# Predicting bone formation in MSC seeded hydrogels using Experiment based mathematical modelling

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## Supporting information

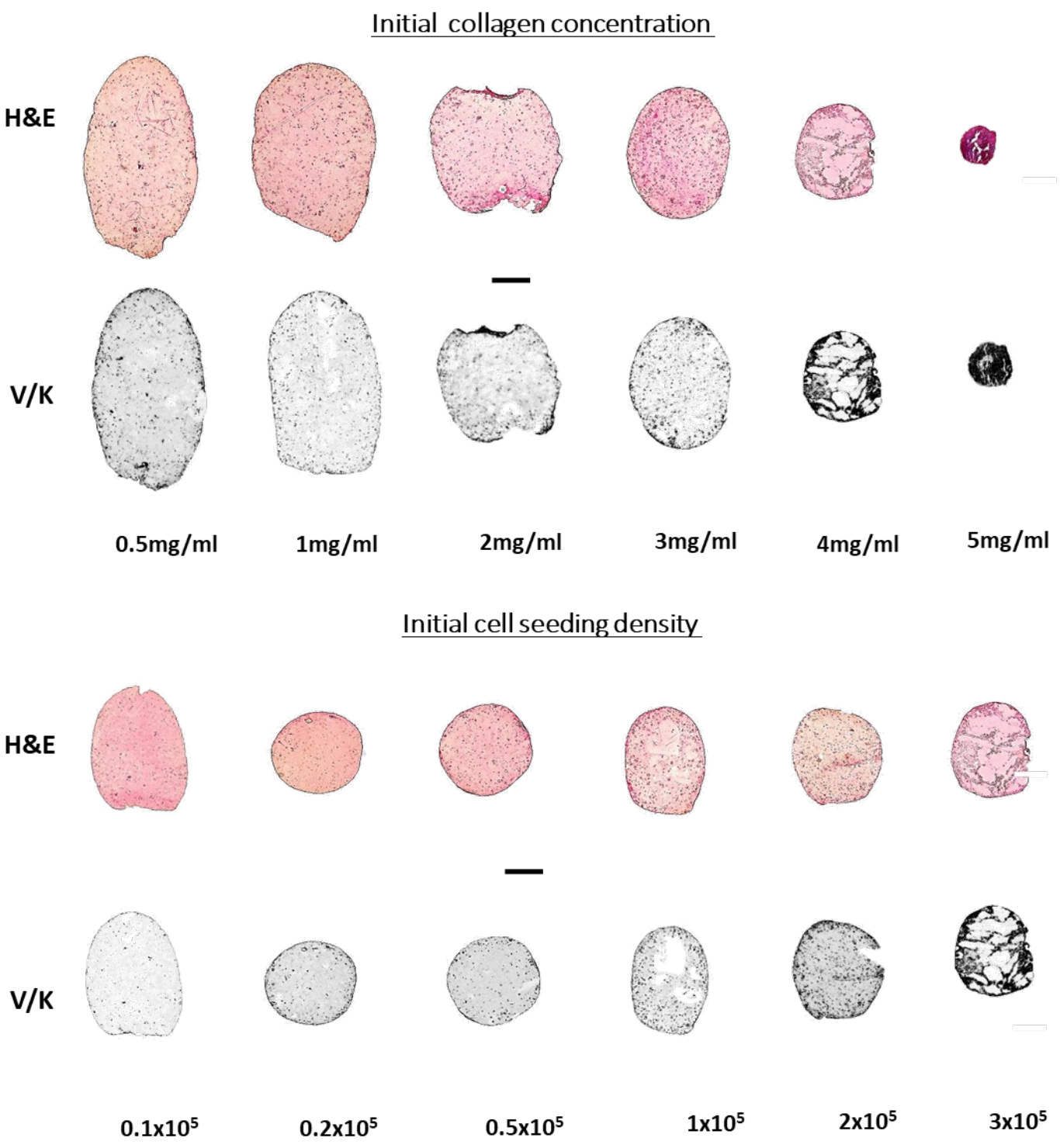
*Histological staining and imaging*

Microscopic analysis of sections was achieved by embedding the hydrogels paraffin wax using an automated vacuum tissue processor (Kedee). 10μm sections were cut using a microtome after which they were transferred to poly-lysine coated microscope slides (Thermo Fisher). Sections were deparaffinised in xylene and rehydrated in serial ethanol dilutions in $dH_2O$. Mineralisation was quantified using The Von Kossa method and staining to confirm the presence of calcium phosphate deposition by cells. Sections were incubated in 5% silver nitrate in dH2O for 30 minutes at room temperature. Sections were then washed under running tap water for 2 minutes and for V/K staining, exposed to UV irradiation for15 minutes in a Bio rad GenX UV chamber. Cell nuclei and cytoplasm were stained using Harris Haematoxylin solution and eosin for 30 secs each followed by 30 secs emersion in scots tap water (10g MgSO4, 1.75g NaHCO3 in 500mls PBS). Sections were mounted in DPX mounting medium and Imaged using an EVOS® FL Colour Imaging System.

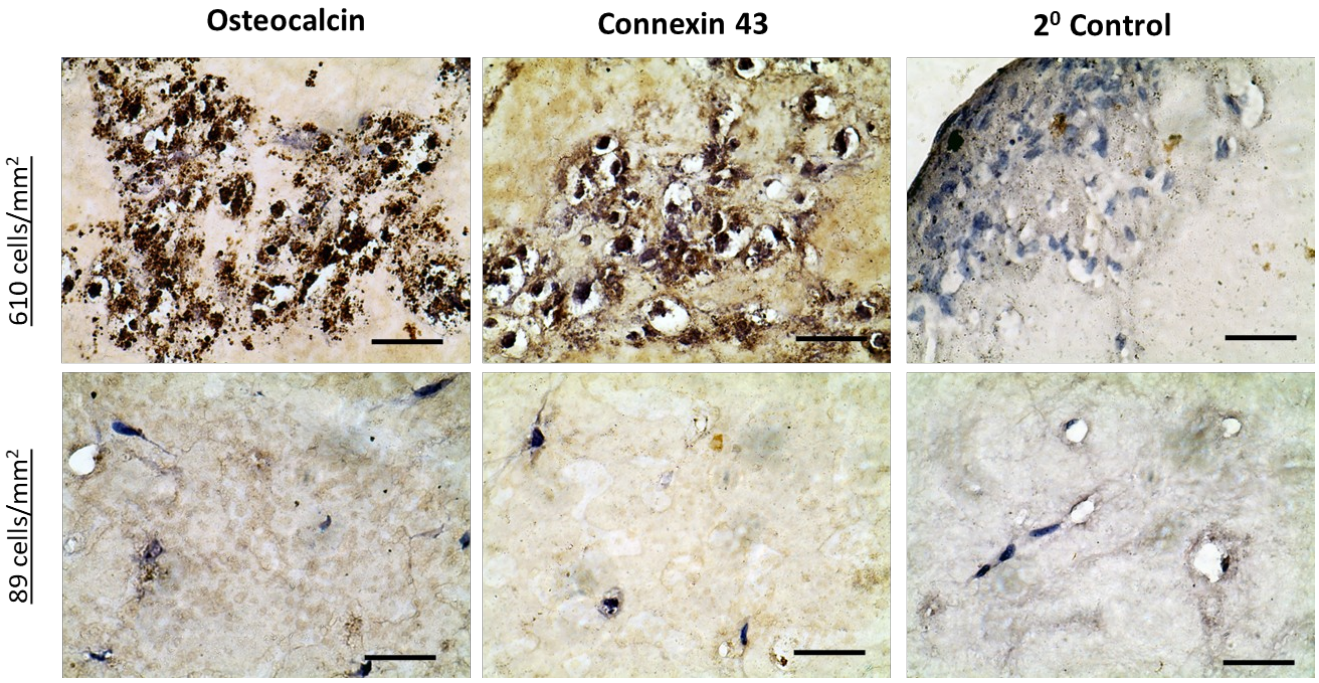
*Immunohistochemistry*

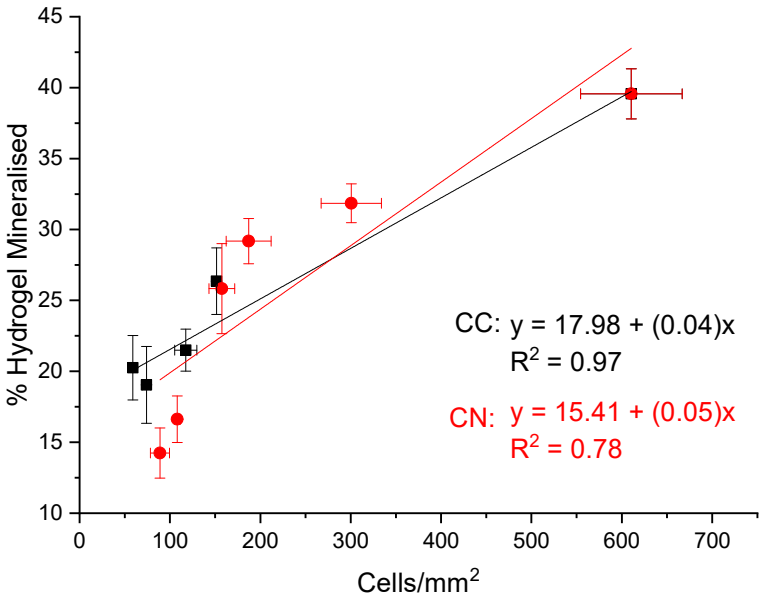
Hydrogels were fixed, paraffin embedded and rehydrated as described previously. 10 μm tissue sections were permeabalised in 0.01% Triton-X 100 for 15 minutes followed by 2 washes in PBS. Antigen retrieval was performed by immersing sections in 20μg/ml pre-warmed Proteinase K solution for 15mins at 37ºC. Sections were then blocked in 1% BSA (Sigma) for 1 hr before overnight incubation at 4ºC in primary antibody solutions (R&D

systems). Human osteocalcin (R&D systems) and Connexin-43(Santa Cruz bio) were diluted to 2μg/ml in 0.1% BSA in PBS with Tween (0.1%) for primary antibody incubation. Secondary antibody staining was performed using an ABC immune-peroxidase method (Santa Cruz bio), following the manufacturer's instructions. Cell nuclei were counterstained using Harris haematoxylin solution before mounting in DPX mounting media.




***Supplementary Figure 1:*** *Histological staining for cell distribution and bone mineral deposition on 10µm sections of MSC collagen hydrogels fixed at day 28. Scale bar = 500µm*




**Supplementary *Figure 2:*** ***A)*** *Immunohistochemistry on 10µm sections of MSC/collagen hydrogels fixed at day 28. Scale bar = 20µm.* ***B)*** *Plot of percentage hydrogel mineralisation against cells/mm² for hydrogels seeded at different CC and CN. n ≥ 4, Error bars in the y-axis represent standard error of mean and propagation of error in the x-axis (refer to main text for details).*