrows).