**A modified "wound healing" and hypoxia model in vitro**

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## Introduction

Closing a wound may resemble some aspects of fusion of embryonic processes during development of face. In addition, both are sensitive to oxygen supply.

## Methods

Human dental pulp stem cells (HDPSC, Celprogen) were grown in alpha MEM with 10% human serum, L-glutamine and penicillin/streptomycin mixture (Invitrogen) and maintained in atmospheric oxygen or 0.5% oxygen (Biospherix chamber).

Strips 2-4 mm wide and 5-6 mm long (Grace Biolabs) were placed in petri dishes, overlayed with HDPSC (1 million cells/ dish) and grown for 2 days in atmospheric oxygen. Then, strips were removed, plates incubated for 12 hours in atmospheric oxygen and placed for 6 hours in 0.5% $O_2$. Controls were incubated only in atmospheric oxygen. Then, all cultures were stained with 1% toluidine blue.

## Results

After three days of cultivation of the control plates, spindle-shaped cells predominated in culture and started filling the gap, while round-shaped cells spread throughout the dish. In the plates exposed to hypoxia for 6 hours and then cultivated for three days in atmospheric oxygen, round-shaped cells in the monolayer outside of the gap margin exhibited a mass detachment after 48 hours, while cells inside the gap margin were spindle-shaped and filled the gap by migration and proliferation.

## Conclusions

We have established the procedure by which our modified "wound healing" combined with hypoxia model can be assembled, followed and evaluated. We observed marked qualitative differences between cultures exposed to transient severe hypoxia and controls in the degree the cell-free areas were filled by cells.