Supplementary information to:

Osteogenic potential of poly(ethylene glycol)-amorphous calcium phosphate composites on human mesenchymal stem cells

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SEM of mineralised cell sheets on scaffolds

Cell-mediated matrix formation and mineralisation in contact with non-mineralised hydrogels and citrate- or zinc-stabilised composites was assessed by culturing cells directly on scaffolds for 21 days. Electron micrographs showed that a dense layer of cells and mineralised cell-produced matrix had formed covering the centre of all scaffolds (Figure S 1a-c). Cells were densely packed and not distinguishable from the ECM. Mineral was present as spherical nodules throughout the ECM, closely resembling observations that have been reported for cells cultured on rigid ceramic scaffolds [1, 2]. Non-mineralised scaffolds exhibited less mineral in the cell-formed ECM. No qualitative differences regarding ECM mineralisation were seen between

the different composite groups (CR and ZR). Closer to the edge of the samples, the cell-ECM sheets were found to be thinner and less densely mineralised in all samples (Figure S 1d-f). Closer to the scaffold periphery, the fibrillar character of the ECM was clearly visible at intermediate magnification. It appears that densely packed cells in the centre of the scaffolds were actively producing ECM and mineralising it. Meanwhile, less dense cell populations spanning from the scaffolds to the surrounding cell culture plates produced a looser, less mineralised matrix, which is in good agreement with findings published elsewhere [2].

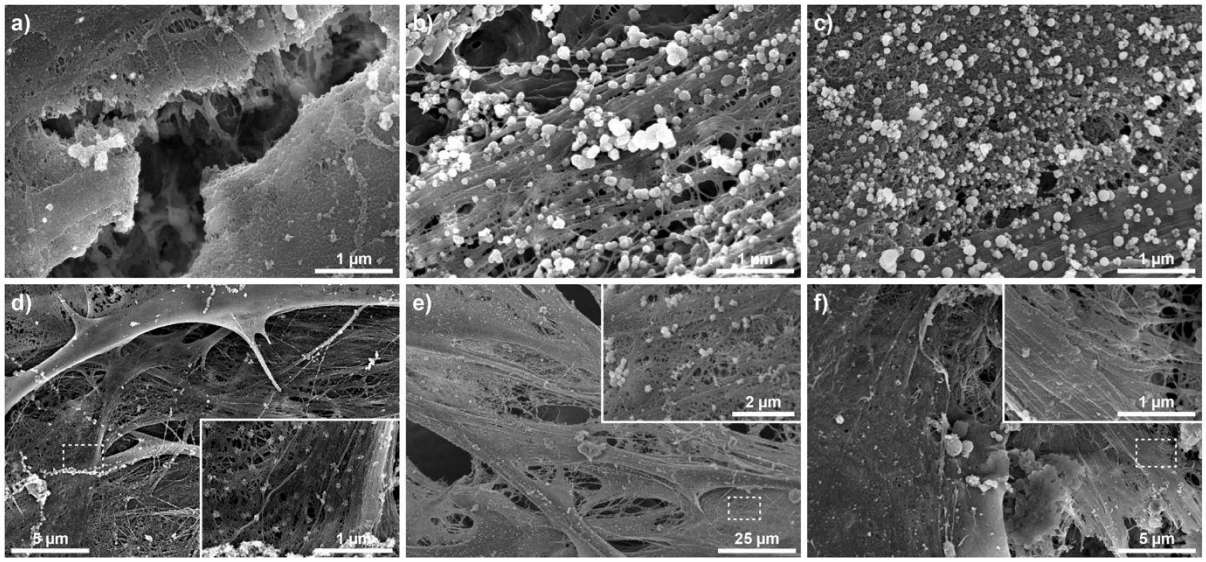

Figure S 1: Representative SEM images of cells cultured for 21 d on **(a, d)** non-mineralised hydrogels, **(b, e)** citrate- and **(c, f)** zinc-stabilised composites show particularly densely mineralised ECM for cells cultured on composite scaffolds. Regions in the centre (a-c) and close to the edge of the scaffolds (d-f) are shown. Inlays show magnified views of the indicated regions.

Mineral dissolution

Mineral dissolution during ageing in cell-free cell culture medium was observed in dark-field microscopy (Figure S 2). The microscopy images reveal clear differences in mineral dissolution between citrate- and zinc-stabilised composites. While no changes could be observed in the mineral distribution of zinc-stabilised ACP, citrate-stabilised ACP gradually dissolved in a directed fashion beginning at the composite-medium interface.

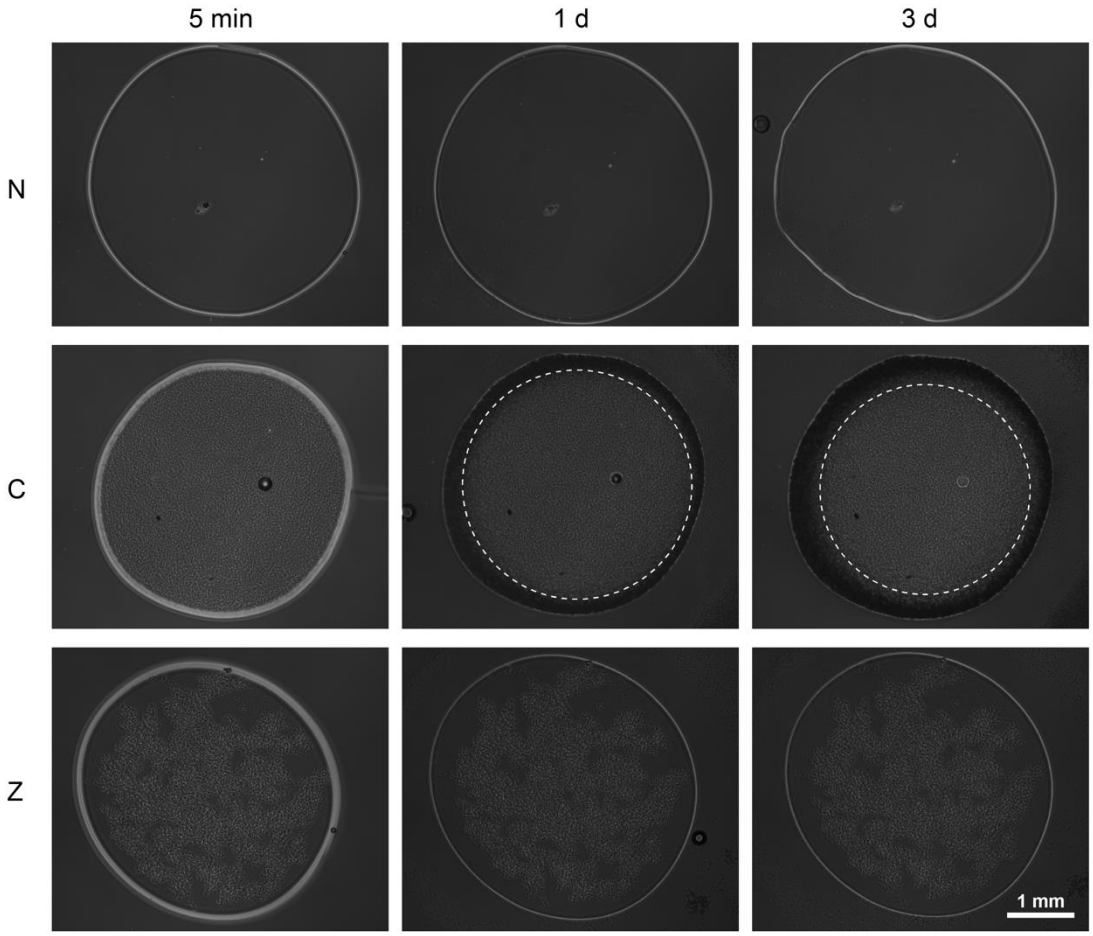

Figure S 2: Dark-field microscopy images of non-mineralised hydrogels (N), citrate-stabilised composites (C), and zinc-stabilised composites (Z) over three days of ageing in cell culture medium in the absence of cells. Mineral slowly dissolves in citrate-stabilised composites at the composite-medium interface as seen in the reduced mineralised areas (dashed circles). No directed dissolution of mineral particles is observed in zinc-stabilised composites.

Phase contrast microscopy of mineralised cell sheets

Phase contrast microscopy images reveal systematic differences in cell sheet organisation and mineralisation among cells exposed to different non-mineralised and mineralised scaffolds (Figure S 3). Mineralised nodules could be observed as early as day 2 for cell sheets exposed to citrate- or zinc-stabilised composites, while phase contrast microscopy did not reveal any mineral depositions in cell sheets exposed to non-mineralised scaffolds.

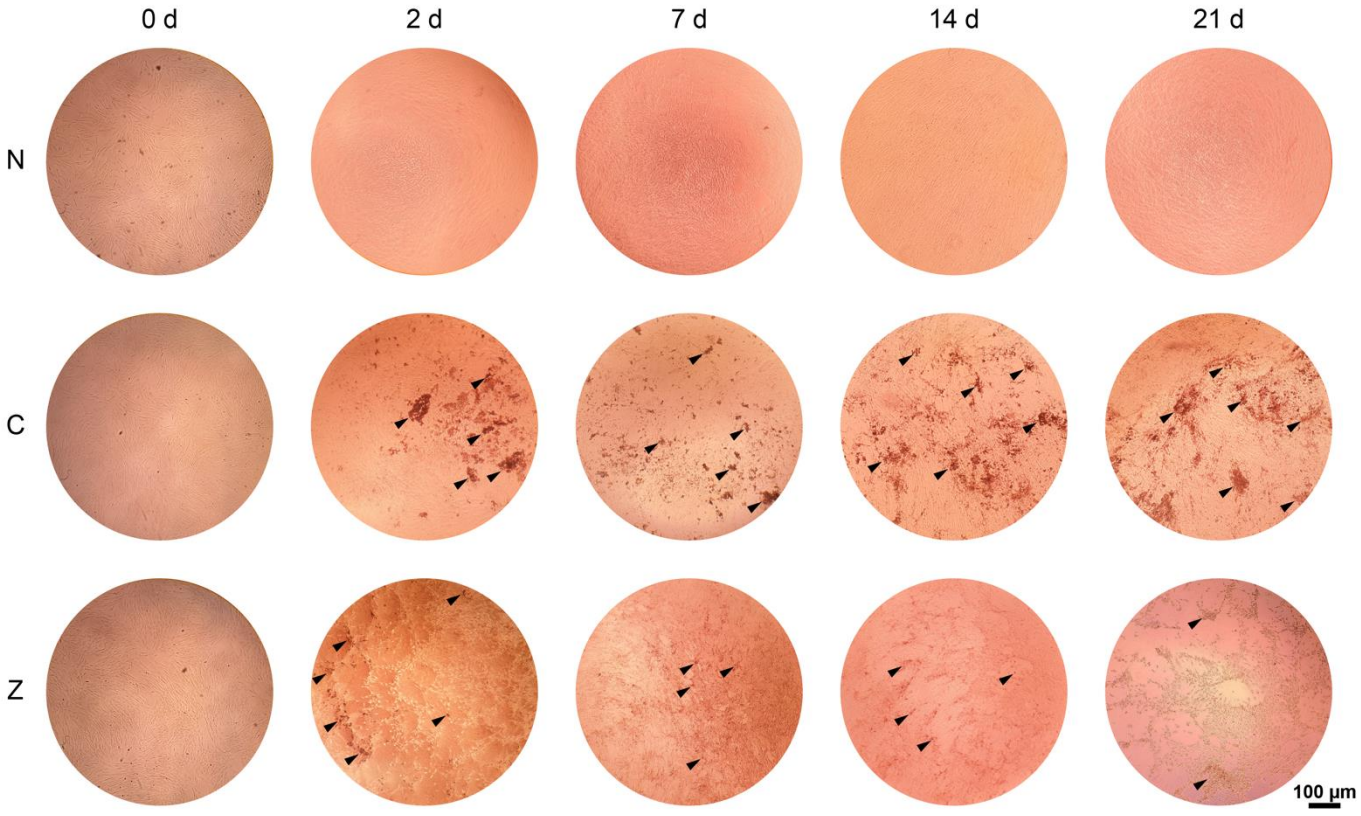

Figure S 3: Phase contrast microscopy images reveal differences in the cell sheets of hMSCs exposed to non-mineralised (N), citrate- (C), and zinc-stabilised composites (Z) for up to 21 d. Arrowheads indicate clusters of mineral particles in the cell sheets.

References

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2. Ros-Tarraga P, Rabadan-Ros R, Murciano A, et al. Assessment of Effects of Si-Ca-P Biphasic Ceramic on the Osteogenic Differentiation of a Population of Multipotent Adult Human Stem Cells. *Materials* 2016; 9: 969-985. 2017/08/05. DOI: 10.3390/ma9120969.