Oxygen Generating Biomaterials for Improving Engineered Tissue Survival

Benjamin S. Harrison, Catherine L. Ward, Daniel Eberli, Se Heang Oh, and James J. Yoo
Wake Forest Institute for Regenerative Medicine, Wake Forest University Baptist Medical Center, Winston-Salem, NC, USA

Statement of Purpose:
Engineering clinically applicable tissues remains a central focus for tissue engineering and regenerative medicine. One challenge to engineering large (>1cm^3) tissue is supplying oxygen to cells within the construct while the vascular support is being established. Because oxygen diffusion is limited to hundreds of microns, cells located within large scaffolds (>1cm^3) typically do not have sufficient oxygen available to remain viable.

One approach we have undertaken to address this challenge is creating biomaterials capable of sustained oxygen generation.[1] These materials are designed to provide controlled release of oxygen for maintaining cell viability in hypoxic environments. These materials consist of an oxygen producing compound such as sodium percarbonate or calcium peroxide encapsulated in a polymeric matrix that allows for the controlled generation of oxygen. We have prepared this material and evaluated its oxygen generating potential and effect on cell viability.

Methods:
Oxygen producing biomaterials were prepared first by dissolving poly(D,L-lactide-co-glycolide) (PLGA 50:50) in methylene chloride. Sodium percarbonate (SPO) or calcium peroxide (CPO) was dispersed in the solution and cast into a mold to form freestanding films. The solvent was allowed to evaporate in air followed by vacuum-drying (5 ~ 10 mbar) for 48 hrs to remove residual solvents. To measure the rate of oxygen release, the films were placed in water and the rate of oxygen release was measured by recording the volume of water displaced with the collected oxygen gas generated.

To assess the effects of the oxygen generating biomaterial on cells experiencing hypoxia, the SPO material were placed in a well plate with keratinocytes and incubated for 24 hours. Keratinocytes, which are sensitive to oxygen concentration, were incubated at 20% or 1% oxygen during that time. Viability was assessed using a neutral red assay.

Results:
We have been developing oxygen generating biomaterials that are designed to provide controlled release of oxygen to maintain cell viability in hypoxic environments. Upon placing in water, the films produced a continuous stream of oxygen. Sodium percarbonate containing films produced oxygen over a period of hours and more rapidly than the calcium peroxide containing films which produced oxygen over several days. (Figure 1)

The neutral red assays demonstrated two things. First, the oxygen generating materials are non-toxic to keratinocytes at normoxia (20% oxygen). Second, under hypoxic conditions (1% oxygen), which is insufficient for keratinocytes to survive, the presence of the oxygen generating material maintained normal cell viability.

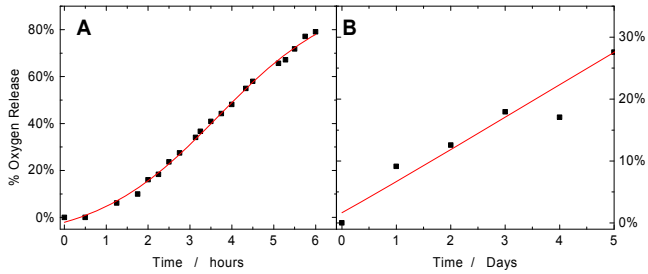

Figure 1. Sustained oxygen release from hours (A) using SPO to days (B) using CPO can be achieved with oxygen rich compounds.

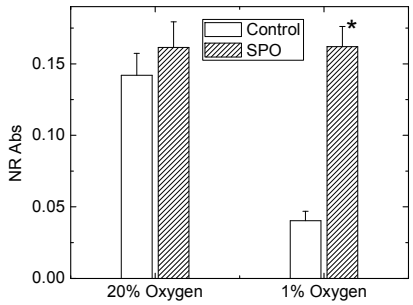

Figure 2. Neutral red assay of keratinocytes incubated for 24 hours in 20% (normoxic) and 1% (hypoxic) O_2 in the presence and absence of oxygen releasing biomaterials.

Conclusions:
A new class of biomaterials has been developed capable of in situ generation of oxygen. Such material may be able to extend cell viability within the typically hypoxic environment found in cell seeded constructs. This may be possible because oxygen could be generated within the normal diffusion range of oxygen inside the scaffold and cells could use the generated oxygen while the vascular structure is being established. These findings indicate that the use of oxygen generating biomaterials may allow for increased cell survivability while vascularization is being established after implantation. Such scaffolds may play an important role in tissue engineering where currently oxygen diffusion limits the engineering of large tissue implants.

References:
1. Harrison BS. Biomaterials. 2007:28: 4628-4634.