Supplementary information

The shape-effect of calcium phosphate nanoparticle based films on their osteogenic properties.

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Experimental

Synthesis of MSN

To synthesise MSNs with amines on the surface a mixture of 1.63 g TEOS (7.82 mmol) and 14.3 g TEA (95.6 mmol) was heated to 90 °C under static conditions (Solution 1). Solution 2 included 100 mg ammonium fluoride (2.70 mmol) dissolved in a solution of 2.41 ml CTAC (1.83 mmol, 25% (wt) in H2O) and 21.7 ml bi-distilled water (1.21 mmol) by heating to 60 °C. Solution 2 was rapidly added to solution 1, and the mixture was stirred vigorously at 700 rpm for 20 min while left to cool. Then, 138.2 mg TEOS (0.922 mmol) was added in four equal increments (34.55 mg each) every 3 min, and the mixture was stirred for another 30 min. Thereafter, 19.3 mg TEOS (92.5 µmol) and 20.5 mg APTES (92.5 µmol) were added, and the solution was stirred overnight at room temperature. The next day, particles were collected by centrifugation at 7800 rpm for 20 min and washed once with ethanol. Template extraction was performed by dispersion into an ammonium nitrate in ethanol solution (2 g NH_4NO_3 in 100 ml ethanol) and refluxed for 45 min at 90 °C. MSNs were collected by centrifugation and washed with ethanol before further template extraction in 100 ml of a 3.7% hydrochloric acid solution in ethanol for 45 min at 90 °C. MSNs were collected by centrifugation, washed twice with ethanol and stored in suspension at -20 °C.

Spherical nHA films stability using particle labelling.

To investigate whether nanoparticles could dislodge from film upon immersion to cell culture media, spherical nanoparticles were labelled with a fluorescent dye (ATTO 647) in their core and spin coated on plasma cover glass slide to create nanoparticles film. Core-labelled nHA films were immersed in cell culture medium (αMEM with 10% v/v 100x penicillin and streptomycin) and incubated at 37 °C, 5% CO2 in a humidified atmosphere in the dark. Supernatant solution were taken for analysis at 7, 14 , 21 days upon nHA film immersion. Media without nanoparticles and media containing same amount of labelled nanoparticles as film were keep in the same condition and used as negative and positive control respectively.

Long-term nHA Films stability using ICP-MS.

To investigate the stability of nanoparticles thin films under cell culture condition, nHA films were immersed in cell culture medium (αMEM with 10% v/v 100x penicillin and streptomycin) and incubated at 37 °C, 5% CO2 in a humidified atmosphere. Media were taken for analysis every 3 days after nHA film immersion. New cell culture media were then refreshed. The concentration of calcium and phosphorus was quantitatively studied by inductively coupled plasma mass spectroscopy (ICP-MS, iCaP Q, Thermo Scientific). To this end, aliquots were diluted 1:200 in aqueous 1% HNO3 containing 20 ppb Sc as internal standard and analysed using He as collision gas in standard mode. Element quantification was based on calibrations with element standards of calcium and phosphorus.

Supplementary Figures

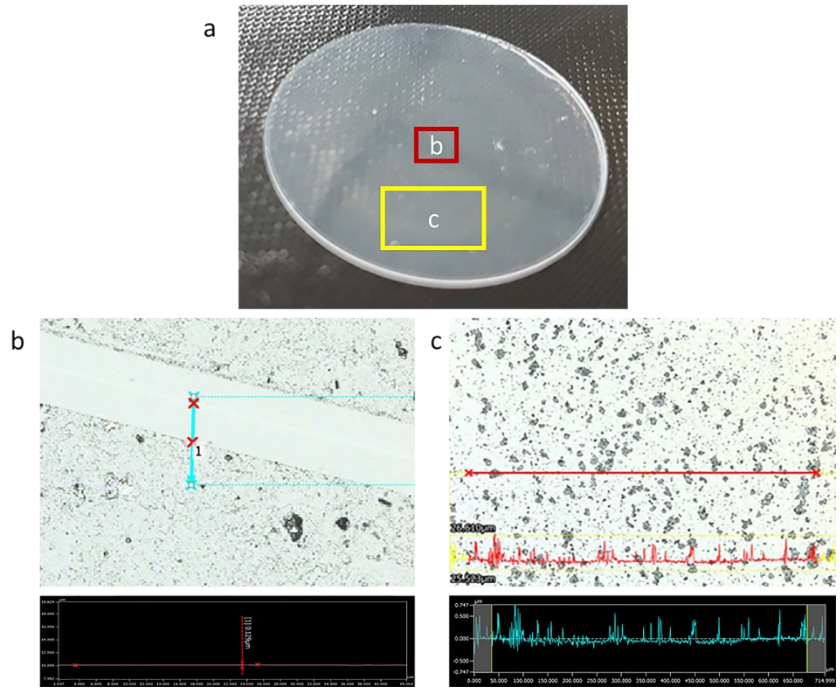

Fig. S1 Film thickness and roughness profile. (a) Example of nHA film (b) Film thickness measured at centre of the cover slip by 3D laser scanning microscopy. A scratch was made and the minimal point of scratched area was used as a reference. The difference in height shown in this example is 0.129 µm. c) Arithmetic average roughness (Ra) of each nanoparticles films was calculated using the Multiprofiler program from 3D laser scanning microscopy images. Ra is the squared root of the mean squared distance between peak and valley within the measuring length.

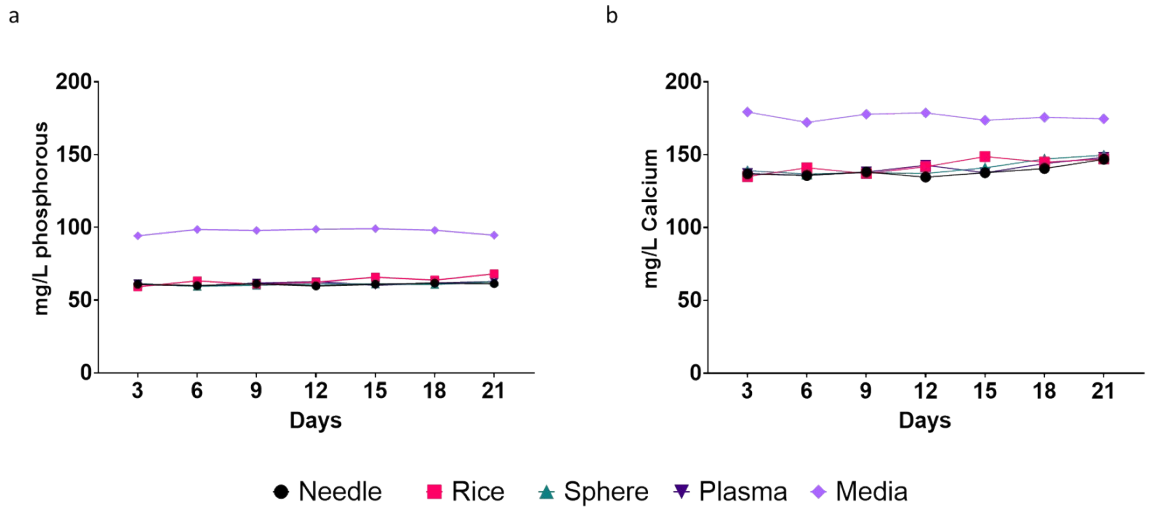

Fig. S2. (a) Calcium and (b) phosphorus concentration profiles in cell culture media after immersion of the nHA films at 37 degrees celsius. Cell culture media were exchanged every 3 days and the amount of calcium and phosphorous within the incubated cell culture media was quantified using ICP-MS analysis.

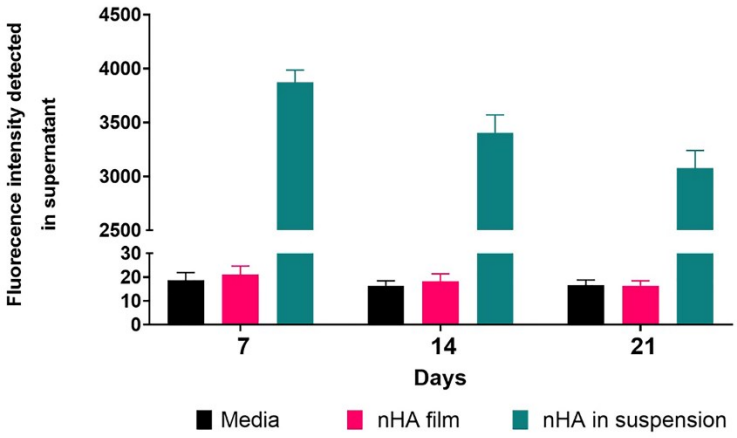

Fig. S3 Spherical nHA films stability using particle labelling. Spherical nanoparticles were labelled with a fluorescent dye (ATTO 647) in their core and spin coated on plasma cover glass slide to create nanoparticles film. Nanoparticle release in media was followed over 7, 14 and 21 days. Low fluorescence signal in supernatant in comparison to negative control (media without nanoparticles) and positive control (media with labelled nanoparticles) indicated no major dislocation of labelled nanoparticles from film.

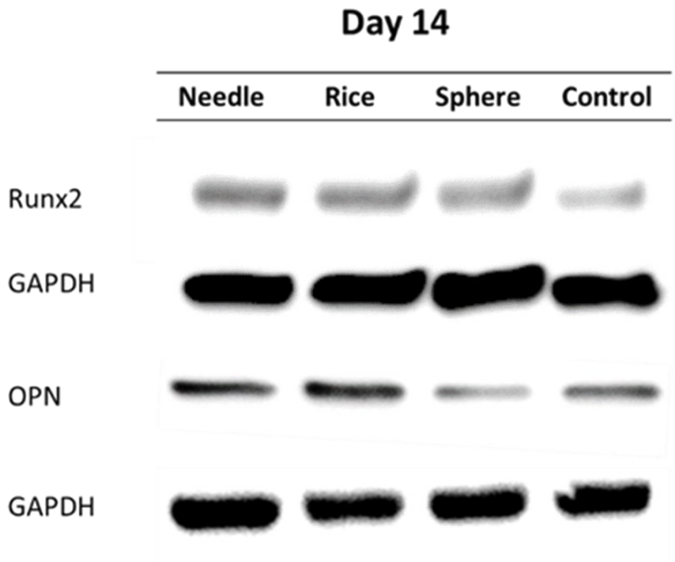

Fig. S4 The protein expression of Runx2 protein and OPN protein of hMSCs cultured on needle-, rice-, sphere-shaped nanoparticles films and control group after 14 days of culturing in basic medium.

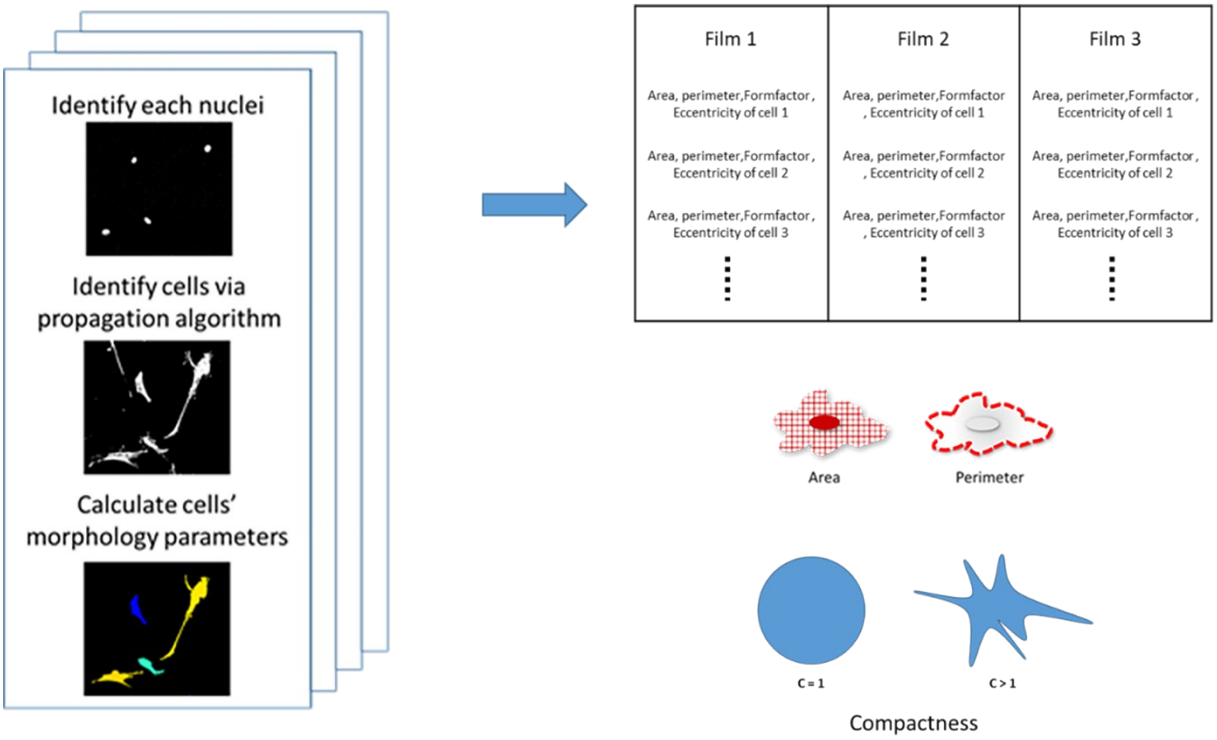

Figure S5. Image analysis workflow including: segmentation (identification of objects, measurements and tracking) in multiple frames; assembly of data in tables; and final merge of the data into groups representing cell morphology measurements as a function cell area, cell perimeter and compactness.

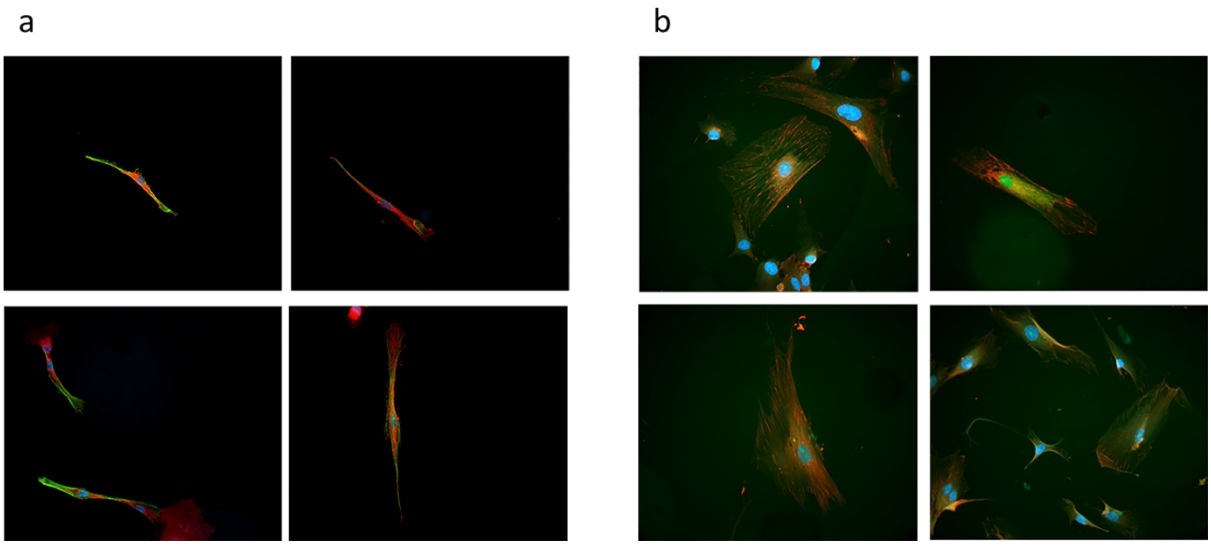

Fig. S6 Fluorescence microscopy images of hMSCs cultured on plasma treated control slides after 3days of culture with (a) basic media and (b) osteogenic media; green represents actin (phalloidin), red represents focal adhesions (vinculin) and blue represents nuclei (DAPI) staining.