

Supplementary Information

Osteomatrix as a personalized 3D tissue-specific invasion test-bed for oral carcinoma

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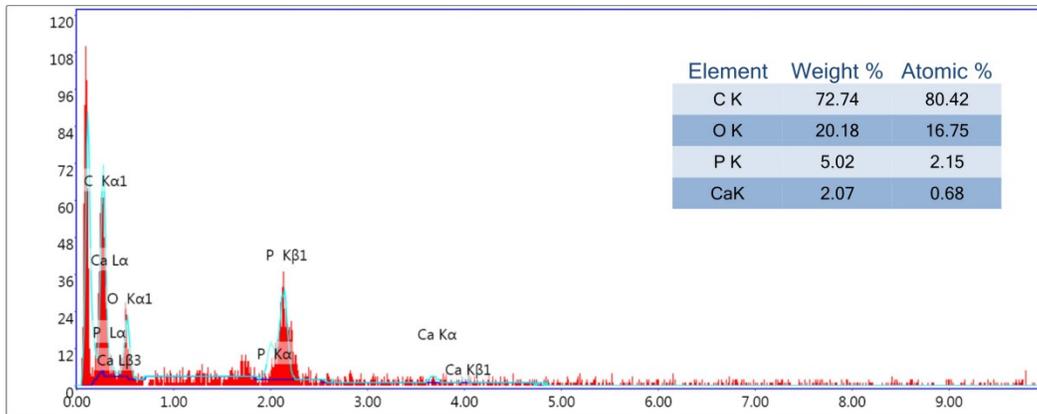


Figure S1 Elemental analysis of osteomatrix from energy dispersive X-ray spectroscopy.

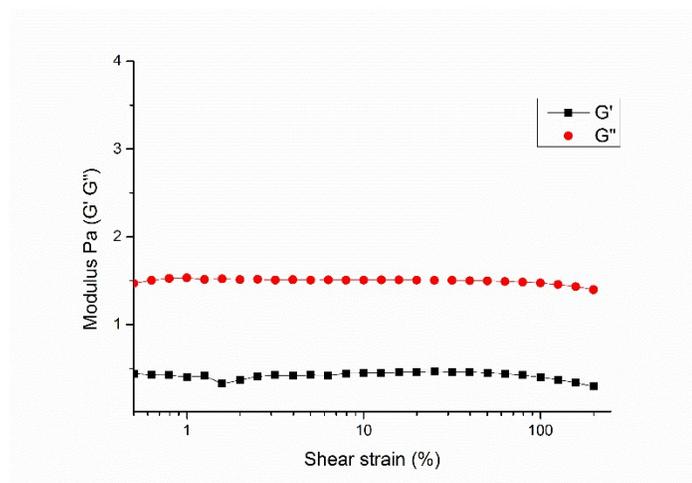


Figure S2 Amplitude sweep of osteomatrix (1%) subjected to 0.01–100% strain at a constant angular frequency. Data represent mean \pm standard deviation for $n = 4$.

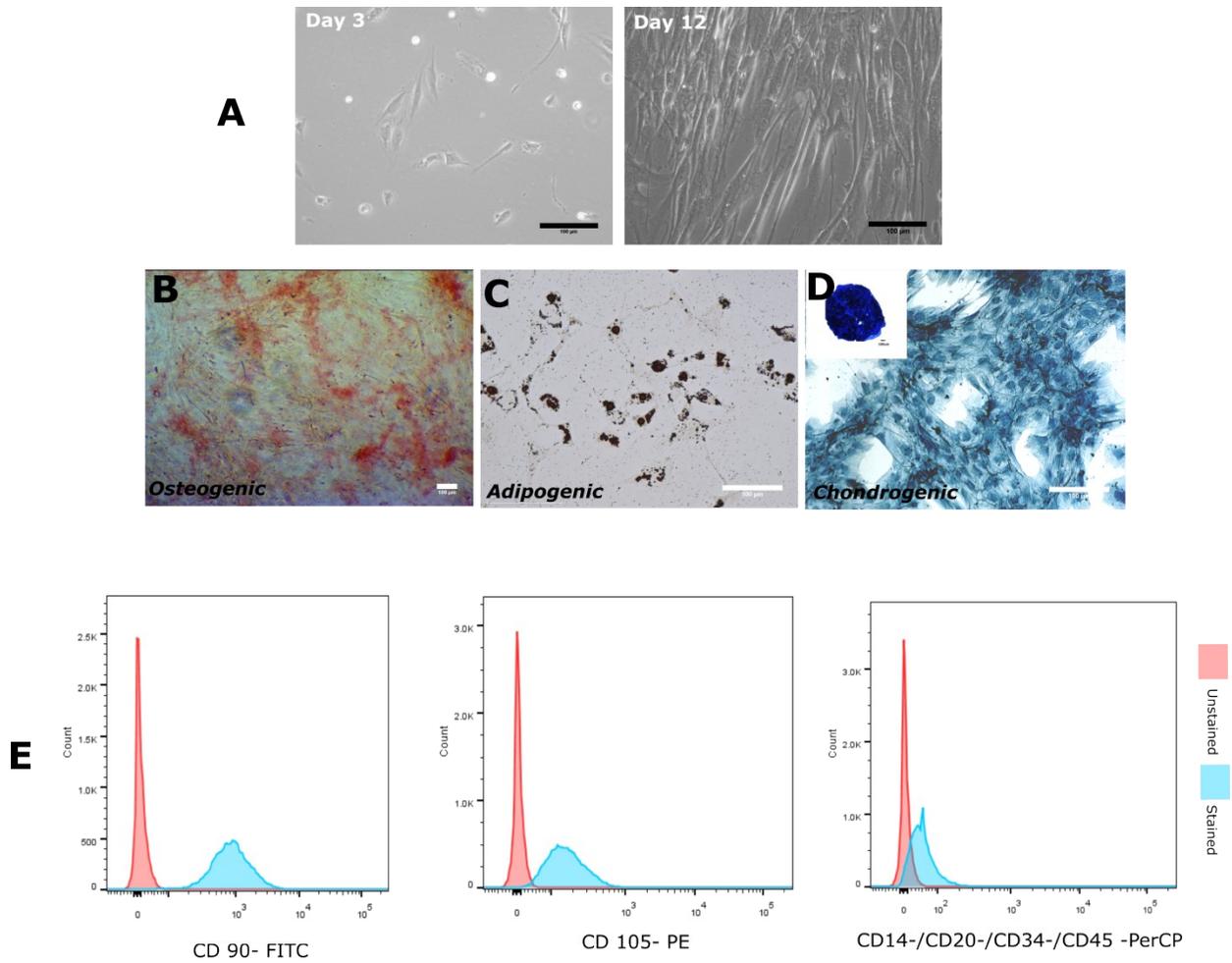


Figure S3 Characterization of isolated human adipose-derived stem cells (ADSCs) (A) ADSCs in culture showed adherent, spindle-shaped fibroblast-like cells. Trilineage differentiation potential confirmed by staining (B) Alizarin red (C) Oil red O and (D) Alcian blue. (E) Flow cytometric analysis of surface markers as recommended by the International Society for Cellular Therapy (ISCT). Representative graphs of a population with most of the cells positive for CD90 and CD73 and negative for CD 14, CD 20, CD34 and CD 45. Scale: 100 μ m.

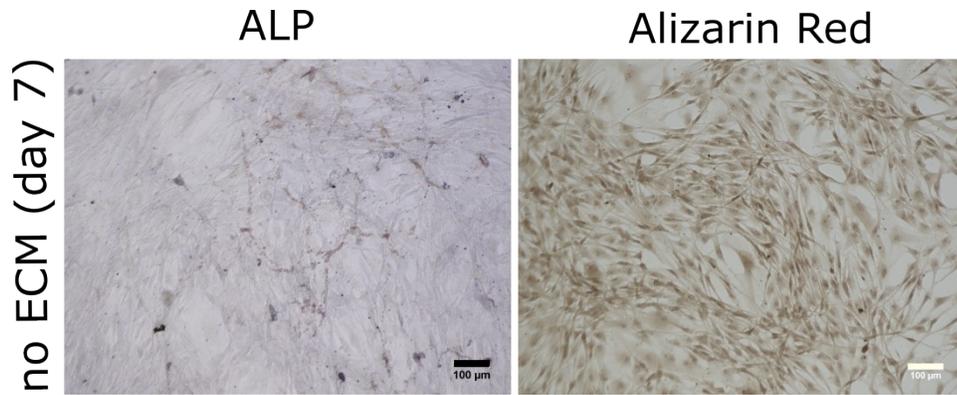


Figure S4 Representative images of ALP and Alizarin Red staining of ADSCs cultured on non-coated control (no ECM) for day 7. Scale: 100 µm.

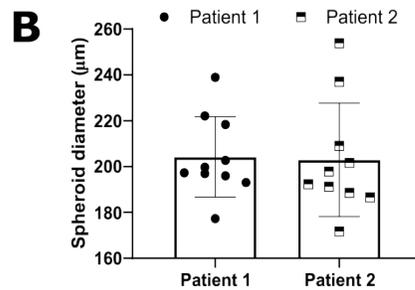
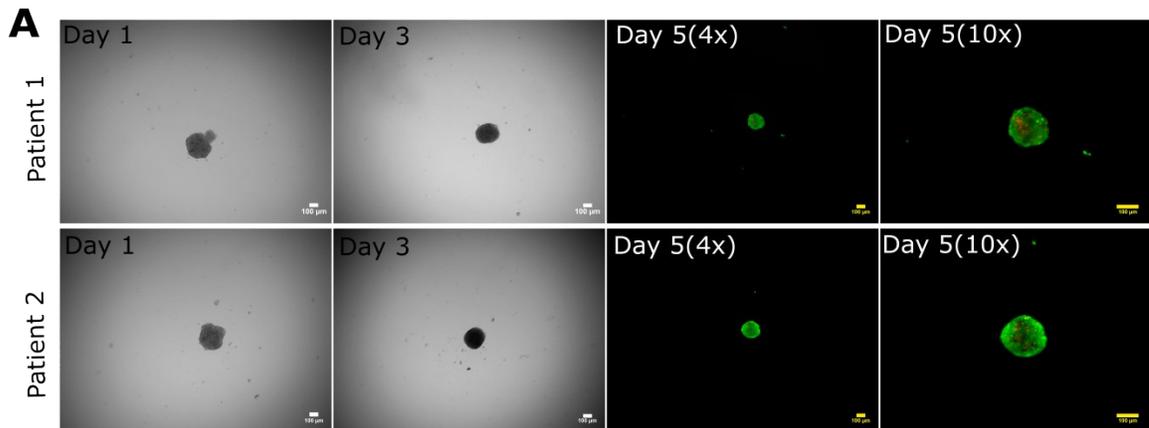


Figure S5 Formation and growth of CSC-enriched spheroid in culture. (A) Image analysis of spheroids from patients over time (5 days). Green channel: Calcein-AM, Red channel: PI. Scale: 100 µm. (B) Spheroid size distribution obtained from measuring spheroid diameter using ImageJ. Average diameter of spheroids is 200 ± 10.59 µm. Error bar represents spheroids of random spheroids of 96-well plate.

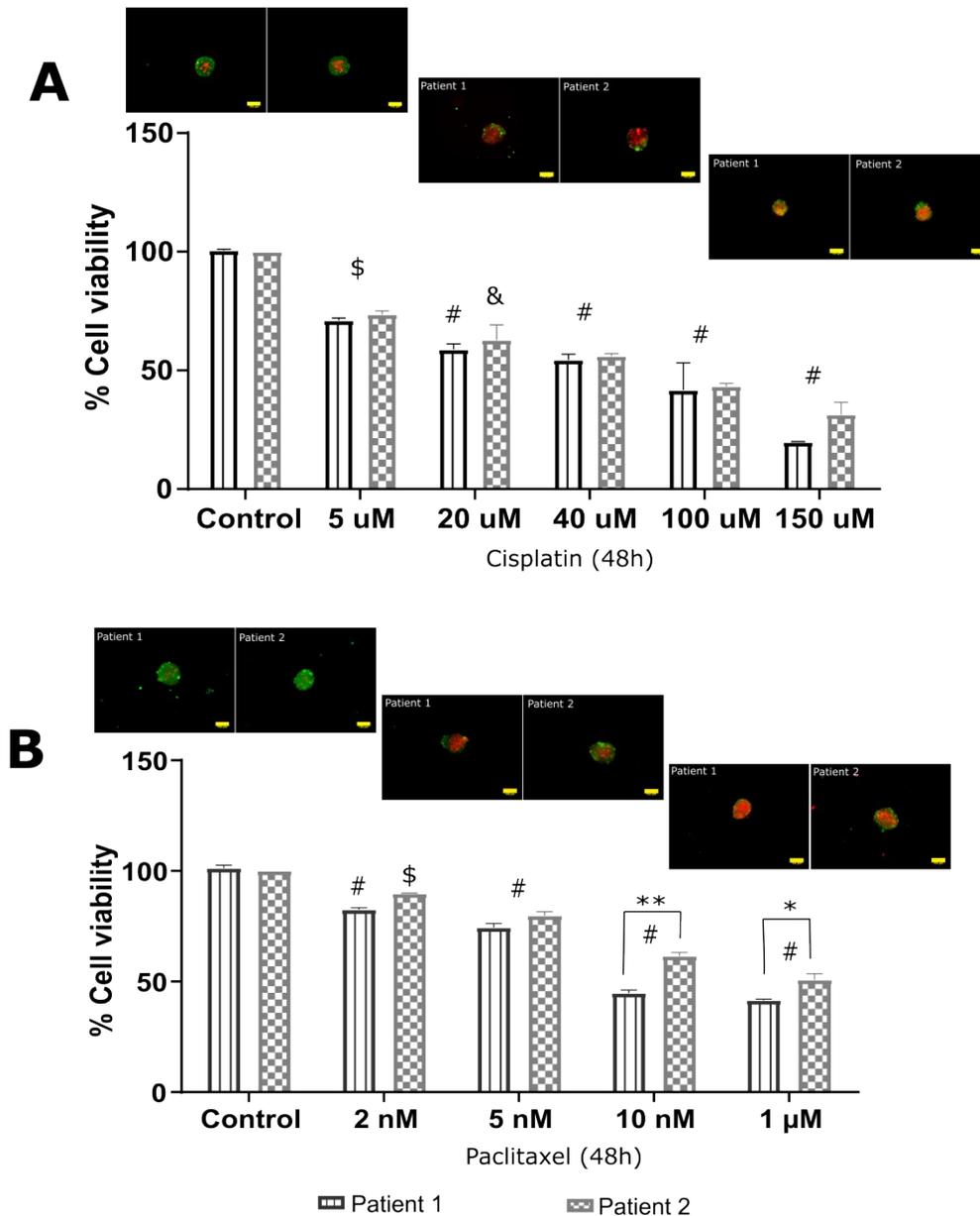


Figure S6 Cell viability of spheroids in suspension culture. Image-based quantification of cell viability and corresponding representative live/dead fluorescence micrographs of drug treated (48h) spheroids in suspension (A) Cisplatin treatment at doses ranging from 5 μ M to 150 μ M. (B) Paclitaxel treatment at doses ranging from 2 nM to 1 μ M. Green channel: Calcein-AM, Red channel: PI. All data normalized to control. Data represent mean \pm standard deviation for n = 6. *p < 0.05, **p < 0.01, ***p < 0.001. '\$' shows p < 0.05, '&' shows p < 0.01, and '#' shows p < 0.001 with control. Scale: 100 μ m.

Table S1. List of Primers for RT-qPCR Analysis

Gene	Forward primer	Reverse primer
GAPDH	CTCTCTGCTCCTCCTGTTCG	TTTCTCTCCGCCCGTCTTC
COL1	AAATGGAGCTCCTGGTCAGA	GTAGCACCATCATTTCACGA
RUNX2	TTGCCTTCAAACCCTAACGG	GAGTCAGGATGAATTCCAAGGG
OPN	TGAAACTGTGCCAGCCAAAC	AGTCTAATTGCAGTGACCCCC

Table S2. Clinical and pathological findings of patients in the study

Patient	Sex	TNM staging	Diagnosis	Invasion
Patient 1	Female	T3N1M0	Well differentiated squamous cell carcinoma	Exfoliative growth
Patient 2	Female	T3N1M0	Moderately differentiated squamous cell carcinoma	Alveolar gingival growth

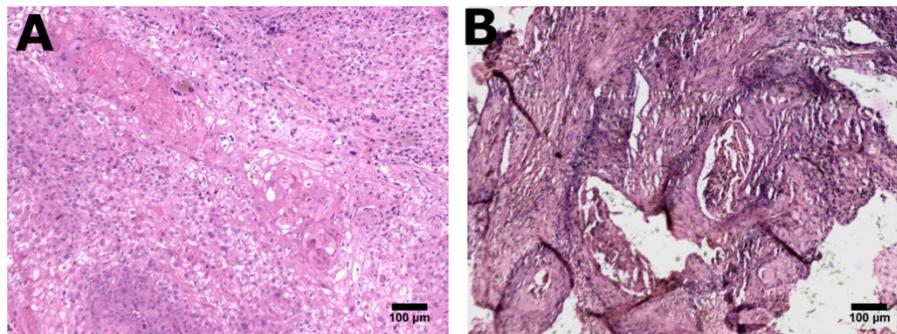


Figure S7 H & E stained section of (A) Patient 1 (B) Patient 2. Scale bar: 100 µm.