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CHARACTERIZATION OF A NOVEL CROSS LINKED PRF UNDER COMPRESSION INTENDED FOR PULP TISSUE ENGINEERING

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ABSTRACT Knowledge of biological and mechanical properties of native tissues is critical for biomaterial design and synthesis for tissue engineering. So far, selection of biomaterials for dental pulp regeneration has been done randomly or based on experience mainly due to the scarcity of data of biomechanical properties of human dental pulp tissue. This study, for the first time, characterizes the physical properties of human dental pulp tissue harvested from wisdom teeth, under compression and compared it with PRF and a novel crosslinked PRF (C-PRF) prepared by crosslinking PRF with tannic acid. The results revealed that conventional PRF compressive sterength is very less compared to dental pulp tissue and comparable compressive strength of the novel crosslinked PRF(C-PRF). Taken collectively, crosslinked PRF will better suit for pulp tissue engineering purposes.

KEYWORDS :

I. INTRODUCTION

Regeneration of dental pulp will certainly avoid complications associated with the endodontically treated tooth. Endodontically treated tooth leads to considerable structural loss due to removal of part of enamel, dentin, and pulp. Such loss may result the tooth vulnerable to fracture. Because of the lost pulpal sensation and inability of the tooth to detect microbial challenges, it can also be more susceptible to re-infections [1]. Studies have shown that the interactions between the cells and their niche are closely related to physicochemical properties of the scaffold materials [2, 3]. In this regard, biomaterial selection, and development for pulp tissue engineering require the understanding of biomechanical behavior of the native pulp tissue. Such properties can be utilized for the selection and tailoring of biomaterials to act as scaffolds for dental pulp tissue engineering.

PRF in now been widely used in dentistry in various clinical regenerative situations. Although this regenerative modality still remains unfamiliar to many clinicians, the evidence supporting its use has accumulated over the years, demonstrating its ability to improve tissue regeneration. The combination of PRF with regenerative therapy has been shown to be most promising for periodontal regeneration of intrabony and furcation defects, as well as soft tissue root coverage of gingival recessions. Evidence from the medical literature suggests that PRF is able to decrease infection following tooth extraction and may further limit dimensional changes of alveolar ridge following tooth loss. Nevertheless, ease of use of PRF, combined with its low cost and autologous source, makes it an ideal biomaterial worth further investigation across a variety of surgical procedures in dentistry [4].

Studies comparing different materials with PRF used in coronal pulpotomy techniques in context of pulp tissue regeneration have proved some promising results [5,6] As PRF compressive strength is very less, it was assumed that crosslinking PRF will improve its compressive strength and better suit for pulp tissue engineering purposes.

II. AIMS AND OBJECTIVES

Aim of our study was to prepare a very economical and autologus biomaterial for pulp tissue engineering.

Our primary objective was to characterize human dental pulp tissue under mechanical compression.

Our secondary objective was to prepare a crosslinked PRF with tannic acid to improve compressive strength to human dental pulp tissue level.

III. METHODS AND MATERIALS

Preparation Of PRF

The protocol followed is that of Choukroun *et al*.[7] Written consent was taken from the donors. The PRF preparation protocol is very simple. Around 10 ml of whole venous blood is collected in sterile glass tubes of 10 ml capacity without anticoagulant. Another 10 ml tube with normal saline was taken as counterbalance in centrifugal machine. The tubes were then placed immediately in a centrifugal machine [Figure 1] and are immediately centrifuged 2700 rpm for 12 minutes. Three distinct layers were appeared in the tubes: Lower red layer containing red blood cells, upper straw colored cellular plasma and in the middle segment the turbid fibrin clot PRF [Figure-2]. We can obtain the PRF extracting the matrix from the tube with forceps and removing the red clot. The success of this technique depends entirely on the blood collection and the transfer speed in the centrifuge.

Preparation Of The Novel Crosslinked PRF

Tannic acid (TA) powder was purchased from Sigma–Aldrich (Bangalore, India). PRF was prepared freshly as described above. PRF samples were crosslinked in freshly prepared TA solution in dapendish for 10 minutes at room temperature. Two different concentrations of TA (1 wt %) was used for preparing two different samples. After crosslinking, the gels were washed with normal saline for 5 min. to ensure that all excess TA was removed.

Dental Pulp Tissue Harvesting

Pulp tissue was obtained from the wisdom teeth of human

donors following a protocol approved by the JIS University, Kolkata ethical committee. Briefly, after the application of local anesthesia, the wisdom teeth were extracted and stored immediately in 3% penicillin–streptomycin solution. The crown was removed from the dentin–cementum junction using diamond disc in clinical micromotor under constant water cooling while immersed in sterile phosphate-buffered saline (PBS), and the fresh pulp tissue was removed as an intact tissue by gently pulling the tissue out using forceps and barbed broaches.

Compression Test of Human Dental Pulp Tissue and PRF and Novel C-PRF

The compression tests of four samples were carried out at the compression speed of 0.05 mm/min using a micro-controlled electronic universal testing machine (10 × 10 × 12 mm). Three specimens of each group were examined. The force and deformation at each position were obtained. Through the analysis of the data, the stress-strain curves of each group of samples were drawn.

IV. RESULTS

The stress-strain curve of the scaffolds obtained by static compression test was shown in Figure. It can be seen from the figure that the two groups of scaffold i.e., dental pulp tissue and 10% crosslinked PRF (C-PRF) samples had similar static properties. The elastic modulus and yield strength of PRF scaffold was significantly lower than those of the other three groups.

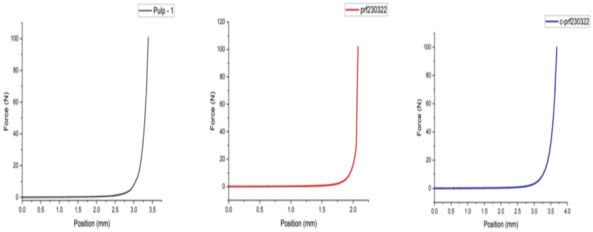

Fig- Stress-strain graph of A. Human dental pulp tissue B. PRF and C. Crosslinked PRF(C-PRF) respectively

V. DISCUSSION

The purpose of present study was to investigate physical characteristics of normal human dental pulp tissue and to prepare a crosslinked PRF with tannic acid to improve compressive strength to human dental pulp tissue level so that it can be used as scaffold for dental pulp tissue engineering. Preparation of different biomaterials was previously done by crosslinking tannic acid with collagen, chitosan etc.[8.9] but no studies in our knowledge that performed PRF crosslinking with tannic acid.

Burak Ozcan et. al.[10] previously characterize human dental pulp tissue under oscillatory shear and compression. But we studied only compression because engineered pulp tissue will be present in the pulp chamber and over its some biomimetic restorative material will take the compressive stress mainly during mastication and that compressive stress will be transmitted to the underlying engineered pulp tissue.

Raji Viola Solomon et. al. did coronal pulpotomy technique analysis as an alternative to pulpectomy for preserving the tooth vitality, in the context of tissue regeneration. Their study was correlated clinical study across four adult permanent molars using PRF. They conclude the potential scope of regenerative pulpotomy approaches in acute irreversible pulpitis in adult permanent teeth. Their study of pulp regeneration was based upon tissue engineering concept.[5] But they did not assured the physical nature of their used PRF material is similar to intended human pulp tissue. So we studied dental pulp tissue and PRF physical nature first under compression and have seen that there a big difference. Then we decided to improve the physical character of the PRF with a low cost natural crosslinker like tannic acid. After testing with different concentrations of tannic acid in a clinically acceptable chair side time of 10 min. we got the comparable concentration of TA of 10wt%.

The case report of Haridas Das Adhikari et. al. described an immature nonvital 12 with apical pathology which was treated via revascularization using 3% NaOCl and 17% ethylenediaminetetraacetic acid as irrigants; Ca(OH)2 as intracanal medicament and platelet-rich fibrin (PRF) as scaffold. On their follow-up at 6 and 12 months they noted healing of periapical lesion, dentinal thickening, and apical closure with a canal exit forming distally. The tooth, however, got fractured and was subsequently extracted at 12 months and sent for histopathological examination. It was revealed that a collagenous matrix for forming hard tissue similar to acellular cementum with a diffuse area of calcification in pulp space being attached with root canal dentin walls. So they concluded that that PRF may yield favorable outcomes in regenerative endodontic procedures.[11] So we set our goal to improve physical property of PRF for pulp tissue regeneration.

Ceren Çimen et. al. reported a case series on regenerative endodontic treatment (RET) using PRF. The purpose of their case series was to evaluate the clinical and radiological results of RET using platelet-rich fibrin (PRF) in 4 immature teeth with necrotic pulps. They concluded that PRF was successful as a scaffold and can be recommended for revascularization protocol of necrotic immature teeth. However, the prognosis of tooth can be attributed to many factors such as the duration of pulp necrosis, pretreatment status of the periapical region, and the viability of living tissues. But they pointed out the disadvantages of PRF is its difficult handling and long term clinical follow up.[12] Our novel C- PRF handability was improved with its improved stiffness and as the scaffold stiffness becomes close to dental pulp tissue follow up period should become shorter.

TA is an established crosslinking agent that utilizes hydrogen bonding and hydrophobic effects to crosslink collagen fibrils [13]. Previously TA was used as a topical treatment for skin burns due to its resistance to bacteria and ability to stabilize damaged tissue. [14] The results from the study by Christopher J. Bridgeman [15] showed that TA improved the strength and stiffness of ECC collagen gels through the merging of collagen fibrils. TA is an established cross-linking agent and fibrin of PRF is a biopolymer with some open chains and it crosslinked the PRF and converted it a stiffer biomaterial.

Of course, this may require more rigorous proof. These characteristics provide certain guidance for the subsequent further optimization of the scaffold and better biological performance. Of course, there were also some shortcomings in our research. FTIR analysis of this novel C-PRF should be done which is our next research focus and direction.

VI. CONCLUSION

In the current study the tannic acid acts as a natural crosslinker reacts with fibrin of PRF to afford a stable and tougher hydrogel network. The compressive strength of PRF was improved comparable to human dental pulp tissue by 10 wt% tannic acid cross linking in order to be an adequate novel scaffold (C-PRF) for dental pulp tissue regeneration.

VII. REFERENCES

[1] Yuan, Z., Nie, H., Wang, S., Lee, C. H., Li, A., Fu, S. Y., Zhou, H., Chen, L., and Mao, J. J., 2011, "Biomaterial Selection for Tooth Regeneration," Tissue Eng., Part B, 17(5), pp. 373–388

[2] Mintz, B. R., and Cooper, J. A., Jr., 2014, "Hybrid Hyaluronic Acid Hydrogel/Poly(Varepsilon-Caprolactone) Scaffold Provides Mechanically Favorable Platform for Cartilage Tissue Engineering Studies," J. Biomed. Mater. Res., Part A, 102(9), pp. 2918–2926.

[3] Cameron, A. R., Frith, J. E., Gomez, G. A., Yap, A. S., and Cooper-White, J. J., 2014, "The Effect of Time-Dependent Deformation of Viscoelastic Hydrogels on Myogenic Induction and Rac1 Activity in Mesenchymal Stem Cells," Biomaterials, 35(6), pp. 1857–1868.

[4] Richard J. Miron1,2 & Giovanni Zucchelli3 & Michael A. Pikos 4 & Maurice Salama1,5,6 & Samuel Lee7 & Vincent Guillemette 8 & Masako Fujioka-Kobayashi 1,9,10 & Mark Bishara11 & Yufeng Zhang12 & Hom-Lay Wang2 & Fatiha Chandad8 & Cleopatra Nacopoulos 13 & Alain Simonpieri 14,15,16 & Alexandre Amir Aalam17 & Pietro Felice3 & Gilberto Sammartino18 & Shahram Ghanaati19 & Maria A Hernandez1 & Joseph Choukroun20 Use of platelet-rich fibrin in regenerative dentistry: a systematic review Clin Oral Invest DOI 10.1007/s00784-017-2133-z

[5] Solomon RV, Faizuddin U, Karunakar P, Sarvani GD, Soumya SS. Coronal pulpotomy technique analysis as an alternative to pulpectomy for preserving the tooth vitality in the context of tissue regeneration: a correlated clinical study across 4 adult permanent molars. Case Rep Dent, 2015, 1-12.

[6] Suryasowjanya Doranala, Jayaprada Reddy Surakanti, Harikumar Vemisetty, Suraj Reddy Loka, Keerthi Sudireddy, Rajani Punna ; Comparative assessment of titanium-prepared platelet-rich fibrin, EndoSequence root repair material, and calcium hydroxide as pulpotomy agents in permanent teeth with irreversible pulpitis: A randomized controlled trial ;Journal of Conservative Dentististry; **Year** : 2021 | **Volume** : 24 | **Issue** : 6 | **Page** : 606610

[7] Choukroun J, Adda F, Schoeffler C, Vervelle A. Une opportunite´ en paro-implantologie: le PRF. Implantodontie 2001;42:55-62. (in French)

[8] Natarajan V, Krithica N, Madhan B, Sehgal PK. 2012. Preparation and properties of tannic acid cross-linked collagen scaffold and its application in wound healing. J Biomed Mater Res Part B 2012:00B:000–000.

[9] A. Sionkowska, B. Kaczmarek, K. Lewandowska. CHARACTERISATION OF CHITOSAN AFTER CROSS-LINKING BY TANNIC ACID: Progress on Chemistry and Application of Chitin and Its Derivatives, Volume XIX, 2014

[10] Burak Ozcan, Ece Bayrak, Cevat Erisken : Characterization of Human Dental Pulp Tissue Under Oscillatory Shear and Compression; Journal of Biomechanical Engineering JUNE 2016, Vol. 138 /0610063

[11] Haridas Das Adhikari, Aparna Gupta Report of a case of platelet-rich fibrin-mediated revascularization of immature 12 with histopathological evaluation:Journal of Conservative Dentistry; Year 2018 | **Vol.** 21 **Issue** : 6 **Page** : 691-695

[12] Çimen C, Şen S, Şenay E, Bezgin T. Platelet-Rich Fibrin Used as a Scaffold in Pulp Regeneration: Case Series. Cumhuriyet Dent J 2021;24:1:113-120.

[13] Heijmen, F., Du Pont, J., Middelkoop, E., Kreis, R., and Hoekstra, M. Crosslinking of dermal sheep collagen with tannic acid. Biomaterials 18, 749,19

[14] De Maquinarias, V., and Monografias, M. The use of tannic acid in the local treatment of burn wounds: intriguing old and new perspectives. Wounds 13, 144, 2001.

[15] Christopher J. Bridgeman: Effect of Tannic Acid Crosslinking on the Mechanical Properties of Collagen Scaffolds and Melanoma Cell Proliferation; https://repository.lib.fit.edu/handle/11141/1606