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A new era in fundamentals of bone homeostasis: biocompatibility of bone mineral doped fluoride ions with osteoblast cells in the balance of calcium and phosphate metabolism

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ABSTRACT

Aim: The use of biocompatible bone tissue grafts, filling materials, bone minerals, and implants, particularly in medicine and dentistry studies, has expanded significantly in recent years, as have expectations from the materials. We aimed to test the biocompatibility and wound and tissue biocompatibility of many grafts and similar materials used in medicine and dentistry with tests such as cytotoxicity, scratch assay, cell adhesion, and hemolysis.

Material and Method: In this study, the interaction of fluorine ions with a dental material was investigated by biological activity experiments. In addition, studies were carried out on important osteoblast cells for tissue regeneration control. For this process, cell migration analysis, which we do not encounter frequently in the literature, was used to examine the interaction of cells with biomaterials more sharply.

Results: Flor ions do not create a cytotoxic effect and also increase the viability of osteoblasts which is important for tissue regeneration and are bone precursor cells.

Conclusions: In this study, in which the efficiency of osteoblast cells was discussed, it was concluded that 2% fluorine added material had more effective biological results compared to the increase in fluorine ion ratio.

Keywords: Tooth minerals, hydroxyapatite, osteoblast, cell migration, hemolysis

INTRODUCTION

The use of biocompatible bone tissue grafts, filling materials, bone minerals and implants, especially in dentistry studies, has increased considerably in recent years and the expectations from these materials have increased proportionally (1,2). These grafts, which are used to repair and regenerate bone tissue; It is expected to have a structure that is biocompatible, has a cell viability-enhancing effect, has high mechanical strength, and triggers osteogenesis, cementogenesis and functional periodontal ligament formation (2). Bone grafts, which are used both in dentistry and in many areas of medicine, are divided into many groups as autogenous bone allografts, xenografts and alloplastic grafts in order to induce bone tissue regeneration or to make implants more compatible. Hydroxyapatites, the primary mineral compound of bone, which is in the alloplastic group and whose use has increased in recent years, is the group that provides advantage in its design and application with its compatibility and positive results with different mineral additives (3-7). In the process of bone fractures, injuries and regeneration; The osteoinduction process is the process of differentiation of mesenchymal stem cells into osteoblasts, one of the bone precursor cells, and thus, osteoblast cells in the bone graft provide a three-dimensional skeleton for vascularization and cell migration. As the rate of osseointegration, which is a direct structural and functional link between living bone and the surface of the designed implant or graft, increases, the biological compatibility also increases (8). Osteogenesis occurs by mesenchymal stem cells and their differentiation by osteoblasts, and thus bone formation is stimulated (9).

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While there are osteoblasts, osteoclasts, and bone lining cells outside the bone tissue, there are osteocytes in the mineralized interior (10). They are fully differentiated cells responsible for the production of the bone matrix, secreting collagen type I and non-collagenous proteins of the bone matrix by osteoblast cells. Osteoblasts are also extremely important cells for bone, responsible for the production of factors that affect the differentiation and function of osteoclasts (11). Hydroxyapatite, which is frequently preferred in alloplastic grafts with hydroxyl ions (OH-) in its chemical structure and consisting of high carbonate ions, also serves as a three-dimensional skeleton in dentin tissue regeneration and repair (12). Increasing its effectiveness with different additives shows that it can be used in different areas (13). In this study, Hydroxyapatite was added with fluoride and its compatibility with cells, its effect on cell migration, and its biocompatibility for human use were evaluated. For this purpose, cell viability analysis according to the 10993-5 standard, cell migration to evaluate the progression potential of cells, and hemolysis tests to evaluate their interaction with blood was performed. In addition, the adhesion of the cells to the biomaterial surface was examined with a scanning electron microscope.

MATERIAL AND METHOD

Chemicals and Reagents

Osteoblast cell, 25 cm^2 and 75 cm^2 cell culture flasks, 96 well plates, serological pipettes and other plastic materials (Corning, NY, USA). DMEM, Dulbecco Modified Medium (DMEM) L-glutamine, Penicillin-streptomycin (PS), fetal bowine serum (FBS), and Trypsin-EDTA (Biological Industries Ltd. Kibbutz Beit Haemek, Israel).

Characterization of F- Doped HAp

Cell culture: After the cells previously frozen in the liquid nitrogen tank were thawed at 37°C in a short time, they were transferred to a 15 mL falcon tube in a sterilized Class II cabinet, and some medium was added. The prepared falcon was centrifuged at 2500 rpm for 2 minutes to allow the cells to collapse. After the supernatant was discarded, 3 mL of DMEM medium (containing 10% FBS, 1% PS) was added to the falcon, and after it was homogenized, it was cultivated into 25cm^2 and 75cm^2 flasks. The cultivated flasks were incubated at 37 °C in a 5% CO_2 incubator. After the cells were 80% confluent, they were passaged at least three times and prepared for the MTT cytotoxicity test and wound healing tests. After cell analysis, osteoblast cells were implanted on the surface of the material and the frequency of cell attachment was tried to be determined by imaging with a scanning electron microscope (14).

Preparation of extracts: EN ISO 10993-12 is used for extraction of medical devices and biomaterials. In this way, it is known that the properties of the materials pass into the extraction liquid when the materials that cannot be applied directly on the cell come into contact with suitable liquids at suitable temperatures under certain conditions. Extraction of test materials as specified in ISO 10993-12, the guideline for biological characterization testing of medical devices and materials requiring biocompatibility; It was incubated at 37°C with 1X DMEM for 24 hours, corresponding to 0.1 g/mL. Then, a suitable sterilization was performed to remove the possibility of possible contamination and material residue and to remove it from the environment. 10% FBS, 1% ps (penicillin/streptomycin) and 1% L-glutamine were added to the extract medium and made ready for testing. The prepared extract was used within 24 hours. The prepared extract directly represents the sample according to the standard and is named as 1/1(full) concentration.

Cytotoxicity assay: After the cells previously frozen in the liquid nitrogen tank were thawed at 37°C in a short time, they were transferred to a 15 mL falcon tube in a sterilized Class II cabinet, and some medium was added. The prepared falcon was centrifuged at 2500 rpm for 2 minutes to allow the cells to collapse. After the supernatant was discarded, 3 mL of DMEM medium (containing 10% FBS, 1% PS) was added to the falcon, and after it was homogenized, it was cultivated into 25 cm^2 and 75 cm^2 flasks. The cultivated flasks were incubated at 37 °C in a 5% CO_2 incubator. After the cells were 80% confluent, they were passaged at least three times and prepared for the MTT cytotoxicity test and wound healing tests.

Scrach assay (cell migration): This test method is a useful in vitro method to detect cell migration. Cell migration and spreading abilities of osteoblast cells were evaluated using a scratch wound assay that measures the expansion of a population of cells on surfaces. Cells were seeded in 24-well cell culture dishes at 2.5×105 cells/mL, incubated at 37 °C for 24 hours. After the cells were confluent and covered the plate, a linear wound sample was created on the layer in one go with a sterile 100 µl plastic pipette tip, the plates were washed 2 times with PBS (phosphate buffered saline) to remove the raised cells and media residue. As the control group, only the medium prepared with DMEM and the extract of the material (1/1 (100%)) as the test substance were applied and incubated at 37 °C containing 5% CO_2. While the created scar was incubated for 24 hours, the wound areas were photographed at 0, 12 and 24 hours.

Hemolysis test: To assess the blood compatibility of the samples in the test groups, they were incubated in a tube with Mg/Ca Free PBS at 10.2 g/mL for 24 hours below 37 °C as specified by ISO-10993-12. Equal volumes of blood were collected from 3 donors in 0.13 M citrate tubes and 7 mL of extraction liquid for each test sample was divided

into tubes. The standard curve, the constant F (calibration coefficient) was determined for all products by dilution of the hemoglobin standard. The required hemoglobin concentration for the test is specified as 10±1 in the standard. In the present experiment, the concentration value of hemoglobin was calculated as 9.8. For each sample, 7 mL of extract was added to 1 mL of blood and incubated at 37°C for 3 hours. Blood and control groups interacting with the sample were centrifuged at 700-800 G for 15 minutes. The supernatant plasma and drabkin are mixed at a ratio of 1:1 and measured in a spectrophotometer at 540 nm after 15 minutes of incubation (15).

Statistical Analysis

All experiments were performed in triplicate. Data were analyzed using one-factor analysis of variance to determine equality of population means and Student's t-tests were performed between respective populations where $P< 0.05$ was considered statistically significant.

RESULT

Biological Activity

Biomaterials, which enable living tissues in the human body to perform their functions or restructure, are used not only as implants and prostheses, but also in extracorporeal devices, diagnostic purposes, and wound treatment (16). Some biomaterials are also included in the medical device class, and in order for these biomaterials to be suitable for human use, they should not be toxic and carcinogenic at first, depending on the characteristics of the target area to be used; it should be mechanically durable and non-corrosive and should not cause reaction other than routine reactions of the body (17). For all these requirements, there is the "EN ISO 10993-1: Biological Evaluation of Medical Devices Part 1- Evaluation and testing" standard, which is accepted in all countries of the world. In our study, we carried out biological activity tests by taking this standard and the standards it references as a guide.

Cytotoxicity Assay

The most important parameter for the use of the material is its suitability for human use, and in vitro cytotoxicity testing under laboratory conditions is the first and most important step for this suitability. In this experimental system designed on the effect of drugs or materials applied on cells on cell viability, application materials that do not affect the viability of cells, do not reduce them below 70% or increase viability are found suitable for use in vitro. In this test method, "EN ISO 10993-5: Extracorporeal cytotoxicity tests" standard was taken into consideration. In its qualitative evaluation, the effect of extracts on cells was examined microscopically. MTT cytotoxicity assessment was done both quantitatively and qualitatively. Qualitative evaluation results are indicated in **Table 1**. Quantitative evaluation results are shown in **Table 2**. Calculation of the % vitality was made using the equation below.

Table 1. Qualitative morphological grading of the cytotoxicity of the extracts

Test material	Reaction	Status of cultures
Negative control	0	Discrete intra-cytoplasm granules, no cell destruction, no decrease in cell proliferation
Positive control	4	All or almost all of the cell layers were destroyed
1 F (1/1)	0	Discrete intra-cytoplasm granules, no cell destruction, no reduction in cell proliferation
2 F (1/1)	0	Discrete intra-cytoplasm granules, no cell destruction, no reduction in cell proliferation
3 F (1/1)	0	Discrete intra-cytoplasm granules, no cell destruction, no reduction in cell proliferation

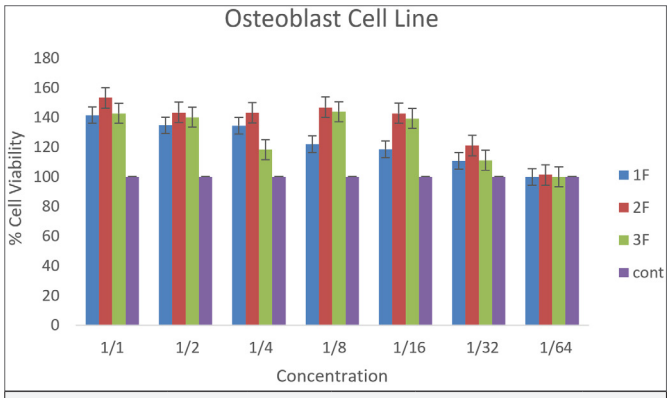
Table 2. %Hemolysis values of 1F, 2F and 3F samples after application

Groups	Abs (540 nm)	SHC	% hemolys	Color	Clarity	Particulates present
Negative control	0.0474	0.0267	0.2645	Colorless	Clear	No
1 F extract	0.0482	0.0271	0.2710	Colorless	Clear	No
2 F extract	0.0545	0.0298	0.3018	Colorless	Clear	No
3 F extract	0.0508	0.0267	0.2921	Colorless	Clear	No
1 F direct contact	0.0483	0.0267	0.2605	Colorless	Clear	No
2 F direct contact	0.0552	0.0327	0.3176	Colorless	Clear	No
3 F direct contact	0.0496	0.0293	0.2774	Colorless	Clear	No
Positive control	1.7018	0.1927	18.672	Colorless	Red	No

Cell viability %=Optical Density, OD570 sample/Optical Density, OD570 (control) ×100

Accordingly, while the negative control did not show any toxic effect on cells (0), natural rubber used as a positive control showed a high degree of toxicity as expected (4). When the cytotoxic effect of the sample extracts was examined, it was evaluated as zero because it was not seen as toxic (0). There was no decrease in cell destruction and cell proliferation. All details can be seen in **Table 1**. According to the standard used, a rating value greater than (2) as indicated in **Table 1** is considered a cytotoxic effect. "TS EN ISO 10993-5 /C MTT Cytotoxicity Test" is used as a quantitative evaluation method and the results are evaluated statistically. **Figure 1** also shows the results obtained from the negative and positive controls used, and it is seen that the test validity criteria are met. In this experiment, the effects of 1/1 to 1/64 dilutions of the sample extract on osteoblast cells were investigated. The viability obtained from the 1, 2 and 3 % F doped samples were determined as 141.45±1.46, 153.30±4.49 and 142.82±6.46 for osteoblast cells, the immature cell type (bone cell precursor), with complete

dilution (1/1) of the samples. Each sample was seeded to evaluate the osteoblast cell adhesion status. As a result of this cultivation process, cell entities were examined by scanning electron microscopy. The visuals of the analysis in which the status of the cell assets according to the contribution rate are evaluated are shown in **Figure 2**.



Sample Concentration	1F		2F		3F	
	%	±	%	±	%	±
1	141.45	1.46	153.30	4.49	142.82	6.46
1/2	134.59	3.25	143.27	1.93	140.09	1.49
1/4	134.51	5.51	143.13	1.19	138.20	1.75
1/8	122.01	2.41	146.74	3.60	143.89	6.18
1/16	118.66	1.55	142.72	1.84	139.16	4.74
1/32	110.81	0.13	121.18	4.05	111.04	1.16
1/64	99.86	5.08	101.26	1.22	99.91	3.03

Figure 1. Percent cell viability value graphs of Osteoblast cells after 24 hours of application. The application was made between 1/1 (full concentration) and 1/64 concentration range

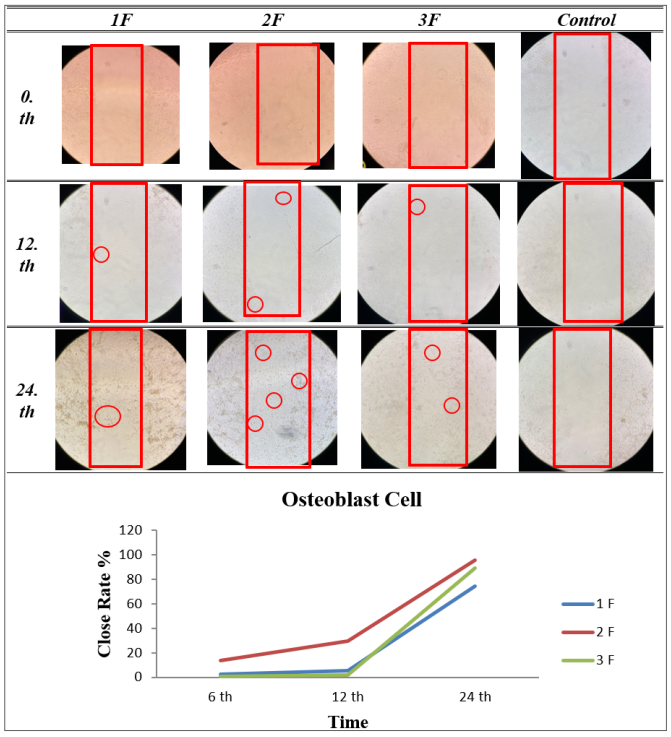


Figure 2. Fluoride-doped cell migration and wound closure analysis on osteoblast cell. (The areas marked at 0 hour are the areas where the wound was formed, and the areas where the cells 12 and 24 were migrated and the wound was closed are shown in the circle.)

Osteoblast cells, which are the immature support cells that are the precursors of the bone cell, are the cell group preferred in this study because of their potential to transform into immature bone cells and their high metabolic activity. Osteoblast cells are involved in the formation and regeneration of bones (18-21).

Osteoblasts are bone cells that are primarily responsible for synthesizing bone matrix proteins and minerals during early bone formation in the embryo, but also control bone formation and mineralization throughout life. Since they are located in areas of high metabolism where new bone formation occurs, the results of osteoblast cells were carefully examined in the study content. The cell analysis results obtained according to all three F ratios were found to be well above the 70% value defined for viability in the EN 10993-5 standard. This result showed that it is a product that supports cell proliferation effectively and has been interpreted as producing a matrix covering the old bone surface, leading to the formation of new bone cell layers and mineralization of the bone to regulate the balance of calcium and phosphate ions in the developing bone.

The increase in osteoblast cell presence increases both maturation and mineralization of the extracellular matrix. When the cell viability values were carefully examined, it was concluded that the cell viability values of the 2% F added sample had a statistically significant difference. As observed in a previous study, in the fluoride doped Hydroxyapatite medium, cell proliferation increased according to the doping ratio and decreased after the critical value. Similarly, the rate of 2% was determined as the most effective rate in this study. This finding helped us to conclude that the produced biomaterial could be well correlated with the feasibility study. According to the viability values obtained, a significant increase in cell viability and a decrease after a certain value were obtained with the increase in fluoride supplementation. The common result for all three contribution ratios is positive contribution to cell proliferation. Consistent with a study by Erdem et al. (7) it was concluded that fluoride-doped HAp ceramics did not cause toxic effects. At the same time, it is thought that these different ratios determined in the study content are sufficient values to examine the effect on the differentiation of primary osteoblast cells.

According to the contribution ratio, different results were obtained in osteoblast cell viability values. Although the cell viability value seems quite good in all three values, this value is at a better point for 2%. This shows that 2% F additive will contribute more to the remineralization of demineralized bone. Increasing the F ratio improved the osteogenic potential of these samples, as expected.

Hydroxyapatite, which has been used for bone development in dentistry and orthopedics for many years, is an inorganic component in bones and teeth and has a very similar structure to biomineralized tissues (22). It is stated that especially ion-added coatings are a good alternative to reduce implant infections. In addition, it is suggested that hydroxyapatite coatings containing fluoride, silver, etc. ions on implants can effectively inhibit bacterial adhesion and growth without affecting the activity of osteoblasts and epithelial cells (23-25). The cell study results obtained from the product in question help to conclude that hydroxyapatite will be an effective dental treatment material when its known antibacterial properties are considered. The test results show that the synthesized 1.2% and 3% F doped materials are biocompatible. It was also found that the test material showed no toxic effects on osteoblasts (bone precursor cells). The test results show that there will be no toxic effects if the sample is used in the body, or if it is broken/cracked out from the area to be applied, or if it is used as a drug synthesis substance.

The periosteum is a thick layer that surrounds the bone from the outside and is rich in collagen fibrils and fibroblasts. The inner layer contains osteoprogenitor cells (26). In the test results, it was determined that this structure of bone tissue increased the viability of 2% F material by 50% more in addition to normal proliferation. This means that the synthesized material can heal quickly on the outside in the repair of bone tissue, due to the rapid adaptation and support to the bone. Osteoblasts are present in 4-6% of bone tissue (27,28). Cytotoxicity results show that the material in the osteoblast is non-toxic, does not inhibit viability, and causes excessive proliferation. Thus, if the material is applied, osteoblasts will continue their normal biological functions and will not disrupt their natural structure.

Ramises et al. (29) and Chen et al. (30) reported that the use of titanium implants coated with HA had a viability-enhancing effect, while HA nanoparticles did not have a negative effect on cell viability. has also been shown by. In our study, HA did not have a cytotoxic effect and increased cell viability with fluoride supplementation. This shows that HAs doped with fluoride can also be used in different fields.

Student's t-tests were conducted between populations of interest, with $P<0.05$ considered statistically significant. Statistical significance for 1/1 concentration for cell line written in **Table** shown together with **Figure 1**'s captions. P value; P 0.213929711>α (0.05) (1F-2F), 0,020677283 <α (0.05) (1F-3F) and 0.123522342>α (0.05) (2F-3F). According to or by means of statistics, the most significant increase in cell viability was at 2% F.

Scratch Assay (Cell Migration) Test

One of the most important steps in wound healing is the migration of cells to the wound area, proliferating and initiating tissue restructuring. Instead of in vivo methods for wound healing, in vitro methods are more preferred in the early stages of synthesis in terms of low cost, animal welfare and fast results. This method is based on the creation of an artificial cavity by scraping on the confluent cell monolayer. The cells remaining at the edge of the wound cavity due to the effect of the applied drugs or materials move towards the opening to close that gap (in vitro wound model) and have the capacity to close the opening. The method is simple to implement and offers several advantages: (I) it mimics cell migration to some extent in vivo, and (II) is suitable for studying the regulation of cell migration by the extracellular matrix (ECM) and cell-cell interactions (31). The size of the wound cavity formed is scaled by SEM and the migration of cells into the cavity is recorded. In vitro wound healing and cell migration were calculated by measuring the areas from which field images were taken, using ImageJ, an open source image processing program. Calculations for osteoblast (**Figure 2**) cell groups are shown in detail.

The visuals and occlusion rates obtained as a result of the cell migration study performed in the light of these data are shown in **Figure 2**. Closure rates were recorded at the end of the 6, 12 and 24 hour periods. It was observed that for the best closure rate at these times, it clearly belonged to 2% F. The values obtained for 1 and 3 % F were recorded similarly to each other. At the end of the 24-hour period, a significant difference is obtained in each of the 3 additive ratios compared to the control group, but the 2% F closure rate in the 6 and 12 hour periods is remarkable. Considering the importance of the first 6 hours for compliance in dental operations, the presence of cells in the first hours is important. For this reason, the result obtained in the first 6 hours was considered valuable and it was concluded that the 2%F ratio was more effective. The capacity of filling the gap and cell migration in Osteoblast cells of the 2% F doped test samples improved compared to the control group. It was also observed that after 24 hours the cells became identical to the non-wound areas (their original state). Although the cell migration of the 1% and 3% F-added test materials also improved compared to the control group, it was found that the increase did not make a statistical difference for the two of them, but increased continuously compared to the control group. This indicates that the applied samples induce cell migration. Osteoblasts have Platelet Derived Growth Factor (PDBF) receptors, thus playing a role in cell division and chemotaxis of applied materials (32). It is thought that the finding of in vitro wound closure and cell migration of the applied materials is by this mechanism. Considering the developments in osteoblast cells and

wound healing potential of the materials planned to be used in bone tissue treatment, the promising potential of the examined materials in bone damage can be mentioned. The fact that the material has wound closure potential shows that both the cells adapt to the material and the effectiveness of the material is fast. It is predicted that if it is used in the clinic for patient health and comfort, it will save time and provide success in treatment.

Cell Adhesion

It is extremely important that the materials to be placed in the body can interact with the tissues, establish a bond with the tissue area where they are implanted, and be integrated. The adhesion of the cells to the prepared biomaterials causes the formation of cell layers and their integration with the tissue over time. Thus, it will begin to participate in the normal flow of the body as well as receive support from the material. Although cell adhesion or non-adhesion differs according to the area planned to be used, the interaction of the material with the cell will contribute to the bone tissue in our study. For this reason, cell adhesion to materials is one of the important points. Since non-biocompatible materials are generally not preferred by cells and cause cell death in contact, the adhesion of cells increases the probability that a material that is biocompatible in vitro will also be compatible in in vivo tests. It is planned that the synthesized fluoride added materials will be used to eliminate and treat bone tissue damage. For this reason, with the cell adhesion test; Scanning Electron Microscope (SEM) examination was performed as an indicator of cell morphology status and evaluation of proliferation potential. Osteoblasts grown on fluoride-doped hydroxyapatites are morphologically diverse, usually cubic, round or flat, cylindrical, and 20~50 μm in diameter. Cell sizes in the images obtained in SEM analysis are around 10-40 μm (**Figure 3**). The growing osteoblasts appeared to form a thin, single layer of squamous cells on the surface of the biomaterial. At the same time, it was observed that highly fibroblast-like and partially round cells were attached to the material by cytoplasmic extensions. As such, osteoblasts have a dendritic and flat morphology. Scanning electron microscope images also show close intercellular contacts. It shows that two cells are interconnected to form a dense layer of flat and spinocellular cells (33). Cell studies, as well as the antibacterial properties of hydroxyapatite, the presence of extracellular matrix proteins and a growth image helped us to demonstrate its osteoinductive and osteoconductive properties well (34). Although the cell presence observed in this study is considered promising for the formation of bone nodules, it does not preclude the need for future in vivo studies to support this assessment. Cell presences were seen in each of the images obtained from osteoblast cells grown on these three biomaterials. Consistent with the cytotoxicity results, cell adhesion was evaluated to be higher at the 2% additive rate compared to the additive rate. This study evaluated the biocompatibility and mineralization ability according to the increase in fluoride additive ratio. According to the results obtained, although all three biomaterials were evaluated as suitable as hard tissue repair material, it was concluded that the cells were overexpressed at the 24th hour of the experiment, especially in the areas where 2% F added samples were applied. This suggests that if the implant to be applied to the bone tissue is covered with material, it will result in an increase in bone precursor cells and the corresponding implant will provide a rapid adaptation to the body. It is also promising because the cytoskeleton prepared with 2% F doping level for bone tissue engineering will adapt quickly and support the formation of new tissue.

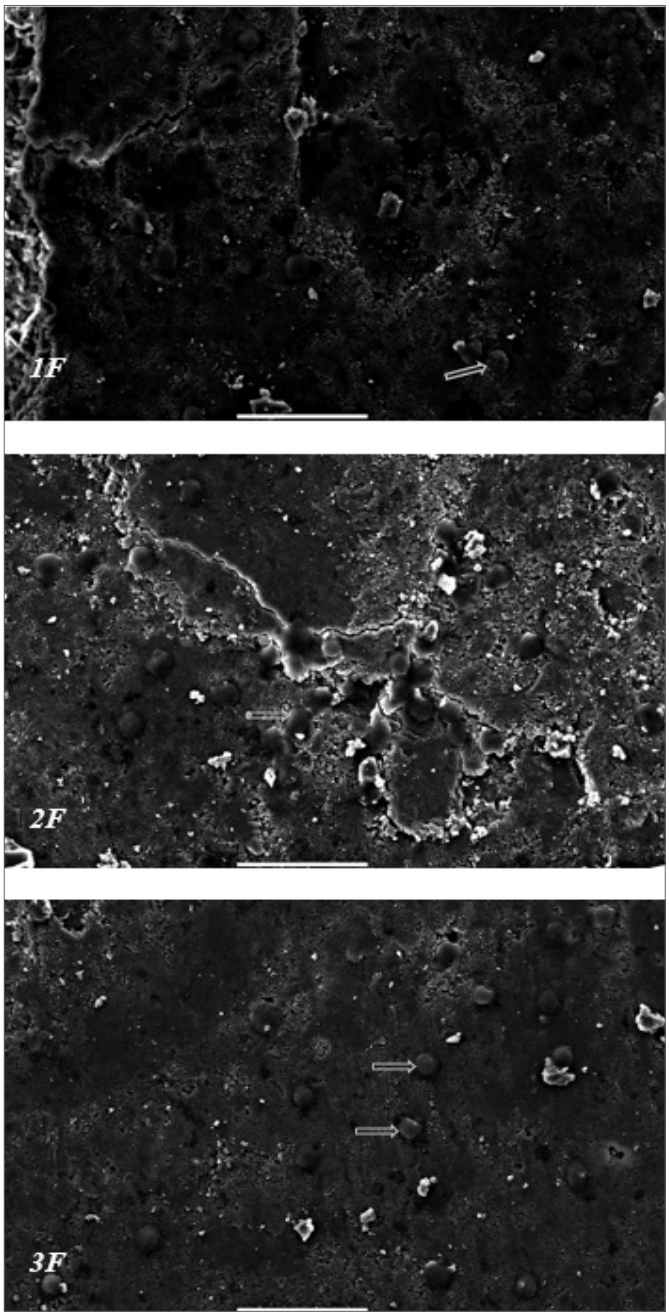


Figure 3. SEM images of fluorinated materials on which cells were applied (arrows indicate adherent cells). (Scale bar 50μm)

Hemolys Test

After the medical device or material is produced, the material must be subjected to some tests according to its intended use. In order to ensure the safety of the material used in devices that come into contact with blood (such as vascular prostheses), the compatibility of the synthesized material with blood should be evaluated. Hemolysis testing is required for all devices and device materials, except for materials that come into contact with intact skin and/or mucous membranes. The general principle of the test is to measure the damage of red blood cells exposed to the substance. Although there are many studies on the mechanism of hemolysis, there is no clear information on how exactly it occurs (35). For this reason, if the material to be used affects the mechanism hemolytically, it is not possible to intervene and repair it. The breakdown of red blood cells in the blood can cause both the normal flow of blood to change and the increase in erythrocyte production in the bone marrow, leading to enlargement of the bone marrow. For all these reasons, it is desired that the materials to be applied should not have a hemolytic effect. The application steps of the test as well as the material and method are specified in the 8th article. The test method applied is "TS EN ISO 10993-4 Biological evaluation of medical devices - Part 4: Selection of blood interaction tests" standard. It is valid because the test acceptance criteria specified in the standard are met. The experimental results obtained show that the sample does not have a hemolytic effect due to the absence of a value greater than 5% at the Hemolytic index levels given in **Table 1**. has been tested and the test results reveal that no hemolytic effect was observed in either case. However, in such a case, it is recommended to perform other (blood-interacting) tests such as coagulation, platelet, hematology and complement. The hemolytic index calculation results are tabulated in **Table 2**, and it was determined that both the extracts and the direct interactions with the blood of HAp samples with F partial substitutes produced in this study did not show any hemolytic effect in both cases (**Table 2**). In this way, this material does not show a hemolytic effect in case of any interaction with the blood in the targeted area. However, in such a case, it is highly recommended to perform other tests.

CONCLUSION

When the biological activity tests were evaluated, it was observed that the results of all tests (cytotoxicity, scratch assay, cell adhesion and hemolysis) were consistent with each other. It has been determined that the applied materials do not have a cytotoxic effect, and they also support the increase of the viability of osteoblasts, which are bone precursor cells. In particular, it was determined that 2% F has no toxic effect and increases cell viability by 50%, the rate of wound closure in the scratch assay test is higher than other materials, and it tends to close the area completely after the 12th hour and supports the complete closure of the wound area at the end of 24 hours. 1, 2, 3 F added materials have no hemolytic effect; indicates the suitability of contact with the skin or blood vessels. It is found that 2% F is suitable for use in materials evaluated in vitro. In order for human use to be possible, in vivo studies in which all body system functions can be evaluated, and cell increase and growth factors such as PDBF (Platelet Derived Growth Factor), EGF (Epidermal Growth Factor), FGC (Fibroblast Growth Factor), VEGF (Vascular Endothelial Growth Factor) It is recommended to examine the effect on support elements such as collogen type I and collogen type III.

ETHICAL DECLARATIONS

Ethics Committee Approval: Ethics committee approval is not required for this study.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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