Comparative Evaluation of Fibrin Gel and Platelet-Rich Fibrin on Stem Cells of the Apical Papilla Fate Using a Dentin-Based Model

Pegah Sarraf
Tehran University of Medical Sciences
Razieh Sadat Moayeri
Tehran University of Medical Sciences
Noushin Shokouhinejad
Tehran University of Medical Sciences
Mehrfam Khoshkhounejad
Tehran University of Medical Sciences
Roya Karimi
Tehran University of Medical Sciences
Abdollah Ghorbanzadeh (dr.a.gh.37@gmail.com)
Tehran University of Medical Sciences
Jafar Ai
Tehran University of Medical Sciences

Research Article

Keywords: Regenerative endodontic treatment, Fibrin gel, Platelet rich fibrin, Dentin

Posted Date: January 7th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1204014/v1

Abstract

Background: PRF as one of the favorable scaffolds in Regenerative Endodontic Treatment (RET), has several limitations such as the need for blood sampling and special equipment. High available commercial scaffolds such as fibrin are able to meet all the necessary requirements of dentin tissue engineering. The present study was designed to evaluate the effect of PRF and fibrin gel, with and without the presence of EDTA-treated radicular dentin segments on SCAP viability, proliferation, migration, and differentiation.

Methods: Radicular dentin were prepared from extracted teeth and treated by EDTA 17% .The samples were divided into 6 groups: Dentin/PRF/Cell, Dentin/Fibrin/Cell, Dentin/Cell, PRF/Cell, Fibrin/Cell and Cell (Control). SCAP viability was assessed using MTT assay. Gene expression levels of odontogenic markers [Dentin sialophosphoprotein (DSPP), Dentin matrix protein 1(DMP1), Collagen type I Alpha 1(COL 1A1) and Alkaline phosphatase (ALP) were assessed using qrt-PCR. Cell migration were also evaluated by means of scratch test.

Results: The results of MTT assay at showed that the viability of SCAP significantly increased after 7 days for both groups containing fibrin ($P < 0.05$). The viability of SCAP seeded on Dentin/PRF and PRF significantly decreased after 7 days ($P < 0.001$). The odontogenic markers were significantly expressed for both scaffolds in the presence of dentin segment ($p<0.05$). Significant decrease in scratch area was seen in Fibrin/Dentin group ($p < 0.001$)

Conclusions:

Fibrin beside EDTA-treated dentin showed great ability in survival, proliferation, differentiation, and migration of SCAP rather than PRF.

Background

Endodontic therapy as a highly predictable treatment approach meets challenges in immature teeth with necrotic pulp and apical periodontitis due to the large open apices and thin root walls (1). Traditional method(apexification) is basically unable to induce root development, causing teeth susceptible to root fractures (2).

Revascularization or RET has been proposed to enhance the hopes of inducing root development and normal physiologic reaction of immature teeth (3). RET is relying on tissue engineering concepts and need a proper scaffold to maintain high viability of stem cells and keep signal molecules (4). In conventional method, a blood clot (BC) scaffold is proposed for tooth revascularization by stimulating the periapical tissue through the insertion of an endodontic file beyond the apical foramen(3). This method is technical-sensitive and a little amount of bleeding will result in an insufficient volume of scaffold (5). Low quality of formed BC and the possibility of injury to the inferior alveolar or mental nerve are other disadvantages of BC procedure (6, 7).

Further studies suggest platelet concentrates (PC) such as platelet-rich plasma (PRP) and PRF, as proper scaffolds to support cellular proliferation during stimulating bleeding process (8). Despite the promising outcomes, there is an important gap in knowledge regarding modifying the RET for best enhancing of root tissue regeneration.

High potential ability of PCs as scaffold in tissue regeneration has been demonstrated due to the high amount of growth factors (GFs) such as transforming growth factor-beta (TGF-β), vascular endothelial growth factor (VEGF), and platelet-derived growth factor (PDGF)(9). Studies have been demonstrated the effectiveness of PRP(first-generation PC) in periapical regeneration and dentinal wall thickening (5, 10). PRF (Second-generation PC) was proposed to overcome the limitations of PRP. PRF has superior properties such as simplicity in preparation, possibility to encapsulate the platelets and leukocytes in the organized structure of fibrin and great mechanical properties(11, 12). However, using PRF has several disadvantages such as the blood sampling especially in the case of children and necessity of special equipment for preparation.

Fibrin is a widely used biopolymer in tissue engineering, drug and cell delivery systems (13, 14). Fibrin gel formation using thrombin enzyme provides a 3D mesh which can support cell survival and ingrowth. Further advantages such as high adhesive nature, suitable plasticity, great elastic resistance and maintaining biocompatibility of fibrin during gel formation makes it a favorable scaffold for cell delivery and growth factors entrapping in RET (9, 15, 16)

It has been shown that mesenchymal stem cells (MSCs) can provide the cellular basis for dentin tissue formation during RET in immature (17) and mature (18) teeth. These cells are assumed to be extracted from apical papilla (19), but they can also be derived from different apical tissues such as bone and periodontal ligament. SCAP extracted from dental tissues have been extensively used in RET (20). These cells have great potential to proliferate and differentiate as the major source of undifferentiated cells during root tissue growth (21).

Several studies have shown the great effect of PRF on cell proliferation, differentiation, and migration (22–24). However, to the best of our knowledge, the presence of a radicular dentin sample, as a substrate and source of GFs and morphogens affecting the stem cells have not been the matter of discussion in ex-vivo studies. Thus, this study aims to compare the effect of fibrin hydrogel and PRF on SCAP viability, differentiation, and migration using a dentin-based model.

Methods

Dentin segment preparation

This study was performed according to the protocols approved by the ethical committee of Tehran University of Medical Sciences, Tehran, Iran.

Single-root canaled teeth that were extracted due to periodontal diseases or orthodontic treatments and distinguished by radiographic images, were obtained. After removing the periodontal tissues and immersing in 0.5% chloramine-T (Merck, Germany) solution for 24h at ambient temperature, the crown was removed and 5-mm of root length was cut below the CEJ by a sterile diamond disc (Prodont-Holliger, Vence, France). The canal preparation was performed using ProTaper Universal rotary files (Densply-Maillefer, Ballaigues, Switzerland) up to #F3 and #3-6 Peeso Reamers (Largo; Densply-Maillefer, Ballaigues, Switzerland) under continuous irrigation by sterile normal saline. To simulate the immature tooth, a sterile high-speed diamond fissure bur (Jota, Rüthi, Switzerland) was employed to enlarge the canal to reverse cone-shape (lower and upper ends as 3 mm and 2 mm, respectively). The obtained root segments (5 6 mm^2) was longitudinally middle cut by the diamond disc and were kept in PBS at atmosphere temperature for further use.

SCAP isolation and culture

All patient donors (19- 25 years old) with the potential of extraction of non-carious and non-inflamed third molar teeth signed the informed consent forms. Teeth of patients with open apices (>1.5 mm) were chosen. Before SCAP harvesting, extracted teeth were washed with cold sterile Hank's Balance Salt Solution (HBSS, Gibco, USA) three times. The apical papilla tissues of extracted teeth were obtained by a sterile surgical blade in HBSS. Then, they were minced into ~ 2 1 1 mm segments and incubated with 3 mg/mL collagenase type I (Worthlington Biomedical, Lakewood, NJ) and 4 mg/mL dispase (Sigma, St Louis, MO) for 40 min followed by homogenizing by passing through a sterile glass Pasteur pipette. Suspended cells were collected by centrifuging at 1400 rpm for 3 min and then were cultured in a media composed of alpha-minimum essential medium(α-MEM; Gibco, Grand Island, NY) supplemented with 10% fetal bovine, 1 L-glutamine(Gibco), penicillin(100 U/mL; Gibco), and streptomycin(100 mg/mL; Gibco). A population of ~1600 cells from each apical papilla was achieved by cells plating at 10cm in diameter of culture plates (BD Biosciences, Bedford, MA). Cells were allowed to expand in culture to 70%–80% confluency. The isolated cells were kept at an atmosphere of 37 °C with 5% CO2 and the media was refreshed every 3 days.

Characterization of SCAP with flow cytometry

SCAP cultures were characterized by flow cytometry for identifying several markers known to be expressed on endothelial(CD105), mesenchymal(CD90, CD24, CD34, STRO-1), and hematopoietic(CD 45) stem cells as described before(25). To this, after trypsinization of the cells, they were washed with PBS and stained using following mouse fluorochrome-conjugated antibodies (BioLegend, Fell, Germany): CD90-fluorescein isothiocyanate (CD90-FITC), CD24- allophycocyanin(CD24-APC), CD34-APC, STRO-1-FITC, and CD45- phycoerythrin (CD45-PE)(BD Biosciences, Heidelberg, Germany). The analysis was carried out using a flow cytometer (BD Bioscience) and evaluated by Summit 5.1 software.

PRF preparation

PRF was obtained according to the protocol (26). Briefly, blood was transferred into a 10 mL A-PRF tube (PRF^{TM}, Nice, France) and immediately was centrifuged by a centrifuge machine (DUO QuartroCentrifuge, Nice, France) at 1300rpm for 8min. After centrifugation, the formed PRF was collected using sterile scissors, pressed and covered by a compressor cover (Process, Nice, France). The collected hydrated PRF membranes were then cut into ~ 4 4 mm^2 pieces.

Samples preparation

A total of 6 groups were evaluated in this study:

Control group: only cells were cultured in 24-well cell culture plates (~105 cells per each well)

Dentin/PRF/Cell: 105 SCAP were seed on the surface of the Dentin/PRF.

Dentin/Fibrin/Cell: 105 SCAP were mixed with 3mg/mL fibrinogen solution carefully and transferred to the 24-well culture plate on the surface of the prepared dentin segment. Next, 50 µL of FBS with 15 µL of thrombin solution (with a concentration of 120 U/mL in 1M sodium buffer) was added to fibrinogen/cells solution. For gel formation, the solution was placed in an incubator at 37 °C for ~ 2h. In the following, 0.5 mL of M199 containing FBS 10% was added to the plate and the mixture was placed in the incubator.

Dentin/Cell: 105 SCAP were seed on the surface of prepared dentin.

Fibrin/Cell: SCAP immobilized in fibrin gel was prepared as similar as Dentin/Fibrin/Cell group without the presence of dentin segments.

PRF/Cell: 105 SCAP were seed on the surface of prepared PRF.

Evaluation of gene expression by Quantitative Real-Time Reverse Transcription Polymerase Chain Reaction (qRT-PCR)

Variations of gene expression from SCAP contacting the samples were evaluated by qRT-PCR. The used oligonucleotides are shown in Table 1. Briefly, total RNA from seeded and encapsulated SCAP were extracted after 7 and 21 days using the RNeasy mini kit following supplier's instructions (Qiagen, USA) and stored at – 80 ºC until further use. Furthermore, complementary DNA was synthesized using a cDNA synthesis kit (Takara). qRT-PCR was performed with the 7500 RT-PCR System (Applied Biosystems, Lincoln, CA) in a reaction volume of 20 µL. Briefly, 12 µL Power SYBRH Green PCR Master Mix (Takara, USA), 1 µL of primer sequences, 6 µL PCR-grade H2O and 1 µL of template 10 ng cDNA were added to each sample. The reaction was carried out with an annealing temperature at 60 °C for 30 s. The relative expression ratio of mRNA was calculated by the 2−ΔΔCT method.

Cell viability evaluation using MTT assay

After 1, 3, and 7 days of cell culture, media at the top of samples was taken out and the samples were incubated with 200 µL of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) solution

for about 3h. Next, 600 µL of dimethyl sulfoxide was mixed gently with the obtained solution. After dissolving formazan, 100 µL of supernatant was removed and transferred to 96-well microplate. The absorbance was read at 570 nm with an Awareness Technology microplate-reader.

Cell migration assay

1 10^5 SCAP were cultured in a 24-well plate and maintained overnight to obtain a monolayer cell in each well. After that, a 1mm wide gap scratch was made onto monolayer cell using a glass Pasteur pipette tip and floating cells were taken out by twice washing with PBS. Then, the media for each group was replaced and the sample was soaked in the media at the top of each well without contacting to the bottom of the plate. To evaluate the effect of each sample on the cell migration, samples were continually imaged after 48h by a microscope and the images were evaluated using ImageJ (ImageJ, NIH, Bethesda, MD, USA) software from the perspective of cell migration that the fraction of cells which have transferred from the edges of the scratch toward the center of it. All experiments were carried out in triplicate for each condition. The data are shown as means ± standard deviation (SD). Statistical analysis was done by one- and two-way ANOVA with Duncan test. Significant differences were shown as *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

Results

Immunophenotypic behavior of cultured SCAP

Cultured SCAP were highly positive for Mesenchymal Stem cells (MSCs) markers such as CD90 (100%) and SCAP related CD24 marker (98.9%)(Fig.1). Lower expression was seen for MSCs markers including STRO-1 (31.8%) and CD34 (1.64%). The CD105 endothelial stem cells markers were highly positive (100%). The hematopoietic stem cell marker CD45 was not significantly expressed (0.308%). Considering the high expression of CD24 marker, total characterizations of SCAP could be concluded.

Cell viability assay

The results of MTT assay at 1, 3, and 7 days post SCAP culturing showed that the viability of SCAP encapsulated fibrin gel significantly increased after 7 days(for both Dentin/fibrin ($P < 0.001$) and fibrin ($P < 0.05$) samples)(Fig.2). The viability of SCAP seeded on the Dentin-PRF had not significantly changed at day 3 compared with day 1. However, the viability of SCAP contacting Dentin-PRF sample significantly decreased after 7 days ($P < 0.001$). Even though the viability of cells seeded on the dentin increased significantly at day 3 compared to day 1 ($P < 0.001$), it notably decreased after 7 days of culture. A gradually significant decrease in viability of SCAP seeded on the PRF was observed.

Gene expression assay by qRT-PCR

All examined biomaterials increased mRNA expression of the odontogenic marker dentine sialophosphoprotein (DSPP) compared to control sample, with the greatest and lowest increase observed

by Dentin/Fibrin and Dentin samples, respectively (Fig.3). DSPP gene expression level from the Cell/Dentin-PRF sample was significantly higher than Dentin, PRF, and Fibrin samples. The expression level from SCAP/Fibrin sample was significantly higher than both PRF ($P < 0.01$) and dentin samples ($P < 0.001$).

The highest level of ALP expression was seen in Cell/Dentin/PRF and Cell/Dentin/fibrin samples without a difference between these two. However, the lowest ALP expression is related to the Cell/Dentin sample. SCAP/Fibrin has significantly higher ALP expression compared to both PRF and dentin samples ($P < 0.001$).

VEGF expression for the Dentin/PRF and Dentin/Fibrin samples was significantly higher than other samples ($P < 0.001$). Our results also showed significant VEGF expression from SCAP/Fibrin compared with Cell/Dentin and Cell/PRF samples.

The COL IA1 gene expression from the Cell/Dentin and Cell/Fibrin was significantly higher than others ($P < 0.001$). As similar as DSPP, ALP and VEGF genes expression, the lowest COL I expression was seen in Cell/Dentin sample. The COL I expression for the Dentin/Fibrin sample was significantly higher than both Fibrin and PRF samples (Fig 3).

Cell Migration

Cells cultured in the media containing dentin, fibrin, PRF, Dentin/PRF, and Dentin/Fibrin samples were used to evaluate cell migration (Fig.4). Initial captured picture from the scratched cells was used as control for measuring gap distance of each group. In Dentin/fibrin sample, fast cell migration were observed toward the opening area and significant decrease in scratch area (***$p < 0.001$)(Fig 4a-4b). Furthermore, almost whole distance gap was covered eventually for this sample. On the other hand, the efficient migration was not seen when only dentin sample was used.

Discussion

Dental pulp reconstruction is a part of RET which significantly affected by proliferation and migration of stem cells inside the prepared root canal space. The chosen biomaterials is dependent on several factors such as the effect of the materials on the SCs fate, the capability to express some related genes and their effect on cell migration.

The important role of dentin as a main source of GFs and potent morphogens in the reconstruction and regeneration of dental pulp cannot be ignored (27). Thus, the effect of dentin on the SC fate and their differentiation ability should be evaluated. Evaluating the role of mid-coronal dentin segment on the SCs proliferation and differentiation in the presence of developed biomaterials has been the matter of discussion, in recent years (28–30). In this study, radicular dentin segments were prepared with a root canal in the shape of immature teeth to simulate the clinical conditions in in-vitro studies. The segments were treated with EDTA to activate GF releasing and signaling molecules close to the clinical study. The

materials were placed in EDTA-treated dentin segments and the viability, differentiation, and migration abilities of SCs were evaluated.

Even though PRF has been extensively used as a proper scaffold in dentistry (31), it has several disadvantages such as time-consuming in blood collection and centrifuging. Furthermore, it is recognized as an age-dependent and experience-related process. Therefore an alternative biocompatible, adhesive and high-strength biomaterial such as fibrin gel is suggested in the present study for using in RET.

Intragroup evaluation of the samples showed that even though SCAP encapsulated in the samples containing fibrin gel has low initial viability at days 1 and 3, they dramatically show high viability at day 7. This can be justified with the effect of 3D cell culture on cell viability and proliferation (32). Even though 3D cell culture has several advantages over 2D culture due to providing more similarity to the physiological conditions, it cannot show high cell viability and proliferation at initial cell culturing days (32) as seen in this study. The results of present study also showed that Dentin/Fibrin sample exhibited the highest cell viability among all examined samples at day 7. Although the Fibrin sample showed high SCAP viability, it is not as a favor as Dentin/Fibrin sample. It seems that released GFs from EDTA-treated dentin segments has an important role in SCAP viability and proliferation.

DSPP is a dentin-specific marker and express significantly in odontoblasts and has an important role in the mineralization of dentin (33). Furthermore, COL IA1 is the main part of the extracellular matrix of the dentin-pulp complex which is significantly expressed at the last stage of odontoblastic differentiation (34). In this study, the DSPP and COL I expressions of cells encapsulated in the Dentin/Fibrin sample were significantly higher than those seeded on the Dentin/PRF sample. It seems that encapsulation of SCAP inside fibrin gel significantly increased the potential ability of odontoblastic differentiation. Furthermore, COL I expression show the SCAP differentiation to fibroblast-like cells which are the main component of connective tissue of the pulp(35) .

ALP is one of the main markers for evaluation of mineralization of hard tissues such as bone, dentin, and cementum. Equal ALP and lower DSPP and COL I markers expressions in Cell/Dentin/PRF sample compared to Cell/Dentin/Fibrin sample may suggest that even though PRF is capable of SCAP differentiation into hard tissues, its potential ability for odontoblasts- and fibroblasts-like cells differentiation is lower than fibrin gel. Both Dentin/PRF and Dentin/Fibrin samples showed an equally favorable condition for VEGF genes expression as a main proangiogenic growth factors which may strongly affect vascular operation like viability, migration and proliferation at the treated site (36, 37). It has been reported that VEGF secretion activates cascades signaling and induce angiogenesis (38), which was released from SCAP encapsulated inside and seeded on the different samples in this study.

On the other hand, VEGF shows the vascular system level of differentiation and the viability and proliferation of endothelial cells (39). The significant role of the new blood vessels formation has been proved in pulp regeneration and homeostasis. The highly VEGF-expressed SCAP inside Fibrin/Dentin sample may be considered as the high potential ability of fibrin in RET. However, VEGF expression was lower for fibrin sample compared to fibrin/dentin in this study. An interesting point of qRT-PCR results is

the better performance of PRF and fibrin beside dentin sample in the expression of all evaluated genes. It seems that dentin may perform an important role in cell proliferation and differentiation.

The scratch assay showed the high ability of SCAP migration in the presence of dentin/fibrin sample. However, the migration ability of cells in the presence of the sole dentin is very low. The migration ability of SCAP in the presence of dentin/fibrin may suggest its high potential ability in RET due to the structural properties of fibrin that provide slow-releasing of dentin GFs(40). The results of this research potentially open new aspects of fibrin gel instead of PRF scaffold in pulp regeneration.

Conclusion

The present study showed that fibrin beside EDTA-treated radicular dentin segments promoted great ability in survival, proliferation, differentiation, and migration of SCAP. PRF scaffold did not promote cell viability, differentiation, and migration as efficient as fibrin gel. Thus fibrin gel can be considered as an appropriate alternative biomaterial instead of PRF for chairside use in RET.

Abbreviations

PRF
Platelet-Rich Fibrin
SCAP
Stem Cell of Apical Papilla
RET
Regenerative Endodontic Treatment
DMP1
Dentin matrix protein 1
DSPP
Dentin sialophosphoprotein
COL 1A1
Collagen, type I, alpha 1
ALP
Alkaline phosphatase

Declarations

Ethics approval and consent to participate

This manuscript was approved by the Ethical Committee of Research in Tehran University of Medical Sciences (TUMS), School of Dentistry (ID: IR.TUMS. DENTISTRY.REC.1397.616.

All methods were carried out in accordance with relevant guidelines and regulations. Informed consent was obtained from all subjects and/or their legal guardian.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interest.

Funding

This research is financially supported by Tehran University of Medical Sciences (grant no: 97-03-69-39872).

Authors' Contributions

PS: Concept and design, critical and scientific revision of the manuscript, data analysis/interpretation. RM: Concept and design, critical and scientific revision of the manuscript, data analysis/interpretation. NS: Consulting and design, data analysis/interpretation MK: Consulting and design. RK: design, supervision of laboratory tests. AG: Concept and design of the study, data analysis/interpretation, drafting the manuscript JA: Concept and design, data analysis/interpretation. All authors read and approved the final manuscript.

Acknowledgements

Our grateful thanks are extended to Dr. Kerstin Galler for the valuable guidance and Dr. Ali Farzin for his assistance in statistical analysis. This research is supported by Tehran University of Medical Sciences (grant no: 97-03-69-39872). The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors Information

[1] **Department of Endodontics, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran**

Pegah Sarraf,Razieh Sadat Moayeri, Noushin Shokouhinejad, Mehrfam Khoshkhounejad, Abdollah Ghorbanzadeh*

[2] **Department of Tissue Engineering. Faculty of Advanced Technologies,**

Tehran University of Medical Sciences, Tehran, Iran

Roya Karimi, Jafar Ai

References

1. Trope M. Treatment of the immature tooth with a non–vital pulp and apical periodontitis. Dental Clinics. 2010;54(2):313-24.
2. Jung C, Kim S, Sun T, Cho Y-B, Song M. Pulp-dentin regeneration: current approaches and challenges. Journal of tissue engineering. 2019;10:2041731418819263.
3. Banchs F, Trope M. Revascularization of immature permanent teeth with apical periodontitis: new treatment protocol? Journal of endodontics. 2004;30(4):196-200.
4. Hargreaves KM, Giesler T, Henry M, Wang Y. Regeneration potential of the young permanent tooth: what does the future hold? Pediatric dentistry. 2008;30(3):253-60.
5. Bezgin T, Yilmaz AD, Celik BN, Kolsuz ME, Sonmez H. Efficacy of platelet-rich plasma as a scaffold in regenerative endodontic treatment. Journal of endodontics. 2015;41(1):36-44.
6. Philips JL, Weller RN, Kulild JC. The mental foramen: Part II. Radiographic position in relation to the mandibular second premolar. Journal of endodontics. 1992;18(6):271-4.
7. Fishel D, Buchner A, Hershkowith A, Kaffe I. Roentgenologic study of the mental foramen. Oral Surgery, Oral Medicine, Oral Pathology. 1976;41(5):682-6.
8. Hong S, Chen W, Jiang B. A Comparative Evaluation of Concentrated Growth Factor and Platelet-rich Fibrin on the Proliferation, Migration, and Differentiation of Human Stem Cells of the Apical Papilla. J Endod. 2018;44(6):977-83.
9. Mehta S, Watson JT. Platelet rich concentrate: basic science and current clinical applications. Journal of orthopaedic trauma. 2008;22(6):432-8.
10. Jadhav G, Shah N, Logani A. Revascularization with and without platelet-rich plasma in nonvital, immature, anterior teeth: a pilot clinical study. Journal of endodontics. 2012;38(12):1581-7.
11. Mishra N, Narang I, Mittal N. Platelet-rich fibrin-mediated revitalization of immature necrotic tooth. Contemporary clinical dentistry. 2013;4(3):412.
12. Keswani D, Pandey R. Revascularization of an immature tooth with a necrotic pulp using platelet-rich fibrin: a case report. International endodontic journal. 2013;46(11):1096-104.
13. Navaei-Nigjeh M, Amoabedini G, Noroozi A, Azami M, Asmani MN, Ebrahimi-Barough S, et al. Enhancing neuronal growth from human endometrial stem cells derived neuron-like cells in three-dimensional fibrin gel for nerve tissue engineering. Journal of Biomedical Materials Research Part A. 2014;102(8):2533-43.
14. Ivica A, Ghayor C, Zehnder M, Valdec S, Weber FE. Pulp-Derived Exosomes in a Fibrin-Based Regenerative Root Filling Material. J Clin Med. 2020;9(2).
15. Ju Y-E, Janmey PA, McCormick ME, Sawyer ES, Flanagan LA. Enhanced neurite growth from mammalian neurons in three-dimensional salmon fibrin gels. Biomaterials. 2007;28(12):2097-108.
16. Kjaergard HK, Weis-Fogh US. Important factors influencing the strength of autologous fibrin glue; the fibrin concentration and reaction time--comparison of strength with commercial fibrin glue. European

surgical research Europaische chirurgische Forschung Recherches chirurgicales europeennes. 1994;26(5):273-6.

17. Lovelace TW, Henry MA, Hargreaves KM, Diogenes A. Evaluation of the delivery of mesenchymal stem cells into the root canal space of necrotic immature teeth after clinical regenerative endodontic procedure. Journal of endodontics. 2011;37(2):133-8.
18. Chrepa V, Henry M, Daniel B, Diogenes A. Delivery of apical mesenchymal stem cells into root canals of mature teeth. Journal of dental research. 2015;94(12):1653-9.
19. Chrepa V, Pitcher B, Henry MA, Diogenes A. Survival of the apical papilla and its resident stem cells in a case of advanced pulpal necrosis and apical periodontitis. Journal of endodontics. 2017;43(4):561-7.
20. Huang GT-J, Sonoyama W, Liu Y, Liu H, Wang S, Shi S. The hidden treasure in apical papilla: the potential role in pulp/dentin regeneration and bioroot engineering. Journal of endodontics. 2008;34(6):645-51.
21. Sonoyama W, Liu Y, Yamaza T, Tuan RS, Wang S, Shi S, et al. Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: a pilot study. Journal of endodontics. 2008;34(2):166-71.
22. Wong C-C, Chen C-H, Chan WP, Chiu L-H, Ho W-P, Hsieh F-J, et al. Single-stage cartilage repair using platelet-rich fibrin scaffolds with autologous cartilaginous grafts. The American journal of sports medicine. 2017;45(13):3128-42.
23. Nagaveni N, Kumari KN, Poornima P, Reddy VS. Management of an endo-perio lesion in an immature tooth using autologous platelet-rich fibrin: a case report. Journal of Indian Society of Pedodontics and Preventive Dentistry. 2015;33(1):69.
24. Karimi K, Rockwell H. The Benefits of Platelet-Rich Fibrin. Facial Plast Surg Clin North Am. 2019;27(3):331-40.
25. Bakopoulou A, Leyhausen G, Volk J, Tsiftsoglou A, Garefis P, Koidis P, et al. Comparative analysis of in vitro osteo/odontogenic differentiation potential of human dental pulp stem cells (DPSCs) and stem cells from the apical papilla (SCAP). Archives of oral biology. 2011;56(7):709-21.
26. Choukroun J, Diss A, Simonpieri A, Girard M-O, Schoeffler C, Dohan SL, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part V: histologic evaluations of PRF effects on bone allograft maturation in sinus lift. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 2006;101(3):299-303.
27. Smith AJ, Duncan HF, Diogenes A, Simon S, Cooper PR. Exploiting the bioactive properties of the dentin-pulp complex in regenerative endodontics. Journal of endodontics. 2016;42(1):47-56.
28. Miller AA, Takimoto K, Wealleans J, Diogenes A. Effect of 3 bioceramic materials on stem cells of the apical papilla proliferation and differentiation using a dentin disk Model. Journal of endodontics. 2018;44(4):599-603.
29. Sakai VT, Cordeiro M, Dong Z, Zhang Z, Zeitlin BD, Nör JE. Tooth slice/scaffold model of dental pulp tissue engineering. Advances in dental research. 2011;23(3):325-32.

30. Aksel H, Mahjour F, Bosaid F, Calamak S, Azim AA. Antimicrobial Activity and Biocompatibility of Antibiotic-Loaded Chitosan Hydrogels as a Potential Scaffold in Regenerative Endodontic Treatment. J Endod. 2020;46(12):1867-75.
31. Hotwani K, Sharma K. Platelet rich fibrin-a novel acumen into regenerative endodontic therapy. Restorative dentistry & endodontics. 2014;39(1):1-6.
32. Edmondson R, Broglie JJ, Adcock AF, Yang L. Three-dimensional cell culture systems and their applications in drug discovery and cell-based biosensors. Assay and drug development technologies. 2014;12(4):207-18.
33. D'souza R, Cavender A, Sunavala G, Alvarez J, Ohshima T, Kulkarni A, et al. Gene expression patterns of murine dentin matrix protein 1 (Dmp1) and dentin sialophosphoprotein (DSPP) suggest distinct developmental functions in vivo. Journal of Bone and Mineral Research. 1997;12(12):2040-9.
34. Pavlin D, Lichtler AC, Bedalov A, Kream BE, Harrison JR, Thomas HF, et al. Differential utilization of regulatory domains within the alpha 1 (I) collagen promoter in osseous and fibroblastic cells. The Journal of cell biology. 1992;116(1):227-36.
35. Abrahão IJ, Martins MD, Katayama E, Antoniazzi JH, Segmentilli A, Marques MM. Collagen analysis in human tooth germ papillae. Braz Dent J. 2006;17(3):208-12.
36. Bagri A, Kouros-Mehr H, Leong KG, Plowman GD. Use of anti-VEGF adjuvant therapy in cancer: challenges and rationale. Trends in molecular medicine. 2010;16(3):122-32.
37. Ricci-Vitiani L, Pallini R, Biffoni M, Todaro M, Invernici G, Cenci T, et al. Tumour vascularization via endothelial differentiation of glioblastoma stem-like cells. Nature. 2010;468(7325):824.
38. Gerwins P, Sköldenberg E, Claesson-Welsh L. Function of fibroblast growth factors and vascular endothelial growth factors and their receptors in angiogenesis. Critical reviews in oncology/hematology. 2000;34(3):185-94.
39. Mattuella LG, Bento LW, de Figueiredo JAP, Nör JE, de Araujo FB, Fossati ACM. Vascular endothelial growth factor and its relationship with the dental pulp. Journal of endodontics. 2007;33(5):524-30.
40. Spicer PP, Mikos AG. Fibrin glue as a drug delivery system. Journal of controlled release. 2010;148(1):49-55.

Tables

Table 1: Forward and reverse primers of genes used for RT-PCR experiments.

Primer	Forward	Reverse
DSPP	AGCAGGCAACATCACAACC	TGACAGAGTAGATGAGTGGAGTG
ALP	ACCATTCCCACGTCTTCACATTTG	AGACATTCTCTCGTTCACCGCC
COL 1A1	AGGGCTCCAACGAGATCGAGATCCG	TACAGGAAGCAGACAGGGCCAACG
VEGF	GTGCCCCCTAGCAGTACCG	GACGTGCCCCTACAAGTTGG
GAPDH	TATGGCGACCCGCAGCCCT	CATCTCGAGCAAGACGTTCAG

Figures

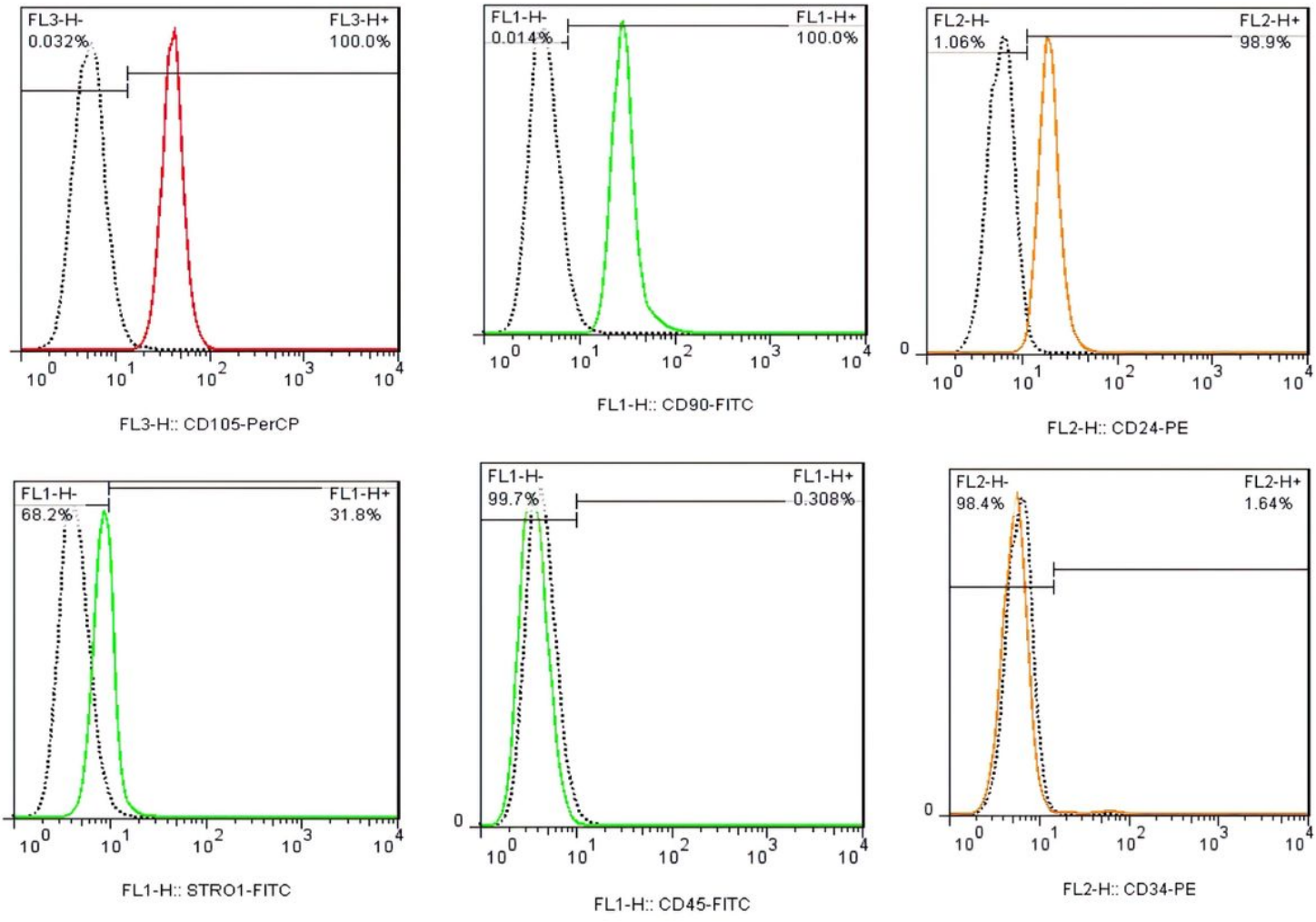

Figure 1

Flow cytometric analysis of cultured SCAP revealed expression of CD 105(100%), CD 90 (90%), CD 24 (98.9%), STRO1 (31.8%) but was negative for surface molecules CD45 and CD 34.

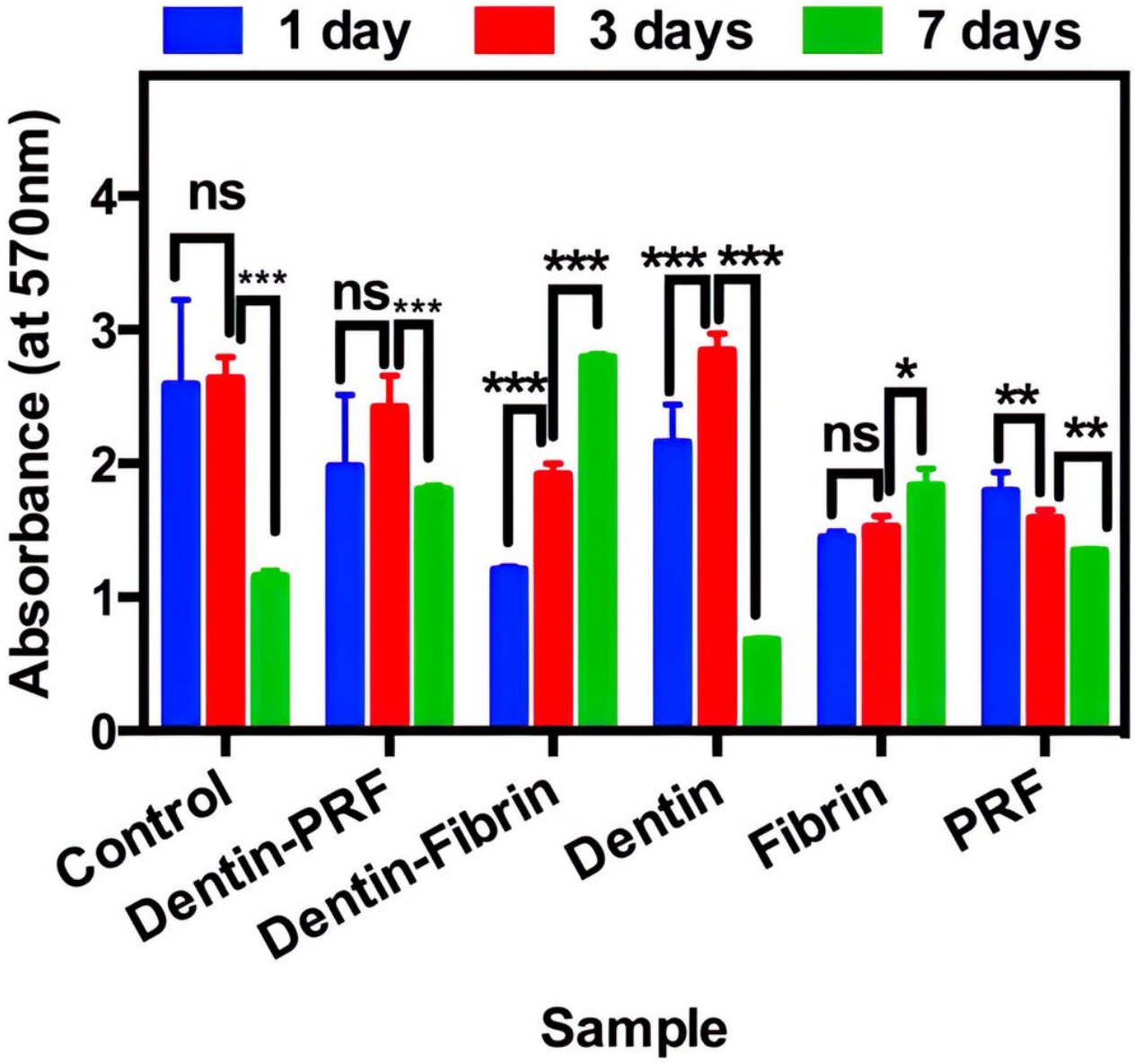

Figure 2

MTT cell viability of different prepared samples (*p < 0.05, **p < 0.01, and ***p < 0.001).

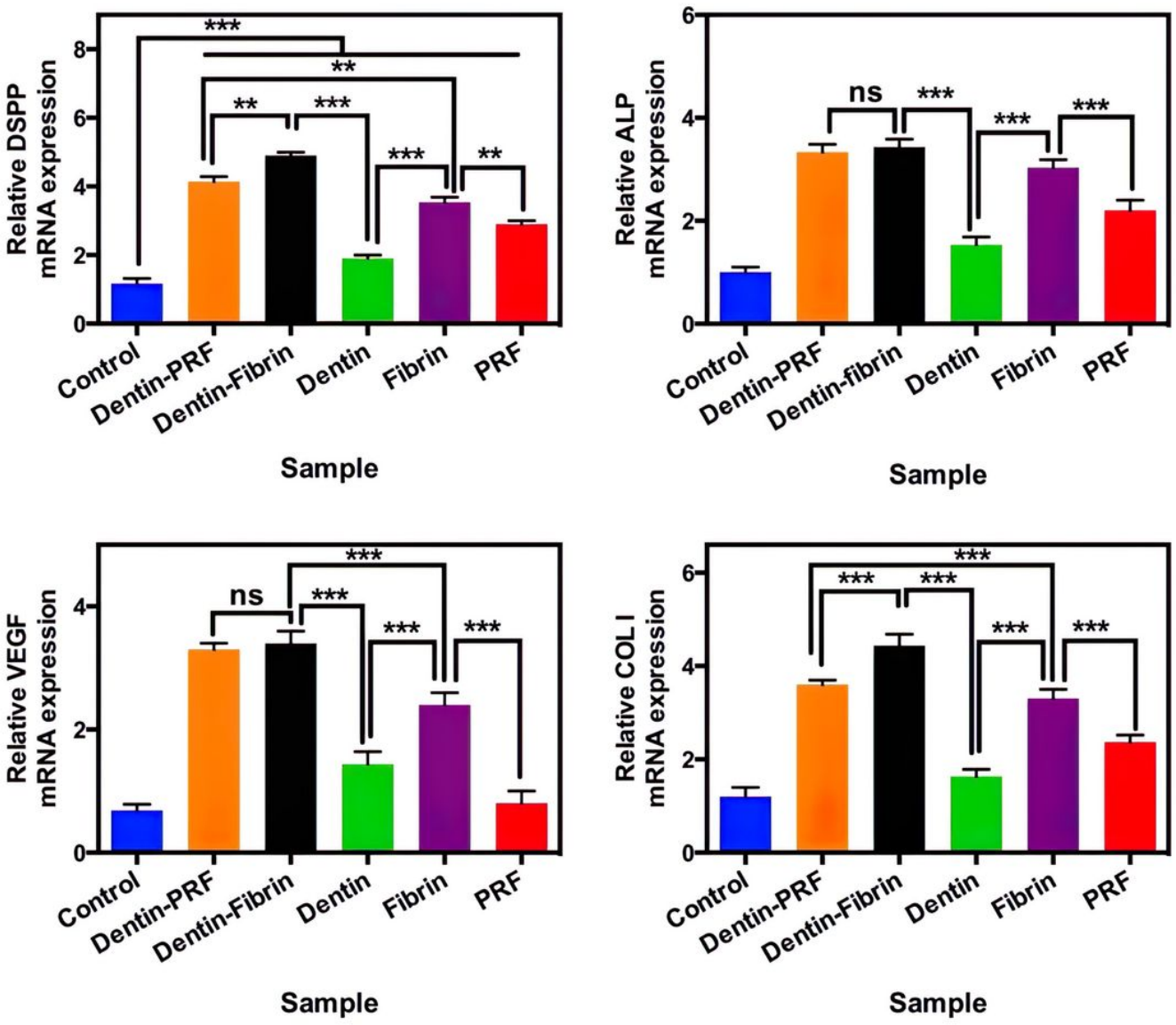

Figure 3

The gene expression differentiation markers in SCAP cultured directly onto different developed biomaterials (*p < 0.05, **p < 0.01, and ***p < 0.001).

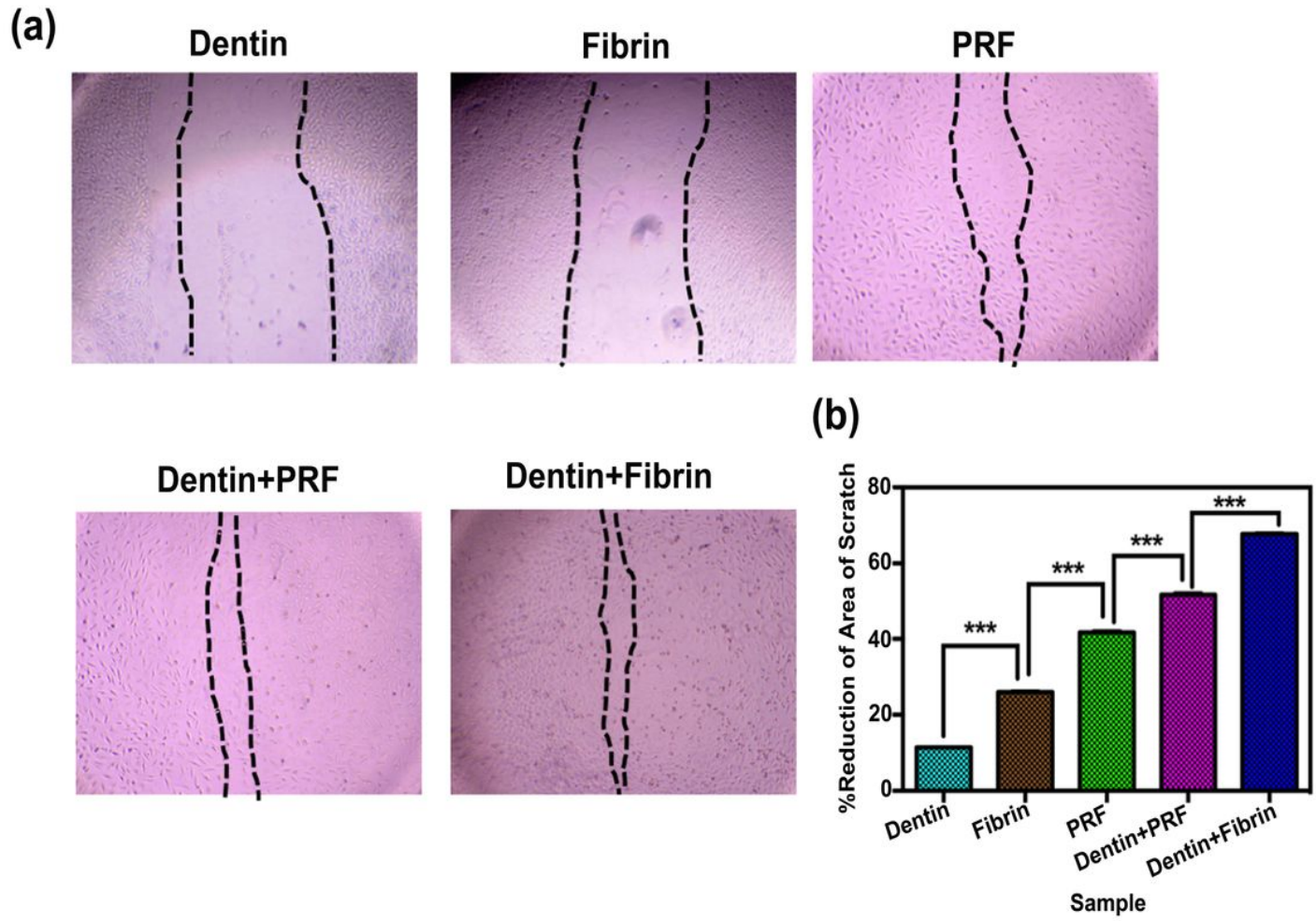

Figure 4

Scratch test assay test for evaluation of SCAP migration in the presence of different samples (*p < 0.05, **p < 0.01, and ***p < 0.001).