Oxygen Generating Materials for Muscle Regeneration

Catherine L. Ward, Ben Corona, Venu Kesireddy, Masood Machingal, Weixin Zhao, George Christ, Benjamin S. Harrison
Wake Forest Institute for Regenerative Medicine, Wake Forest University Baptist Medical Center, Winston-Salem, NC, USA

Statement of Purpose: Loss of functional skeletal muscle due to congenital and acquired conditions, such as traumatic injury, tumor excision, etc., produces a physiological deficit for which there is still no effective clinical treatment. Tissue engineering of skeletal muscle *in vitro* for functional tissue replacement *in vivo* may provide a potential therapeutic solution to this unmet medical need. Skeletal muscle requires an adequate source of oxygen that is usually provided by a vascular network of blood vessels and capillaries.[1] However, in replacement tissue, this supply is not present in the early stages and the avascularized tissue is difficult to integrate into the native, healthy muscle tissue. Particulate oxygen generators (POGs) are particles that have the ability to release oxygen when placed in aqueous environments such as the body. They have previously been shown to aid in providing oxygen in an ischemic skin flap model in mice.[2] We hypothesize that a scaffold containing POGs could provide a supplemental source of oxygen for skeletal muscle *in vitro,* generating oxygen for cells in environments with various oxygen tensions, similar to those seen in avascularized skeletal muscle constructs.

Methods: POGs were created by electrospinning a polymer-based mesh with the incorporation of peroxide-based particles. Meshes were cut to the appropriate size. For *in vitro* testing, rat muscle precursor cells (MPCs), primary skeletal muscle cells, were isolated and grown in both normoxic (20%) and hypoxic (<1%) environments. POGs were added to the culture dishes to provide a source of oxygen. At specified timepoints, cultures were removed to determine viability and differentiation of the skeletal muscle cells over time. Cells were stained and quantified using immunocytochemistry and Western Blot analysis. Control groups with no POGs present were also analyzed.

Results: Typically, skeletal muscle cells experiencing hypoxia do not differentiate down a myogenic pathway. It was observed, that in the presence of POGs, MPCs were able to both thrive and differentiate under hypoxia, proving that the supplemental oxygen was adequate for the cells. According to past studies, addition of the POGs increases the oxygen tension by 2-5%, which is enough for MPCs to thrive. It was also observed that the addition of POGs at normoxic levels facilitated faster and more defined myogenic maturation. Overall, POGs demonstrated the ability to advance the development of skeletal muscle *in vitro* at varying oxygen levels.

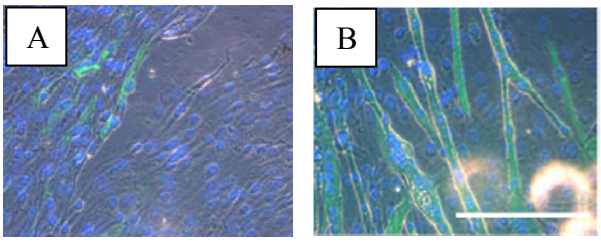

Figure 1. Effect of POGS on skeletal muscle cells
Skeletal muscle cells (an immortalized line) were grown with (B) and without (A) the presence of POGs in a normoxic environment for 14 days. Cells with POGs present were able to differentiate more markedly, as reflected by staining with myosin heavy chain, a marker of myogenic differentiation seen by green fluorescent staining. Differentiation was also evident by the multinucleated myofibers, where nuclei were stained with DAPI (blue). The scale bar represents 500um.

Conclusions: Skeletal muscle cells and tissue require a specialized amount of oxygen to both thrive and to differentiate, which lead to proper functioning tissue. We have determined that this amount of oxygen can be provided to the cells from a biomaterial that can be optimized to produce a particular amount of oxygen when placed in a culture dish at a range of oxygen tensions. These results suggest that POGs may act as an enabling technology for creation of large tissue constructs, providing oxygen in areas with diffusion limitations. The supplement will be able to provide enough oxygen to keep the tissue viable and in a healthy state while aiding in the skeletal muscle maturation until the constructs can be infiltrated with native vasculature. Our overall goal is to apply these findings to the *in vivo* environment. In that scenario, the POGs are incorporated into a biodegradable scaffold which will serve as a construct for muscle regeneration, generating oxygen as the scaffold is integrated into the native tissue. Studies are ongoing in a rodent model where 50% of the latissimus dorsi has been surgically removed and replaced with a POG construct. Future work will include testing the POGs in a larger and more clinically relevant muscle defect to determine the extent of the ability of the POGs to provide enough oxygen for large three-dimensional tissue.

References:

1. Di Carlo A. J Biol Chem. 2004;16:279:16332-16338.
2. Harrison BS. Biomaterials. 2007;28:31:4628-4634.

Acknowledgements:
This project is funded by NIH Grant AR05735.