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ORIGINAL ARTICLE

Patient-Derived Primary Cultures in Breast Cancer: A Comparative Study of Isolation and Culture Methods

Meme Kanserinde Hastadan Türetilen Primer Kültürler: İzolasyon ve Kültür Yöntemlerinin Karşılaştırmalı Çalışması

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Abstract

Introduction: Breast cancer is the most common cancer among women worldwide. Primary culture methods play a pivotal role in understanding cancer's heterogeneity. Improving experimental conditions is essential for advancements in treatment. This study aims to compare two different isolation methods for primary culture derived from breast cancer patient tissues.

Methods: Breast tissues surgically excised from breast cancer patients were cultured under sterile conditions using explant culture and enzymatic digestion. In the explant culture group, parallel lines were drawn in the wells to examine cell adhesion, while the effect of collagen on cells was assessed by coating the wells and evaluating them on days 0, 5, and 10. Enzymatic digestion was analyzed using two different methods: short-term and long-term. The morphology of cultured cells was examined under an inverted microscope.

Results: Long-term enzymatic digestion resulted in better cell adhesion and proliferation compared to short-term, leading to higher cell counts and more fibroblast-like morphology. However, explant culture yielded the best results in terms of cell count and morphology for both tissues. The cells in explant cultures exhibited epithelioid morphology from day 5 onward, maintaining this characteristic through day 10. Additionally, no significant effects were observed on collagen-coated surfaces in our study.

Discussion and Conclusion: The findings highlight the importance of selecting appropriate primary culture methods in breast cancer research. While long-term enzymatic digestion improves proliferation, explant culture remains the superior approach for preserving cell morphology and mimicking the tumor microenvironment. Optimizing culture techniques is essential for enhancing translational cancer research and advancing therapies.

Keywords: Breast cancer; Explant culture; Patient derived primary culture

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Cancer is defined as the formation of abnormal cell clusters that differ from normal cells in terms of genetic mutations, morphology, and behavior.[1] Breast cancer is the most common cancer type among women globally and a leading cause of cancer-related mortality.[2]

The breast cancer microenvironment comprises local factors, cancer cells, immune cells, and stromal cells from both local and distant tissues.[3] The interaction between cancer cells and their microenvironment plays a critical role in tumor proliferation, spread, and response to treatment.[2] The tumor microenvironment, influenced by extracellular matrix components such as collagen, plays a critical role in cell attachment and proliferation.

Breast cancer is a heterogeneous disease that exhibits significant variability both between patients and within a tumor. [4] Thus, selecting a model that accurately reflects the tumor system is one of the challenges of cancer research. [5] Currently, *in vitro* and *in vivo* models are valuable tools for developing cancer therapies and investigating the molecular mechanisms of tumor growth and metastasis. While there is no universally ideal cancer model, these models serve the transition from laboratory research to clinical application.

In the context of *in vitro* studies, most research relies on cancer cell lines; however, these cells exhibit genetic or phenotypic differences from the original tumor.[6] Many of these cell lines, such as MCF-7 and MDA-MB-231, are derived from metastatic sites rather than primary tumors and their use in studying the onset and progression of breast cancer may be limited, as they may not fully capture the tumor biology seen in primary lesions.[7] *In vivo* animal models are another commonly used tool and they have various advantages/ disadvantages. Patient-derived primary cultures offer a relevant approach for studying tumor biology, making the selection of an optimal isolation method crucial.

Primary cancer cells serve as powerful tools for investigating cancer biology, gene expression, or amplification. Since primary cells maintain genetic stability, normal morphology, and essential cellular functions, they can provide biologically relevant representations of complex cellular processes that cannot be achieved using cell lines. Consequently, primary cell culture remains the gold standard *in vitro* model and is widely advocated for research. Moreover, the ability of primary cultures to preserve the stem-like phenotype of cancer cells offers a significant advantage, particularly in preclinical stages of drug resistance research.[8]

The concept of "personalized medicine" has become widely recognized in cancer treatment.[9] At this point, primary human breast cancer cultures serve as valuable tools for investigating various aspects of cancer biology. These intercellular behaviors play a crucial role in carcinogenesis, progression, metastasis and are also involved in responses to treatments.[10] Primary cells directly isolated from tissues are considered more suitable for studying human and animal biology.[6]

The organization of epithelial cells plays a crucial role in the development and sustainability of primary tumor cultures, as it influences their characteristics and responses to treatments. Epithelial cells are key components of the tumor microenvironment, contributing to tumor progression through cell signaling and interactions with stromal elements. Another important aspect of the primary culture phase is separating tumor cells from the remaining non-malignant cells, including tumor-associated fibroblasts, immune cells, and blood vessels.[11] Diverse enzymes such as trypsin and hyaluronidase are commonly used for these steps. [12] However, the use of these enzymes will also affect epithelial cells, which are sensitive to them. As noted in the study, enzymatic activity can notably affect the behavior of epithelial cells.[13] Given the impact of these enzymes, using explant culture methods in disease modeling may provide more reliable results by minimizing alterations in epithelial cell behavior. In this study, healthy and tumor tissues obtained from breast cancer patients after surgical operations were cultured under sterile conditions using explant culture and enzymatic digestion. While various enzymes were utilized for enzymatic digestion, the explant culture method involved dissecting the tissue into small fragments. This study aims to compare the efficiency of explant culture and enzymatic digestion in generating viable patient-derived primary breast cancer cultures, focusing on their effects on cell yield and morphology.

Materials and Methods

The tissues used in this study were obtained from four breast cancer patients during surgery at the Department of General Surgery, Erciyes University in Kayseri, Türkiye. The tissues were collected under supervision of pathologists, and the experimental stages were carried out at Erciyes University Gevher Nesibe Genome and Stem Cell Center.

These were obtained as two separate samples: healthy and tumor tissue. Informed consent was obtained from the breast cancer patients. Inclusion criteria for patients:

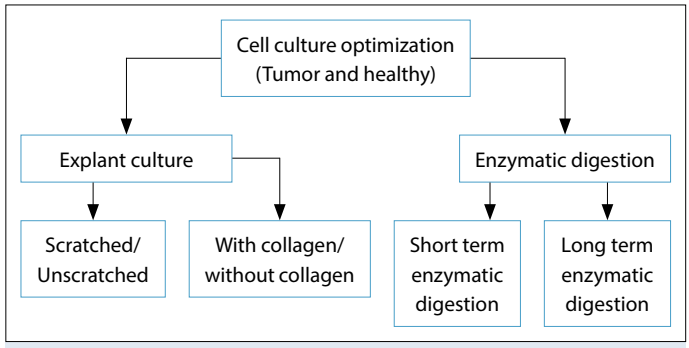

Figure 1. Schematic Representation of the Workflow Model. This flowchart visually illustrates how the experiment will be conducted.

Women diagnosed with invasive ductal breast cancer, aged 18 years or older, with a tumor diameter of 3 cm or larger, who underwent mastectomy or breast-conserving surgery.

Both samples were evaluated using explant and enzymatic methods. In the explant culture, the effect of collagen on cells in primary culture was also examined. In the enzymatic method, two approaches, short-term and long-term, were compared. The workflow is shown in Figure 1.

This study was approved by Erciyes University Clinical Research Ethics Committee with the decision number 2023/56 and dated 18/01/2023. The study was conducted in accordance with the Declaration of Helsinki.

The tumor group criteria included patients aged over 18 years old, 4 patients diagnosed with invasive ductal breast cancer, with tumors measuring 3 cm or larger, and who underwent either mastectomy or breast-conserving surgery, were included in the study. The healthy group consisted of patients who underwent biopsy due to suspected breast cancer but were not diagnosed with the disease.

Primary Cell Culture: Based on studies conducted in primary cell culture, the most suitable method for laboratory conditions was selected by considering various parameters such as cell viability, application efficiency, and cell morphology.

To collect healthy and tumor tissue from the operating room, a transport medium was prepared by adding 10% FBS (Thermo Fisher, USA), 1% Penicillin-Streptomycin (Thermo Fisher, USA), 1% Amphotericin B (Thermo Fisher, Waltham, Massachusetts, USA), and 1% L-Glutamine (STEM CELL, Canada) to RPMI 1640. Transfer process was conducted in a cold environment. Once the tissues were transferred to the laboratory, the procedures continued in a laminar flow hood (Telstar, Barcelona). Prior to starting, the washing solution was prepared by adding 1% Amphotericin and 1% pen-strep to a 50 ml Falcon tube containing Hank's Balanced Salt Solution (HBSS) (STEM CELL, Canada) with 10 mM HEPES and without phenol red. After adding a washing solution to a 100 mm round dish, the tissue was transferred onto the washing solution. After the tissue was cut into small pieces with a scalpel, it was washed with the washing solution to remove blood vessels, necrotic parts, and mammary glands, and then cut into 1 mm pieces. The same steps were followed for both tissuees, as shown in Figure 2.

Explant Culture

Explant culture applied to both tissues as represented in Figure 3. 1 mm tissue pieces were cut and placed into a six-well plate. The plate was then incubated for 20 minutes at 37°C with 5% CO_2 conditions to allow the tissues to adhere to wells. Once the tissues adhered to the bottom of the six-well plate, they were cultured in two different growth media to determine which medium would be more effective. The first medium, referred to as Primary Breast Cancer Medium (PBM), consisted of 20% FBS, 1% pen-strep, 1% amphotericin, 1% L-glutamine, 0.005 mg/ml insulin, and 10 ng/ml EGF in RPMI. The second medium was EpiCult-C Human Media Kit (STEM CELL, Canada), which contained 5% FBS, 1% pen-strep, 1% amphotericin, and 1% L-glutamine. PBM and EpiCult media were added to the wells containing the explants. As the results obtained with PBM were more successful than those obtained with EpiCult. Procedure continued with PBM.

Explant culture tumor cells were seeded at 106 cells per well in a collagen coated 6-well culture plate and incubated for 24 h to allow adherence to the culture plate. Morphological differences and viability between collagen-coated and non-coated groups were examined on days 0, 5, and 10 using an inverted microscope.

Enzymatic Digestion Method

After conducting the mechanical dissociation, both tissues were exposed to two different enzymatic digestion processes: short-term (two hours) and long-term (overnight) enzymatic digestion. A 1:1 collagenase/hyaluronidase (STEM CELL, Canada) solution was added to the tissue.

Short-Term Enzymatic Digestion

After a two-hours incubation of healthy and tumor tissues, the cell fragmentation was applied using sequential filtration. After mechanical fragmentation, the tissue fragments were collected into a 50 mL falcon tube. Then,

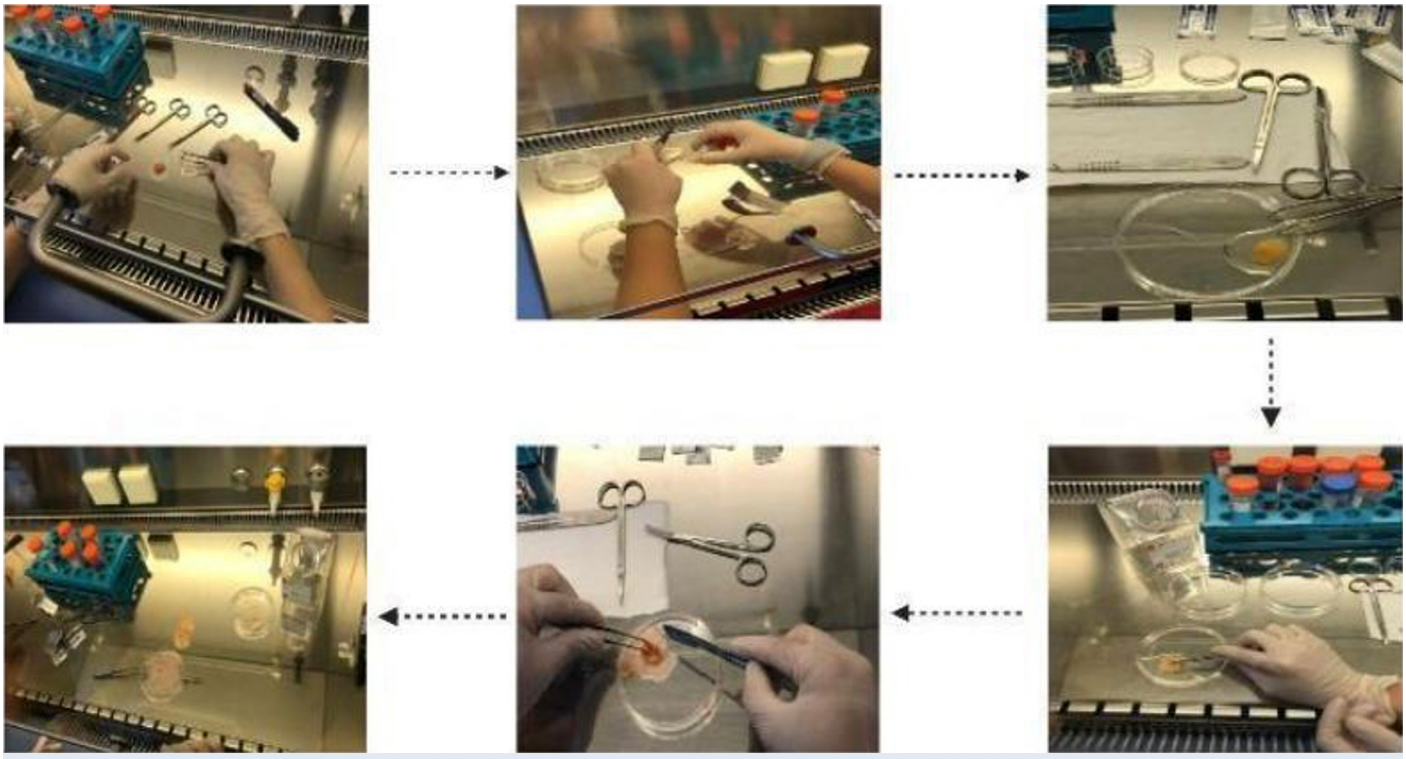

Figure 2. Preparation of healthy and tumor tissue for primary culture. The figure illustrates the mechanical dissociation of the tissue sample in the laminar flow.

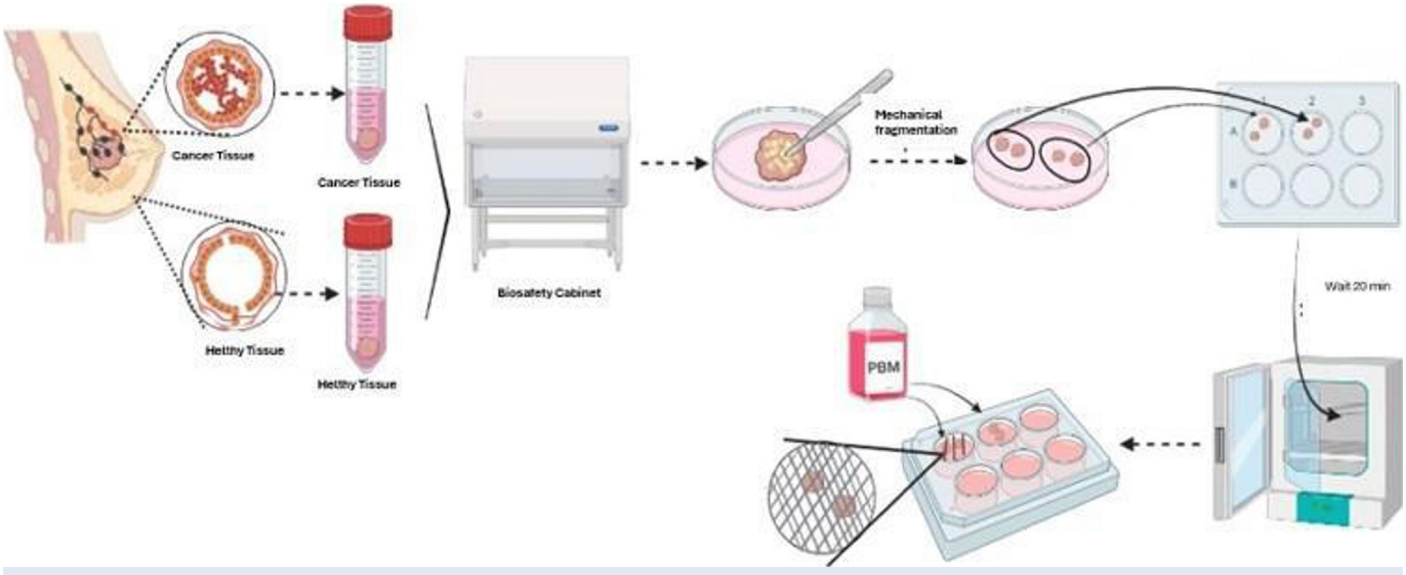

Figure 3. Schematic representation of the explant culture method. The figure illustrates the fragmentation and culture of tissues under a laminar flow.

1 mL of collagenase/hyaluronidase was added to the tube, and the mixture was incubated in Thermomixer (Eppendorf, Germany) for two hours at 37°C and 300 RPM. Afterward, the solution was passed sequentially through 100 µm, 70 µm, and 45 µm filters (Corning, USA), and the cells were plated in PBM medium.

Long-Term Enzymatic Digestion

Long term enzymatic digestion was applied as shown in Figure 4.

After mechanical fragmentation, the tissue fragments were collected into a Falcon tube, and collagenase was added.

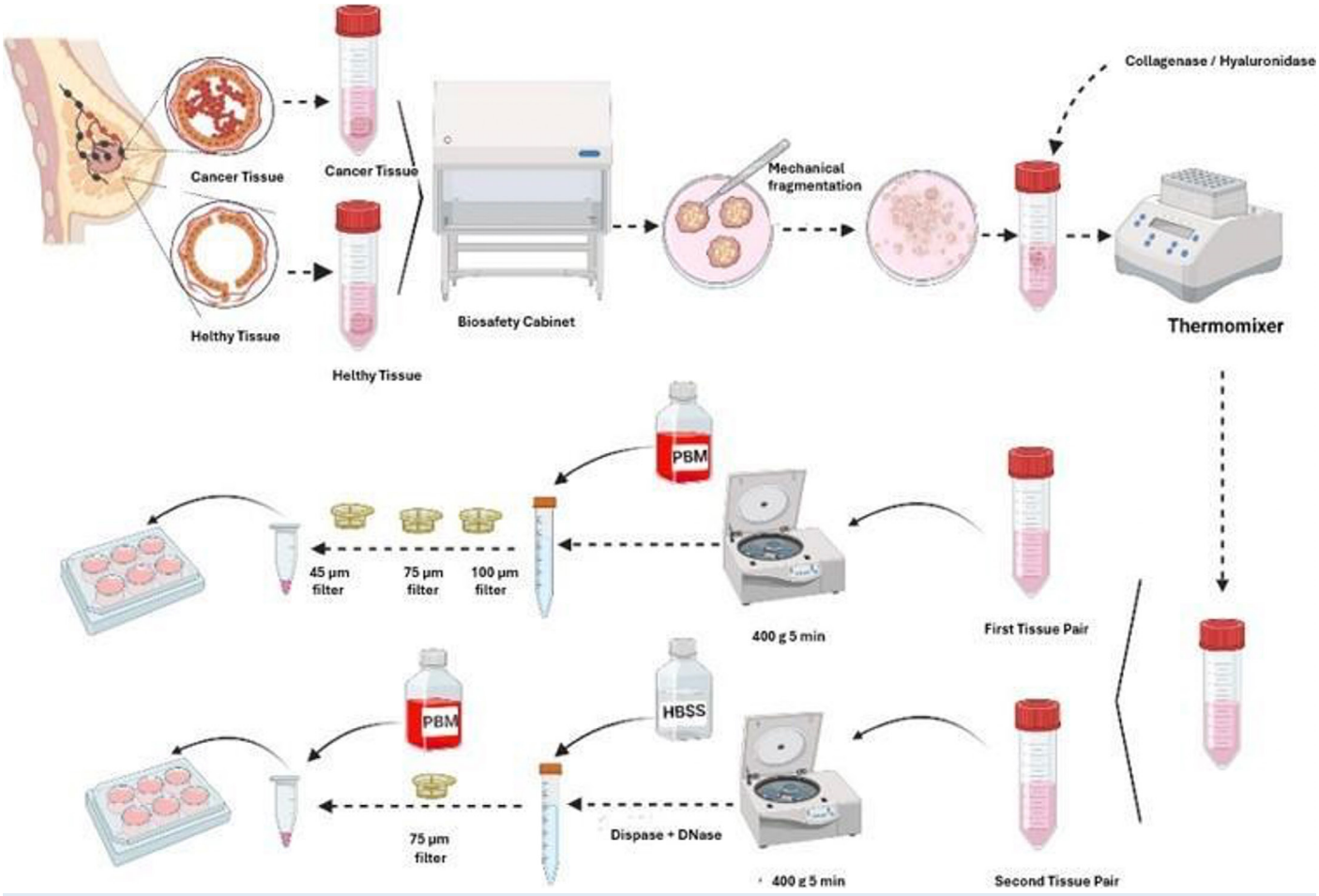

Figure 4. Schematic representation of the long-term enzymatic digestion method. The figure provides a detailed illustration of the long-term enzymatic method.

The mixture was incubated overnight at 37°C and 300 RPM in a Thermomixer (Eppendorf, Germany). The samples were then transferred into Falcon tubes.

First sample: Tissue sample was centrifuged at 400 g for 5 minutes. The supernatant was removed, and PBM medium was added to the pellet. The cells were then passed through 100 µm, 70 µm, and 45 µm filters and plated.

Second sample: Tissue pieces were centrifuged at 400 g for 5 minutes. The supernatant was discarded, and 2 mL of Dispase (5 U/mL) and DNase-I (1 mg/mL) were added. An inhibition solution was prepared using HBSS containing 2% FBS, and 10 mL of this solution was added to each sample. The mixture was filtered through a 70 µm filter and then plated in PBM growth medium.

Results

Clinical Information on Breast Cancer Patients

Four female patients aged 18 years or older, diagnosed with invasive ductal breast cancer, with tumors measuring 3 cm or larger, and who underwent either mastectomy or breast-conserving surgery, were included in the study.

Short-Term and Long-Term Enzymatic Digestion

Tumor cells isolated from breast cancer patient-derived tissues using short term enzymatic digestion exhibited minimal attachment to the culture flask at days 0 and 5, with only a limited number of adherent cells observed. At these times, the cells predominantly displayed round, irregular, and polygonal morphologies, with a substantial presence of free-floating cells (Fig. 5a-1, 2). By day 10, while loosely attached round cells were still present, a notable increase in the number of adherent cells was observed. Furthermore, cell-cell adhesion, a critical factor in supporting cell proliferation and survival, appeared to have been initiated among the cells (Fig. 5a-3).

In tumor cells obtained through long-term enzymatic digestion, on day 0, a greater number of cells adhered to the surface of the flask and began to form colonies,

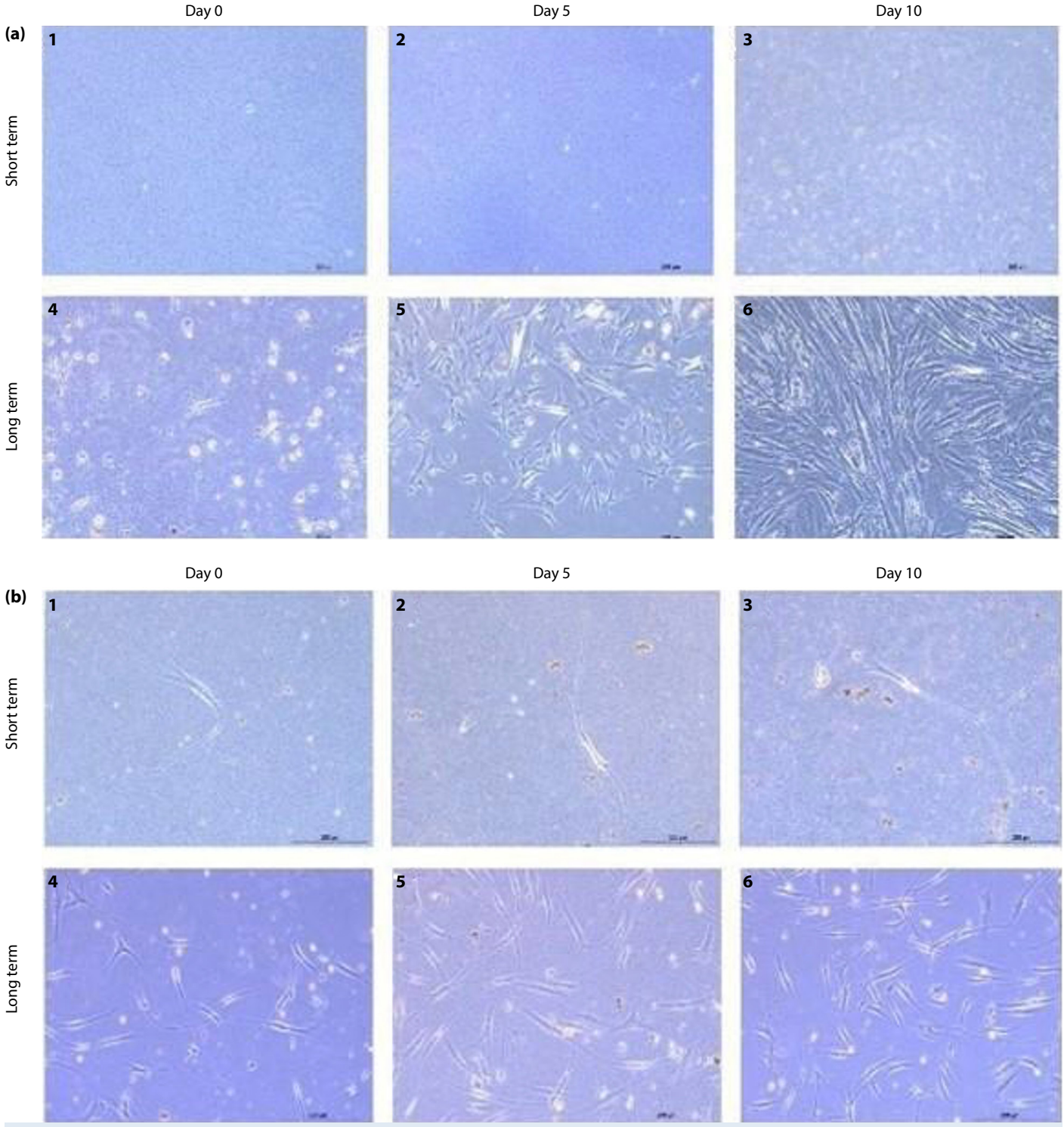

Figure 5. Short-term and long-term enzymatic digestion results for tumor **(a)** and healthy **(b)** tissues. Short-term enzymatic digestion results for tumor tissue on day 0 (a-1), 5 (a-2) and 10 (a-3). Long-term enzymatic digestion for tumor tissue on day 0 (a-4), 5 (a-5) and 10 (a-6) representations. Short-term enzymatic digestion results for healthy tissue on day 0 (b-1), 5 (b-2) and 10 (b-3). Long-term enzymatic digestion for tumor tissue on day 0 (b-4), 5 (b-5) and 10 (b-6) representations. 10x light microscopy image.

alongside loosely attached round cells and floating cells (Fig. 5a-4). On day 5, there was a significant increase in the number of cells attached to the flask, and as expected, fibroblast-like morphology was observed. Loosely attached round cells were still present among the adherent cells (Fig. 5a-5). By day 10, a substantial increase in the number

of cells adhering to the surface of the flask was observed, along with a noticeable increase in cell proliferation (Fig. 5a-6). Long-term enzymatic digestion of healthy and tumor tissues demonstrated enhanced outcomes in terms of cell count and cell morphology compared to short-term enzymatic digestion (Fig. 5a).

Cells derived from healthy tissues through short-term and long-term enzymatic digestion exhibited a morphology like those obtained from tumor tissues. In the short-term group, on days 0 and 5, loosely attached round cells and predominantly floating cells were observed (Fig. 5b-1, 2). Compared to day 0, an increase in cell proliferation was evident on day 10 (Fig. 5b-3). In cells derived from healthy tissues with long-term enzymatic digestion, the proportion of cells adhering to the flask was higher than in the other groups starting from day 0. The cell morphology on days 0 and 5 included fibroblast-like cells attached to the flask, along with loosely attached and floating cells. On day 10, an increase in cell proliferation was observed compared to days 0 and 5, as well as the short-term enzymatic digestion group (Fig. 5b-1–3).

Compared to long-term enzymatic digestion, explant culture demonstrated better results in terms of cell count and cell morphology for both tissues. In the explant culture method, it was observed that the cells exhibited an epithelioid character starting from the 5th day of culture and maintained this characteristic through the 10th day (Fig. 6a).

Cells obtained from tumor tissue and healthy tissue through explant culture exhibited loosely attached round and floating cells on day 0 (Fig. 6 a-1, 4). On days 5 and 10, particularly in the tumor cell group, a regular increase in cell proliferation was observed (Fig. 6a-2, 3). This was accompanied by a decrease in floating cells and an increase in the number of cells adhering to the flask. Morphologically, epithelial-like cells were present (Fig. 6a-2, 3). Compared to short-term and long-term enzymatic digestion methods, cells obtained through the explant culture method were more abundant, exhibited faster proliferation, showed a reduction in floating cells, and demonstrated epithelial cell characteristics (Fig. 6a).

To assess the effect of the surface on cell morphology and proliferation in the explant culture method, the flask bottom was coated with collagen. In both collagen and non-collagen groups, an increase in cell number was observed over time, with only a minimal presence of floating cells. Morphologically, no significant differences were observed between the groups (Fig. 6b). In the explant culture method, it was determined that drawing parallel lines on the bottom of six- well plates did not have a significant impact on cell culture.

Similarly, coating the well bottom with collagen, another parameter evaluated, was found to have no significant effects on cell culture (Fig. 6b).

Discussion

Cancer development is a multi-step process in which various oncogenic mutations lead to cancer cells with different genetic defects that may vary even within a single tumor.[14] Breast cancer is the second leading cause of cancer-related deaths among women.[15] Efforts to develop effective approaches for cancer treatment are traditionally conducted using *in vitro* and *in vivo* models.[16] In breast cancer cell lines, which are part of *in vitro* modeling systems, the inability to reflect tumor heterogeneity remains a significant issue to be addressed. Additionally, since these cell lines are not patient-specific, their use in this field will not be feasible.

Primary cell culture serves as an effective model for developing personalized therapies for cancer, which vary from person to person. The realization of personalized treatments and the translation of *in vitro* findings into *in vivo* models and clinical settings require patient-derived primary tumor cells. Selecting the most suitable techniques for different tumors is crucial to establishing primary cultures.[17] The most common methods for obtaining a uniform cell population include enzymatic digestion, chemical processing, or mechanical fragmentation.[17] Primary cancer cultures are crucial for conceptualizing therapeutic targets that could support future drug development studies or personalized cancer treatments. In this study, primary culture methods, which are among the most suitable approaches for developing personalized treatment options, were investigated in a comparative fashion.

The results obtained from long-term enzymatic digestion were found to be more successful, which aligns with findings in the literature. The higher success rate of long-term enzymatic digestion may be due to its less aggressive nature, enabling higher cell yield.[8]

Although long-term enzymatic digestion produced a higher cell yield compared to short-term digestion, the morphology of the cells obtained deviated from the desired epithelial characteristics, displaying a more fibroblastic appearance (Fig. 5a, b).

Comparison of two different primary culture techniques from the literature revealed that explant culture yields higher

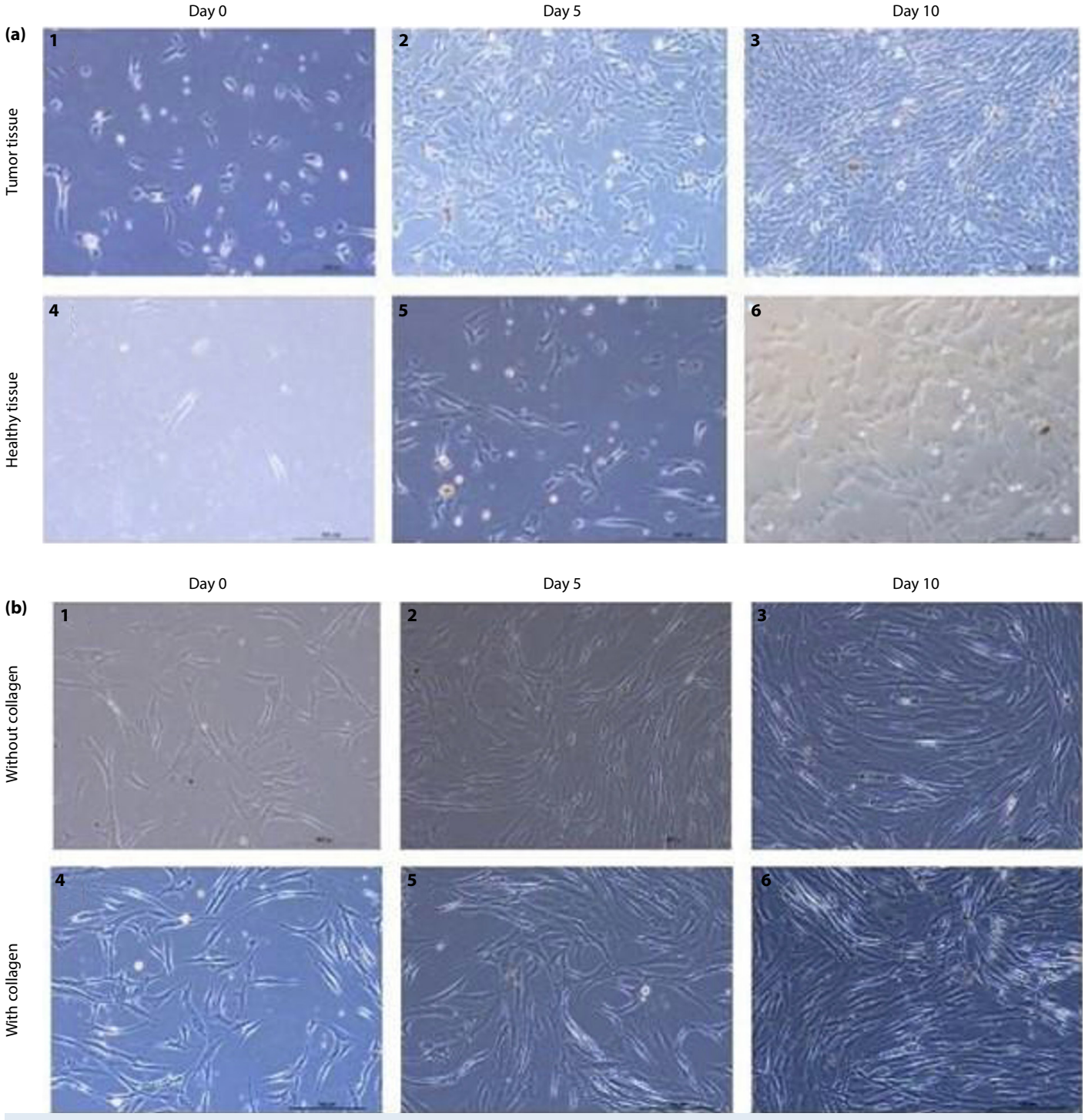

Figure 6. Explant culture results for tumor and healthy tissue and non-coated collagen coated wells. Explant culture results for tumor and healthy tissue and non-coated/ collagen coated wells. explant culture for tumor tissue (**a**-A, B, C) and healthy tissue (**a**-D, E, F) and comparison of non-coated wells (**b**-A, B, C) and collagen-coated wells (**b**-D,E,F) in cell culture days 0-5-10 during explant culture an. 10x light microscopy images.

efficiency in cell isolation rather than enzymatic digestion from breast cancer tissue. This finding is consistent with studies in the literature on breast cancer culture methods. [18] The morphological characteristics of the cells obtained from explant culture were more epithelial (Fig. 6a).

The ability of the explant model to allow comprehensive evaluation of individual tumors within their natural tumor microenvironment (TME), preserving the integrity of tumor tissue morphology, viability, and endogenous hormonal signaling, may have contributed to this

outcome. Also, studies have shown that the explant method significantly impacts the purity and characteristics of isolated cells. Additionally, explant techniques can yield higher cell proliferation rates and maintain better cell vitality compared to enzymatic methods[19] These findings underline that tumors are not solely composed of tumor cells but also emphasize the significance of the tumor microenvironment. Our results demonstrate that explant culture is the most effective method for identifying and culturing breast cancer cells in terms of protocol efficiency and cell survival rate.

Studies have shown that surface structure plays a significant role in regulating cell adhesion. For instance, Lawrence et al.[20] demonstrated that silk structures with grooved surface features enhanced initial cell adhesion compared to smooth surfaces. Surface features like parallel lines can also influence cell differentiation or how cells interact with the substrate. Surface coatings can optimize the contact area between cells and the substrate, thereby increasing adhesion strength.[21] Studies have shown that collagen coating leads to a lower apoptotic rate in breast cancer cells compared to plastic surfaces.[1] In contrast, Maquoi et al.[22] found that type I collagen within a three-dimensional (3D) matrix can induce apoptosis in weakly invasive luminal-like breast carcinoma cells. This finding suggests that, instead of promoting growth, collagen may limit cancer cell expansion by activating apoptotic pathways, particularly in certain breast cancer subtypes. The presence of parallel lines may enhance tissue adhesion by increasing the contact area for cell binding and influencing mechanotransduction pathways that regulate cellular behavior. In this study, collagen coating of the culture dish were applied to the explant culture group of cells derived from breast cancer tissue. However, no significant differences were observed in terms of cell count and morphology (Fig. 6b). Although previous studies have demonstrated that collagen coating can enhance adhesion and reduce apoptosis, no significant effects were observed in our study. This could be due to differences in breast cancer subtypes, variations in extracellular matrix composition, or culture duration. Future studies should investigate these factors to determine the optimal role of collagen in breast cancer primary cultures.

The higher number of cells observed from the fifth day of culture in the explant method (Fig. 6) compared to the enzymatic method (Fig. 5) indicates that explant culture is a more appropriate technique for the primary culture stage.

Limitations

The limited sample size of this study constrains its overall impact. Future studies involving larger sample groups will likely enhance the inclusivity and generalizability of the findings.

Conclusion

In conclusion, this study compared explant culture and enzymatic digestion methods for the establishment of patient-derived primary breast cancer cultures. Long-term enzymatic digestion was found to be more effective than short-term digestion in terms of cell proliferation. However, explant culture demonstrated superior outcomes in preserving cell morphology and maintaining epithelial characteristics, making it the more advantageous approach. These findings highlight the critical role of preserving the tumor microenvironment in primary culture processes. Furthermore, primary cultures obtained via explant methods more accurately reflect tumor biology, suggesting their potential as a reliable model for drug development, disease pathogenesis studies, and personalized medicine applications. Future studies with larger patient cohorts will further validate the reliability and applicability of this method in clinical and translational research.

Ethics Committee Approval: The Erciyes University Clinical Research Ethics Committee granted approval for this study (date: 18.01.2023, number: 2023/56).

Authorship Contributions: Concept: SY, MDS, MSK, EY, NMÖ, MA, MG, FÖ; Design: SY, MDS, MSK, EY, NMÖ, MA, MG, FÖ; Supervision: SY, MDS, MSK, EY, NMÖ, MA, MG, FÖ; Materials: MG, FÖ; Data Collection or Processing: MDS, MSK, EY, NMÖ; Analysis or Interpretation: SY, MDS, MSK; Literature Search: SY, MA, MDS, MSK, EY, NMÖ; Writing: SY, MA; Critical Review: SY, MDS, MSK, EY, NM.

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References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA 2018;68(6):394-424. [CrossRef]
2. Huerta-Reyes M, Aguilar-Rojas A. Three-dimensional models to study breast cancer: A review. Int. J. Oncol 2021;58(4):331-343. [CrossRef]

3. Li JJ, Tsang JY, Tse GM. Tumor microenvironment in breast cancer: Updates on therapeutic implications and pathologic assessment. Cancers 2021;13(16):4233. [CrossRef]
4. Turashvili G, Brogi E. Tumor Heterogeneity in Breast Cancer. Front Med 2017;8(4):227. [CrossRef]
5. Breitenbach M, Hoffmann J. Editorial: Cancer Models. Front Oncol 2018;3(8):401. [CrossRef]
6. Faridi N, Bathaie SZ, Abroun S, Farzaneh P, Karbasian H, Tamanoi F, et al. Isolation and characterization of the primary epithelial breast cancer cells and the adjacent normal epithelial cells from Iranian women's breast cancer tumors. Cytotechnology 2018;70(2):625-39. [CrossRef]
7. Shen C, Gu M, Liang D, Miao L, Hu L, Zheng C, et al. Establishment and characterization of three new human breast cancer cell lines derived from Chinese breast cancer tissues. Cancer Cell Int 2009;9:2. [CrossRef]
8. Richter M, Piwocka O, Musielak M, Piotrowski I, Suchorska WM, Trzeciak T. From donor to the lab: A fascinating journey of primary cell lines. Front Cell Dev Biol 2021;9:711381. [CrossRef]
9. Blay J, Lacombe D, Meunier F, Stupp R. Personalised medicine in oncology: questions for the next 20 years. Lancet Oncol 2012;13(5):448-449. [CrossRef]
10. Bremnes RM, Dønnem T, Al-Saad S, Al-Shibli K, Andersen S, Sirera R, et al. The role of tumor stroma in cancer progression and prognosis: emphasis on carcinoma-associated fibroblasts and non-small cell lung cancer. J Thorac Oncol 2011;6(1):209-217. [CrossRef]
11. Tsai MJ, Chang WA, Huang MS, Kuo PL. Tumor microenvironment: a new treatment target for cancer. ISRN Biochem 2024;10:351959. [CrossRef]
12. Piwocka O, Musielak M, Ampuła K, Piotrowski I, Adamczyk B, Fundowicz M, et al. Navigating challenges: optimising methods for primary cell culture isolation. Cancer Cell Int 2024;24(1):28. [CrossRef]
13. Cuevas EP, Moreno-Bueno G, Canesin G, Santos V, Portillo F, Cano A. LOXL2 catalytically inactive mutants mediate epithelial-to-mesenchymal transition. Biol Open 2014;3(2):129-37. [CrossRef]
14. Nushtaeva AA, Stepanov GA, Semenov DV, Juravlev ES, Balahonova EA, Gerasimov AV, et al. Characterization of primary normal and malignant breast cancer cell and their response to chemotherapy and immunostimulatory agents. BMC Cancer 2018;18(1):728. [CrossRef]
15. Zhu J W, Charkhchi P, Adekunte S, Akbari MR. What Is Known about Breast Cancer in Young Women? Cancers 2023;15(6):1917. [CrossRef]
16. Nanou A, Lorenzo-Moldero I, Gazouleas KD, Cortese B, Moroni L. 3D Culture Modeling of Metastatic Breast Cancer Cells in Additive Manufactured Scaffolds. ACS Appl Mater Interfaces 2022;14(24):28389-28402. [CrossRef]
17. Mitra A, Mishra L, Li S. Technologies for deriving primary tumor cells for use in personalized cancer therapy. Trends Biotechnol 2013;31(6):347-354. [CrossRef]
18. RS Arum. Deriving Breast Cancer's Primary Cultures from Patients' Tumor Biopsies in Indonesia Using Explant and Enzymatic Methods. J Hunan Univ Nat Sci 2022;49(9):11-16. [CrossRef]
19. Shwetha HR, Kotrashetti VS, Babu NC, Kumbar V, Bhat K, Reddy R. Ex vivo culture of oral keratinocytes using direct explant cell culture technique. JOMFP 2019;23(2):243-247. [CrossRef]
20. Lawrence BD, Pan Z, Liu A, Kaplan DL, Rosenblatt MI. Human corneal limbal epithelial cell response to varying silk film geometric topography in vitro. Acta Biomater 2012;8(10):3732-3743. [CrossRef]
21. Yi X and Gao H. Cell interaction with graphene microsheets: near-orthogonal cutting versus parallel attachment. Nanoscale 2015;7(12):5457-5467. [CrossRef]
22. Maquoi E, Assent D, Detilleux J, Péqueux C, Foidart J, Noël A. Mt1-mmp protects breast carcinoma cells against type i collagen-induced apoptosis. Oncogene 2011;31(4):480-493. [CrossRef]