



AN EVALUATION OF A TOPICAL DISSOLVED OXYGEN DELIVERY DEVICE ON DERMAL CELL VIABILITY

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Introduction

Several key processes in wound healing are dependent upon an adequate supply of oxygen. Oxygen is used in the respiratory burst to kill bacteria, synthesis and hydroxylation of collagen, proliferation of fibroblasts, and oxidative pathways for ATP formation. We have developed a topical oxygen delivery device in a dressing format that is designed to deliver oxygen by release of pre-formed oxygen contained in a closed cell foam matrix. These materials deliver >300 mmHg of oxygen for an intermittent duration using this model. In the current study we show that the dressing delivers physiologically relevant oxygen for recovery and maintenance of dermal cells cultured under hypoxic conditions. The dressing's delivery of oxygen to a hypoxic environment allows cells to maintain metabolic processes. This dressing offers a tool for the topical transfer of oxygen into hypoxic tissue associated with chronic wounds in order to facilitate cellular mechanisms involved in healing.

Materials and methods

O₂ penetration of live skin samples

Oxygenesys™ Continuous releases pre-formed oxygen from a closed cell foam matrix. The delivery of dissolved oxygen by these dressings into physiologic saline (0.85% NaCl) was measured using an *in vitro* human skin model(*). All recordings were performed at 37°C. Human skin samples were provided by Community Tissue Services, Portland, Oregon

Cell Cultures

Adult Human dermal fibroblasts were obtained from Cascade Biologics (C-013-5C) and maintained as monolayers in DMEM (Gibco) supplemented with 10% bovine serum (Sigma) and Penicillin-Streptomycin (Gibco) at 37°C and 5% CO₂.

Cell Viability Assay

Cell viability was monitored at time points of exposure to test conditions using a tetrazolium compound (MTS) reduced to a colorimetric product by active mitochondrial dehydrogenase enzymes. Cell media was aspirated from the cell layer and 1ml of DMEM with out phenol red and 10% MTS/PMS was added to each well. After incubation at 37°C and 5% CO₂, the absorbance was read at 490nm in a microplate reader (EMax, Molecular Devices).

Protein Assay

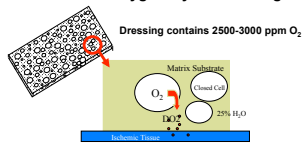
After completion of the viability assay, media/dye solution was aspirated and 0.25 ml of cell lysis solution (250nM Tris pH 7.4, 0.1% Triton X-100) was added to each well. After 15 min. incubation 0.25 ml of saline was added to each well. 0.05 ml of sample was added to 0.15 ml Bradford Reagent (Sigma) in a 96-well plate and absorbance read at 590 nm.

Materials and Methods cont.

Experimental Treatments

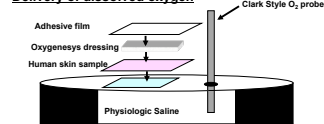
Fibroblasts were seeded at 10,000/cm² in 12-well plates. After adhering overnight cells were washed with PBS. DMEM without serum was added at 1.5 ml per well. Experiments were started 24 hrs after addition of serum free media. For samples containing Oxygenesys dressings 8mm punches of dressings were added to each well. Dressing punches were exchanged every 48h. Hypoxic conditions were performed by incubating cells inside a hypoxic chamber (Billups-Rothenberg) in a mixture of 5% CO₂ and 95% N₂ following the manufacturer's instructions to attain a 0.1% oxygen concentration and incubated at 37°C.

Schematic of Oxygenesys™ Dressings



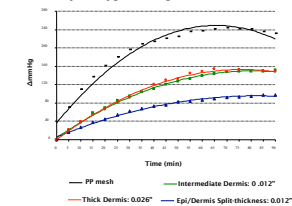
Results

Delivery of dissolved oxygen

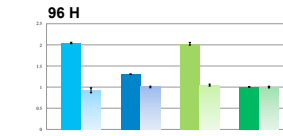
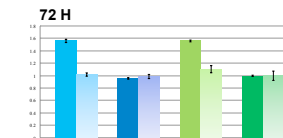
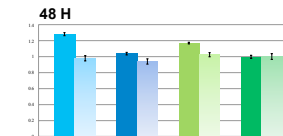
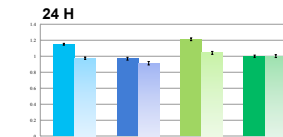


* apparatus for measurement of transdermal oxygen delivery

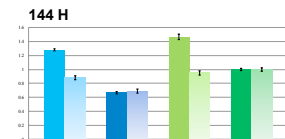
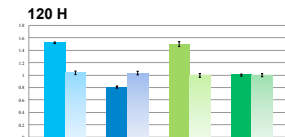
Delivery of oxygen through human skin substrates



Relative Fibroblast Viability and Protein Content



Relative Fibroblast Viability and Protein Content cont.



Data Interpretation:
MTS: metabolic state/viability of cells
Protein content: gross estimate of cell number

Conclusions

- Oxygenesys dressing delivers oxygen into dermal tissue up to 600 microns
- Oxygenesys dressing increases metabolic activity of fibroblast above cells cultured under normoxic conditions alone within 24 h and up to 144 h.
- Oxygenesys dressing protects fibroblast from metabolic deficiency and eventual cell loss due to hypoxia.

*AcryMed, Inc. is a subsidiary of I-Flow Corporation, Lake Forrest, Ca.