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Oxygen Diffusion Rates of Particulate Oxygen Generators (POGs) on Cell Viability

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Abstract: Oxygen diffusing biomaterials provide a controlled release of oxygen while minimizing moisture loss, which is a major complication in the application of topical oxygen to wounds. We have devised an oxygen releasing biomaterial using a combination of sodium percarbonate (SPO), calcium peroxide (CPO), and polycaprolacetone (PCL) to supply a quick diffusion of oxygen delivery to hypoxic cells. Results indicate oxygen levels released via treatment enhanced cell viability despite hypoxic conditions as compared with normoxic environmental control.

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Keywords: Oxygen Producing Biomaterials, Hypoxia, Oxygen diffusion

1. Introduction

Oxygen concentrations play a vital role in the survival and growth of damaged or regenerating cell tissue. Efficient oxygenation is critical for tissue growth in any cell-based healing process because cells do not have a functioning supply of oxygen available during the days following trauma - causing hypoxia [1]. Tissue trauma can be susceptible to low oxygen environments due to ischemia, which often results in severe physiological changes and tissue death in as little as four hours [2]. Particulate oxygen generators (POGs) have been studied for uses in engineered tissue as well as for other applications such as wound dressings [3,4]. It has been shown that hyperoxic conditions increase the expression of keratinocyte growth factor, which is the primary growth factor for these cells [5]. With this in mind we set out to engineer an oxygen generating biomaterial that would enhance cell growth by diffusing oxygen into a hypoxic cell environment. In order to determine the maximum effectiveness of our POGs it was necessary to determine how most effectively to distribute them into tissue scaffolds, wound dressings, or other structures in order to enhance cell viability and proliferation. This was determined by calculating the optimal diffusion distance between the POGs and their target cell tissue through a collagen matrix, then assessing cell viability with a CellTiter 96 Aqueous One Solutions Cell Proliferation (MTS) assay.

2.Material and Methods

2.1 Electrospinning

Sodium percarbonate (SPO) sheets were electrospun using 5% hexafluroisopropanol/ polycaprolacetone (w/v) and 10% 40μm SPO (w/v). This substance was spun at 20 kV with a flow rate of 1.0 mL/hr. Polycaprolactone (Polysciences, Warrington, PA, USA) was dissolved in Hexafluoro-2-propanol (Triad Scientific, Manasquan, NJ, USA) at a 5% w/v then dissolved at 37℃ overnight. Fifty percent w/v of sodium percarbonate (Acros Organics, Waltham, Massachusetts, USA) was then added and vortexed for three minutes to evenly disperse the SPO particles. This substance was electrospun into sheets using 15ml of POG solution using syringe infusion pumps (Medfusion, Lewisville, TX, USA) with a maximum load limit of 5ml creating a thickness between 100-150 μm. Spinning parameters were set to a 3ml/hr flow rate using 20KV power supply. The rotating mandrel (4 cm in width and 10 cm in height) was placed approximately 10 cm from the tip of the needle (0.838 mm inner diameter, 18-gauge). The electrospun POG sheet was then stored in a 15cm^2 cell tissue plate.

2.2 Seeding Keratinocytes

Keratinocytes were seeded in 2-mL glass vials on a 150µL layer of collagen. Additional collagen was aliquoted onto the cells at 3mm, 5mm, and 7mm respectively (Fig. 1).

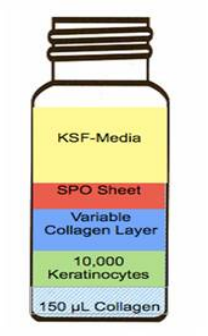

Fig. 1 Collagen levels are layered below cells above cells at 3 mm, 5mm and 7 mm.

Kerotinocyte Serum Free (KSF) media supplemented with epidermal growth factor and bovine pituitary extract (Gibco #37000-015, Grand Island, NY, USA) was added as a growth supplement. Vials designated as negative control contained normoxic media, while positive and experimental controls contained media stored in hypoxic chamber set at 2% oxygen. Experimental vials contained an 8 mm-diameter sample of the 44% ± 5% SPO electrospun sheet. This system was given 48 hours to stabilize prior to analysis.

2.3 MTS Assay

Cell Titer 96 Aqueous One Solution Cell Proliferation Assay (MTS Assay Kit purchased from Promega Corporation, Madison WI, USA) was performed for each group at 2-day, 4-day and 6-day time points. Absorbances were obtained at 490nm using a spectrometer.

3. Results and Discussion

Polycaprolacetone (PCL) was used to homogeneously disburse SPO particles throughout the electrospun sheet. Particle characterization was assessed via scanning electron microscope (SEM). Fibers were shown to have a diameter of 0.28µm ± 0.12µm with film thickness of 117µm ± 22µm (Fig. 2).

Oxygen displacement was assessed using a dressing composed of 44% ± 5% SPO along with a hydrogel layer. When submerged in water 90% of the oxygen was released from the SPO within 48 hours (Fig. 3).

Collagen was used to determine the range of diffusion for the POGs. Results of MTS analysis for keratinocyte viability over a 6-day period showed maximum diffusion range between POGs and cells to be between 3-5 mm as compared with hypoxic control (Fig. 4).

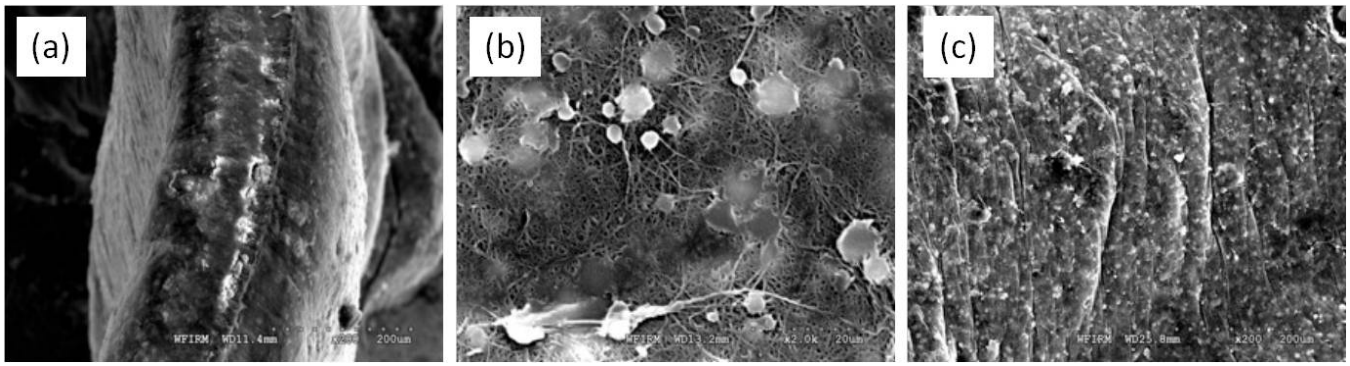

Fig. 2 a) *Scanning Electron Microscope (SEM)* of SPO (44% ± 5%) electrospun sheet, b) SPO particles were encapsulated by PCL and homogeneously dispersed, c) Fibers show a diameter of 0.28 µm ± 0.12µm with film thickness of 117µm ± 22µm.

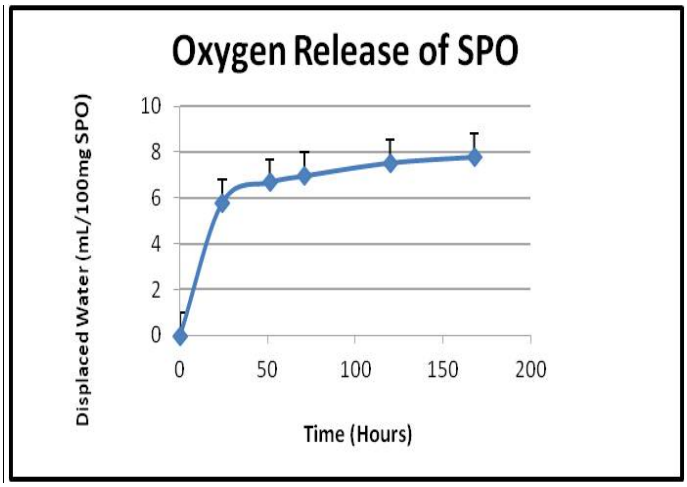

Fig. 3 Oxygen displacement with respect to time shows 90% of the oxygen is released within 48 hours.

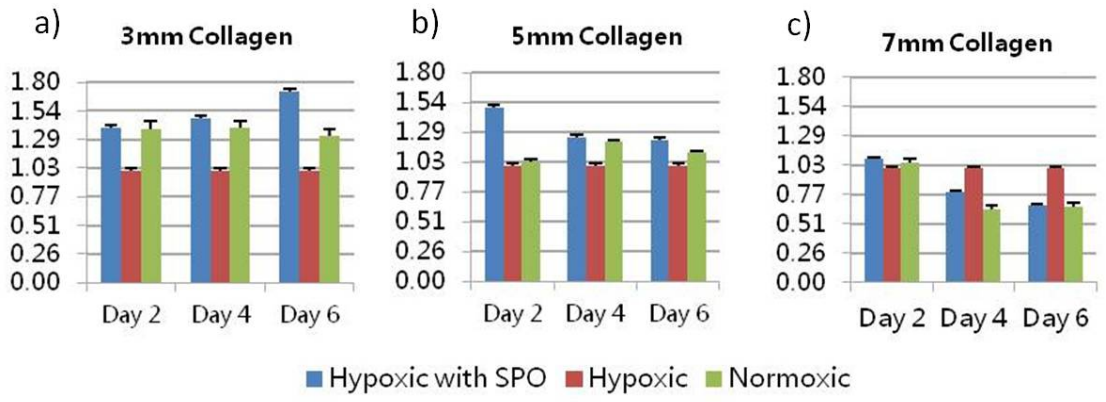

Fig. 4 a) Cell viability over a 6-day time period shows proliferation for normoxic and experimental condition is not enhanced beyond 5mm in the collagen.

4. Conclusion

Results indicate oxygen levels released from POGs enhanced cell viability despite hypoxic conditions as compared with normoxic controls. Here we show that 3-5mm of collagen is the maximum diffusion range between POGs and cells in order to maintain cell viability and proliferation under hypoxic conditions. In this range oxygen diffusion was effective, but beyond 5 mm it was no longer beneficial in maintaining cell viability. Only after 48 h, while oxygen is still rapidly generating, is it capable of diffusing through a 5 mm level of collagen to the cells. These results suggest that POGs can maintain cell viability and proliferation in a hypoxic environment at well as or greater than the same cells in normoxic conditions.

Acknowledgements

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