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Derleme

A New Dimension in Periodontal Regenerative Therapy: 3D Cell Culture

Periodontal Rejeneratif Tedavide Yeni Bir Boyut: Üç Boyutlu Hücre Kültürü

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ABSTRACT

Therapies established with three-dimensional (3D) culture environments, particularly developed by using Mesenchymal Stem Cells (MSCs), have come to the fore in recent years. Functions of tissues and organs with cell cultures, their behaviour in the case of an illness, and their interactions with drugs can be evaluated *in vitro*. This review examined the methods of creating 3D culture environments, their advantages, and disadvantages, as well as their use in periodontal regenerative therapy.

Keywords: Cell culture techniques; Guided tissue regeneration; Periodontal diseases; Periodontology

ÖZET

Periodontal rejeneratif tedavide özellikle Mezenkimal Kök Hücrelerden (MKH) yararlanılarak geliştirilen üç boyutlu (3B) kültür ortamları ile oluşturulan tedaviler son yıllarda ön plana çıkmaktadır. Hücre kültürleri ile doku ve organların fonksiyonları, hastalık durumunda göstermiş oldukları davranışları ve ilaçlarla olan etkileşimleri *in vitro* olarak değerlendirilebilmektedir. Bu derlemede 3B kültür ortamların oluşturulma metotları, avantaj ve dezavantajlarının yanı sıra periodontal rejeneratif tedavide kullanım alanları incelenmiştir.

Anahtar kelimeler: Hücre kültürü teknikleri; Periodontal hastalıklar; Periodontoloji, Yönlendirilmiş doku rejenerasyonu

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INTRODUCTION

Periodontium is composed of the gingiva, periodontal ligament (PDL), cementum, and alveolar bone, which are the basic tissues of the tooth. Biofilm accumulated on the tooth surface is an important etiological factor that induces pathological changes in periodontal tissues.[1]

Periodontal regeneration aims to reconstruct the original form and function of all components of the periodontium damaged by disease.[2] The goal of regenerative periodontal therapy is to regenerate alveolar bone and cementum and induce new periodontal ligament formation.[3]

Techniques for achieving periodontal regeneration may include root surface disinfection with chemical agents following Phase-I treatment, various graft materials (autogenous, allogeneic, synthetic), membranes as a physical barrier method, and the use of polypeptide growth and attachment factors.[2]

Another technique targeting the regeneration of the periodontium is the directed tissue regeneration technique which uses grafts and membranes together. With this technique, an increase in root surface regeneration can be achieved by giving priority to the cell populations that will provide periodontal regeneration.[4] Even though this technique has provided successful results, the degree of predictability of the results is low. After the use of graft materials or the placement of barrier membranes, an infection may develop in the tissue during the healing period, negatively affecting the regeneration. Although the purpose of the membrane barrier is to prevent epithelial migration, the majority of histological examinations have found epithelial tissue between the membrane and the tooth surface.[1]

These studies aiming at periodontal regeneration, unfortunately, could not achieve the goal of providing a complete regeneration of the tissues to their pre-disease state with these treatment methods. In this context, therapies created with two-dimensional (2D) and three-dimensional (3D) culture environments, particularly developed by using Mesenchymal Stem Cells (MSCs), are considered an important option.[5]

2D AND 3D CULTURE ENVIRONMENTS AND THEIR FEATURES

Tissue engineering is a field of science that aims to develop new tissue production techniques using biocompatible scaffolds and growth factors, as well as structures consisting of dissociated cells to replace damaged tissues. In tissue engineering studies, cell culture systems are used to keep cells alive in extracorporeal environments, thus solving the mechanisms and behaviors of various diseases and are based on the principles of cell biology, developmental biology, and biomaterial sciences.[6]

Controlled artificial environments (*in vitro*), which are created for research on growth and differentiation capacities, proliferation amounts, and monitoring of functions of cells in normal and abnormal conditions, are called cell cultures. In addition to the evaluation of cell-cell and cell-matrix interactions in the created cell culture environments, there are also studies such as evaluating the efficacy of drug administration for the treatment of disease models.[7]

Dr. Harrison first developed the 2D culture technique in 1907 to investigate the origins of nerve fibers explanted from frog embryos.[8] With this traditional technique, which has been used in cell culture studies since the early 1900s, studies were analyzed by ensuring the adhesion, displacement, and spread of cells in cell culture dishes with a flat surface.[9]

In a study on chickens conducted by Roux in the early 20th century, *in vitro* environments were created for the extraction and growth of animal cells by isolating them from the tissue, providing the reproduction and development of cells by giving the necessary medium and growth factors.[10]

Cell culture studies aim to reproduce cells *in vitro* by creating experimental conditions similar to the specialized functions of tissues and performing relevant analyses. The data obtained in the culture environment created for targeted regenerative therapy are provided by evaluating the quantitative and semi-quantitative results of analyses such as immunohistochemical stains, Real-Time PCR (RT-PCR), and Flow Cytometry.[11]

In monolayer 2D culture systems, one of the classical techniques to study the molecular mechanisms and

behavior of cells, cells are cultured by being placed on various planar surfaces or by being suspended in a liquid medium.[12] However, these systems do not fully reflect the behavior of cells and tissues in the body. Three-dimensional (3D) culture systems were developed at the beginning of the 20th century to eliminate the disadvantages of 2D culture and to imitate the natural environment of cells as in the body.[13] These systems are artificial systems in which cells can grow and interact in all directions, as in the *in vivo* environment.[14]

Three-dimensional cell culture is a model created to mimic the protein and other biological molecules present in the Extracellular Matrix (ECM) of tissues. Vital and physical features such as cell polarization, proliferation changes, RNA, and gene expressions observed in cells cultured with this modeling are to mimic the *in vivo* environment.[15]

In 2D cell culture, cells grow as a monolayer and are in direct contact with nutrients and gases in the culture medium. For this reason, they are equally exposed to all nutrients, growth factors, and drugs placed in the culture medium. In 3D experiments, on the other hand, cells are mostly in contact with other cells, while nutrient and gas exchange occurs by diffusion in the culture medium[16] (Figure 1).

The comparison of proliferation rates of cells in culture media has demonstrated contradictory findings depending on the cell type and the characteristics of the 3D culture environment created. For example, some researchers have reported faster proliferation of tumor cells in 2D cultures compared to 3D cultures.[17] On the other hand, MSCs have been found to proliferate more slowly in 3D cultures.[18]

Considering the life cycles of cells during culture, the cells forming the 2D culture environment are simultaneously in the same cycle stage, while some of the cells in the 3D culture medium proliferate, others may be in a hypoxic and necrotic state.[19]

A study examining the drug susceptibility of cells in different culture media showed that the drug interactions of cells cultured in 3D media were parallel to those *in vivo*, reporting that this interaction rate was higher than that of cells cultured in 2D media.[20] In addition, 3D cell culture systems were reported to increase the differentiation capacity of stem cells.[21]

Another study to determine the resistance of cells to drugs in 2D and 3D cultures reported that the gene and protein expression levels of cells in 3D culture were more consistent with the results obtained *in vivo*. The researchers interpreted this result as that the behavior of cells in 3D culture is more similar to *in vivo* conditions.[22]

Three-dimensional cell cultures can be modeled with different methods based on the cell type to be assessed and application techniques. Although each model has its differences and advantages and disadvantages, no technique has been proven to be superior to others. While performing 3D cell culture modeling, multicellular spheroids, organoids, scaffolds and hydrogels, chip organs, and 3D bioprinters are used.[23,24]

In the spheroid technique developed by Sutherland *et al*.[25] in 1970, the researchers studied the functional phenotype of human tumor cells and their response to radiotherapy.Cell layers made up of free-floating cell types of the same origin in culture dishes or co-cultures are called spheroids. Spheroid cultures

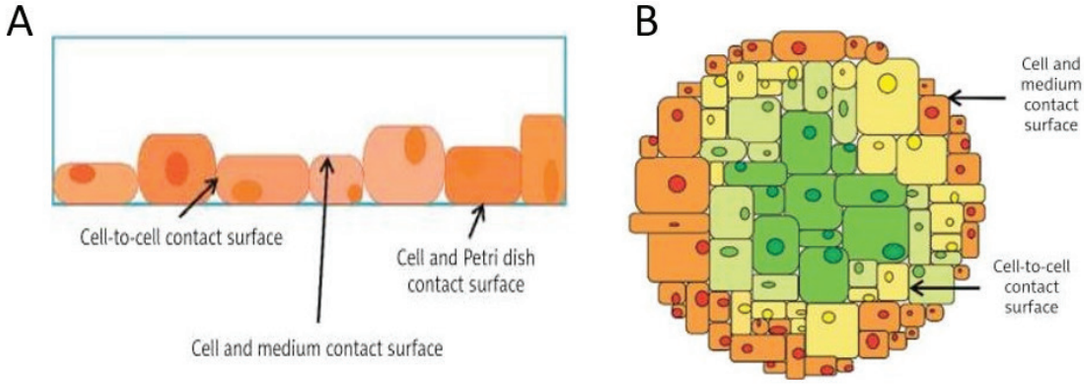

Figure 1: A. 2D Cell Culture, **B.** 3D Cell Culture.[16]

can be created using four different techniques. The first of these approaches is the use of low-adhesive culture dishes to allow the cells to self-attach to the spheroids. The second is the technique called the hanging drop technique. In this technique, cells are slowly placed on the tops of the middle droplets and form aggregates at the tops of the droplets. This approach produces more effective results for studies to be carried out with multiple co-cultures.[26] The third approach is to aggregate cells using a bioreactor.[27] The last approach regarding spheroids is to create scaffold structures with specific properties on micro and nano surfaces.[28] Scalability and easy repeatability in different formats are considered the advantages of spheroids in the 3D culture environment. In addition, different ratios are used for different cell types to develop spheroids of appropriate size, while the inability to ascertain these ratios is one of the disadvantages of the technique.[29] Researchers have reported a positive contribution of 3D culture created with appropriate environmental factors to the osteogenic differentiation of MSCs.[30]

Another model used for 3D cell culture is organoids. Organoids are three-dimensional *in vitro* culture systems that develop from embryonic or adult stem cells, reflecting the structural and functional properties of tissues and acting as an organ.[31] Organoids, which are mostly used for disease modelling in modern medicine, modulate the signal communication of cells with biochemical effects and modify cellular capacity for proliferation, differentiation, and self-renewal. They ensure the adhesion and survival of cells by using supportive elements such as collagen and fibronectin.[32]

In another 3D culture model called scaffold and hydrogel, the microenvironment created is biocompatible, mechanical supportive elements that can mimic the matrix property of a particular tissue, support the adaptation of cells to the environment, proliferation, and differentiation, and have different permeability and chemical properties.[33] They can be studied with a large number of cells and media. Moreover, hydrogels can be designed in accordance with the subject to be studied and supplied commercially.[34]

Chip organs are structures that mimic an organ or disease model of the human body. Thanks to these structures, *in vitro* modeling of organs of the human body and analysis of studied subjects have been possible more safely and easily. This modeling technique is an alternative method that will contribute to the reduction of animal testing for the screening of drug candidates and preclinical drug development.[35]

The latest techniques used for 3D tissue modelling are bioprinters. Bioprinters are devices that use the growth factors and biomaterials of cells to form tissue-like structures by layering these materials on top of each other. The advantages of this modelling system include fast prototyping, low production cost, and the ability to be designed in the desired form. Three-dimensional bioprinters have challenges with tissue maturation and functionality compared to other 3D cultures.[36,37]

3D CULTURE SYSTEMS IN PERIODONTAL REGENERATIVE TREATMENT

In periodontal regenerative therapy, cells have been reported to better adapt to the microenvironment created by 3D culture environments. However, it has been reported that the behaviour, morphology, differentiation degree, polarity, proliferation rate, and gene expression levels of cells are better evaluated *in vivo* conditions.[38]

A study evaluating the osteogenic differentiation levels of periodontium-derived stem cells reported the positive effect of a 3D culture environment on osteoblast differentiation.[39]

An animal study investigating the effect of 3D culture environments on osteogenic differentiation in periodontal regenerative treatment found significantly higher expression levels of Bone Differentiation Protein (BMP), Alkaline Phosphatase (ALP), Collagen I (COL-1), and Osteocalcin in the cells in the 3D experimental groups compared to the 2D experimental groups.[40]

In dentistry, 3D cell culture environments are used in regenerative studies created with stem cells, cell-based drug tests, cancer research, gene, and protein expression studies.[41]

A 2014 study by Dolati *et al*.[42] evaluated the viability of endothelial cells in a 3D culture environment created using alginate and reported a rate of over 83% as a result of the experiment.

In another study evaluating periodontal regeneration, three different tissue scaffolds with different sizes of microchannels containing polycaprolactone-hydroxyapatite (90:10 wt%) were designed on 3D bioprinters. The designs, called Phase A, Phase B, and Phase C, were created for the cement/dentin interface, PDL, and finally the alveolar bone, respectively. In the experimental groups cultured with differentiation medium, dental pulp-derived MSC in Phase A, PDL-derived MSC in Phase B and alveolar bone progenitor stem cells in Phase C were cultured for 4 weeks. The researchers reported regeneration in all three phases, with stem cells differentiating into dentin/cement, PDL, and alveolar bone complexes.[43]

Another study investigating the effect of experimental models created using spheroids in a 3D culture environment on PDL-derived MSCs compared proliferation and gene expression levels of cells with a 2D culture environment. The researchers stated that the gene expression levels of the cells in the 3D culture environment were at a higher level of significance compared to the 2D culture environment, but they reported a decreased proliferation ability of the cells in the 3D medium. This result was interpreted as better preservation of physiological properties by PDL-derived stem cells in the 3D spheroid culture.[44]

In an animal study investigating the efficacy of 3D tissue culture using MSCs in guided periodontal regeneration, an experimental bone loss model was created around the teeth. While graft material was used in one of the experimental groups created in the study, scaffolds containing MSCs were used in the other group. At the end of the experiment, the researchers reported that although PDL-like tissues were created in both groups, more COL-1 and connective tissue were synthesized in the tissue created in the group using a scaffold containing MSCs.[45]

In another study with PDL-derived stem cells, the cells were cultured in centrifuge tubes. The study reported the spontaneous formation of spheroid structures in this culture medium, which is called pellet culture, with the size of the structures being inversely proportional to the culture period. The reason for this shrinkage observed in the spheroid structure was explained by cell apoptosis. The researchers also reported increased expression of anti-inflammatory genes such as COX-2 and angiogenesis genes such as Vascular Endothelial Growth Factor (VEGF) and Human Growth Factor (HGF) in cells in 3D culture, which promoted periodontal regeneration.[46]

Elango *et al*.[47] evaluated the effect of human periodontal ligament fibroblasts (HPLF) on osteogenic differentiation in 2D and 3D culture environments. They reported significantly higher osteocalcin expression levels in the cells in the 3D culture than in the cells in the 2D culture, stating that this result was a promising approach for periodontal regenerative therapy.

Another study evaluated the effect of stem cells on osteogenic differentiation capacity of the created 3D culture environment by histochemical analyses and determination of gene expression levels. The results of the study showed the key role of hydrogels containing alginate and gelatine in osteogenic differentiation.[48]

Recent studies on periodontal regenerative treatment have reported that drugs, growth factors, and nanosystems added to the 3D culture environment make the regenerative effect of stem cells more effective. Increased osteogenic differentiation potential of periodontium-derived stem cells, as well as immunomodulatory effects, are considered the advantages of these environments.[49,50]

CONCLUSION

It is necessary to better understand the properties of cells in the 3D environment in order to create implantable artificial tissues and organs for humans and to use them in the field of regenerative medicine.

Due to the optimization cost, application, and reproducibility difficulties of 3D culture environments, studies are limited. It is anticipated that studies will expand, contributing to the development of new techniques and methods in many subjects such as disease modelling, stem cell use, drug therapies, organ transplants, and toxicology and eventually replacing animal studies in the future by providing an ideal *in vivo* environment.

Considering this information, the data to be obtained from studies in 3D culture environments will contribute to the development of periodontal regenerative therapies.

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