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RESEARCH ARTICLES

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RESEARCH ARTICLE

Comparison of Dental Follicle Stem Cells and Dental Pulp Stem Cells in a Translational Bone Tissue Engineering Protocol

Translasyonel Kemik Doku Mühendisliği Protokolünde Diş Folikül Kök Hücreleri ve Diş Pulpa Kök Hücrelerinin Karşılaştırılması

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ABSTRACT

Purpose: This study aims to establish and refine a translational protocol and compare the osteogenic potential of dental pulp stem cells (DPSC) and dental follicle stem cells (DFSC) on nano mesh containing polycaprolactone (nmPCL) and plain polycaprolactone (m) scaffolds in vitro and contribute the translational medicine protocols in bone regeneration.

Materials and Methods: DPSCs and DFSCs were osteogenically differentiated on plain polycaprolactone (m) and nano mesh containing polycaprolactone (nm) scaffolds and four groups were examined for cell proliferation and type I collagen formation rates after two weeks of culture. Following immunofluorescence labeling, nonparametric (Kruskal Wallis) and multiple comparison tests were used to compare the groups.

Results: Among all groups, mean cell counts on scaffolds ranged from 30.8 to 82.6 cells/0.0915 mm^2, and total collagen formation ranged from 2.79% to 17,9%. DFSC and nmPCL complex showed significantly higher cell counts ($p<0.01$) and collagen formation rates ($p<0.01$) in comparison to other groups.

Conclusion: The DFSCnm group is found to show superior properties on cell proliferation and bone matrix formation. This complex is a promising tool for maxillofacial tissue engineering applications.

Keywords: Dental Follicle Stem Cell, Dental Pulp Stem Cell, Polycaprolactone, Tissue Engineering, Bone, Translational Medicine

ÖZET

Amaç: Bu çalışmada, DPSC ve DFSC'lerin nanomesh içeren (nmPCL) ve düz (PCL) polikaprolakton iskeleler üzerindeki osteojenik potansiyellerini in vitro olarak karşılaştırmak ve kemik rejenerasyonunda translasyonel tıp protokollerine katkıda bulunmak amaçlanmıştır.

Gereç ve Yöntemler: DPSC'ler ve DFSC'ler PCL ve nmPCL iskeleleri üzerinde osteojenik olarak farklılaştırılmış ve iki haftalık kültür sonrasında dört grup hücre proliferasyonu ve tip I kollajen oluşum oranları açısından incelenmiştir. İmmünofloresan etiketlemenin ardından, dört grubu karşılaştırmak için parametrik olmayan (Kruskal Wallis) ve çoklu karşılaştırma testleri kullanılmıştır.

Sonuçlar: Tüm gruplar arasında, iskelelerdeki ortalama hücre sayıları 30.8 ila 82.6 hücre/0.0915 mm^2 arasında ve toplam kolajen oluşumu %2.79 ila %17,9 arasında değişmektedir. DFSC ve nmPCL kompleksi diğer gruplara kıyasla anlamlı olarak daha yüksek hücre sayısı ($p<0,01$) ve kolajen oluşum oranları ($p< 0,01$) göstermiştir.

Sonuç: DFSC/nmPCL grubunun hücre proliferasyonu ve kemik matriksi oluşumu üzerinde üstün özellikler gösterdiği bulunmuştur. Bu kompleks maksillofasiyal doku mühendisliği uygulamaları için umut verici bir araçtır.

Anahtar Kelimeler: Diş Folikülü Kök Hücresi, Diş Pulpası Kök Hücresi, Polikaprolakton, Doku Mühendisliği, Kemik, Translasyonel Tıp

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Repair and regeneration of bone defects is one of the main research areas of maxillofacial surgery. Bone tissue diseases, injuries and congenital malformations often require treatment with grafting approaches. Allogenic, xenogenic, alloplastic and synthetic materials, as well as autogenous bone grafts, are currently used for reconstruction of maxillofacial complex. Providing the most efficient vascularization and regeneration of bone tissue within the defect area is one of the main objectives for craniofacial tissue engineering. Although autologous bone grafts are accepted as the gold standard for reconstruction of bone defects, a limited amount can be considered as an important problem. Since autologous bone graft harvesting also has the disadvantage of donor site morbidity and all the other materials lack osteogenic potential, tissue engineering methods need to be studied as an alternative to conventional grafting.[1] With the aim of efficient reconstruction of large defects, studies on tissue scaffolds have gained importance in recent years.[2,3]

Polycaprolactone (PCL) is a biodegradable and biocompatible synthetic material that is reported to have suitable chemical and physical properties in osteogenic differentiation and bone tissue engineering research.[4] With the goal of autologous bone regeneration, allogeneic and xenogeneic stem cell transplantation, have been studied and obtained successful results.[5-7] However the success of treatment also varies according to the characteristics of the tissue scaffolds and type and source of transplanted stem cells[8,9]. Evaluation of the osteogenic differentiation and regeneration capacity of stem cell colony types is an important criterion in the specific reconstruction of bone defects.[10]

Stem cells have been identified as clonogenic cells which have the ability to self-renewal, differentiating into various cell types and forming new cell lines.[11] In the area of bone tissue engineering, bone-marrow derived stem cells are one of the most reported sources in the literature.[3,12,13] Due to the lack of donor site morbidity and the presence of strong osteogenic potential, investigations on dental pulp (DPSC) and dental follicle (DFSC) derived stem cells are recently increased and satisfactory results on bone regeneration are reported.[14-19]

In order to apply successful clinical translation of the tissue engineering techniques, tissue specific differentiation potential of stem cell types is needed to be carefully evaluated. The studies that directly compare the results of osteogenic potential of different mesenchymal stem cell colony types are rare in the literature.

Instead of bone marrow-derived cell lines, mesenchymal stem cell (MSC) colonies that are cultured in this study can be obtained from tissues that will be discarded as medical waste at the end of an oral surgical operation. Hence, clinical translation of DPSC and DFSC studies will result in improved patient comfort by eliminating donor site morbidity and complications.

The purpose of our study is to compare the osteogenic potential of DPSC and DFSC colonies which are cultured on polycaprolactone mesh and polycaprolactone nanomesh scaffolds.

MATERIALS AND METHODS

1. Study Groups

For 10 scaffolds for each of four different groups (DPSCm, DPSCnm, DFSCm, DFSCnm), a total of 40 scaffolds were examined. For each group, two additional scaffolds were cultured as negative controls.

2. Isolation and Expansion of DPSCs and DFSCs

This comparative experimental study was approved by Medipol University Research Ethics Committee (number of approval: 10840098-153). The authors have read the Helsinki Declaration and have followed the guidelines in this investigation. A fully impacted wisdom tooth with its follicle was extracted in aseptic conditions and cracked in sterile conditions with an osteotome. Removed pulp tissue and follicle tissues were finely minced with a scalpel and transported into a 15 ml falcon tube. 2 ml of collagenase type I was added (1:500, ab 34710; Abcam, Cambridge, UK) to each sample and mixture was incubated at 37°C for 1 hour. Samples were then filtered through a 70 µm cell strainer and washed two times with equal volumes of phosphate buffered saline (PBS). After washing, supernatant was removed and 1 ml of culture medium was added to the pellet. Then, cells were seeded into the cell culture flasks and incubated. Culture media was changed every three days and cells were passaged when 80% of confluency was achieved.

3. Characterization of DPSCs and DFSCs

Analyses were performed in every sub-culture from passage 1 to passage 5 using flow cytometry. Characterization of the DPSCs and DFSCs was done with regard to described

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characteristic MSC markers including CD45, CD14, CD34, CD25, CD28, CD105, CD146, CD90, CD73 and CD29.

For osteogenic differentiation, culture medium was supplemented by 50 µg/ml ascorbic acid (Sigma, USA), 10 mm β-glycerophosphate (Sigma, USA) 100 nmol/L dexamethasone (Sigma, USA). In the fourth week of culture, samples were stained with Alizarin red to examine the formation of mineralized nodules within the culture. For differentiation into adipocytes, cells were cultured in adipogenic differentiation media. Adipocytes were identified by inverted microscopy examination and Oil red O (Sigma, USA) staining. For differentiation into chondrocytes, culture medium was supplemented with chondrogenic differentiation media. Chondrocytes were characterized by Alican blue staining.

4. Scaffolds, Cell Seeding and Culture

Mesenchymal stem cells were seeded on sterile polycaprolactone scaffolds (3D Biotek, USA). Before the cell seeding procedure, cell colonies were washed with PBS (phosphate buffer saline), and incubated with alpha-MEM with 10% fetal calf serum. Then, DPSC and DFSC suspensions were seeded into the 5x1.5 mm PCL mesh (m) and 5x1 mm PCL nanomesh (nm) scaffolds in 96 well plates. For 10 scaffolds for each of four different groups (DPSCm, DPSCnm, DFSCm, DFSCnm), total of 40 scaffolds were examined. For each group, two additional scaffolds were cultured as negative controls. A number of $2.0x10^5$ cells in 25 µl of suspension were transferred into each well. For higher seeding efficiency, careful manipulation was applied in order to avoid the contact of the pipette tip with the walls of the wells. After three hours of incubation in 5% CO_2 and 37° C, 175 µl of medium containing 10% FCS and 1% Penstrep was added to the wells. After examining the cell morphology by microscopic examination, cells were taken into the incubator. After adhesion of the cells to the tissue scaffolds for two days, cell-scaffold complexes were supplemented with alpha-MEM, gentamycin (50 µg/ml) and 15% FCS containing 50 µg/ml ascorbic acid (Sigma, USA), 10 mm β-glycerophosphate (Sigma, USA) 100 nmol/L dexamethasone (Sigma, USA) for osteogenic differentiation. Culture medium was changed in every 2 or 3 day of intervals. Cell-scaffold complexes were incubated in 5% CO_2 and 37° C. On 14^{th} day of culture, scaffolds were fixed for immunofluorescence staining.

5. Type I Collagen Formation and Cell Count Analysis

Scaffolds were fixed in 0.05% PFA + 4° C for overnight, then washed with PBS for 2 minutes. After incubation with 70% cold ethanol for a period of 15 minutes, permeabilization was performed for 15 minutes with 0.1% PBS Tween. Cells were blocked with 10% goat serum for 1 hour. Scaffolds were incubated with primary antibody (1: 500 by rabbit anti-collagen I, EU 34710; Abcam, Cambridge, UK) for overnight at + 4° C. A conjugated goat anti-rabbit IgH antibody (DyLight488, Abcam, Cambridge, UK) was used as the secondary antibody. Nuclear staining was performed with 4',6-diamidino-2-phenylindole (DAPI, Sigma) for 5 minutes. Examples were maintained at 4° C until examination. For total cell count, seven representative images were captured using a phase contrast fluorescent microscope at 20x and 40x magnification (Leica, Germany). For each scaffold, seven random representative sections were obtained by confocal laser scanning microscopy (CLSM) (Leica, Germany). The total area of the collagen formation was quantified and total cell counts were calculated for each slide with ImageJ software (National Institutes of Health, Bethesda, MD) and also confirmed manually by the same observer.

6. Statistical Analysis

Datasets were analyzed by using GraphPad Prism 5 software (GraphPad Software, Inc., CA, USA). Comparisons of the multiple groups were performed with nonparametric Kruskal-Wallis test and Bonferroni correction was used when comparing the groups. For all analyses, a P value less than 0.05 was considered statistically significant.

RESULTS

1. Characterization of DPSCs and DFSCs

Characterization of DPSCs was performed using flow cytometry analysis and differentiation potentials of the cell colonies. Flow cytometric analysis demonstrated that DPSCs express stem cell markers CD73, CD90, CD105, CD146 and CD29 and do not express hematopoietic cell markers CD45, CD34, CD25, CD28 and CD14 on their cell surface. With supplementation of the culture by the according differentiation media, isolated DPSC colonies were shown to be capable of in vitro adipogenic, chondrogenic and osteogenic differentiation.

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2. Cell Counts

At the conclusion of the culture period, no deformation was observed in the scaffold structures. Immediately following the seeding process, the cells exhibited a round morphology.

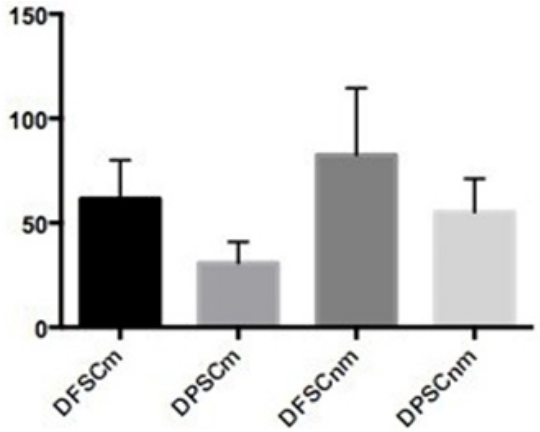

Fig. 1. Cell counts were significantly higher in DFSCnm group.

However, as the culture progressed, a transition to a spindle-like morphology was observed. This change occurred in response to the initiation of adhesion and tissue bridge organization on the PCL fibers.

The mean cell count values for the group DPSCm, DFSCm, DPSCnm, and DFSCnm were 30.8 cells/ 0.0915 mm^2, 61.6 cells/ 0.0915 mm^2, 55.2 cells/ 0.0915 mm^2, and 82.6 cells/ 0.0915 mm^2, respectively (Fig. 1-3).

The results of the statistical analysis indicated that the DFSCnm group exhibited a statistically significant increase ($p<.001$) in cell count compared to the other groups. Furthermore, observations of DAPI-stained samples revealed the most frequent and uniform cell organization throughout the DFSCnm group. A comparative analysis of the cell counts in the DPSCnm/DFSCnm, DPSCm/DPSCnm, and DPSCm/DFSCm groups was performed. The analysis revealed statistically significantly higher cell counts in the latter groups ($p<0.05$). An analysis of the data yielded no statistically significant differences between the DFSCm and DFSCnm groups.

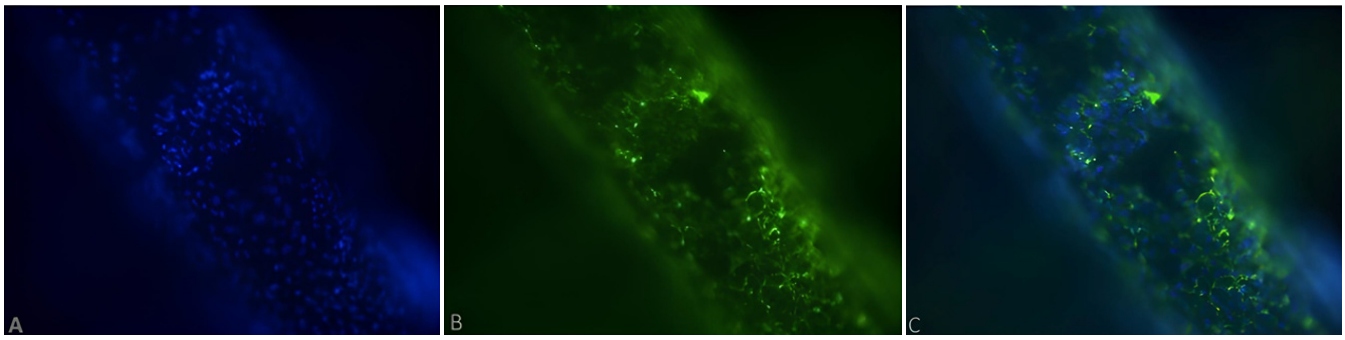

Fig. 2. DAPI (A), Type I Collagen (B) and merged (C) images of DFSCs on PCL fiber structure (X20).

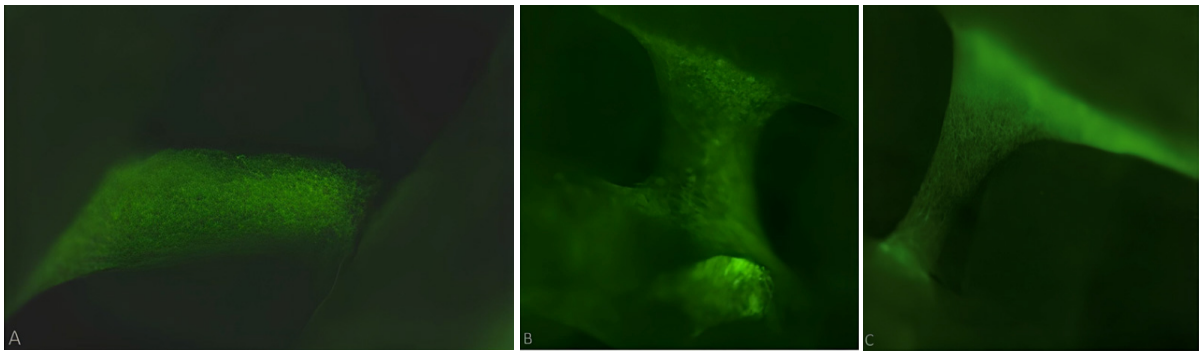

Fig. 3. A-C: Tissue bridging formations of DFSCs on nanomesh scaffold structures.

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3. Type I Collagen Formation

The mean values of collagen formation ratio for the DPSCm, DFSCm, DPSCnm, and DFSCnm groups were 2.79%, 3.93%, 12.7%, and 17.9%, respectively. In the statistical analysis, the rate of type I collagen formation in the DFSCnm group was found to be significantly higher than in the other groups ($p < .001$). Evaluation via confocal microscopic analysis revealed that the DFSCnm group exhibited the most profound and uniform distribution of collagen organization (Fig. 4-6). A comparison of type I collagen formation rates in DPSCm/DPSCnm and DFSCm/DFSCnm groups revealed statistically significantly higher rates in the latter groups ($p<0.05$). The comparison of the groups, DFSCnm/DPSCnm and DFSCm/DPSCm, revealed no significant statistical difference.

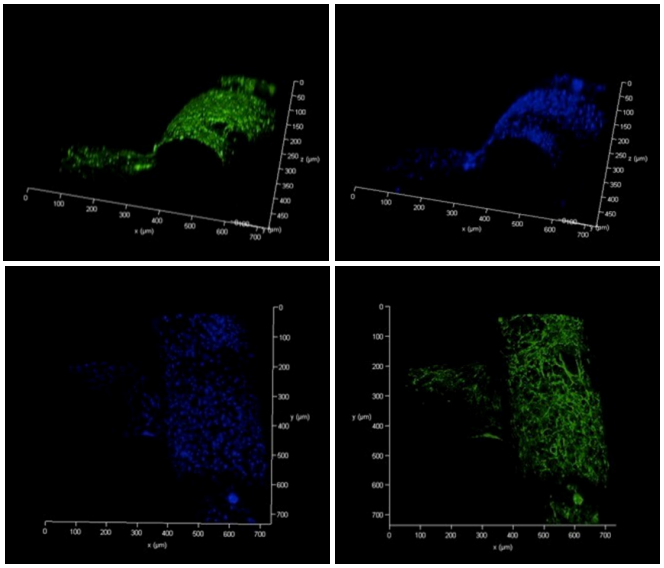

Fig. 4. DAPI (B, C) and Type I Collagen (A, D) confocal microscopy images of tissue bridge forming DFSC lines on nanomesh PCL fibers.

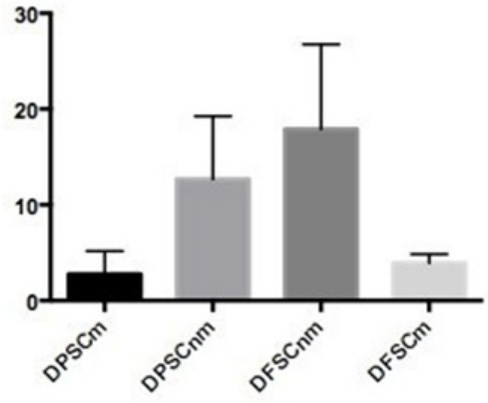

Fig. 5. Type I collagen formation rates were significantly higher in DFSCnm group.

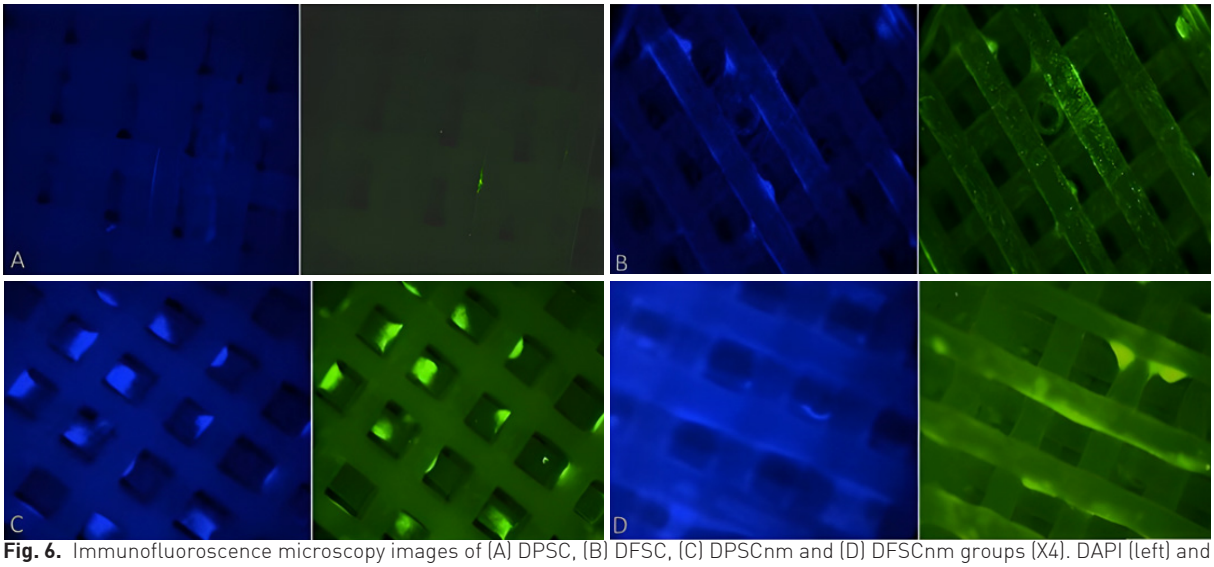

Fig. 6. Immunofluoroscence microscopy images of (A) DPSC, (B) DFSC, (C) DPSCnm and (D) DFSCnm groups (X4). DAPI (left) and Type I Collagen (right).

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DISCUSSION

In the context of bone tissue engineering, the successful organization of bone tissue in qualitative and quantitative aspects is critically dependent on the utilization of scaffold materials to provide support for cells and expressed extracellular matrix. Also, these materials are able to provide guidance to the geometric shape of the tissue growth. The most important task of the material to be used as scaffold is to help fulfill tissue function by providing a temporary support to the cell colonies and an environment that will create the biological orientation of the cells.[4,20-23] Abukawa et al. studied pig mandibular condyle reconstruction by culturing mesenchymal stem cell colonies on PLGA (polylactic-co-glycolic acid) scaffolds, and reported that the bone formation was observed only on the surface of the construct[24]. In a subsequent study, Abukawa et al. cultivated porcine bone marrow stem cells on a channeled PLGA scaffold for a period of 10 days. The cells were then implanted into a porcine mandibular critical-sized defect. In the second, fourth, and sixth weeks of healing, histologic sections were obtained, and there was observation of more intensive, uniform, and highly vascularized bone formation on channeled-PLGA scaffolds. In the case of non-channeled scaffolds, the formation of bone tissue was observed exclusively on the surface area. The addition of channels and micropores to the scaffold structure was found to enhance the permeability and transport of the culture medium, cell number and cell distribution, thereby facilitating the formation of bone tissue. In addition, the maximum cell count was documented to occur during the second week of the culture, and a substantial decline in cell counts after this period was reported.[25]

Scaglione et al. indicated the significance of total porosity, fully connected interior structure, and chemical composition of a scaffold. They further suggested a new "open-pore" tissue scaffold architecture. The formation of tissue and vascular infiltration in both in vitro and in vivo models was found to be satisfactory when using mesh-formed, calcium phosphate-coated hydroxyapatite polymer structures.[26]

Polycaprolactone structures can be made by three-dimensional fabricating technologies without exposure to chemical solvent materials. Porter et al. evaluated short term biocompatibility and long-term bioactivity assays of PCL nanomesh structures that were produced with three-dimensional printing methods. In this study, the cultivation of rat bone marrow-derived mesenchymal stem cells on PCL structures was conducted. The results indicated that there was an enhancement in cell adhesion, viability, and elevated levels of bone tissue biochemical markers on nano surfaces during the first, second, and third weeks in comparison to the control groups. In this study, the findings suggest that three-dimensionally printed, solvent-free PCL scaffolds have a positive impact on the biological performance of mesenchymal stem cells and can be utilized as an effective form of tissue scaffolds for bone regeneration.[27]

Binulal et al. evaluated the adhesion and proliferation potential of human mesenchymal stem cells on nanofibrous and microfibrous electro-spun PCL scaffolds. Adhesion, organization, proliferation and osteoblastic differentiation features of the stem cells were observed to be superior on nanofibrous structures.[28]

After the implantation of a cultured cell-scaffold complex into a defect area, viability and successful fusion depends on the angiogenesis activity within the first three days. In in-vitro conditions, mineralization of the extracellular matrix and formation of bone nodules is observed from the beginning of the fourth week of culture. In the studies on bone tissue engineering that have been documented, the cell-scaffold complex is predominantly indicated to be implanted subsequent to the observation of mineralization in the tissues. In the present study, the interconnected multilayer mesh scaffold design and culture duration were selected to ensure optimal nutrient and oxygen diffusion, vascular penetration, and uniform bone formation, thereby facilitating the clinical translation of the technique.

Jensen et al. compared three scaffold models for osteogenic differentiation of DPSCs on the 1st, 7th, 14th and 21st days. Cell proliferation, migration, osteoblastic activity and calcium deposition were observed to be increased at day 21 in nano-structure hyaluronic acid / TCP modified scaffold group when compared to control group. According to the results, DPSC/PCL scaffold complexes were stated to be a suitable implementation method for in vivo bone regeneration studies[29].

Studies that directly compare the osteogenic potential of DPSCs and DFSCs are rare in the literature. Shoi et al. evaluated the cell proliferation, colony forming capacity, gene expression,

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cell surface markers and differentiation capacity of DPSCs and DFSCs isolated from supernumerary incisors. Due to the increased obtainable tissue amount, DFSCs are indicated to be a more accessible stem cell source for isolation protocols. The rate of cell proliferation and colony forming capacity of DFSCs were found to be significantly higher in comparison to the DPSCs. In the appropriate culture medium, osteogenic differentiation potential of both cell lines was shown. Despite the similar stem cell characteristics of the DPSCs and DFSCs, due to easier access and higher proliferation rate of DFSC, it is indicated that DFSCs are a more favorable source of stem cells in regenerative applications.[30-33]

Surface topography is one of the main factors in determination of the differentiation of mesenchymal stem cell lines. This process is based on cell-cell, cell-extracellular matrix and cell-biomaterial interactions via signaling mechanisms.[34] Osteogenic differentiation is reported to be more effective on fibrillar nanostructured constructs.[35-37]

In our study, the highest cell count and type I collagen formation rates were observed in DFSCnm group. The effect of the electro-spun nanomesh base membrane structure was found to have a positive impact on cell spreading, adhesion, and proliferation. The observed variations in morphology, cell counts, and type I collagen expression rates may have resulted from the distinct osteogenic differentiation potentials of the cell types, as well as the asynchronous differentiation of cell lines.

In conclusion, using nanomesh PCL scaffold and DFSC complexes is found to be a suitable and promising method for bone tissue engineering applications. In vitro characteristics of stem cell-tissue scaffold complexes are needed to be correlated with in vivo bone regeneration studies. Based on these results, an experimental orthotopic critical size defect model should be studied in order to elucidate the impact of the technique on in situ osteogenesis.

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COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

ETHICAL APPROVAL

This study was performed in line with the principles of the Declaration of Helsinki. This study was approved by Medipol University Research Ethics Committee (number of approval: 10840098-153).

CONSENT TO PARTICIPATE

Informed consent was obtained from individual participants included in the study.

CONSENT TO PUBLISH

Non applicable.

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RESEARCH ARTICLE

Comparison of Marginal Bone Loss Between Titanium and Titanium-Zirconium Implants at Least 5 Years at Function

En Az 5 Yıllık Fonksiyon Süresiyle Titanyum ve Titanyum-Zirkonyum İmplantlar Arasındaki Marjinal Kemik Kaybının Karşılaştırılması

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ABSTRACT

Titanium (Ti) is the leading material in implant dentistry for the treatment of partial or full edentulism.. More recently, Titanium- Zirconium (TiZr) alloy has been developed for more demanding clinical conditions, as it shows greater mechanical and biological features than commercially pure Ti Grade 4. Survival of dental implants is based on the relationship between implant and oral tissues. Marginal bone level stability around implants has been used as one of the main criteria for implant success. Implant failures are often associated with implant mobility due to marginal bone loss. The aim of this study was to compare Titanium and Titanium-Zirconium implants marginal bone loss values which were at function more than 5 years. Titanium and 13-17% Zr containing TiZr alloy implants included in this study. Marginal bone loss measurements were performed digitally in computer software programme. TiZr alloy implants showed less marginal bone loss compared to traditional titanium implants but this difference statistically was not significant. TiZr alloy may become the dominant material in implant material choice in order to increase clinical implant success.

Keywords: dental implants, marginal bone loss, titanium zirconium implants

ÖZET

Titanyum (Ti), kısmi veya tam dişsizlik tedavisinde implant diş hekimliğinde en yaygın kullanılan materyaldir. Son zamanlarda zorlu klinik koşullar için, ticari olarak saf Ti Grade 4'e kıyasla daha üstün mekanik ve biyolojik özellikler sergileyen Titanyum-Zirkonyum (TiZr) alaşımı geliştirilmiştir. Dental implantların başarısı, implant ile ağız dokuları arasındaki ilişkiye dayanmaktadır. İmplant çevresindeki marjinal kemik seviyesi stabilitesi, implant başarısının temel kriterlerinden biri olarak kabul edilmektedir. İmplant kayıpları sıklıkla, marjinal kemik kaybına bağlı implant mobilitesi ile ilişkilidir. Bu çalışmanın amacı, 5 yıldan daha uzun süredir fonksiyonda olan Titanyum ve Titanyum-Zirkonyum implantlarının marjinal kemik kaybı değerlerini karşılaştırmaktır. Çalışmaya Titanyum ve %13–17 oranında Zr içeren TiZr alaşımlı implantlar dahil edilmiştir. Marjinal kemik kaybı ölçümleri bilgisayar destekli yazılım programı kullanılarak dijital olarak gerçekleştirilmiştir. TiZr alaşımlı implantlar, geleneksel Titanyum implantlara kıyasla daha az marjinal kemik kaybı göstermiştir, ancak bu fark istatistiksel olarak anlamlı bulunmamıştır. Klinik implant başarısını artırmak amacıyla TiZr alaşımının ilerleyen dönemlerde implant materyali seçiminde dominant hale gelebileceği düşünülmektedir.

Anahtar Kelimeler: dental implantoloji, marjinal kemik kaybı, titanyum zirkonyum

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INTRODUCTION

Since Branemark introduced the concept of osseointegration in 1977, dental implants have become a popular method for the treatment of total or partial edentulism.[1] According the AAID (American Academy of Implant Dentistry) implant treatment have been shown almost %95 success rate.[2] In a systematic review and meta-analysis including 23 studies performed in 2015, the success rate of implants was 94.6% in a total of 7711 implants (mean follow-up year 13 years).[3]

Titanium is a biocompatible material that causes little or no reaction in the tissues in which it is loaded[4] It has also been found that titanium is a material that is resistant to corrosion and allows osseointegration.[5] Branemark proved that titanium forms a permanent connection with the bone due to the titanium oxide layer and this created the concept of osseointegration today[1]. Titanium have been recognized as the gold standard in implant materials.[6] However, the stress/strength ratio of narrow diameter titanium implants were found higher than regular diameter implants, indicating higher risk of fatigue failure.[7] Implants made of titanium alloys containing zirconium (TiZr; commercially known as Roxolid; Institut Straumann AG, Switzerland) have been introduced to the market showing better mechanical properties than pure titanium while maintaining its biocompatibility.[8] This particular TiZr alloy dental implant manufactured from mixing Ti with 13%–17% of Zr.

The ICOI's (The International Congress of Oral Implantologists) Pisa Consensus defined implant success criteria as pain, mobilization, radiographic marginal bone loss and pocket depth and peri-implant disease.[9] Regarding of bone loss, Albrektsson et al. recommended that a successful implant should have a marginal bone loss of less than 1 mm in the first year and less than 0.2 mm in the following years.[10] The aim of this study was to compare the marginal bone loss between conventional titanium and TiZr implants under function at least 5 years

MATERIALS AND METHOD

This study was approved by Baskent University Institutional Review Board (Project no: D-KA24/07) and supported by Baskent University Research Fund. This retrospective study was conducted using clinical and radiographic records of patients who received dental implants between 2015 and 2018 at the Department of Oral and Maxillofacial Surgery, Başkent University Faculty of Dentistry.

Patients with posterior single tooth, same brand (Institut Straumann AG, Switzerland) tissue level implants in 4.1 mm diameter and 10 mm length were included. Patients divided into two groups with 40 implants in titanium and 40 implants in Ti-Zr group. Of the 80 implants included in the study, 44 were located in the mandible and 36 in the maxilla. Patients with uncontrolled systemic diseases, active periodontal diseases, keratinized mucosa less than 2 mm, implants required bone augmentation were excluded from the study.

Panoramic radiographs were taken via Veraviewepocs 2D (J. Morita Corp., Japan) device. Digital panoramic radiographs which were taken after the implant placement (T1) and after minimum 5 years in function (T2) were used in peri-implant bone loss evaluations. Measurements were performed digitally in ImageJ software programme (ImageJ, USA). Measurements of one of the digital panoramic radiography are shown in Figure 1 . The values were obtained by measuring the distance from the apical end of the implant to the marginal bone level separately on the mesial and distal sides, and then subtracting these values from the total implant length. Twenty percent of the radiographs were randomly selected and remeasured in order to ensure intra-observer reliability and the the Intraclass Correlation Coefficient (ICC) was calculated.

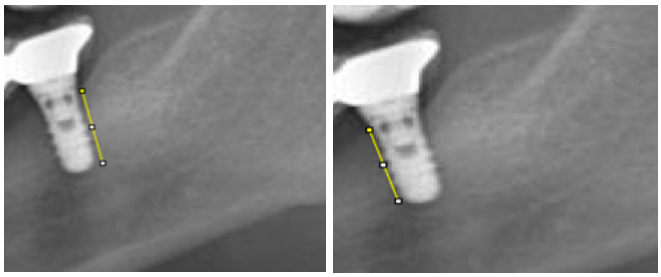

Fig 1: Distally and mesially measurements

Statistical analyses were performed with SPSS version 25.0 program. The conformity of the variables to normal distribution was examined by Shapiro-Wilk test. Mean, standard deviation, median, minimum and maximum values were used to present descriptive analyses. Mann Whitney U Test was used to evaluate the variables that were not normally distributed between Ti and Ti-Zr groups. p-values below 0.05 were considered as statistically significant results.

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RESULTS

Thirty nine patients with a total of eighty implant included in this study.Patient demographic characteristics were summarized as; 16 male (45 %) and 20 female (55 %), with an average age of 41.75 (ranging between 24 and 60 years).

The mean marginal bone loss was 1.46 ±1.038 mm in the Ti group and 1.11±0.698 mm in the Ti-Zr group. There was no statistically significant difference between the Ti group and the Ti-Zr group (p=0.101) regarding of peri-implant bone loss values. Ti and Ti-Zr implants did not show different results in the follow-up of marginal bone loss over 5 years (Table 1).

Table 1: No statistical difference between Titanium and Titanium Zirconium Group

	Titanium		Titanium - Zirconium		
	Mean±sd	**Median(Min-Max)**	**Mean±sd**	**Median(Min-Max)**	**p**
Bone loss	1,464±1,038	1,212 (0-6,289)	1,113±,698	1,11 (0-2,377)	0,101

DISCUSSION

The marginal bone around the implant is considered as a significant indicator of implant health. In the literature the most common method to measure bone loss is by radiographic evaluation. However, conventional two dimension radiographics allow to monitor only the mesial and distal aspect of bone loss. It was suggested that the peri-implant bone level measurements should be related to the original marginal bone level which was measured at implant insertion, rather than to a previous measurement.[9]

The use of Ti–Zr dental implants is well published in several in vitro and animal studies, showing similar results to Grade IV titanium dental implants.[11]

In a clinical trial by Quirynen et al., Ti–Zr alloy implants were compared to titanium grade IV implants. The marginal bone level values were found similar in the two groups, which is compatible with the results of this study.[12]

In our study, slightly less marginal bone loss was observed in titanium-zirconium implants compared to titanium implants. In their in vitro study, Sista et al. showed that more osteoblasts adhered to the 50% Ti-zr surface compared to the titanium surface and more alkaline phosphatase and osteocalcin were released. This may lead to better osseointegration and thus indirectly to less marginal bone loss.[13]

A study by Ghazal et al. in 2019, the 5-year mean bone loss of ti-zr standard implants was measured as -0.48 ± 0.67 mm, which was less than the average marginal bone loss in the ti-zr group of our study. This may be due to the fact that Ghazal et al. included only single tooth implants or excluded heavy smokers (more than 10 per day).[14]

Carlson et al, measured the 10-year periimplant marginal bone loss of conventional titanium implants as 0.9 mm .This result was close to the marginal bone loss of titanium implants in our study, but slightly less than our result.[15]

CONCLUSION

The relatively new alloy implants appear as a reliable treatment option. Ti-Zr implants showed slightly less bone loss compared to Ti group. Although this difference clinically is not significant, Ti-Zr alloy may be the dominant material in implant material choice in order to increase clinical implant success. Due to the fact that peri-implant bone loss depends on many factors, multiparameter studies with larger sample size are needed.

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RESEARCH ARTICLE

İliak Kemik Grefti ile Ogmente Edilmiş Kretlere Yerleştirilen İmplantların Uzun Dönem Sağkalımlarının Değerlendirilmesi

Long-Term Survival Assessment of Dental Implants Placed in Atrophic Ridges Augmented with Iliac Bone Grafts

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ÖZET

Bu retrospektif çalışma, atrofik çenelerin rekonstrüksiyonunda iliak kemik grefti ile ogmente edilen kretlere yerleştirilen dental implantların uzun dönemli sağkalımını ve komplikasyon profilini değerlendirmeyi amaçlamaktadır. 2008–2018 yılları arasında Baskent Universitesi Adana Turgut Noyan Uygulama ve Arastirma Hastanesi'nde tedavi edilen, en az 5 yıllık takibi tamamlanmış 12 hasta çalışmaya dahil edilmiştir. Toplam 67 implant yerleştirilmiş, hastaların ortalama yaşı 62,8 ve takip süresi 83,5 ay olarak hesaplanmıştır. Greft başarı oranı %100 olup, alıcı bölgede cerrahi sonrası komplikasyon izlenmemiştir. Ancak donör bölgede 5 hastada çeşitli komplikasyonlar gözlenmiştir. Bu komplikasyonlar arasında en sık karşılaşılanlar yürüme güçlüğü ve hematomdur. Toplam 6 implant (%9,2) kaybedilmiş olup, bunların 4'ü osseointegrasyon başarısızlığı nedeniyle, 2'si ise protetik tedavi sonrasında kaybedilmiştir. Bulgular, iliak kemik greftleriyle ogmente edilen alanlara yerleştirilen implantların uzun vadeli sağkalım oranlarının literatürle uyumlu olduğunu göstermektedir. Ancak iliak kemiğin yüksek rezorpsiyon oranı ve embriyolojik köken farkı nedeniyle bazı implant kayıpları yaşanmıştır. Greft stabilitesini artırmaya yönelik tekniklerin değerlendirilmesi, gelecekteki çalışmalarda klinik başarının artırılmasına katkı sağlayabilir.

Anahtar Kelimeler: İliak Kemik Greftleme, Dental İmplant, Osteointegrasyon

ABSTRACT

This retrospective study aimed to evaluate the long-term survival and complication profile of dental implants placed in alveolar ridges augmented with iliac bone grafts for the reconstruction of atrophic jaws. Twelve patients treated at Baskent University Adana Turgut Noyan Application and Research Hospital between 2008 and 2018, with a minimum follow-up period of 5 years, were included. A total of 67 implants were placed, with a mean patient age of 62.8 years and an average follow-up duration of 83.5 months. The graft success rate was 100%, with no postoperative complications observed at the recipient sites. However, donor site complications were noted in five patients, including gait disturbances and hematomas. A total of six implants (9.2%) failed—four due to lack of osseointegration before prosthetic loading and two after prosthetic use. The findings indicate that the long-term survival rates of implants placed in iliac bone-grafted areas are consistent with existing literature. Nevertheless, some implant losses were attributed to the high resorption potential and embryological origin differences of iliac bone. Future studies assessing techniques to enhance graft stability may contribute to improved clinical outcomes.

Keywords: Iliac bone graft, Dental implant, Osseointegration

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Son yıllarda diş implantları kısmen veya tamamen dişsiz olan hastaların rehabilitasyonu için rutin bir tedavi yöntemi haline gelmiştir.[1] Birçok vakada dental implantlar herhangi bir zorluk veya komplikasyon olmadan yerleştirilebilmektedir. Ancak bazı durumlarda, şiddetli periodontal hastalıklar, uzun süreli dişsizliğe bağlı alveolar kemik atrofisi, travma, enfeksiyon ya da kemik patolojileri kaynaklı kemikte ileri dereceli defektler oluşması implantın doğru açı ve uzunlukta yerleştirilmesini imkansız hale getirebilir. [2,3]

Atrofik kretlerin rekonstrüksiyonunda otojen kemik greftleri kullanılarak implantın ideal pozisyonda ve biomekanik açıdan uygun şekilde yerleştirilmesi sağlanabilir. Otojen kemik greftleri, osteokondüktif, osteoindüktif ve osteojenik özellikleri bir arada barındırması nedeniyle kemik rejenerasyonunda altın standart olarak kabul edilmektedir.[4]

Otojen kemik grefleri intraoral ve ekstraoral bölgelerden elde edilebilir. İntraoral kemik greftleri düşük rezorpsiyon oranlarına sahip olmakla beraber sağlayabilecekleri kemik hacmi sınırlıdır. Geniş kemik defektlerinin rekonstrüksiyonunda kalvarial kemik, kosta, tibia ve iliac kemikler gibi ekstraoral donör bölgelerden greft temin etmek gerekebilir.[5]

İliak kemik, yüksek osteojenik potansiyele sahip olması, vaskülarizasyonunun hızlı gerçekleşmesi ve ortalama 50 cm^3 kortikokansellöz greft materyali temin edilebilmesi nedeniyle, greftleme işlemleri için optimal donör sahalardan biridir.[6]

İliak kemikle yapılan rekonstrüksiyonda greftin uzun vadeli sağkalım oranı ve stabilitesi, özellikle kemik rezorpsiyon oranlarına ilişkin belirsizlikler nedeniyle halen tartışmalıdır.[3] Bu çalışmanın amacı, kliniğimizde atrofik çenelerde gerçekleştirilen iliak kemik grefti augmentasyonu sonrasında uygulanan dental implantlar ile implant üstü protetik rehabilitasyonun en az 5 yıllık kümülatif başarı oranlarını, erken ve geç dönem komplikasyon profillerini, ayrıca radyografik ve klinik takip bulgularını değerlendirmektir.

MATERYAL METOT

Bu çalışma, Baskent Universitesi Adana Turgut Noyan Uygulama ve Arastirma Hastanesi Kurumsal Etik Kurulu tarafından onaylanmıştır (Protokol No: KA25/14). Çalışma protokolü, Dünya Tıp Birliği'nin Helsinki Bildirgesi etik ilkelerine uygun olarak tasarlanmış ve tüm katılımcılardan yazılı bilgilendirilmiş onam alınmıştır.

Bu retrospektif çalışmaya Baskent Universitesi Adana Turgut Noyan Uygulama ve Arastirma Hastanesi Ağız, Diş ve Çene Cerrahisi kliniğinde 2008-2018 yılları arasında iliak greft ile alveolar augmentasyon uygulanarak dental implant yerleştirilmiş ve implant üstü protetik rehabilitasyonu tamamlanmasının ardından en az 5 yıl süre geçmiş, tüm tedavi süreci ile takip ziyaretlerine ait klinik muayene bulguları ve radyografik görüntüleri hasta kayıtlarında eksiksiz olarak bulunan 18 yaşından büyük bireyler dahil edilmiştir.

Sistemik hastalık öyküsü olan, kemik metabolizmasını etkileyen ilaç kullanan, greft bölgesinde postoperatif komplikasyon (enfeksiyon vb.) gelişen, düzenli takipleri bulunmayan, klinik veya radyografik verileri eksik olan, tedavi süreci tamamlanamayan, parafonksiyonel alışkanlık (ör. bruksizm) öyküsü olan ve 18 yaş altı bireyler çalışma dışı bırakılmıştır

Greft Başarısı ve İmplant Sağkalımının Değerlendirilmesi: Greftin başarılı kabul edilmesi için gerekli görülen kriterler greftleme işleminden sonra ve implant yerleştirilme aşamasında greft bölgesinde enfeksiyon bulunmaması, radyografik incelemelerde greftlenen sahada radyolüsensi gözlenmemesi, greftin alıcı bölgede entegre ve hareketsiz olması, implant yerleştirilmesi sırasında greftten kanama gözlenmesi ve implant yerleşimi için yeterli miktarda kemik dokusu bulunmasıdır.[7]

İmplant sağkalımı; implantın ağızda tutulabiliyor olması, hareketli olmaması, fonksiyon sırasında ağrı oluşturmaması kriterlerine göre değerlendirildi.[8]

Veriler tanımlayıcı istatistiklerle değerlendirildi. Sürekli veriler ortalama ± standart sapma (SD), kategorik veriler sayı ve yüzde (%) olarak sunuldu.

SONUÇLAR

Bu çalışmaya toplam 67 adet implant uygulanmış olan 12 hasta (6 kadın, 6 erkek) dahil edildi (Tablo 1). Hastaların yaş ortalaması 62,8± 4,9 yıl (54-72 yıl) ve takip süresi ortalama 83.5 ±12.1 ay olarak hesaplandı.

Yedi hasta tam dişsizlik ve 5 hasta kısmi dişsizlik nedeniyle tedavi edildi. Mandibulaya toplam 11 adet, maksillaya 54 adet implant yerleştirildi.

Greft başarı oranı %100'dü. Ameliyat sonrası alıcı bölgede herhangi bir komplikasyon görülmedi. Çalışmada incelenen 12 hastanın donör bölge komplikasyonlarına ilişkin

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değerlendirmesinde, toplam 5 hastada çeşitli komplikasyonlar gözlenmiştir. En sık rastlanan komplikasyon yürüme güçlüğü olup 4 hastada bildirilmiştir. Hematom ise 2 hastada izlenmiştir. Geriye kalan 7 hastada ise herhangi bir donör bölge komplikasyonu gözlenmemiştir (Tablo 2).

Toplam 6 implant (%9,2) kaybedilmiştir. Bu implantlardan 4'ü, protez için ölçü alınmadan önce osseointegrasyon başarısızlığı nedeniyle; 2'si ise protez kullanımı başladıktan sonra kaybedilmiştir.

Tablo 1: Hastaların Yaş, Cinsiyet, Uygulanan Protez Türü, İmplant Sayısı ve Takip Süresi Dağılımı

Yaş	Cinsiyet	Protez Türü	Uygulandığı Çene	İmplant Sayısı	Süresi(ay)
72	Kadın	All on Four	mandibua	4	84
56	Erkek	Hibrit Protez	maksilla	6	92
64	Erkek	All on Four/Hibrit	maksilla/mandibula	6	68
63	Kadın	All on Six	maksilla	6	86
63	Kadın	Hibrit Protez	maksilla	4	110
61	Kadın	All on Six	maksilla	6	74
67	Erkek	All on Four	mandibula	4	79
58	Erkek	All on Six	maksilla	6	69
61	Kadın	All on Six/Hibrit	maksilla/mandibula	8	82
64	Erkek	All on Six	mandibula	6	76
61	Kadın	Hibrit Protez	mandibula	5	81
68	Erkek	All on Six	maksilla	6	105

Tablo2: İmplant Kaybı Nedenleri ve Donör Bölge Komplikasyonlarının Sıklığı

	İmplant Kaybı	İmplant Kaybı	Donör Bölge
Hastalar	Protez Öncesi	Protez Sonrası	Komplikasyonları
1	0	0	Yok
2	0	0	Yok
3	0	0	Yok
4	2	1	hematom, yürüme güçlüğü
5	0	0	Yok
6	0	0	yürüme güçlüğü
7	1	0	Yok
8	1	0	Yok
9	0	1	hematom
10	0	0	Yok
11	0	0	yürüme güçlüğü
12	0	0	yürüme güçlüğü

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TARTIŞMA

Dental implantlar, eksik dişlerin tedavisinde sabit veya hareketli protez uygulamalarını kolaylaştıran etkili bir tedavi seçeneğidir. Ancak implant yerleştirilecek bölgedeki alveol kemiğinin aşırı rezorbe olması, implantın ideal açıda ve yeterli uzunlukta yerleştirilmesini zorlaştırabilir. Bu gibi durumlarda, uzun vadede fonksiyonel ve estetik açıdan başarılı bir sonuç elde etmek için yetersiz kemik hacminin artırılması gerekir. Böylece implantın stabilitesi ve dayanıklılığı sağlanarak hastanın hem işlevsel hem de estetik beklentileri karşılanabilir. [9]

Yakın zamanda yayınlanmış olan bir sistematik derlemede iliak kemikten elde edilen greftler ile rehabilite edilen kretlere uygulanan implantların sağkalım oranlarının, greft uygulanmamış bölgelere veya intraoral kaynaklı kemik greftleri ile ogmente edilen kretlere uygulanan implantların sağkalımına kıyasla belirgin şekilde daha düşük olduğu gösterilmiştir. Bu çalışmada iliak kemik bölgesine yapılan implantların 5 yıllık sağkalım oranı % 90 olduğu rapor edilmiştir. [10] Çalışmamızın sonuçları iliak greft ile ogmente kretlere yerleştirilen implantların sağkalım oranları bakımından literatür ile benzerlik göstermektedir.

Çene kemikleri ile iliak kemik greftleri arasında embriyolojik köken açısından belirgin farklılıklar bulunmaktadır. Çene kemikleri intramembranöz ossifikasyon yoluyla, ilium ise endokondral ossifikasyon ile gelişir. Bu gelişimsel farklılık, greftlerin rezorpsiyon oranları ile greft ve implant sağkalımı üzerinde önemli bir etkiye sahip olabilir. Özellikle, iliak kemik greftlerinin en önemli dezavantajlarından biri, yüksek rezorpsiyon oranlarına sahip olmalarıdır. Yapılan çalışmalarda, iliak greftlerde altı ay sonrasında hacimsel olarak %24 ila %49 oranında azalma meydana geldiği bildirilmiştir. [11,12,13]

Literatürde, erken dönem kemik rezorpsiyon miktarını azaltmak amacıyla greft ve implantın aynı zamanda yerleştirildiği tek aşamalı tedavi ve bariyer membran kullanımı önerilmektedir. [14,15] Tek aşamalı yöntemin işlem sayısı ve tedavi süresini kısaltma gibi avantajlarının yanısıra bu yöntemde implantların primer stabilitesinin sağlanmasında zorluk yaşanabilir. Çalışmamızda değerlendirilen hiçbir olguda greftleme ile eş zamanlı implant yerleştirilmemiş veya iliak greft üzerine bariyer membran uygulanmamıştır.

Bu çalışmanın bulguları, iliak kemik greftleri ile ogmente edilen kretlere yerleştirilen dental implantların uzun dönemli sağkalım oranlarının literatürle uyumlu olduğunu göstermektedir. Her ne kadar greft başarı oranı yüksek olsa da, iliak greft kaynaklı implantların bir kısmında erken dönemde osseointegrasyon başarısızlığı ve ileri derecede periimplant kemik rezorpsiyonu nedeniyle implant kayıpları gözlenmiştir. Bu sonuçlar, greftin embriyolojik kökeni ve yüksek rezorpsiyon potansiyelinin implant başarısı üzerinde etkili olabileceğini düşündürmektedir. İleriye dönük çalışmalarda, greft stabilitesini artırmaya yönelik tekniklerin değerlendirilmesi klinik başarıyı artırmada faydalı olabilir.

Bu çalışmamızın bazı sınırlamaları bulunmaktadır. Çalışmanın retrospektif olması, verilerin hasta kayıtlarından elde edilmesi nedeniyle bazı parametrelerin standardizasyonunda sınırlamalar bulunmaktadır. Ayrıca, çalışmaya dahil edilen hasta sayısının ve implant sayısının nispeten düşük olması, istatistiksel gücü ve sonuçların genellenebilirliğini etkileyebilir. Ayrıca donör bölge komplikasyonları (yürüme güçlüğü, hematom) hasta bazında değerlendirilmiş olsa da, komplikasyonların şiddeti ve süresine ilişkin nicel verilerin hasta epikriz notlarından ve hastalara geçmişe dair sorulan sorulardan elde edilmiştir. Bu durum bulguların objektif olarak karşılaştırılmasını kısıtlamaktadır.

Bu kısıtlılıklara rağmen çalışmanın bulguları, iliak kemik greftleri ile ogumente edilen atrofik kretlerde yerleştirilen dental implantların uzun dönemli sağkalım oranlarının klinik açıdan kabul edilebilir düzeyde olduğunu göstermektedir.

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CASE REPORT

Gunshot Injury to The Mandible of a Patient Due to Domestic Violence With Tough Treatment Process: A Case Report

Aile İçi Şiddet Sebebiyle Bir Hastanın Mandibulasına Oluşan Ateşli Silah Yaralanmasının Zorlu Tedavi Süreci: Bir Olgu Raporu

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ABSTRACT

Gunshot wounds (GSWs) are a significant cause of trauma in the maxillofacial region, particularly in the mandible. Following the establishment of a treatment plan, patients may experience various complications, which often necessitate a secondary surgical intervention to correct the patient's concerns. This case study presents the details of a 36-year-old female patient who experienced a gunshot injury six years ago, that resulted in a fracture of the mandibular ramus. As a consequence of the injury, the mandibular condyle and coronoid process migrated into the infraorbital fossa, causing difficulty with mastication and mouth opening. To correct this issue, a surgical procedure was performed that included submandibular and preauricular incisions. The condyle was replaced and fixed with rigid internal fixation and maxillomandibular fixation techniques. Additionally, an abdominal fat graft was harvested and transplanted into the mandible. Postoperative follow-up was carried out; however, two weeks later, the patient developed an infection, requiring transfer to the infectious diseases department. In the follow-up period, the patient has acceptable function and well-being in the other health condition.

Keywords: Gunshot, wound, trauma, mandible, infection, fat graft.

ÖZET

Ateşli silah yaralanmaları (ASY), özellikle mandibula bölgesinde, maksillofasiyal bölgede önemli bir travma nedenidir. Tedavi planının belirlenmesini takiben, hastalarda genellikle çeşitli komplikasyonlar gelişebilir ve bu durum hastanın şikayetlerini gidermek amacıyla ikincil cerrahi müdahaleleri gerekli kılar. Bu olgu sunumunda, altı yıl önce ASY geçirmiş 36 yaşındaki bir kadın hastanın durumu ele alınmaktadır. Yaralanma sonucunda mandibular ramusta bir kırık oluşmuş ve mandibular kondil ile koronoid çıkıntı infraorbital fossaya göç etmiştir. Bu durum hastada çiğneme ve ağız açmada zorluklara neden olmuştur. Bu sorunu düzeltmek amacıyla submandibular ve preauriküler insizyonları içeren bir cerrahi işlem gerçekleştirilmiştir. Kondil, yerine yerleştirilmiş ve rijid internal fiksasyon ile maksillomandibular fiksasyon teknikleri kullanılarak sabitlenmiştir. Ayrıca, karın bölgesinden yağ grefti alınarak mandibula bölgesine transplant edilmiştir. Ameliyat sonrası takip süreci yürütülmüş; ancak iki hafta sonra hastada enfeksiyon gelişmiş ve enfeksiyon servisinde tedavi görmesi gerekmiştir. Enfeksiyon bir ay içerisinde başarıyla tedavi edilmiş ve hasta sağlıklı bir şekilde taburcu edilmiştir. Takip sürecinde, hastanın genel sağlık durumu iyi olup fonksiyon açısından kabul edilebilir düzeyde iyileşme gözlemlenmiştir.

Anahtar Kelimeler: Ateşli silah, yara, travma, mandibula, enfeksiyon, yağ grefti

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INTRODUCTION

Gunshot wounds (GSWs) frequently result in traumatic injuries to the maxillofacial region, with the mandiblebeing particularly vulnerable. Patients may experience various complications, including limited mouth opening, fibrous or bony ankyloses, scarring, trismus, paresthesia, and facial asymmetry.[1-4] These complications often necessitate a secondary surgical intervention to correct the patient's concerns. This case aims to illustrate the surgical procedures employed for the replacement and reconstruction of a migrated condyle resulting from a GSW. Additionally, it highlights the technique of harvesting a fat graft from the abdominal dermis to replenish the incision site. The case also sheds light on the topic of domestic violence and its impacts.

CASE REPORT

A 36-year-old female with no known systemic illnesses applied to Ondokuz Mayis University, Six years ago, the patient suffered a gunshot to her right mandible, which was inflicted by a family member. She had several operations in other clinics with unacceptable outcomes. She reported experiencing pain while opening her mouth and expressed difficulty in chewing food. After the examination of the patient; pain while opening mouth, trismus, and facial asymmetry were noted.

At the radiological exam computed tomography (CT), panoramic and posterioanterior x-rays were obtained. The radiographs clearly showed that the mandibular condyle and coronoid process had migrated into the infratemporal fossa after gunshot injury (GSI).

The fracture was unfavorable, so masseter muscle's movement helped in the displacement. As a firearm wound, the fracture was in multi-piece which requires rigid fixation in the treatment plan.

A comprehensive operation under general anesthesia was planned to reconstruct the condyle, The potential complications associated with the surgery were explained to the patient, and all the necessary preparations were made.

Preauricular and submandibular incisions were made during the surgical procedure. The mandibular condyle was separated from the coronoid process, inserted into the glenoid fossa, and fixed with a reconstruction plate. In addition, an intraoral closed reduction fixation of the mandible (MMF) was performed to provide stability to the dental arch (Figure 1).

To close the gap in the incision area, a dermis fat graft was harvested from the patient's abdomen. The harvested fat was then used to fill the mandibular area in the same surgical session. Subsequently, the wound was sutured to facilitate proper healing.

The patient received prophylactic antibiotics for a duration of two weeks. This included amoxicillin 1000mg taken twice daily, metronidazol 500mg taken twice daily, and cefuroxime 500mg taken once daily.

After two weeks, the patient returned for a follow-up examination and presented with symptoms of swelling, redness, and pain in the surgical site. Therefore, she was

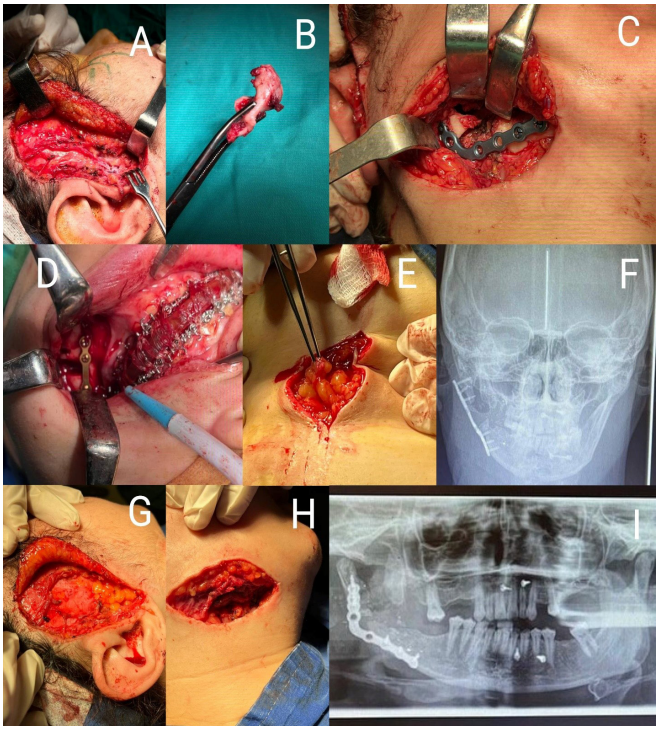

Figure 1 (A) Preauricular incision and access to the infratemporal fossa. (B) The mandible condyle after separating from coronoid process. (C) reconstructing condyle to the mandible corpus using reconstruction palate. (D) closed reduction maxillomandibular fixation. (E) Harvesting fat graft from abdominal dermis. (F) A post-Operative posterioanterior radiography. (G) Filling the preauricular region by the fat autograft. (H) Filling the region of submandibular incision by the fat autograft. (I) A Post-operative panoramic radiography showing the reconstruction plate.

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transferred to the infectious diseases department for further evaluation and treatment. After one month, the infection was successfully eliminated, and the patient was discharged in a good health. The follow-up period is ongoing and there is no discomfort reported so far.

DISCUSSION

GSWs) can affect all parts of the body, with approximately 6% involving the face, and an incidence of 87–91.5% occurring in men. [5-8]

The mandible is one of the most affected bones in the maxillofacial region following a GSW, with involvement reported in up to 65% of cases.[9] Mandibular fractures are the most common consequence, occurring in approximately 92% of cases[7], and these fractures are often comminuted in nature.[10] Factures can also be classify according to their line and vector of muscle traction into favorable and unfavorable, in favorable (stable) type muscle can support the fracture while unfavorable (unstable) type cause displacement. [11] Angle of mandible is one of the weakest regions of the mandible[12], so the percentage of angle fractures in total mandibular fractures is 33.4%.[13]

Recent findings by the World Health Organization (WHO) and its collaborators show that violence against women remains alarmingly widespread and commences at a young age. Data show that approximately one-third of women, or about 736 million individuals, experience violence during their lifetime. Shockingly, this number has remained relatively constant over the past decade.[14]

In Turkey, a study has demonstrated that domestic violence is a significant issue. Almost half of the women surveyed reported cases of domestic violence, and the majority of them were victims of violence.[15] In this case, the patient endured violence perpetrated by her own family members, resulting in her being injured by a shotgun.

The structure of the infratemporal fossa is formed by osseous boundaries located inferiorly and medially to the zygomatic arch. It is situated deep to the ramus of the mandible. So when high violent injury such as a gunshot targets the ramus, it's expected to migrate into infratemporal fossa, and that is what happened in this case.[16]

Preauricular and submandibular surgical incisions were chosen to access the wound area. The preauricular incision is placed in the preauricular skin fold, which is posterior to the superficial temporal artery and the auriculotemporal nerve. This incision provides maximal lateral and anterior access to the joint. The submandibular incision is a curved incision that is made at least 2 cm inferior to mandible and is very close to the facial vein, facial artery, parotid gland, and marginal mandibular nerve. Therefore, the surgeon must protect these anatomical structures to avoid complications. For example, in the preauricular approach, injury to the auriculotemporal nerve, branch beside parotid gland, can lead to Frey's syndrome, and any pluck to artery can extend surgical time by hours in some cases.[11]

Open reduction internal fixation (ORIF) and closed reduction MMF are used as treatment method in such situations , both have advantages and disadvantages; for example, ORIF has a higher rate of infection, which can be reduced by antibiotics, careful debridement and surgeon skill and experience. However, fewer complications occur with ORIF, such as non-union, mal-union, and facial asymmetry.[17, 18, 19, 20]

In this case, an infection developed. Notably, the infection rate of GSWs in the maxillofacial region is significantly higher than that of GSWs in other parts of the body.[21] And in some cases, it is difficult to predict because it depends on the patient's immunity, environmental factors, and prophylactic medication administered. Therefore, an antibiotic is routinely administered in this type of surgery.

The decision of which antibiotic to use should be based on the area of injury and the degree of wound contamination. Empiric broad-spectrum antibiotics are administered over a period of 7 to 14 days to control potential pathogens.

Additionally, the chosen antibiotic should encompass coverage against staphylococci, Clostridium perfringens, and Acinetobacter baumannii.[22,23]

Fat grafting is a highly effective technique for the treatment of maxillofacial deformities resulting from trauma. It has the advantage of providing long-term clinical monitoring and can serve as an alternative to traditional reconstructive procedures. The use of fat grafts reduces complications and healthcare costs.[24] Consequently, fat grafting was performed to restore the surgical site and correct facial asymmetry in this case.

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"In conclusion, this case study involved gunshot-induced displaced fractures of the mandibular ramus and condyle, which had previously resulted in unsuccessful treatment outcomes in other clinics. However, in an oral and maxillofacial surgery clinic, the case was managed through a challenging yet ultimately successful treatment process, particularly due to recurrent infections. This case may serve as a valuable reference for similar cases in other clinical settings.

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