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ENGINEERING AND NANO-ENGINEERING APPROACHES FOR MEDICAL DEVICES

Rapid Communication

Nano-titanium coating on glass surface to improve platelet-rich fibrin (PRF) quality

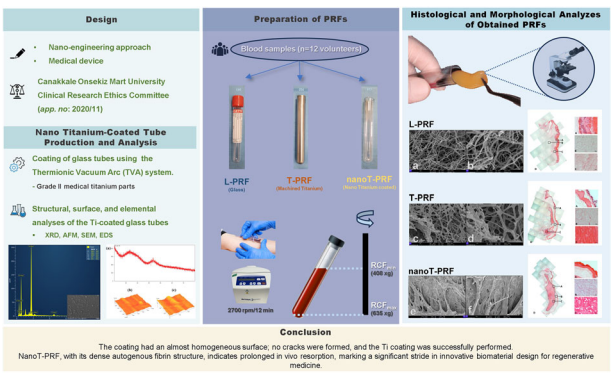
Mustafa Tunalı[1] · Esra Ercan[1] · Suat Pat[2] · Emrah Sarıca[3] · Aysel Güven Bağla[4] · Nilüfer Aytürk[4] · Duygu Sıddıkoğlu[5] · Vildan Bilgin[6]

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Abstract

The quality of platelet-rich fibrin (PRF) is contingent on the surface characteristics interfacing with blood. Titanium's superior platelet activation, surpassing silica, has made Titanium-platelet-rich fibrin (T-PRF) a favored autogenous bone graft material due to its extended degradation time. Pioneering a novel approach, this study aims to achieve an enhanced fibrin structure using glass tubes coated with nano-titanium, marking the surface's debut in our PRF production endeavors. Employing a rapid thermionic vacuum arc (TVA) process under high vacuum, we conducted comprehensive analyses of the tubes. Comprehensive analyses, including X-ray diffraction (XRD), atomic force microscopy (AFM), scanning electron microscopy (SEM), and energy dispersive X-ray spectroscopy (EDS), were conducted on the nano-titanium-coated glass tubes. Three PRF types were formulated: silica-activated leukocyte- and platelet-rich fibrin (L-PRF, control group), machined-surface titanium tubes (T-PRF), and nano-titanium-coated tubes (nanoT-PRF). Analyses unveiled denser fibrin areas in nanoT-PRF than T-PRF, with the least dense areas in L-PRF. Cell distribution paralled between nanoT-PRF and T-PRF groups, while L-PRF cells were embedded in the fibrin border. NanoT-PRF exhibited the densest autogenous fibrin structure, suggesting prolonged in vivo resorption. Additionally, we explore the potential practicality of single-use production for nanoT-PRF tubes, introducing a promising clinical advancement. This study marks a significant stride in innovative biomaterial design, contributing to the progress of regenerative medicine.

Graphical Abstract



✉ Esra Ercan
esraercan@comu.edu.tr

[1] Department of Periodontology, Faculty of Dentistry, Canakkale Onsekiz Mart University, 17110 Canakkale, Turkey

[2] Department of Physics, Faculty of Science, Eskişehir Osmangazi University, 26004 Eskişehir, Turkey

[3] Department of Electrical and Electronics Engineering, Faculty of Engineering, Baskent University, 06790 Ankara, Turkey

[4] Department of Histology and Embryology, Faculty of Medicine, Canakkale Onsekiz Mart University, 17110 Canakkale, Turkey

[5] Department of Biostatistics, Faculty of Medicine, Canakkale Onsekiz Mart University, 17100 Canakkale, Turkey

[6] Department of Physics, Faculty of Science, Canakkale Onsekiz Mart University, 17110 Canakkale, Turkey

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1 Introduction

Currently, a variety of grafts, membranes, biological materials, and their combinations are utilized for the regeneration of soft and hard tissues [1]. However, an ideal material has not yet been produced. Autogenous graft materials, which are the gold standard, also have significant disadvantages, such as a second surgical area, increased postoperative morbidity and limited availability [2]. Although nonautogenous materials have osteoconductive potential in bone healing, they have some disadvantages such as nonosteogenicity, aggravating the resorptive effect, which can promote the inflammatory response, and susceptibility to infection [3].

Blood products have been used in regenerative medicine and dentistry for many years [4]. Despite being autogenous, platelet-rich plasma products have significant limitations, as they contain foreign substances (anticoagulants and artificial coagulants) [5]. As with nonautogenous materials, they are affected by resorptive inflammatory mechanisms. For this reason, their effects are limited when used in soft and hard tissue regeneration [6]. Since 2001, platelet-rich fibrin (PRF) has attracted attention as a completely autogenous (second generation) blood product [7]. After 2006, it started to be used for the regeneration of soft tissues and in combination with other grafts for bone regeneration [8]. Although successful clinical results have been obtained with PRF, recent studies have shown that silica in glass and silica-coated plastic tubes can pass into PRF clots, which negatively affects fibrin formation and may have harmful health effects [9].

During PRF clot formation, the primary mechanism for activating blood is through endogenous coagulation pathways triggered by the blood's contact with the centrifuge tube walls. Initially, platelets in the blood adhere to the tube walls, leading to their activation [10]. Since the hydrophilic or hydrophobic nature of the tube surface plays a key role in protein and cell adhesion, altering the surface properties and its chemical charge can be crucial for controlling platelet activation during PRF membrane preparation. Therefore, enhancing the tube surface may be a promising strategy to improve the formation of a more effective clot structure by promoting platelet adhesion and activation through the tube walls [11, 12]. This can be achieved by modifying the surface characteristics. Characteristics of the surface in contact with blood has critical importance in determining the quality of PRF. For example, PRF obtained with glass/silica tube has a looser structure compared to PRF obtained with titanium tube [13]. This directly affects the degradation time in the body. Additionally, PRF has limited use in bone regeneration due to its short resorption time (1–2 weeks) [14]. Some studies have shown that titanium platelet-rich fibrin (T-PRF), which was introduced by our study group in 2013 and is activated with titanium instead of silica, can be used alone in bone regeneration due to its tighter fibrin carpet structure and longer in vivo degradation time [13, 15, 16]. It has been shown that titanium activates platelets better than silica and forms a tighter natural fibrin network; furthermore, the in vivo resorption time of titanium-activated fibrin is longer (more than 30 days) [13, 17]. Despite this, researchers have continued to develop new fibrin materials by imitating natural coagulation mechanisms [18].

A new idea in this area is to coat conventional PRF tube surfaces with nano-titanium, thus increasing the titanium surface area in contact with blood in the tube and enabling platelets to be activated with titanium even more strongly during centrifugation. With this method, a tighter fibrin structure with a long in vivo resorption time can be obtained. In addition, this fibrin structure can be used as a bone graft alone because it can create an adequate osteoconductive effect to promote bone healing.

2 Material and methods

2.1 Nano-titanium-coated tube production and analysis

Before the coating process, the glass tubes were cleaned by ethyl alcohol and made ready for coating. Hot air flux was applied of all sample before the coating approximately 4 min. The titanium coating process was carried out quickly under high vacuum with a thermionic vacuum arc (TVA). A TVA system is a physical vapor deposition technology, which is used to high-quality thin film. The coatings realize with arc coating process and no gases were used because of the TVA no needs the buffer gas or process gas. It can use only the pure material plasma in TVA coating. The TVA system consists of two electrodes as the anode and cathode. The cathode is an electron gun containing a Wehnelt cylinder, and the anode is the tungsten crucible material in which the material to be evaporated or coated is placed. According to Wehnelt and filament positions, ejected electrons from the filament were focused on the top of the evaporated material. Medical-grade 2 Ti parts were used in the coating process of the tubes. The coated medical-grade 2 Ti slugs were placed into the anode boat. In the TVA process, the titanium parts were first heated at a high current value of 19 A with an electron gun inside the cathode, and then plasma was produced using a high voltage of approximately 1000 V as the acceleration voltage. In that time, a green plasma was created by using a material which located into anode boat. Discharge current was jump to higher value suddenly and applied voltage value decreased to 200 V.

While producing titanium plasma with TVA, the coatings were carried out under a vacuum of approximately 5×10^{-5} Torr, and the coating process was completed within 5 min. In the coating process, 12 tubes were coated by pure Ti material. In this way, glass tubes coated with nano-titanium were obtained. A total of 12 glass tubes underwent a nano-titanium coating process using the Thermionic Vacuum Arc (TVA) system. The geometrical parameters of TVA are electron gun angle, distance of electrodes and distance of the tube position. The angle was 45°, 5 mm and 100 mm. The tube length is 160 mm and diameter of the tube was 16 mm. After the coating, coted tubes were controlled the quality of the samples. Especially, adhesion is an important quality for the sample and no pin hole was not observed on the coated tubes. Coated layers were observed of the bottom of the tubes and coating locations were observed alongside of each tube sample.

Structural, surface, and elemental analyses of the Ti-coated glass tubes were conducted using X-ray diffraction (XRD), atomic force microscopy (AFM), scanning electron microscopy (SEM), and energy dispersive X-ray spectroscopy (EDS). A Rigaku Ultima-IV X-ray diffractometer was used to obtain XRD patterns of the Ti-coated glass. Measurements were taken between the limit values of $10° < 2\theta < 60°$ using a CuKα beam with a wavelength of 1.5405 Å. All measurements were realized at room temperature.

2.2 Obtaining PRF

The study received ethical approval from the Canakkale Onsekiz Mart University Clinical Research Ethics Committee (approval number: 2020/11; approval date: August 26, 2020. Written informed consent was obtained from all study participants. Twelve volunteers were included in the study. Thirty milliliters of blood were collected from the antecubital vein of each volunteer, quickly transferred into tubes without anticoagulant (10 ml per tube) and centrifuged at 2700 rpm for 12 min (angled system; RCFclot 408 × *g* and RCFmax 635 × *g*) according to the IntraSpin L-PRF centrifugation protocol (Intra-Lock International). The tubes used to obtain PRF are shown in Fig. 1a–c.

The L-PRF, T-PRF and nanoT-PRF samples were kept for 20 min in a sterile petri dish to release their serum and then transferred to containers containing formaldehyde. A small piece of each sample was separated for SEM analysis and placed in glutaraldehyde solution.

Venous blood of the 12 volunteers was divided equally into three groups: Leukocyte- and platelet-rich fibrin (L-PRF), Titanium platelet-rich fibrin (T-PRF), and Nano-titanium platelet-rich fibrin (nanoT-PRF).



Fig. 1 **a** Glass tube (L-PRF); **b** Titanium tube (T-PRF); **c** Nano-titanium coated tube (nanoT-PRF)

2.3 Histomorphometric evaluation

For histomorphological evaluation, the prepared platelet-rich fibrin samples were placed in 10% neutral buffered formaldehyde. The tissue samples were then fixed in formaldehyde for 48 h followed by paraffin blocking for histological analysis.

The samples were incubated in increasing alcohol concentrations and soaked in xylene. Paraffin blocks were prepared after placing the samples in molten paraffin in an oven at 70 °C. Eight consecutive sections of 6 µm thickness were taken from each block at intervals, and the sections were deparaffinized and stained with hematoxylin-eosin stain. The sections were covered with biomount closure medium, and imaging was performed with a light microscope (Zeiss Axio, Scope A1 Carl Zeiss, Göttingen, Germany). For fibrin area measurement, all sections from each block were examined, and the dense fibrin areas present in each section were photographed with a light microscope

(Carl Zeiss Primo Star) at x4 magnification. Light microscope images were transferred to the ImageJ analysis program, and morphometric area measurements (μm^2) were made.

Histomorphometric scoring was modified from Hamed and Hasouni [19]:

- Border width (buffy coat): thin (1), medium (2), thick (3).
- Border layout: regular (1), irregular (2).
- Placement of cells in the border: above the border (1) or embedded in the border (2).
- Fibrin density condition touching the inner surfaces of the tube: thin (1), medium (2), thick (3).

All histomorphological evaluations were performed by 2 histologists (A.G.B. and N.A.) in a double-blinded manner. Before the evaluation, the method to be used for measurement and scoring was discussed and a consensus was reached. The results were obtained through a consensus.

2.4 Histological procedures for scanning electron microscopy evaluation

The morphologic evaluation of the L-PRF, T-PRF, and nanoT-PRF fibrins was conducted with a scanning electron microscope (LEO 440, Leica and Zeiss Co.). The fibrin samples were fixed in 2.5% glutaraldehyde for 1 h and then desiccated. The specimens were sputter-coated with 20 nm gold/palladium and subsequently examined under the scanning electron microscope. Photographs were taken at 20 kV using a magnification from 51 to 10,000.

2.5 Statistical analysis

SPSS for Windows version 20 (IBM Corp.) was used to analyze the data obtained in the study. The normality of the obtained data was assessed using the Shapiro–Wilk test. The dense fibrin area measurements were compared using the Kruskal–Wallis test, and post hoc comparisons were performed. For categorical data (border width, border arrangement, cell location, fibrin density touching the inner surfaces of the tube), Fisher's exact test was used to analyze the distributions between groups.

3 Results

3.1 Analysis of the coated surface

According to the XRD analysis, the Ti coating exhibited an amorphous structure (Fig. 2a). Because it was an amorphous structure, it could not be entered into the peak analysis. Three-dimensional surface photographs of Ti-coated glass were taken with an Ambios Q-scope model AFM device (Fig. 2b, c).

Examining the images revealed that the coating had an almost homogeneous surface; no cracks were formed, and the Ti coating was successfully performed. SEM and EDS analyses of the Ti coating were conducted using a Hitachi Su5000 field emission scanning electron microscope (FE-SEM). The SEM images showed that the Ti coating surface was homogeneous, and no gaps or cracks were observed along the surface (Fig. 3a, b).

The EDS spectrum showed that in addition to the Ti coating material, there were peaks belonging to other elements from the glass. Because the Ti coating was very thin, the spectral intensities and percentage weights (wt%) of the elements from the glass were observed more frequently than the Ti peak (Fig. 3c).

3.2 Scanning electron microscopy analysis

The PRF samples were photographed at ×5000 and ×10,000 magnifications (Fig. 4).

Mature and well-organized fibrin networks were observed in all three groups. However, a tighter and thicker fibrin carpet structure with more complex and denser fibrin clusters was observed in the nanoT-PRF group than in the other two groups and in the T-PRF group than in the L-PRF group.

3.3 Histomorphometrically analysis

Because the dense fibrin area measurement results are not normally distributed, the data are given as the median (interquartile width; 25th–75th percentile). The measurements of the groups are as follows (in μm^2): L-PRF group: 0 (0–7785.65); T-PRF group: 338,388.37 (7899.68–768,171.24); nanoT-PRF group: 694,474.02 (30,932.33–2,109,370.32). There were statistically significant differences in the dense fibrin area measurements among the groups ($p < 0.001$). The groups were also compared in pairs by post hoc comparison. The dense fibrin area was significantly higher in the T-PRF and nanoT-PRF groups than in the L-PRF group ($p = 0.003$ and $p = 0.043$, respectively). Although a denser fibrin area was observed in the nanoT-PRF group than in the T-PRF group, the difference was not significant ($p > 0.05$).

The results for border thickness, border layout, placement of cells, and fibrin density condition touching the inner surfaces of the tube are given in Supplementary Information and Fig. 5. The border thickness significantly differed among the groups ($p < 0.001$). In the L-PRF group, the border thicknesses were mostly scored as thick, while the T-PRF and nanoT-PRF groups were similarly scored as

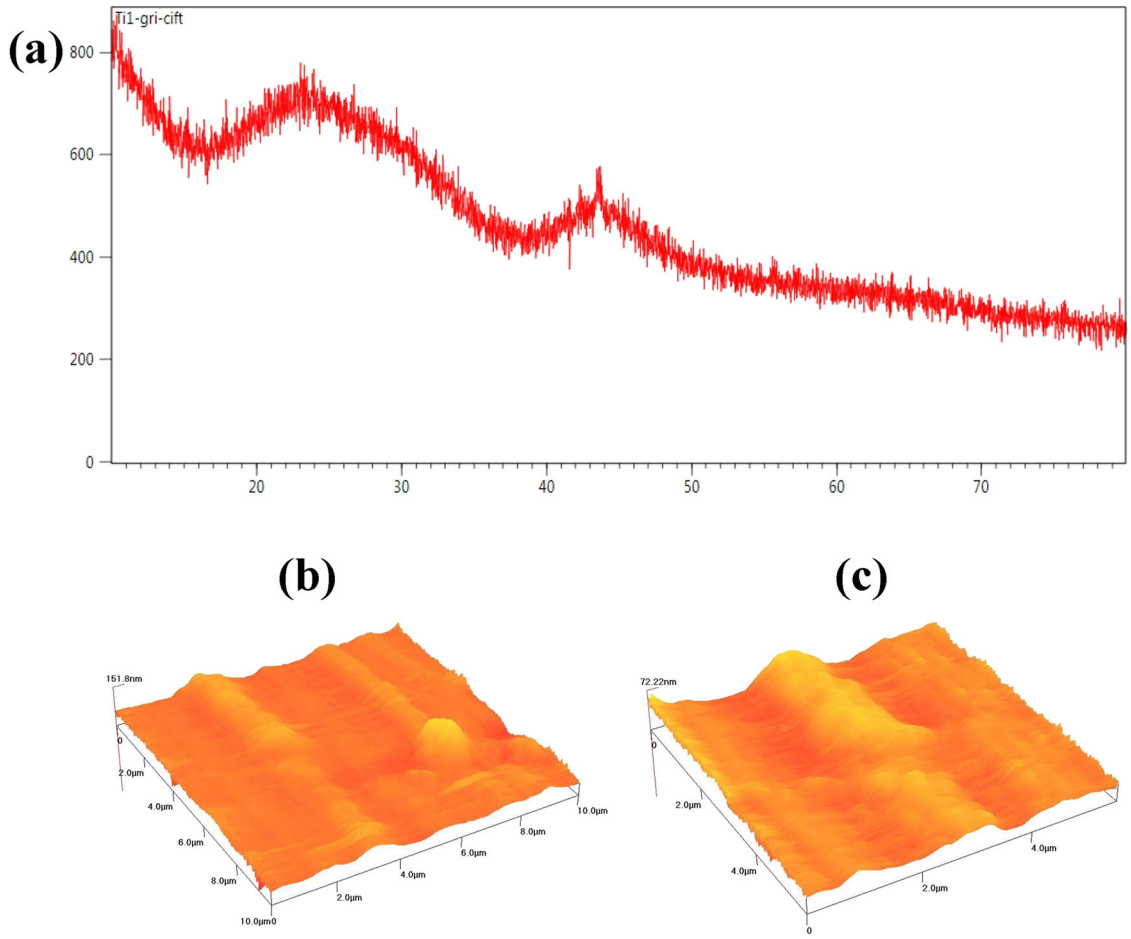


Fig. 2 **a** The XRD results of the Ti-coated glass surface; **b**, **c** 3D surface photographs of Ti-coated glass surface

moderate. No significant difference was observed among the groups in terms of border layout ($p > 0.05$). However, most samples in the nanoT-PRF and T-PRF groups were scored as regular. The placement of cells significantly differed among the groups ($p < 0.001$). In the T-PRF and nanoT-PRF groups, cells were observed on the border. Cells were observed on the border in 91.7% of the samples in the nanoT-PRF group and 75% of the samples in the T-PRF group. In the L-PRF group, the cells were embedded in the border for all samples. There were no significant differences among the groups in terms of fibrin density touching the inner surfaces of the tube ($p > 0.05$).

4 Discussion

The interactions between blood and the material surface require a different kind of events like platelet adhesion, activation, protein adsorption, and coagulation [20]. It was showed that activation and aggregation of platelets increased with the diameter of the nanotubes due to the increasing roughness [21]. In a recent study, it was showed that the adsorption of plasma proteins on titanium surfaces of different structures is also different. This directly affects platelet adhesion and activation [22]. Platelet activation on titanium oxide nanotubes was enhanced according to flat titanium surface. However, they used PRP (platelet-rich plasma). PRP is a first-generation autogenous blood product. The preparation protocol is more complicated compared to PRF and it is necessary to add chemicals in this process. The resulting product also has very weak biological effects compared to PRF [5, 23].

This is the first study that investigated the effects of nano-titanium surface coating on PRF quality. In our study, nano-titanium-coated surface provides a dense and high-quality PRF. This may be attributed to surface morphology with nano structure.

We found that the nanoT-PRF fibrin network structure was more tightly structured and well organized than the L-PRF fibrin structure. There was a difference in the buffy coat border structure of T-PRF and nanoT-PRF compared to L-PRF. The border was thinner in T-PRF and nanoT-PRF than in L-PRF, and the nonerythrocyte blood cells were on the fibrin side of the border. By contrast, in L-PRF,

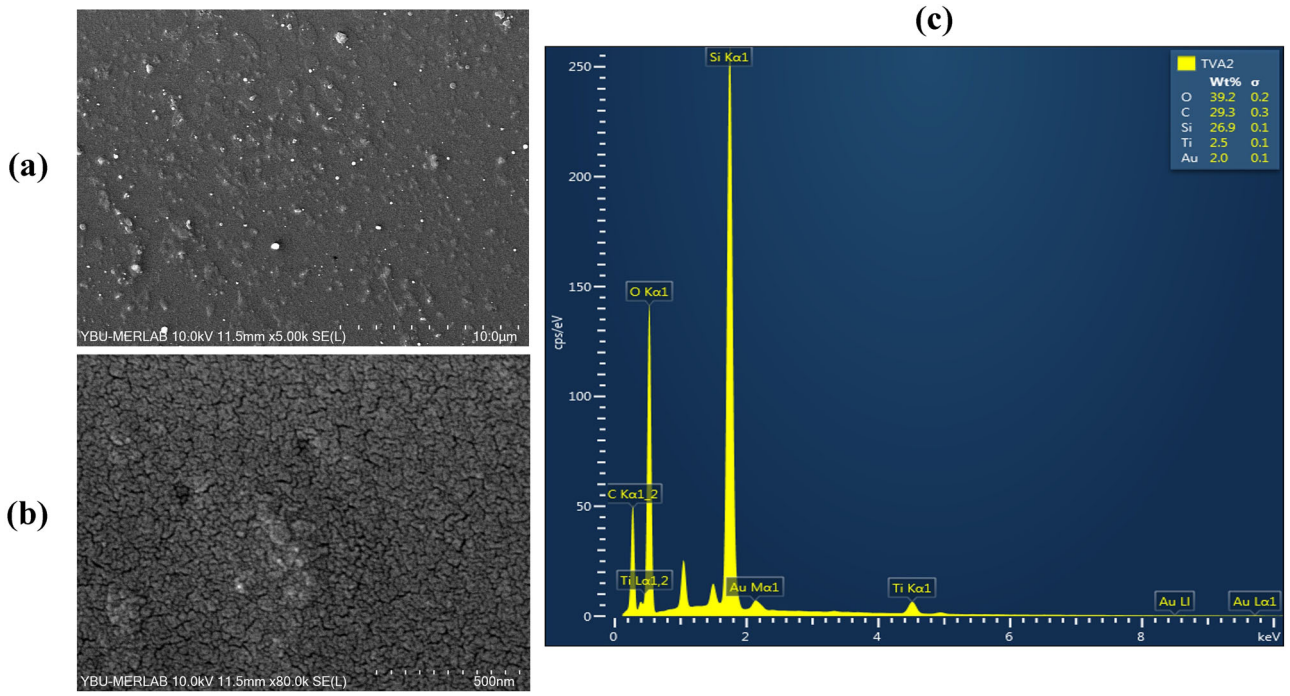


Fig. 3 SEM images of the Ti-coated glass surface **a** x5.00k; **b** x80.0k; **c** EDS spectrum of Ti-coated glass surface

nonerythrocyte cells were found to be embedded in the thick border. It was observed that nanosizing the titanium surfaces of the tubes created a tighter PRF with the activation of platelets, and through this mechanism, the fully autogenous fibrin matrix became stronger.

Recent studies have highlighted the significance of the matrix structure overgrowth factors in biological materials [24, 25]. The completely autogenous fibrin matrix structure does not cause nonphysiological resorptive inflammation. Thus, it can act as a good scaffold for the surrounding tissue that is to be regenerated, and it induces regeneration with the angiogenic factors and growth factors within it.

Three basic functions can be expected from second-generation blood concentrates such as T-PRF and nanoT-PRF, which have promising prospects, especially in bone regeneration. The first is the graft material function. Owing to this function, these materials can replace autogenous and nonautogenous graft materials, inducing rapid and high-quality regeneration with long resorption times (more than 30 days for T-PRF) and fully autogenous structures [18, 26]. A clinical study found that 4 months after a sinus lifting operation with T-PRF, the T-PRF was replaced by fully functional autogenous bone [18]. However, it has been shown that nonautogenous bone graft materials, despite their good placeholder function, can remain unabsorbed for a long time, may have fibrous encapsulations form around them, and may form areas prone to periimplantitis in future implant applications [27].

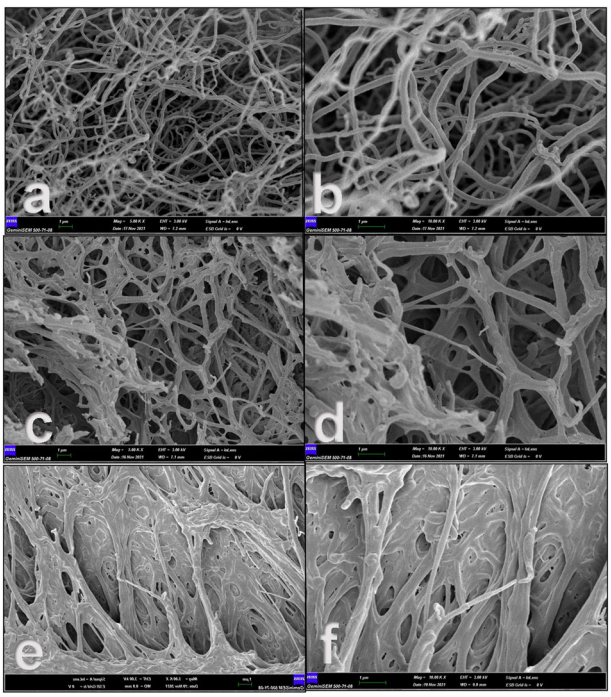


Fig. 4 SEM images of the samples **a** L-PRF ×5000; **b** L-PRF ×10,000; **c** T-PRF ×5000; **d** T-PRF ×10,000; **e** nanoT-PRF ×5000; **f** nanoT-PRF ×10,000

The second basic function is the autogenous membrane function. Second-generation blood concentrates with tight network structures, such as T-PRF and nanoT-PRF, can also be used as membranes for Guided Tissue Regeneration

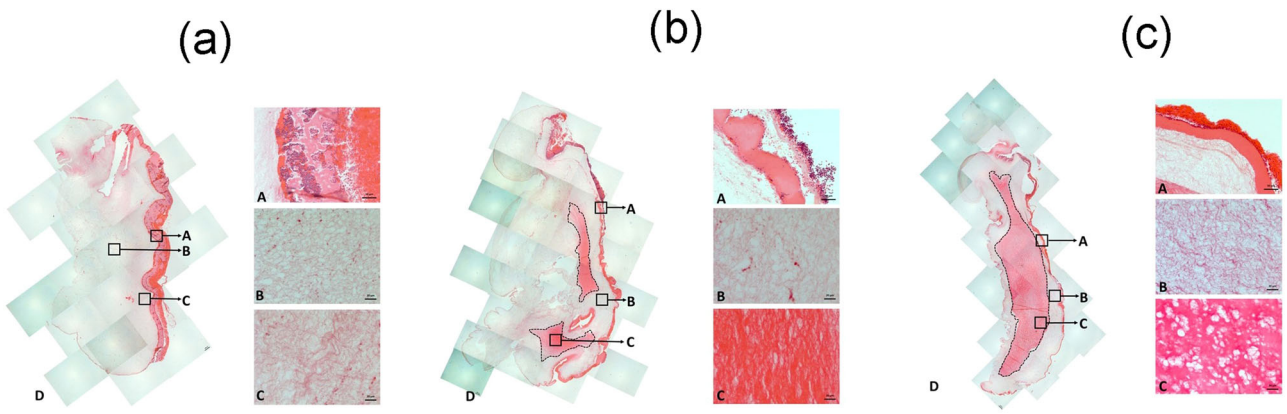


Fig. 5 Haematoxylin-eosin staining images of groups: **a** L-PRF; **b** T-PRF; **c** nanoT-PRF Border area (A), Loose fibrin area (B), Dense fibrin area (C), Entire cross-sectional surface (D)

(GTR) and Guided Bone Regeneration (GBR), considering that their in vivo resorption times exceed 30 days. In addition to acting as a barrier for soft tissues, they can provide faster and higher quality regeneration in the region without restricting the functions of the periosteum. The mechanical properties and degradation time are also crucial for tissue regeneration. These properties of T-PRF, L-PRF and A-PRF were investigated in a recent study, and T-PRF was found to contain the highest tensile strength and modulus of elasticity [15]. The largest amount of degradation was found for L-PRF, followed by A-PRF and T-PRF. Recent findings suggest that these materials, which are essentially condensed autogenous clots, offer enhanced clotting and wound stabilization.

The third basic function is the biological material function of T-PRF and nanoT-PRF fibrin membranes. Like all second-generation fully autogenous blood products, they contain many autogenous growth factors present in the blood. Not only do they contain these growth factors, but their appropriate amount and combination might provide a suitable rate of release. In a recent study, a T-PRF membrane provided significantly higher concentrations of growth factors and a lower receptor activator of nuclear factor kappa-B ligand (RANKL)/osteoprotegerin (OPG) ratio in gingival crevicular fluid for 4–6 weeks after periodontal surgical treatment and improved periodontal healing compared to open flap sites [28]. Owing to these three basic functions, the T-PRF and nanoT-PRF membranes are likely to have a local antibacterial effect in the wound area, and the immune system cells they contain might help regulate inflammation. Furthermore, these materials may be able to perform other blood functions that have not been identified.

In this study, differences in fibrin structure between PRF produced from nano-titanium-coated tubes and conventional PRF were examined; however, the biological relevance of these findings was not thoroughly explored. Future research should investigate the impact of these structural differences on functional properties, such as growth factor release (e.g., PDGF, TGF-β) and the ability to stimulate cell proliferation and differentiation. Additionally, cell culture experiments using different cell types relevant to tissue regeneration, such as mesenchymal stem cells or osteoblasts, should be conducted to evaluate cellular responses to PRF produced from nano-titanium-coated tubes. These experiments could include assays for proliferation, migration, and gene expression related to tissue regeneration markers. Finally, assessing the degradation kinetics of PRF produced from nano-titanium-coated tubes compared to conventional PRF, both in vitro and in vivo, would provide valuable insights into the potential for prolonged in vivo resorption. These additional experiments will strengthen the clinical significance of the findings and further highlight the unique advantages of this innovative biomaterial.

5 Conclusions

Thermionic vacuum arc (TVA) system was successfully coated the glass surface with medical titanium. When the surfaces coated with this system encountered blood, it was successful in obtaining high-quality PRF because of the centrifugation process. In this study, it was showed that nanoT-PRF has a stronger structure and improved histological appearance compared with T-PRF and L-PRF. We believe that future in vivo experimental and clinical studies of nanoT-PRF will contribute to a full understanding of the potential of this new product and will help to determine its optimal application areas. This enhanced fibrin structure is particularly significant for regenerative medicine applications, such as guided tissue regeneration and bone augmentation, where extended resorption time and improved tissue scaffolding are crucial. Future research should investigate how these structural differences influence

growth factor release, cell proliferation, and differentiation, particularly in osteogenesis and angiogenesis. Such studies would further highlight the advantages of this innovative biomaterial, emphasizing its broad potential in regenerative medicine, particularly in enhancing tissue regeneration and repair.

Data availability

Data will be made available on request.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s10856-024-06838-3.

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Author contributions Dr. Mustafa Tunalı: conceptualization, funding acquisition, project administration, resources, supervision, validation, writing—review & editing; Dr. Esra Ercan: conceptualization, funding acquisition, project administration, supervision, visualization, writing—review & editing; Dr. Suat Pat: investigation, methodology; Dr. Emrah Sarıca: data curation, investigation, methodology; Dr. Aysel Güven Bağla: conceptualization, formal analysis, methodology, resources, software, validation; Dr. Nilüfer Aytürk: data curation, formal analysis, methodology, visualization; Dr. Duygu Sıddıkoğlu: formal analysis, software, writing—review & editing; Dr. Vildan Bilgin: conceptualization, data curation, investigation, methodology, resources, software, supervision, validation, visualization.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

Ethical approval Ethical approval was obtained from the Canakkale Onsekiz Mart University Clinical Research Ethics Committee (number: 2020/11; date: August 26, 2020) at the beginning of this study. The written informed consent was obtained from study participants.

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