HUMAN DENTAL PULP STEM CELL CULTURE MODEL FOR STUDIES ON ETIOPATHOGENESIS OF NON-SYNDROMIC CLEFT LIP WITH OR WITHOUT CLEFT PALATE ANOMALY

Tyler Starley[1], Jonathan Starley[2], Harrison Truman[1], Mahboube Hasheminasab[3]; Solaleh Amirpour[3]; Marie M. Tolarova[3], Miroslav Tolar[3,4]

[1]DDS student; [2]Department of Orthodontics Resident; [3]Department of Orthodontics; [4]Department of Biomedical Sciences, University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, CA, USA.

INTRODUCTION

Nonsyndromic cleft lip with or without cleft palate (NSCLP) occurs approximately in 1 of 700 live births. It has a serious impact on patient's life. NSCLP can be prevented by maternal supplementation of folic acid (FA). However, much more needs to be known about etiopathogenetic factors and molecular mechanisms leading to NSCLP in order to personalize its prevention.

Basic science information on etiology and pathogenesis of NSCLP was obtained from animal experiments. Clinical studies and trials showed efficacy of prevention by FA supplementation. A cell culture model is needed for studies on cellular and molecular mechanisms leading to development of NSCLP and underlying its prevention.

In etiology of NSCLP, genetic and environmental factors interact. Exposure of embryo to environmental toxins or malnutrition contributes to compromise of neural crest cells that are essential for development of face[1,2,3]. Human dental pulp stem cells (HDPSC) are descendants of neural crest cells. We isolate HDPSC from extracted teeth of patients with or without NSCLP (IRB2021-80).

OBJECTIVE

The purpose of this poster is to present preliminary data obtained with a model of cultured HDPSC exposed to external stressing conditions. Then, behavior of HDPSC isolated from patients with or without NSCLP will be compared.

MATERIALS and METHODS

- HDPSC (Celprogen) were cultured in growth media composed of 88% alpha MEM, 10% human serum, 1% penicillin and streptomycin and 1% L-glutamine.
- Cells were incubated at 37 ℃, 5% CO2 and atmospheric oxygen, until a cell monolayer was formed.

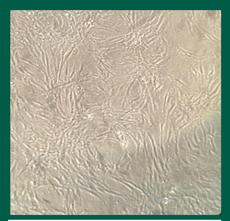


Figure 1: Monolayer of HDPSC

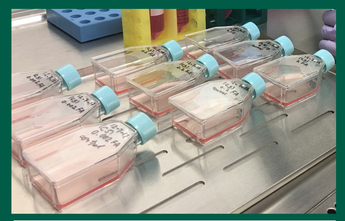


Figure 2: Flasks with HDPSC exposed to 0.002 µg/ml, 0.2 µg/ml, 2 µg/ml, and 20 µg/ml folic acid.

Seeded flasks were randomly divided in test and control groups**.**

Media with different concentrations of added FA (0.2 µg/ml, 2 µg/ml, and 20 µg/ml) were added to the test flasks. The controls contained 0.002 µg/ml FA. (**Figures 1, 2).**

The hypoxia group was put in the hypoxia chamber for 2 hours in 0.5% oxygen **(Fig. 3),** the normoxia group stayed in the incubator.

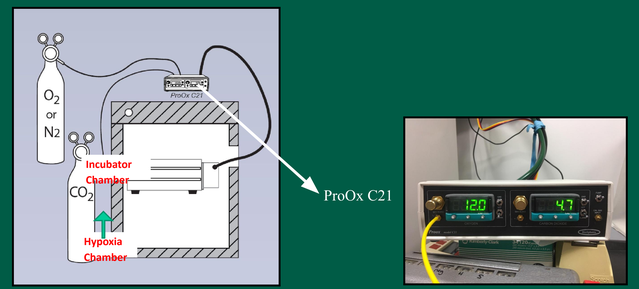


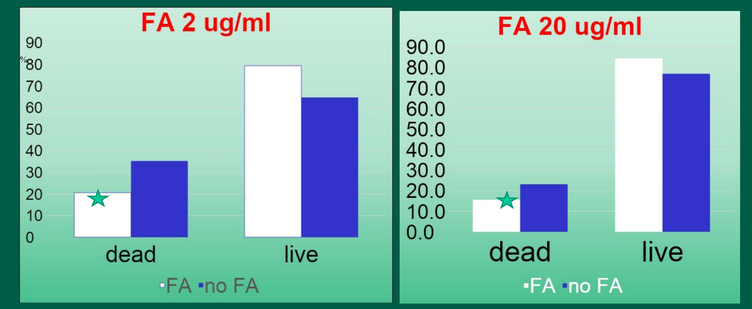
Figure 3: BioSpherix Proox Model C21 O2/CO2 Controller. The unit continuously controls and monitors O_2 and CO_2 levels.

Cells were then harvested using 0.25% trypsin solution, stained with 0.4% trypan blue for 10 minutes and counted in a hemocytometer.

RESULTS

FA Concentration	Percentage of Dead Cells		Percentage of Live Cells		P-Value
	Test group	Control Group	Test group	Control Group	
20 µg/ml	15.6	23.1	84.4	76.9	**0.18465**
2 µg/ml	20.6	35.3	79.4	64.7	**0.0001**
0.2 µg/ml	23	24.5	77	75.5	0.60822

Table 1. Live and dead (stained with trypan blue) cells were counted, and their frequencies were compared using Chi square test. Three experiments were performed. The optimal protective concentration was 2 µg/ml FA (P = 0.0001).



Graph 1: White columns show percentages of dead and live cells after 0.5% oxygen hypoxia for 2 hours when FA was added to cultivation medium (2 µg/ml and 20 µg/ml). Blue column is control.

A *statistically significant* decrease in the number of dead cells occurred in the flasks containing 2 µg/ml FA (P < 0.001) and 20 µg/ml FA (P < 0.05).
The difference was not statistically significant for the group with 0.2 µg/ml FA.

Graph 2: Percentages of dead and living cells after hypoxia in presence of 0.2 µg/ml FA.

CONCLUSIONS

- Supplemented FA increased survival of HDPSC exposed to transient severe hypoxia in a cell culture model.
- A future study will compare properties of HDPSC isolated from extracted teeth of patients with or without NSCLP and their sensitivity to folate supplementation.

REFERENCES

1. Bishop T, Ratcliffe PJ. HIF hydroxylase pathways in cardiovascular physiology and medicine. Circ Res. 2015;117(1):65–79. doi: 10.1161/CIRCRESAHA.117.305109
2. Fonseca SG, Burcin M. Endoplasmic reticulum stress in beta cells and development of diabetes. *Curr Opin Pharmacol.* 2009 December; 9(6): 763–770. doi:10.1016/j.coph.2009.07.003
3. Kim P. Effect of folate on endoplasmic reticulum stress caused by hypoxia. MSD thesis 2018

AKNOWLEDGEMENT

This study was partially supported by DRES03-115 Research Award from the Research Committee of the Dugoni School of Dentistry, UOP