**Oxygen Imaging of Biomaterials**
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**Statement of Purpose:** Insufficient oxygen delivery to metabolic cells is a crucial hurdle in creating three dimensional functional and viable artificial tissues that are more than a few mm of thickness. Different tissue types thrive under different oxygen conditions. Therefore, monitoring of partial oxygen pressure (pO₂) in three-dimensional tissue starting from cell seeding is important and is expected to lead to viable functional artificial tissues. Electron paramagnetic resonance oxygen imaging (EPROI) is an established method of mapping pO₂. EPROI works on a principle similar to magnetic resonance imaging (MRI) where instead of manipulating nuclear spins, unpaired electrons are introduced into the biological sample via the use of water-soluble trityl radicals (OX063-D24) as contrast agent, whose spin-lattice relaxation rates indicate the average local oxygen concentration. In this study, we tested the feasibility of oxygen mapping using EPROI in commonly used acellular biomaterials. This is the first step in the monitoring of the viability of artificial tissue grafts with active cells.

**Materials and Methods**

**Biomaterial Preparation:** Three different biomaterials, Agarose, Gelatin, and VitroGel were tested. These are commonly used biomaterials in tissue engineering and regenerative medicine. The gels were created using standard or vendor-supplied method. The samples were prepared in a 10 mm OD tube that has 8 mm inner diameter and 15 height. Vitrogel was also tested using a configuration similar to the standard 96-well plate, a 3D printed single-well with the dimensions identical to the 96-well plate. The samples were cycled through hypoxic (0 torr) to normoxic (160 torr) conditions using air and N₂ gas flow. The ability of biomaterials to go from hypoxic to normoxic conditions was measured using oxygen maps.

**Oxygen Imaging Experiments:** All oxygen imaging experiments were performed using O2M’s instrument JIVA-25 (Model V) (Figure 1). The vertical orientation instrument has a 25 mT magnet with 11 cm bore with about 5 cm of homogenous volume. The experiments were performed using 1mM of trityl added to the media on top of the biomaterials. All experiments were performed using a 10 mm vertical access resonator (Figure 1B and 1C) that is specially designed for in vitro tissue graft oxygen imaging.

**Results:** Gelatin and Agarose gels demonstrated high oxygen diffusion. Both gels were able to cycle through the hypoxic to normoxic conditions quickly. Vitrogel showed poor oxygen diffusion when the gel thickness was beyond 5 mm. However, in the case of 96-well plate experiments with 5 mm layer, the oxygen diffusion in the gel was excellent as shown in Figure 2.

**Conclusion:** JIVA-25 can produce pO₂ maps and demonstrated the compatibility of EPROI with commonly used biomaterials by tissue engineers. The knowledge of oxygen concentration is expected to be a vital tool for producing viable functional artificial tissues.

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![Figure 1: (A) JIVA-25 Instrument, (B) The 10 mm vertical access resonator for in vitro tissue graft oxygen imaging, (C) Top view of the resonator.](image)

![Figure 2: (top) preparation of VitroGel sample using 96-well plate design, (bottom) pO₂ maps of VitroGel sample showing high oxygen diffusion.](image)