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Nanomagnetic levitation three-dimensional cultures of breast and colorectal cancers

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ABSTRACT

Background: Innovative technologies for drug discovery and development, cancer models, stem cell research, tissue engineering, and drug testing in various cell-based platforms require an application similar to the *in vivo* system.

Materials and methods: We developed for the first time nanomagnetically levitated three-dimensional (3-D) cultures of breast cancer (BC) and colorectal cancer (CRC) cells using carbon-encapsulated cobalt magnetic nanoparticles. BC and CRC xenografts grown in severe combined immunodeficient (SCID) mice were evaluated for N-cadherin and epidermal growth factor receptor expressions. These phenotypes were compared with two-dimensional and 3-D cultures grown in a gel matrix.

Results: The BC and CRC cells grown by magnetic levitation formed microtissues. The levitated cultures had high viability and were maintained in culture for long periods of time. It has been observed that N-cadherin and epidermal growth factor receptor activities were highly expressed in the levitated 3-D tumor spheres and xenografts of CRC and BC cells.

Conclusions: Nanomagnetically levitated 3-D cultures tend to form stable microtissues of BC and CRC and maybe more feasible for a range of applications in drug discovery or regenerative medicine.

1. Introduction

The advancement in cell culture methods from two-dimensional (2-D) to three-dimensional (3-D) is a big leap by demonstrating significant differences in cellular characteristics and behavior enabling the assessment of drug efficacy, pharmacokinetics, and pharmacodynamics [1]. Using 3-D polymeric scaffolds, efforts have been made to achieve 3-D cell matrix structure with enhanced cell growth and improved functions [2], embryonic stem cell differentiation [3], active regulation of multicellular organization [4], cell migration [5], and angiogenic capability [6].

The 3-D polymeric systems have been shown to facilitate 3-D growth of cancer cells and tumors [7–9]. The use of 3-D scaffold materials such as hydrogel, Matrigel, and Alvetex polystyrene produces successful 3-D cultures but they have limitations, and broad practical applications of such methods have not yet been achieved. The use of these 3-D scaffold

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materials tend to act as a mass transport barrier within the tissue construct resulting in impedance in drug delivery to target cells, limits nutrient supply to these cells, and allows the accumulation of metabolic waste [10] resulting in delayed proliferation of cells and establishment of cell–cell interactions.

To overcome these problems, previous studies reported feasibility of magnetically levitated 3-D tissue culture for long-term multicellular studies [11]. The biological application of magnetic forces in clinical diagnostic radiology has long been studied [12–16]. Magnets have also been used to levitate biological samples through the natural diamagnetism of organic material [17]. Internalization of nanoparticles has further supported cell sorting [13], mechano-conditioning of cells [13–15], and cellular micromanipulation [18]. However, development of magnetically levitated 3-D microtissues of breast cancer (BC) and colorectal cancer (CRC) cells using carbon-encapsulated cobalt magnetic nanoparticles has not yet been studied. Therefore, in the present study, using encapsulated cobalt nanoparticles with carbon (C-Co) for biocompatibility and biosafety, we developed the modified, efficient, cost effective, and scaffolds-free nanomagnetic levitation-based 3-D cultures of BC and CRC microtissues for high throughput drug screening and xenograft studies. To compare the biological difference generated by magnetic levitation growth, we evaluated the expression of N-cadherin and epidermal growth factor receptor (EGFR) markers in our 2-D and 3-D cultures and in xenografts of BC and CRC cells. These cultures are so phenotypically different that we considered the possible roles the presence of adhesion proteins such as N-cadherin and EGFR might play in their development. We hypothesized that an optimal *in vitro* tumorigenic model can be derived through improved 3-D cultures using nanotechnology. We have applied magnetic forces to levitate cells while they divide and grow.

2. Methods

2.1. 2-D cultures

BC (MDA-MB-231, MDA-MB-468, and MCF-7) and CRC (HT-29, WiDr, SW-480) cell lines (ATCC, Global Resource Center, Manassas, VA) were tested and found to be without pathogen, including mycoplasma. These cell lines were maintained in flasks with Roswell Park Memorial Institute culture media (RPMI-1640) medium (ATCC) supplemented with 10% fetal bovine serum (FBS; Thermo Scientific HyClone, Logan, UT), 25 mM 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES), penicillin/streptomycin (pen/step) (Mediatech, Manassas, VA), L-glutamine, and sodium bicarbonate, and incubated at 37°C and 5% CO_2. All cell lines were initially grown as 2-D culture monolayers. These cells were then harvested and used for the experimental culture parameters as follows: 3-D cultures in hydrogel and magnetic levitation-based 3-D cell cultures.

2.2. 3-D cultures using SeaPrep hydrogel

BC (MDA-MB-231, MDA-MB-468, and MCF-7) and CRC (HT-29, WiDr, and SW-480) cells were mixed with 1% SeaPrep hydrogel (Lonza, Rockland, ME) in Nunclon petri dishes (5 cm in diameter, Sigma–Aldrich, St. Louis, MO) diameter and allowed to solidify for 30 min at 4°C. Subsequently, the culture medium RPMI-1640 supplemented with 10% FBS, 25 mM HEPES, pen/step, L-glutamine, and sodium bicarbonate was added to the gel with embedded cells and allowed to grow in the incubator at 37°C and 5% CO_2. Tumor spheres developed in the gel matrix. These tumor spheres were used for comparison with microtissue developed in magnetic-levitated 3-D cell cultures.

2.3. 3-D cultures based on magnetic levitation

2.3.1. Preparation of C-Co nanoparticles

Carbon-encapsulated cobalt magnetic nanomaterial (<50 nm particle size; resistivity 6.24 μΩ-cm, 20°C) that has monodisperse magnetic nanoparticle property was sonicated in sterile aqueous solution (Sigma–Aldrich, St. Louis, MO). This nanomaterial (C-Co nanoparticles) was used for internalization by the cancer cells for the purposes of nanomagnetic levitation and formation of 3-D cancer microtissues. The carbon coatings (approximately three graphitic layers used) will endow these magnetic particles with biocompatibility and stability in both biological and nonbiological systems.

2.3.2. Internalization of C-Co nanoparticles

BC (MDA-MB-231, MDA-MB-468, and MCF-7) and CRC (HT-29, WiDr, SW-480) cell lines were subjected to nanomagnetic levitation to assume 3-D growth in cultures by internalization of magnetic cobalt nanoparticles. The cancer cells were suspended in the warm solution of 0.25% SeaPrep hydrogel (Lonza), mixed with 20 μL of sonicated carbon-encapsulated cobalt magnetic nanomaterial (1 μg/μL), and allowed to solidify in the petri dish for 30 min at 4°C. This hydrogel phase has been shown to effectively prevent the aggregation and clumping of the nanoparticles and to facilitate their passage into the cells. Subsequently, the growth medium RPMI-1640 supplemented with 10% FBS, 25 mM HEPES, pen/step, L-glutamine, and sodium bicarbonate was added to the gel with embedded cells and allowed to grow for 12 h at 37°C in 5% CO_2. The cells with internalized magnetic nanoparticles were separated from the very dilute (0.25%) gel, and RPMI-1640 supplemented with 10% FBS, 25 mM HEPES, pen/step, L-glutamine, and sodium bicarbonate was added. A small block of magnet was placed on the petri dish to achieve magnetic forces to levitate cells while they divide and grow.

2.4. Animals and xenografts

Severe combined immunodeficient (SCID) female mice were purchased from Taconic Farm (Taconic, NY) at the age of 4 wk and quarantined for 1 wk before use. The mice were inoculated with 2-D cultures of BC (MCF-7 and MDA-MB-231) and CRC (HT-29 and SW-480) cells to establish primary tumors (xenografts). All food, water, and bedding were sterilized by autoclaving. The mice resided in micro-filtered cages in a room designated for immune-compromised mice. On a daily basis, the animals were evaluated regarding health status and tumor growth. Body weight, nutritional intake, general