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Production of PEX protein from QM7 cells cultured in polymer scaffolds in a Taylor–Couette bioreactor

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ABSTRACT

In recent decades, many practical applications were developed with regard to the Taylor–Couette device, for example, reaction, filtration, extraction and bioreactor. In this study, the Taylor–Couette bioreactor was used to culture cells seeded in a biodegradable porous scaffold and produce PEX protein. Two different cell lines (NIH/3T3 and QM7) were seeded into PLGA sponges, which were fabricated using a solvent-free supercritical gas foaming method, and then cultured in the Taylor–Couette bioreactor. Cell proliferation was characterized using Quant-iT™ PicoGreen® dsDNA assay and the results indicated that high mass transfer rate in the Taylor–Couette bioreactor enhanced cell proliferation. Qualitative distribution of live/dead cells was characterized using LIVE/DEAD® Viability/Cytotoxicity assay and SEM and the results showed that cells cultured in static control mainly proliferated on the outer surface while the cells of Taylor-vortex bioreactor group could penetrate into the scaffold. The production yield of PEX protein, from QM7 cells transfected with pM9PEX, was quantified using PEX ELISA and the results showed a much higher PEX mass per scaffold for bioreactor than the control. As such, there is potential for the use of Taylor–Couette bioreactor in the mass production of PEX protein.

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1. Introduction

PEX is a fragment of matrix metalloproteinase-2 (MMP-2) that contains its non-catalytic C-terminal hemopexin-like domain and it occurs naturally in vivo as a MMP-2 breakdown product [1]. It is established that a recombinant PEX is able to disrupt angiogenesis and tumor growth in vivo, suggesting its potential as a new approach in treating such diseases [1,2]. However, purified commercial PEX human recombinant protein is expensive; thus, it is desired to explore novel methods to mass produce PEX protein economically.

Quail muscle clone 7 (QM7) is an avian myogenic cell line derived from the quail fibrosarcoma cell line QT6 [3]. The in vitro differentiation of QM7 cells greatly resembles that of mammalian myogenic cell line. The differentiation of QM7 cells depends on the serum content; the myoblasts replicate in high-serum medium but in a low-serum medium they stop replicating and fuse into multinucleated myotubes which express genes encoding muscle-specific proteins. These myotubes are able to survive for an extended period of time while synthesizing proteins in a low-serum medium, thus advantageous to express recombinant protein [4]. Moreover, QM7 cells can be transfected with the expression vector pM9PEX in high efficiency for PEX protein production.

A scaffold provides the structure for cell attachment, proliferation, differentiation and formation of extracellular matrix secreted by the cells in the scaffold [5]. It can also act as a carrier for cells, growth factors and biomolecular signals [5,6]. It is ideal for a scaffold to have the following properties: 3D highly porous and interconnected structure for the growth of cells and material transport; suitable surface for cell attachment and growth; mass reproducibility without the use of organic solvents [7].

Common synthetic biodegradable polymers used to fabricate scaffolds include polylactide (PLA), polyglycolide (PGA), their copolymers and polycaprolactone (PCL). Poly(lactide-co-glycolide) (PLGA), a copolymer of PLA and PGA, is biocompatible and biodegradable, approved by Food and Drug Administration (FDA) to host therapeutic devices and drugs, and is often used in the fabrication of porous sponges for biomedical applications [8,9]. Several promising methods have been reported in the literature for applications in scaffold fabrication, such as fiber bonding, solvent casting and particular leaching, phase separation, microspheres adhesion [10,11]. However, residual of organic solvent or uneven pore size distribution could be disadvantages of these methods; which are potentially harmful for the cell proliferation and the cell migration

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and oxygen transport in the scaffold [9]. Therefore, a supercritical CO_2 gas-foaming method was proposed for the scaffold fabrication. The gas foaming technique does not require the use of organic solvents, and process parameters can be varied to obtain different pore sizes and porosities. Polymer sponges fabricated using this method have characteristics of adequate porosity, uniform distribution of pore, moderate interconnectivity and reproducibility [9].

The Taylor–Couette device was proposed as a bioreactor for the cultivation of QM7 cells in this study. The conventional Taylor–Couette device is made up of two coaxial cylinders that can rotate [12,13] and it could be used in many practical applications, for example, reaction, filtration, and extraction [14–16]. The annular space between the cylinders can be filled with media and cells for application as a bioreactor [17–20]. The use of a Taylor–Couette bioreactor offers several benefits. Firstly, it enables an even mixing which results in a uniform nutrient and oxygen concentration throughout the liquid media, thus resulting in a higher mass transfer efficiency of the materials to the cells [18]. As cells are vulnerable to shear forces, the degree of mixing needs to be controlled, requiring a delicate balance between the effects of shear and mass transport. Secondly, it is easy to scale the system due to its simple geometry. Thirdly, it can create uniform flow regimes ranging from laminar to highly turbulent, and the transitions can be controlled to a large extent [18].

Previously, we have carried out several studies on the Taylor–Couette behavior. Deng et al. [21] performed a detailed study on the individual bubble behaviors in a Taylor vortex, the complicated bubble behaviors such as bubble ring, interaction between large and small bubbles, relationship between maximum bubble size and Reynolds number were observed. Besides, the behaviors of very light particle and glass beads were also studied and the results showed that the dispersed phase could be trapped in the Taylor vortex; therefore suggesting a possible novel dynamic cultivation method of mammalian cells. The cells can be seeded in the scaffold first and then the scaffold can be transferred into the Taylor–Couette device for the proliferation of cells and production of recombinant protein. Working under this principle the existence of Taylor vortices can trap the scaffolds and produce a moderate shear stress environment and enable high mass transfer rates. The other significant advantage of the Taylor–Couette device is that the layers of Taylor vortex can be a potential bioreactor for the multiple culture and mass production of bio-products. This is to be investigated in the present study.

2. Materials and methods

2.1. Materials

Poly(D,L-lactic-co-glycolic acid) (PLGA 85:15) was purchased from Sigma–Aldrich (MO, USA). The compressed carbon dioxide (CO_2) was purchased from Soxal (Singapore Oxygen Air Liquid Pte Ltd., Singapore).

Dulbecco's Modified Eagle Medium (DMEM) was purchased from Gibco (Life Technologies, CA, USA). Fetal Bovine Serum (FBS) was from HyClone (UT, USA). Penicillin–Streptomycin solution was from PAN-Biotech (Germany). Trypsin Ethylenediamine tetraacetic acid (EDTA) (10×) was from PAA Laboratories (Germany). Phosphate-buffered saline (PBS) solution (10×) was from 1st BASE (Singapore). 37% formaldehyde solution was from J.T. Baker (PA, USA).

Quant-iT™ PicoGreen® double-stranded DNA (dsDNA) Assay Kit, for DNA quantification, was a product from Invitrogen (USA). LIVE/DEAD® Viability/Cytotoxicity Kit, for LIVE/DEAD® Viability/Cytotoxicity assay analysis, was a product from Molecular Probes (OR, USA).

For PEX ELISA, anti-c-Myc Antibody (9E10), horseradish peroxidase (HRP) conjugated rabbit anti-mouse immunoglobulin G (IgG) antibody and HRP conjugated goat anti-rabbit IgG antibody were purchased from Novus Biologicals (CO, USA). Sodium carbonate was from Nacalai Tesque (Kyoto, Japan), Tween 20 was from Sinopharm Chemical Reagent (PRC) and blocking buffer (10×) was from Thermo Scientific (IL, USA). Sodium bicarbonate, hydrochloric acid (37%) and SIGMA*FAST*™ OPD (*o*-phenylenediamine dihydrochloride) tablet was from Sigma–Aldrich (MO, USA).

2.2. Fabrication and characterization of PLGA sponges

PLGA sponges were fabricated using a solvent-free supercritical gas foaming method outlined by Zhu et al. [8]. As the external surface of the foams was non-uniform with low interconnectivity level, it was removed with a cutting blade for more efficient cell seeding on the inner surface [8]. The fabricated PLGA foams were cut into cubes of dimensions 1 mm × 1 mm × 1 mm to be used as scaffolds for subsequent cell culture studies. The scaffolds were cut at low temperature to avoid deformation during cutting.

The morphology and pore size of the PLGA scaffolds were analyzed with the use of scanning electron microscopy (SEM) (JEOL JSM-5600 LV, Tokyo, Japan); and the internal pore diameters of the scaffold were estimated using the SmileView software (JEOL, Tokyo, Japan).

2.3. Cells and cell culture

The NIH/3T3 cell line used in this study was purchased from ATCC (Manassas, USA), while the QM7 cell line transfected with pM9PEX plasmid was contributed by Peking University School of Oncology. Both cells were cultured in growth medium of DMEM supplemented with 10% FBS and 1% penicillin–streptomycin antibiotic solution. The differentiation medium for QM7 was formulated using DMEM supplemented with 0.5% FBS and 1% penicillin–streptomycin antibiotic solution.

2.4. Scaffold preparation and cell seeding

The cell seeding procedure was modified based on the protocol from Zhu et al. [9]. The scaffolds were sterilized before cell seeding to create a clean environment for cell growth. The scaffolds were immersed in 70% ethanol in a covered cell culture dish and exposed to ultraviolet (UV) light in a biosafety cabinet (BSC) for 30 min. The scaffolds were then air-dried in an open cell culture dish and exposed to UV light in the BSC for 20 min. The remaining ethanol was removed, and the scaffolds were washed twice with PBS dripped drop-wise onto the scaffolds. The scaffolds were incubated overnight in the culture medium at 37 °C to pre-wet them.

The pre-wet scaffolds were immersed in 2 ml cell solution of density 200,000 cells/ml by drop-wise addition to the scaffolds in a well plate, and gently shaken using a XYZ shaker (FINEPCR multi shaker FMS3, Korea) for 2 h at room temperature. They were then placed in an incubator for 4 h. After a total of 6 h of cell seeding process, the scaffolds were washed twice with PBS to remove unattached cells before any further use in cell culture or analysis.

2.5. Taylor–Couette bioreactor

The Taylor–Couette bioreactor used in this study is similar to the device used by Deng et al. [19] and it is made up of two concentric cylinders, where the outer cylinder is stationary and the inner cylinder is rotating and performed as an agitator (Fig. 1). The outer cylinder, made from transparent poly (methyl methacrylate), has a height of 70 mm and an inner radius (R_o) of 30 mm. The inner cylinder, made from stainless steel, has a height (H) of

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