Acta Biomaterialia xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Oxygen-sensing scaffolds for 3-dimensional cell and tissue culture

James Jenkins [a,1], Ruslan I. Dmitriev [a,*,1], Karl Morten [b], Kieran W. McDermott [c], Dmitri B. Papkovsky [a]

[a] School of Biochemistry and Cell Biology, University College Cork, Ireland
[b] Nuffield Department of Obstetrics and Gynaecology, University of Oxford, United Kingdom
[c] Department of Anatomy and Neuroscience, University College Cork, Ireland

ARTICLE INFO

Article history:
Received 15 September 2014
Received in revised form 21 January 2015
Accepted 22 January 2015
Available online xxxx

Keywords:
3D tissue model
Oxygen
Phosphorescence quenching microscopy
Porous membrane scaffold
PLIM

ABSTRACT

Porous membrane scaffolds are widely used materials for three-dimensional cell cultures and tissue models. Additional functional modification of such scaffolds can significantly extend their use and operational performance. Here we describe hybrid microporous polystyrene-based scaffolds impregnated with a phosphorescent O_2-sensitive dye PtTFPP, optimized for live cell fluorescence microscopy and imaging of O_2 distribution in cultured cells. Modified scaffolds possess high brightness, convenient spectral characteristics (534 nm excitation, 650 nm emission), stable and robust response to pO_2 in phosphorescence intensity and lifetime imaging modes (>twofold response over 21/0% O_2), such as confocal PLIM. They are suitable for prolonged use under standard culturing conditions without affecting cell viability, and for multi-parametric imaging analysis of cultured cells and tissue samples. We tested the O_2 scaffolds with cultured cancer cells (HCT116), multicellular aggregates (PC12) and rat brain slices and showed that they can inform on tissue oxygenation at different depths and cell densities, changes in respiration activity, viability and responses to drug treatment. Using this method multiplexed with staining of dead cells (CellTox Green) and active mitochondria (TMRM), we demonstrated that decreased O_2 (20–24 μM) in scaffold corresponds to highest expression of tyrosine hydroxylase in PC12 cells. Such hypoxia is also beneficial for action of hypoxia-specific anti-cancer drug tirapazamine (TPZ). Thus, O_2 scaffolds allow for better control of conditions in 3D tissue cultures, and are useful for a broad range of biomaterials and physiological studies.

© 2015 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.

1. Introduction

3D tissue models are increasingly used to study tissue function [1], behavior [2], morphology [3], (patho)physiological and disease states [4], responses to drugs and stimuli [5–7]. Compared to conventional 2D models they more closely mimic the microenvironment and cell–cell interactions in animal tissue in vivo, ensure normal signaling (regulated by cell adhesion molecules), gene expression and post-translational protein modifications. In such systems cell function can be manipulated by the addition of physiologically relevant signaling molecules, growth factors and peptides, while internal gradients of nutrients, growth factors, drugs and O_2, mediated by diffusion and cellular demand can be adjusted to resemble those found in vivo [8–10].

3D tissue models can be categorized into scaffold-based and scaffold-free systems. Scaffold-free systems include spheroids [11], organoids and tissue slices and explants [12]. Scaffold-based tissue models allow cultivation of cells in the presence of extracellular matrix (collagen, Matrigels) [13,14], self-assembling peptides [15,16] or synthetic biocompatible polymers [17,18]. Scaffold materials are designed to be porous for efficient gas and mass exchange, possess mechanical strength and solid structures which encourage adhesion and growth of cells, proliferation, differentiation and migration properties, and normal function superior over 2D cell models [19]. One such example is Alvetex™ scaffolds comprising highly porous polystyrene-based membranes having thickness of 200 μm, pore size 36–40 μm and thin walls. These materials have been used in drug screening and toxicity studies

Abbreviations: 3D, three dimensional; DIV, days in vitro; DMEM, Dulbecco's modified Eagle's medium; ECM, extracellular matrix; FBS, fetal bovine serum; HBSS, Hanks' balanced salt solution; HIF-1α, hypoxia inducible factor-1α; HS, horse serum; PBS, phosphate buffered saline; PFA, paraformaldehyde; PLIM, phosphorescence lifetime imaging microscopy; PS, Polystyrene; PtTFPP, Pt(II)-tetrakis(pentafluorophenyl)-porphine; ROI, region of interest; ROS, reactive oxygen species; RT, room temperature; SFM, serum free media; STDEV, standard deviation; TBST, Tris buffered saline with Tween 20; TMRM, tetramethylrhodamine methyl ester; TPZ, Tirapazamine; TCSPC, time-correlated single photon counting.

* Corresponding author at: Cavanagh Pharmacy Building, University College Cork, College Road, Cork, Ireland. Tel./fax: +353 21 4901339.
E-mail address: r.dmitriev@ucc.ie (R.I. Dmitriev).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.actbio.2015.01.032
1742-7061/© 2015 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.

Please cite this article in press as: Jenkins J et al. Oxygen-sensing scaffolds for 3-dimensional cell and tissue culture. Acta Biomater (2015), http://dx.doi.org/10.1016/j.actbio.2015.01.032

[20,21], culturing of brain and spinal cord tissue explants [22,23]. Apart from their mechanical and supporting function, 3D scaffolds may have additional functionalities providing controlled drug release, quantitative measurement of mechanical stress, specific analytes and others parameters [24], e.g., agarose gel with controlled pH affecting metabolic function [25] of chondrocytes or growth factor-release system for cardio-spheres [26].

On the other hand, high thickness and low transparency of such materials limit the use of conventional fluorescence microscopy and access to deep regions during culturing [10]. Use of many fluorescent probes, live cell markers and real-time analysis of viability and physiological processes within scaffolds is difficult.

Molecular oxygen (O_2) is a key metabolite affecting cell function and viability which must be carefully controlled in 3D tissue models [27,28]. O_2 can affect cellular function via energy stress (ATP production by oxidative phosphorylation), generation of ROS (reactive oxygen species), altered activity of metabolic enzymes and signaling pathways [29], transcription, protein production or degradation [30,31]. Low oxygen availability (hypoxia state) can aid progression of cancer, through increased production of specific growth factors (VEGF, angiopoeitin 2), HIF-1α activity [32] and angiogenesis [32–35]. O_2 fluctuations are an indicator of stroke, defective metabolism, neurological disorders such as Alzheimer's disease [36,37], and can influence the efficiency of tumor-, chemo- and radiation therapy.

The phosphorescence quenching method allows quantitative measurement and imaging of O_2 levels using solid-state sensor materials or probes introduced into the vasculature, tissue or cells [22,28,38]. Compared to probe-based methods [22,39], (pre-)staining of scaffold allows for controlled deposition of O_2-sensitive indicator dye, higher brightness and provides opportunity to sense extracellular O_2 gradients in simple manner. Using this approach O_2 gradients in engineered tissue were measured using planar solid-state phosphorescent O_2 sensors [40]. Fiber-optic O_2 microsensors were used to investigate O_2 tension in human dermal fibroblast or human bone marrow derived stromal cells grown on collagen type 1 scaffolds [41]. Scaffolds made of electrospun PLGA fibers with unevenly distributed nanosensors were used for O_2 and pH detection [42]. Optical O_2 sensors have advantages over the invasive and unstable electrode systems [43], however, the Fibre-optic probes [41], needle-type microsensors [44] and planar sensor membranes only allowing spot measurements or O_2 visualization in 2D.

2D and 3D visualization of O_2 distribution with sub-micron spatial resolution can be achieved by fluorescence/phosphorescence microscopy, which can also be combined with live cell imaging and multi-parametric analysis [45]. Fabrication of O_2-sensitive materials from micro-porous polymeric substrates and their studies by phosphorescence lifetime imaging microscopy (PLIM) were recently demonstrated [46,47]. This approach can also be applied to scaffold materials based on polymers with moderate O_2 permeability, such as polystyrene [48], thus enabling imaging analysis of O_2 in 3D tissue models and scaffolding systems.

Here, we applied the commercial polystyrene-based Alvetex™ membranes to prepare hybrid O_2-sensitive scaffold materials to establish 3D tissue models with O_2 levels determined using live cell imaging methods. We optimized staining of the scaffolds with the O_2-sensitive phosphorescent dye, PtTFPP, evaluated their O_2-sensitivity and general usability with several different cell and tissue models. We show that O_2-sensitive 3D scaffold can be used in 3D cell cultures in a similar manner to ordinary scaffolds. They also allow measurement of O_2 over large surface area without chemical or mechanical stress and the need to stain biological samples with O_2 probes. Multi-parametric analysis of tissue samples in fluorescence/phosphorescence imaging modality and microsecond FLIM (PLIM) is also demonstrated, which provides reliable and accurate quantification and mapping of O_2 distribution and its correlation with cell/tissue function.

2. Experimental section

2.1. Materials

Polystyrene scaffold membranes Alvetex™ (12-well inserts) were from Reinnervate (Amsbio, UK). PtTFPP dye was from Frontier Scientific (Inochem Ltd., Lancashire, UK, Cat. No. PtT975). ProLong Gold anti-fade reagent, Tethramethylrhodamine methyl ester (TMRM), Alexa Fluor-conjugated secondary antibodies, B27 serum-free supplement were from Invitrogen (Biosciences, Dublin Ireland). Epidermal growth factor (EGF), fibroblast growth factor (FGF), anti-β3-tubulin were from Millipore (Cork, Ireland). Anti-Tyrosine Hydroxylase antibody was from Abcam, Cambridge UK. CellTox Green assay kit was from Promega (MyBio, Ireland). Goat anti-Nestin antibody was from Santa Cruz biotechnology, Heidelberg Germany. Calcein Green AM probe, Tirapazamine, anti-GFAP antibody and all the other chemicals (HPLC or spectrophotometric grade) were from Sigma–Aldrich (Dublin, Ireland). Other plasticware (cell culture grade) was from Sarstedt (Wexford, Ireland).

2.2. Preparation of O_2-sensitive 3D scaffolds

PtTFPP dye was dissolved at 0.025–0.05 mg/ml in acetone:water (7:3) mixture at room temperature, and 1 ml aliquots of this solution were added to the Alvetex™ scaffold membranes placed individually in wells of a standard 24-well plate. The soaked membranes were incubated for 1 h at 60 °C, then washed sequentially (each time 5 min at 60 °C) with 1 ml of acetone:water (3.5:6.5), acetone:water (1.5:8.5) and lastly with sterile water. The stained "O_2-scaffolds" were dried under sterile laminar air flow and stored at room temperature protected from light and contamination.

2.3. Growing cells in the O_2 scaffolds

Human colorectal carcinoma HCT116 were cultured in McCoy 5A medium supplemented with 10 mM HEPES pH 7.2, 2 mM L-glutamine, 10% FBS and 1% Penicillin–Streptomycin as described previously [22], and then seeded at a density of 100,000 cells per scaffold.

Rat pheochromocytoma PC12 cells were cultured in Phenol Red free DMEM (Sigma D5030) supplemented with 10 mM glucose, 1 mM pyruvate, 2 mM L-glutamine, 10 mM HEPES, 1% Penicillin–Streptomycin, 2% B27, 20 ng/ml EGF, 10 ng/ml FGF and 100 ng/ml NGF to produce suspension cells and multi-cellular aggregates as described in [22] and then seeded at a density of 60–100 aggregates per O_2 scaffold.

Prior to cell seeding, dried O_2 scaffolds were pre-soaked in small amount of 70% ethanol and then rinsed 3 times with 1 ml of sterile PBS. After seeding, cells were allowed to grow inside the scaffold for 1–5 days, then counter-stained with Calcein Green AM (1 μM, 30 min) and analyzed on a microscope in Phenol Red free DMEM.

Cell viability within the O_2 scaffold was assessed by analyzing the membrane integrity (CellTox Green assay, Promega) and number of viable (stained with TMRM) cells, in comparison with control samples (unstained scaffold). Cells were stained with TMRM (20 nM) and CellTox Green (0.1%) for 30 min, washed with fresh media and imaged on the confocal microscope.

For immunofluorescence, cells grown in O_2 scaffolds were fixed in paraformaldehyde (4%, 10 min, RT) and immunostained with anti-tyrosine hydroxylase antibody (Abcam, ab112) as described

Please cite this article in press as: Jenkins J et al. Oxygen-sensing scaffolds for 3-dimensional cell and tissue culture. Acta Biomater (2015), http://dx.doi.org/10.1016/j.actbio.2015.01.032

Download English Version:

https://daneshyari.com/en/article/6483639

Download Persian Version:

https://daneshyari.com/article/6483639

Daneshyari.com