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3-Dimensional Tissue Is Formed From Cancer Cells In Vitro on Gelfoam®, But Not on Matrigel™

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ABSTRACT

Cell and tissue culture can be performed on different substrates such as on plastic, in Matrigel™, and on Gelfoam®, a sponge matrix. Each of these substrates consists of a very different surface, ranging from hard and inflexible, a gel, and a sponge-matrix, respectively. Folkman and Moscona found that cell shape was tightly coupled to DNA synthesis and cell growth. Therefore, the flexibility of a substrate is important for cells to maintain their optimal shape. Human osteosarcoma cells, stably expressing a fusion protein of α_v integrin and green fluorescent protein (GFP), grew as a simple monolayer without any structure formation on the surface of a plastic dish. When the osteosarcoma cells were cultured within Matrigel™, the cancer cells formed colonies but no other structures. When the cancer cells were seeded on Gelfoam®, the cells formed three-dimensional tissue-like structures. The behavior of 143B osteosarcoma cells on Gelfoam® in culture is remarkably different from those of these cells in monolayer culture or in Matrigel™. Tissue-like structures were observed only in Gelfoam® culture. The data in this report suggest a flexible structural substrate such as Gelfoam® provides a more in vivo-like culture condition than monolayer culture or Matrigel™ and that Matrigel™ does not result in actual three-dimensional culture. J. Cell. Biochem. 115: 1362–1367, 2014.

KEY WORDS: MATRIGEL™; GELFOAM®; CULTURE; DIMENSION; HISTOLOGY; α_v INTEGRIN; GREEN FLUORESCENT PROTEIN; OSTEOSARCOMA; REAL-TIME IMAGING; CONFOCAL MICROSCOPY

THE DAWN OF TISSUE CULTURE

The first documented maintenance of tissues out of the body was in 1885 by Roux [Zurlo et al., 1994]. Roux maintained the medullary plate of an embryonic chicken in a warm saline solution for several days. This experiment established the principle that tissues could live outside the body. In 1907, Harrison placed fragments of a tadpole spinal cord in a clot of lymph in a hollow-ground glass slide. Nerve fibers grew out from the explants from the nerve fiber tips [Harrison, 1907]. Carrel transferred (passed) tissue cultures from medium to medium in order to maintain them long term. The culture was then sealed on a hollow slide, put in an incubator, and allowed to grow. In 1923, Carrel introduced the first practical cell culture flask [Carrel, 1923].

THE BEGINNING OF MODERN CELL CULTURE

Earle was among the first to establish cell lines that could grow indefinitely, including the L-cell line [Sanford et al., 1948]. HeLa cells were derived from a human cervical tumor [Gey et al., 1954]. Defined cell culture media were first developed by Earle [Earle, 1962] and Ham [McKeehan et al., 1977]. Eagle developed a medium with

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over 25 ingredients [Eagle, 1955]. These defined media had to be supplemented with serum such as fetal bovine so that cells could proliferate.

SERUM-FREE CELL CULTURE

Growth factors such as nerve growth factor (NGF), epidermal growth factor (EGF), and platelet-derived growth factor (PDGF) were discovered to promote the growth of certain cells in culture. Due to the work of Sato, some cell types can be cultured in completely defined media without serum [Hayashi and Sato, 1976; Sato, 1980, 1981].

HISTOCULTURE

Fragments of tissue as well as cells were cultured on a collagen sponge matrix that was developed by Leighton et al. [1967]. This was the beginning of modern histoculture. Leighton made a number of important early observations on the advantages of histoculture; for example, when C3HBA mouse mammary adenocarcinoma cells were grown in sponge-matrix histoculture, he found that the cells aggregated in a manner similar to that in the original tumor. Distinct structures were formed within the tumors such as lumina and stromal elements, with some of the structures similar to the original tumor.

In the current century, articles have appeared about "the new dimensions" in cell culture [Jacks and Weinberg, 2002; Abbott, 2003] due to the use of a mouse cancer-cell extracellular matrix preparation called "Matrigel™."

Matrigel™ contains proteins, such as collagen, elastin, and laminin, which may help to organize communication between cells embedded within the matrix.

Matrigel™ [Kleinman et al., 1982, 1986], was first isolated from a murine tumor as a crude protein mixture that was a liquid at 4°C and gelled at 24–37°C. Matrigel™ is composed mainly of laminin-111, collagen IV, heparin sulfate proteoglycan, various growth factors, and additional components [Benton et al., 2011]. In Matrigel™, cancer cells formed branched and invasive structures while the less malignant cells formed small aggregates. Invasive morphology correlated with the metastatic ability of the cancer cells in vivo [Benton et al., 2011].

Folkman and Moscona [1978] observed that cell shape is critical for DNA synthesis and cell proliferation. It would seem important that a culture substrate should let cells acquire their natural shape. Therefore, we directly compared cancer cell growth on plastic, Matrigel™, and on Gelfoam®.

We report here the use of green fluorescent protein (GFP) imaging to visualize and compare the behavior of osteosarcoma cells in monolayer culture, in Matrigel™ culture, and on sponge-matrix Gelfoam® culture and observed that each substrate provided a very different result with three-dimensional tissue-like structures formed on Gelfoam® and not Matrigel™ or plastic.

MATERIALS AND METHODS

CELLS

The 143B osteosarcoma cell line [Uehara et al., 2013] was maintained with RPMI 1640 medium (Irvine Scientific, Santa Ana, CA) containing 10% fetal bovine serum (FBS) (Omega Scientific, San Diego, CA) and 1% penicillin/streptomycin at 37°C in a humidified incubator with 5% CO_2.

ESTABLISHMENT OF HUMAN OSTEOSARCOMA CELLS EXPRESSING α_V INTEGRIN-GFP

The pCMV6-AC-ITGAV-GFP vector, containing a fusion of α_v integrin and GFP, was obtained from OriGene Technologies (Rockville, MD). 143B cells were seeded at 1×10^6 per 100-mm dish. At 80% confluency, cultures were transfected with the α_v integrin-GFP fusion vector pCMV-AC-ITGAV-GFP using Lipofectamine LTX (Invitrogen, Carlsbad, CA) according to the manufacturer's instruction. After transfection, cells stably expressing α_v integrin-GFP were selected with G418 (800 μg/ml) (Sigma-Aldrich, St. Louis, MO) starting at 24 h after transfection. Stable colonies were selected and maintained in RPMI 1640 containing 10% FBS and 500 μg/ml G418 (Sigma-Aldrich) [Tome et al., 2013; Uehara et al., 2013].

IMMUNOFLUORESCENCE STAINING TO CO-LOCALIZE ENDOGENOUS α_V INTEGRIN AND α_V INTEGRIN-GFP

Immunofluorescence staining was performed based on the protocol provided by Cell Signaling Technology (Danvers, MA). The cells were seeded on two-well glass chamber slides (Thermo Fisher Scientific, Pittsburgh, PA) the day before immunofluorescence staining. The cells were fixed in 4% paraformaldehyde for 10 min. After washing with PBS, the cells were incubated in blocking buffer containing 5% normal goat serum and 0.3% Triton X-100 for 60 min. Then, the cells were incubated with a 1:250 dilution of a primary antibody (anti-CD51) (Invitrogen) overnight at 4°C. After washing with PBS, the cells were stained with a 1:200 dilution of a secondary antibody (Alexa 555, Invitrogen) for 2 h, followed by staining with 4′6′-diamidino-2-phenylindole (DAPI) (Invitrogen) and imaged by confocal laser-scanning microscopy (FV1000, Olympus, Tokyo, Japan) [Tome et al., 2013].

MONOLAYER CULTURE

For monolayer culture, 143B cells (1×10^4) stably expressing α_v integrin-GFP were seeded in 35 mm culture dishes [Tome et al., 2013].

MATRIGEL™ CULTURE

Cell culture plates (35 mm) were coated with 600 μl Matrigel™ (BD Biosciences) and incubated at 37°C for 30 min. Matrigel™ (5%) was added on top of the cell culture after cells were allowed to spread for 2–3 h. Cells were incubated at 37°C in a humidified incubator with 5% CO_2 [Benton et al., 2011].

GELFOAM® HISTOCULTURE

Sterile Gelfoam® sponges (Pharmacia & Upjohn, Kalamazoo, MI), prepared from porcine skin, were cut into 1 cm cubes. The Gelfoam® cubes were placed in six-well tissue-culture plates. RPMI 1640 medium was added and Gelfoam® was incubated at 37°C in order that the Gelfoam® absorbed the medium. 143B cells (1×10^6) expressing α_v integrin-GFP were then seeded on top of the hydrated Gelfoam® and incubated for 1 h. Medium was carefully added up to the top of the Gelfoam®. Cells were incubated at 3°C in a humidified

incubator with 5% CO_2 [Freeman and Hoffman, 1986; Hoffman, 2010, 2013; Mii et al., 2013a,b].

IMAGING

Cultures were imaged with an Olympus FW 1000 laser-scanning confocal microscope (Olympus) with a XLUMPLFL 20× (0.95 NA) water-immersion objective. GFP was excited at 488 nm and Z-slices were acquired every 1 μm. Images were produced with FV10-ASW Fluoview software (Olympus) and ImageJ and were not modified beyond the standard adjustment of intensity levels [Uchugonova et al., 2011].

RESULTS AND DISCUSSION

CANCER CELL GROWTH IN MONOLAYER CULTURE

143B cells expressing α_v integrin-GFP have bright fluorescence in the cytoplasm in monolayer culture (Fig. 1). The cells grew as a monolayer without structure.

CANCER CELL GROWTH IN MATRIGEL™ CULTURE

143B α_v integrin-GFP cells were embedded within Matrigel™ (Fig. 2). Colonies of cells were formed within 24 h after seeding. Colonies were seen by 72 h after seeding, but no three-dimensional tissue-like structures were formed (Fig. 3B).

CANCER CELL GELFOAM® HISTOCULTURE

To investigate the behavior of α_v integrin-GFP on a sponge matrix, cancer cells were seeded on Gelfoam® (Fig. 3). The cancer cells formed three-dimensional tissue-like structures along the Gelfoam® within 72 h.

IMAGE RENDERING

Images of 143B α_v integrin-GFP cells on each of these substrates were rotated over 85° to visualize tissue-like morphology as well as three-dimensional structures (Fig. 4). The 143B α_v integrin cells on plastic were observed to behave as individual cells lying flat on the plastic surface of the culture plate. The 143B α_v integrin-GFP cells in Matrigel™ aggregated but did not appear tissue-like. The 143B α_v integrin-GFP cells on Gelfoam® appeared to have a tissue-like structure, which was three-dimensional. The relatively undifferentiated appearance of the tissue-like structure resembles 143B tumors formed in vivo [Luu et al., 2005].

The behavior of 143B osteosarcoma cells in Gelfoam® culture is remarkably different from those of the cells in monolayer culture [Cukierman et al., 2001] or in Matrigel™. Tissue-like 3-dimensional structures were observed only in Gelfoam® culture. Gelfoam® culture is different than Matrigel™ culture despite the presence of extracellular matrices in Matrigel™.

Despite announcements by Jacks and Weinberg [2002] and Abbott [2003] about "biology's new dimension" with the use of

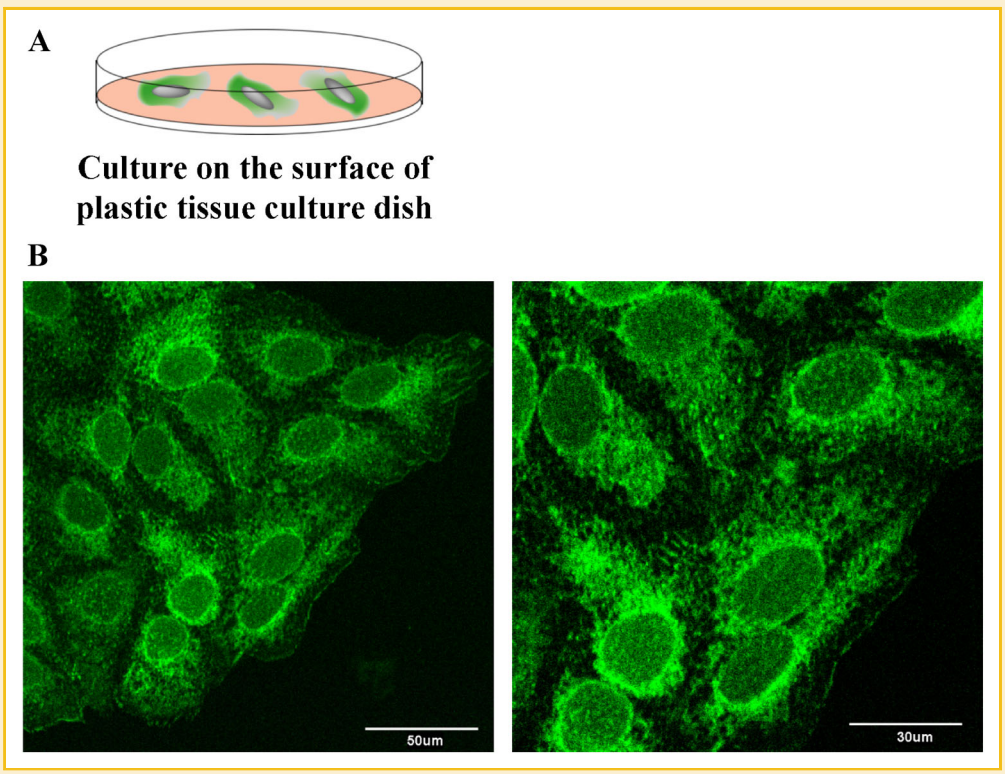

Fig. 1. Behavior of 143B cells expressing of α_v integrin-GFP in monolayer culture. A: Schematic of culture of 143B human osteosarcoma cells on flat, plastic substrates. B: Confocal fluorescence micrographs of 143B human osteosarcoma cells, expressing α_v integrin-GFP, in monolayers on plastic plates.

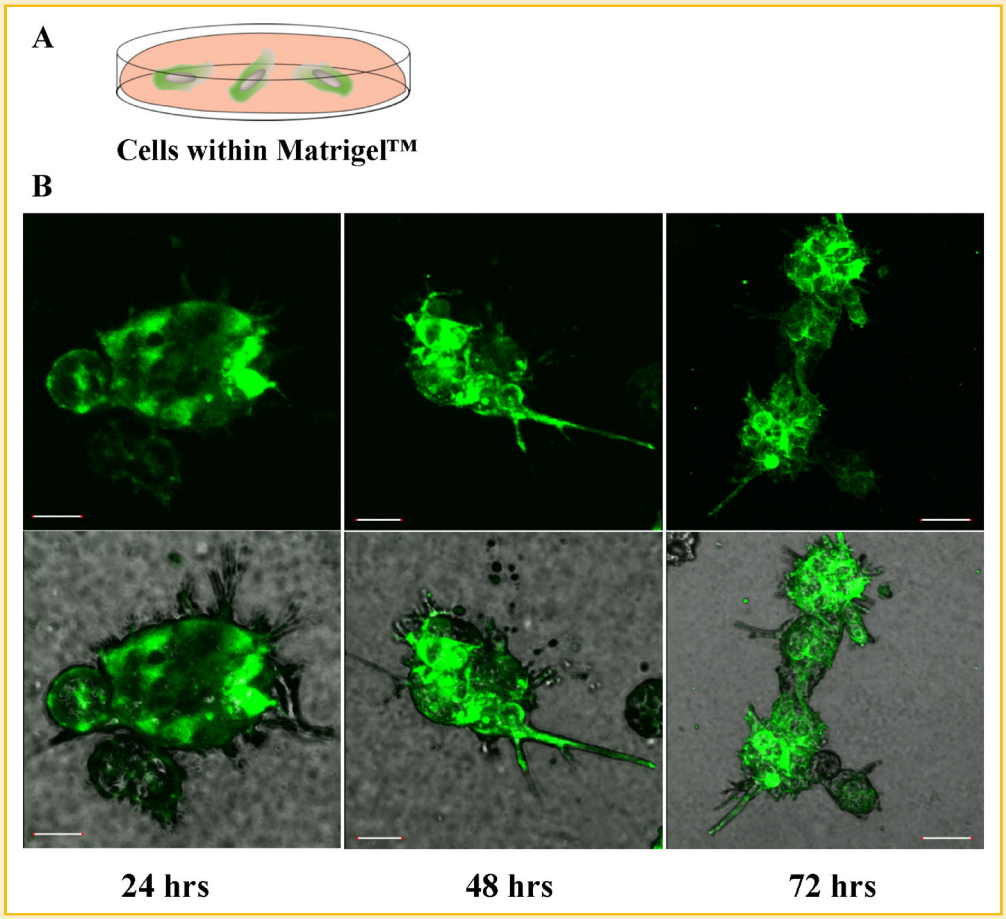

Fig. 2. Behavior of 143B cells expressing α_v integrin-GFP in Matrigel™ culture. A: Schematic of culture of 143B cells sandwiched between a Matrigel™-coated surface and a layer of Matrigel™. B: Phase contrast (lower row) and fluorescence (upper row) micrographs of live human osteosarcoma cells expressing α_v integrin in Matrigel™ culture. FV1000 confocal fluorescence microscopy. Bar: 20 μm (left panels). Bar: 30 μm (middle panels). Bar: 50 μm (right panels).

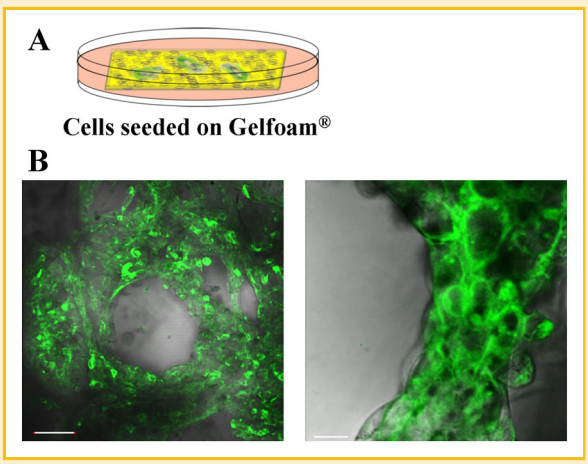

Fig. 3. Behavior of 143B cells expressing α_v integrin-GFP on Gelfoam®. A: Schematic of 3-D Gelfoam® culture of 143B cells. B: Phase contrast and fluorescence merged images of live 143B human osteosarcoma cells expressing α_v integrin in 3-D Gelfoam® culture. FV1000 confocal fluorescence microscopy. The cancer cells formed three-dimensional complexes and gland-like structures within the Gelfoam® within 72 h (left panel). Bar: 100 μm. Diffuse expression of α_v integrin-GFP in the cancer cells in the Gelfoam® culture (right panel). Bar: 20 μm.

Matrigel™, sponge-gel matrix histoculture, of which Gelfoam® histoculture is an example, was conceived by Leighton way back in the 1950s [Leighton, 1951], and allows three-dimensional tissue-like structures to form. This report distinguishes Matrigel™ culture, where only colonies of cells form, from Gelfoam® sponge-gel matrix culture, where three-dimensional tissue-like structures are formed by cancer cells. The cancer cells are readily visualized by α_v integrin GFP expression.

Gelfoam® has also been used to maintain tissue over relatively long periods of time in order to obtain biologically relevant information concerning tumor biology and drug response [Vescio et al., 1987; Li et al., 1992; Furukawa et al., 1995; Kubota et al., 1995]. Additional uses of Gelfoam® histoculture include skin and hair growth [Li et al., 1992], immune tissue histoculture and HIV infection [Glushakova et al., 1995], and nerve growth from stem cells [Mii et al., 2013a,b].

The results in this report suggest that further studies be undertaken to determine an optimal general substrate for culture of cancer cells such that they can have as much fidelity as possible to in vivo tumor growth, and that Gelfoam® and similar flexible sponge matrix substrates are promising candidates to achieve this goal.

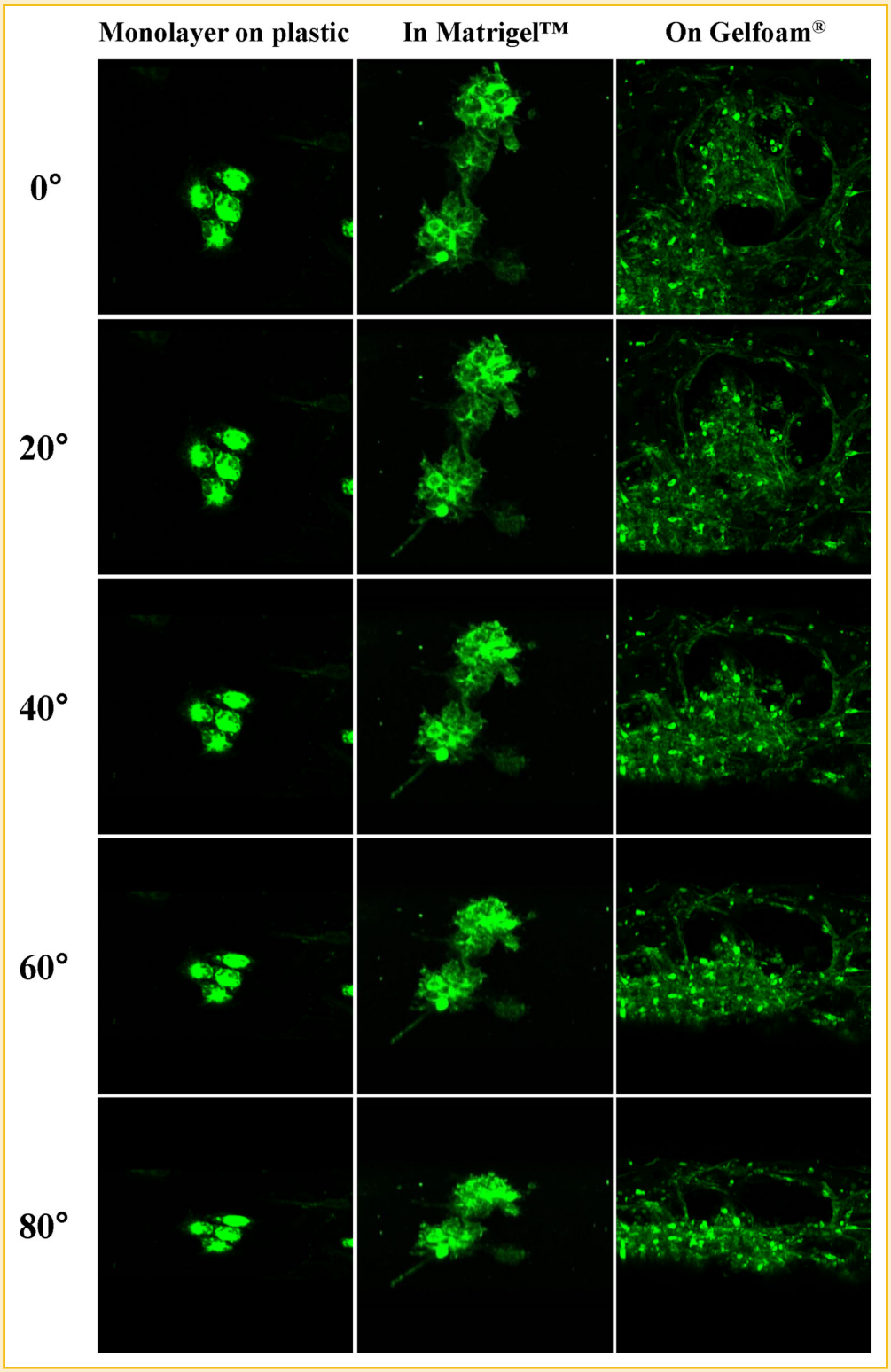

Fig. 4. Image rendering. Phase contrast fluorescence micrographs and rotating images of 143B human osteosarcoma cells expressing α_v integrin on plastic plates (left panels), in Matrigel™ culture (middle panels), in Gelfoam® culture (right panels) using the Olympus FV1000. Z-stack in 1 μm steps. The depths of images are on plastic surface 20 μm; Matrigel™ 70 μm; Gelfoam® 180 μm. These images are rotated Z axes from 0° to 80° in 5 sections, using the FV-10 ASW software system.

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