



# SAĞLIK HİZMETLERİ VE EĞİTİMİ DERGİSİ

## JOURNAL OF HEALTH SERVICES AND EDUCATION

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# Teaching Opticianry Students Empathy for Patients with Hemianopia and Strabismus

## Optisyenlik Öğrencilerine Yönelik Hemianopsi ve Strabismus Hastalarına Karşı Empati Öğretimi

Erdoğan ÖZDEMİR<sup>1</sup>, Hatice Semrin TİMLİOĞLU İPER<sup>2</sup>, Onur YARAR<sup>3</sup>

### ABSTRACT

**Introduction and Objective:** Strabismus occurs as a result of the coordination disorder of the six muscles in the outer part of the eye. In case of hemianopia; half of the visual field is not seen and the same half area is perceived from each half of both eyes. The aim of this study is; to mimic these conditions artificially for the students to make them experience the patients' difficulties and create empathy as well as to teach them the use of prisms in correcting these conditions. In accordance with this purpose, simulation goggles which mimics the condition were designed.

**Method and Materials:** In this study, two different simulations were developed. One of them is about strabismus and the other one is about hemianopia. Simulations were arranged to simulate these disorders. The mixed-method was used in this research. This study was attended by 25 students studying in the second year of the optician program of a university in Istanbul. The effects of the developed simulations on the students' empathy with patients were evaluated. As a teaching process, opticianry students worn these simulation goggles and complete some activities. These activities have chosen among the situations in which patients with strabismus and hemianopia have difficulties to complete. After using these simulations for empathy teaching, empirical data concerning

“Self-Assessment Survey for Simulators” and “Writing Essay” were collected.

**Results:** Students' view on the simulations is that they are convenient tools for empathy training. In addition, it was determined that the students using the simulations suggested compensatory strategies which also shows that, they created awareness and empathy among themselves towards the patients.

**Conclusion:** It is important for health program students to empathize with patients related to their field of study. It is recommended that the simulations developed in this research be used in the education of opticians and optometry students.

**Keywords:** Strabismus, Hemianopia, Prism, Simulation, Empathy.

### ÖZ

**Giriş ve Amaç:** Strabismus, gözün dış kısmındaki altı kasın koordinasyon bozukluğu sonucu oluşur. Hemianopi durumunda ise görme alanının yarısı görülmez ve her iki gözün her iki yarısından da aynı yarım alan algılanır. Bu çalışmanın amacı; optisyenlik öğrencilerine bu hastaların yaşadıkları zorlukları yapay olarak yaşatarak, öğrencilerin hastalar ile empati kurmalarını sağlamak ve bu hastalıkların rehabilitasyonunda prizmaların nasıl kullanıldığını açıklamaktır.

**Gereç ve Yöntem:** Araştırmada strabismus ve hemianopi ile ilgili birer tane simülasyon gözlüğü geliştirilmiştir. Simülasyonlar bu hastalıkları canlandırarak biçimde kalibre edilmiştir. Araştırmada karma model kullanılmıştır. Araştırmaya, İstanbul'daki bir üniversitenin optisyenlik programında öğrenim gören 25 öğrenci katılmıştır. Öğrencilere çalışma için geliştirilen simülasyon gözlükler kullanılarak empati eğitimi verilmiştir. Araştırmaya katılan öğrenciler simülasyon gözlükleri takarak çeşitli etkinlikler gerçekleştirmiştir. Bu etkinlikler strabismus ve hemianopi hastalarının güçlük çektiği durumlar arasından seçilmiştir. Araştırmada ölçme aracı olarak “Simülasyonlar için Değerlendirme Anketi” ve kompozisyonlar kullanılmıştır.

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**Bulgular:** Araştırma sonuçları öğrencilerin, hastalarla empati kurma açısından simülasyonları başarılı bulduğunu göstermektedir. Ayrıca simülasyonu kullanan öğrencilerin hastalara yönelik telafi edici stratejiler önerdikleri, hastalara yönelik farkındalık oluşturdıkları ve empati kurdukları da tespit edilmiştir.

**Sonuç:** Sağlık programı öğrencilerinin çalışma alanları ile ilgili hastalara karşı empati kurmaları önemlidir. Bu çalışmada geliştirilen simülasyonların optisyenlik ve optometri öğrencilerinin eğitiminde kullanılması önerilmektedir.

**Anahtar Kelimeler:** Strabismus, Hemianopia, Prizma, Simülasyon, Empati

## INTRODUCTION

Optical devices like prism is used frequently for corrections and rehabilitations of ophthalmic disorders. Refractive environments surrounded by lateral surfaces which are non-parallel to each other are called as prisms. The peak edge of prism is referred to as the prism apex. The place against the prism apex is referred to as prism base. Prisms change the direction of the light towards its base. In this case, the image slides towards the prism apex (1).

The image-shifting ability of a prism is very useful. Prisms are used in the treatment and rehabilitation of various ophthalmic disorders, especially strabismus and hemianopia (2). Strabismus is caused by the effect of six muscles which are located in the externum of the eye. As the strength increases or decreases in one or more of these muscles, the position of the eye changes as well (3). In the case of strabismus, one eye may look straight and the other may slide in, out, up or down. Prisms are used for treatment and rehabilitation of strabismus, in addition it is used for visual field expanders in patients with hemianopia (4,5). In case of hemianopia; half of the visual field is missing and this missing is perceived from both eyes. A loss of vision in these two areas is called homonymous hemianopia (6). Peli prisms are used for hemianopia and are actually a special fresnel prism designed to expand the visual field (7).

Empathizing with patients is very important for health professionals. Empathy, a skill that can be learned, which means intellectual and emotional understanding of other people's feelings, thoughts and behaviors is very important for health professionals (8). One of the most effective methods used in empathy teaching is simulation (9,10,11). Thus, there are various studies using simulations in empathy teaching (12,13,14).

In this research, simulations were developed and applied to enable the opticians and optometrists to empathize with patients have hemianopia and double vision problems.

## MATERIAL AND METHOD

The mixed-method was used in this research. This method means that combining qualitative and quantitative data collection tools using together (15). Data collection instruments were administered to the students after the course. Participants, data collection tools and data analysis of the research are explained in the following.

*Participants:* This study was attended by 25 students studying in the second year of the optician program of a university in Istanbul. The ages of these students vary between 18 and 45. While 13 of the students are female and 12 of them are male. Students participating in the research have basic information about eye anatomy, physiology and eye health. Participants succeeded "anatomy of the eye", "diseases of the eye", "visual optics", "basic physics" and "optical physics" courses in the optician program.

*Data Collection Tools:* The effects of the simulations on empathy towards patients were evaluated with the students. These students' evaluations about the simulations were collected with "Evaluation Survey for Simulations". The survey is set as a five-point Likert and consists of seven items. It was created by DeCarlo and Shechtman (9). Survey scales were adapted according to hemianopia and diplopia. The survey items are included in Table 1. Another data collection tool of the research is "Essays". The students who participated in the research wrote essays on three subjects after course. The essays topics were; "Balancing Strategies for Patients with Homonym Hemianopia and Double Vision", "Awareness of Patients with Homonym Hemianopia and Double Vision" and "Empathy for Patients with Homonym Hemianopia and Double Vision".

*Data Analyses:* In the study, the data of the "Assessment Survey for Simulations" were analyzed by descriptive analysis. Furthermore, the essays were evaluated by the researchers and student's opinions in essays are coded and then categorized.

### *Developing Simulations:*

*Simulation for Strabismus:* To simulate double vision (strabismus), a goggles with a temple length of 140 mm, a bridge size of 19 mm and a diagonal length of 56 mm was used. On the right side of the goggles 4.00D (base up) prismatic lens was mounted. A glass without an optical power was mounted on the left side of the frame.

When this goggles is worn, the objects appear double. The image in the right eye is located below the image in the



left eye. As in the Figure 1, when 4.00D Fresnel (bottom down) are placed on the right side of this frame, the double image turns a single image.



**Figure 1.** Simulation for strabismus and double vision and correction of double vision with Fresnel prism.

This simulation was tested by one student. Firstly, the student was asked to read a word document containing 542 words, written in size 10 Times New Roman without simulation goggles. The student completed reading this text in 2 minutes and 39 seconds. Secondly the student was asked to read same document with simulation goggles. In this condition, the student completed reading this text in 2 minutes and 47 seconds. The student stated that reading with simulation goggles was difficult due to her double vision problem. It was observed that the student read more slowly when wearing simulation goggles. It was also observed that she could not read some words and her fluency decreased and she had difficulty in reading. She stated that it was very difficult to read with simulation because of double vision. Finally, 4.00D fresnel prism (bottom up) was fitted right part of frames and she said that she started to see the objects one by one again. In this condition, the student was asked to read the same text again. The student completed reading the text in 2 minutes and 40 seconds. She indicated that she did not see a double vision with simulation goggles with fresnel. This application shows that the simulation goggles created double vision. Also, the results concerning the usage of goggles with fresnel shows that the simulation goggles have corrected the double vision.

*Simulation for Homonymous Hemianopia:* To simulate homonymous hemianopia, a goggles frame with a temple length of 140 mm, a bridge size of 19 mm and a diagonal

length of 56 mm was used. Half of the glasses, which has not refractive power, of this goggles are painted to be opaque. The sides of the frames are covered with a leather material to narrow the visual field of view.



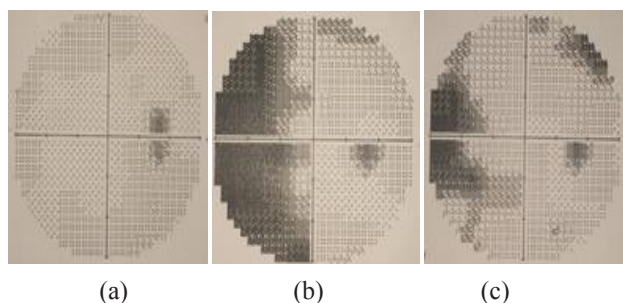
**Figure 2.** Simulation for homonymous hemianopia

The simulation in figure 2 was arranged according to the pupillary center of each student. Firstly, the pupillary center of the students was determined. After that, glasses were painted as per focal point of each student. In other words, the simulations were designed specifically for each student's pupillary centers.



**Figure 3.** Visual field measurement (The vision field measurement by Zeiss Humphrey 850)

As seen figure 3, this simulation was tested by one student. At first, the student's visual field was tested without simulation goggles and then the test repeated with simulation goggles for homonymous hemianopia. Finally, the visual field of the student was tested with Peli prisms attached to simulation goggles in figure 5. The results obtained from these examinations are given below.



**Figure 4.** (a) Normal visual field, (b) Hemianopic visual field with simulation goggles (the outer half of the visual field is black) (c) Peli prisms attached to simulation goggles

Figure 4(a) shows the normal visual field while figure 4(b) represents the narrowing of the visual field with simulation goggles. This situation is similar to the visual field of a patient with Homonim hemianopia. Changes in the visual field after the application of Peli prism are shown in figure 4(c).



**Figure 5.** Visual field testing with Peli prism attached to simulation goggles

As a result of these tests, it can be claimed that a student using this simulation experienced homonymous hemianopia condition and is able to understand the correctional use of Peli prisms in enlargement of the visual field.

**Teaching Process:**

After the development of the simulations, the teaching phase was started. Empathy training with simulation goggles was carried out in 2018-2019 academic year in accordance with the following steps.

*Empathy training for double vision:* The students participating in the research first practiced reading text using double vision simulation goggles (542 words, written in 10 points Times New Roman size). They also took a short walk between the desks in a classroom with these goggles. Students also performed the same activities with fresnel mounted simulation goggles. Since it was uncomfortable for the students to wear these goggles for a long time, no other activity was done.

*Empathy training for homonymous hemianopia:* 25 students wore the goggles simulations separately for homonymous hemianopia. All students walked individually for 250 meters across the university campus wearing hemianopic simulation goggles. This predetermined track is arranged to include crowded areas. For the safety of the student using simulation goggles, another student accompanied her/him. In addition to this activities, students wore this simulation goggles for 3 hours during their daily activities at home.

After the empathy training, research data were collected by measurement tools. Students evaluated both simulations by Evaluation Survey for Simulations. Furthermore, the students who participated in the research wrote essays on three subject.

**RESULTS**

In this section, the data obtained from the survey and essays are presented. The Table 1 below shows the distribution of students’ responses to the “Evaluation Survey for Simulations”.

**Table 1.** The result of students’ evaluation of simulations

Survey Items	5	4	3	2	1
Simulators are effective in representing the vision of hemianopia and diplopia patients.	11	11	2	1	0
The tasks I performed with simulators taught me to empathize with hemianopia and diplopia patients.	20	2	2	1	0
The tasks I performed with simulators taught me compensatory strategies that could be useful for hemianopia and diplopia patients.	19	3	2	1	0
Using simulators increased my awareness of the difficulties experienced by that hemianopic and diplopic patients.	20	2	1	1	1
After this simulation I feel capable to adjust goggles for hemianopia and diplopia	11	9	2	2	1
Consequently, this experience with simulators is a valuable part of the courses.	17	5	2	1	0
After using these simulators, I had the idea that hemianopia and diplopia could not be taught by traditional teaching methods.	9	8	5	1	2

5=I strongly agree, 4=I agree, 3=I am undecided, 2= I disagree, 1= I strongly disagree.

Considering the answers for Table 1, it shows that the students found the simulations very successful in terms of empathizing with patients, suggesting strategies for patients, and awareness of diseases. The Table 2 contains the themes from student essay.

**Table 2.** Themes related to strategy, awareness and empathy

Themes for Compensatory Strategies	Frequency	Themes for awareness	Frequency	Themes for empathy	Frequency
Security	12	Difficulty doing housework	7	Panic, fear and stress	11
Ergonomics	7	Hobby difficulties	7	Insecurity	6
Using other senses	3	Walking difficulties	7	Environmental barriers	5
Act independently	2	Basic maintenance difficulty	3	Building a relationship	4
Amount of light	2	Reading and vision difficulties	2	Enjoying life	3
Using auxiliary equipment	1	Difficulty building a relationship	1	Despair	1
Contrast	1	Suffering from pain	1	Irritability	1
		Driving difficulties	1		

According to themes, students frequently emphasized the patients' difficulties about housework involving movements. It was understood that students mostly shared their feelings of panic, fear and stress. In addition, students offered security strategies to patients who have hemianopia and double vision.

## DISCUSSION

As a result of the research, students having simulation experience suggested especially safety-themed strategies for patients. It was determined that they noticed patients' difficulties they experienced in housework. In addition, it was understood that the students empathized with the patients especially about their panic, fear and stress. Both hemianopia and diplopia patients do not feel safe in environments with movement. These patients feel fear, anxiety and stress in these places. They also have difficulty in doing work involving movement. Therefore, it is also important for students to understand this situation after the application.

When the students' opinions about the developed simulations were examined, it was understood that the students found the simulations quite successful (9-12) in terms of empathizing with patients, awareness of disorders and they suggested strategies for patients. This result was achieved other studies about health professional educations. Chua et al. recommended to use simulations in the education of health care students. Effectiveness of simulation-based interventions in improving empathy among health-care students was examined in this study. In this review study, sixteen researches were included and collected data with meta-analysis. It was indicated that using regular simulation-based interventions develop health-care student's empathy (16). Campbell et al. investigated the effect of simulation based education for empathy of Alzheimer disease on nursing students. 163 under-graduate baccalaureate nursing

students were attended this study. After the 44-minute interactive simulated virtual reality dementia experience with Virtual Dementia Tour (VDT®), increasing of student perceptions of awareness, knowledge and sensitivity of Alzheimer disease were detected in the study (17). Wang and Zhang were developed low vision simulation in virtual reality for training empathy within eye care providers. This computer based virtual reality simulation can support hazy vision, contrast difficulty, peripheral vision loss, night blindness and central vision loss. They emphasized that the simulation particularly useful for eye care providers such as optometrists, ophthalmologists, vision rehabilitation therapists, mobility specialists, and potential eye care providers such as students related to specific areas (18).

Finally, from the results of this research and other related studies, it was concluded that it is necessary to use and develop various simulations to increase health professions students' empathy towards different patients.

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RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ

## In Vitro and in Vivo Compliance of 3D Printed PLA Scaffolds with Differentiated Mesenchymal Stem Cells

3D Baskılı PLA-İskelelerin Üzerinde Farklılaştırılmış Mezenkimal Kök Hücrelerin in Vitro ve in Vivo Uyumları

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### ABSTRACT

**Aim:** 3D printing made a rapid entry as a means of production of biocompatible and biodegradable materials for tissue engineering applications. 3D printing made it possible to create custom biodegradable implants and polylactic acid is one of the most promising polymers. In this study, we aimed to form both bone and cartilage differentiated from bone marrow stromal mesenchymal stem cells on 3D printed polylactic acid polymers.

**Materials and Methods:** The polylactic acid scaffolds were designed, 3D printed and sterilized in dedicated university facilities. Mesenchymal stem cells were collected from rat bone marrow and were then differentiated to either osteoblasts or chondroblasts. The characterization of cells was analyzed using Alizarin Red, Alcian blue staining and osteonectin and collagen II by indirect immunocytochemistry. Differentiated cells were seeded on the 3D scaffold, cultured for 2 weeks. For in vivo tests, 3D scaffolds with or without differentiated bone marrow stromal mesenchymal stem cells were implanted into subcutaneous connective tissue. After the four-month implantation, the rats were sacrificed, and all samples were histochemically and immunohistochemically analyzed.

**Results:** Osteogenic and chondrogenic differentiation from bone marrow stromal mesenchymal stem cells were performed after 2 weeks culture condition. They were positively stained both histochemically and immunohistochemically. After transfer of the cells onto 3D polylactic acid scaffold, their differentiation continued and both bone and cartilage formation were observed after histochemical and immunohistochemical analyses both under in vitro and in vivo conditions.

**Conclusion:** 3D printed polylactic acid scaffolds supported both bone and cartilage formation, therefore, it may be conveniently used for experimental cell in vivo studies.

**Keywords:** Bone, Cartilage, 3D Printing, Mesenchymal Stem Cells,

### ÖZ

**Amaç:** Üç boyutlu baskı, doku mühendisliği uygulamaları için biyolojik olarak uyumlu ve biyolojik olarak parçalanabilen malzemelerin üretim aracı olarak hızlı bir giriş yaptı. Üç boyutlu baskı, özel biyobozunur implantlar oluşturmayı mümkün kıldı ve polilaktik asit (PLA) en umut verici polimerlerden biridir. Biz bu çalışmada, 3D baskı PLA polimerleri üzerinde kemik iliği stromal mezenkimal kök hücrelerinden (BMSC) hem kemik hem de kıkırdığa farklılaştırılan hücreler oluşturmayı amaçladık.

**Gereç ve Yöntem:** PLA iskeleleri tasarlanmış, Solidworks Yazılımı kullanılarak 3D olarak basılmış ve Yakın Doğu Üniversitesi'nin özel tesislerinde sterilize edilmiştir. Mezenkimal kök hücreler sıçan kemik iliğinden toplandı ve daha sonra osteoblast veya kondroblastta farklılaştırıldı. Hücrelerin karakterizasyonu Alizarin kırmızısı, Alcian mavisi boyama, osteonektin ve kollajen II kullanılarak indirekt immünohistokimya ile analiz edildi. Farklılaştırılmış hücreler, 3D yapı iskelesine ekilerek 2 hafta boyunca kültüre edildi. İn vivo test için, farklılaştırılmış BMSC'leri olan veya olmayan 3D yapı iskelesi, subkutan alanın bağ dokusuna implante edildi. Dört aylık implantasyondan sonra sıçanlar sakrifiye edildi ve tüm numuneler histokimyasal ve immünohistokimyasal olarak incelendi.

**Bulgular:** BMSC'den osteojenik ve kondrojenik farklılaşma 2 haftalık kültür koşulundan sonra gözlemlendi. Hücreler hem histokimyasal hem de immünohistokimyasal olarak pozitif boyandılar. Hücreler 3D PLA iskelesine aktarıldıktan sonra farklılaşmaları devam etmiş ve hem in vitro hem de in vivo koşullarda histokimyasal ve immünohistokimyasal analizlerden sonra hem kemik hem de kıkırdak oluşumu gözlemlenmiştir.

**Sonuç:** 3D baskılı PLA iskeleleri hem kemik hem de kıkırdak oluşumunu desteklediği ve bu nedenle bu hücrelerin in vivo çalışmalar için rahatlıkla kullanılabileceği gözlemlenmiştir.

**Anahtar Kelimeler:** Kemik, Kıkırdak, 3D baskı, Mezenkimal Kök Hücre

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## INTRODUCTION

In tissue engineering, 3D printing became an alternative method for in vivo delivery of custom sized and shaped biomaterials seeded with in vitro produced cells. In recent years, research on stem cell interaction with the biocompatible and biodegradable materials obtained by 3D printing and their potential for tissue regeneration has gained evident speed (1).

Stem cells derived from different sources are being continuously investigated as possible “drugs” for cell therapy applications. Mesenchymal stem cells (MSCs) can be isolated easily from bone marrow, adipose tissue, umbilical cord, placenta and dental pulp tissues; therefore, they are preferred for use in tissue engineering with high in vitro exchange capacity (2-6). The compliance of biomaterials with cells is an important issue to provide crosslinking between the biomaterials and the cells in the microenvironment and maintain the integrity for the possible tissue formation and remodeling (7). Biomaterials play an important role in the particular fields by providing matrices for cellular growth, proliferation, and new tissue formation.

For this purpose, polycaprolactone (PCL), poly(l-lactide-co-glycolide) (PLGA), collagen and laminin have been investigated in a variety of studies to determine their supporting capacity of cell differentiation (8-9).

Poly(lactic acid (PLA) has been variously used in biomedical applications due to its biocompatibility, processability and good mechanical properties (10-15). PLA seeded with different cells has been studied regarding muscle tissue, neural tissue, cartilage, and bone regeneration in the fields of cardiovascular surgery, orthopedics and neurology (11).

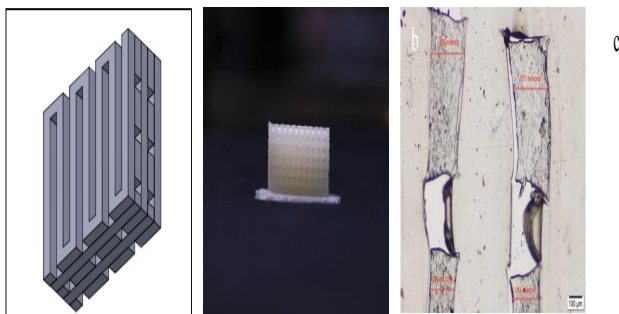
3D printing technologies help to create structures using biomaterials with or without cells. The technology itself can control the shape and size of the printed materials. It is therefore advantageous to be able to tweak the properties of 3D printed scaffolds to fully mimic the structure which is aimed to be replaced or augmented. Furthermore, this technique requires a good design, manufacture and viable cells in the model providing the possibility to merge in an appropriate environment (16-19).

In this study, we aimed to investigate to compatibility of 3D-printed PLA bioscaffolds with bone marrow mesenchymal stem cells, and their differentiation potential to osteoblasts and chondroblasts in vivo.

## MATERIALS AND METHODS

All procedures were approved by the Ethics Committee on Animal Research, Manisa Celal Bayar University, Manisa, Turkey (Approval #77.637.435-72).

**PLA Scaffold Design and Printing:** Design and manufacturing of the scaffolds were performed at NEU3D Laboratories (Near East University, 3D Printing Facilities) (Figure 1a). Scaffolds were designed using Solidworks 3D Design Software (Dassault Systemes S.A., MA, USA). Layers consisted of 0.4 mm wide connected parallel bars. Each layer was positioned in perpendicular manner to form a cage like structure in order to capture maximum number of cells in suspension. The spacing between the bars was arranged as 0.4 mm (Figure 1b). The designs were exported as “.stl” files and printed at 215 °C using 1.75 mm polylactic acid filament (Colorfabb B.V, Belfeld, NE) in MakerBot Replicator 5th Generation 3D printer (MakerBot Industries, New York, USA) (Figure 1c). According to the data provided by manufacturer the density of the polylactic acid filament was 1.2-1.3 g/cm<sup>3</sup>, tensile strength was 45 MPa and tensile modulus was 3400 Mpa. Vaporized hydrogen peroxide sterilization (low temperature plasma sterilization) of scaffolds was performed in Near East University Hospital, Sterilization Unit. Scaffolds with a solution of hydrogen peroxide, were kept for 30 min in a solution of peroxide at room temperature, then the peroxide was removed, and scaffolds were washed 3 times with a phosphate buffer solution (pH 7.4) (20).



**Figure 1:** 3D design (a), 3D printed version (b) and sectioned view (c) of PLA scaffold.

### **Culture and Differentiation of Bone Marrow Stromal Cells:**

Male Wistar Albino rat bone marrow stromal cells (BMSC) which were stocked in Department of Histology and Embryology, Manisa Celal Bayar University, were cultured in standart culture medium containing 10% fetal bovine serum (F4135, Sigma-Aldrich, MO, USA), 1 % L-glutamine (G7513, Sigma-Aldrich, MO, USA), 1 % penicillin-streptomycin (P0781, Sigma-Aldrich, MO, USA), 1 % gentamycin (G1397, Sigma-Aldrich, MO, USA), and 0,1 % amphotericin B (A2942, Sigma-Aldrich, MO, USA) in  $\alpha$ -MEM (M4526, Sigma-Aldrich, MO, USA) until 80 % confluency. The osteoblastic differentiation of stem cells was performed using osteogenic stimulatory mesencult™ Kit (05504, StemCell Technologies, MA, USA) chondrogenic differentiation was performed using Stempro® Chondrogenesis Differentiation Kit (A10071-01, Gibco/Life Technologies, MD, USA). Cells were incubated in a humidified atmosphere of 95% air, 5% CO<sub>2</sub> at 37°C (Hera Cell, Kendro Laboratory, Germany). The medium was changed every other day. Cells were microscopically observed under inverted microscope with a phase-contrast attachment and photomicrographs were obtained (21).

### **Characterization of Osteoblasts and Chondroblasts**

**Alizarin Red Staining:** Alizarin red staining was done for identification of osteoblast cells. The culture medium from osteoblast differentiated cell layers was removed, and the cells were rinsed with phosphate buffered saline (PBS) for 3 times before fixation in 4% paraformaldehyde for 1 hour at 4°C. Then, they were washed with deionized water and allowed to dry. The fixed cells were stained with 0.1% of Alizarin Red's (A5533, Sigma-Aldrich, MO, USA) solution

in distilled water (pH 7.2) for 1 hour at 37°C. They were then washed with deionized water and observed under inverted phase-contrast (IX-71, Olympus, Tokyo, Japan) microscope (22).

**Alcian Blue Staining:** Alcian blue staining was done to identification of chondroblast cells. The culture medium from chondroblast differentiated cell layers was removed, and the cells were rinsed with PBS for 3 times before fixation in 4% paraformaldehyde (P6148, Sigma-Aldrich, MO, USA) (pH 7.4) for 30 minutes at room temperature. They were then washed with PBS and stained with 1% of Alcian blue solution (1263395-3, Carlo Erba, Val-de-Reuil, France) in 0.1 N HCl for 30 min in the dark at room temperature. They were washed 3 times with 0.1 N HCl and distilled water for neutralization (23).

### **Immunocytochemical Methods**

Differentiated cells were washed with PBS and they were fixed with 4% paraformaldehyde (P6148, Sigma-Aldrich, MO, USA) for 30 min at room temperature. They were then washed with PBS for 3 times, 3% of hydrogen peroxide solution (H<sub>2</sub>O<sub>2</sub> solution, 3%, H1009, Sigma-Aldrich, MO, USA) was added for 10 minutes to inhibit of the endogen peroxidase activity. After washing with PBS for 3 times, the cells were incubated with blocking solution (85-9043, Invitrogen, ThermoFisher Scientific, MA, USA) for 1 hour and primary antibodies; anti-collagen II (sc-52658, Santa-Cruz, CA, USA), anti – osteonectin (33-5500, Invitrogen, ThermoFisher Scientific, MA, USA) were added and incubated overnight at 4 °C. After washing with PBS, they were incubated with biotinylated secondary antibodies for 30 min and streptavidin hydrogen peroxidase for 30 min (85-9043, Invitrogen, ThermoFisher Scientific, MA, USA). They were washed again with PBS and stained with diaminobenzidine (DAB, 85-9043, Invitrogen, ThermoFisher Scientific, MA, USA) for 5 min. They were mounted with mounting medium (107961, Merck Millipore, Darmstadt, Germany) and examined under light microscope (BX-40, Olympus, Tokyo, Japan) (22).

### **Culture, Characterization and in vivo Transplantation of Differentiated Cells in PLA Scaffolds**

Sterile 3D PLA scaffolds were conditioned for 12 hours in medium before seeding with the cells. Differentiated cells (1x10<sup>6</sup> cells / cm<sup>3</sup> density of either osteoblast or chondroblast)



were seeded on PLA and cultured for 14 days. After the culture time they were then either fixed in 10 % formalin solution for histochemical and immunohistochemical analyses at 24-48 hours or transferred animals for in vivo studies.

A total of eighteen male Wistar rats were used for the in vivo studies. Implantation area was shaved and sterilized with iodine solution at the dorsal side of each rat after anesthesia with ketamine (60 mg/kg, Ketalar, 002 038, Eczacıbasi) and xylazine (5 mg/kg, Alfazyme, 0804125-11, Alfasan) (Figure 2). Animals were sacrificed 4 months after the in vivo implantation of PLA scaffolds with or without cells. For light microscopic analyses, biomaterials with or without differentiated cells were incubated for 14 days in borax-sodium carbonate buffer (0.01 M Na<sub>2</sub>CO<sub>3</sub> and 0.3 mm Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>, pH:11) to achieve softening of PLA (20). All samples were embedded in paraffin and 5µ sections were taken. For histochemical analyses, sections were stained with alcian blue and alizarin red (see above). For immunohistochemical analysis, distributions of osteonectin and collagen II were evaluated using standart protocol of indirect immunoperoxidase staining (see above).



**Figure 2:** PLA scaffolds with or without cells were implanted subcutaneously in the rat's neck.

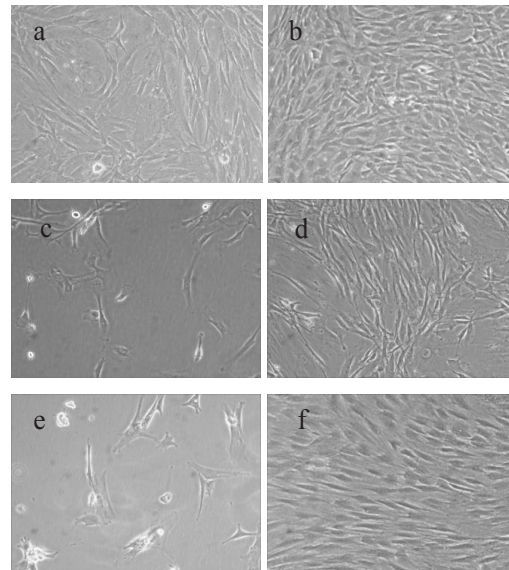
## RESULTS

### Culture of Bone Marrow Stromal Cells and Differentiation of Bone Marrow Stromal Cells to Osteogenic or Chondrogenic Lineage

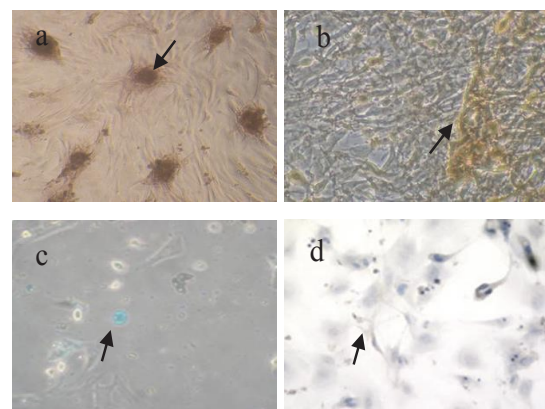
BMSCs had characteristic fibroblast-like morphology under standart culture medium condition (Figure 3a). Culture of the cells was continued until 80 % confluency was reached after 14 days in culture (Figure 3b).

The BMSCs were cultured in either osteogenic or chondrogenic medium for 14 days. After the differentiation, cuboidal osteogenic-like cells (Figure 3c-d) and epitheloidal chondrogenic-like cells (Figure 3e-f) were identified. While BMSCs maintained fibroblast morphology in standart

culture condition at 14 days of culture, the morphology of the cells differentiated into osteogenic or chondrogenic lineage were different. After 14 days in culture, osteogenic and chondrogenic cells were positively stained with Alizarin Red (Figure 4a). and Alcian blue (Figure 4c), respectively. They also expressed osteonectin (Figure 4b) or collagen II (Figure 4d) after differentiation of the cells.



**Figure 3.** Photographs of BMSCs at 7 (a) and 14 (b) days of culture time. Differentiation of BMSC to osteogenic (c, d) or chondrogenic (e, f) lineage after 3 (c, e) and 14 (d, f) days of culture. Original Magnification X400.



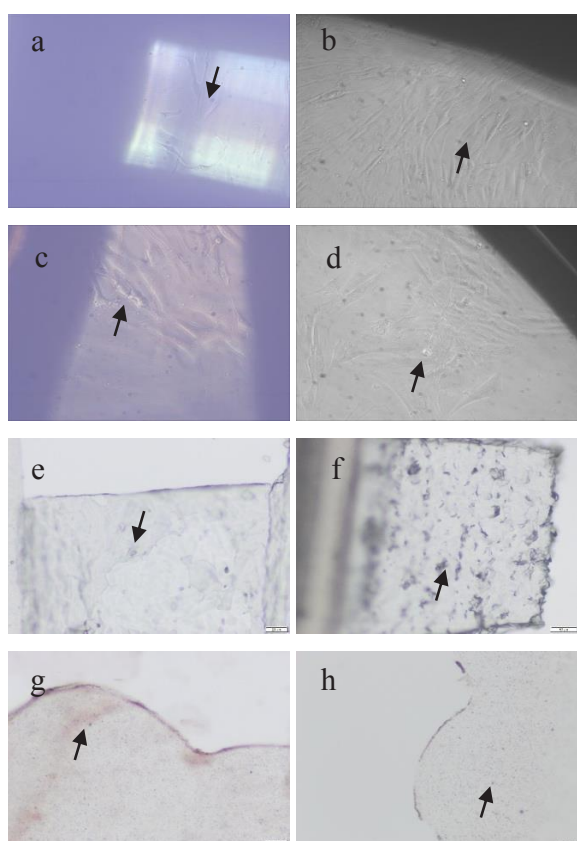
**Figure 4:** Osteogenic (a, b) or chondrogenic (c, d) differentiated cells were identified after 14 days in culture. a: Alizarin Red (arrow), b: Osteonectin (arrow), c: Alcian blue (arrow), d: Collagen II (arrow). Original Magnification X400.

### Identification of Differentiated Cells in PLA Scaffolds

The differentiated cells were cultured in PLA scaffold for 14 days. During culture periods, the proliferation of both

osteogenic and chondrogenic cells were observed in 3D PLA scaffolds. The differentiation and proliferation were continued until 14 days of cell culture. Osteogenic (Figure 5a, b) and chondrogenic (Figure 5c, d) cells were highly affected by the geometry of individual pores within the scaffold

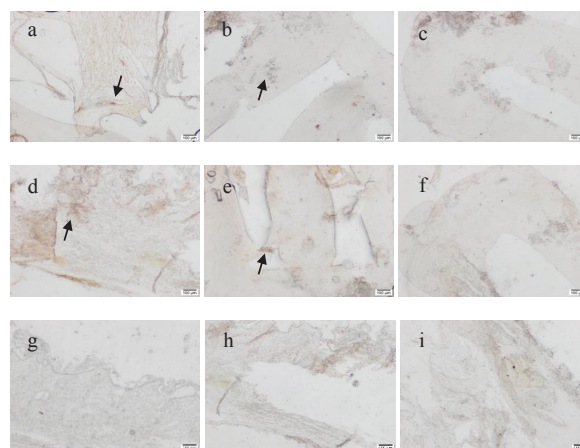
Indeed, differentiated cells had long projections onto surface of 3D PLA scaffolds. These results showed that differentiated cells grow and proliferate on 3D PLA scaffolds. Alizarin calcium deposits in areas with red staining was observed in reddish brown color (Figure 5e) In alcian blue stained areas, the chondrogenic accumulation was also observed with blue (Figure 5f). The cells expressed osteogenic or chondrogenic markers such as osteonectin or collagen II, respectively (Figure 5g, h).



**Figure 5:** Culture of differentiated cells in 3D PLA Scaffolds at 14 days. a-b: Osteogenic differentiation (arrow), c-d: Chondrogenic differentiation (arrow). Characterization of differentiated cells on 3D PLA scaffolds. e: Alizarin Red staining (arrow), f: Alcian Blue staining (arrow), g: Osteonectin (arrow), h: Collagen II (arrow). Scale Bars: 20 µm.

### In vivo Implantation of 3D PLA Scaffold

The 3D PLA scaffolds with or without differentiated cells were implanted in the subcutaneous area of the rat necks and they were examined after 4 months. All of the rats stayed alive until sacrifice without any obvious inflammatory response. Upon histochemical observation, the cells or connective tissue which covered the scaffolds with differentiated cells were seen (Figure 6 a, b, d, e). No cells or extracellular matrix were detected in the unseeded scaffolds (Figure 6c, f). During immunohistochemical evaluation, immunoreactivities for osteonectin and collagen II were stained positively in 3D PLA scaffolds with differentiated cells. (Figure 6). No staining was observed in negative controls (Figure 6 g-i).



**Figure 6:** In vivo implantation of osteogenic (a, b) (arrow), chondrogenic (d, e) (arrow) and without cells (c, f) on 3D PLA scaffolds. a, b, c: Osteonectin, d, e, f: Collagen II. Scale Bars: 100 µm. Negative control (g: osteogenic, h: chondrogenic, i: without cells).

### DISCUSSION

3D printed scaffolds may help to solve the problems during in vivo experimental studies with cells as different types of the scaffolds provide a three-dimensional environment for cell attachment and growth by mimicking the in vivo environment. Three-dimensional printing allows production of bioscaffolds of variable sizes and shapes to serve the needs of the patients. Obtaining an optimal high resolution 3D printed bioscaffold is a complex process which needs careful selection of both software and hardware (16). Currently, there are some in vitro culture studies, aiming production of bone graft materials seeded with osteoblasts and osteoclasts (7).

Biocompatibility and mechanical properties of the PLA has attracted great interest to this material, and it is being widely investigated in medicine. Our results demonstrated that 3D printed PLA scaffolds supported the differentiation, proliferation and attachment of BMSCs. In addition, the osteonectin which is a marker of osteogenic differentiation and collagen II which is a marker of chondrogenic differentiation continued their expressions on 3D PLA scaffolds. Therefore, we may state that PLA is not a toxic material for differentiated cells.

The structure and porosity of scaffolds are extremely important for adequate cell attachment during the culture process. Accurate pore size of scaffolds may provide appropriate signaling to encourage the differentiation of stem cells. Currently, three-dimensional printing allows the scientists to determine the size, shape, mechanical strength, and porosity of the intended scaffold. Mechanical performance of a 3D scaffold affects the response of the cells, and it may also affect the nutrient diffusion. Mesenchymal stem cells from differentiated cells in the osteoblastic differentiation and proliferation have been reported to be effective in the geometric structure of the pier (17). However, some biomechanical studies showed that PLA implants are not suitable for use in the weight bearing bones (18). In our study, 3D PLA scaffolds provided in vivo cell survival and it was possible to achieve BMSCs' proliferation and differentiation.

Intercellular communication is related directly to scaffold. For good adhesion and proliferation of cells on synthetic scaffolds, natural polymers extracted from the native extracellular matrix (ECM) have been used to modify the surface of PLA scaffolds (24, 25).

Polymer degradation rate is determined by the water content of the environment, temperature, humidity and lactic acid concentration. Several studies examined endochondral and intramembranous ossification in implanted biodegradable scaffolds seeded with cultivated osteogenic stem in the bone defects (26,27). The possibility to create 3D culture system with PLA was also shown (28). In our study, the implanted PLA scaffolds stayed for 4 months without apparent signs of major degradation. This finding shows that PLA scaffolds are probably more suitable for the implants which need long term mechanical stability instead of rapid degradation. But we should also keep in mind that polylactic acid is not strong enough to be used in weight bearing bones.

Biomaterials with or without cells can be used for implantation. In regenerative medicine, different kind of biomaterials has been tested for clinical use. In the early 1990's, the focal articular cartilage defects were treated with autologous chondrocytes with biomaterials (19). PLA is shown to be non-toxic in bone or cartilage repair applications. PLA was successfully used to accelerate the rat tibia bone healing and bone formation. At the same time absence of infection or inflammation with PLA was shown in the studies regarding articular healing in osteochondral defects in the rabbit (11). There were no differences between osteogenic and chondrogenic differentiation on 3D PLA scaffolds in our study. Therefore, we can also say that 3D printed PLA scaffolds may be safely used both in bone and cartilage studies.

One of the concerns regarding PLA is that it may cause inflammatory reaction in long term (29, 30). Some studies have shown that the implanted PLA was found to be well tolerated with no chronic inflammation for up to 39 weeks in rats (11). In our study, inflammatory response of tissue to PLA was not detected in 4 months' period, however, the long-term effects of PLA with stem cells have to be investigated.

Among the limitations we should note that we did not perform thermal, spectroscopic, and elemental analysis of our 3D printed scaffolds because it was not among the primary focus of our study.

## CONCLUSION

3D printed PLA scaffolds were used as three-dimensional environment during differentiation of bone marrow stromal mesenchymal stem cells to osteoblast or chondroblast cells showed excellent support for both bone and cartilage cell formation. In addition, there were no toxic effects of PLA scaffold during differentiation period of mesenchymal stem cells. Therefore, patient-based 3D printed PLA scaffolds with different type of mesenchymal stem cells may be conveniently used for in vivo and further clinical studies.

## Ethics

There are no ethical issues after the publication of this manuscript.



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# First-Trimester Missed Abortion: A Case Report

## Birinci Trimesterde Missed Abortus: Olgu Sunumu

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### ABSTRACT

According to World Health Organisation (WHO), spontaneous abortion generally occurs in 10% to 15% of clinically recognized pregnancies, although many spontaneous abortions occur before the woman recognises she is pregnant. Traditionally, treatment using surgical management which involves dilation and curettage and the manual vacuum aspiration of the product of conception is preferred for incomplete and missed abortion. Additionally, medical management with the use of prostogladin analogues such as misoprostol and dinoprostol with or without antiprogesterone (mifepristone) to expel the products of conception has also been in use. However, recently, the expectant management, defined as waiting for the foetus remains to be naturally expelled from the uterus, is being researched and experimented with more. In this case report, the case of a 35-year-old woman who experienced missed abortion during the 7<sup>th</sup> week of pregnancy is reported and the possible best management options is discussed.

**Keywords:** Expectant management; first trimester; missed abortion; pregnancy

### ÖZ

Dünya Sağlık Örgütü'ne (WHO) göre, spontan abortus, genellikle klinik olarak tanınan gebeliklerin % 10 ile % 15'inde meydana gelir, ancak birçok spontan abortus, kadın gebe olduğunu fark etmeden önce gerçekleşir. Geleneksel olarak, tamamlanmamış ve missed abortuslar için dilatasyon/ kürtaj ve gebelik ürünlerinin manuel vakum aspirasyonu ile yapılan cerrahi tedavi tercih edilir.

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Ayrıca, gebelik ürünlerini atmak için misoprostol ve dinoprostol gibi prostogladin analoglarının, antiprogesteronla (mifepriston) veya antiprogesteron olmadan kullanımı da tıbbi tedavi de yer almaktadır. Ancak son zamanlarda fetüsün doğal olarak uterustan atılmasını beklemek olarak tanımlanan bekleme yönetimi, daha fazla araştırılmakta ve denenmektedir. Bu olgu sunumunda gebeliğinin 7. haftasında missed abortusu geçiren 35 yaşındaki bir kadın hastada olası en iyi tedavi seçenekleri tartışılmaktadır.

**Anahtar Kelimeler:** Bekleme tedavisi; birinci trimester; gebelik; missed abortus

### INTRODUCTION

Abortion is the death and expulsion of the foetus from the uterus either spontaneously or by induction before the foetus is viable (more than 20 weeks/ more than 500g) (1). The specific number of weeks may vary from one country to another. According to World Health Organization (WHO), abortion generally occurs in 10% to 15% of pregnancies, but many spontaneous abortions occur before the woman realizes that she is pregnant (1,2). Abortion may be caused by embryonic/foetal, maternal, paternal and unknown factors. Genetic abnormalities account for 55% of all spontaneous abortion with the most common chromosomal defects being autosomal trisomies, polyploidy and monosomy X. Endocrine factors also play a role in spontaneous abortion where corpus luteum failure causes insufficient progesterone. Additionally, polycystic ovarian syndrome, uncontrolled diabetes and untreated thyroid diseases have been found to have a relation with spontaneous abortion (1).

Maternal illnesses and infections, for example, influenza, pyelitis, and malaria; congenital abnormalities of uterus, cervical incompetence, autoimmune diseases, thrombophilic effects and alloimmune diseases are some maternal factors as well. Paternal factors include teratospermia and oligospermia. Increased maternal age, alcohol use, heavy caffeine use, chronic maternal diseases, cigarette smoking,

cocaine use, conception within 3 to 6 months after delivery, intrauterine device use, medications, multiple previous elective abortions, previous spontaneous abortion, toxins have been found to be risk factors for spontaneous abortion as well (1,3).

The types of spontaneous abortion include the following. Threatened abortion (*abortus imminens*); this is the situation where the pregnancy is complicated by bleeding before the foetus is considered viable. This is characterised by fresh blood and pelvic pain. Fetal heart rate (FHR) is positive if the pregnancy is between the 8-10th week (4,5).

Inevitable abortion (*abortus incipiens*); it occurs when the cervix dilates (more than 3 cm) but the products of conception have not yet been passed. During ultrasonography, FHR is undetectable, endometrial integrity is disturbed. Beta human chorionic gonadotropin (B-HCG) is below normal for that week of pregnancy. Incomplete abortion; some but not all of the products of conception have passed. This is characterised by contractions and heavy bleeding. Complete abortion; it occurs when all the products of conception have passed without surgical or medical intervention. This is characterised by bleeding which reduces with time, and the pain stops which later on. Missed abortion; it is the death of the foetus without any uterine activity to expel the foetus. This is characterised by loss of pregnancy symptoms, reduced size of uterus, and reddish brown spotting. Pain is absent septic abortion; this is a spontaneous abortion complicated by uterine infection and is characterised by a temperature of 38 degrees or more. Recurrent spontaneous abortion; this the situation where the pregnancy has been lost 3 or more consecutive times (1,4,5).

In the management of abortion, surgical treatment which involves dilation and curettage (D&C) and manual vacuum aspiration is traditionally used. Medical management involves the use of prostaglandin analogues such as misoprostol and dinoprostol with or without antiprogesterone (mifepristone) to expel the products of conception. Yet there is the more modern expectant management which involves waiting for the foetus remains to be naturally expelled from the uterus (1,6).

The role of a midwife in abortion care involves taking records, providing bed rest, closely monitoring bleeding and vital signs, preparing the patients for D&C (stopping food intake), administering prescribed medication, preparation of blood for transfusion, providing information and emotional support for the patient (3,5).

Similar to the other managements for abortion, the midwife plays an essential role in the expectant management

of spontaneous abortion in addition to their basic roles. Particularly in the expectant management, there is a requirement for robust counselling, support for treatment, follow-up and support for grief (7). Especially in cases where the midwife is the primary caregiver, thorough counselling which includes why the expectant management has been indicated, what the advantages and the disadvantages of this management are, the success rate of this management, the option to opt out of this whenever the patient wants to, what will happen during this period (e.g. pain, bleeding, the expected size of the products of conception that will be passed etc), the availability of painkillers to manage pain during this period, and the signs of infection and haemorrhage to watch out for in order for the patient to make an informed decision is always necessary. Follow-up after 7-10 days and a pregnancy test three weeks after miscarriage to confirm a successful miscarriage should also be encouraged. As a provider of individualised care, the midwife also plays an important role in supporting grief based on the culture, religion and personal beliefs of the patient. What usually happens during the grieving season and how best to manage grief should be explained as well. This case report aims at deliberating how best to manage this case (1,4,5).

## CASE

This case report was carried out according to the principles of the Declaration of Helsinki and voluntary consent was obtained from the patient for the obstetric history. A 35-year old patient, who was 7 weeks and 3 days pregnant based on her last menstruation date (01/09/2018), presented to the obstetrics and gynaecologic clinic with 7 weeks of amenorrhea after her last menstruation, fatigue, itchiness and brownish spotting. Her obstetric history was gravida 2 and para 1 (cesarean section).

Her vitals were found to be normal: temperature, pulse, respiration rate, blood pressure and oxygen saturation were measured as 36.4°C, 90 beats/min, 20 breaths/min, 100/70 mmHg, and 99% respectively. Upon physical examination and interrogation, it was discovered that symptoms of pregnancy in breasts were disappearing and that there was no pain. An ultrasound revealed a gestational sac with an intact foetus but without any foetal heartbeat. b-HCG less than 7650 mIU/ml but more than 1500 mIU/ml. Laboratory tests showed haemoglobin, haematocrit and leucocytes were 12.2 gr/dL, 37.8% and 315 respectively. Her blood type was O Rh+

After analysing the laboratory results, physical examination and ultrasound, she was diagnosed with missed

abortion and dilatation and curettage was decided as the treatment option.

## DISCUSSION

A presentation of reddish brown spotting after 7 weeks of amenorrhoea has differential diagnoses to include implantation bleeding especially if the patient was on contraceptives, infection, abortion, ectopic pregnancy and molar pregnancy. A diagnosis of missed abortion was confirmed based on the absence of FHR and the presence of an intact foetus, although there were changes in the size of the uterus, amniotic cavity, and an intact embryo during ultrasound; and b HCG level lower than what corresponds to the level for 7 weeks in addition to physical examination findings. Infection was ruled out due to a normal temperature and the absence of signs of infection in the laboratory test results. Rh immunoglobulin administration was not necessary since the mother was Rh(D) positive (3, 8).

In the management of the various cases of abortion including missed abortion, prompt surgical evacuation has been the traditional management of choice due to the risk of infection and coagulation disorders and also the absence of ultrasonography until about 50 years ago (3). However, the surgical method is without its disadvantages. Surgical evacuation has been associated with complications including bleeding, infection, uterine and cervical perforation, bowel damage and decreased fertility. Considering these complications, with the availability of ultrasonography and antibiotics, successful outcomes for expectant and medical management and a significant percentage of failure of D&C, there is the question of whether surgical treatment should continue to be the first option of management, especially if the patient is in a stable condition (if there is no heavy bleeding, unacceptable pain or infection) (8).

Luisse et al. (2002) report 81% and 76% successful outcome for expectant management in all abortions and missed abortion respectively (5). Rafi et al. (2014) report 54% success rate for the expectant management and also reported that 74% of patients upon detailed counselling opted for the expectant method (4). Expectant treatment had an overall success rate of 92.5% based on 9 studies representing 545 pooled patients (8). It has been reported that women who were given the opportunity to choose their treatment had a better subsequent mental health indicating that if a complete spontaneous abortion should be safe, effective, acceptable to patients and available at the lowest cost and risk, then the expectant management should be the

first choice of treatment (3, 8, 9, 10). The case discussed here was stable without heavy bleeding, unacceptable pain or infections, therefore, the expectant management could be used upon detailed counselling, however, surgical management was the treatment of choice.

Although the Health Ministry recommends the use of expectant management especially for abortus imminens and incomplete abortion the normal practice in most hospitals is the use surgical management as first choice of treatment (11). Inadequate counselling on the expectant management of abortion, unwillingness to lose time and avoidance of any risks involved in this method are thought to be reasons for this situation.

## CONCLUSION

The patient who had been diagnosed with missed abortion is in a stable condition. It is recommended that she be given detailed counselling about the expectant management and encouraged to choose it because it is effective, safer, and less costly.

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# Mitophagy and Obesity

## Mitofaji ve Obezite

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### ABSTRACT

Overfed and high-fat diets lead to many metabolic changes such as deterioration in energy homeostasis and aerobic respiration, increase ROS (reactive oxygen species) products, and inflammation status in obesity. Obesity-mediated occurred oxidative stress and low-level chronic inflammation can result in impaired mitochondrial homeostasis, which has been involved in the pathophysiological of metabolic syndrome-related diseases such as Type 2 Diabetes Mellitus. The mitochondria organelle plays an effective role in energy and calcium homeostasis, cell metabolism, and even cancer cell metabolism. Mitophagy known as a mitochondrial-recover operation has been thought as a critical factor in supporting mitochondrial hemostasis, which can make a great contribution to recovery from cellular abnormalities in obese patients. This review's aim is to ensure a current and comprehensive summary of the role of developing mitochondrial-related dysfunction and protective effect of mitophagy in the pathophysiology of obesity.

**Keywords:** Obesity, Mitophagy, Mitochondrial Dysfunction, Adipose Tissue

### ÖZ

Obezitede aşırı beslenme ve yüksek yağlı diyetler, enerji homeostazında ve aerobik solunumda bozulmalara, reaktif oksijen türlerinin (ROS) üretiminde artışa ve inflamasyon durumu

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gibi bir çok metabolik değişikliğe sebep olmaktadır. Obezite aracılı meydana gelen oksidatif stres ve düşük seviyeli kronik inflamasyon, Tip 2 Diabetes Mellitus gibi metabolik sendromla ilişkili hastalıkların patofizyolojisinde rol oynayan mitokondriyal homeostazın bozulmasına neden olabilir. Mitokondri organeli enerji ve kalsiyum homeostazında, hücre metabolizmasında hatta kanser metabolizmasında etkin rol oynamaktadır. Mitokondriyal operasyonu olarak bilinen mitofaji, obez hastalarda mitokondriyal hemostazın korunmasına ve hücre anormalliklerin iyileşmesine büyük katkı sağlayan kritik bir faktör olarak düşünülmektedir. Bu derlemenin amacı, obezite patofizyolojisinde mitokondriyal disfonksiyon gelişiminin ve mitofajinin koruyucu etkisinin güncel ve kapsamlı bir özetini sunmaktır.

**Anahtar Kelimeler:** Obezite, Mitofaji, Mitokondriyal Disfonksiyon, Yağ Dokusu

### INTRODUCTION

Obesity has an epidemiological profile with a major trend worldwide and is characterized as abnormal weight gain or excessive overfat accumulation that may harm health, resulting from impaired energy hemostasis (1). Considering the increase in obesity prevalence from an evolutionary perspective, increasing the fat and sugar content in the diet and adopting a sedentary lifestyle are considered, obesity is multifactorial, which is due to genetic causes as well as environmental factors (2). Increasing adipose tissue in obesity occurs critical and different changes such as oxidative stress, low-level inflammation, mitochondrial dysfunction, apoptosis, insulin resistance (IR), glucose intolerance, and dyslipidemia (3). Furthermore, obesity plays an important role in the development of serious diseases such as Type 2 Diabetes Mellitus (T2DM), heart diseases, atherosclerosis, coronary artery disease, hypertension, and even some types of cancer (4). The World Obesity Federation reported that obesity is approved an epidemic and is accepted as a significant public health issue facing that is correlated with a grown risk of mortality rate and morbidity rate around

the world (5). Obesity leads to inflammation in related to a progressive increase in reactive oxygen species (ROS) and oxidative stress in adipose tissue. Moreover, Increasing inflammation can result in mitochondrial imbalance and mitochondrial dysfunction (6,7). Mitochondria play an effective role in fuel and energy hemostasis and all cell metabolism, including, beta-oxidation of fatty acids, adenosine triphosphate (ATP) synthesis from nutrition substrates, ROS, amino acid metabolism, iron metabolism, heme and steroid hormone process, and calcium metabolism (8,9). In addition, the mitochondrial respiratory chain complexes are related to the synthesis and elimination of ROSs during ATP production in mitochondria. The mitophagy pathway is described as a system controlling mitochondria function and content (1,10–14). We discuss the features of pathophysiological changes in adipose tissue and how trigger mitochondrial dysfunction by focusing on the role of mitophagy.

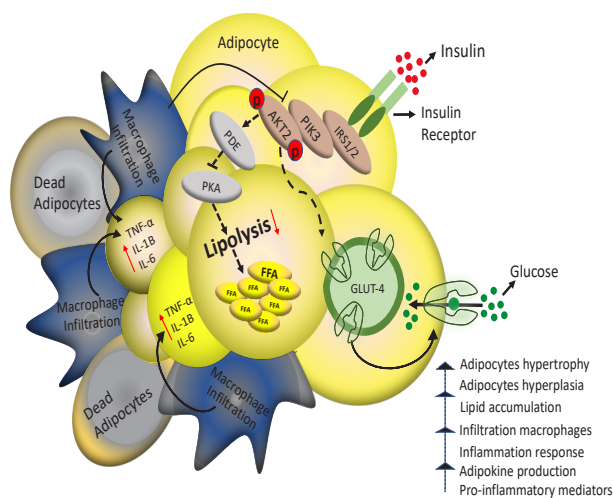
### Inflammation in Adipose Tissue

Adipose tissue plays an effective and critical role in fuel metabolism for energy hemostasis and is considered an important endocrine organ. Consuming excessive calories causes adipocyte hypertrophy and hyperplasia, which leads to dysfunctional adipose tissue formation in obesity (6,15). Adipose tissue secretes several bioactive mediators described as pro-inflammatory cytokines, (as well as it is called adipocytokine or adipokines) and it serves both as controllers of the metabolism and immunomodulatory properties (11,16).

In obesity, there are several mechanisms triggering low-level chronic inflammation of the adipose tissue. Increased accumulation of fatty acid causes adipocyte hypertrophy and impaired bloodstream by triggering the production of proinflammatory cytokines, particularly via the nuclear factor kappa B (NF-κB) signaling pathway. As well as enlargement of the volume of infiltrated macrophages causes synthesis of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), adiponectin, visfatin, interleukin-1β (IL-1β), and interleukin-6 (IL-6), in adipocytes that are secreted from infiltrated macrophages that lead to the process described as low-grade chronic inflammation (7,17,18). Low-level chronic inflammation in the long-term may turn into chronic systemic inflammation and lead to the occurrence and advancement of metabolism-related disorders, such as IR, hyperlipidemias, hypercholesterolemia, T2DM, cardiovascular diseases, and atherosclerosis (10–12).

Tumor Necrosis Factor-alpha (TNF-α) leads to lipolysis, which causes the release of saturated free fatty acids that are capable of binding Toll-like receptor 4 on both the adipocyte and the infiltrated macrophages leading to NF-κB activation and secretion of IL-1β and IL-6 via macrophages (as seen in Figure 1). Moreover, TNF-α and IL-1β increase impairs the insulin signaling pathway and may lead to the development of IR. Then, this process is attended by the adipocyte release of monocyte chemoattractant protein–1 (MCP-1) resulting in macrophage aggregation and activation (10).

Consequently, both impaired insulin signaling pathways and increased lipolysis prevent insulin-responsive glucose transporter (GLUT-4) translocation across the plasma membrane and glucose uptake and lead to glucotoxicity and lipotoxicity (19,20). The decrease in glucose oxidation and oxidative phosphorylation (TCA) leads to reduced electron flow during the electron transport chain (ETC), which trigger electron outflow toward oxygen and production of ROS such as superoxide, hydrogen peroxide, and hydroxyl ions followed by oxidative stress (9,21).



**Figure 1.** Overfed and a high-fat diet leads to inflammation in obesity. PKA: Protein kinase A, FFA: Free fatty acids, GLUT4: Glucose transporter 4, IRS1/2: Insulin receptor substrate 1/2, PDE: Phosphodiesterase, AKT2: Protein kinase B, PIK3: Phosphoinositide-3 kinase, PKA: protein kinase A

### Oxidative Stress and Mitochondrial Dysfunction in Obesity

The antioxidant defense system can both decrease ROS levels and enhance control of cell damage. The antioxidant defense mechanisms consist of enzymatic (e.g., glutathione peroxidase enzymes, catalase, and superoxide dismutase) and non-enzymatic (e.g., products from nutrients such as vitamins, carotenoids, uric acid, and polyphenols) (21,22).



with dysregulations of mitochondrial autophagy process (1,13,14).

There are two ways for inducing mitophagy; firstly, by an accumulation of mitochondrial defects that trigger ETC impairment, and secondly, by dissipation of mitochondrial membrane potential. The molecular mechanism of mitophagy is usually divided into two pathways (as seen in Figure 2): Ubiquitin-dependent PINK1/Parkin-mediated mitophagy pathway and Ubiquitin-independent receptor-mediated mitophagy pathway (19,36).

The most-studied pathway is mediated by the phosphatase and tensin homolog (PTEN)-induced kinase 1 (PINK1) and the E3-ubiquitin ligase PARK2 (Parkin). PINK1 and Parkin's genes are related to autosomal recessive Parkinson's types (37). The PINK1/Parkin mitophagy pathway is activated by oxidative stress, mitochondrial dysfunction and damage (as seen in Figure 2). Under unstress and healthy mitochondrial conditions, PINK1 is replaced in intermembrane via TOM/TIM protein complex and degraded via matrix processing peptidase (MPP) and presenilin-associated rhomboid-like (PARL) protease (19). Increased oxidative stress in the cell causes disruption of mitochondrial membrane potential and inhibition of TOM/TIM complex in mitochondrial intermembrane space (14). PINK1 isn't transported to the mitochondria and accumulates in outer membrane (MOM) and this situation leads to starting Parkin activation to ubiquitination process. Active Parkin protein plays an important role as a signal to autophagy receptors optineurin (OPTN), and nuclear domain 10 protein 52 (NDP52) and leads to polyubiquitination process in MOM proteins such as mitofusin 1/2 (MFN1/2), voltage-dependent anion-selective channel 1 (VDAC), and mitochondrial fission 1 protein (FIS1). Ubiquitin activation via Parkin resulting accumulation of polyubiquitinate MOM proteins such as the autophagosome adaptor protein p62, which initiates autophagosome interactions with autophagy-related 16-like 1 (ATG16L1) complex and LC3 in MOM. The autophagy core complexes such as class III PI 3-kinase (VPS34) and unc-51 like autophagy activating kinase (ULK1/2) are the source of phagophore membrane of the ATG16L1 complex and LC3 that followed the formation of autophagosomes (14,19,38).

Mitochondrial homeostasis is necessary for a balance between mitochondrial biogenesis and mitophagy. Elongated mitophagy leads to bio-energetic setback while excess upregulation of mitochondrial biogenesis cause increased levels of ROSs and supports apoptosis. Although

recent studies comprehensively revealed the role of PINK1/Parkin-mediated pathway in mitophagy, the involvement and connection of PINK1/Parkin pathway has been not enough enlightened in obesity and needs future studies. The first studies included that decreased mitochondrial content and impaired activity of mitochondrial enzymes in obese and/or T2DM patients (39,40). Recent studies mostly relate to the expression of genes and proteins associated with mitophagy in metabolic diseases (9,41–44). Wu et al. showed that PINK1/Parkin genes expression were activated and increased as in response to metabolic stress in diabetic obese mice (41). Another study reported that reduction of mitochondrial function is in relationship with IR and mitochondrial dysfunction and is accompanied by downregulation in mitofusin 1/2 (Mfn1/2) expression in skeletal muscle in obesity (9).

Furthermore, another study showed that knockout PARK2 leads to raised regulation of basal macroautophagy/autophagy flux, reduced mitochondrial respiration capacity, and upregulation of dynamin-related protein 1 (DRP1) that increases mitochondrial damage in skeletal muscle (42). Similarly, the study reported that Parkin gene knockout that regularly exercised mice increased of mitochondria amount of skeletal muscle and decreased ROSs production lead to impaired in mitochondrial respiration chain and reduced of mitochondria quality (43). Chen et al. reported that mice fed a HFD have increased gene expression of PINK1/Parkin in adipose tissue but levels of high concentrations of lipid can be inhibited gene expression of PINK1/Parkin in long term (45).

Obesity leads to hypertrophy and hyperplasia of the adipocytes in adipose tissue. Adipocyte hypertropia leads to impaired blood flow and cardiac outflow, increased local necrosis and macrophage infiltration, and proinflammatory responses that result in hypoxia and adipocyte death. Hypoxia-inducible factor-1 $\alpha$  is defined as a transcription factor that is secreted under hypoxic conditions and causes mitophagy receptors' activation by dephosphorylation (12,14,19).

The receptor-mediated mitophagy pathway (as seen in Figure 2) is activated as an adaptive response under hypoxia conditions to remove dysfunctional or damaged mitochondria and favors mitochondrial remodeling. The proteins found in the mitochondrial membrane play a crucial role in the receptor-mediated mitophagy pathway such as FUN14 domain-containing protein 1 (FUNDC1), BCL2 interacting protein 3 (BNIP3), and BNIP3-like protein



(BNIP3L, also known as Nip3-like protein X, NIX) (38). These receptors include the conserved LC3 interacting-region (LIR) domain thereby they interact directly via LC3 and other autophagy-related proteins (ATGs) for formation of autophagosomes (14,46). The study reported that these receptor proteins can also coordinate mitochondrial fission via DRP1 by causing its localization to mitochondria (47). Greene et al. investigated how the typical western diet (WD) was affected by obesity of mitochondrial autophagy control mechanisms and found that p62 and BNIP3 gene expression levels were decreased in WD-induced obesity (48). Fu et al. showed that FUNDC1 plays an influential role as a mitophagy receptor protein in preventing HFD-induced obesity, and mitochondrial energy homeostasis is impaired and the LC3-mediated mitophagy pathway is inhibited in FUNDC1 knockout mice fed HFD (13).

## CONCLUSION

Taken as a whole, the existence of low-level chronic inflammation and prolonged hypoxia conditions in obesity may lead to oxidative stress and increased ROS products, which in turn, can cause metabolic inflexibility, mitochondrial defect, mitochondrial dysfunction, and mitophagy pathway activation. The investigation of mitophagy pathway involved in obesity is a recent interest field as mitochondrial abnormalities and dysfunction are the foundation of metabolism-related diseases. The important role of mitochondria in regulating fuel homeostasis strengthens the opinion that metabolic diseases such as obesity are the most affected by mitochondrial dysfunction and damage.

Mitochondrial autophagy/mitophagy plays a crucial role in controlling the detrimental effect of mitochondrial abnormalities, hypoxia conditions, and oxidative stress by regulating mitochondrial quality and quantity. Furthermore, impairment of the mitophagy pathway can result in decreased glucose oxidation and thus aggravating lipid accumulation in fat, liver, and muscle tissue. Transcriptional regulatory mechanisms of mitophagy-related genes may be hopeful and quite target-driven therapies for metabolic diseases and for preventing age-related diseases.

In this context, more studies are needed to ameliorate obesity and better understand the mitochondrial processes of the mitophagy pathway and diet-induced mitochondrial degeneration.

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## Conflict of interests

The authors declare no conflict of interests.

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