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PATHWAY OF REGENERATION-DENTIN AS A NOVEL BARRIER MEMBRANE

Dental Science

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ABSTRACT

Context: Correction of periodontal bone loss using GTR is an accepted approach. But absorbable membrane lacks flexibility and has limited osteo-conductive and osteo-inductive capability.These aspects require further improvement to better utilize barrier materials with natural source and contents similar to bone, non-invasive attainability, bio-compatibility, low risk of rejection and infection.

Aims: To evaluate proliferation and attachment of dental pulp stem cells on demineralized dentin to be used as semi-rigid barrier for GTR.

Methods and Material: Dentin was harvested from caries free extracted teeth and were stored in 10% formalin. They were sliced in the occluso-apical direction in cuboid form to acquire a thin block(2x2x8mm) of medial dentin. Three specimens from each group- untreated dentin, acid treated dentin, and collagen membrane were included. MTT assay was done to detect cell attachment and proliferation of dental pulp cells on each specimen. Pulp stem cells were incubated on the each specimen for 3 days and observed under microscope.

Results: Cell attachment and proliferation of dental pulp stem cells were observed on acid treated dentin.

Conclusions:Hence, we conclude demineralized dentin is bio-compatible and can be used in future as novel barrier membrane for guided tissue regeneration.

KEYWORDS

Demineralized dentin ; GTR; MTT assay; Periodontitis.

INTRODUCTION:

In 1976, Melcher suggested that the nature of cell attachment is determined by the type of cell repopulating the root surface after periodontal surgery.After flap surgery the curetted root surface may be repopulated by four different types of cells: Epithelial cells, cells derived from the gingival connective tissue, cells derived from the bone and cells derived from the periodontal ligament.[1]It is recognized as a validated and reliable procedure and is widely accepted by dental practitioners. However, barrier membranes are not sturdy enough to support the covering soft tissue, thus, compression of space between barrier membrane and debrided root surface is inescapable.[2] Accordingly, different regenerative strategies such as the use of growth factors and biomaterials have been merged with GTR to aid periodontal tissue restoration. However, the limited effectiveness of regeneration of periodontal soft and hard tissues still leaves room for further up gradation.[3]

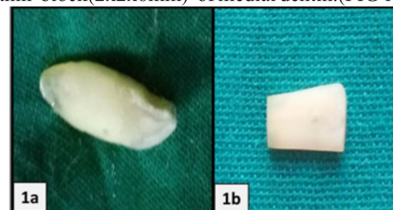
Dentin, the chief component of tooth, which content 70% mineral, 20% organic components, and 10% water, quite similar to that of bone tissue.[4] Viewing properties such as bio-security, non-invasive attainability, low risk for rejection and infection, should guide us to take full advantage of this natural source for periodontal applications. Yeomans and Urist (1967) were the first to find the regenerative properties of autogenous demineralized dentin matrix. It was also observed that demineralized dentin exhibited plasticity and calcifying capability to some extent, which amplifies the possibility of utilizing demineralized dentin as a barrier material for GTR in the treatment of periodontitis.[5] Acid etching treatment is used for dentin modification. Its advantages are high manoeuvrability, low cost and non-toxity.[6] Irrespective of what kind of acid and how long the duration of etching were tried previously, they all showed reduction in intrafibrillar mineralization while increasing collagen fibrils exposure.[14]

Gronthos et al (2000) was the first to isolate stem cells from human dental pulp. Stem cells from human exfoliated deciduous (SHED) teeth are immature, unspecialized cells that are able to grow into specialized cell types.Miura et al (2003) observed that SHED represent a population of postnatal stem cells capable of extensive proliferation and multipotential differentiation into neurons, adipocytes, myocytes, chondrocyte and,osteoblasts.[7]

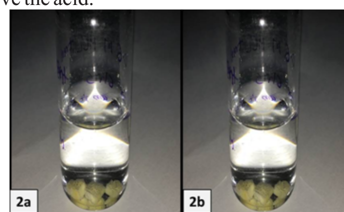
Taking into consideration the regenerative properties of demineralized dentin and dental pulp stem cells provides extensive research scope. In this present study we have evaluated dental pulp stem cells proliferation on demineralized dentin to be used as semi-rigid barrier membrane for GTR.

MATERIALS AND METHODS:

Six caries-free teeth which were extracted from healthy adult subjects for orthodontic reasons were used for this study with patient's consent. All extracted teeth were stored in a solution of 10% formalin immediately after extraction. They were scaled to remove the debris and sliced in the occluso-apical direction in cuboid form to acquire a thin block(2x2x8mm) of medial dentin.(FIG 1a , b)



Five specimens were divided into four groups each- untreated dentin (S1), 0.6 M hydrochloric acid treated dentin[6] (S2) (FIG 2a), 2% nitric acid acidtreated dentin[8] (S3)(FIG 2b) and collagen membrane (S4). Acid treated dentin was then rinsed with ample amount of distilled water to remove the acid.



In order to culture dental pulp stem cells, dental pulp wereextirpated from caries free deciduous teeth indicated for extraction for

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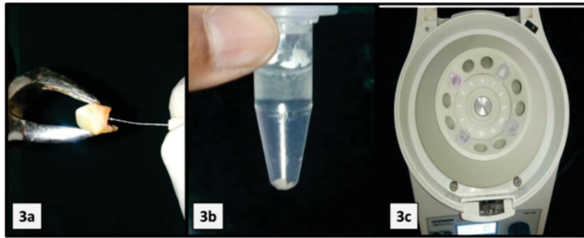
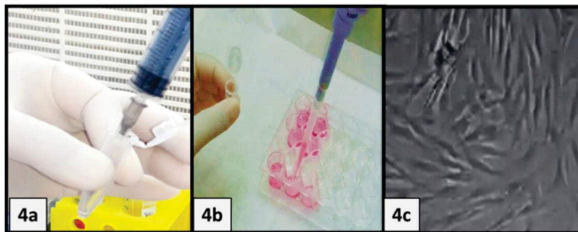
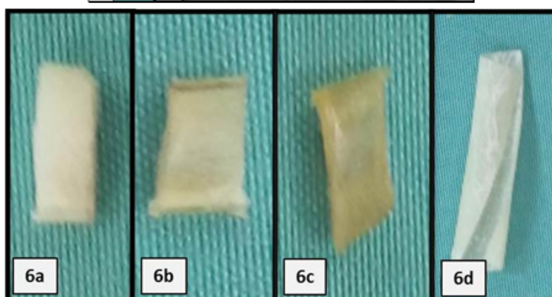
orthodontic purpose.(FIG 3a) Once the dental pulp was extirpated it was transfer to phosphate buffer saline solution (PBS) and then centrifuged at 254 rpm for three minutes (FIG 3b, c).



Serum layer was removed for centrifugated sample (FIG 4a) and was transferred to 3 consequent wells of 12 – well culture plate containing minimum essential media (MEM)(FIG 4b) then the culture plate was incubated for 4 days in CO2 incubator. Minimum essential media(MEM) was changed alternate days. After 4 days samples were observed under inverted microscope and dental stem cells were obtained. (FIG 4c)



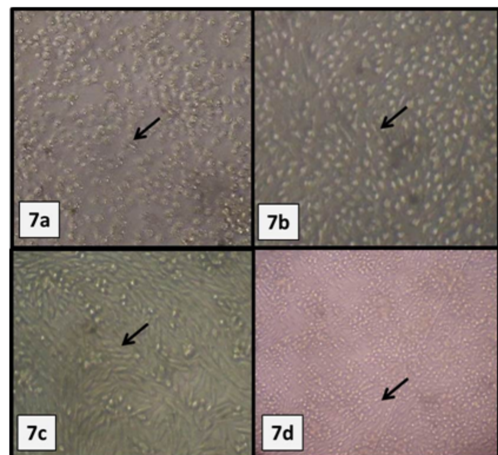
Once the pure lines of dental pulp stem cells were obtained MTT ASSAY was performed. MTT ASSAY [3-(4,5-Dimethylthiazol-2-yl)2,5-Diphenyltetrazolium Bromide] measures the cell proliferation rate and reduction in cell viability when metabolic events lead to apoptosis or necrosis. For MTT assay 96-well culture plate was used (FIG 5d). All samples were placed into wells and named as S1,S2, S3 and S4 (FIG 6a, b, c, d) along with MEM, 10%FBS, Antibiotic solution(FIG 5b) and dental pulp stem cells and incubated in 5% CO2 incubator at 37°C for 96 hours. Then treated with MTT solution (FIG 5a) for 4 hrsuntil purple precipitation was visible, then 100µl detergent reagent was added(FIG 5c). After dissolution of formazan crystals, optical density of the solution was read at 540-690 nm wavelength using an ELISA Reader. The intensity of colour generated correlated with the percentage of viable cells. The samples were also stained with haematoxylin and eosin stain and observed under inverted microscope.



RESULTS:

The results showed that the difference in the mean percentage of viable cells between the four study groups and the control. Collagen membrane group (FIG 7d) showed highest cell viability (100%) when compared to untreated and demineralized dentin. Untreated dentin (FIG 7a) showed 90% cell viability, HCL treated demineralized dentin (FIG 7b) showed 83% cell viabilityand HNO_3 treated demineralized dentin (FIG 7c) showed 87% cell viability, when subjected to MTT assay.

No. samples	Sample	Absorbance (nm)(OD value)	Mean	Cell viability
1.	S1	1.166	1.164	90%
2.		1.161		
3.		1.211		
4.		1.142		
5.		1.140		
1.	S2	1.044	1.072	83%
2.		1.105		
3.		1.001		
4.		1.102		
5.		1.111		
1.	S3	1.014	1.133	87%
2.		1.144		
3.		1.164		
4.		1.152		
5.		1.191		
1.	S4	1.345	1.289	100%
2.		1.384		
3.		1.244		
4.		1.246		
5.		1.256		
1.	Control group	1.301	1.291	100%
2.		1.289		
3.		1.265		
4.		1.304		
5.		1.300		



DISCUSSION:

This study was conducted to assess the cytotoxicity of demineralized dentin on Stem cells from human exfoliated deciduous (SHED) teeth using MTT assay. Ideal properties of barrier membrane are biocompatibility, ability to create space, cell occlusiveness, tissue integration and handling as well as the resorption time.[9]

In the present study dentin demineralization was done with 0.6 N HCl. It results in the elimination of the major part of the minerals and immunogenic components, while retaining a very low fraction of minerals, and the majority of type I collagen and non-collagenase proteins, providing an osteoconductive and osteoinductive scaffold.[6] Two percent HNO_3 has also been successfully used for dentin decalcification.[8]

Stem cells from human exfoliated deciduous (SHED) teeth were used. Since, SHED can also undergo osteogenic differentiation and generate bone in vivo, making them an interesting model for bone tissue regeneration.[7] Y. Zheng et al (2009) highlighted that when SHED was mixed with β-tricalcium phosphate carrier, they were able to promote bone regeneration in jaw defects in swines compare to the carrier without cells which failed to induce the same.[10]MTT is well set up for

cytotoxicity analysis of materials, being used initially for cell viability analysis . This method assesses the ability of viable cell in changing the water-soluble tetrazolium salts to the insoluble formazan crystals *via* the activity of mitochondrial dehydrogenase enzymes. Shed were observed viable on demineralized dentin when subjected to MTT assay.[11] This showed biocompatilbilty of demineralized dentin.

Due to acid action on demineralized dentin, the artificial macropores throughout of 300–400 μm in diameter were observed. Such porosity can influence the osteoconductive characteristics of the scaffold by creating spaces for osteoblast attachment, differentiation, and growth and vascular invasion from surrounding tissues.[12] Surface demineralized root dentin induces new bone formation, slow mineral resorption, and release of recombinant human bone morphogenetic protein-2 (rhBMP-2). Murata *et al (2005)* showed that rhBMP-2 increased the bone-inductive potential of DDM in rat subcutaneous tissues and suggested that human recycled DDM is a unique, absorbable and osteoconductive matrix that could be an effective scaffold for BMP-2 delivery.[13] Kim YK et al (2013) was first to publish clinical report using autogenous DDM blocks for socket preservation demonstrated excellent bone formation and strong integration of the DDM block into the recipient bone, in 12 patients. The alveolar bone volume was observed well maintained both vertically and horizontally and the formed bone was not resorbed during the early stages.[12]

CONCLUSION:

Semi-rigid dentin due to acid treatment could benefit periodontal tissues formation. Concept of stem cells from exfoliated teeth could be an unexpected unique resource for stem-cell therapies including autologous stem-cell transplantation and tissue engineering and demineralised dentin bank might be meaningful in order to provide as a source of periodontal regeneration.

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