Dental Pulp Stem Cell (DPSC) Differential Media Selection And Analysis

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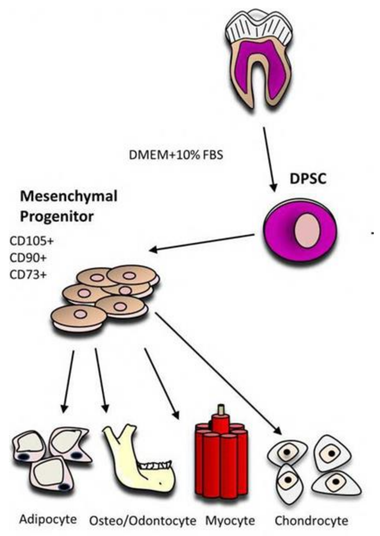
BACKGROUND

- Dental pulp stem cells (DPSC) are pluripotent mesenchymal stem cells found in the interior of the "pulp chamber" within healthy intact teeth.
- Evidence has emerged that DPSC may be capable of biologic regeneration and tissue repair and are therefore the subject of intense research into these new and emerging fields of clinically applied biotechnology.
- Despite the progress made in recent years to demonstrate these potential applications, much remains unknown regarding the most effective and appropriate methods for isolation, expansion and culture techniques for DPSC and whether these vary by DPSC phenotypes or biomarker expression.

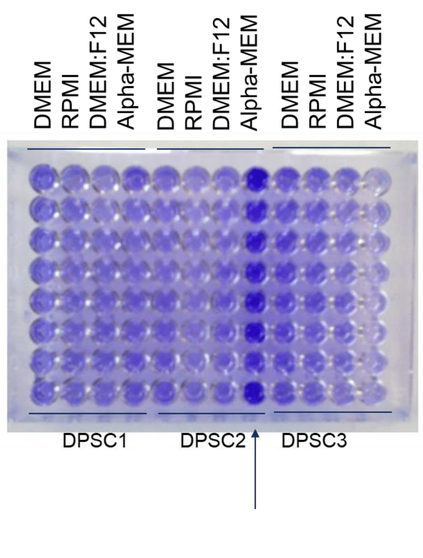


STUDY OBJECTIVE

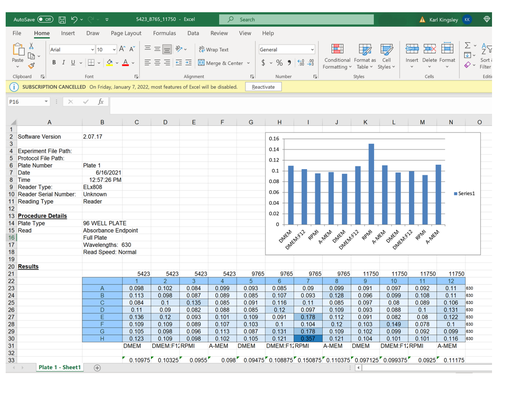
- To address these deficiencies, the primary objective of this study is to evaluate any effects of the major, commercially available cell culture medias on DPSC phenotypes, such as growth, viability and expression of pluripotent stem cell biomarkers, including:
- Dulbecco's Modified Eagle's Medium or DMEM
- Roswell Park Memorial Institute or RPMI
- DMEM:F12
- alpha-MEM

METHODS

- IRB approval was not needed for this in vitro (cell-based) study.
- Several previously collected and cryopreserved DPSC isolates were identified, thawed and cultured for this study, n=18.
- Each DPSC isolate was plated into 96-well assays under each of the experimental conditions (DMEM, DMEM:F12, RPMI, alpha-MEM) to determine any effects on cellular growth and viability.
- Cells were fixed with formalin and stained with Gentian (crystal) violet and read using a BioTek 808 Microplate reader using absorbance at A630 nm (corresponding to violet wavelengths); darker = more cells, more growth.



- Absorbance reading were exported into Excel
- Data are continuous (measured on a scale)
- Can use descriptive statistics to give averages
- Can use Student's t-tests to compare media
- Statistical significance, $p<0.05$



RESULTS

- Growth and viability assays revealed differential responses among DPSC isolates.
- Some responded best to one media (DPSC-5653 to DMEM:F12; top row), p=0.018
- Some responded to two different medias (DPSC-9765 to DMEM, DMEM:F12 bottom row), p=0.021
- Others did not respond to any media tested (DPSC-3882), p=0.366

	DMEM	DMEM:F12	RPMI	alpha-MEM
rDT				
5653 d1	35	81	54	37
5653 d2	45	77	69	48
ave.	40	79	61.5	42.5
% change	0.2857142857	-0.04938271605	0.2777777778	0.2972972973
8604 d1	62	54	41	39
8604 d2	66	39	42	57
ave.	64	46.5	41.5	48
% change	0.06451612903	-0.2777777778	0.0243902439	0.4615384615
7089 d1	75	64	51	75
7089 d2	62	36	55	62
ave.	68.5	50	53	68.5
% change	-0.1733333333	-0.4375	0.07843137255	-0.1733333333
3924 d1	65	50	73	69
3924 d2	63	46	47	63
ave.	64	48	60	66
% change	-0.03076923077	-0.08	-0.3561643836	-0.0869565217
3882 d1	45	69	65	43
3882 d2	40	37	36	52
ave.	42.5	53	50.5	47.5
% change	-0.1111111111	-0.4637681159	-0.4461538462	0.2093023256
9765 d1	63	67	50	72
9765 d2	58	61	61	43
ave.	60.5	64	55.5	57.5
	-0.07936507937	-0.08955223881	0.22	-0.4027777778

Optimal condition with low variability		
Potential condition with variability		
Potential condition with high variability		
DMEM	3 green	1 yellow
DMEM:F12	2 green	
RPMI	1 green	
alpha-MEM	1 green	1 yellow

Table 1. Total RNA isolation and cDNA synthesis analysis.

DPSC isolate	RNA concentration	cDNA concentration	A260:A280
4595	311.1 ng/ul	1660.4 ng/ul	1.81
1089	447.7 ng/ul	1667.7 ng/ul	1.81
11418	296.2 ng/ul	1617.8 ng/ul	1.84
8124	424.6 ng/ul	1735 ng/ul	1.84
5653	213.1 ng/ul	1903 ng/ul	1.82
11750	140.8 ng/ul	1632.9 ng/ul	1.81
17322	528.9 ng/ul	1326.6 ng/ul	1.83
3924 a-MEM	157.8 ng/ul	1937.6 ng/ul	1.82
9765 F12	256.8 ng/ul	2105.7 ng/ul	1.82
9765 DMEM	323 ng/ul	1858.2 ng/ul	1.81
11832	291.3 ng/ul	1196.7 ng/ul	1.80
3924 DMEM	289.7 ng/ul	1377.7 ng/ul	1.82
8604	216.9 ng/ul	947.6 ng/ul	1.82

Table 1. RNA extraction using phenol: chloroform was successfully performed with concentrations ranging from 140 – 528 ng/uL. cDNA synthesis was done using the High Capacity cDNA reverse transcription kit from ThermoFisher, with concentrations ranging between 1326 - 2105 ng/uL and purity (A260:A280 ratio) > 1.80.

Analysis of samples was completed using the NanoDrop spectrophotometer at absorbances of A260 and A280 nm, which allows for purity and concentrations to be calculated

Table 2. qPCR biomarker screening results

DPSC isolate	SOX2	OCT4	NANOG	Nestin
4595				
7089				
11418				
8124				
5653				
11750				
17322				
3924 a-MEM				
9765 F12				
9765 DMEM				
11832				
3924 DMEM				
8604				

Table 2. Analysis revealed DMEM responses for n=7 (viability ranging between 50.5% - 68.5%), DMEM:F12 for n=6 (viability 52% - 72.5%), RPMI for n=5 (viability 52% - 68%), and alpha-MEM for n=5 (55,5% - 74.5%). To see if these results correlated with DPSC biomarkers, mRNA was screened using qPCR.

qPCR revealed most DPSC isolates continued to express one or more of the pluripotent stem cell biomarkers (Oct4, Sox2, Nestin, NANOG);

However, no clear pattern of growth and viability with the optimal media type correlated with these biomarkers.

CONCLUSIONS

This study determined that out of the n=52 potential combinations (n=13 DPSC isolated x 4 media types) only n=21 (DMEM, n=7; DMEM:F12, n=4; RPMI, n=5; alpha-MEM, n=5) resulted in viability with the top n=13 selected for further culture and RNA isolation.

Screening of mRNA using qPCR revealed most DPSC isolates continued to express one or more of the pluripotent stem cell biomarkers (Oct4, Sox2, Nestin, NANOG) – but no clear pattern of growth, viability or biomarker expression was found with the optimal media type.

These results strongly support the hypothesis that differential growth media screening may be necessary to ensure the highest viability and growth potential for DPSC isolates, but also suggests that biomarker expression or growth rate may not be sufficient to determine which media will work best.

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