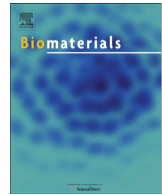
Biomaterials 114 (2017) 34–43

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials



Development of three-dimensional collagen scaffolds with controlled architecture for cell migration studies using breast cancer cell lines

Jonathan J. Campbell [a, 1, 2], Anke Husmann [a, *, 2], Robert D. Hume [b, 2], Christine J. Watson [b], Ruth E. Cameron [a]

[a] Cambridge Centre for Medical Materials, Department of Materials Science and Metallurgy, University of Cambridge, Cambridge CB3 0FS, UK
[b] Department of Pathology, University of Cambridge, Cambridge, CB2 1QP, UK

A R T I C L E I N F O

Article history:
Received 14 July 2016
Received in revised form
27 October 2016
Accepted 28 October 2016
Available online 1 November 2016

Keywords:
Breast cancer
Three dimensional migration
Collagen 1
Ice-templating technique
Scaffold architecture
Invasion

A B S T R A C T

Cancer is characterized by cell heterogeneity and the development of 3D *in vitro* assays that can distinguish more invasive or migratory phenotypes could enhance diagnosis or drug discovery. 3D collagen scaffolds have been used to develop analogues of complex tissues *in vitro* and are suited to routine biochemical and immunological assays. We sought to increase 3D model tractability and modulate the migration rate of seeded cells using an ice-templating technique to create either directional/anisotropic or non-directional/isotropic porous architectures within cross-linked collagen scaffolds. Anisotropic scaffolds supported the enhanced migration of an invasive breast cancer cell line MDA-MB-231 with an altered spatial distribution of proliferative cells in contrast to invasive MDA-MB-468 and non-invasive MCF-7 cells lines. In addition, MDA-MB-468 showed increased migration upon epithelial-to-mesenchymal transition (EMT) in anisotropic scaffolds. The provision of controlled architecture in this system may act both to increase assay robustness and as a tuneable parameter to capture detection of a migrated population within a set time, with consequences for primary tumour migration analysis. The separation of invasive clones from a cancer biomass with *in vitro* platforms could enhance drug development and diagnosis testing by contributing assay metrics including migration rate, as well as modelling cell-cell and cell-matrix interaction in a system compatible with routine histopathological testing.

© 2016 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction

The process of metastasis, whereby cancer cells are able to disengage from a primary tumour and seed and colonise distant sites of the body, remains the primary key contributor to cancer lethality. Cell migration, together with the ability to degrade extracellular matrix is a critical requirement for invasion [1] and could form a potential metric for assessing drug potency alongside indices of cell death. However, to date the development of robust migratory 3D models has received little attention [2]. In addition, cellular heterogeneity is a defining feature of the cancer microenvironment, and *in vitro* test systems that can distinguish or even separate cell types dependent on their migratory or invasive ability could contribute enhanced platforms for diagnosis or aid drug development [3]. *In vitro* cellular assays for examining cancer invasiveness and migratory potential within 3D extracellular matrices remain potent tools for examining features of this process [4,5], however such models now need adaptation into standardized and reproducible formats suitable for high-throughput applications [6]. If migration is to form a useful parameter for studies of cell behaviour and drug efficacy in this context, 3D models should be consistent with regard to fabrication and incorporate deterministic architecture to minimise random cell migration patterns.

The Boyden chamber assay, exploiting a chemokine gradient between upper and lower chambers to drive cell invasion and migration, can be readily adapted to model specific extracellular matrix (ECM) chemistries by simple coating procedures. However, a comprehensive assessment of three-dimensional cell invasion or migratory mechanisms using histological techniques is difficult to achieve in these systems, with metrics usually limited to an end-point summation of cell numbers within the lower chamber.

* Corresponding author.
E-mail address: ah492@cam.ac.uk (A. Husmann).
[1] Current address: LGC, Queen Road, Teddington, Middlesex, TW11 0LY, UK.
[2] Equal first authors.

http://dx.doi.org/10.1016/j.biomaterials.2016.10.048
0142-9612/© 2016 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Controlling the spatial distribution of cells on extracellular proteins at the 2D interface is challenging and we anticipate that with development, biomaterial platforms with controlled *x,y* and *z* internal dimensions could prove a valuable advance. Such platforms could additionally be engineered to confine cells in a more physiological context at the point of seeding, with better control of substrate chemistry. Furthermore, there is a physiological basis for the provision of anisotropic matrices in studies of this type. Other groups have shown previously that ECM surrounding human breast tumours can be remodelled to display spatially anisotropic features and a high degree of fibre orientation [7,8]. Further studies have shown that anisotropy enhances the invasion of cancer cells into the surrounding stroma and supports their eventual metastasis [9–11]. Therefore, whilst allowing for more controlled analyses of migration, the internal properties of our anisotropic scaffolds may recapitulate *in vivo* ECM conditions.

This group has previously described the formation of complex mammary tissue architectures [12,13] using mixtures of epithelial and stromal cells within collagen-based porous biomaterials with controlled architecture, achieved through an ice-templating process [14]. In the present study, we hypothesize that 3D collagen scaffolds with radially aligned anisotropic pores enhance the directed migration of invasive breast cancer cell lines over non-invasive phenotypes, thus providing a 3D natural substrate for interrogating invasive and migratory properties of cancer. To test our model, we generated scaffolds with splayed radially aligned pores or random isotropic porous architecture by a controlled freeze drying procedure [15,16]. We propose that this anisotropy could be achieved by modifying a previously established freeze drying method and manipulating ice crystal growth to produce scaffolds with orientated pores emanating from a central nucleation point. The freeze drying strategy utilised a spatially controlled thermal gradient from a single point source, acting both as a point of origin for ice growth and producing a moulded feature within the 3D structure allowing spatially repeatable seeding of a cell inoculate. We tested the capacity of the system to support directional cell migration by seeding a range of breast cancer cell lines exhibiting varied invasive characteristics on the basis of luminal or basal epithelial phenotype. Aliquots of MCF-7 (luminal A, non-invasive), MDA-MB-231 (triple negative, basal, claudin-low, invasive) or MDA-MB-468 (triple negative, basal, invasive) were used to evaluate scaffolds exhibiting either isotropic and anisotropic pore orientations. MDA-MB-468 cells were also treated to undergo an epithelial-to-mesenchymal transition (EMT) to verify whether this would influence migration potential in anisotropic scaffolds. As well as assessing the distribution of cells in a temporal context we also monitored cell proliferation within these systems by both EdU and Ki67 staining.

2. Materials and methods

2.1. Scaffold synthesis

All materials were obtained from Sigma Aldrich (Poole, UK), unless otherwise stated. Scaffolds were prepared according to a modified previously published method [15,16,17]. Collagen from bovine achilles tendon was dispersed overnight in 0.05 M acetic acid at 4° C to make a 1 wt% collagen slurry. The slurry was homogenised at about 10,000 rpm for 30 min using an overhead homogeniser, keeping the container in an ice water bath, followed by centrifugation at 2500 rpm for 5 min to remove air bubbles.

The slurry was carefully aspirated within the scaffold moulds (Fig. 1), taking care to completely cover the copper pins with minimal bubble formation before a plate of glass was applied to the top of the mould chamber. For anisotropic scaffolds, see also Table 1, the freeze dryer shelf was cooled prior to use ensuring a shelf temperature of -40 °C. The moulds were placed so that the copper pins were in direct contact with the metal shelf. For isotropic scaffolds, the freeze dryer shelf was cooled from 20 °C to -40 °C over 1 h. The copper pins were thermally insulated with a thin rubber foam mat of less than 1 mm thickness so that they were not in direct thermal contact with the metal shelf. The freezing protocol was chosen to produce pore sizes around 100 μm away from the funnel [16]. Pore sizes in this range have been successfully used before [12,13].

Following freeze-drying, scaffolds were removed from the mould by carefully lifting the glass cover slide to which the collagen scaffolds stick very well. Scaffolds were immediately submerged in a cross-linking solution (70% ethanol + 33 mM 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride + 6 mM *N*-hydroxysuccinimide) for 30 min with constant agitation on a rotating plate. Following cross-linking, the scaffolds were removed to fresh 70% ethanol and degassed under vacuum (approximately 10 kPa) for 5 min. Samples were then stored in 70% ethanol to ensure sterility until needed.

2.2. Scanning electron microscopy

Scanning electron microscopy micrographs were used to visualise the pore structure of the scaffolds at various magnifications. Prior to imaging, collagen scaffolds were sputter coated with gold for 2 min at a current of 20 mA. All micrographs were taken on a JEOL 5800, with a tungsten source, operated at 10 kV.

2.3. X-ray micro-computed tomography

X-ray micro-computed tomography (μCT), Skyscan 1172, scans were taken of the whole scaffolds (25 kV, 140 μA). Reconstructions were performed with the software program NRecon (Skyscan), with a resolution of 6 μm.

2.4. Human breast cancer cell line culture

All cell lines were maintained between passages 5 to 15 in complete media as follows. MCF7 cells (ATCC) were cultured in DMEM media (Gibco, Life technologies) supplemented with 10% foetal bovine serum (FBS) (Gibco) in a humidified 5% CO_2 incubator at 37 °C. MDA-MB-468 and MDA-MB-231 were cultured in Leibovitz L-15 media (Gibco, 11415-064) supplemented with 10% FBS in

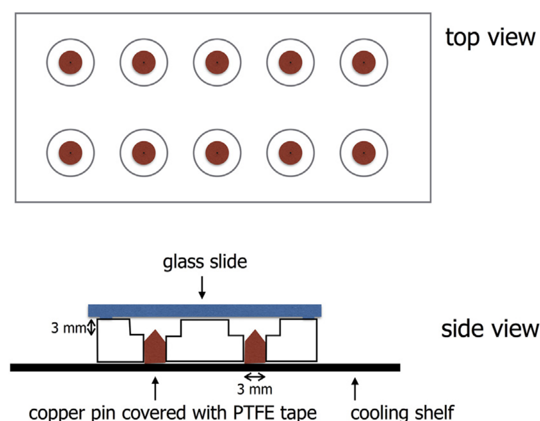


Fig. 1. The main body of the mould was machined from 9.6 mm thick polycarbonate sheet with evenly spaced 7 mm diameter by 3 mm deep troughs. In the centre of each trough, a 3 mm diameter hole was added in which a copper pin with a conical tip was inserted. The tip was covered by PTFE tape.

Download English Version:

https://daneshyari.com/en/article/6451022

Download Persian Version:

https://daneshyari.com/article/6451022

Daneshyari.com