Probing Oxygen Tension of Tissue Engineered Grafts using Oxygen Imaging

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Statement of Purpose: The goal of this study is to examine the sensitivity of electron paramagnetic resonance (EPR) oxygen imaging (EPROI) technique to map oxygen tension in three-dimensional and porous scaffolds. EPROI has been widely used for mapping tumor hypoxia, tumor response to drugs, and improving radiation efficiency [1]. However, its use in tissue engineering and regenerative medicine is relatively new [2]. In this study, biodegradable polymer scaffolds with gradually increasing porosity were mapped for oxygen tension using EPROI non-invasively. The results suggest that the oxygen probing technique can be effectively used to image oxygen tension inside a tissue engineering matrix in vitro.

Materials and Methods:

Scaffold preparation: Poly (85 Lactide-co-15 Glycolide), PLGA 85:15 (Evonik), was dissolved in methylene chloride and fabricated into microspheres using an oil-in-water emulsion procedure. Microspheres, with a size range of 425-600μm, were collected and mixed with 0, 20 or 40 weight percent NaCl porogen (size range 200-300μm). The combinations were placed in a 5mm diameter x 10mm height scaffold mold and were allowed to thermally sinter at 100˚C for 1 hour. Porogen leaching with water was used to create low porous (0% NaCl), medium porous (20% NaCl), and highly porous (40% NaCl) scaffolds, respectively. The low porosity scaffold has most pores in the range of 100-300 μm, while the medium and highly porous scaffolds have increasingly bigger pores (300- 500 μm) [3].

Cell culture: Bone marrow derived human mesenchymal stem cells (hMSCs) were isolated using Magellan ® and MACS ® technologies and cultured in regular basal media (DMEM/F-12 + GlutaMAX, with 10% FBS and 1% P/S) at 5% CO_2 and 87% humidity. Cells were frozen at passage 3 and used at passage 5. Low, medium, and highly porous scaffolds were sterilized in a 70% ethanol solution (15 minutes) followed by UV treatment for 15 minutes on each side. Once sterilized, passage 5 hMSCs were extracted and 500k cells were seeded onto the top end of each scaffold. The cells were allowed to attach to the matrix for 1 hour prior to the addition of media. The scaffold constructs were cultured for 14 days in basal media; media was changed every 2-3 days.

Oxygen imaging experiments: The EPROI experiments were performed on 250 MHz home built pulse EPR imager at the University of Chicago [4]. The sample was placed in a 1.5 ml Eppendorf tube and trityl (OX063) spin probe was added to media to achieve the final concentration of 1 mM. The tubes were open to ambient air throughout the experiments. A series of 20 min long spin-lattice relaxation images were taken and oxygen concentration was inferred from the linear relation between the relaxation rate and absolute pO_2.

Results: Figure 1 shows the pO_2 maps of PLGA scaffolds with low, medium and high porosity. As shown in the figure, the mean pO2 for these scaffolds were 123.7 (± 10.6) torr, 127.0 (± 11.8), and 133.0 (± 8.44) torr, respectively. The oxygen tension in all scaffolds was lower than in adjacent media.

Conclusion: This study confirms that EPROI is a sensitive tool to map local oxygen pressure in three-dimensional porous scaffolds. It is sensitive technique that can distinguish scaffolds with different porosity. The knowledge of local oxygen pressure in tissue engineering and regenerative medicine can be potentially used to enhance the scaffold graft design, optimize cell and tissue performances and understand cell metabolism.

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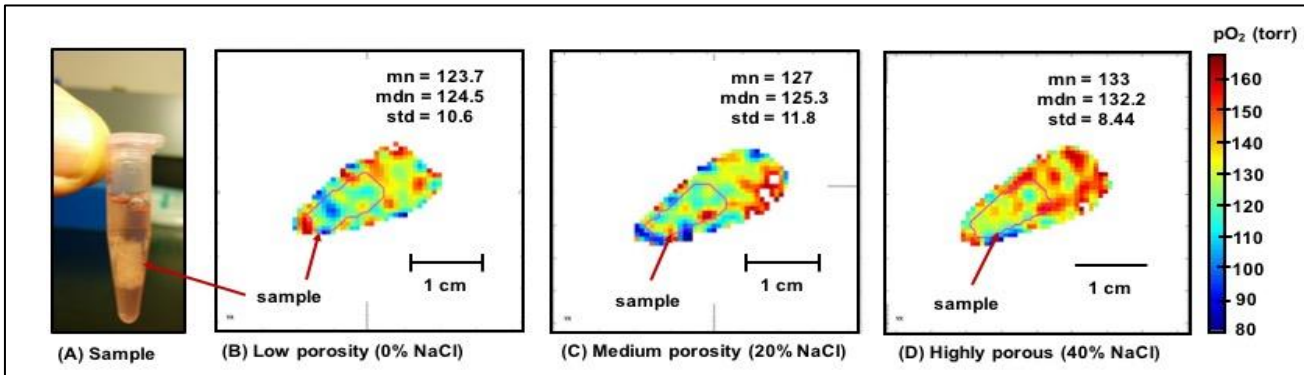

Figure 1: (A) A sample in an Eppendorf tube with media, pO_2 images covers samples and small portion of media on top. (B-D) pO2 images of PLGA scaffolds with gradually increasing porosity.