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A three dimensional micropatterned tumor model for breast cancer cell migration studies

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ABSTRACT

Breast cancer cell invasion is a highly orchestrated process driven by a myriad of complex microenvironmental stimuli, making it difficult to isolate and assess the effects of biochemical or biophysical cues (i.e. tumor architecture, matrix stiffness) on disease progression. In this regard, physiologically relevant tumor models are becoming instrumental to perform studies of cancer cell invasion within well-controlled conditions. Herein, we explored the use of photocrosslinkable hydrogels and a novel, two-step photolithography technique to microengineer a 3D breast tumor model. The microfabrication process enabled precise localization of cell-encapsulated circular constructs adjacent to a low stiffness matrix. To validate the model, breast cancer cell lines (MDA-MB-231, MCF7) and non-tumorigenic mammary epithelial cells (MCF10A) were embedded separately within the tumor model, all of which maintained high viability throughout the experiments. MDA-MB-231 cells exhibited extensive migratory behavior and invaded the surrounding matrix, whereas MCF7 or MCF10A cells formed clusters that stayed confined within the circular tumor regions. Additionally, real-time cell tracking indicated that the speed and persistence of MDA-MB-231 cells were substantially higher within the surrounding matrix compared to the circular constructs. Z-stack imaging of F-actin/α-tubulin cytoskeletal organization revealed unique 3D protrusions in MDA-MB-231 cells and an abundance of 3D clusters formed by MCF7 and MCF10A cells. Our results indicate that gelatin methacrylate (GelMA) hydrogel, integrated with the two-step photolithography technique, has great promise in the development of 3D tumor models with well-defined architecture and tunable stiffness.

1. Introduction

Metastatic dissemination of cancer cells is a highly complex and multi-step biological process starting with the invasion of cancer cells through the extracellular (ECM) matrix of the stroma toward the blood vessels [1–5]. Cancer cell invasion through the tumor stroma is governed by diverse factors including biochemical signals and biophysical cues [2]. There are many *in vivo* animal models used to conduct biological studies on cancer invasion, but despite their significance, most of these models present an abundance of confounding variables, which create difficulties in attributing specific microenvironmental cues to cellular invasion [6]. In this regard, physiologically relevant *in vitro* tumor models, with precise control over microenvironmental cues, are crucial to better understand cancer cell invasion within a native-like breast tumor microenvironment.

In the past few years, there has been a tremendous initiative to develop *in vitro* models to study cancer cell behavior within three-dimensional (3D) microenvironments. For instance 3D surface topographies have been widely used to study cancer cell behavior in response to various geometrical features [5,7–10]. Despite their significance, these platforms have been limited in terms of tuning native-like parameters such as stiffness and matrix architecture. Alternatively, a wide variety of 3D hydrogel-based matrices such as

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Matrigel® [11], fibrin [12], collagen [13], and polyethylene glycol (PEG) [14] have shown great promise to recapitulate cancer cell invasion in a 3D matrix and assess cellular behavior in response to various biophysical and biochemical cues. Such 3D hydrogel-based matrices enable cells to retain accurate phenotypes and, consequently, exhibit physiological responses to microenvironmental stimuli in addition to displaying accurate cell–cell and cell–matrix interactions [15]. Although these models have resulted in outstanding biological findings, they lack specific patterned features that would enable precise control over cellular distribution, matrix architecture and stiffness to conduct fundamental studies on tumor biology.

The integration of microengineering technologies and advanced biomaterials (e.g. hydrogels) has offered great promise to develop well-defined microenvironments for fundamental biological studies. These technologies are appealing because they enable tight control over the cellular microenvironment [16]. Particularly, through the use of photocrosslinkable hydrogels and micropatterning techniques, it is possible to generate biologically relevant constructs for tissue engineering and cancer related studies. However, there are still very few studies on the use of photocrosslinkable hydrogels in the development of biologically relevant tumor models [17–20].

In this study, we explore the use of a novel, two-step photolithography technique and gelatin methacrylate (GelMA) hydrogel to develop a highly organized micropatterned breast tumor microenvironment model. GelMA has been demonstrated to be an excellent candidate for generating biologically relevant tissue constructs [21] since cells have readily adhered to, proliferated within, and migrated throughout the 3D matrix when encapsulated within the hydrogel [22–24]. More importantly, the use of GelMA enables the creation of arrays of specific cell-laden features with high precision and fidelity [25]. Previous studies using GelMA hydrogel have been largely focused on tissue engineering and regenerative medicine applications [22,23,26,27], with very few focused on cancer studies [17,28]. The proposed platform, presented herein, has unique advantages through the ability to independently decouple different cell-embedded regions within the tumor model and independently tune their stiffness. Furthermore, the microfabricated model enables precise visualization of cancer cell migration within a 3D matrix in response to microenvironmental cues. In order to validate the proposed microengineered tumor model, we primarily assessed the morphology and proliferation of highly invasive human breast cancer MDA-MB-231 cells, non-invasive, tumorigenic human breast cancer MCF7 cells, and non-tumorigenic mammary epithelial MCF10A cells. In addition, we analyzed migration patterns and cytoskeletal organization of the cells within different regions of the micropatterned breast tumor models.

2. Materials and methods

2.1. Synthesis of GelMA hydrogels

GelMA preparation was completed similar to prior studies [21,25]. First, a 10% (**w**/**v**) solution of type A porcine skin gelatin (Sigma–Aldrich) was prepared in Dulbecco's phosphate buffered saline (DPBS; Gibco). This solution was then dissolved at 60 °C before proceeding to subsequent steps. Afterward, methacrylic anhydride (MA) was added drop-wise to the gelatin solution. Next, the mixture was stirred vigorously for three hours to ensure the completion of the reaction. In order to shift the equilibrium and stop the reaction, the mixture was diluted (5X) with warm (40 °C) DPBS. This crude prepolymer GelMA was dialyzed for one week in distilled water (replaced twice a day) using dialysis membranes (MWCO 12000-14000) at a constant temperature (40 °C) to filter out any salt byproducts created from the reaction between gelatin and MA. The desired degree of methacrylation was achieved by precisely controlling the proportion of MA to gelatin during synthesis (92 ± 2% methacrylation confirmed based on ^{1}H NMR). The gelatin methacrylate solution was lyophilized for one week to create a large quantity of dehydrated, porous macromer, which could be preserved for future experiments.

2.2. Cell culture

The invasive breast cancer MDA-MB-231 cell line, non-invasive tumorigenic breast cancer MCF7 cell line, and normal mammary MCF10A cell line were used in this study. All cell lines used during the course of the experiments expressed red fluorescence. Specifically, MDA-MB-231 cells expressing tdTomato were provided by the McCarty lab (Oregon Health & Science University), and MCF7/MCF10A cells were transduced with lentivirus to stably express cytosolic mCherry fluorescence. Cancer cells were maintained in 1X DMEM supplemented with 10% (**w**/**v**) fetal bovine serum (FBS), 1% (**w**/**v**) L-glutamine, and 1% (**w**/**v**) 1:1 penicillin:streptomycin. Normal mammary cells were maintained in DMEM:F12 supplemented with 1% (**w**/**v**) L-glutamine, epidermal growth factor (20 ng/mL), cholera toxin (100 ng/mL), insulin (10 μg/mL), hydrocortisone (0.5 mg/mL), and 5% (**w**/**v**) horse serum. All media and media supplements were provided by Life Technologies. Cells were kept at standard physiological conditions (humidified, 37 °C, 5% CO_2), were passaged weekly, and their media was changed every three days.

2.3. Microfabrication of the tumor model

In order to promote adherence of the GelMA hydrogel constructs, glass slides were functionalized with 3-(trimethoxysilyl) propyl methacrylate (TMSPMA, Sigma–Aldrich) as described in previous protocols [22,23]. Subsequently, a 7 μL drop of 20% (**w**/**v**) PEG prepolymer solution with 0.5% (**w**/**v**) photoinitiator (PI) (2-hydroxy-1-(4-(hydroxyethoxy)phenyl)-2-methyl-1-propanone) was placed onto cut (area: <1 cm^2), sterilized glass slides. Next, an untreated coverslip was placed on top of the PEG prepolymer and was then exposed to ultraviolet (UV) light (360–480 nm, 800 mW) for 50s to form a layer of PEG coating on top of the TMSPMA-treated glass slides.

To microengineer the tumor model, GelMA macromer was dissolved in DPBS containing 0.5% (**w**/**v**) PI to create the prepolymer solution. Cells were encapsulated in the prepolymer solution through resuspension of pelleted cells (cell density: 6 × 10^6 cells per mL of GelMA). The tumor model was patterned by first pipetting a 15 μL droplet of cell-laden GelMA onto a spacer (depth: 100 μm). A PEG-coated glass slide was then inverted on top of the spacer thereby spreading the prepolymer solution to cover the area of the glass slide and fill in the 100 μm depth of the spacer (Fig. 1A,B). A photomask (designed with AutoCAD software and printed by CAD/Art Services Inc., Oregon) was then placed on the inverted PEG-coated glass slide and exposed to UV light for 12s (Fig. 1C). The photomask had a layout as described in Table 1 where an 11 × 11 array of transparent circles (radius: 500 μm) was surrounded by a black unpatterned area. After UV exposure, the patterned glass slide was washed with DPBS to remove the remaining uncrosslinked prepolymer, revealing an array of circular constructs. Next, the glass slides were stored in warmed DPBS until all constructs were microfabricated. Following that, a 13 μL drop of pristine GelMA (no cells) was placed onto the spacer and the patterned glass slide was inverted on top of it (Fig. 1D–E). The empty areas between circular constructs were filled by the pristine GelMA. This assembly was exposed to UV light for another 5s in order to crosslink the gel filled in between the circular constructs