Monitoring Tissue-Engineered Graft Oxygenation *In Vivo* by Fluorine-19 Magnetic Resonance Spectroscopy

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Purpose: Transplantation of tissue-engineered grafts (TEGs) has the potential to treat numerous debilitating diseases. Non-invasive monitoring of the oxygen partial pressure (pO_2) in TEGs is critically important because of the harmful effects of prolonged or even short-term exposure to hypoxia and anoxia. This monitoring is particularly important in the early post-transplant period (before revascularization) or if a TEG is immunoisolated (and does not revascularize). Additionally, *in vivo* tissue pO_2 measurements may enable detection of events that would alter oxygenation of a TEG such as fibrous capsule formation, vascularization, inflammation, or cell proliferation, as well as the estimation of cell or tissue viability[1]. The spin relaxation mechanisms of some biocompatible fluorine compounds are very sensitive to dissolved oxygen (and temperature) and insensitive to other external factors[2]. Fluorine-19 nuclear magnetic resonance spectroscopy (19F-MRS) can, therefore, be used to quantify the oxygenation status of a TEG. This pilot study evaluated the utility of 19F-MRS for the non-invasive measurement of *in vivo* pO_2 within macroencapsulated TEGs following implantation in the murine model.

Methods: TEGs were constructed by injecting an emulsion of perfluoro-15-crown-5-ether (PFCE) and porcine plasma into a 40-µL TheraCyte® immunoisolation device (TheraCyte, Inc. Laguna Hills, CA), which was then crosslinked using 5% v/v bovine thrombin solution. An image of the encapsulation device and the distribution of fluorine in the device are shown in Figure 1. A linear correlation between the spin-lattice relaxation rate (R_1) and oxygen concentration was established before implantation of a TEG (Figure 2), and this correlation was used to convert measurements of R_1 to local pO_2 within the TEG following implantation. Nine male Lewis rats were implanted with a TEG into a dorsal subcutaneous pocket and assigned to groups for serial evaluation of oxygen content in relation to implant day +1 (n=3), +8 (n=3), and +15 (n=3). On the day of evaluation, rats were anesthetized with inhalation isoflurane, stabilized at a core body temperature of 37 °C, and imaged using a 16.4 T horizontal-bore magnet (Agilent Technologies Santa Clara, CA). Then, 19F-MRS was performed using a single-loop surface coil and an inversion-recovery sequence was used to obtain the R_1 from the singlet PFCE resonance within the TEGs. R_1 measurements within the TEG were then converted to pO_2 values using the linear calibration. Following imaging, animals were promptly euthanized using CO_2 for device explant and gross evaluation.

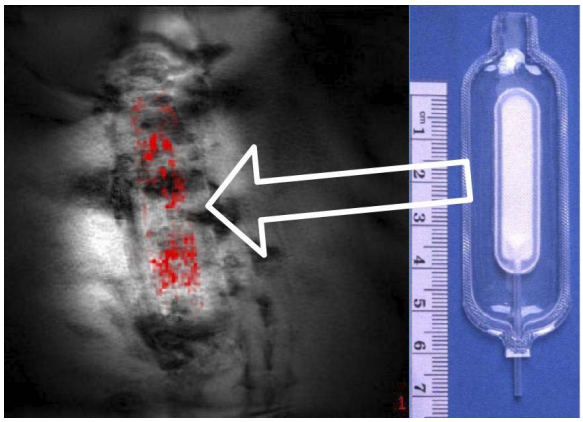

Figure 1: A photograph of the encapsulation device in sterile packaging and a 19F/1H fusion MRI showing the distribution of fluorine in the device implanted in a rat.

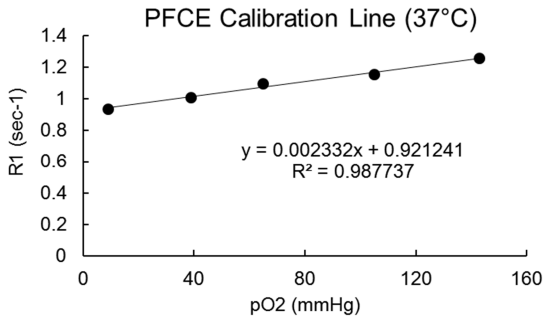

Figure 2: R_1 was confirmed to be linear with pO_2 at 37 °C. This relationship was used to quantify the oxygen status of the TEGs.

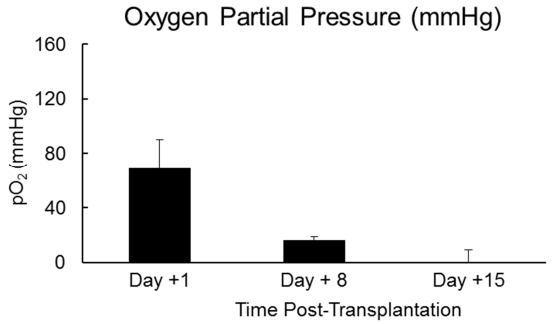

Figure 3: The results of the *in vivo* experiments. While initially at room oxygen (160 mm Hg), after implantation the TEGs quickly become hypoxic. They are measured to be anoxic two weeks post-implantation.

Results: The mean pO_2 in the encapsulated TEG 1 day post-implantation was 69±22 mm Hg. The mean pO_2 fell over time to 16±3 mm Hg at 8 days post-implantation and 0±10 mm Hg at 15 days post-implantation (Figure 3).

Conclusion: These preliminary studies confirm that 19F-MRS can be used to non-invasively measure pO_2 *in vivo* within a macroencapsulated TEG. Preliminary data suggest that the encapsulated TEG becomes hypoxic 1 week post-implantation despite the oxygen permeable membrane and may require supplemental oxygenation to sustain cells and tissue. Future studies will attempt to correlate pO_2 measurements with changes in TEG viability and oxygenation both pre- and post-transplantation.

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References:

1. Suszynski TM, Avgoustiniatos ES, Stein SA, et al. Assessment of Tissue-Engineered Islet Graft Viability by Fluorine Magnetic Resonance Spectroscopy. *Transplant Proc*. 2011; 43(9):3221-3225.
2. Mason RP, Shukla H, and Antich PP. *In vivo* Oxygen Tension and Temperature: Simultaneous Determination Using ^{19}F NMR Spectroscopy of Perfluorocarbon. *Mag Res Med.*1993; 29:296-302.