

Sample preparation for the Scanning Electron Microscope

For SEM examination, the cell-scaffolds were first fixed with 3-5% glutaraldehyde (w/v, in pH 7.4 PBS). After fixation, the cells were dehydrated by soaking the scaffolds subsequently in ethanol solutions with gradient concentrations of 25%, 50%, 75%, 90% and 100% (v/v, in pH 7.4 PBS), respectively. The dehydrated cell-scaffolds were stored in 100% ethanol. Prior to SEM imaging, the samples were treated by CPD (critical point drying) and coated with a thin layer of gold and platinum through sputter coating process.

Mice tumor models

MCF-7 breast cancer cells both cultured on commercial 2-D plates and on 3-D microfibrillar silica scaffolds were injected into mice to test their in vivo proliferation rate. The cells were detached separately from the two different substrates and both were resuspended to a concentration of 2×10^8 cells/ml. The viability of cells was checked, by Typan Blue (ATCC) staining, to be more than 95%. Six female athymic nude mice (from the JAX laboratory) were adopted for housing for 3 days under IVC system (Tecniplast) after arrival. 2×10^7 MCF-7 cells were injected in the armpits of mice after being anesthetized by intraperitoneal (ip) injection of 1.25% (w/v) avertin (Sigma). The cells from the 2-D and 3-D pre-culture were injected into the left and right armpits, respectively. After 10 days, 3 mice were randomly selected to be sacrificed after being anesthetized by ip injection of 1.25% (w/v) avertin. Tumors from both armpits were taken out to be weighed and photographed. The remaining 3 mice were treated in the same way after being fed for another 10 days. The total tumor weight was presented as mean value \pm standard deviation ($M \pm SD$). All the mice had free access to the food and water during the experimental course.

Biocompatibility study of the silica scaffold

To study the biocompatibility, the silica microfibrillar scaffolds, without cells, were implanted into the mice. C3D2F1 mice (from the JAX laboratory) were anesthetized and implanted with the as-treated scaffolds. After being adopted to breed for 3 days, the scaffolds were subcutaneously implanted into the nuchal region. After 21 days, the implanted scaffolds and surrounding organs were taken out for pathological inspection. Mice not receiving any treatment were used as control. All the mice had free access to the food and water during the experimental course.

Pathological inspection

After an appropriate period of culture, the scaffolds occupied by MCF-7 cells were rinsed thoroughly with PBS (pH 7.4) and then fixed in 3% glutaraldehyde (in PBS, pH 7.4) for 30 minutes at 4 °C. Following the fixation, the samples were placed in 2% liquid agarose at 45 °C. The scaffold-agarose mixture was solidified at 4 °C for at least one hour. The agarose gel-filled scaffolds were then placed in a Tissue-Tek cassette (Sakura FineTek; Zoeterwoude, the Netherlands), and embedded in paraffin using an automated tissue processor (Tissue-Tek VIP150; Sakura) under standard conditions for surgical biopsies. The paraffin embedded samples were cut to 4 μ m sections, mounted on SuperFrost/Plus glass slides (Menzel-Gläser, Germany), and dried overnight at 56 °C. For cytomorphological examination, sections were stained with H&E. For tumors, organs and other tissues, 10% formalin, instead of 3% glutaraldehyde, was applied in the fixation step. All of the rest of the treatments were the same as those for cell-scaffolds. The protocols applied to the experimental animals were approved by Institutional Animal Care and Use Committee (IACUC) of the University of Oklahoma.

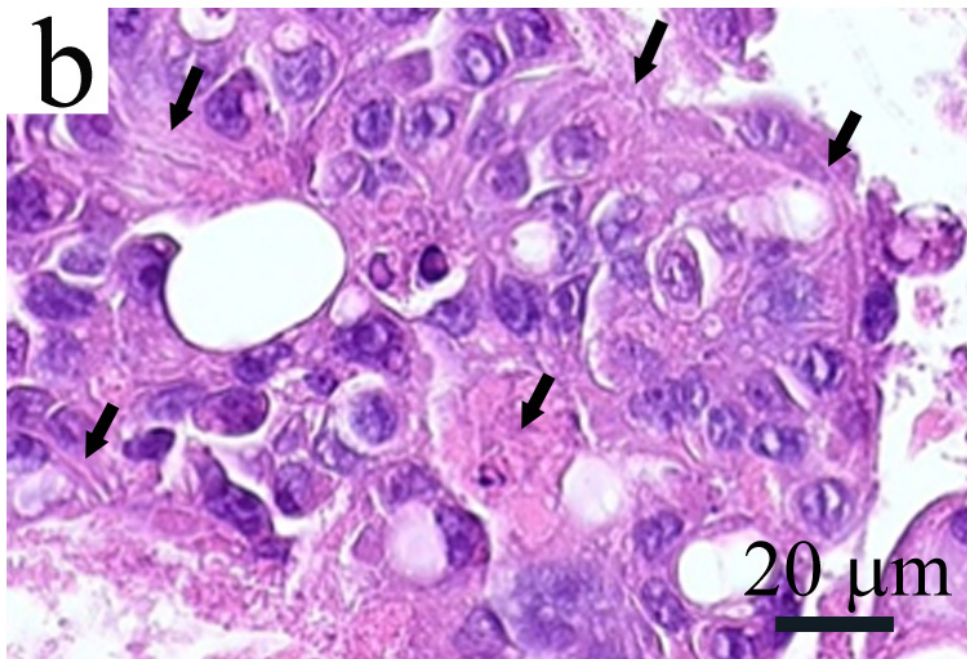
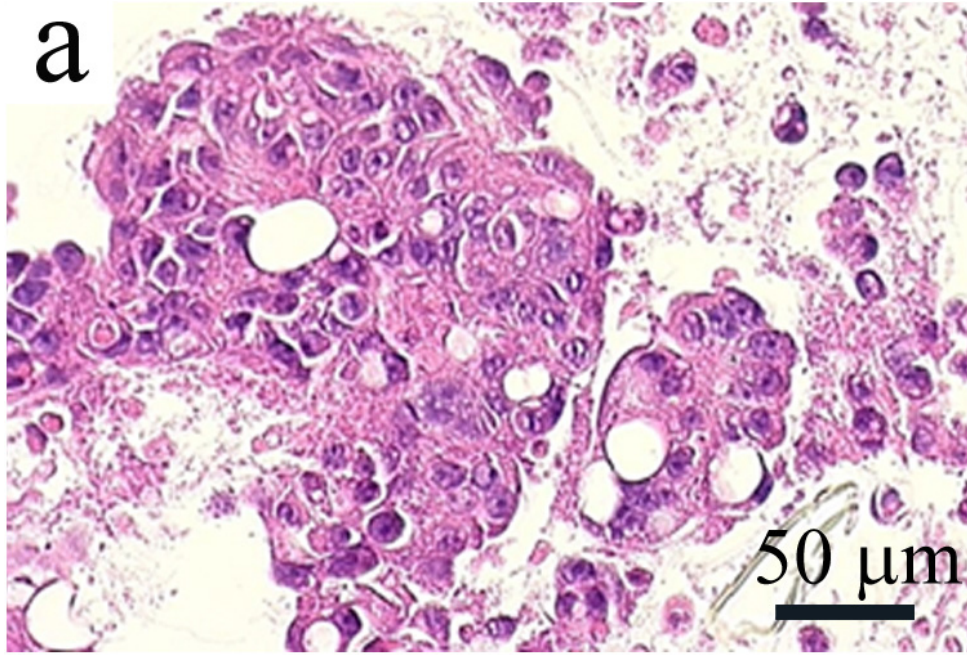


Figure S1. Low (a) and high (b) magnification HE staining results of MCF-7 multi-cellular spheroids cultured on the 3D silica fibrous scaffolds. Arrows: microfibers of the silica scaffold.

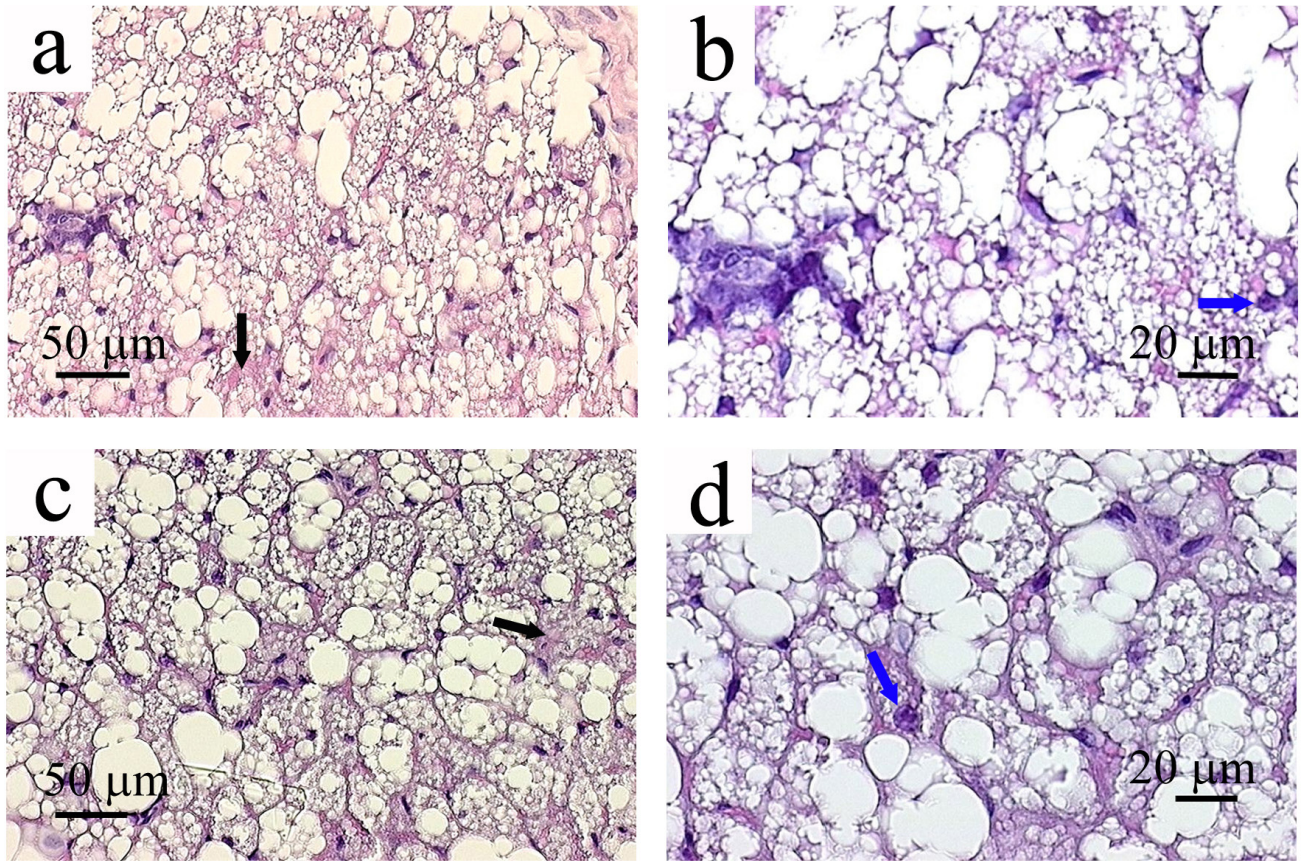


Figure S2. Low and high magnification HE staining images of normal loose connective tissue as control (a, b) and silica scaffold implanted into mice (c, d) for the biocompatibility examination. The adipose cells appeared as white bubbles in all images. Black arrows: extracellular matrix (ECM); Blue arrows: phagocyte.