

Antibacterial bioadhesive LbL coatings for orthopedic applications

Ana L. Carvalho^{a,b}, Ana C. Vale^{a,b}, Maria P. Sousa^{a,b}, Ana M. Barbosa^{b,c}, Egídio Torrado^{b,c}, João F. Mano^{a,b},

Nátalia M. Alves^{a,b}

^a 3B's Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, 4805-017 Barco, Guimarães, Portugal.

^b ICVS/3B's PT Associate Laboratory, Guimarães, Portugal.

^c Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga, Portugal

Modeling of QCM-D data

According to Voinova and co-workers²⁶, the changes in the resonant frequency and in the dissipation factor, were determined with the eqn.(S1) and eqn. (S2), respectively:

$$\Delta f(\text{Hz}) \approx -\left(1/2\pi\rho_0 h_0\right)\left(\eta_B/\delta_B\right) + h_L \rho_L \omega - 2h_L \left(\eta_B/\delta_B\right)^2 \left(\eta_L \omega^2 / \omega^2 \eta_L^2\right) \quad (\text{S1})$$

$$\Delta D(\times 10^{-6}) \approx -\left(1/\pi_f \rho_0 h_0\right)\left(\eta_B/\delta_B\right) + 2h_L \left(\eta_B/\delta_B\right)^2 \left(\eta_L \omega / 2\omega^2 \eta_L^2\right) \quad (\text{S2})$$

where ω is the angular frequency of the oscillation, and ρ_0 and h_0 are the density and thickness of the crystal, respectively. The viscosity of the bulk liquid is η_B , $\delta_B (=2(\eta_B/\rho_B\omega)1/2)$ is the viscous penetration depth of the shear wave in the bulk liquid and ρ_B is the liquid's density. The thickness, density, viscosity and elastic shear modulus of the adsorbed layer are represented by h_L , ρ_L , η_L and μ_L , respectively.

Surface characterization.

Atomic force microscopy imaging was performed to study the topography of the antibacterial nanostructured films, in air and at room temperature, using a Multimode STM microscopy controlled by the NanoScope III from Digital Instruments system. Several images were obtained in different areas on each scanned sample ($5 \times 5 \mu\text{m}^2$), using a silicon probe (TESP, Bruker) in tapping mode at a frequency of 1 Hz.

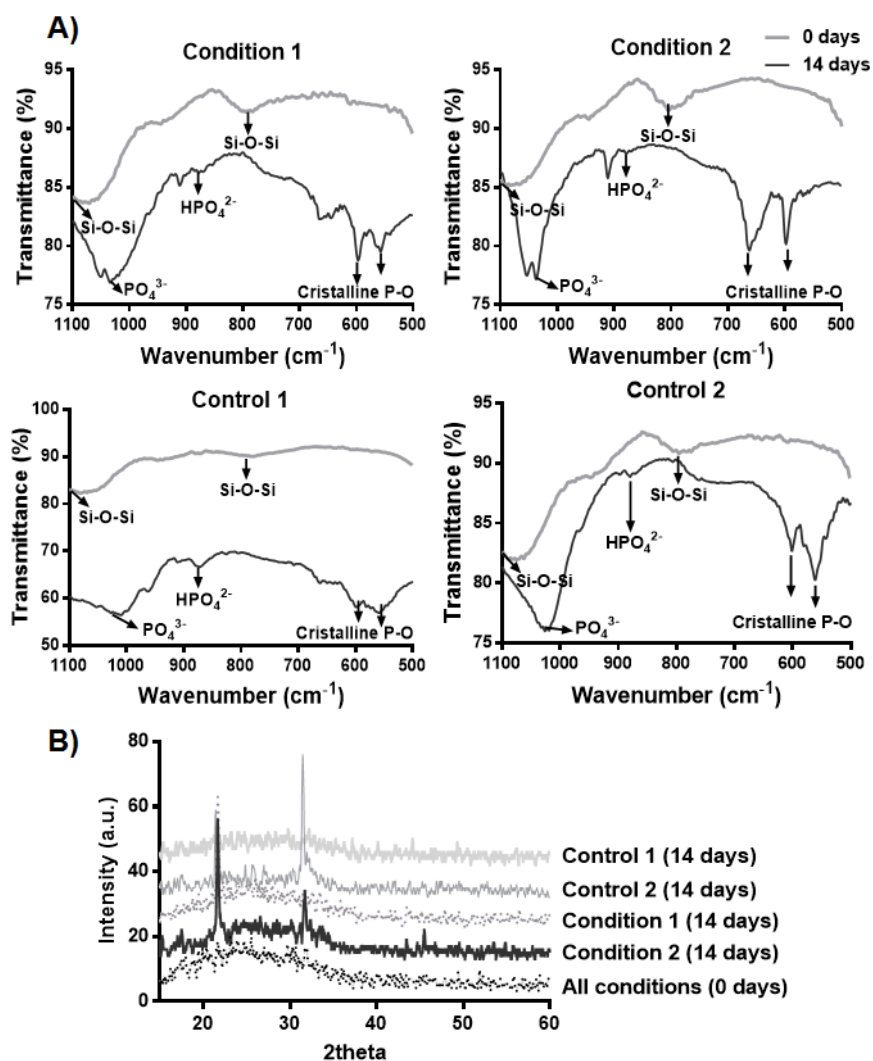


Fig. S1. *In vitro* bioactivity studies: A) FTIR spectra obtained before and after the immersion in SBF for 14 days for the distinct film configurations (condition 1:[CHT/HA-DN/CHT/AgBG]₅+CHT/HA-DN), condition 2:[CHT/HA-DN/CHT/AgBG]₅, control 1, [CHT/HA/CHT/AgBG]₅+[CHT/HA], control 2: [CHT/HA/CHT/AgBG]₅); and respective B) XRD diffractograms obtained before and after 14 days of SBF immersion.

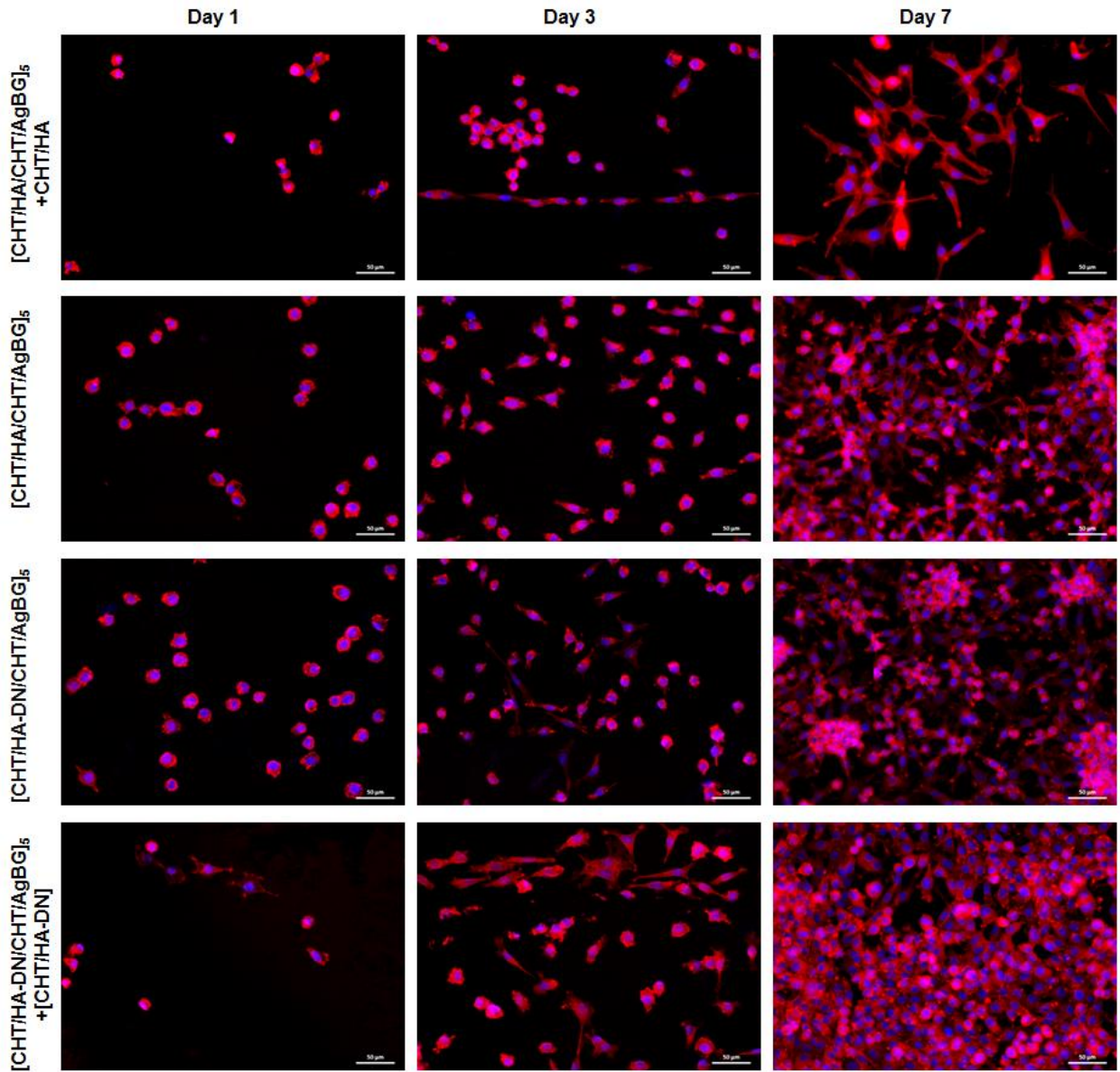


Fig. S2. Fluorescence images of cells stained with DAPI (blue) and phalloidin (red) for L929 at 1, 3 and 7 days of culture above the different multilayer coatings (condition 1:[CHT/HA-DN/CHT/AgBG]₅+CHT/HA-DN), condition 2:[CHT/HA-DN/CHT/AgBG]₅, control 1, [CHT/HA/CHT/AgBG]₅+ [CHT/HA], control 2: [CHT/HA/CHT/AgBG]₅): the nuclei of the cells was stained with blue and cytoskeleton of the cells with red. All images are representative for each condition and time point. Scale bar represents 50 μ m.

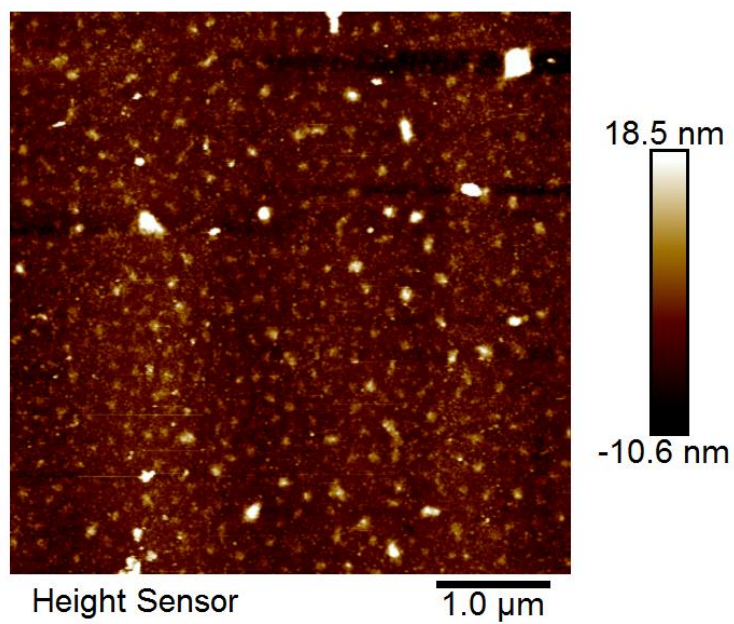


Fig. S3 Representative image of atomic force microscopy of LbL film configuration - condition 1 ([CHT/HA-DN/CHT/AgBG]₅+CHT/HA-DN). Scanned area of 5x5μm², using a silicon probe (TESP, Bruker) in tapping mode at a frequency of 1Hz.