Agarose floor technique: a simple scaffold-free method for 3D cell culture and multicellular tumor spheroids formation.

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To the editors:

3D cell cultures can close the gap between *in vitro* experiments used for discovery and *in vivo* designs used for efficacy and safety assessment before proceeding to clinical studies ¹. 3D multicellular tumor spheroids reflect better the tumor environment in terms of phenotypic heterogeneity, nutrient and oxygen gradients, intervascular domains, and micrometastases ².

This letter presents the agarose floor technique to obtain single-cell suspensions from oral cancer cells in nonadherent conditions. SCC-9 cells (American Type Culture Collection, Manassas, VA, USA) were cultured in culture plastic wares with nonadhesive surface. 150 mm dish are made of nonadhesive for cells by coating with 3.2% sterile agarose (Analytical Grade, Promega, Madison, WI, USA) thin films (5-8 mL/dish). This agarose floor is allowed to dry before addition of medium (DMEM/Ham's F12, supplemented with 10% fetal bovine serum, antibiotics and 0.4 µg/mL hydrocortisone) containing 6×10^5 live cells/dish. The cells were maintained at 37°C in a 5% CO₂ atmosphere and the culture medium was changed every 2 days until the sphere formation (8 days). With the passing of the days multicellular tumor spheroids are obtained. The spheres were examined with microscope and digital camera (**Figure 1**).

Multicellular tumor spheroids are easily generated by the liquid overlay technique that prevents matrix deposition. Tumor cells are placed on tissue culture plastic covered with a thin layer of inert substrate such as agarose ³. Agarose is frequently used in molecular biology for the separation of large molecules, especially DNA, by electrophoresis. The pore size of a 3% agarose gel has been estimated in ~290 nm ⁴. The low porosity, along with being an inert material, make the agarose a good candidate to obtain a non-adherent surface to epithelial cells.

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Figure 1. Three-dimensional multicellular tumor spheroids formation. Oral cancer cells (SCC-9) grown in non-adhesive liquid-based culture tend to form cell clusters (panels from day 2 to 8). With tumor spheroids, the cells appear fused together as “*floating popcorns in the night sky*”. Scale bar, 1mm.