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Differentiated Protein and Lipid Composition Profiles of Breast Cancer Cells Grown on Various ECM Proteins

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Abstract: The development of malignant cancer cells is dependent on the function of extracellular matrix (ECM) proteins. As cells undergo malignant transformation, they must overcome specific barriers, such as basement membranes, in order to migrate to other tissues and organs. These barriers are primarily composed of ECM proteins. It is therefore evident that the interaction between cancer cells and these ECM proteins is of critical importance in the development of malignancy. In this study, MCF7 breast cancer cells were cultivated on surfaces coated with three distinct ECM proteins: fibronectin, collagen, and laminin. Subsequently, the cells were harvested and examined to ascertain the impact of interaction with distinct ECM proteins on their molecular composition, utilising attenuated total reflection infrared spectroscopy (ATR-FTIR). The results of the study demonstrate a significant enhancement in the process of lipid biosynthesis and/or a substantial reduction in lipid degradation when cells are cultivated on collagen and fibronectin proteins. The cells grown on fibronectin exhibited a markedly elevated protein and nucleic acid content in comparison to those grown on laminin and the control cells. However, the ratio of lipids to proteins was significantly lower in cells grown on fibronectin than in the control group and in cells grown on collagen and laminin. The principal component analysis (PCA) demonstrated that there were considerable spectral discrepancies between the cells grown on ECM proteins and those not grown on ECM proteins. The experimental treatments were successfully differentiated from the control.

Keywords: Breast cancer, fibronectin, collagen, laminin, ATR-FTIR spectroscopy.

Çeşitli ECM Proteinleri Üzerinde Büyüyen Meme Kanseri Hücrelerinin Farklılaşan Protein ve Lipid Kompozisyon Profilleri

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Öz: Kötü huylu kanser hücrelerinin gelişimi, hücre dışı matris (ECM) proteinlerinin işlevine bağlıdır. Hücreler malign transformasyona uğradıkça, diğer doku ve organlara göç edebilmek için bazal membranlar gibi belirli bariyerleri aşmaları gerekir. Bu bariyerler öncelikle ECM proteinlerinden oluşur. Bu nedenle, kanser hücreleri ile bu ECM proteinleri arasındaki etkileşimin malignite gelişiminde kritik öneme sahip olduğu açıktır. Bu çalışmada, MCF7 meme kanseri hücreleri fibronektin, kolajen ve laminin olmak üzere üç farklı ECM proteini ile kaplanmış yüzeylerde yetiştirilmiştir. Daha sonra, hücreler hasat edilmiş ve zayıflatılmış toplam yansıma kızılötesi spektroskopisi (ATR-FTIR) kullanılarak farklı ECM proteinleriyle etkileşimin moleküler bileşimleri üzerindeki etkisini belirlemek için incelenmiştir. Çalışmanın sonuçları, hücreler kolajen ve fibronektin proteinleri üzerinde yetiştirildiğinde lipid biyosentezi sürecinde önemli bir artış ve/veya lipid yıkımında önemli bir azalma olduğunu göstermektedir. Fibronektin üzerinde yetiştirilen hücreler, laminin ve kontrol hücreleri üzerinde yetiştirilenlere kıyasla belirgin şekilde yüksek protein ve nükleik asit içeriği sergilemiştir. Bununla birlikte, lipidlerin proteinlere oranı fibronektin üzerinde büyütülen hücrelerde kontrol grubuna ve kolajen ve laminin üzerinde büyütülen hücrelere göre önemli ölçüde daha düşüktü. Temel bileşen analizi (PCA), ECM proteinleri üzerinde yetiştirilen hücreler ile ECM proteinleri üzerinde yetiştirilmeyen hücreler arasında önemli spektral farklılıklar olduğunu göstermiştir. Deneysel uygulamalar kontrolden başarılı bir şekilde ayırt edilmiştir.

Anahtar Kelimeler: Meme kanseri, fibronektin, kolajen, laminin, ATR-FTIR spektroskopisi.

INTRODUCTION

Breast cancer represents the most common form of cancer diagnosed in women, accounting for approximately 30% of all cancers in this demographic (Siegel, Miller, & Jemal, 2020). Breast cancer often has an insidious onset, making it difficult to detect. Timely detection is crucial for successful treatment and survival. Clinical methods like core biopsy, X-ray mammography, ultrasound, MRI, and CT are used when the disease has already progressed (Sitnikova et

al., 2020). Fourier transform infrared spectroscopy (FTIR), alternatively designated as FTIR spectroscopy, signifies a technologically advanced approach for the analysis of breast tissue samples. This technique facilitates the chemical composition analysis of tissue samples by enabling the identification of chemical bonds and functional groups therein (Movasaghi et al., 2008). The identification of chemical changes at the tissue level can be beneficial in the correlation of different disease states, including breast cancer.

Infrared spectroscopy (IR) is an excellent and preferred technique for reflecting the structural and functional alterations triggered by diseases or environmental factors in diverse biological systems, extending from individual cells to entire organisms (Baker et al., 2014). IR has been employed to discern alterations in protein structure that may be indicative of the progression from healthy epithelial tissue to cancerous epithelial tissue (Elshemey et al., 2016). To distinguish between malignant and benign lesions in various tissues, the composition of biomolecules such as lipids, proteins, and nucleic acids is examined (Zhou et al., 2006). Furthermore, chemical characterization can facilitate the acquisition of additional information pertaining to cellular processes, including the cell cycle, differentiation, transcription and apoptosis (Eckel et al., 2001).

Genetic alterations occur at various stages of cancer cell development, influencing metabolic pathways and phenotypic characteristics. These changes enhance the cells' adaptability to their environment, facilitate metastasis, and render them resistant to chemotherapy (Lochter & Bissell, 1995).

During these stages of development and change, cancer cells alter both their own structures and their surrounding environment. In this context, the extracellular proteins with which they interact become significance. These interactions ultimately alter the metabolic activities of the cancer cells, thereby enhancing their capacity to adapt and develop resistance (Bonnans et al., 2014; Schedin et al., 2007).

In this study, the impact of different extracellular matrix (ECM) proteins on the molecular structure of MCF7 breast cancer cells were characterized utilizing ATR-FTIR spectroscopy.

MATERIAL AND METHOD

Cell Culture: The MCF7 cells were cultivated in a cell culture medium comprising DMEM GlutaMax (Thermo Fisher) and 10% FBS (Thermo Fisher). During routine cell culture, cells were allowed to proliferate until all cell types occupied 80% of the available space in T-25 and T-75 culture dishes. The cells were cultured at a temperature of 37°C within a controlled atmosphere comprising a 5% CO_2 concentration and observed for optimal growth and morphology. The cell culture medium was replaced three times per week, and a solution of trypsin and ethylenediaminetetraacetic acid (EDTA) was used for cell passaging. The cells were utilized until a maximum of 30 passage numbers were reached, and a sufficient number of cells at early passage numbers were frozen in liquid nitrogen tanks for use in subsequent experiments.

Coating of T-25 cell culture flasks and 35 mm petri dishes with collagen-I, fibronectin, and laminin: A 30 µg/ml collagen solution was prepared by taking 0.5 ml of collagen-I (thermo-fisher) stock (3 mg/ml) and adding dH_2O until the total volume was 50 ml. A total of 2 ml (30 µg/ml) of the diluted collagen I solution was added to each T-25 culture flask. The flasks were incubated overnight at room temperature to permit the collagen to adhere to the plastic. Following the incubation period, the excess solution remaining in the flasks was removed, and the collagen-I-coated T-25 cell culture flasks were utilized for cell seeding.

To obtain a stock solution with a concentration of 1 mg/ml, 5 mg of powdered fibronectin (Thermo Fisher) was added to 5 ml of dH_2O. A solution of 1.5 ml was prepared by adding dH2O to a total volume of 50 ml, resulting in a fibronectin solution of 30 µg/ml. A volume of 2 ml (30 µg/ml) of the diluted fibronectin solution was added to each T-25 culture flask and incubated overnight at room temperature to permit the fibronectin to adhere to the plastic. Following the incubation period, the remaining solution was removed from the flasks, and the fibronectin-coated T-25 cell culture flasks were utilized for cell seeding.

A solution of 10 µg/ml diluted laminin was prepared by adding 50 ml of dH_2O to 0.5 mg of powdered laminin (thermo-fisher). A volume of 2 ml (10 µg/ml) of the diluted laminin solution was added to each T-25 culture flask and incubated overnight at room temperature to permit the laminin to adhere to the plastic. Following the incubation period, the excess solution remaining in the flasks was removed, and the laminin-coated T-25 cell culture flasks were utilized for cell seeding.

Data Collection for ATR-FTIR Spectroscopy: The MCF7 cells, which had been grown on three distinct matrix proteins, were harvested from the culture medium and transferred into 15-ml Falcon tubes. Without any preliminary treatment, one million cells were placed on a ZnS attenuated total reflectance (ATR) crystal in 10 µl of PBS. The PBS solution in which the cells were collected and the water in the cells was removed by applying nitrogen gas. The spectra in the 4000-650 cm^{-1} region were then collected with a resolution of 4 cm^{-1} and a scan number of 100. The experiments were repeated at least five times for each of treatment groups.

Analysis of Spectrum Data: The spectral bands in the 3050-2800 cm^{-1} C-H region and 1800-900 cm^{-1} fingerprint region were subjected to analysis using Perkin-

Elmer Spectrum (Version 10.03.06.0100) as shown in Figure 1. Band area analyses were conducted on spectra that had undergone baseline correction in order to monitor changes that were sensitive to molecular concentration.

Additionally, principal component analysis (PCA) was conducted using OPUS 5.5 software (Bruker Optics, GmbH) to differentiate between MCF7 cells based on spectral variations. PCA provides insight into the relationships between spectral data and the degree of spectral similarity.

Statistics: The results obtained are expressed as the mean ± standard error of the mean (SEM). A Mann-Whitney U-test was conducted to ascertain the statistical significance of the discrepancies between the MCF7-Collagen, Fibronectin, Laminin, and Control groups. In the context of statistical analysis, P-values lower than or equal to 0.05 (or 0.01) were regarded as statistically significant, indicating a substantial difference from the control group. The magnitude of statistical significance was denoted as follows: * $p < 0.05$, ** $p < 0.01$.

RESULTS AND DISCUSSION

The C-H stretching region is primarily comprised of vibrations derived from lipids, including CH_3 antisymmetric, CH_2 antisymmetric, CH_2 symmetric, and =CH stretching vibrations (Cakmak et al., 2012; Movasaghi et al., 2008). The impact of diverse ECM proteins on saturated lipid content of cells was examined through analysis of CH_2 antisymmetric and symmetric stretching bands. Figure 2 displays the band area values for CH_2 antisymmetric, CH_2 symmetric, and CH_3 symmetric stretching bands in control, collagen, fibronectin, and laminin-treated MCF7 cells.

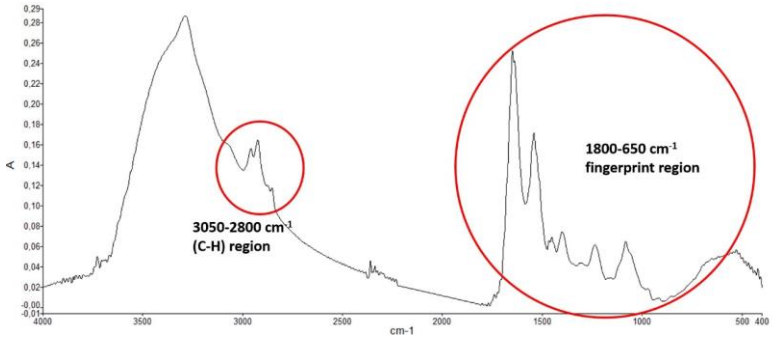

Figure 1. A sample ATR-FTIR spectrum of MCF7 cells that were grown on collagen type-I ECM protein in the spectral region of 4000-650 cm^{-1}.

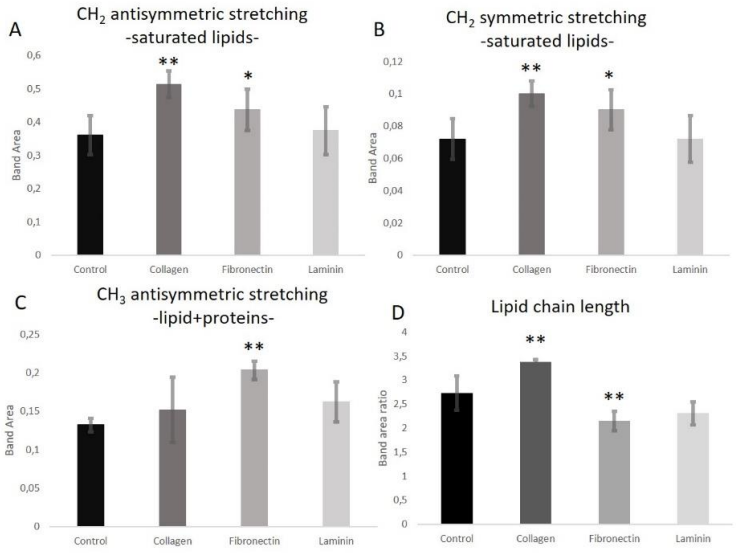

Figure 2. The band area values of the CH_2 antisymmetric (A), CH_2 symmetric (B), and CH_3 antisymmetric (C) bands are presented. The ratios of the band area values of the CH_2 antisymmetric to CH_3 antisymmetric bands are represented in (D). The results are expressed as the mean ± SEM, with * indicating $p < 0.05$ and ** indicating $p < 0.01$.

In the domain of absorption spectroscopy, the magnitude or the area under the curve of a band exhibits a direct proportionality to the quantity of the sample, in accordance with the Beer-Lambert law (Cakmak et al., 2012; Garip et al., 2010). The band area demonstrates a notable increase in the collagen and fibronectin-treated groups when compared to the control group for CH_2 antisymmetric, CH_2 symmetric stretching, and CH_3 antisymmetric bands, as illustrated in Figure 2. This increase in lipid band areas indicates an increase in saturated lipid content in cell lines grown on surfaces coated with fibronectin and collagen type-I ECM proteins

(CH_2 antisymmetric stretching and CH_2 symmetric stretching bands, Figure 2-A and Figure 2-B, respectively). These findings suggest an augmentation in lipid biosynthesis and/or a reduction in lipid degradation in cells cultivated on these ECM proteins. In the cell group grown on the surface treated with laminin, no statistically significant change was observed in the band area values compared to the control group. These increases in saturated lipid content may be attributed to an increase in lipid biosynthesis or a decrease in lipid degradation due to a decrease in lipid peroxidation (Cakmak et al., 2012; Yonar et al., 2022). The findings in the present study on the increased band area values for CH_2 antisymmetric, CH_2 symmetric stretching, and CH_3 antisymmetric bands in breast cancer cells grown on collagen type-1 and fibronectin align with previous studies that have highlighted the role of ECM proteins in modulating cancer cell behavior (Rizwan et al., 2015). For instance, a study by Zhao et al. (2021) emphasized the dynamic nature of the ECM and its significant impact on breast cancer progression (Zhao et al., 2021). They noted that ECM proteins, including collagen and fibronectin, play crucial roles in cancer cell invasion and metastasis (Zhao et al., 2021). The observed increase in saturated lipid content in MCF7 cells also indicates a rapid cell growth behavior (Hedegaard et al., 2010). Similarly, research by (Nazemi et al., 2024) demonstrated that ECM components, particularly collagen I, support breast cancer cell growth under nutrient-deprived conditions by promoting tyrosine catabolism. This suggests that ECM proteins not only influence the structural integrity of cancer cells but also their metabolic pathways (Nazemi et al., 2024). Our observation of increased saturated lipid content in cell lines grown on fibronectin and collagen type-I ECM proteins is consistent with the findings of Haukaas et al. (2016), who identified distinct metabolic clusters in breast cancer tissues. They reported that ECM-related genes were upregulated in certain clusters, potentially contributing to altered lipid metabolism.

Furthermore, alterations in lipid biosynthesis and/or lipid degradation can be tracked by monitoring the ratio of changes in the CH_2/CH_3 antisymmetric band areas (Figure 2-D). An increase or decrease in the ratio indicates an increase or decrease in the length of the hydrocarbon chains, respectively (Gok et al., 2021; Wang et al., 2005). Additionally, alterations in the length of lipid hydrocarbon chains were discerned in cells grown on collagen type-I and fibronectin-treated surfaces (Figure 2-D). The significantly higher ratio observed in collagen-treated cells indicates a longer hydrocarbon chain length, whereas a lower ratio in fibronectin-treated cells suggests a shorter hydrocarbon chain length. Conversely, this ratio remained largely unchanged in the presence of laminin, indicating that laminin does not exert any significant influence on hydrocarbon chain lengths. The ratios of CH_2 antisymmetric stretching and CH_2 symmetric stretching band areas suggest that breast cancer cells grown on fibronectin-treated surfaces revealed a significant shortening of lipid hydrocarbon chains compared to those grown on collagen type-I protein-treated surfaces. This observation aligns with previous studies indicating that the extracellular matrix (ECM) composition can influence cellular behavior and biochemical properties (Belbachir et al., 2009; Hunter et al., 2014). For instance, fibronectin has been shown to promote cell adhesion, migration, and invasion, which may contribute to alterations in lipid metabolism and membrane composition (Hunter et al., 2014). In contrast, collagen type-I is known to provide structural support and stability, potentially leading to a more rigid cellular environment (Hunter et al., 2014). The differences in lipid hydrocarbon chain lengths observed in this study suggest that fibronectin may induce a more dynamic and flexible membrane environment, which could be associated with enhanced cellular processes such as proliferation and metastasis. Comparatively, other studies have also reported variations in lipid composition in response to different ECM components (Verdonck et al., 2016). For example, Verdonck et al. (2016) utilized FTIR imaging to characterize human breast cancer tissues and found distinct spectral characteristics in the stroma close to the tumor, indicating ECM-dependent molecular alterations. Similarly, another study conducted by Jun et al., 2020 highlighted the role of fibronectin in promoting breast cancer metastasis, further supporting the notion that ECM composition can modulate cellular properties (Jun et al., 2020).

The band observed at approximately 1635 cm^{-1} (amide I) is a well-characterized protein band that can be utilized for the analysis of protein content (Mega Tiber et al., 2011; Yonar et al., 2022). The amide I band area demonstrated a notable elevation in the collagen, fibronectin, and laminin-treated groups in comparison to the control, as illustrated in Figure 3-A. The vibrations from PO_2^- functional groups are primarily derived from the phosphodiester groups of nucleic acids. Consequently, the augmented absorption observed in these bands indicates an elevated nucleic acid content in malignancies (Fujioka et al., 2004). As illustrated in Figure 3-B, the band area of PO_2^- symmetric stretching bands exhibits a notable increase in the collagen, fibronectin, and laminin-treated groups in comparison to the control. These observations imply that there is an increase in protein and nucleic acid biosynthesis in response to growth on these ECM proteins. The ratio between the Amide I and Amide II bands can be employed as an informative indicator of conformational modifications within protein secondary structures

(Bunaciu et al., 2014; Yonar et al., 2022). The increase may indicate a shift in the protein's secondary structure, such as an increase in α-helical or β-sheet content (Garip et al., 2010). Our findings indicate that breast cancer cells cultured on fibronectin and laminin extracellular matrix proteins exhibit significant alterations in their protein secondary structures compared to the control group (Figure 3-D). These findings are corroborated by the observation that the lipid/protein ratio was found to be reduced in cells undergoing growth on fibronectin and laminin in comparison to the others (Figure 3-C). An increase in the band area values of the amide I and PO^{2-} symmetric stretching bands indicates an increased protein and nucleic acid content, respectively (Fujioka et al., 2004; Mega Tiber et al., 2011; Yonar et al., 2022). The results of our analysis indicated that the band area values for both bands were markedly elevated in MCF7 breast cancer cells grown on collagen type-1 and fibronectin-coated surfaces relative to the control group and cells grown on laminin-coated surfaces (Figure 3). Comparatively, other studies have also reported similar findings. For example, Verdonck et al. (2016) used FTIR imaging to identify distinct spectral characteristics in breast cancer tissues, which were attributed to variations in protein and nucleic acid content. The ECM of a normal mammary gland undergoes substantial alterations in cases of breast cancer, with a desmoplastic reaction being a hallmark of the disease. These alterations are driven by increased secretion of fibrous further supporting the notion that ECM composition can modulate cellular properties. This observation indicates that ECM proteins, such as collagen type I and fibronectin, exert a dual influence on both protein biosynthesis and nucleic acid biosynthesis in breast cancer cells by means of activating gene expression. Conversely, the action of enzymes that remodels the ECM, such as matrix metalloproteinases (MMPs) and heparanase, results in the degradation of the basement membrane that encircles the mammary gland epithelium. Normal breast myofibroblasts secrete Laminin-111 (LM-111), which is an essential constituent of basement membranes and plays a critical role in their maintenance. The maturation of breast epithelial cells is induced by prolactin, and this process is promoted by LM-111 (Streuli et al., 1995). In breast tumors, the expression of LM-111 is frequently lost, and it has been associated with alterations in cell polarity (Gudjonsson et al., 2002). In 3-dimensional cultures of breast cancer cells, LM-111 has been shown to reduce the expression of DNA methyltransferase 1 (DNMT1), thereby hindering DNA methylation of the E-cadherin promoter and enhancing the levels of E-cadherin expression. This phenomenon leads to an increased expression of cell-cell adhesion proteins, which suggests that LM-111 might act as a potential inhibitor of breast cancer proliferation and metastasis (Benton et al., 2009).

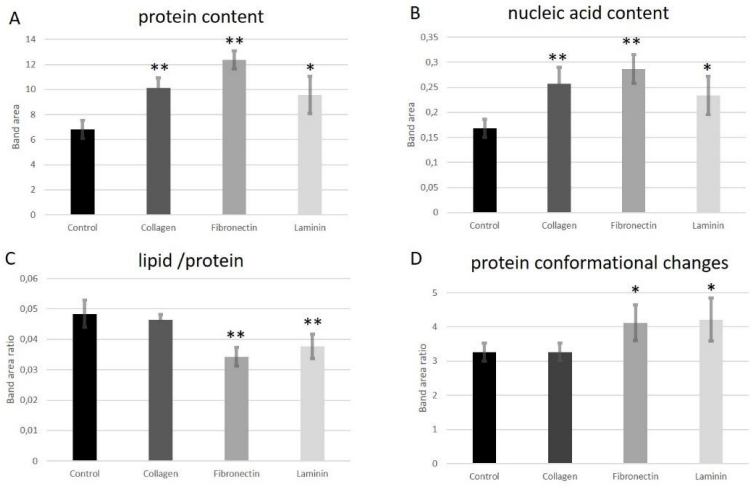

Figure 3: The band area values of the Amide I (A) and PO_2^- symmetric stretching (B) bands are presented. The ratio of the sum of the band area values of CH_2 antisymmetric + CH_2 symmetric stretching to the sum of the band area values of Amide I and Amide II are represented in (C). The ratio of the band area values of Amide I to Amide II are represented in (D). The results are expressed as the mean ± SEM, with * indicating $p < 0.05$ and ** indicating $p < 0.01$.

Figure 4 illustrates the principal component analysis (PCA) results of the study. Prior to conducting the PCA analysis, the full region of the spectrum was initially baseline offset corrected, followed by peak normalization with respect to the peak at 1635 cm^{-1}. The PCA analysis performed were able to distinctly differentiate the spectra of cells cultivated on collagen and fibronectin from the control cells, whereas the spectra of cells grown on laminin exhibited a greater degree of dispersion across a more extensive region (Figure 4). This finding aligns with recent studies that have explored the interaction between ECM components and breast cancer progression. These studies suggest that ECM components, such as collagen type I and fibronectin, induce distinct metabolic alterations in cancer cells, enabling them to proliferate and migrate (Bhattacharya et al., 2023). The Hippo pathway, for

instance, has been shown to regulate ECM composition and its impact on tumor invasiveness and metastasis (Yang et al., 2024). Additionally, research by the Zhao et al. (2021) has highlighted the role of ECM proteins in promoting resistance to therapeutic interventions.

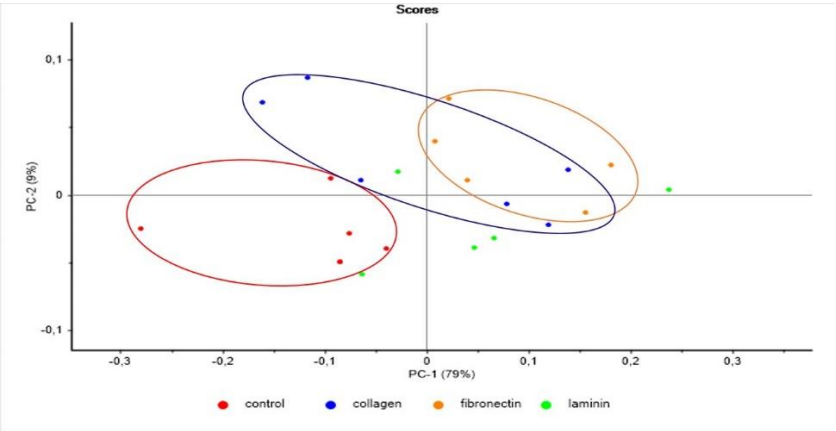

Figure 4. A scores plot in the PC2 plane has been presented for the full spectral region, encompassing all samples: those designated as Control, Collagen, Fibronectin, and Laminin.

CONCLUSION

Overall, our results contribute to the growing body of evidence that ECM proteins significantly influence the biochemical and metabolic landscape of breast cancer cells. The findings of the present study underscore the importance of ECM interactions in cancer progression and highlight potential avenues for therapeutic intervention. These findings also highlight the potential of FTIR analysis as a tool for investigating these interactions. Future studies could explore the mechanistic pathways through which fibronectin and collagen type-I influence lipid metabolism and membrane dynamics, providing deeper insights into their roles in cancer progression.

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Authors' Contributions: Ediz Sarıışık designed and conducted the experiments, carried out the data analysis, and was the primary author of the manuscript. Fatma Küçük Baloğlu contributed to the execution of the experiments and assisted in the analysis of the data.

Statement of Research and Publication Ethics: The author declares that this study complies with Research and Publication Ethics.

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