Oxygen Generating Biomaterials for Three-Dimensional Cell Growth

Catherine Ward, Se Heang Oh, Anthony A. Atala, James J. Yoo, Benjamin S. Harrison
Wake Forest Institute for Regenerative Medicine, Wake Forest School of Medicine, Winston Salem, NC

Statement of Purpose: Many current regenerated organs that are in clinical trials are limited to thin-walled, hallow structures, such as the bladder, urethra, blood vessel, etc. The reason for this limitation is that currently, it has only been possible to engineer tissue with a thickness limitation of a few hundred microns, due to oxygen constraints[1]. Three-dimensional tissue regeneration of thick organs and tissues is limited by the ability of oxygen to diffuse throughout in order to create an oxygenated environment for viability. There is a need to accelerate the quantity of regenerated organs by providing oxygen to the entire organ. Ideally, a regenerated tissue would be able to maintain sufficient cell viability with appropriate oxygen concentrations until the body can take over the construct with native vasculature.. Our strategic approach to this problem is preparing tissue scaffolds capable of sustained release of oxygen. It is hypothesized that oxygen generating scaffolds can provide oxygen to the cells and maintain three-dimensional cell growth *in vivo*.

Methods: Three-dimensional scaffolds of oxygen generating biomaterials were prepared by dissolving poly(D,L-lactide-co-glycolide) (PLGA 50:50, i.v. 0.89 dl/g in HFIP at 30°C, Lactel Absorbable Polymers, Pelham, AL) in dimethyl sulfoxide and mixing the solution with calcium peroxide and 300-500 μm paraffin particles. The solutions were placed in a circular disk mold with a diameter of 1 cm and a thickness of 0.5cm. The paraffin particles were removed in a leeching process and the scaffolds were dried under vacuum. Control scaffolds were also created with PLGA alone. Oxygen production was quantified *in vitro* by placing the scaffolds in media in a hypoxic environment and recording the increase in dissolved oxygen using a BloodGas Analyzer. Fresh three-dimensional scaffolds were also tested *in vivo* by seeding the scaffolds with smooth muscle cells and implanting them in the subcutaneous space of a mouse. Animals were sacrificed at 1 and 2 weeks to determine three-dimensional cell viability over time. The constructs were sectioned and stained with hematoxylin and eosin to observe viable cell thickness.

Results: The oxygen generating materials provided significant oxygen release over a 14-day period. When compared to a solution without the oxygen generating material, the oxygen producing scaffolds were able to elevate the dissolved oxygen concentration approximately 1.5mm Hg.. This made it possible for the scaffolds to support cell growth *in vivo*. Celll seeded scaffolds that were implanted in the subcutaneous space of mice showed varying cell growth between the control and experimental scaffolds. At 1 week, the control and experimental groups showed similar cell growth inside the constructs. However, at 2 weeks, there was significant cellular ingrowth in the oxygen producing scaffold. Results indicate that the oxygen generating materials are capable of maintaining cell growth, where the controls showed very limited viability at the same timepoints.

Conclusions: This study shows that three-dimensional tissue viability can be accomplished with an oxygen supplement for a 2 week period. The *in vivo* studies suggest that at 2 weeks, three-dimensional cell growth is possible, which is obvious in the hematoxylin and eosin stains. This is very promising since the scaffolds produced three-dimensional growth of approximately 0.5cm thickness. We envision this as a beginning of a generation of biomaterials that will allow for thicker organs and tissue to be regenerated and self-sustain until the body can incorporate a blood supply into the area. Such scaffolds of specific tissue geometries could be used for the regeneration of several thick organs such as skeletal muscle, kidneys, liver, etc. Ideally, a oxygen generating scaffold could be created, seeded with cells, implanted in the body and maintain viability, and finally be taken over with vasculature from the surrounding areas. Future work will be analyzing the ability of the body to incorporate vasculature into the constructs for permanent acceptance and fabricating new biomaterials that exhibit the oxygen generating material for other applications such as wound dressings.

References: 1. Kellner K, *et al*. Biotechnology and Bioengineering, 2002; 73-83