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Citation for final published version:

Yang, Yu, Knight, Rob , Stephens, Phil and Zhang, Ying 2020. Three-dimensional culture of oral progenitor cells: effects on small extracellular vesicles production and proliferative function. Journal of Oral Pathology and Medicine 49 (4) , pp. 342-349. 10.1111/jop.12981

Publishers page: http://dx.doi.org/10.1111/jop.12981

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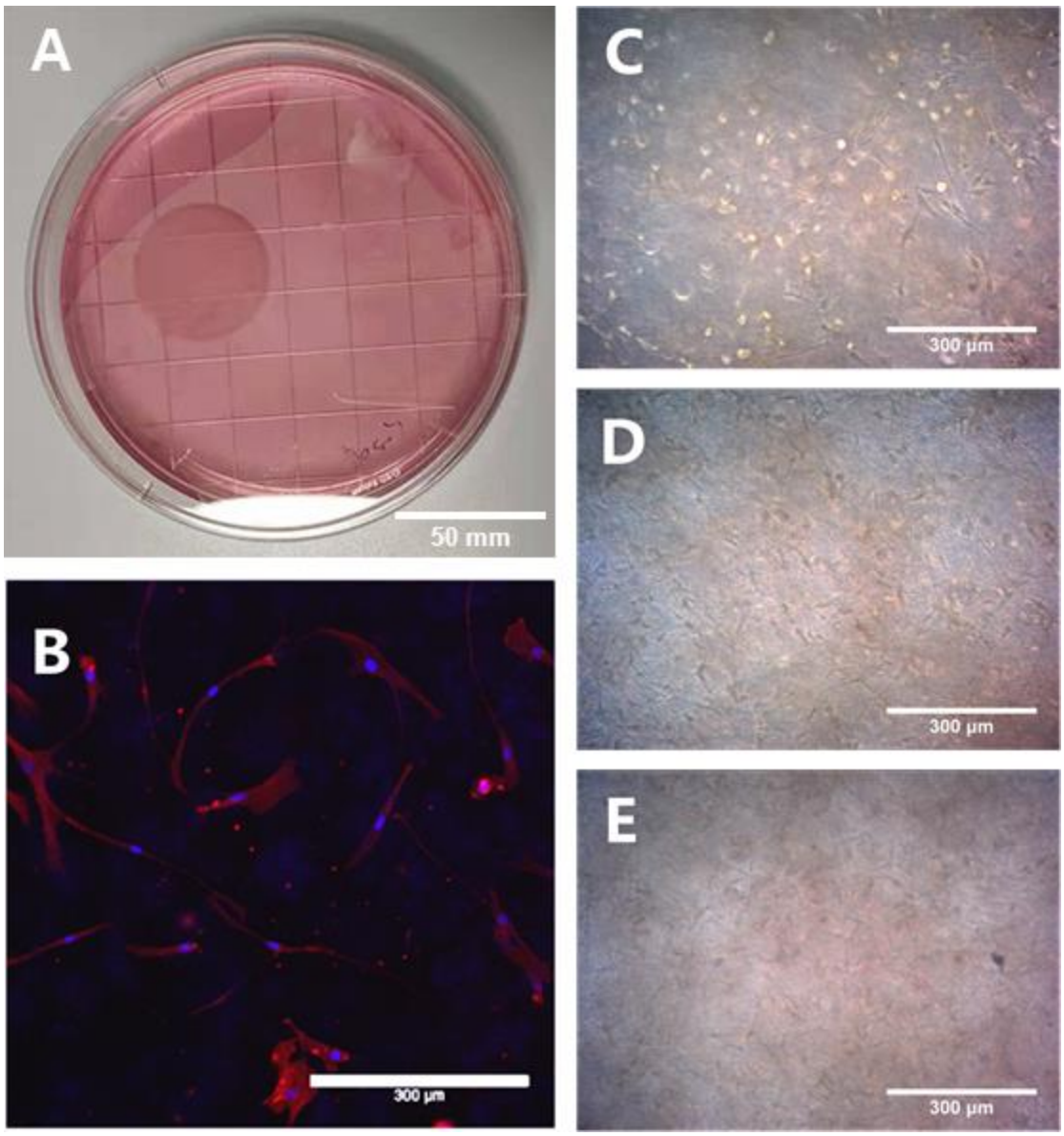

Figure 1: OMLP-PCs were cultured in type I collagen lattices and cell status was assessed over 72 hrs. The diameter of lattice reduced over the initial 24 hr period indicating that the cells were viable and had attached to the surrounding 3D matrix (A). Cells were stained with Phalloidin-Atto-594 and a Laser scanning confocal microscope was used to capture confocal images. Cells could be observed spreading out extensively within the matrix indicating viable cells after 72 hrs; nuclei = blue, actin = red (B). Images of cells were captured using a microscope equipped with a digital camera at 24 hrs (C), 72 hrs (D) and 120 hrs (E). Cells visualized under the light microscope demonstrated spindle like morphologies as expected for cells within a 3D matrix.

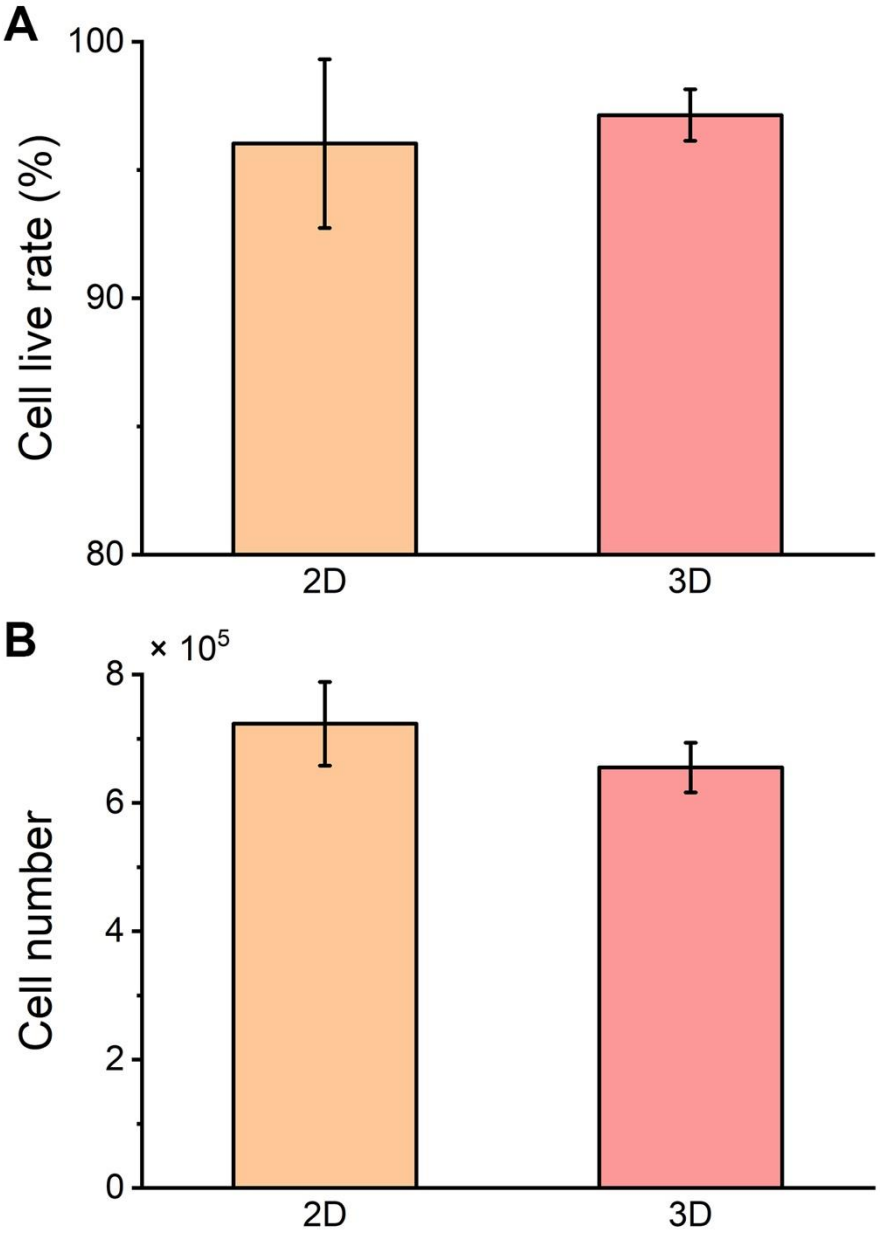

Figure 2: After 72 hrs of culture under 2D or 3D conditions, there was no significant difference in cell viability (A; P>0:05) or cell number (B; P>0.05) between monolayer (2D) and collagen lattice (3D). Mean ± SD.

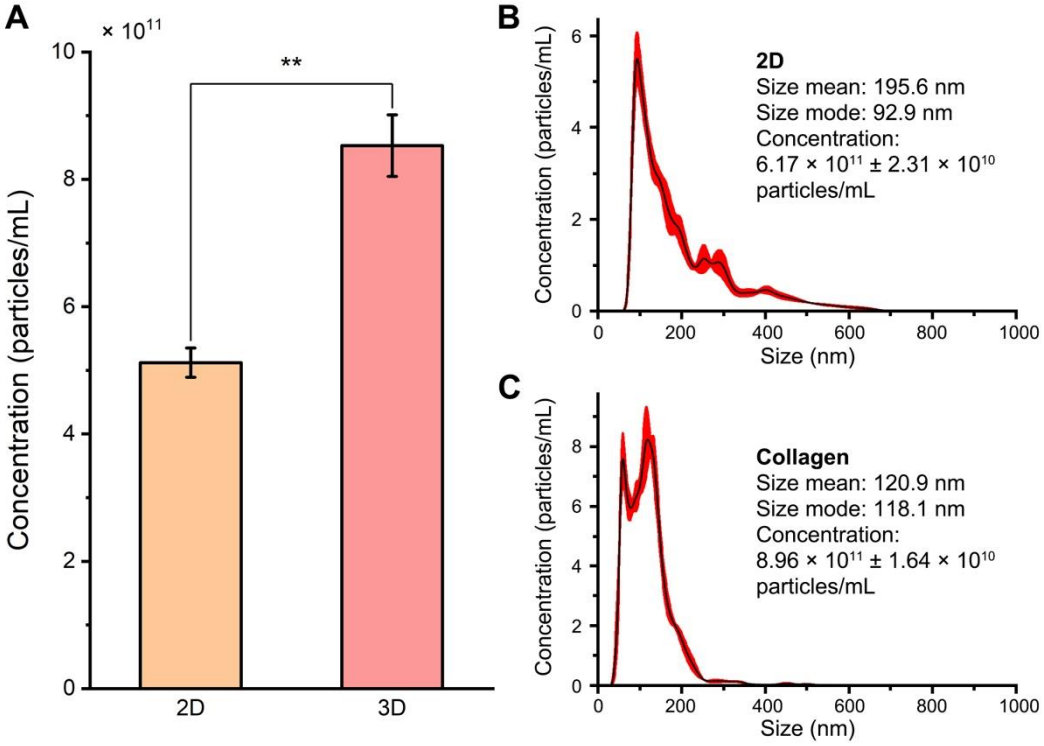

Figure 3: The size and concentration of particles derived from 3D and 2D cultures were assessed by NTA. (A) Particle concentration of EVs derived from 3D culture was significantly greater (**P<0.01) than that derived from 2D culture. (B) For both 2D and 3D cultures the particle mode size was in the range of 30-150 nm. Mean ± SD.

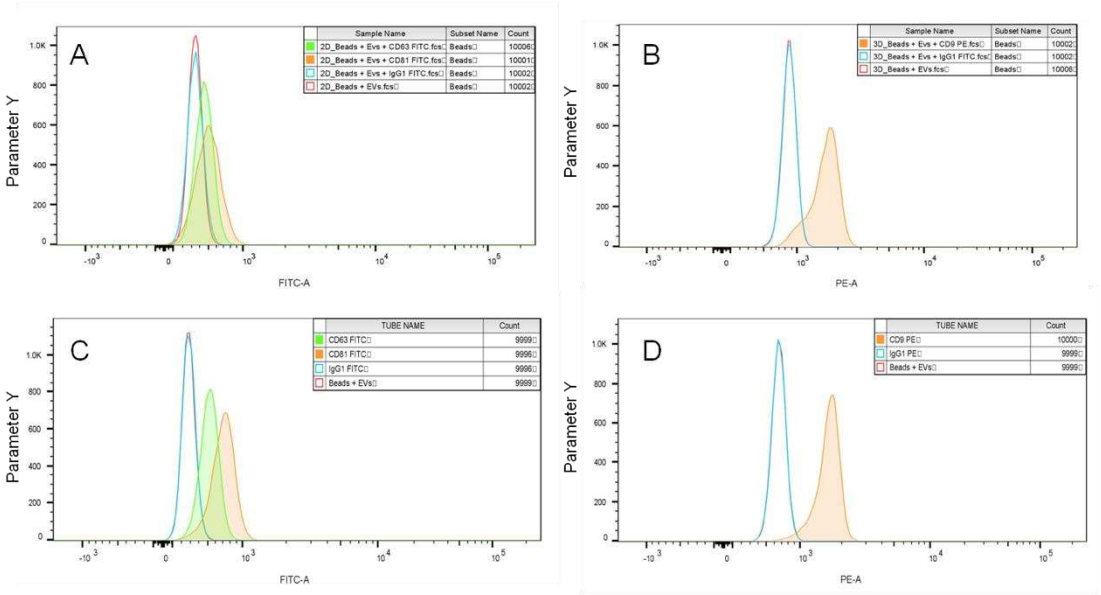

Figure 4: Flow cytometry demonstrated that SEVs isolated from 3D (A&B) and 2D (C&D) cultures were positive for the tetraspannins CD9, CD81 and CD63.

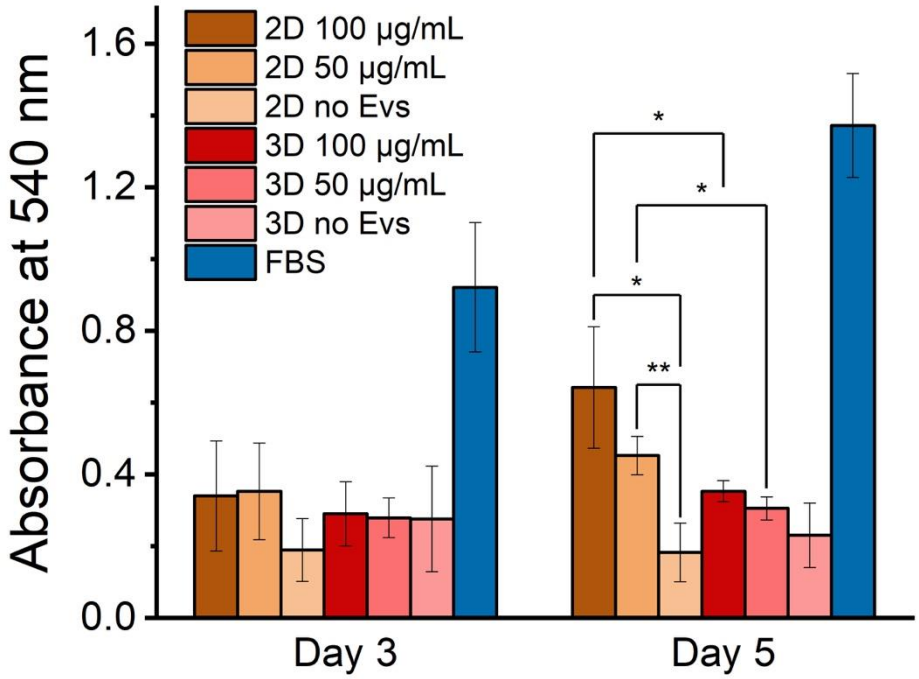

Figure 5: Proliferation of skin fibroblasts when cultured with or without SEVs derived from 2D or 3D cultures as determined by a MTT assay. The results demonstrated that at day 5, growth in the presence of 3D exosomes was decreased compared to 2D (*P<0.05, **P<0.01). Mean ± SD.