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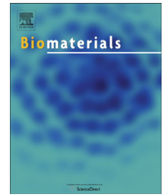

Human decellularized adipose tissue scaffold as a model for breast cancer cell growth and drug treatments

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ABSTRACT

Human adipose tissue extracellular matrix, derived through decellularization processing, has been shown to provide a biomimetic microenvironment for adipose tissue regeneration. This study reports the use of human adipose tissue-derived extracellular matrix (hDAM) scaffolds as a three-dimensional cell culturing system for the investigation of breast cancer growth and drug treatments. The hDAM scaffolds have similar extracellular matrix composition to the microenvironment of breast tissues. Breast cancer cells were cultured in hDAM scaffolds, and cell proliferation, migration, morphology, and drug responses were investigated. The growth profiles of multiple breast cancer cell lines cultured in hDAM scaffolds differed from the growth of those cultured on two-dimensional surfaces and more closely resembled the growth of xenografts. hDAM-cultured breast cancer cells also differed from those cultured on two-dimensional surfaces in terms of cell morphology, migration, expression of adhesion molecules, and sensitivity to drug treatment. Our results demonstrated that the hDAM system provides breast cancer cells with a biomimetic microenvironment *in vitro* that more closely mimics the *in vivo* microenvironment than existing two-dimensional and Matrigel three-dimensional cultures do, and thus can provide vital information for the characterization of cancer cells and screening of cancer therapeutics.

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1. Introduction

For decades, it has been well accepted that two-dimensional (2D) cell culture systems do not fully recapitulate the *in vivo* microenvironment. To bridge the gap between 2D culture and the *in vivo* scenario, three-dimensional (3D) cell culture techniques have been increasingly utilized to improve the physiologic relevance of assays using cells cultured *in vitro* [1,2]. These 3D cell culture methods include the generation of 3D cellular spheroids [3–6], and the culture of cells using membranes [7,8], microcarriers [9,10], hydrogels [11,12], and 3D scaffolds [13–15]. Compared with 2D cultures, 3D cultures more closely resemble *in vivo* tissues in terms of integrin expression, cell migration, and cell mechanics [16–19]. For example, breast cancer cells cultured in basement membrane-based scaffolds more closely resembled to *in vivo* cells in cell morphology, organization, gene expression, protein expression, and cell signaling than breast cancer cells in 2D cultures did [4,19,20]. Additionally, multiple malignant cancer cell lines have exhibited more drug resistance in 3D cultures than in 2D cultures, and drug resistance in the 3D cultures was similar to that observed in *in vivo* models [21–25].

The interaction between cells and the extracellular matrix (ECM) *in vivo* plays a pivotal role in cellular behaviors such as proliferation and migration. To mimic the *in vivo* ECM, biomimetic scaffolds have recently been utilized in tissue engineering and drug screening applications [26,27]. Even though many 3D cell culture systems have been developed for cancer cells, there is still no ideal 3D cell culture system that can fully reproduce the tissue-specific architectures, mechanical and biochemical cues, and cell–cell interactions present in *in vivo* models. Breast cancers usually originate from epithelial cells in the ducts or lobes of the breast, which are surrounded by adipose tissues; this microenvironment plays critical roles in the proliferation and metastasis of breast cancer [28–32]. Fat grafts

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(composed mainly of adipose tissues) and abdominal flaps (composed of skin, subcutaneous adipose tissue, and vascular structures) have been widely utilized in breast reconstruction surgeries because they are highly analogous to the native breast tissues [33,34]. Recently, abdominal adipose tissue ECM has been derived through a process of decellularization and has been shown to provide a biomimetic microenvironment for adipose tissue regeneration [35,36].

In this study, we utilized human adipose tissue-derived ECM (hDAM) to recapitulate the microenvironment of the mammary adipose tissues surrounding breast cancer cells. This hDAM platform was used to investigate breast cancer cell proliferation, migration, and response to drug treatments. Breast cancer cells (MCF-7, BT474, and SKBR3) cultured in hDAM scaffolds were compared with those on 2D surfaces or Matrigel and *in vivo* xenografts. It's hypothesized that breast cancer cells cultured in hDAM scaffolds would more closely resembled *in vivo* xenografts than those cultured on 2D surfaces or Matrigel with respect to cell proliferation, migration, cell morphology, and drug response. The goal of this study was to provide an *in vitro* platform for the characterization of breast cancer cells and screening of cancer therapeutics.

2. Materials and methods

2.1. Cells, antibodies, and other reagents

Human breast cancer cell lines MCF-7, BT474, and SKBR3 were obtained from the American Type Culture Collection (Manassas, VA), were maintained in supplier-specified media containing 10% fetal bovine serum (FBS, Thermo Scientific HyClone, Logan, UT), and the antibiotics penicillin and streptomycin (Life Technologies, CA), and were cultured in an incubator with 5% CO_2 at 37 °C.

Monoclonal antibodies targeting AKT1 (1:2500) and phospho-AKT1 (1:5000) were from Abcam (Cambridge, UK); those targeting phospho-epidermal growth factor receptor (p-EGFR, 1:1000), E-cadherin (1:1000), N-cadherin (1:1000), claudin (1:1000), and vimentin (1:1000) were from Cell Signaling Technology (Danvers, MA). Anti-beta-actin antibodies (1:2500) were from Santa Cruz Biotechnology (Santa Cruz, CA). Fluorescein isothiocyanate (FITC)-dextran (3000 Da) was from Sigma–Aldrich (St. Louis, MO). Lapatinib was from LC Laboratories (Woburn, MA), and doxorubicin was from Sigma–Aldrich.

2.2. Human adipose tissue decellularization

All procedures were conducted under institutional review board approval and in accordance with research guidelines at The University of Texas MD Anderson Cancer Center. Patients provided informed consent for the use of their tissues for basic research. Adipose tissue samples (i.e., subcutaneous adipose tissue in the abdominal wall area) were collected from three patients undergoing reconstructive surgery, stored in saline on ice, and delivered to the laboratory for processing within 4 h after excision [35]. Briefly, tissue pieces were re-frozen at −80 °C and thawed at room temperature (three cycles) and then processed with ultrapure water, NaCl solution (0.5 M and 1 M), 0.25% trypsin/EDTA, 1% Triton X-100, and isopropanol. Samples were lyophilized, sterilized using 70% ethanol, and rinsed in phosphate-buffered saline (PBS). Sterile samples were stored at 4 °C in PBS containing 1% penicillin/streptomycin until use.

2.3. Histology and immunohistochemistry

hDAM scaffolds ($n = 3$) were fixed in 10% formalin, embedded in paraffin, and sectioned into 5-μm slices. Slides cut from the paraffin-embedded samples were processed for histologic and immunohistochemical staining as previously described [35,37]. Slides underwent hematoxylin and eosin staining, Oil Red O staining, and Masson trichrome staining. Cell nuclei were stained using DAPI. Slides were imaged using an Olympus IX71 microscope (Olympus America, Center Valley, PA).

2.4. Scanning electron microscopy

hDAM scaffolds were frozen at −80 °C and lyophilized for scanning electron microscopy [35,37]. These dry scaffolds were coated in a vacuum with a platinum alloy to a thickness of 25 nm using a Balzer MED 010 evaporator (Techno Trade International, Manchester, NH) and were immediately flash-coated with carbon in a vacuum. Scaffolds were examined with a JSM-5910 scanning electron microscope (JEOL, Peabody, MA) at an accelerating voltage of 5 kV. Fiber size was measured with ImageJ software (National Institutes of Health, Bethesda, MD).

2.5. Porosity measurement

The porosity values of hDAM samples ($n = 6$) were measured by liquid displacement [38,39]. Ethanol was used because it could easily penetrate into pores without causing shrinkage or swelling.

2.6. Diffusivity measurement by fluorescence recovery after photobleaching (FRAP)

Diffusion coefficients of small molecular weight compounds were measured with FITC-dextran (3000 Da) [38,40]. hDAM scaffold were incubated with FITC-dextran (30 μM) at 4 °C overnight. Before testing, samples were equilibrated at room temperature for 30 min, and all tests were performed at room temperature (25 °C). FRAP experiments were carried out in the middle of the scaffolds. All photobleaching experiments were performed using a 405-nm laser and a 488-nm laser at 90% power for 300 frames (at 1.64-s intervals). Images of the recovery process were obtained with a 488-nm laser at 3% power for 300 frames (at 1.64-s intervals). Diffusion coefficients were calculated from the FRAP experiments. Briefly, the mean fluorescence in the bleached region over time was converted to normalized fractional fluorescence intensity

$$f = \frac{F(t) - F(0)}{F(\infty) - F(0)}$$

where $F(t)$ is the fluorescence intensity at time t, $F(0)$ is the fluorescence intensity immediately after bleaching, and $F(\infty)$ is the fluorescence after complete recovery. The fractional fluorescence intensity was plotted versus time and fitted with a logarithmic curve. The equation for the curve was used to determine the half-recovery time ($\tau_{1/2}$) at $f = 0.5$. The bleaching parameter (γ_D), which describes the relationship between the half-recovery time and the characteristic diffusion time [40,41]. The half-recovery time, the initial spot radius (ω), and the bleaching parameter were used to determine the diffusion coefficient, D:

$$D = \gamma_D \omega^2 / 4\tau_{1/2}$$

2.7. Cell viability

Cell viability in hDAM scaffolds was studied using live cell staining with calcein AM (Biotium, Hayward, CA) as described previously [35,37]. Samples ($n = 3$ for each condition) were examined with an Olympus IX71 fluorescence microscope on days 1, 3, 7, and 14 after cell seeding.

2.8. Cell proliferation

Cells were seeded onto 2D surface of cell culture dishes, onto Matrigel (BD Biosciences, San Jose, CA), and into hDAM scaffolds at a density of 2×10^4 cells/cm^2. For 2D cultures, cells were seeded into each well of 96-well clear-bottom black plates (Thermo Fisher Scientific, Hampton, NH) with each well containing 100 μL media and cultured overnight at 37 °C in 5% CO_2. To grow 3D spheres on Matrigel, cells from 2D cultures were trypsinized and added on top of the Matrigel: medium mixture (1:1). To integrate cells with hDAM scaffolds, 5×10^5 cells were added onto each hDAM scaffolds (1 cm^2) which were placed in 24-well plates, incubated for 5 min at room temperature, and transferred to an incubator at 37 °C, 5% CO_2. The Medium was replaced every 3–4 days, and cells were imaged with an Olympus DP72 phase contrast microscope (Olympus, Japan). AlamarBlue assays (Life Technologies, Carlsbad, CA) were employed to measure cancer cell proliferation on the 2D surface, on Matrigel, and in hDAM scaffolds ($n = 5$). At different time points following cell seeding, 10 μL of alamarBlue solution was added to each sample and incubated at 37 °C, 5% CO_2 for 4 h. Fluorescence signals were measured with an excitation wavelength at 530 nm and an emission wavelength at 590 nm using a plate reader (Molecular Probes, Eugene, OR).

2.9. Cell migration

To study cancer cell migration in transwell assays, MCF-7, BT474, and SKBR3 breast cancer cells (5×10^5 cells/well) were seeded in the top chambers of the transwell migration plates (8-μm pores; Corning, NY) in Roswell Park Memorial Institute (RPMI) medium containing 10% FBS. At 24 h after seeding, cells that migrated to the underside of the transwell were stained with 0.5% crystal violet for 5 min and imaged in ten random fields (10× magnification). To investigate the invasion and migration of breast cancer cells in hDAM scaffolds, the MCF-7, BT474, and SKBR3 cells (5×10^5 cells/sample) were injected at designated sites within the hDAM scaffolds (1 injection/scaffold, $n = 5$) with a 27 gauge needle (8.25 μm in diameter) and the cell migration away from the injection sites was monitored. Cells within the hDAM scaffolds were visualized with fluorescence staining of calcein AM. The cells that migrated away from the injection sites were counted and quantified using ImageJ.

2.10. Cell organization and morphology

Cells grown in 2D culture, on Matrigel, or in hDAM scaffolds were washed with PBS, fixed with 4% paraformaldehyde for 12 min, permeabilized in 0.1% Tween 20 in PBS for 5 min three times, and blocked in Tris-buffered saline containing 5% bovine serum albumin for 1 h. Cells were stained with rhodamine-conjugated phalloidin for

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