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The Effects of TGF-β3 on the Proliferation and Function of Encapsulated Costal Cartilage Chondrocytes in Alginate Scaffold

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Abstract

Introduction: Damages to cartilage are one of the most challenging issues of orthopedist in medicine as the healing of defects in the tissue has a very slow process and is extremely difficult. Tissue engineering (using scaffold), cells and growth factors can be used as alternatives to improve healing. Alginate is an ideal scaffold which has been also approved by the Food and Drug Administration (FDA). The transforming growth factor β3 (TGF β3) increases the viability of the chondrocytes and secretion of extra cellular matrix. The aim of this study was to evaluate the effects of TGF β3 in the viability and production of glycosaminoglycan (GAG) and aggrecan by rib chondrocytes.

Materials and Methods: In this experimental study, isolated costal chondrocytes were encapsulated in alginate and cultured for 3 weeks. Then, samples were divided into 2 groups: TGF-β3 treated and control groups. Finally, the viability of chondrocytes and production of GAG and aggrecan in both groups were evaluated by MTT, GAG and enzyme-linked immunosorbent assay (ELISA).

Results: By 14 days, the results of the MTT showed that viability had significantly increased in the control group in compared to the TGF-β3 treated group. This is while by 21 days, the TGF-β3 treated group the viability had increased After 14 and 21 days, the GAG production in the TGF-β3 treated group had significantly increased in compared to the control group. The ELISA technique revealed that the production of aggrecan significantly increased in the TGF-β3 treated group at 14 days.

Conclusions: Results indicate that TGF-β3 can increase the growth of costal cartilage and the production of extracellular matrix (ECM). Accordingly, TGF-β3 is necessary for the regeneration of cartilage.

Keywords: TGF-β3, Costal Cartilage, Chondrocyte

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Introduction

Cartilage is a specialized connective tissue containing extracellular matrix (ECM) rich in GAG and proteoglycans. This matrix can cause the cartilage to withstand mechanical pressure without permanent deformation. Also, these compounds create an appropriate smoothness and sliding in joints.[1] Cartilage tissue does not have a high regenerative power due to its lack of blood flow unlike the bone, which has a more efficient mechanism for repairing itself.[1] The damaged or eroded cartilage has limited ability to repair itself. It has also less power in malicious and wider damage.[2-6] Nowadays, cartilage diseases such as osteoarthritis are the most common skeletal diseases. These diseases are especially common in older people or athletes. According to a report, over 39 million people over 25 years in Europe and 26 million people in America experience osteoarthritis and have estimated this number to be doubled by 2020.[7,8] Today, the treatment of cartilage diseases is one of the biggest problems in the medical society. A series of new therapies has been taken into consideration based on tissue engineering in recent years for the treatment of cartilage diseases. The procedure requires proper scaffold, repairing cells, and growth factors. Tissue engineering of cartilage using stem cells is to either produce cartilage in the laboratory or to produce cartilage producing cells in the body. In the occasion that the cartilage is synthesized in the laboratory, it must be transplanted into the body and in the joints. In this type of treatment, the patient will not need artificial joints anymore. Among the appropriate treatment

methods, transplanted chondrocytes in combination with a special scaffold or alone can be very effective.[7,8] Chondrocyte transplant was firstly performed in 1968, but its successfulness was only 40%. The chondrocyte transplant faces problems such as the movement of the cell from the lesion site, and placing in the joint space. Thus, using a special scaffold like alginate can help improve these problems.[9,10] One of the problems associated with chondrocyte transplantation is the required cell source that is normally extracted from rib cartilage or joints that do not weigh much but this method has limitations as well. For example, due to the limitation in the achieved tissue, access to a sufficient number of cells is not easily possible. Also, it has been observed in previous studies that chondrocytes proliferation by single layer culture would lose their differentiation and secretory properties. In addition, it would need continuous use of immunosuppressive drugs that have their own side effects which is considered as another problem for patients.[11-13] Alginate is a natural biopolymer that is mainly extracted from brown algae and bacteria. This material includes less than 40% of the dry weight of algae. In the ECM of the algae, alginate exists in combination with calcium, sodium and magnesium cations. Alginate is a dual polymer of glucuronic acid and monotonic acid that are arranged like chains in a counter-clockwise pattern. Alginate can be used as a suitable ECM because this gel prepares a three-dimensional scaffold that provides a proper place for cell proliferation and on the other hand facilitates the release of the nutrient materials.[9] In addition, the scaffold should have the ability to absorb in the body after transplantation. For this purpose, the speed of absorption should coordinate the speed of matrix production. Also, the used scaffold should not cause an immune and inflammatory response in the body and should be biodegradable. Alginate has all these characteristics and other studies have shown that chondrocytes retain their differentiated phenotype within it and its ability to be secreted [13,14] Paige et al's study which was conducted in 1995 took alginate on chondrocytes and grafted it to the damaged cartilage.[15] Kurth et al also studied the transformation of mesenchymal cells taken from the knee joint into the chondrocytes in alginate scaffold in 2007.[16] In addition, in the study of Eslaminejad et al mesenchymal cells seeded in alginate gel differentiated after subcutaneous transplantation of hyaline cartilage cells where there were signs of bone formation process.[17] In addition to the scaffold and appropriate culture environment, various growth factors are required to regulate the differentiation process. The TGF-β protein families are multi-functional proteins and control the proliferation, secretion of ECM and chondrogenesis process. Cals et al showed the effect of TGF-β on the in vitro differentiation of bone marrow-derived mesenchymal cells into chondrocytes in a study in 2012.[18] In another study it has also been shown that when mesenchymal cells are exposed to high doses of TGF-β; it differentiates into chondrocytes that help in vivo cartilage repair.[19] Since achieving the best conditions for chondrocytes' growth and proliferation is taken into consideration by researchers, this study aimed to survey and compares the viability and ECM secretion of cultured rib chondrocytes with and without TGF-β3.

Materials and Methods

Study Design

The present study was designed as an experimental study. The cells were divided into 2 treatment groups. The TGF-β3 treated group and a control group. The MTT GAG and ELISA assays were used for the viability and secretion of ECM.

Isolation of Chondrocytes From Costal Cartilage

Samples of costal cartilage of human beings were removed by arthroscopy from three patients. The patients had signed a written consent form before the beginning of the experiment. The cartilaginous samples were cut to 1 mm pieces. Type II collagenase enzyme solution (Sigma, Germany) 350 µg/mL) was applied to digest the tissue for 4 hours at 37°C. The resultant suspension was centrifuged at 1400 rpm for 10 minutes and the chondrocytes were cultivated in DMEM/F12, penicillin/streptomycin 1%.and FBS 10% medium. After trypsinization, 10^6 cells of passage 2 cultured in 1.5% alginate solutions in 2 groups: chondrogenic medium containing TGF-β3 growth factor and without TGF-β3 cells and produced extracellular matrix evaluated in 14 and 21 days.

MTT Assay

The MTT assay has been applied for investigating the cell viability of encapsulated chondrocytes in alginate at 0, 14 and 21 days. Cells were transferred in 12 wells for 24hr. The medium was removed and washed by Phosphate buffered saline (PBS). After on, the medium was added with MTT to each well for 4hr and incubated in CO_2 5% and 37°C. In the next step, the medium was removed and DMSO and pipetting were added. The rate of absorbance read by the ELISA reader was 570 nm.

GAG Assay

For the evaluation of GAGs the GAG assay was used. The samples were homogenized and digested in papain solution containing phosphate buffer, cysteine, EDTA in 60°C for 18 hours. Concentration of GAG obtained by adding of DMMB dye to samples and compared with standard curve of chondroitin sulfate.

Investigation of the Level of Produced Aggrecan by ELISA

Human direct ELISA kit was used for investigating the level of produced aggrecan in culture medium. The aggrecan in the supernatant media produced by chondrocytes was measured by the ELISA kit (Invitrogen Cat. No. KAP1461) according to their manufacturers' protocols. Finally, samples were read in the ELISA reader (Hyperion, Micro plate reader) with a 450 nm wave length.

Results

MTT Assay

The cell viability was evaluated with the MTT assay. Figure 1 shows the percent of cell viability for the TGF-β3 treated group in comparison to the control group at 1, 14 and 21 days post seeding. The TGF-β3 treated group had a significant increase of cell viability at 21 days compared to 1 and 14 days. The cell viability in the control group was significantly

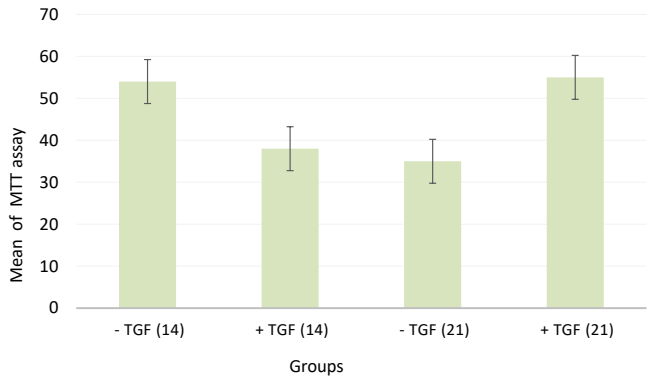

Figure 1. Mean percentage of alive cell numbers in different groups. + TGF-β3 14: Mean percentage of alive cell numbers in the TGF-β3 treated group at day 14. +TGF-β3 21: Mean percentage of alive cell numbers in the TGF-β3 treated group at day 21. TGF-β3 14: Mean percentage of alive cell numbers in the control group at day 14. TGF-β3 21: Mean percentage of alive cell numbers control group at day 21.

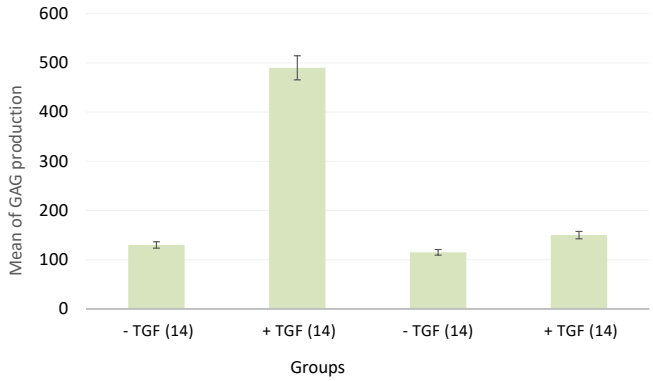

Figure 2. Results of the produced GAG in different groups. + TGF-β3 14: Mean of GAG production in TGF-β3 treated group at day 14. + TGF-β3 21: Mean of GAG production in TGF-β3 treated group at day 21. TGF-β3 14: Mean of GAG production in the control group at day 14. TGF-β3 21: Mean of GAG production in the control group at day 21.

decreased in day 21 compared to 1 and 14 days.

GAG Assay

The total GAG production was determined at 14 and 21 days using the GAG assay. Result indicated that the TGF-β3 treated group, produced GAG was significantly increased than none treated group ($P \leq 0.05$) (Figure 2). The produced GAG in the TGF-β3 treated group significantly decreased at 14 to 21 days ($P \leq 0.05$). At 14 to 21 days, the produced GAG had none significantly decrease in none treated group ($P \geq 0.05$).

ELISA Assay

The results of ELISA assay show that the TGF-β3 treated group had significantly produced more aggrecan compared to the control group ($P \leq 0.05$) (Figure 3).

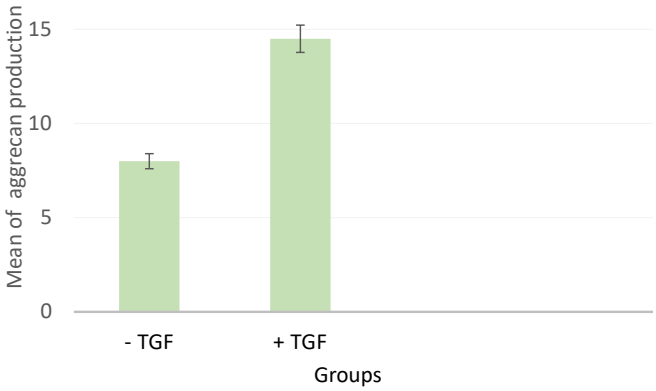

Figure 3. Results of total production aggrecan at day 14: - TGF-β3. Mean of produced aggrecan in the control group at day 14. + TGF-β3. Mean of produced aggrecan in TGF-β3 treated group at day 14.

Discussion

Cartilage tissue does not have the ability to repair due to the lack of blood vessels and nerves. Therefore, new therapies for the treatment of cartilage lesions need to achieve the cartilage tissue in vitro. Research has shown that high cell density is required for the development of cartilage tissue.[1-6] So, the best conditions for the growth and proliferation of chondrocytes in the laboratory is an important issue. The pellet culture system and 3D scaffold provide ideal conditions for cell interaction, but different growth factors are required to achieve acceptable results.[20-22] Many studies have used natural and synthetic scaffolds for chondrogenic differentiation. These scaffolds have some disadvantages such as lack of biodegradability and reduced cell adhesion.[22,23] Natural scaffolds also have problems including short-term stability, absence of appropriate mechanical characteristics and a high speed destruction of them.[24,25] In this study, an alginate scaffold that converts into gel in the laboratory without the need for organic solvents and has a porosity that will facilitate the release of macromolecules was used.[25,26] Due to its hydrophilic properties, alginate could play the role of extracellular material .The other advantages of alginate scaffolds include the fact that it makes the cells not de-differentiate. This issue has been examined in previous studies under different conditions. For example in the study conducted by Domm et al, it was shown that type 1collagen in the chondrocytes encapsulated on alginate can be synthesized at low oxygen pressure.[27] Growth factors in tissue engineering are needed for the proliferation and differentiation of chondrocytes. In this study, the TGF-β3 growth factor was used for evaluating its effects in viability and ECM production of rib chondrocytes. The TGF-β3 family consists of 5 groups; its first 3 groups have the ability to induce the synthesis of type 2 collagen and proteoglycans in chondrocytes.[28] Barry et al also showed that TGF-β2 and β3 cause accumulation of glycosaminoglycan's more than type 1 collagen in the process of chondrogenesis.[29]

In this study, the effect of TGF-β3 on the viability of the rib chondrocytes and the secretion of proteoglycan and aggrecans was assessed. The MTT results showed significant differences between the TGF-β3 treated and control groups but some studies reported that the viability of chondrocytes in

the alginate increases over time.[30,31] This is while other group of studies revealed a decrease of chondrocytes encapsulated in alginate over time.[32] In addition, a group of researchers reported an increase in the proliferation of chondrocytes, influenced by TGF-β but some also observed a decrease in the percent of cells by TGF-β.[33] Another study has reported that the proliferation of encapsulated nucleus pulposus of intervertebral disc in alginate significantly increases in the early days of cultivation and significantly decreases through time but secretion of extracellular matrix significantly increases.[34] Bahramian Renani et al seeded the nucleus pulposus (NP) cells of intervertebral disc in alginate and chitosan-gelatin scaffolds and reported that the percent of growth and proliferation of NP significantly decreased over time while the secretion of extracellular matrix significantly increased. They reported this decrease of growth and increased secretion due to both inherent properties of the cells and lack of nutrient exchanges which is due to the buried cells in their secretion matrix.[34] The results of this study are similar to the findings of the present study. Many studies have suggested that TGF-β increases the secretion of extracellular matrix in chondrocytes, but it is controversial whether this growth factor increases or decreases cell growth and proliferation.[30-32] In the present study, it has been observed that at 21 days, the secretion of glycosaminoglycan's and aggrecan significantly increased in the TGF-β3 treated group.

Conclusions

According to the results of the present study it can be concluded that TGF-β3 has positive effects on viability and secretion of costal chondrocyte proteins. This is while, since the tissue engineering treatment process needs high cell density, other growth factors in addition to TGF-β3 are required to increase the growth and proliferation of the harvested cells to obtain better results. It is advised to evaluate the effect of alginate without TGF-β3 in separate groups in further studies.

Authors' Contributions

BH designed, coordinated, conceived and analyzed the experiments. MG performed and wrote the first draft of the manuscript. MMA, AK, MG, PN and MA performed the study. All the authors reviewed the results and approved the final version of the manuscript.

Conflict of Interest Disclosures

The authors declare they have no conflicts of interest.

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