Supporting Information

Tissue Mimetic 3D Scaffold for Breast Tumor-derived Organoid Culture Toward Personalized Chemotherapy

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Detailed Methodology

Preparation of porous PCL scaffolds

Porous scaffolds of PCL (Average molecular weight = 80,000 g/mol, Sigma) were fabricated in 96-well plates by salt leaching technique. Sodium chloride (Sigma) crystals were sieved to a defined size range of 250-425 µm. A total of 0.13 g of the salt was added to the desired wells of a 96-well polypropylene plate (Sigma). A volume of 45 µL of 10% (weight/volume) PCL solution in chloroform was added, centrifuged at 2000 rpm for 2 min and air dried for 24 h. The salt was leached in deionized water for 72 h, with the water changed after every 24 h. The plates were removed from the water and completely air dried.

Isolation of human mammary cancer cells from primary tumors

Single breast cancer cells were isolated from breast tumor tissues as follows. The tumor tissues obtained from patients during surgery were transported to the lab in a transporting media (DMEM, with streptomycin sulfate (200 µg/ml), benzyl penicillin (200 U/ml), gentamycin (10 µg/ml) and amphotericin B (2 µg/ml)). The tissues were mechanically minced into small pieces using a sterile scalpel and enzymatically digested with 1 mg/ml Collagenase (Sigma Aldrich) and 100 U/ml of Hyaluronidase for 8-12 h with media of composition similar to the transportation media at 37°C. The digested tissue was centrifuged at 800 rpm for 1 min, to pellet the organoids. They were then resuspended in culture media and single cells were obtained by differential trypsinization.
Figure S1: 3D PCL scaffolds showed good porosity and pore interconnectivity
Representative SEM micrographs of 3D porous scaffolds at different magnifications (A, B and C). SEM micrograph revealed a uniform open porous structure (A, B) with good pore interconnectivity (indicated by yellow arrows in C). (D) X-ray Micro CT image of a scaffold. Scale bar represents 1 mm.
Figure S2: Primary patient-derived cancer cells (Sample 16061T) show increased attachment and generate 3D tumoroids in hybrid scaffolds

Fluorescent images of primary cancer cells cultured on the polymer and hybrid scaffolds for 10 days, stained for actin (red) and nuclei (green). Imaging was performed using a confocal microscope and maximum intensity projections are shown. Scale bar represents 200 µm in (A) and 30 µm in (B).