Journal Name

ARTICLE

Soluble silicon patterns and templates calcium phosphate nanocrystal deposition in collagen type 1

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

*G. Birdi-Chouhan[a], R.M. Shelton[b], J. Bowen[c], P. Goldberg-Oppenheimer[a], S.J. Page[d], J.V. Hanna, A. Peacock[e], A. J. Wright[e], L.M. Grover[a]**

Supporting Information

Supporting Figure 1

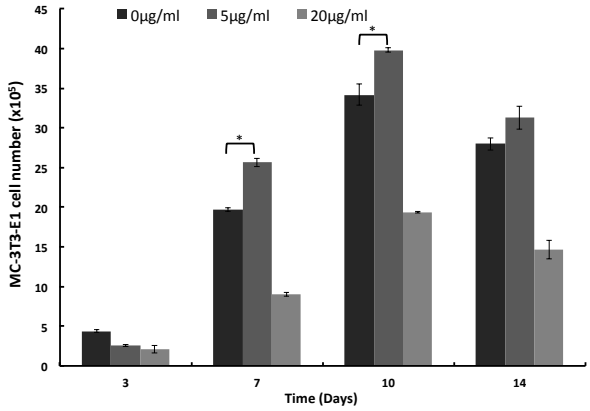

Figure S1: The effect of 0, 5 and 20µg/ml of OSA on cell viability and proliferation, assessed using a proliferation assay. 5µg/ml OSA significantly increased cell proliferation on days 7 and 10. 20µg/ml showed to reduce proliferation in the cell cultures

Supporting Figure 2

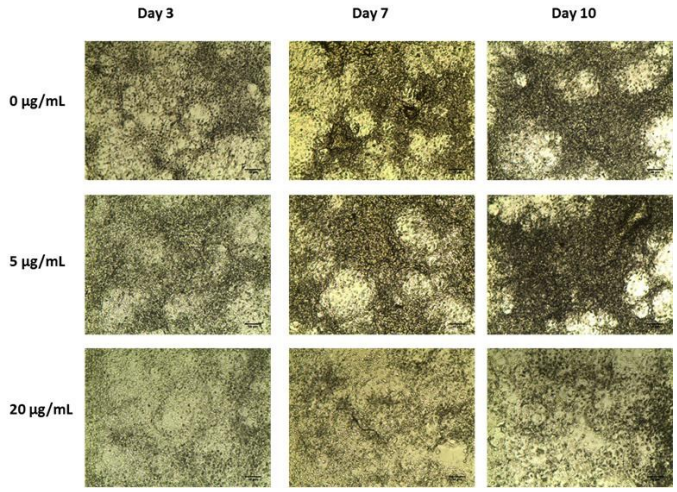

Figure S2: Bone mineral formation was determined using Von Kossa staining in MC-3T3-E1 cultured in the presence of 0, 5 and 20µg/ml OSA. An increase in staining was noted in cultures with 5µg/ml OSA when compared with cultures containing 20µg/ml OSA. Scale bar denotes 200µm

[a] *School of Chemical Engineering, University of Birmingham, Birmingham, B15 2TT, UK*
[b] *School of Dentistry, University of Birmingham, Birmingham, B4 6NN, UK*
[c] *Department of Engineering and Innovation, The Open University, Milton Keynes, MK7 6AA, UK*
[d] *Department of Physics, University of Warwick, Coventry, CV4 7AL, UK*
[e] *School of Chemistry, University of Birmingham, Birmingham, B15 2TT, UK*
† Footnotes relating to the title and/or authors should appear here.
Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

Supporting Figure 3

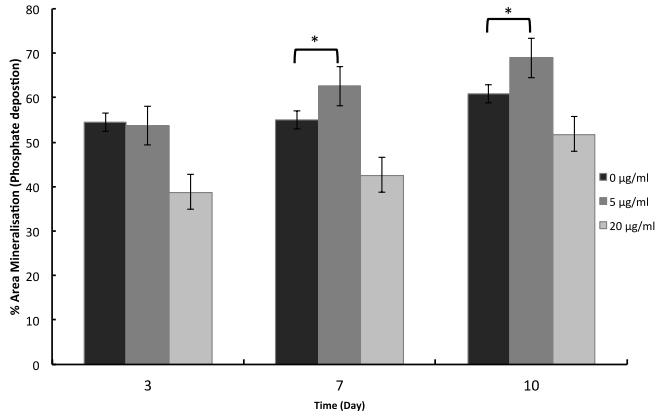

Figure S3 Area of mineralisation (%) was quantified with Image-J analysis of phosphate deposits in MC-3T3-E1 cultures in the presence of OSA. Semi quantitative analysis indicated that mineral formation was increased ($p<0.05$) in the presence of 5µg/ml OSA

Supporting Figure 4

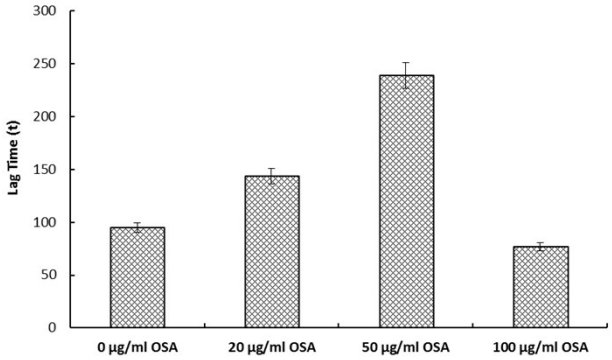

Figure S4 The effect of various concentrations of OSA on the lag phases in collagen fibrillogenesis. 20 and 50 µg/ml of OSA have increased lag times when compared to the control and highest OSA concentration. Changes in the lag phase indicate that OSA is most likely modifying the collagen helices and/or microfibrils before the start of the self- assembly process

Experimental

Proliferation and viability studies*:* Transformed mouse calvarial osteoblasts, MC-3T3-E1 were obtained from LGC, ATCC CRL- 2594™ (Middlesex, UK) and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (v/v) foetal bovine serum (FBS), 2.5% (v/v) 1M HEPES, 1% (v/v) 10mg/ml penicillin streptomycin and 2.5% (v/v) 200mM L-glutamine. MC-3T3-E1 cells were cultured at a seeding density of $2x10^4$ cells/ml in 12 well plates and left to attach overnight. The following day, supplemented DMEM containing 5µg/ml or 20µg/ml of sodium metasilicate was added to the cells. Trypan Blue staining (Sigma, UK) was used to determine cell viability and proliferation. Trypsinised cells were suspended in a small amount of culture medium and mixed with 0.2% w/v solution of trypan blue (Sigma, UK) in a ratio of 1:1 and viewed on a Neubauer haemocytometer (Beckman Coulter Ltd, UK). The numbers white cells (live cells) were counted and the dilution factor taken into account.

Von Kossa Assay Cell cultures for analysis were washed with PBS and fixed with 10% (v/v) paraformaldehyde for 30 minutes. The cultures were serially dehydrated in 70%, 95% and 100% ethanol and air dried. 2% (v/v) silver nitrate solution was added and the samples were exposed to UV light for 45 minutes. The samples were further rinsed with distilled water and sodium thiosulphate (5% v/v) was added to the cultures and left to stand for 2 minutes. The samples were then rinsed twice with distilled water and 95% ethanol and left to dry for image analysis (L F Bonewald et al. 2003). Digital images were captured using an inverted light microscope with a digital camera attached (CETI, Medline Scientific Limited, UK), for quatitative analysis the % area of mineralisation was determined using the image processing and analysis software ImageJ 1.43 (National Institutes of Health, USA).

Calculating the Lag Phase Type 1 collagen kinetics profiles were determined by carrying out turbidity measurements. The lag phase was calculated using Equation 1. Whereby t_o is the inflection point = 0, A_o is the initial absorbance and A_f is the final absorbance, Δt is the time constant.

$$Lag(t) = to = 2(Ao + Af)\Delta t / (Ao - Af)$$

Equation 1