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Porous microscaffolds for 3D culture of dental pulp mesenchymal stem cells

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ABSTRACT

The collective power of stem cells due to their evident advantages is incessantly investigated in regenerative medicine to be the next generation exceptional remedy for tissue regeneration and treatment of diseases. Stem cells are highly sensitive and a 3D culture environment is a requisite for its successful transplantation and integration with tissues. Porous microscaffolds can create a 3D microenvironment for growing stems cells, controlling their fate both in vitro and in vivo. In the present study, interconnected porous PLGA microscaffolds were fabricated, characterized and employed to propagate human dental pulp mesenchymal stem cells (DPMSCs) in vitro. The porous topography was investigated by scanning electron microscopy and the pore size was controlled by fabrication conditions such as the concentration of porogen. DPMSCs were cultured on microscaffolds and were evaluated for their morphology, attachment, proliferation, cell viability via MTT and molecular expression (RT-PCR). DPMSCs were adequately proliferated and adhered over the microscaffolds forming a 3D cell-microscaffold construct. The average number of DPMSCs grown on PLGA microscaffolds was significantly higher than monolayer 2D culture during 5th and 7th day. Moreover, cell viability and gene expression results together corroborated that microscaffolds maintained the viability, stemness and plasticity of the cultured dental pulp mesenchymal stem cells. The novel porous microscaffold developed acts as promising scaffold for 3D culture and survival and transplantation of stem cells for tissue engineering.

1. Introduction

Stem cells have created a revolution in tissue engineering due to their immense potential of self-renewal, plasticity and ability to differentiate and generate numerous tissue lineages. The exciting field of stem cell research, however, is driven back due to many scientific hinges such as source availability and ethical concerns, obtaining and maintaining pure culture populations, differentiating into the desired lineage, regenerated viability of tissue, transplantation and graft rejection (King and Perrin, 2014; Nadig, 2009). One of the breakthroughs of stem cell research which have created a paradigm shift in its research is isolating stem cells from dental tissues (Saito et al., 2015). Dental pulp stem cells are multipotent mesenchymal stem cells capable of differentiating into numerous cell lineages in the presence of suitable signaling growth factors. Dental pulp mesenchymal stem cells (DPMSCs) offer characteristic advantages over conventionally isolated mesenchymal stem cells (MSCs) from bone marrow such as convenient, accessible and available source, capable of producing abundance number of cells and less ethical concerns (Kabir et al., 2014; La Noce et al., 2014). Conventional cell culturing on two-dimensional flat surfaces is an unnatural approach and leads to flattening of cells without the presence of natural extracellular matrix (ECM). Three-dimensional (3D) cell culture models closely mimic the in-vivo structure and facilitate their differentiation, intercellular signaling and expression. Utilization of porous microspheres as microscaffolds for 3D cell culture provides several distinct advantages. Microscaffolds aid in the rapid expansion of cells and scale up with bioreactors. Porous microscaffolds owing to their geometry provide a large surface area, minimum utilization of media and mimic the in-vivo microenvironment for cell growth. Biodegradable microscaffolds can be directly transplanted in vivo

Abbreviations: DPMSCs, human dental pulp mesenchymal stem cells; MSCs, mesenchymal stem cells; ECM, extracellular matrix; PLGA, poly (lactic-co-glycolic acid); PVA, polyvinyl alcohol; SSA, specific surface area; BET, Brunauer-Emmett-Teller; ESEM, environmental scanning electron microscopy; RT, reverse transcript; PCR, polymerase chain reaction; DMEM, Dulbecco's modified eagle's medium; PBS, phosphate buffered saline; FBS, fetal bovine serum; ITS, insulin, transferrin and sodium selenite solution; Penstrep, penicillin-streptomycin solution; TGF, transforming growth factor; GAG, glycosaminoglycan; DMMB, dimethyl methylene blue; ALP, alkaline phosphatase.

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and preserve proliferated cell proteins in their 3D native form (Chen et al., 2013; Martin et al., 2010). There are numerous microscaffolds reported in the literature for 3D cell culturing. PLGA is one of the most abundantly explored scaffold material due to its biocompatibility, biodegradability, FDA approval and mechanical strength (Pan and Ding, 2012). 3D culturing of stem cells and preserving their viability is a technical challenge. Only few research papers have been reported which have produced a viable PLGA stem cell constructs. The main issue is the hydrophobicity of PLGA resulting in low adherence and reduced growth of cells (Wei et al., 2015). A porous uniform shaped geometry promotes cell adhesion and accelerates their growth. The goal of the study is to culture and maintain the viability of DPMSCs on uniform interconnected porous microscaffolds of PLGA in 3D environment for tissue engineering and transplantation. Few similar studies have been reported to culture adipose tissue mesenchymal stem cells and ovarian cancer cells on porous PLGA microscaffolds (Kang et al., 2008; Zhang et al., 2014). However, till date, there are no reports regarding culturing human dental mesenchymal stem cells on porous PLGA microscaffolds.

2. Materials and methods

2.1. Materials

Poly (lactic-co-glycolic acid), RG 504H Mw 38,000–54,000 was obtained as gift sample from Evonik industries. Poly (vinyl alcohol) (PVA) (hydrolysis degree 87–89% and Mw 31,000 50,000) was purchased from Sigma-Aldrich. Ammonium bicarbonate (NH_4HCO_3) (analytical grade) was obtained from SD fine chemicals. Pluronic F68 was obtained as gift sample from BASF. Dulbecco's modified eagle's medium (DMEM), 1% Penicillin-Streptomycin, Insulin, Glutamine was purchased from Hi-Media, phosphate buffered saline (PBS) and Fetal bovine serum (FBS) was obtained from GIBCO.

2.2. Preparation of microscaffolds

A modified double emulsion solvent evaporation technique was used to prepare porous PLGA microscaffolds (Kim et al., 2006a,b). Briefly, 0.5 ml of an aqueous phase containing various concentrations of ammonium bicarbonate (W_1) was emulsified into 8 ml dichloromethane solution consisting of 400 mg PLGA and 100 mg Pluronic F68 using a homogenizer to produce primary W_1/O emulsion. The W_1/O emulsion produced was added to 100 ml of 0.5% aqueous PVA (outer aqueous phase, W_2) to generate a $W_1/O/W_2$ emulsion. The emulsion was stirred overnight on a magnetic stir plate at stirring speed 400 rpm to evaporate the organic solvent. The microscaffolds obtained were washed with distilled water and lyophilized.

2.3. Characterization of porous microscaffolds

2.3.1. Particle size and optical microscopy

The measurements were performed in triplicates using a Malvern Mastersizer particle size analyzer at $25 \pm 2\,^{\circ}C$ and at 90° to the incident beam applying the principle of photon correlation spectroscopy. Microscaffolds were dispersed in double distilled water and were diluted to ensure that sample obscuration was within the instrument's sensitivity range. Visual observation of spherical microscaffolds was done by observing under an optical microscope equipped with a digital camera.

2.3.2. Specific surface area determination by BET (Brunauer–Emmett–Teller) isotherm

The specific surface area (SSA) was determined by nitrogen adsorption single-point measurement using BET analyzer SAA-2000 (SP consultants, Mumbai). 30% nitrogen in helium was used as the adsorbate gas. For physisorption isotherm determination, the samples were first degassed for 24 h at room temperature under vacuum. After complete degassing, samples were placed on the surface area analyzer in the sample cell with a dewar flask filled with liquid nitrogen $-195\,^{\circ}C$ underneath the sample cell to start the adsorption process. Desorption isotherm was produced by placing a dewar flask filled with distilled water underneath the sample after completion of the adsorption process. After generation of the adsorption/desorption isotherm, the surface area of all the microscaffolds formulations was evaluated by using modified BET isotherm for rapid single point determination of surface area. Sample sizes between 0.3–0.5 g were analyzed, to ensure accurate and reproducible results.

2.3.3. Morphological characterization of microscaffolds by scanning electron microscopy

Surface morphology of microscaffolds was characterized by Environmental Scanning Electron Microscope (ESEM), FEI Quanta 200 ESEM equipped with the imaging tool: Phenom operating at an accelerating voltage of 10–30 kV. The average particle diameter and pore diameter was determined from the software using imaging tool.

2.4. Human dental pulp mesenchymal stem cell culture and its maintenance

Human dental pulp mesenchymal stem cell line (DPMSCs) was obtained from Jaslok Hospital and Research Centre, Mumbai. The DPMSCs were fed with freshly prepared DMEM, which was supplemented with 10% FBS, 1% Penicillin-Streptomycin, 1 μl/ml insulin, 2 μl/ml glutamine and 0.04 μl/ml ascorbic acid. They were cultured as a monolayer on T-25 tissue culture flasks (Nunc, USA) and were sub-cultured thrice a week at 37 °C and 95% relative humidity in a CO_2 incubator (Thermo Scientific, USA). When the cells reached 80% confluency, they were trypsinized with 0.25% trypsin containing 0.02% EDTA ethylene-diamine-tetraacetic acid (Himedia, India) and counted by a hematocytometer (Chemometec, Denmark) prior to being used in the experiments.

2.5. Cell seeding and attachment over porous microscaffolds

PLGA porous microscaffolds were sterilized using graded concentrations of ethanol followed by 10 min sterilization under UV. Prior to seeding cells, the microscaffolds were soaked in fetal bovine serum for 24 h to acclimatize microscaffolds with the in-vivo environment. For loading the cells on sterilized microscaffolds, DPMSCs (1.0×10^5 cells/ml) were trypsinized and seeded on microscaffolds (1 mg/ml) and were cultured on 24-well tissue culture plate in DMEM supplemented media for 7 days.

2.6. Evaluation of stem cell adhesion, attachment and expansion over microscaffolds by phase contrast fluorescence microscopy

The adhesion and growth of the DPMSCs on PLGA microscaffolds surface were observed under Zeiss phase contrast fluorescence microscope and their morphological features were photographed. The DPMSCs–PLGA microscaffolds constructs were stained using Giemsa.