Matrix Mechanics Dictate Odontoblast Responsiveness to Photobiomodulation Treatments

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Introduction

Regenerative dentistry aims to promote directed differentiation of stem cells. While the predominant focus of these efforts investigate genetic manipulations, the epigenetic roles of the extracellular matrix (ECM) have been poorly investigated.

Hypothesis

Simulating wound-like microenvironment by altering matrix mechanics and biochemical conditions, PBM responses on Odontoblasts can be better directed to promote dentin regeneration.

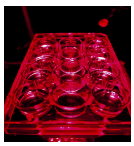
Methods

Polymer Scaffolds

- Polydimethylsiloxane (Sylgard 184 silicone elastomer) was used in various proportions (2:1, 20:1, and 40:1) in 12-well polystyrene plates that were cured for 48 hours at 105°C in an oven.
- The resulting surface stiffness was measured using a Shore A Durometer (Insize).

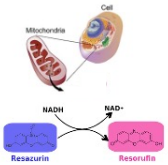
Sterilization and Seeding

- Each plate was sterilized with 70% ethanol, PBS, and UV-A treatments for 15 minutes each. Following sterilization, the plates were serum-coated with FBS. Odontoblasts (MDPC) cell lines may then be incubated in each well.



Cell Viability Assay

- Alamar Blue Assay performed at 24 hours
- Fluorescence assessed using a spectrophotometer
- Data organized with Graph Pad Prism



Growth Factors/Inhibitors and Altered 'Wound' Conditions

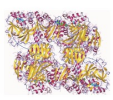
TGF-β1 (Transforming Growth Factor)

Extrinsically drives cell growth/development, tissue repair, and differentiation

FAK Inhibitor (Focal Adhesion Kinase Inhibitor)

Intrinsically inhibits focal adhesion kinase, preventing cell adhesion, reduce cell growth

SB-431542 (TGF-β1 Inhibitor)



Blocks the promoting effects of TGFβ1, reduces cell growth, migration, etc.

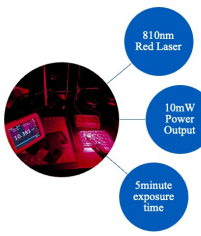
Hypoxic Media (Cobalt Chloride in DMEM)

- Using cobalt chloride, a hypoxic media condition is created;
- This emulates a lack of oxygen mimicking a wound-like scenario

Serum Starvation (0.2% FBS DMEM)

- FBS is a growth supplement within cell media;
- A low serum condition imitates wound-like conditions where cells are deprived of serum

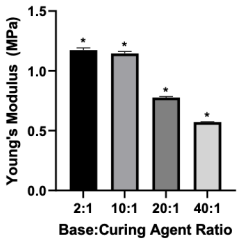
Photobiomodulation Treatments have shown to



- **Activates latent TGF-β1**
- **Increases cell viability**
- **Promotes osteodentin induction**

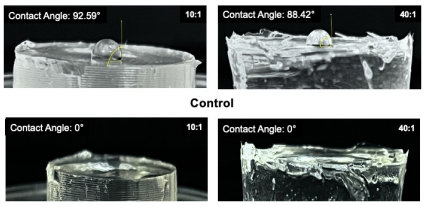
Results

Varying Polymer Ratio Correlates with Substrate Stiffness



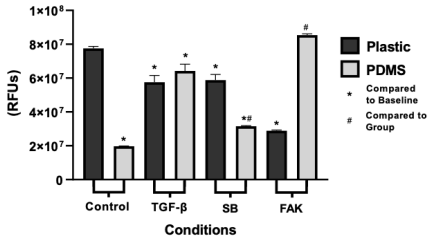
- Shore A values were converted to MPa. Plastic wells were noted to have durometer reading of 100 or 5.5MPa (saturation), but the reported polystyrene has a value of ~3000 MPa.

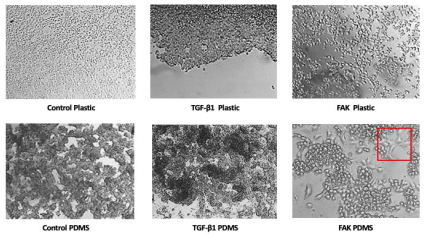
Serum Conditioning Enables Cell Attachment

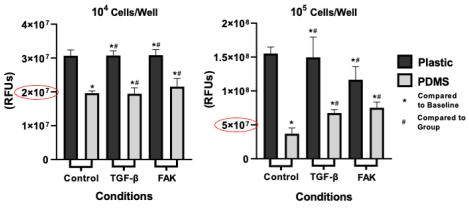


- Serum conditioning increased the contact angle and wettability of polymer surfaces, enabling cell adhesion and growth.

Substrate Stiffness and Signaling Pathways Modulate Cell Viability & Morphology Independent of Cell Density



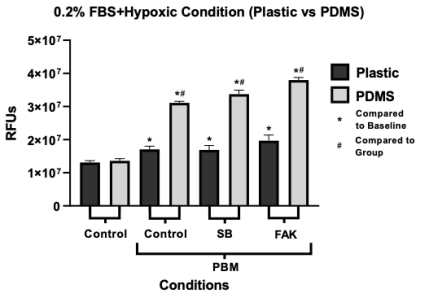


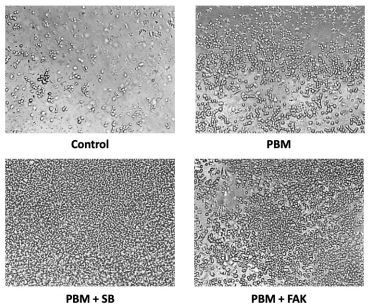


Soft matrix reduces cell viability which was rescued by both TGF-β1 addition and FAK inhibition at high cell density. Cells appear to cluster in PDMS conditions, but a migratory phenotype is observed following FAK inhibition. Surprisingly, there were no effects on cell adhesion (data not shown, *manuscript in preparation*).

Results

PBM Treatment Rescued Cell Viability in 'Wound' Scenario





- In simulated wound conditions, 'soft matrix, hypoxic, low serum,' PBM treatment rescued Odontoblast viability. This appeared to be further enhanced by TGF-β1 and FAK inhibition.

Conclusions

By altering the matrix stiffness and generating hypoxic and low serum conditions, a wound scenario can be simulated where odontoblasts have poor survival. These effects appear to be mediated by TGF-β1 and FAK signaling pathways. We observed that PBM treatments can rescue Odontoblast survival in simulated wound conditions.

Future Directions

- To analyze cell survival and migration by inhibiting ERK and AKT signaling after PBM treatments;
- To investigate if increased cell survival is due to increased Odontoblast proliferation or enhanced resilience (fitness).

This study aims to generate a mechanistic understanding of Odontoblast responses for human clinical translation of PBM treatments for Endodontic Regeneration.

References

1. Evans ND et *al.* Substrate Stiffness Affects Early Differentiation Events in Embryonic Stem Cells. J Eur Cells and Mat 18, 1 – 14 (2009).
2. Ponnusamy S et *al.* Redox signaling induces laminin receptor ribosomal protein-SA expression to improve cell adhesion following radiofrequency glow discharge treatments. *Sci Rep* 12, 7742 (2022).
3. R. Seghir, S. Arscott. Extended PDMS stiffness range for flexible systems. Sensors and Actuators A: Physical, Elsevier, 230, 33-39 (2015).
4. Yoon H, Dehart JP, Murphy JM, Lim ST. Understanding the roles of FAK in cancer: inhibitors, genetic models, and new insights. J Histochem Cytochem. 2015 Feb;63(2):114-28. doi: 10.1369/0022155415614498. Epub 2014 Nov 7. PMID: 25380750; PMCID:PMC430551
5. Arany PR. Photobiomodulation-Activated Latent Transforming Growth Factor-β1: A Critical Clinical Therapeutic Pathway and an Endogenous Optogenetic Tool for Discovery. Photobiomodulation Photomed Laser Surg. 2022 Feb;40(2):136-147. doi: 10.1089/photob.2021.0109. Epub 2021 Dec 14. PMID: 34905400.