How to Cite:
Saeed, M. A., Alfotoh, M. M. A., Mubarak, R. T., & Grawish, M. E. (2022). Surface properties and elemental composition of human treated dentin matrix nano-scaffold as direct pulp capping material. *International Journal of Health Sciences*, *6*(S2), 6079–6087. https://doi.org/10.53730/ijhs.v6nS2.6559

Surface properties and elemental composition of human treated dentin matrix nano-scaffold as direct pulp capping material

Mahmoud A. Saeed
Ph.D. candidate at Department of Oral Biology, Faculty of Dentistry, Cairo University, Egypt & lecturer assistant at Department of Oral Biology, Faculty of Dentistry, Menufia University, Egypt
Corresponding author email: dr.mahmoudsaeed2014@gmail.com

Mona M. Abo Alfotoh
Professor at Department of Oral Biology, Faculty of Dentistry, Cairo University, Egypt
Email: monaabofotoh@dentistry.cu.edu.eg

Rabab T. Mubarak
Professor at Department of Oral Biology, Faculty of Dentistry, Cairo University, Egypt
Email: Rabab_mubarak@hotmail.com

Mohammed E. Grawish
Professor at Department of Oral Biology, Faculty of Dentistry, Mansoura University, Egypt.
Email: Grawish2005@gmail.com

***Abstract*---**Objective The aim of the study was investigating surface topography, roughness, and wettability of the TDM semi-rigid nano-scaffold. In addition to its chemical composition to prove the principle of using as direct pulp capping (DPC) material. Materials and methods: Forty sound and fully erupted premolars scheduled to be extracted for orthodontic treatment were involved for the TDM scaffolds preparation. The surface and chemical properties were investigated in comparison with UTD. The surface topography was investigated using SEM while AFM was used for studying SR. Surface wettability was analyzed via sessile-drop goniometry protocol. However, the elemental composition was analyzed using EDX. Result: The surface characterization of the TDM scaffolds revealed a nano-tubular pattern with high surface wettability. However, the elemental analysis revealed its optimum partial demineralization. Conclusion:

Manuscript submitted: 27 Feb 2022, Manuscript revised: 18 March 2022, Accepted for publication: 09 April 2022

the surface and elemental composition of TDM semi-rigid scaffold are promising to be a reasonable DPC material.

***Keywords*---**direct pulp capping, TDM scaffold, dentin-pulp complex regeneration.

Introduction

The development of the current direct pulp capping (DPC) materials was on empirical basis rather than understanding the molecular cascade of the pulp-self healing mechanism [1]. Therefore, they have some limitations such as inducing reparative dentinogenesis with an uncontrolled manner toward the residual pulp tissue leading to intra-pulpal calcification. In addition, the formed dentin bridge is not a true dentin; it is just dentin like tissue/scar dentin [2]. Therefore, many recent studies have focused on dentin-pulp complex regeneration through regenerative technologies using a biological triad for regenerating the injured pulp tissue as well as maintaining its functions. This triad is a combination of stem cells, signaling molecules, and scaffolds. Scaffolds are three-dimensional constructs that hold the cells together to produce the physical form of the damaged tissue [3]. The scaffold surface topography, roughness, and wettability affect dental pulp stem cell (DPSC) adhesion and differentiation. Therefore, the surface of the ideal scaffold should allow chemical, physical, and bio-molecular modification to enhance the cells-scaffold interaction [4]. Several studies reported the high healing potentiality of the human treated dentin matrix (TDM) in dentin-pulp complex regeneration at the pulp exposure site [5][6]. But, there is a lack of information about its surface topography, roughness, and wettability properties. Therefore, this study investigated the surface properties and elemental composition of the human TDM semi-rigid scaffold.

Material and methods

This study was performed according to the CRIS (checklist for reporting in-vitro studies) and guidelines of the Ethical Committee of Faculty of Dentistry, Cairo University, Egypt. Good manufacturing practice (GMP) were associated in the preparation steps of TDM scaffold. The scaffolds were fabricated from forty intact upper and lower premolars. The extraction procedure was planned for orthodontic treatment of ten patients aged between 19 and 22 years. The patients were free from any systemic or local diseases that affect the dental tissues.

Fabrication of TDM scaffold

After extraction, the cementum was removed by grinding through a high-speed fissure carbide bur under an effective water coolant system and the crowns were separated from their root using wheel-shaped discs. The pulp and pre-dentin tissues were removed by rough mechanical instrumentation and irrigation with 10% ethylene diamine tetra-acetic acid (EDTA) [7]. The root dentin was cut in cuboids of 2 x2 x 1 mm using isomet cutting machine (precision cutter, PICO 155, USA).

The surface treatment of the dentin cuboids were carried out following the method described by Chen et al. [6]. In brief, soaking root dentin cuboids in deionized water for 5h and cleaning cycles of 20 min were performed every 1h using an ultrasonic cleaner (VGT 1200H, China). Then soaking in consecutive gradient concentrations of 17%, 10% and 5% EDTA (Sigma, USA) and the time frame for each concentration was 5 min, 5 min and 10 min, respectively. Using the ultrasonic cleaner, the cuboids were washed once with deionized water for 10 min between each concentration application. The untreated dentin (UTD) cuboids were used as control group.

Surface properties of TDM scaffold

Surface topographic analysis

The ultra-morphologic surface structure of the TDM scaffolds was investigated by scanning electron microscope (SEM, JEOL JSM 6510 lv). Scaffolds were washed three times with sterile PBS, fixed at 0 oC with 2.5% glutaraldehyde [8], dried in a critical point dryer (Tousimis Autosamdri – 815 Coater), and coated with gold in a sputter coating evaporator (SPI Module - Sputter Carbon / Gold Coater). Finally, the scaffolds were scanned with the SEM at ×2000 magnification.

Surface roughness analysis

Atomic force microscopy (AFM, FlexAFM3, USA) was used to analyze the surface roughness (SR) of five TDM scaffolds in comparison to five UTD cuboids. It operated at contact mode using a nonconductive silicon probe. Scaffolds were dried in a vacuum oven (EQ-DZF-6050, USA) at 80 oC for 5 min and then fixed on the AFM substrate using double face sticky tape [9]. The scan area was 10×10 μm2, and the number of data points was 256 × 256 at a 1 Hz scan rate. The scanning results were analyzed by Nano surf C3000 (version 3.5.0.31) software.

Surface wettability analysis

The surface wettability (SW) of TDM scaffolds was analyzed by sessile-drop goniometry protocol [10]. This protocol was performed by capturing a photo of a water drop on the TDM scaffold surface using a professional camera (Canon, DC7.4 V, Taiwan). The contact angle between the water drop and the surface was measured by image J (version 1.8.0) software. The experiment was repeated five times.

Elemental analysis

Energy-dispersive X-ray spectroscopy (EDX) was used for the elemental analysis of the TDM scaffold and UTD to investigate the effect of EDTA treatment on dentin partial demineralization [11]. The elemental analysis was carried out for five TDM scaffolds and UTD cuboids.

Statistical analysis

The statistical analyses were performed using SPSS soft-ware (version 20). Studying the SR, SW, and elemental composition of TDM were statistically analyzed using independent samples T-test.

Results

Surface topography results

The three-dimensional SEM images of the UTD surface showed complete obliteration of the dentinal tubules openings by the smear layer of dentin debris (Fig. 1A). However, the topographic analysis of the TDM surface showed opened and intact dentinal tubules (Fig. 1B).

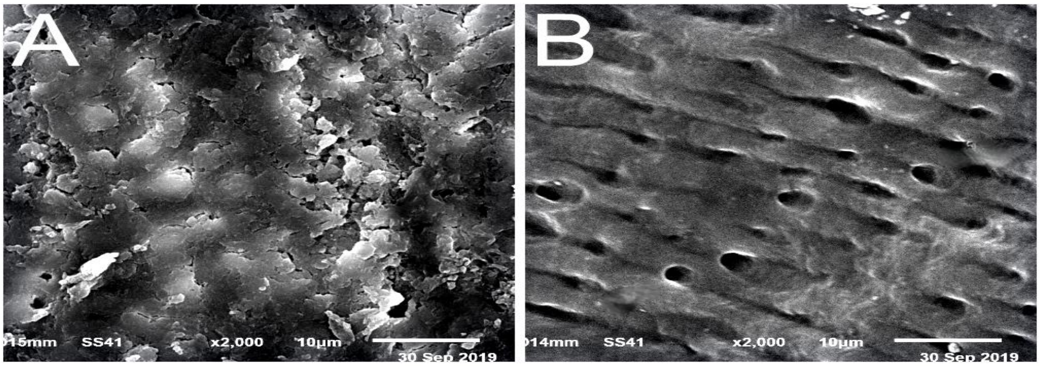

Figure 1: SEM photomicrographs showing A; the surface topography of UTD with the smear layer obliterating the dentinal tubules openings and B; the surface topography of TDM with opened dentinal tubules, ×2000

Surface roughness results

The atomic force microscopy aspect of UTD and the profile of a 10 µm section demonstrated a wide range in the roughness dimensions that ranged between 10 – 273 nm (Figs. 2A, 2B). However, AFM aspect of TDM and the profile of the same area demonstrated the roughness dimensions that ranged between 39.1- 90.8nm (Figs. 2C, 2D). The descriptive statistics for the SR showed that the mean and SD of the surface roughness average (SA) of UTD and TDM were 112.80 ± 0.83nm and 64.80 ± 0.44nm, respectively. The results of the independent samples T-test revealed a high significant difference ($P = 0.001$) between the two groups (Table 1).

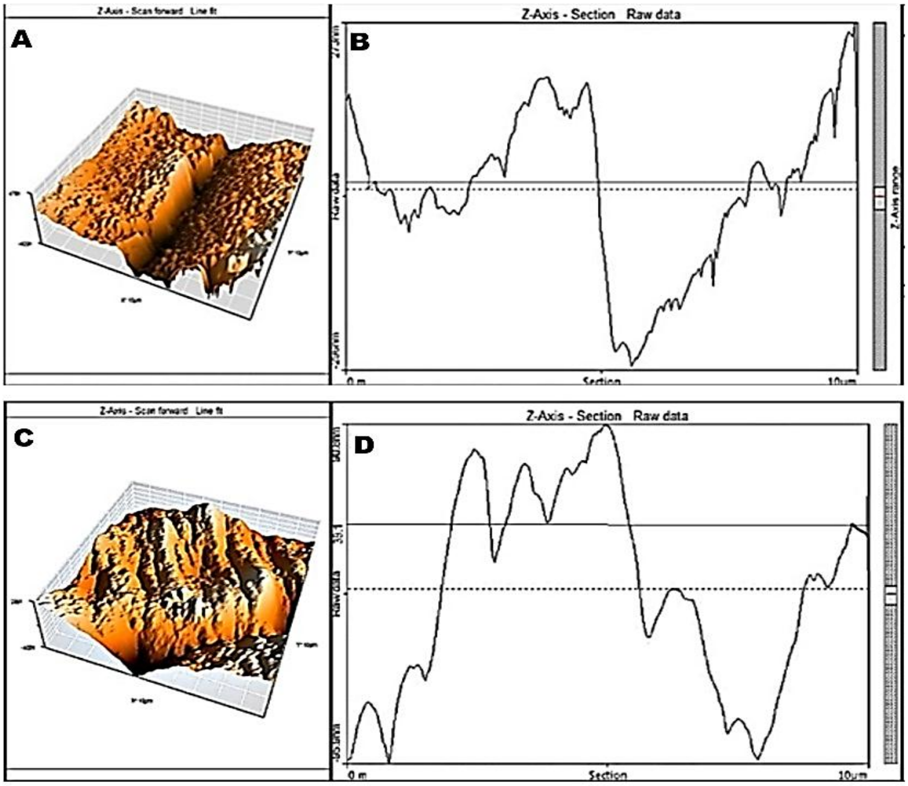

Figure 2: AFM photomicrograph showing A; the 3D surface texture of UTD, B; linear chart showing inhomogeneous SR of UTD at a profile of 10 μm, C; the 3D surface texture of TDM and D; linear chart showing homogeneous SR of TDM at a profile of 10 μm.

Surface wettability analysis

The value analysis of the contact angles between the water drop and the UTD and TDM surfaces revealed a higher contact angle for the UTD that indicated lower SW than the TDM. The descriptive statistics for the mean and SD showed that the mean of the contact angles for UTD were 60.20 ± 0.56, while they were 29.20 ± 0.34 for the TDM. The results of the independent samples T-test showed a significant difference (P = 0.001) between the two groups (Table 1).

Table 1: Results of the independent sample t-test and its statistical significance for SA and SW between UTD and TDM.

Test	Mean ± SD		Lower	Upper	T	df	*P* value†
	UTD	TDM					
SR	112.80 ± 0.37	64.80± 0.44	48.97	47.02	113.10	8.00	0.001
SW	60.20 ± 0.56	29.20 ± 0.34	36.04	25.96	14.00	8.00	0.001

Elemental analysis results

The value comparison of the elemental analysis between UTD and TDM revealed lower minerals' percentage by weight in TDM than UTD (Fig. 3). The higher percentage means of CaCo3 (35.14 ± 0.30), Ca (41.41 ± 0.36), P (21.66 ± 0.20), Na (1.84 ± 0.15), and Mg (0.96 ± 0.03) were for UTD. However, the lower percentage means of CaCo3 (17.12 ± 1.04), Ca (25.95 ± 0.548), P (6.48 ± 0.30), Na (1.38 ± 0.16), and Mg (0.66 ± 0.05) were for TDM. The results of the independent samples T-test revealed a significant difference (P = 0.001) in the percentage by weight of the CaCo3, Ca, Na, Mg, and P between the two groups (Table 3).

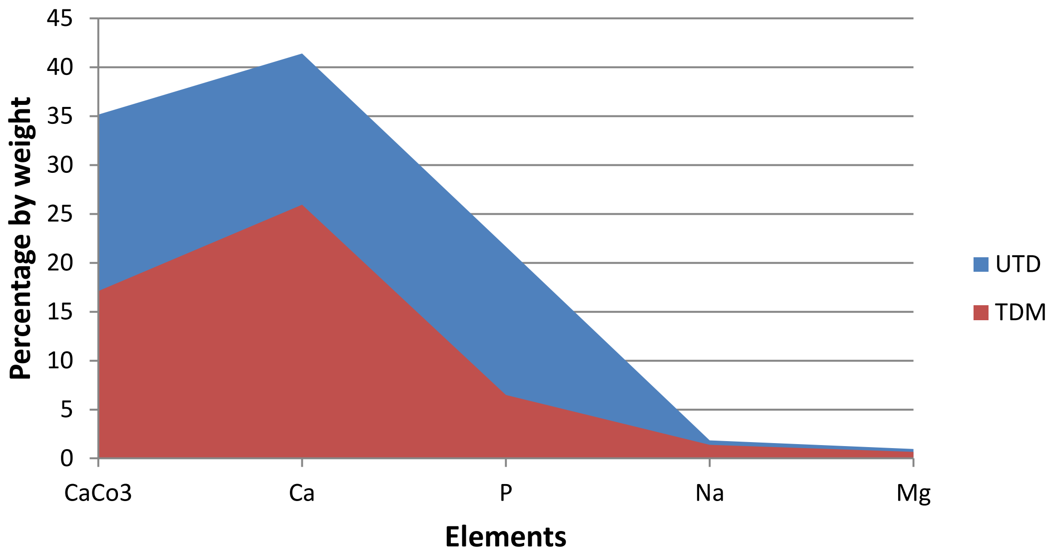

Figure 3: Area chart of the elemental analysis showing high minerals' percentage by weight for UTD and a decrease in the minerals' percentage by weight for TDM after treatment with gradient concentrations of EDTA.

Table 2: Results of independent sample T-test and its statistical significance for the elemental values between UTD and TDM

Element	Mean ± SD		*P* value
	UTD	TDM	
$CaCo_3$	35.14 ±0.30	17.12 ± 1.04	0.001
Ca	41.41 ± 0.36	25.95 ± 0.54	0.001
P	21.66 ± 0.20	6.48 ± 0.30	0.001
Na	1.84 ± 0.15	1.38 ± 0.16	0.001
Mg	0.96 ± 0.03	0.66 ± 0.05	0.001

Discussion

Surface properties of the scaffold modulate DPSCs proliferation and differentiation. Mechano-transduction mechanism via integrin-focal adhesioncytoskeleton pathway can explain the biological mechanism by which the stem cells can sense the surface topography of the scaffolds [8]. In the present study, the TDM surface topography was analyzed by SEM in comparison to the UTD to evaluate the effect of the surface treatment using gradient concentration of EDTA. The SEM photomicrographs of UTDM surface showed obliterated dentinal tubules by smear layer of denatured cutting debris that created on the dentin surface during cutting by isomet. The cutting debris were forced into variable distances inside dentinal tubules. These smear plugs together with the smear layer decrease dentin permeability and SW [12]. However, the SEM photomicrographs of the TDM surface showed removal of the smear layer and opened dentinal tubules. This finding could be attributed to the efficacy of EDTA in modulating the surface topography. These results were in consistent with the study of Sadaghiani et al. who reported that treatment of the dentin surface with EDTA remove smear layer and open dentinal tubules. This tubular nano-pattern increased the attachment and spreading of DPSCs and altered their morphology as they stretched towards the opened dentinal tubules. Besides, they developed long cytoplasmic processes with many granules [8].

The results of SR analysis using AFM revealed that treating dentin surface with EDTA is a fabrication technique of dentin scaffold with nano-SR. The size range that holds great interest for nanotechnologies is typically ranging from 100 nm down to the atomic level [13]. The surface roughness of TDM scaffold was 64.80 nm. That is involved in the nano-scale range. However, the SR of UTD was 112.8 nm that isn't involved in the nano-scale range. The profile of the 10 µm section of TDM demonstrated a narrow range of the roughness dimensions that reveal homogenous SR. However, the wide range in the roughness dimensions of UTD revealing non-homogenous SR. Material with homogenous nano-SR mimic the natural microenvironment that enhances cellular attachment, proliferation, and differentiation [14] that ensured a high healing potentiality of the TDM scaffolds. The nano-tubular pattern of the TDM affects its SW and therefore, dentin SW had analyzed in the present study. The results of SW test revealed higher SW of the TDM than that of the UTD. This indicted a high healing potentiality of the TDM [15]. These results were in agreement with the findings of Galler et al. [16] who reported the efficacy of a high SW on enhancing the cellular attachment and so the healing potentiality.

The present study revealed a significant difference between the elemental analysis of UTD and TDM that confirmed partial demineralization of the dentin by EDTA. These findings were in agreement with the results of Holiel et al. [5][17] who reported the importance of the dentin partial demineralization as an optimal condition for dentin regeneration. The partial demineralization of dentin matrix explains the semi-rigidity of TDM scaffolds enabling them to withstand the masticatory forces.

Conclusion

The surface and elemental analysis of TDM scaffold revealed that it is a promising substitute for the traditional DPC materials.

References

1. M. Okamoto, Y. Takahashi, S. Komichi, P. R. Cooper, and M. Hayashi, “Dentinogenic effects of extracted dentin matrix components digested with matrix metalloproteinases,” *Sci. Rep.*, 2018, doi: 10.1038/s41598-018-29112-3.
2. J. C. Farges *et al.*, “Dental Pulp Defence and Repair Mechanisms in Dental Caries,” *Mediators of Inflammation*, vol. 2015. 2015, doi: 10.1155/2015/230251.
3. J. G. Najdanović *et al.*, “Effects of bone tissue engineering triad components on vascularization process: comparative gene expression and histological evaluation in an ectopic bone-forming model,” *Biotechnol. Biotechnol. Equip.*, 2016, doi: 10.1080/13102818.2016.1213662.
4. B. Nasiri and S. Mashayekhan, “Fabrication of porous scaffolds with decellularized cartilage matrix for tissue engineering application,” *Biologicals*, vol. 48, pp. 39–46, 2017, doi: https://doi.org/10.1016/j.biologicals.2017.05.008.
5. A. A. Holiel, E. M. Mahmoud, W. M. Abdel-Fattah, and K. Y. Kawana, “Histological evaluation of the regenerative potential of a novel treated dentin matrix hydrogel in direct pulp capping,” *Clin. Oral Investig.*, pp. 2101–2112, 2020, doi: 10.1007/s00784-020-03521-z.
6. J. Chen *et al.*, “Treated dentin matrix paste as a novel pulp capping agent for dentin regeneration,” *J. Tissue Eng. Regen. Med.*, 2017, doi: 10.1002/term.2256.
7. P. Mehrvarzfar, P. V. Abbott, F. Mashhadiabbas, M. Vatanpour, and S. Tour Savadkouhi, “Clinical and histological responses of human dental pulp to MTA and combined MTA/treated dentin matrix in partial pulpotomy,” *Aust. Endod. J.*, vol. 44, no. 1, pp. 46–53, 2018, doi: 10.1111/aej.12217.
8. L. Sadaghiani, H. B. Gleeson, S. Youde, R. J. Waddington, C. D. Lynch, and A. J. Sloan, “Growth factor liberation and DPSC response following dentine conditioning,” *J. Dent. Res.*, vol. 95, no. 11, pp. 1298–1307, 2016, doi: 10.1177/0022034516653568.
9. S. Vahabi, B. N. Salman, and A. Javanmard, “Atomic force microscopy application in biological research: a review study,” *Iran. J. Med. Sci.*, vol. 38, no. 2, p. 76, 2013.
10. T. Huhtamäki, X. Tian, J. T. Korhonen, and R. H. A. Ras, “Surface-wetting characterization using contact-angle measurements,” *Nat. Protoc.*, vol. 13, no. 7, pp. 1521–1538, 2018, doi: 10.1038/s41596-018-0003-z.
11. D. F. Sanchez *et al.*, “2D/3D Microanalysis by Energy Dispersive X-ray Absorption Spectroscopy Tomography,” *Sci. Rep.*, vol. 7, no. 1, pp. 1–12, 2017, doi: 10.1038/s41598-017-16345-x.
12. B. Buldur and A. Kapdan, “Comparison of the EndoVac system and conventional needle irrigation on removal of the smear layer in primary molar root canals.,” *Niger. J. Clin. Pract.*, vol. 20, no. 9, pp. 1168–1174, Sep. 2017, doi: 10.4103/1119-3077.181351.
13. A. Konala and K. K. R. Tetala, “Nanomaterial grafted Microfluidic Columns for Biomolecule Separation at Analytical Scale,” in *Nanomaterials in Chromatography*, Elsevier, 2018.
14. G. K. Thakral, R. Thakral, N. Sharma, J. Seth, and P. Vashisht, “Nanosurface-the future of implants,” *J. Clin. Diagnostic Res.*, vol. 8, no. 5,

pp. 7–10, 2014, doi: 10.7860/JCDR/2014/8764.4355.
15. L. Jiao *et al.*, “Cryopreserved dentin matrix as a scaffold material for dentin-pulp tissue regeneration,” *Biomaterials*, vol. 35, no. 18, pp. 4929–4939, Jun. 2014, doi: 10.1016/j.biomaterials.2014.03.016.
16. M. Widbiller *et al.*, “Cell Homing for Pulp Tissue Engineering with Endogenous Dentin Matrix Proteins,” *J. Endod.*, 2018, doi: 10.1016/j.joen.2018.02.011.
17. A. A. Holiel, E. M. Mahmoud, and W. M. Abdel-Fattah, “Tomographic evaluation of direct pulp capping using a novel injectable treated dentin matrix hydrogel: a 2-year randomized controlled clinical trial.,” *Clin. Oral Investig.*, vol. 25, no. 7, pp. 4621–4634, Jul. 2021, doi: 10.1007/s00784-021-03775-1.