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Tissue engineering scaffolds containing embedded fluorinated-zeolite oxygen vectors

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ABSTRACT

Efficient oxygen supply is a continuing challenge for the fabrication of successful tissue engineered constructs with clinical relevance. In an effort to enhance oxygen delivery we report the feasibility of using fluorinated zeolite particles embedded in three-dimensional (3-D) polyurethane scaffolds as novel oxygen vectors. First, 1H,1H,2H,2H-perfluorodecyltriethoxysilane was successfully coupled to zeolite framework particles to examine the dose-dependent dissolved oxygen concentration. Following this, the fluorinated-zeolite (FZ) particles were embedded in 3-D tissue engineering polyurethane scaffolds. Our data demonstrates an even distribution of FZ particles in the 3-D scaffolds without affecting the scaffold porosity or pore size. Human coronary artery smooth muscle cell (HCASMC) proliferation on FZ-containing polyurethane (PCU-FZ) scaffolds was significantly greater than on control scaffolds ($P = 0.05$). Remarkably, cell infiltration depths on the PCU-FZ scaffolds was double that on PCU control scaffolds. Taken together, our data suggest the potential of PCU-FZ scaffolds for tissue engineering with enhanced oxygen delivery to cells.

1. Introduction

Tissue engineering strategies to potentially replace conventional therapies for the repair and regeneration of diseased or damaged tissues and organs is a continued active area of research and innovation. In addition to the anticipated clinical uses, engineered tissues that can capture both the three-dimensional (3-D) organization and multicellular complexity of the native system could provide a powerful tool for screening the effects of therapeutic candidates [1]. Although progress has been made in fabricating engineered tissues in vitro, the inability to deliver sufficient oxygen to the growing constructs (due to its poor solubility in culture media) remains a formidable task [2,3]. This is exemplified by early studies showing that cellular spheroids generally contain a hypoxic and necrotic center surrounded by a rim of viable cells [4,5]. Similar observations have been reported for osteoblasts, hepatocytes, and cardiomyocytes cultured in 3-D scaffolds under static conditions [6–8].

Previous attempts to overcome oxygen transfer limitations in tissue engineering relied on perfusion bioreactors where oxygen dissolved in the culture medium diffuses to the scaffold interior. However, the high flow rate required to maintain an adequate oxygen concentration for cell viability often surpasses the shear stress tolerance of the cells [9,10]. As an alternative, the use of perfluorocarbon (PFC) emulsions as an oxygen carrier in tissue engineering has been investigated, where the oxygen unloading of the PFC emulsion was facilitated by the increased surface area due to the emulsion droplet size [11]. In spite of reported encouraging data, the high density of PFC emulsions meant that the droplets could easily settle, either in the culture well or in the medium reservoir [12]. This, in turn, implies that PFC emulsions may not be effective oxygen carriers. In view of the above it would be advantageous if the oxygen carrier molecules could be bound to the scaffold. In this regard, Harrison and co-workers reported calcium peroxide-based oxygen generating particles incorporated into a scaffold to provide sustained oxygen release over an extended period of time [13]. The reaction products, $Ca(OH)_2$ and H_2O_2, are, however, strong base and strong oxidizing agents, respectively, the drawbacks of which could outweigh the benefits. This drawbacks are exemplified by a recent study which demonstrated a significant reduction in fibroblast viability when calcium peroxide was incorporated in fibrous polycaprolactone scaffolds [14]. In addition, the calcium peroxide loaded on the scaffold will eventually be depleted, limiting this approach to short-term cultures.

In this study we report a novel approach to enhanced oxygen delivery to cells seeded in 3-D scaffolds by incorporating fluorinated porous zeolite particles as an integral part of the scaffolds.

2. Materials and methods

2.1. Preparation of fluorinated zeolite (FZ)

Analytical grade chemicals and reagents used for the preparation of FZ were purchased from Sigma–Aldrich (Milwaukee, WI)

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unless otherwise stated, and were used as received. Zeolite Y powder was crystallized from a synthetic gel with a molar composition of $4Na_2O{:}1Al_2O_3{:}6SiO_2{:}200H_2O{:}5(TMA)_2O$, where $(TMA)_2O$ designates tetramethylammonium hydroxide (25%) (Merck KGaA, Darmstadt, Germany) used as a organic template or structural directing agent. The synthetic gel was prepared by dissolving appropriate amounts of sodium aluminates ($NaAlO_2$) as Al source and sodium hydroxide (NaOH) in a mixture of water and $(TMA)_2O$ under vigorous stirring until a homogeneous solution was obtained. Silica gel (as Si source) was then added to the resulting solution in a 30 ml Teflon reactor and mixed for 10 min. The resulting synthetic gel was aged for 48 h under static conditions. The zeolite Y product was allowed to crystallize from the aged gel at 100 °C in an oven for 48 h. When the reaction was complete the pure crystalline zeolite powders were recovered from the suspension by centrifugation at 10,000 r.p.m. The solid particles were redispersed in deionized water and centrifuged again. The washing and centrifugation process were repeated three times. The solid products were dried at 50 °C for 24 h, followed by calcination at 500 °C for 5 h in a programmable furnace at a heating and cooling rate of 1 °C min^{-1} to activate the zeolite by removing the organic residues and water. The resulting zeolite Y was then fluorinated with a 1 wt.% methanol solution of 1H,1H,2H,2H-perfluorodecyltriethoxysilane (PTES) for 1 h, filtered and then heated at 140 °C for an additional 1 h. The particle sizes of the zeolite were in the range 850–1000 nm.

2.2. Fabrication of PCU-FZ scaffolds

A medical grade poly(carbonate urethane) (PCU) (Bionate® 55D) was kindly donated by the Polymer Technology Group (Berkeley, CA). 3-D scaffolds were fabricated by a solvent casting and particulate leaching method as previously described [15], with some modifications. Ground and sieved NH_4Cl porogens (180–250 μm) were packed into a 6 mm diameter cylindrical infiltration chamber together with 2 wt.% FZ particles, and the polymer solution (15 wt.% PCU dissolved in *N*,*N*-dimethylformamide) was subsequently poured over the porogen bed and pressurized to infiltrate it. Following this, the scaffolds were removed from the assembly and the solvent was allowed to evaporate in a fume hood. Finally, the NH_4Cl porogen was leached out using deionized water and, the scaffolds were sectioned into 1.5 mm height disks using a microtome. This height (or thickness) of scaffold was chosen to mimic the thicknesses of many soft tissues, such as cartilage [16], skin [17], cardiac [18], and blood vessels [19] that are the subject of tissue engineering. Two different control scaffolds, namely PCU scaffolds without FZ particles and scaffolds that contained non-fluorinated zeolite particles, were fabricated in a similar way.

2.3. Dissolved oxygen measurement

A NeoFox fiber optic oxygen sensor (Ocean Optics, Dunedin, FL) equipped with NeoFox software was used to measure dissolved oxygen. The sensor uses ruthenium(II) complexes suspended in a support matrix and attached to the tip of the fiber optic cable. When excited by a light-emitting diode at 475 nm the ruthenium complex fluoresces, with emission at 620 nm. When the excited ruthenium complex encounters an oxygen molecule the emission is quenched, allowing the intensity of the fluorescence to be related to the oxygen concentration. Accordingly, the more oxygen present the lower the emission intensity, and vice versa. In the absence of oxygen the maximum fluorescent intensity of emitted light is observed. FZ particles at concentrations of 0.5%, 1%, and 2% were suspended in deionized water and continuously stirred at 37 °C while the assembly was left open to atmospheric air. Deionized water and non-fluorinated zeolite particles (non-FZ) suspended in deionized water (at 2% concentration) were used as controls. A 300 nm diameter oxygen probe was used to measure the dissolved oxygen following a two point calibration with 20.9% oxygen at standard temperature and pressure and 0% oxygen in 100% nitrogen.

2.4. Chemical composition and morphological studies of FZ and PCU-FZ scaffolds

The crystallinity of the zeolite particles was measured by powder X-ray diffractometry (XRD) (Rigaku RINT 2500, Tokyo, Japan) with CuK_α radiation (λ = 1.54 Å) at 40 kV and 50 mA with a scan rate of 0.02 s^{-1} over a 2θ range of 2–40°. The zeolite particle pore size and surface area were determined by the BET (Brunauer, Emmett and Teller) method using nitrogen adsorption–desorption isotherms and an ASAP2010 instrument (Micromeritics Instrument Corp., Norcross, GA). The zeolite pore size was also calculated by the advanced Barrett–Joyner–Halenda (BJH) method using the adsorption–desorption branches of the isotherms. Prior to these measurements the samples were degassed at 170 °C for 24 h under vacuum. To investigate the surface and cross-sectional morphology of the scaffolds, high resolution scanning electron microscopy (SEM) images were captured using a model FIB/SEM LEO 1540XB microscope (Carl Zeiss, Oberkochen, Germany) operating at an electron beam voltage of 1 keV. Scaffolds were affixed to a carbon sample holder and coated with 4 nm osmium vapor before imaging. In addition, energy dispersive X-ray spectroscopy (EDX) was used to map the elemental composition and distribution within the scaffolds. Elemental compositions of the fluorinated zeolite particles and the scaffolds were determined by X-ray photoelectron spectroscopy (XPS) (Perkin Elmer, Waltham, MA) and EDX. The XPS analyses were carried out with a Kratos Axis Ultraspectrometer using a monochromatic AlK_α source (15 mA, 14 kV).

2.5. Scaffold porosity measurements

Mercury porosimetry measurements were made using an Autopore IV porosimeter (Micromeritics, Norcross, GA). Samples of PCU and PCU-FZ scaffolds were cut into cylindrical disks 1.5 mm high and 6 mm in diameter, before being placed in the penetrometer. Care was taken to place adequate sized samples in the penetrometer to achieve significant measurable intrusion volumes while maintaining unimpeded access by the mercury to the entire surface of the samples. Prior to mercury intrusion the penetrometer was degassed to approximately 4 kPa to remove air from the system. Mercury filling of the penetrometer was performed at 3.5 kPa. Logarithmically spaced data points were taken at pressures ranging from 0.35 to 410 kPa. An equilibrium intrusion rate threshold was set at 0.003 ml g^{-1} s^{-1}. For the purposes of data analysis the surface tension of mercury and the intrinsic contact angle with the scaffolds were taken to be γ_{Hg} = 480 N m^{-1} and θ = 140°, respectively. Average pore areas were corrected for deformation of the scaffold under elevated pressure, as described elsewhere [20].

2.6. Cell culture on PCU and PCU-FZ scaffolds

Primary human coronary artery smooth muscle cells (HCASMC) and smooth muscle growth medium (SmGM®-2 BulletKit) were purchased from Lonza Walkersville Inc. (Walkersville, MD, USA). Cells were cultured according to supplier's instructions in smooth muscle growth media (SmGM®-2 Bullet Kit), supplemented with 100 U ml^{-1} penicillin G sodium and 100 μg ml^{-1} streptomycin sulfate (Invitrogen, Burlington, Canada). All cultures were maintained in a humidified incubator at 37 °C under an atmosphere containing 5% CO_2. HCASMC were passaged every 7 days at a split ratio of 1:3 and used between passages 4 and 7. Cylindrical scaffolds were affixed to glass coverslips using silicone grease, sterilized with 70% ethanol for 30 min and allowed to dry under germicidal UV light