Electronic Supplementary Information

Ion-doping as a strategy to modulate hydroxyapatite nanoparticle internalization

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Characterization of co-doped Mg/carbonate nanoparticles

Characterization of the NPs was performed using various techniques. The phase composition was determined by X-ray powder diffraction (XRD), using a D8 Advance Diffractometer (Bruker, Karlsruhe, Germany) with CuKα radiation at 40 kV and 40 mA. XRD spectra were recorded in the range 10–80 °, with a step size of 0.02 and a counting time of 1 s (Figure 1). The morphology of the NPs was assessed by transmission electron microscopy (TEM, JEOL 1010). Samples for TEM examination were prepared by soaking a 300 mesh carbon-coated copper grid in the solution of interest, blotted to remove the excess liquid and air dried (Figure 2).

Figure 1: X-ray diffraction of various NPs: non-doped (HA), 10C and co-doped Mg/carbonate NPs.
Figure 2: TEM images of different NPs: non-doped (HA), 10C and co-doped Mg/carbonate NPs.

Dose dependent cytotoxicity of 10M NPs on MG63

Cells were exposed different doses of 10M NPs dispersed in water in cell culture media without FBS. The appropriate volume of NPs from the stock (1 wt% NPs in water) was added to the corresponding cell culture media to make the desired concentration. Before adding the suspensions to the cells the mixture was vortexed to homogenise it. The incubation period was set to 24 h.

For the cytotoxicity evaluation of the various NPs the cell culture medium was removed after the 24 h incubation time and then viable cells attached on the surface of polystyrene tissue culture plates were lysed adding 100 μL of mammalian protein extraction reagent (M-PER, Thermo Scientific Inc., USA). Upon lysis cells released lactate dehydrogenase (LDH) that was measured using a commercially available kit (Cytotoxicity Detection KitPLUS, Roche, USA) following the manufacturer instructions. The absorbance at 492 nm was quantified on a micro spectrophotometer (PowerWave XS, Bio-Tek Instruments, USA) and the percentage of viability was calculated by the following equation:

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\text{viability\%} = \frac{\exp. \text{ value} - \text{negative control}}{\text{positive control} - \text{negative control}} \times 100 \quad \text{Eq.(2)}
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Where the ‘positive control’ was the absorbance value of cells incubated in cell culture medium under the same conditions of the experimental value but without NPs and the ‘negative control’ corresponded to the absorbance of the well without NPs and without cells. The studies done in triplicate were expressed as mean ± standard error of the mean.
Figure 3: Dose dependent cytotoxicity for 10M on MG63 cells cultured in cell culture media without 10% v/v FBS.