Human Mesenchymal Stem Cells Osteogenic Differentiation on Dental Barrier Membrane
İnsan Mesenkimal Kök Hücrelerinin Dental Bariyer Membran Üzerinde Osteojenik Farklılaşması

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***Abstract*—In the field of tissue engineering, there are biodegradable bone implants with biocompatible synthetic polymers that provide successful results in many areas. Dental barrier membranes are bioabsorbable polylactide (PLA) membranes designed for use in many applications of guided bone regeneration (GBR). It provides a structure designed to attract, capture and retain fibroblasts and epithelial cells while protecting the area around the tooth for the development of bone and periodontal supporting tissue. The aim of this study was to evaluate the properties of dental barrier membranes that inhibit cell migration and promote bone formation differentiation using bone marrow stem cells (BMSCs) with high differentiation and proliferation properties. As a result of the study, characterization studies and cell viability experiments of the Synthetic Barrier Membrane product were carried out, and it was observed that it had a positive effect on the adherence and viability of the BMSCs.**

***Keywords*—dental barrier membrane; bone marrow mesenchymal stem cell (BMSC); bone regeneration**

***Özetçe*—Doku mühendisliği alanında birçok alanda başarılı sonuçlar veren biyouyumlu sentetik polimerlere sahip biyobozunur kemik implantları bulunmaktadır. Dental bariyer membranlar, kılavuzlu kemik rejenerasyonunun (KKR) birçok uygulamasında kullanım için tasarlanmış biyolojik olarak emilebilir polilaktid (PLA) membranlardır. Kemik ve periodontal destek dokusunun gelişimi için dişin etrafındaki alanı korurken fibroblastları ve epitel hücrelerini çekmek, yakalamak ve tutmak için tasarlanmış bir yapı sağlar. Bu çalışmanın amacı, yüksek farklılaşma ve çoğalma özelliklerine sahip kemik iliği kök hücreleri (BMSC'ler) kullanılarak hücre göçünü önleyen ve kemik oluşumu farklılaşmasını destekleyen dental bariyer membranlarının özelliklerini değerlendirilmesidir. Çalışma neticesinde, Sentetik Bariyer Membran ürünün karakterizasyon çalışmaları ve hücre canlılık deneyi yürütülerek, kemik iliği kök hücresinin tutunumu ve canlılığına pozitif etki ettiği gözlemlenmiştir.**

***Anahtar Kelimeler*—dental bariyer membran; kemik iliği kök hücresi; kemik rejenerasyonu**

I. INTRODUCTION

Tissue engineering (TE) is an interdisciplinary science that works by coordinating many subfields of science with engineering principles to ensure tissue regeneration. It aims to provide organizational restrictions by applying for both engineering principles and medicine. Bone tissue problems or bone defects can result from infections, tumors, osteolysis, osteomyelitis, periodontitis, or traumatic fractures. There are studies aimed at addressing and solving many clinical problems such as spinal fusion, joint replacement, tumor treatment, pathological bone loss, and fracture healing. Therefore, bone tissue regeneration is becoming a requirement for bone tissue engineering (BTE) applications. Bone induction is the ability to induce osteoblast formation through bone growth from the tissue surrounding the graft host site [1-3]. Bone conduction is the promotion of bone growth on the surface of the graft material. Factors such as the number of grafts available and the harvesting method limit the use of autologous grafts. These limiting factors allowed the formation of other types of grafts. Allografts also called allogeneic, homologous, or homografts, consist of material from another individual of the same species. Xenografts, also known as heterografts or xenogenic grafts, are materials from another species [4]. Very popular in bone tissue research, stem cells have the ability to maintain the population and regenerate themselves to produce more stem cells. One of the most common types of stem cells with these general characteristics is mesenchymal stem cells (MSCs). MSCs are a type of stem cell that forms the basis of stromal cells found in connective tissue and can differentiate in any environment and be easily transferred from the tissue in which they reside to the damaged tissue [5-7]. Due to the high proliferation rate, differentiation, and regeneration properties of MSCs, BMSC is often the preferred cell line for BTE studies, especially laboratory studies of bone tissue biomaterials. Specifically for the development of bone reconstruction techniques, MSCs have been observed to be first placed in three-dimensional (3D) biomaterials, leading to bone formation after transplantation and directly contributing to the repair of many bone defects [8].

Biomaterials are known to be used in trauma, fractures, loss of quantity and quality of bone structure, surgical support of bone tissue due to tumor reasons, orthopedics, spine, dentistry, and accident surgery. Especially in the field of biomaterials, it has been shown to help direct cell behavior by providing 3D space for cell proliferation, interaction, and differentiation. The 3D structure of biomaterials and the microenvironment they provide to cells are important for cells to attach, proliferate, perform their functions, and exhibit differentiation functions. 3D systems with synthetic or natural biocompatible scaffolds have been shown to support bone formation, hematopoiesis, and neural differentiation [9,10]. In addition to the biocompatibility of biomaterials for BTE, their mechanical strength and specific mechanical properties support the bone formation and differentiation of cells. The quality of biomaterials used for effective periodontal tissue regeneration can control the formation of new tissue by providing stem cell proliferation and differentiation.

In biomaterials, the concepts of osteoinduction and osteoconduction, which BTE researchers especially focus on, also gave direction to biomaterial production studies. Researchers have demonstrated the osteoconductive effect of synthetic absorbable polymer materials. TE applications draw attention to various and effective methods in restructuring damaged tissues in both engineering and medicine fields. One of the effective applications of TE is guided tissue regeneration (GTR). One of the areas where TE applications are highly effective is the studies aiming at repairing the damage to bone tissues caused by trauma, infection, and tumor formation. In bone tissue repair, a very common method in clinical medicine is guided bone regeneration (GBR) applications. It is used in many bone defect applications in clinical areas to support bone cell proliferation and to make an effective application. Guided bone and tissue regeneration applications, like many other TE applications, require a biomaterial to support, stimulate, and direct cell formation. Particularly in dental applications, GBR protocols for dental regeneration are frequently applied clinically today [11]. In dental applications, products called dental barrier membranes are used for periodontal tissue regeneration, especially for bone augmentation associated with implant treatments. In addition, the use of a dental barrier membrane product is a very effective application for providing osteogenesis. It is an effective application used to prevent epithelial cell migration, which can prevent osteopromotion and osteogenesis, and prevent fibroblasts from preventing bone formation with a dental barrier membrane product. Barrier membranes in the medical device market are designed to promote tissue regeneration and can be differentiated according to the biodegradability of the base material. The use of biodegradable barrier membranes has gained momentum in GBR studies [12]. In this study, it was aimed to determine the features of dental barrier membranes that prevent cell migration and encourage bone formation and differentiation by utilizing BMSCs.

II. Materials & Methods

A. Characterization of Synthetic Barrier Membrane

The morphology of the Barrier Membrane was observed using a Scanning Electron Microscope (SEM) (Carl Zeiss 300VP, Germany) at Izmir Katip Celebi University Central Research Laboratory. A thin layer of gold was coated on the surface of the barrier membranes by using an automatic sputter coater (Emitech K550X) to reduce the extent of sample arcing during SEM observation.

For the mechanical characterization experimental study, a tensile test was performed by using a universal testing machine having a 500 N load cell (Shimadzu AGS-X Model, Japan) at Izmir Katip Celebi University Biomechanics Laboratory. The tensile test of the barrier membrane samples was carried out according to the ASTM D638 standard, and the crosshead speed was selected to be 50 mm/min. The test was repeated at least three times to check for repeatability.

B. MSC Cultivation and Proliferation

Human bone marrow mesenchymal stem cells (hMSCs) (HMSCAD500, CLS Cell Line Services, Lot # 102, Eppelheim, Germany) used in bone marrow differentiation studies were obtained and implemented at Izmir Katip Celebi University Faculty of Biomedical Engineering. Cells were cultured in standard polystyrene cell culture dishes in a basal medium containing DMEM: F12, 10 µg fetal bovine serum (FBS), 100 units/ml penicillin, 100 µg /ml streptomycin, 50 µg/ml gentamicin, 250 ng/ml fungizone.

C. Cell Seeding

After sterilization by Ultraviolet radiation, the samples were conditioned in a basal medium for 1 hour, then each sample was seeded with MSC cell suspension (5 x 106 cells / cm2) in a basal medium. After 24 hours of incubation for cell adhesion, the medium was replaced with a bone formation medium (basic medium supplemented with 100 nM dexamethasone, 50 µg / ml ascorbic acid, 10 mM β-glycerophosphate) and in a humidified incubator containing 5

D. Cell Viability Observation

The live/dead cell viability assay was used to assess cell viability on well plates by fluorescence staining and fluorescence microscopy. A double staining kit (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) was used. Briefly, the viable cells (green fluorescence) and dead cells (red fluorescence) were studied using a fluorescence microscope after 45 min of incubation in a culture medium supplemented with Calcein AM/DMSO (used for viable cells) and propidium iodide/purified water (used for dead cells).

III. RESULT AND DISCUSSION

A. Characterization of Synthetic Barrier Membrane

The Synthetic Dental Barrier Membrane product (Ref#PM1520, Bonegraft Biomaterials Co., Turkey) has

double-layer fiber structure with bioresorbable polylactide (PLA) as the main raw material (Fig. 1). Synthetic Dental Barrier Membrane is a uniquely structured bioresorbable PLA membrane designed to be used in many applications within GTR and GBR procedures.

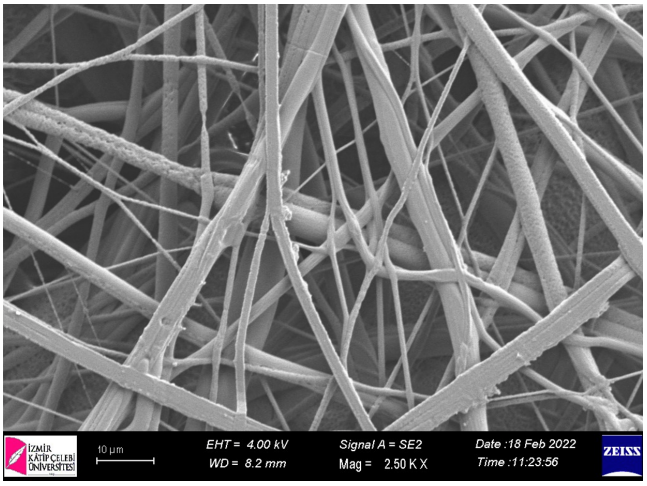

Figure 1: Scanning electron microscopy images of Synthetic Barrier Membranes (scale bar represents 10 µm).

Fiber Diameter (µm)	Measurement Number
1	3.257
2	3.362
3	3.638
4	4.744
5	3.503
6	2.072
7	4.953
8	3.274
9	4.260

Table I: Fiber diameter measurements on SEM image.

According to the fiber diameter measurements in Table I, an average of 3.6 10 µm diameter fibers and a homogeneous structure were observed. The homogeneous fiber structure creates an environment conducive to cell adhesion, proliferation and differentiation.

According to the tensile strength values of barrier membrane as shown in Table II, an average of 1,75337 MPa was measured. The mechanical results of the Synthetic Barrier Membrane show that the product can be applied without any problems despite the loads it will be exposed to during application, since the product will already degrade in the body, mechanical strength is not sought for the continuity of the Synthetic Barrier Membranes.

Sample	Tensile Strength (MPa)
Synthetic Barrier Membrane (Ref#PM1520, Bonegraft Biomaterials Co., Turkey)	1,63710
	2.04724
	1.57579

Table II: Tensile Strength (MPa) of Synthetic Barrier Membranes.

B. Cell Viability Observation

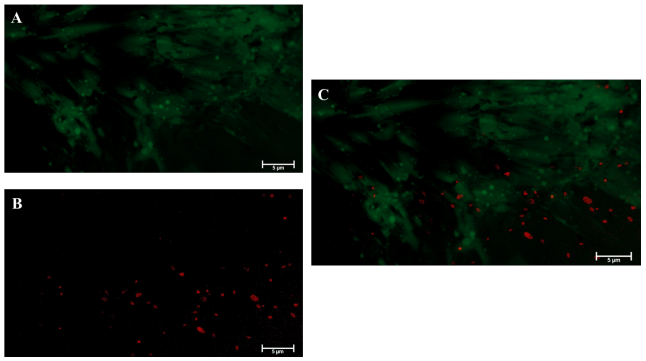

Figure 2: Application of Live & Dead Viability/Cytotoxicity assay on dental barrier membranes. A) Live cells' view, B) Dead cells' view, C) Combined image of Live and Dead cells.

Fig. 2 shows the image of living and dead cells on dental barrier membranes. In the images, the red color shows dead cells, and the green color shows live cells. Separate screenshots of live and dead cells were taken, and two images were combined to give the color intensity of live & dead cells.

As observed in Fig. 3, the viability of the cells shown in green in the experimental group was considerably higher than in the control group, and the red color was quite low. However, in the control group, because there is a film layer and there is no fiber structure, it is seen that both the green cells that appear green are less, and the cell adhesion is low. These findings reveal that cell viability and adhesion were superior in the experimental group due to the fiber structure since the green color intensity in the experimental group was higher than that of the control group. Based on these, we can say that dental barrier membranes in this study can provide a good microenvironment for hMSCs seeded in vitro. As a result of mechanical tests and cell viability studies, it is supported that the surgical application of the Synthetic Barrier Membrane in the dental surgery field has a positive effect compared to the use of non-biodegradable products. The use of biodegradable barrier membranes is increasing nowadays because they do not require a secondary operation. With the raw materials and production methods used, the product is biologically harmlessly degraded in the body by providing the necessary mechanical and biological expectations within the body. It has been observed that the expected mechanical

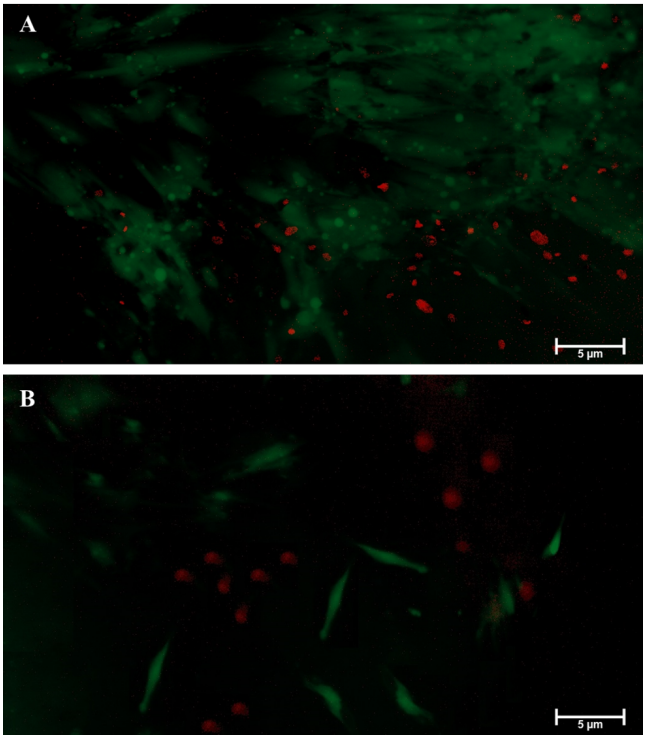

Figure 3: Combined viability images of cells on a 10x magnification scale. A) Combined viability image of the experimental group which is fibrous barrier membrane 5mm x 5mm sample, B) Combined viability image of the control group which is film layer 5mm x5mm sample.

properties of the study and the cell work done, and the cell adhesion are a product that has positive effects thanks to the fiber structures.

IV. CONCLUSION

Based on the performed analyses, we observed that dental barrier membranes showed a positive impact on the viability of hMSCs. Besides, osteogenic differentiation studies of bone marrow stem cells on the dental barrier membrane product are continuing. In conclusion, it is believed that these synthetic dental barrier membranes might be promising biomaterials in BTE applications. Moreover, these barrier membranes might be developed with further studies in the future.

AUTHOR CONTRIBUTIONS

In this study, Bahar Utar reviewed the literature, established, and implemented the experiment procedure, reported the study, Gülşah Sunal conducted the experimental procedure implementation studies, reviewed the writing of the study results, Günnur Onak established the experimental procedure, planned the application of the study experiments, evaluated the results of the study experiments, Ozan Karaman evaluated and approved the report of the study.

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