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Bone Cancer Metastatic Model on Pla Scaffold with Breast Cancer Cell Lines

PLA skafold Üzerinde Meme Kanseri Hücre Hatları ile Kemik Kanseri Metastatik Modeli

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ABSTRACT

Breast cancer (BC) represents the most frequent cancer case in women (29%), with a high mortality rate. Bone metastasis occurs in around 20–50% of the cases, and despite the advances in BC research, the interactions between tumor cells and the metastatic microenvironment remain poorly understood. In vitro 3D models gained a considerable interest in cancer research due to their reproducibility, 3D spatial cues and associated low costs, compared to in vivo and 2D in vitro models. In this study, we investigated the suitability of a poly-lactic acid (PLA) foam as a 3D in vitro model to study and compare the 3D structure of three different breast cancer cell lines (MCF-7, 4T1, MDA-MB-231). PLA foam open porosity (>70%) appeared to be suitable for mimicking the trabecular bone structure (hexagonal structure). The PLA foam showed good mechanical properties under cyclic compression (E = 69–109 kPa), even lower than human trabecular bone. This study presents a new 3D cell culture model for metastatic BC for researchers to construct in vitro bone morphology.

Keywords: *Metastatıc model, breast cancer, 3d modeL*

ÖZET

Meme kanseri (MK), kadınlarda en sık görülen kanser türünü (%29) temsil eder ve yüksek bir mortalite oranına sahiptir. Kemik metastazı olguların %20-50'sinde meydana gelir ve meme kanseri araştırmalarındaki ilerlemelere rağmen, tümör hücreleri ile metastatik mikroçevre arasındaki etkileşimler hala yetersiz anlaşılmaktadır. İn vitro 3D modeller, in vivo ve 2D in vitro modellere kıyasla, tekrarlanabilirlikleri, 3D uzamsal ipuçları ve ilişkili düşük maliyetleri nedeniyle kanser araştırmalarında büyük ilgi görmektedir.Bu çalışmada, poli-laktik asit (PLA) skafoldun, üç farklı meme kanseri hücre hattının (MCF-7, 4T1, MDA-MB-231) 3D yapılarını incelemek ve karşılaştırmak amacıyla bir 3D in vitro model olarak uygunluğunu araştırdık. PLA skafold açık gözenekliliği (%70'ten fazla), trabeküler kemik yapısını (altıgen yapı) taklit etmek için uygun göründü. PLA skafold, döngüsel basınç altında iyi mekanik özellikler göstermiştir (E = 69-109 kPa).Bu çalışma, metastatik meme kanseri için yeni bir 3D hücre kültürü modelini oluşturmaktadır.

Anahtar Kelimeler: *Metastatik model, meme kanseri, 3d model*

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INTRODUCTION

Breast cancer is the most frequent cancer case in women (29%) and globally represents the leading cause of cancer-related death in females [1]. Although several advances in breast cancer (BC) management have been made in the last decades, metastatic cancer still represents the most common cause of cancer-related mortality among breast cancer patients. Specifically, 20–50% of patients with early BC eventually develop metastatic disease [2], which is generally incurable.

Tissue engineering applications have been extensively focused on the in vitro investigations of cells proliferated and grown on three-dimensional (3D) polymeric scaffolds. In recent years, 3-D cell cultures have been suggested as a better study model for investigating complex biological processes than cells grown in monolayers [3,4]. In cancer research, the biology of tumor development in vivo can be mimicked similarly in vitro, using a scaffold to form a tissue-like structure for tumor cells to grow in.

Growth of cells in 3D scaffolds typically involves cell seeding onto a scaffold which, following their adhesion, spreading, and proliferation can develop into a tissue-like structure. One of the essential requirements for promoting the 3D growth of cells in vitro is to design a suitable polymeric scaffold that provides a structural template for cell adhesion and growth. The ideal scaffold is made by a material which biodegraded at the same rate as extracellular matrix disposition therefore no residual polymer is left [6]. It is also necessary that scaffolds do not form any product(s) which may interfere with cell growth.

This study presents a polymeric scaffold which is biodegradable and inert while available to cell adhesion. This scaffold was constructed to mimic bone trabecular morphology (hexagonal structure), which makes it a good candidate to study bone metastatic tumor morphology as a 3D model.

MATERIAL-METHODS

Preparation of Scaffolds by FDM

Scaffolds were prepared with the PLA filaments using a custom FDM printer with a 0.3-mm nozzle. Scaffolds template (Ø = 20.0 mm, thickness = 2.0 mm) were designed using SolidWorks 2017 software and subsequently filled and sliced using and Slic3r 1.2.9 software to obtain hexagonal STL models (Fig x). The total volume was 628 mm^2.

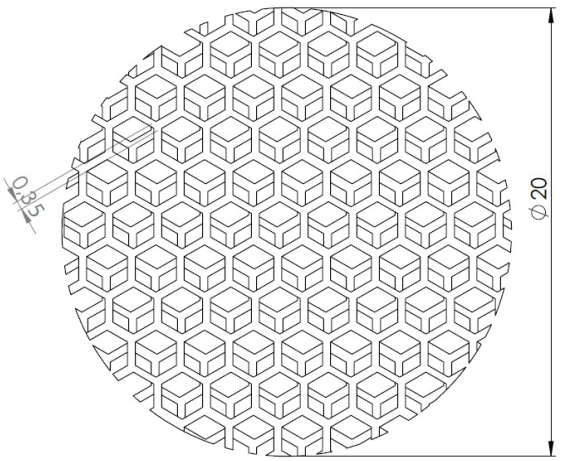


Fig 1. Hexagonal Scaffold Design in Solidworks 2017

Fabrication of a 3D Printed Scaffolds

A scaffold was fabricated according to the fused deposition modeling (FDM) method in a single extruder 3D printer, which uses hot end extruder to print PLA filaments. This printer has a mechanical precision of 100-100-200 µm in the X-Y-Z axis. Primarily, the scaffolds were designed in Soldworks, after which the critical parameters of the distance between columns and the width and height of columns were optimized, followed by the optimization of the printing parameters. The gap speed in the slicer (Slic3r) program was optimized to obtain a uniform porosity in the Z direction. We determined the optimal speed of 15 mm/s for the fill speed and 25 mm/s for the gap speed. The travel speed of the extruder was set to be 100 mm/s. Settings were a layer height of 0.3 mm, a hexagonal infill pattern, a solid top and bottom value of 0 and a perimeter value of 0. The printing bed was heated to 40°C for the first layer of the print to facilitate adhesion. Printing was done

through a 0.3 mm nozzle. The porosity of the bottom of the scaffolds was set to 70%. Polylactic acid (PLA) was used as the printing material at a printing temperature of 250°C. After printing, the scaffolds were carefully removed from the printing bed.

Cell Culture

In this study, three types of breast cancer cell lines as MCF-7 (HTB-22), MDA-MB-231 (HTB-26), and 4T1 were cultured. Breast cancer cell lines MCF-7 (HTB-22), MDA-MB-231 (HTB-26) was incubated in a medium consisting of 10% Fetal Bovine Serum (FBS), 1% L-Glutamine, 1% penicillin&streptomycin solution, Dulbecco's Modified Eagles Medium (DMEM) where 4T1 cell line was incubated in RPMI - 1640 medium containing 10% FBS, 1% L-Glutamine, 1% penicillin&streptomycin solution at incubation conditions, 5% CO2 and 37oC. When incubated flasks reached %80 density, cultures were passaged to culture flasks at a 1:2 ratio, using trypsin/EDTA solution to detach cells from the surface of the flask. Cells have been proliferated via passaging until enough cells or the experiment groups were obtained. Scaffolds were sterically placed into 12 well plates. 2 ml medium (RPMI 1640-DMEM) was added onto scaffolds, and wells were incubated at 5% CO2 and 37oC for an hour. Cells were detached from flasks using tyripsin/EDTA solution and added to well plates paying attention to properly place in PLA pores.

RESULTS

In this study, a bone cancer metastasis model was developed using three different breast cancer cell lines (4T1, MDA-MB-231, MCF-7). Scaffolds in a bone trabecular structure (hexagonal) were produced. Cell structures on the scaffold were demonstrated using light, SEM and confocal microscopy. This research analysed the in vitro bone cancer structure developed by the metastasis of breast cancer. Breast cancer cell lines 4T1, MDA-MB-231 and MCF-7 were cultured in in vitro conditions using scaffolds produced to mimic bone tumor morphology (hexagonal structure). Adhesion and proliferation of cell lines on scaffolds were observed with the light microscope and corresponding images were captured. Breast cancer cells proliferated on scaffolds were also investigated with 3D screening microscopes, SEM and Confocal microscopy. Cells were stained with trypan blue. Observations of cell lines on the PLA scaffold were compared regarding adherence to the scaffold and proliferation rates. Since MCF-7 and 4T1 cells have lineal morphology and are more aggressive, which means the proliferation rate is higher, than the other two cell lines, these cells adhered to the PLA scaffold more efficiently and formed cell colonies. MDA-MB cells were observed to be poorly adhered to PLA scaffold due to their morhological properties. The continuation of this study will involve observation of tumor formation in laboratory animals after implanting them the scaffolds cultured with cancer cells.

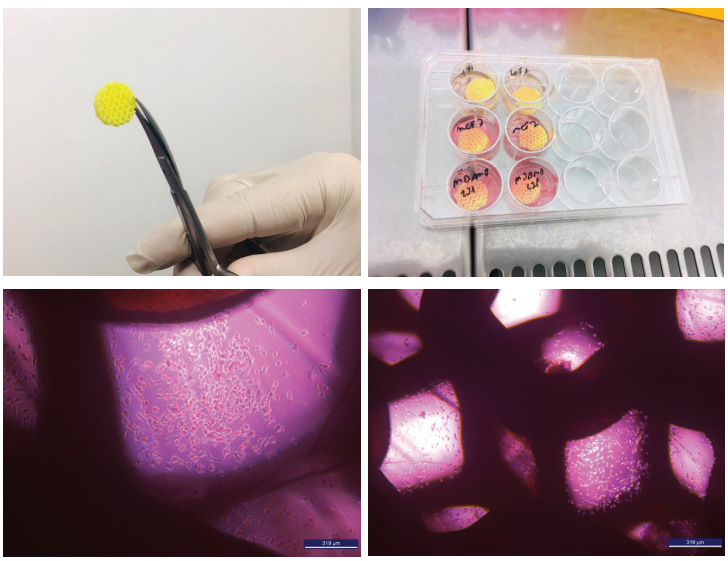


Fig. 2. Light microscope images of cells on PLA scaffold.

SEM Analysis

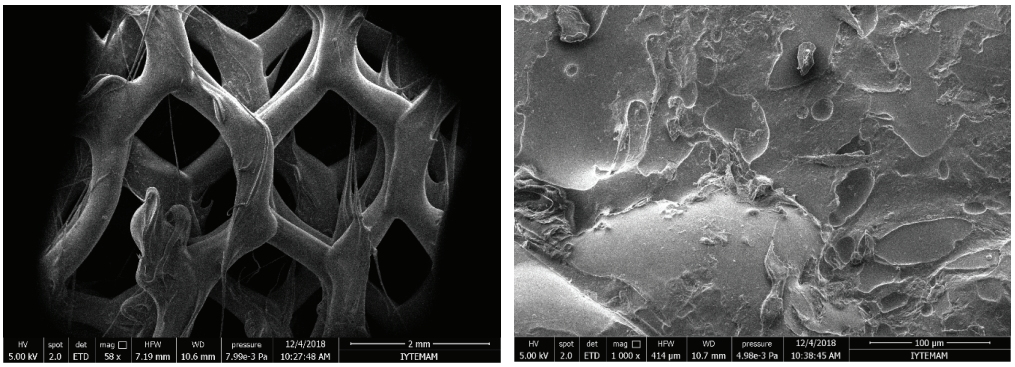
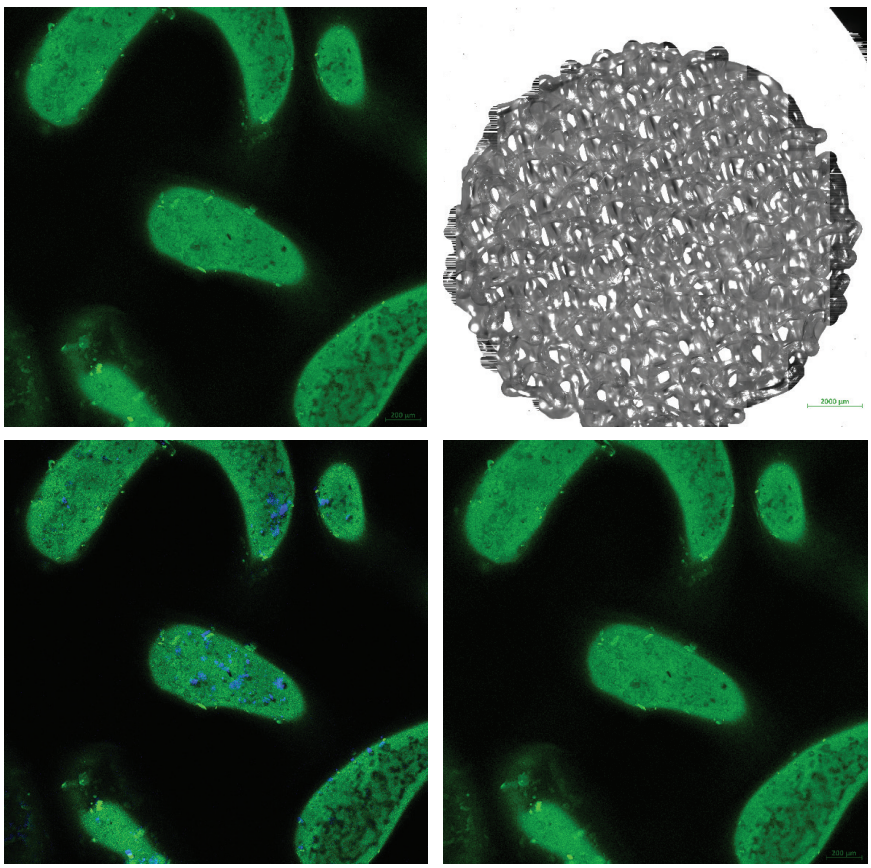


Fig. 3. SEM microscopy images of cells on PLA scaffold

Confocal Microscopy Images



4T1 cell lines on the PLA scaffold showed colony formation

MDA-MB cell lines showed poor adherence and proliferation on PLA scaffold

DISCUSSION

Compared to the traditional 2D condition, 3D co-culture system conditions are more similar to physiological conditions and reflect better the in vivo interactions between tumor and stroma. Thus, these culture systems represent a more genuine method of making predictions [8]. In vitro 3D models provide an efficient combination of demonstrating the complexity of the biological tissue characterizing in vivo models and being experimentally reproducible as 2D in vitro models [9,10]. Advances in tissue engineering have paved the way for the design of scaffold-based (SB) cell culture systems that are more powerful tools to mimic not only the natural structure, with an open porosity percentage over 70% but also a 3D extracellular matrix of trabecular bone tissue. In recent years, in vitro SB models designed specifically for the research of breast cancer bone metastases are still being developed and improved; the design and development of an efficient 3D in vitro model is closely correlated with the procurement of a biomimetic 3D morphology that simulates the metastases development. Pathi et al. [12] reported that a poly-lactide-co-glycolide (PLG) porous scaffold and substantiated the fundamental role of additional hydroxyapatite (HA) in obtaining a more biomimetic morphology. Talukdar et al. [14] utilized an engineered 3D silk fibroin-based scaffold to assess direct and indirect interactions between tumor cells and osteoblasts. The biggest and improvable challenge in designing an efficient 3D in vitro SB culture model is to achieve the biomimetic properties of the tissue to be modeled in terms of morphological, chemical, mechanical and biological cues to be administered to the cultured cells.

Compared to traditional 2D in vitro cultures, which are insufficient to mimic physiological conditions and thus oversimplify the 3D tissue morphology, 3D in vitro models play a critical role in being an exceptional alternative in tumor metastases development studies. Also, these 3D in vitro culture models can be prefferred more than in vivo studies which are affected by low producibility and ethical issues. In this study, we demonstrate the suitability of porous polyurethane foam, synthesized using an appropriate formulation, in mimicking the bone tissue morphology (hexagonal) and reproducing the metastatic colonization derived from human breast cancer.

CONCLUSION

This study presents a new 3D cell culture model for metastatic BC for researchers to construct in vitro bone morphology. Scaffolds used in this study are better for demonstrating bone morphology since they are hexagonally designed and, therefore, reflect bone trabecular structure.

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