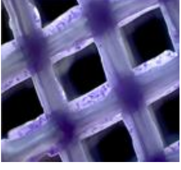

JOMIT

Journal Med. Inv. Tech. 2019; 1 (2): 45-50

Research Article

Investigation of Surface Adhesion of MCF-7 Cells in 3D Printed PET and PLA Tissue Scaffold Models

3B Baskılı PET ve PLA Doku İskele Modellerinde MCF-7 Hücrelerinin Yüzey Adezyonlarının Araştırılması

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Abstract: Tissue scaffolds with a wide range of applications are usually rigid structures made of polymeric materials. Biocompatibility and biodegradability are important properties for scaffold materials to possess, ensuring they support for cell growth and are extremely useful in *in vitro* three-dimensional (3D) cell cultures. Cancer is a disease caused by mutations or abnormal changes in genes responsible for regulating the growth of cells and keeping them healthy. Breast cancer is the most common type of invasive cancer and the second cause of cancer death among women. Two-dimensional (2D) cell cultures have helped to attain important knowledge about cell biology and biochemistry. However, they are not suitable for clinical use. 2D *in vitro* studies do not provide the desired success in *in vivo* applications. The formation of the tumor microenvironment is challenging. Tissue scaffolds are 3D cell culture systems that eliminate this problem with breast cancer cell culture. Cell culture models with 3D tissue scaffold are thought to be more successful in representing *in vivo*. The main objective of this study was to produce biocompatible and suitable porosity scaffolds from polylactic acid (PLA) and polyethylene terephthalate (PET) materials, which enables MCF-7 breast cancer cells to proliferate in three dimensions. Polyethylene terephthalate (PET) and polylactic acid (PLA) are biocompatible, non-toxic dye-free polymers and are used for the production of scaffolds that are rigid structures suitable for 3D cancer cell culture. A custom 3D printer and 1.75 mm PET and PLA filaments were used for the production of tissue scaffolds. Tissue scaffolds are produced with two different filling rates (20% and 40%). The design and production parameters of the scaffolds are defined and optimized by SolidWorks and Slic3r softwares to set the correct printing procedure. Biomechanical tests for mechanical characterization of all scaffolds were performed. MCF-7 breast cancer cell line was used to evaluate tissue scaffolds for 3D cell culture. The ability of the cells to adhere to the scaffold surface was determined by crystal violet fixation and staining method detecting viable cells. 3D cell culture with PET and PLA tissue scaffolds is useful to improve cancer cell culture applications and enhance cell proliferation. 3D tissue scaffolds have shown that MCF-7 cells are more compatible with surface adhesion than 2D cultures. As a result, the data obtained show that porous PET and PLA tissue scaffolds are supportive of the 3D culture and proliferation of MCF-7 breast cancer cells by providing a micro-environment *in vivo* mimic.

Keywords: Breast cancer, MCF-7, PLA, PET, 3-dimensional, scaffold, cell culture

Geyik O.G, Nalbant B, Husemoglu R.B, Yuce Z, Unek T, Havitcioglu H, 2019. Investigation of Surface Adhesion of MCF-7 Cells in 3D Printed PET and PLA Tissue Scaffold Models, *Journal of Medical Innovation and Technology*

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Özet: Doku iskeleleri genellikle, geniş bir uygulama alanına sahip polimerik malzemelerden yapılmış sert yapılardır. Biyouyumlulukları ve biyolojik olarak degradasyonları sayesinde üç boyutlu (3B) hücre büyümesi için gerekli desteği sağlar ve *in vitro* 3B hücre kültürlerinde son derece faydalıdır. Kanser; hücrelerin büyümesini düzenleyen ve onları sağlıklı tutmaktan sorumlu olan genlerin mutasyonları veya anormal değişikliklerinden kaynaklanan bir hastalıktır. Meme kanseri, en yaygın invazif kanser türüdür ve kadınlar arasında ikinci kanser ölüm nedenidir. İki boyutlu (2B) hücre kültürü, hücre biyolojisi ve biyokimyası hakkında önemli bilgiler edinilmesine yardımcı olmuştur. Ancak, klinik kullanım için uygun değildir. 2B *in vitro* çalışmalar, *in vivo* uygulamalar da istenilen başarıyı sağlayamamaktadır. Tümör mikro-ortamının oluşturulması zordur. Doku iskeleleri, meme kanseri hücre kültürü ile ilgili bu problemi ortadan kaldıran 3B hücre kültürü sistemleridir. 3B hücre kültürü modellerinde gerçekleştirilecek çalışmalar, *in vivo*'yu taklit etmede daha başarılı olacaktır. Bu çalışmanın temel amacı, MCF-7 meme kanseri hücrelerinin üç boyutta prolifere olmasını sağlayan polilaktik asit (PLA) ve polietilen tereftalat (PET) materyallerden biyouyumlu ve uygun gözenekli doku iskeleleri oluşturmaktır. Polietilen tereftalat (PET) ve polilaktik asit (PLA), biyolojik olarak uyumlu, toksik boya içermeyen polimer olup, 3B kanser hücre kültürü için uygun rijit yapılar olan doku iskelelerinin üretimi için kullanılmaktadır. Doku iskelelerinin üretimi için özel yapım 3B yazıcı ve 1.75 mm PET ve PLA filamentleri kullanılmıştır. Doku iskeleleri iki farklı doluluk oranı (%20 ve %40) ile üretilmiştir. Doku iskelelerin tasarım ve üretim parametreleri, doğru baskı prosedürünü ayarlamak için SolidWorks ve Slic3r yazılımları tarafından oluşturulmuş ve optimize edilmiştir. Tüm doku iskelelerin mekanik karakterizasyonu için biyomekanik testler gerçekleştirilmiştir. 3B hücre kültürü için doku iskelelerinin değerlendirilmesi amacıyla MCF-7 meme kanseri hücre hattı kullanılmıştır. Hücrelerin iskele yüzeyine adezyon kabiliyeti, canlı hücreleri tespit etmeye yönelik olarak kullanılan kristal viyole ile fiksasyon ve boyama yöntemiyle belirlenmiştir. PET ve PLA doku iskeleleriyle 3B hücre kültürü, kanser hücre kültürü uygulamalarını geliştirmek ve hücre proliferasyonunu zenginleştirmek için faydalı olmaktadır. 2B hücre kültürüne kıyasla 3B doku iskeleleri, MCF-7 hücrelerinin yüzeye tutunma düzeylerinde anlamlı bir artış göstermiştir.
Sonuç olarak, elde edilen veriler gözenekli PET ve PLA doku iskelelerinin, *in vivo* mimiği bir mikro-ortam sağlayarak MCF-7 meme kanseri hücrelerinin 3B kültüre edilmesi ve proliferasyonu için destekleyici olduğunu göstermektedir.
Anahtar Kelimeler: Meme kanseri, MCF-7, PLA, PET, üç boyutlu, doku iskelesi, hücre kültürü

Hüsemoğlu R.B, Geyik Ö.G, Nalbant B, Yüce Z, Ünek T, Havıtçıoğlu, 2019. 3B Baskılı PET ve PLA Doku İskele Modellerinde MCF-7 Hücrelerinin Yüzey Adezyonlarının Araştırılması, *Medikal İnovasyon ve Teknoloji Dergisi*

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1. Introduction

In vitro cell culture is a basic technique for the research of cell behavior mechanisms [1]. By this method, we are able to study various cellular properties such as migration, differentiation, proliferation, and mechanics in biochemical and biomechanical micro-environments. Even though conventionally performed 2D (two dimensional) cell cultures yield useful outcomes for cellular mechanisms, studies failed to reflect the *in vivo* responses. The inability to appropriately model certain types of cancers in 2D is one of the reasons accounts for this problem [2].

Additionally, 2D cell cultures cannot reflect the differentiation and characteristics of cancer cells in vivo [3]. Since the cells exist in an organism embedded in a 3D (three dimensional) ECM (extracellular matrix) that contains various proteins and molecules, it is vital to mimic the *in vivo* environment in order to obtain accurate results. Therefore, 3D cell culture systems equipped with scaffolds have begun to be used. These systems provide more reliable results. There are many 3D cell scaffolds. One of the most widely used materials are biopolymers. Its porous structure allows cells to interact with each other better in terms such as their natural morphology and *in vivo* environment [4]. 3D polymeric cell scaffolds are an increasingly innovative method for

reconstructing tumor microenvironment in the culture medium [5].

Our aim is to determine the optimal three-dimensional cell scaffold characteristics to ensure adhesion and proliferation of the breast cancer cell line MCF-7. For this purpose, we produced cell scaffolds in a 3D printer using polyethylene terephthalate (PET) and polylactic acid (PLA) materials.

PET is a non-toxic, biocompatible, non-carcinogenic biostable linear polymer with good mechanical properties. PET use in cell culture applications and medical applications [6]

PLA is a thermoplastic aliphatic polyester suitable for biomedical and cell culture applications due to its biocompatibility, high strength and biodegradable properties [1,4]. PLA has been found to be beneficial because of the porous scaffold structure that enriches the cell microenvironment and also of increasing the viability and proliferation of breast cancer cells [4].

Here we present an in vitro investigation of adhesion levels of human breast cancer MCF-7 cell line cultures on PET scaffolds with different densities in comparison with PLA. The results obtained here are important for understanding *in vivo* procedures.

An in vitro investigation of adhesion levels of human breast cancer cell line on PLA and PET scaffolds with different densities was performed previously by our team. Here we have continued to test scaffolds made of biopolymers with different characteristics in order to determine the optimum material and density for breast cancer cell adhesion *in vitro*.

2. Materials and Methods

2.1. Design and Manufacturing of Scaffolds

Scaffolds were prepared with the PLA / PET filaments using a custom-made fused deposition modeling (FDM) printer. Scaffolds were fabricated according to the FDM method in a single extruder 3D printer, which uses hot end extruder for printing PLA/PET filaments. This printer has a mechanical precision of 100-100-100 μm in the X-Y-Z axis. 0.3 mm nozzle and 1.75 mm filaments were used for the production of tissue scaffolds. Tissue scaffolds are produced with two different filling rates (20% and 40%). The nozzle temperature was set to 230°C for each materials.

The design and production parameters of the scaffolds are defined and optimized by SolidWorks and Slic3r software to set the correct printing procedure. Scaffolds template (Ø = 4 mm, thickness = 2 mm) were designed using SolidWorks 2017 software and subsequently filled and sliced using and Slic3r 1.2.9 software to obtain cylindrical STL models. Briefly, clump generator software was used to create squared pores into a 3D object in a “stl” file format. The printing head was computer-controlled in three axes (X, Y, Z) while extruding the PLA and PET filaments using the Slic3r software.

The melted PLA and PET was then extruded through a 0.3 mm diameter stainless-steel nozzle on to a printing plate heated at 40°C. Porous scaffolds were printed layer-by-layer in the form of squares surrounded by a dense PLA perimeter. We determined the optimal speed of 15 mm/s for the filling speed and 25 mm/s for the gap speed. The travel speed of extruder was set to be 100 mm/s. We fabricated scaffolds with two different pore sizes (20%-40%).

After printing, the scaffolds were carefully removed from the printing bed. Prior to biological evaluations, printed scaffolds were sterilized by ethylene oxide (EtO).

2.2. Mechanical Characterization

To investigate the possible influence of pore dimensions on mechanical properties of the printed PET and PLA structure, a uniaxial compression test was performed on scaffolds. Five scaffolds were tested for each pore size (20%-40%) for each group. Scaffolds were tested by universal compression test machine (Shimadzu Autograph AG-IS 5kN). Scaffolds were compressed at a speed of 10 mm/min until 1 mm thickness. Maximal strength (F max) was then recorded simultaneously using the Trapezium software.

2.3. Cell Culture and Staining

MCF-7 breast adenocarcinoma cells were used in order to assess the optimum scaffold type and filling rate. MCF-7 cells were cultured in complete RPMI-1640 (10% FBS, 2 mM L-Gln, 1% 10,000 U/mL Penicillin/Streptomycin) (Gibco/Thermo Fisher, MA, USA) and incubated in humidified chamber with 5% CO_2 at 37°C.

After PET and PLA scaffolds with 20% and 40% filling rates were printed, scaffold tablets were placed in 96 well plates under sterile conditions. 150,000 cells were seeded on each sterilized scaffold tablets in 50 µL complete growth medium and cultured overnight. Following the incubation period, media were removed from the wells, rinsed with PBS (Gibco/Thermo Fisher, MA, USA) and adhered cells on scaffolds were detected by crystal violet staining. Images were obtained using Olympus CKX41 microscope mounted with UIS2 camera. Cell counts were determined using ImageJ. Graphs were plotted using GraphPad Prism 7.

3. Results

3.1. Mechanical Characterization of Scaffolds

The mean maximum compression strengths for groups PET 20%, PET 40%, PLA 20%, and PLA 40% were 179.22 N, 455.00 N, 199.82 N, and 732.37 N, respectively. When PLA and PET scaffolds were compared, it was found that PLA had better strength than PET scaffolds (Figure 1).

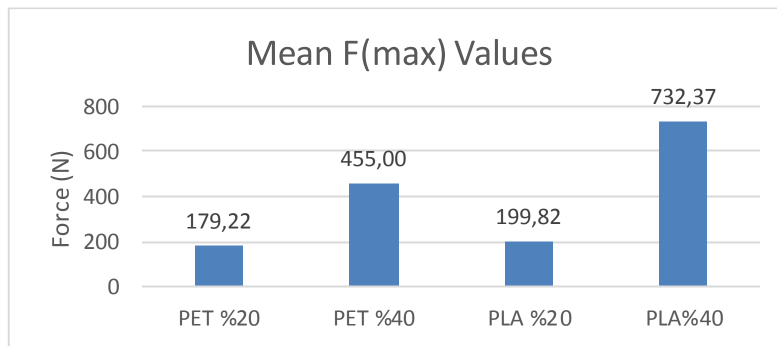

Figure 1.
The mean values of maximum compressive strength of PET and PLA scaffolds in all the groups.

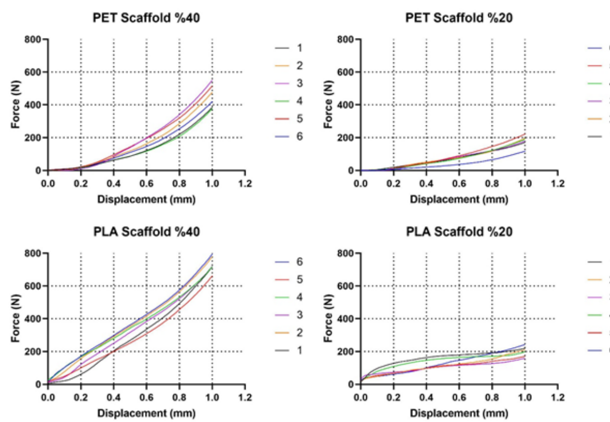

Figure 2.
Curves resulting from the equations for the force-displacement ratio. Force-displacement curves for all groups (n=24) with a displacement rate of 10 mm/min.

3.2. Cell Culture and Staining

This experiment was performed with scaffolds in three replicates. Five different regions and two different layers were captured for each scaffold tablet. Images of all regions of the lower and upper layers of scaffolds were taken with inverted microscope. PLA material showed higher number of stained cells than PET material (Figure 3)

Cell count analysis by ImageJ showed that higher filling rate enabled more cells to adhere on and also showed that the number of cells adhered on the PLA scaffold is higher than the PET scaffold (Figure 4)

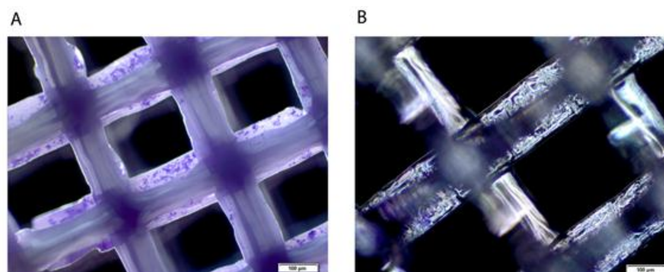

Figure 3.
Images of crystal violet stained MCF-7 cells on **A)** PLA 20% lower layer **B)** PET 20% lower layer

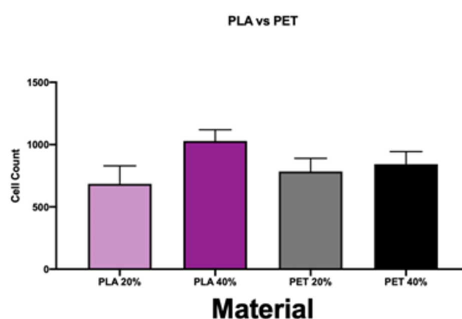

Figure 4.
Cell numbers determined using ImageJ for PLA and PET scaffolds. A significant difference wasn't found between them (n=3, Bars: SD).

4. Discussion

Previously, our group has studied adhesion properties of MCF-7 in cell culture with 3D printed scaffold models including PLA and PCL materials. As a result of this study which was accepted by the Turkish Journal of Biochemistry in October 2019, we found that the PLA scaffold was more preferable than PCL scaffold.

Our aim as the study group is to determine the optimum material to be used in cell culture and also *in vivo* by experimenting with different biomaterials. In this study, we compared PLA and PET scaffolds.

In cancer biology studies, the limitations of 2D cell culture were exceeded by 3D cell culture techniques. These limitations are a hindrance to understanding the mechanisms of cancer. So, we need platforms that provide the closest mimic to the natural environment of cancer cells. For this purpose, various 3D cell culture systems have been developed to overcome the limits of 2D cultures [7].

Diomede et al. showed that 3D printed porous PLA scaffolds enrich the physical and chemical properties for cell culture [8]. Compared to 2D cell culture, in 3D cell culture cells proliferate slower but they have been proven to survive longer. Emma et al. showed that PLA scaffolds make the optimal device for *in vitro* 3D cell culture [9].

Rimington et al., proved that cells cultured on PET and PLA supply a viable metabolic phenotype within a cell culture. They observed no difference in terms of cell viability between

scaffolds [10]. Similar to these studies, we found no significant difference between PET and PLA scaffolds. Both scaffolds provide an appropriate culture environment for breast cancer cells.

5. Conclusion

In conclusion, the data obtained showed that porous PET and PLA tissue scaffolds can be supportive of the 3D culture and for adhesion of MCF-7 breast cancer cells by providing a micro-environment *in vivo* mimic. PLA is a more preferable polymer when compared to PET and a more porous (40% fill rate) surface is better in terms of adhesion than a less porous surface (20% fill rate).

Progress on testing of different biocompatible polymers in vitro would be great contribution in this field.

For developing cell-type specific, optimum for cell adhesion, proliferation, and other growth properties and taking research for a better implantation in patients to a next level, we will continue to our studies with different bio-polymers in the market and different cell lines. That way we will accumulate information for the best conditions to study each cell line in vitro and also best polymer to use in a patient.

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