Investigating Oxygen Dynamics of Stem Cells in a Mineralized Osteogenic Scaffold

Mrignayani Kotecha[1]*, Sriram Ravindran[2], Boris Epel[3], Howard Halpern[3]
[1]Department of Bioengineering, University of Illinois at Chicago, Chicago IL 60612, USA, Email: mkotecha@uic.edu
[2]Department of Oral Biology, College of Dentistry, University of Illinois at Chicago, Chicago IL 60612, USA
[3]Department of Radiation and Cellular Oncology, Center for EPR In Vivo Physiology, The University of Chicago, Chicago IL 60637, Chicago, USA

Statement of Purpose: The purpose of this study is to assess the sensitivity of electron paramagnetic resonance (EPR) oxygen imaging (EPROI) to monitor the oxygen dynamics of stem cells seeded in an osteogenic scaffold. EPROI is a technique conceptually similar to MRI, and with the help of oxygen sensing reporter OX063 spin-probe, it provides three dimensional absolute oxygen maps in tissues with oxygen resolution ~1-3 torr and spatial resolution 1 mm. EPROI has been widely used to map animal tumor hypoxia, however its use in tissue engineering regenerative medicine is relatively new [1]. We monitored the oxygen dynamics of dental pulp stem cells seeded in a mineralized osteogenic scaffold using EPROI. The hypoxic state of cells in the tissue and pO_2 gradient in the sealed tube was observed within two hours of oxygen deprivation. This study suggests that EPROI can be used to monitor oxygen dynamics and calculate oxygen consumption rare (OCR) of cells used for tissue engineering and regenerative therapies.

Materials and Method:

Sample Preparation: Bone graft material with MSCs was created by seeding 1 million human marrow stromal cells (HMSCs) in collagen-chitosan (1 mg/ml: 1 mg/ml) hydrogel. The tissue was cultured for 14 days in mineralization media with 5% CO_2. The media was changed every 2-3 days.

Oxygen Imaging Experiments: The EPROI experiments were performed using the 250 MHz home built pulse EPR imager at the University of Chicago [2]. The sample was placed in a 1.5 ml Eppendorf tube with media filled to the top to limit the amount of available oxygen to the sample and trityl (OX063) spin probe was added to media achieve final concentration of 1 mM. The tube was kept sealed for the first 7 hours of experiment, and then was opened and another hour of imaging was performed. A series of 20 min long spin-lattice relaxation images were taken every 20 min and oxygen concentration was inferred from the linear relation between the relaxation rate and absolute pO_2 in media.

Results: Figure 1(A) shows the bone graft scaffold with MSCs in a 1.5 ml Eppendorf tube. In pO_2 maps (Figure 1(B-C), the sample along with media on top of the sample is visible. Figure 1(B) shows the first pO_2 image directly after the tube was sealed. The pO_2 in the tube is smaller than the ambient air (21% oxygen, pO_2 = 160 torr). The cells start consuming oxygen quickly and the pO_2 gradient becomes clearly visible after approx. 2 hours of measurement. After about 2 hours, cells stay in hypoxic mode with little change in pO_2. The oxygen pressure returned quickly to normal after the tube was opened. The change in local oxygen pressure as a function of time within the sample and the media is shown in Figure 2(D). The calculated OCR was 0.74 torr/min during the active state (first hour) and .0046 torr/min during the dormant state (4th hour) of cells at the site of voxel depicted with a blue circle in Figure 1(C).

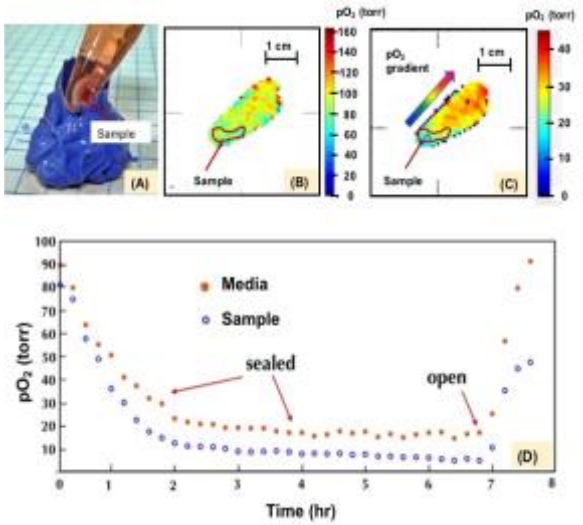

Figure 1: (A) Osteogenic scaffold in a 1.5 ml Eppendorf tube with media, (B) pO_2 map of sample with media after the tube was partially sealed. (C) pO_2 map after approx. 2 hours after first measurement, pO_2 gradient is visible in the sample as well as in the tube as cells are consuming available oxygen, (D) pO_2 dynamics for voxels inside the sample (denoted by a blue circle in C) and adjacent media (denoted by a red star in C) as a function of time (measurement was done with closed tube for 7 hours and then another hour after opening the tube.

Conclusions: This study shows the EPROI is a sensitive technique in assessing oxygen dynamics of stem cells in tissue grafts. Currently, there is no other noninvasive method for deep in tissue oxygen measurement available to tissue scientists. The knowledge of local oxygen pressure in cell and tissue based therapies can be utilized to enhance the graft design, calculate oxygen consumption rate, and modulate oxygen pressure to optimize cell differentiation.

References:

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