Evaluation of Transforming Growth Factor Beta 1 and Curcumin on Proliferation and Differentiation of Nasal-Derived Chondrocyte Seeded on the Fibrin Glue Scaffold

Hossein Ghanepour[1], Mohammad Mehdizadeh[1], Ebrahim Zabihi[2], Mahdieh Ghiasi[3], Mohsen Sheykhhasan[3], Mitra Narmashiri[1*]

1. Department of Oral and Maxillofacial Surgery, School of Dentistry, Babol University of Medical Sciences, Babol, Iran.
2. Cellular and Molecular Biology Research Center, Babol University of Medical Sciences, Babol, Iran.
3. Stem Cell Laboratory, Academic Center for Education, Culture, and Research, Qom Branch, Qom, Iran.

Citation: Ghanepour H, Mehdizadeh M, Zabihi E, Ghiasi M, Sheykhhasan M, Narmashiri M. Evaluation of transforming growth factor beta 1 and curcumin on proliferation and differentiation of nasal-derived chondrocyte seeded on the fibrin glue scaffold. Anatomical Sciences. 2016; 13(1):39-46.

Mitra Narmashiri is the student of Babol university of Medical Sciences in General Dentistry. She has completetd her Bachelor of Dental Surgery (BDS) in RajivGandhi University of Health and Sciences, India.

Article info:
Received: 09 Feb. 2015
Accepted: 15 Nov. 2015
Available Online: 01 Jan 2016

Key Words:
Transforming growth factor beta 1, Curcumin, Chondrocytes, 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-Diphenyl tetrazolium bromide, Real-time PCR

ABSTRACT

Introduction: Natural biomaterials and growth factors are key factors in tissue engineering. The objective of the present study was to evaluate transforming growth factor beta 1 (TGF-β1) and curcumin on proliferation and differentiation of nasal-derived chondrocyte seeded on the fibrin glue scaffold.

Methods: Chondrocytes were isolated from nasal samples. Nasal-derived chondrocytes were seeded on fibrin glue at chondrogenic induction medium for 2 weeks. In this study, the effects of various concentrations of curcumin and TGF-β1 on the survival and proliferation of chondrocytes seeded on fibrin biomaterial were assessed by MTT assays. Also, chondrocyte-specific gene expression was assessed by real-time polymerase chain reaction (PCR).

Results: There were significant differences among the group treated with curcumin 10 µg compared to other groups with regard to cell viability. Also, gene expression of collagen type II, aggrecan, and SOX9 in the chondrocytes seeded on fibrin biomaterial containing the growth factor TGF-β1 significantly differed from those of curcumin and control group.

Conclusion: Our results indicate that TGF-β1 and natural biomaterial of curcumin can be used effectively in chondrogenic viability and differentiation of nasal-derived chondrocyte.

*** Corresponding Author:**
Mitra Narmashiri, MD
Address: *Department of Oral and Maxillofacial Surgery, School of Dentistry, Babol University of Medical Sciences, Babol, Iran.*
Tel: *+98 (11) 32291408*
E-mail: *mitra63@gmail.com*

1. Introduction

Articular cartilage is known as a connective tissue with low cellular activity that limits its potential for regeneration and repair [1, 2]. Tissue engineering (TE) provides a technique to reconstruct the cartilage defects. Identification of the optimal condition for regeneration is one of the major factors in TE [3-6]. Articular cartilage cannot repair by itself. This shortcoming later leads to arthritis. Arthritis is the most common skeletomuscular disease of old age. In 2012, arthritis was reported as the fourth cause of disability [7-10]. There are several treatments such as chondrocyte transplantation and drilling for current disabilities. Although these treatments have advantages, they can produce fibrous tissue instead of hyaline cartilage [11].

Nowadays, cell therapy using tissue engineering has become a promising way for repairing injured articular cartilage, which contains chondrocyte biomaterials and appropriate cultural environment [12]. It is proven that transplantation of monolayer chondrocytes culture helps the chondrocytes looks like fibroblasts. As regards cell choices, use of differentiated cells in TE is limited due to many factors. Despite the fundamental requirement of cell expansion to achieve a sufficient number of cells for TE, differentiated cells have limited proliferation potency in culture. Furthermore, differentiated cells often lose their tissue-forming capacity in vitro and may dedifferentiate in conventional monolayer culture and their phenotype changes in vitro. On the other hand, the researchers showed that maintenance of the chondrocyte phenotype could be achieved by using 3D structure, herbal extracts, and growth factors. These scaffolds provide voided structures for attachment and growth of cells and their suspension with high density and desirable stimulation at these 3D structures [13]. In the last decade, the capacity of natural scaffolds for support of cell growth has been tested. Fibrin obtained from blood has been used in clinical treatments. Several advantages of fibrin include excellent biocompatibility, promotion of the cell adhesion, and lack of immunogenicity [14].

Several studies have been done regarding the effects of some plants in treating osteoarthritis and joint disorders, among which turmeric has proven to be the most effective one [15-17]. Curcumin is the effective component of turmeric with chemical name of feruloylmethane. Curcumin has various water soluble components with anti-inflammation, apoptosis, anti-catabolic, and strong antioxidant effects. It has a high solubility in ethanol, dimethyl solfoxide and other organic solvents. Curcumin molecular weight is 368.37 and its melting point is 183ºC [18, 19]. Commercial curcumin is made of diferuloylomethane (82%) and its derivatives demethoxycurcumin (15%) and bisdemethoxycurcumin (3%). Some of curcumin effects include loading to reduce tumor cell survival, expansion, and secondary inflammation through NF-KB inhibition, as well as suppression of constitutive phosphorylation IKBα via the presentation of IKB kinase [20-24]. In this study, we evaluated the comparative efficacy of TGF-β1 and curcumin on the proliferation and differentiation of nasal-derived chondrocyte seeded on the fibrin glue scaffold.

2. Materials and Methods

Materials

Dulbecco's minimum essential medium (DMEM) for cell culture was purchased from Sigma and fetal bovine serum (FBS) and penicillin-streptomycin were purchased from Gibco (BRL, Gaithersburg, MD, USA).

Isolation of chondrocyte cells

The specimens were obtained by rhinoplasty after informed consents were obtained from patients. Next, the specimens were carried from the hospital to the laboratory (under sterilized condition: phosphate buffered saline containing 2% penicillin streptomycin). They were washed by phosphate buffer saline (PBS) 3 times, then divided into smaller pieces. After that, these pieces were digested by collagenase type II so that nasal-derived chondrocytes got isolated. They were incubated for 4-5 hours at 37ºC and then centrifuged for 10 minutes at 1800 rpm. Then, the supernatant was eliminated and pellet was cultured in DMEM containing 1% penicillin, streptomycin, and 10% FBS at 95% humidity, 5% CO_2, and at 37ºC. After 24 hours, the adherence cells have been removed and cells became ready to use at P3.

Expression analysis of chondrogenic genes by real time PCR

Expression of cartilage-specific genes (type I and II collagen, Sox9 and aggrecan) was evaluated after 14 days as previously described. All of the samples were digested within liquid nitrogen and then total RNA was extracted using an Accuzol™ (BioNEER) in accordance with the manufacturer's protocol. Afterwards, the reverse transcription of RNA was carried to produce a complementary DNA (cDNA) using the Accupower® RTpreM1X (BioNEER). Real-time PCR was performed using SYBR

Green PCR Master Mix and Rotor-Gene™ 6000 Series software version 1.7.65 (Corbett Life Science), real time-PCR performance using SYBR Green PCR Master Mix (Amplicon) and Rotor-Gene™ 6000 Series software version 1.7.65 (Corbett Life Science), and primers of each gene were designed as follows utilizing primer 3 program (Table 1).

The reaction was initiated by heating at 95°C for 15 minutes, followed by 40 cycles of elongation at 59°C for 30 seconds and denaturation at 95°C for 15 seconds. Target gene was normalized based on glyceraldehyde 3-phosphate dehydrogenase reference gene. The level of expression of each target gene was calculated using $2^{-\Delta\Delta CT}$.

Histological examination

Scaffolds from 2 groups containing TGF-β1 and curcumin were analyzed histologically. After fixation with 10% neutral buffered formalin for 24 hours, samples were embedded within paraffin and sectioned at 5 µm thickness. For histological evaluation, sections were deparaffinized, rehydrated through a series of graded ethanol, and stained with hematoxylin/eosin.

Statistical analysis

Significant differences in expression levels of 4 genes in construct were identified by the least significant difference (LSD) test for the real-time PCR analysis. This reaction was repeated 4 times for every sample. Data were analyzed statistically using SPSS software (version 17). Significance was considered at $P<0.05$.

Cell proliferation and viability

Cell viability and proliferation were assessed by MTT at day 14. Our study identified that in 2 intervention groups, viability and proliferation of cells significantly increased compared to the control group (cells without TGF-β1 and curcumin) ($P<0.05$).

Preparation of fibrinogen and thrombin

Fibrinogen and fresh frozen plasma solutions (FFP) and thrombin were obtained from the Iranian Blood Transfusion Organization. FFP was thawed in water bath at 37°C. Then, 15 mL of it was mixed with 10 mL of calcium gluconate (mid 5:3 ratio). The solution was incubated for 1 hour at 37°C and subsequently centrifuged at 2200 rpm for 10 minutes. The supernatant was collected as thrombin. Fibrinogen and thrombin solutions were prepared for use as cell culture. To this end, the chondrocyte cells at a concentration of 1×106 cells per mL were dissolved within fibrinogen and then thrombin was added to them. The chondrogenic medium was prepared as follows: DMEM high glucose, including insulin-transferin-selenium 1%, dexamethasone 100 nM, bovine albumin serum 1%, 1.5 µg linoleic acid, 50 µg/mL ascorbic 2-phosphate and penicillin streptomycin 1%, and 10 ng/ml TGF-β1/10 µm curcumin.

3. Results

Chondrocyte characterization

Human chondrocytes were identified by spindle shape, fibroblast-like morphology in the primary culture (Figure 1). Homogeneous cell population was determined at passage 3 (Figure 1). The cell survival in the group treated with 10 µg TGF-β1 compared to the other groups showed no significant difference (Figure 2). Also, this study showed that a significant difference existed in cell viability among the group treated with curcumin 10 µg compared to other groups (Figure 3). Chondrocyte-specific gene expression was performed in different groups using real-time PCR. In this study, it was shown that gene expression of collagen type II and aggrecan and Sox9 in the chondrocytes seeded

Table 1. Primer sequences used for real-time PCR.

Primer name	Sequences (3'>5')	Reference
Collagen type I (COL2A1) forward Collagen type I (COL2A1) reverse	CGTCCAGATGACCTTCCTACG TGAGCAGGGCCTTCTTGAG	NM_001844
Collagen type II (COL1A2) forward Collagen type II (COL1A2) reverse	CAGGAAACAGCTATGACC CTACTCTCAGCCCAGGAGGTCCTG	NM_000089
Aggrecan (ACAN) forward Aggrecan (ACAN) reverse	AGGCAGCGTGATCCTTACC GGCCTCTCCAGTCTCATTCTC	NM_001135
SOX9 Forward SOX9 Reverse	GTACCCGCACTTGCACAAC TCTCGCTCTCGTTCAGAAGTC	NM_000346
GAPDH Forward GAPDH Reverse	CGCTCTCTGCTCCTCCTGTT CCATGGTGTCTGAGCGATGT	NM_001256799

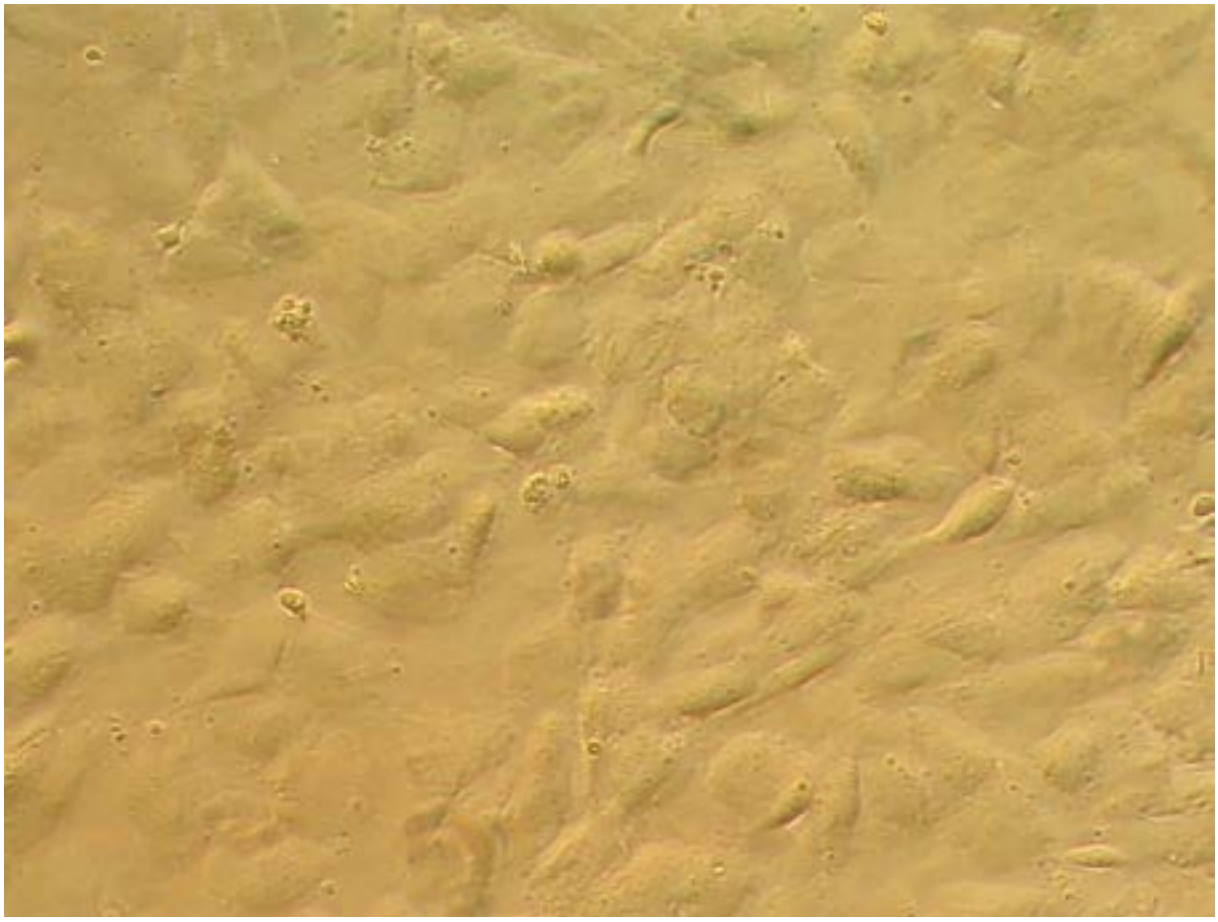

Figure 1. Human nasal-derived chondrocytes of fibroblast-like observed by inverted phase-contrast microscope.

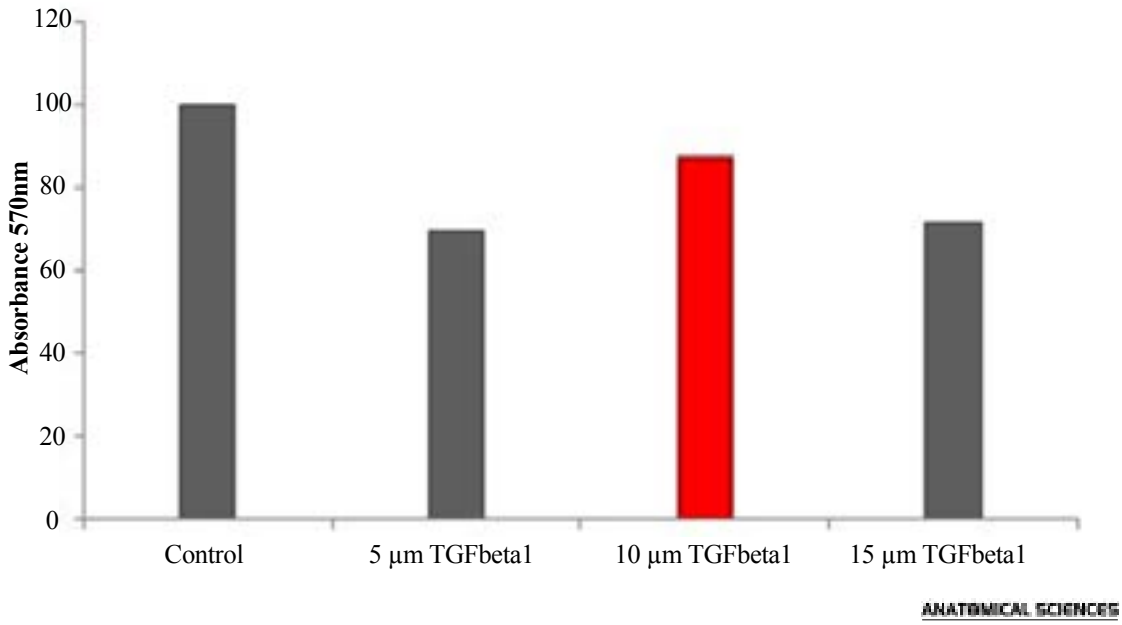

Figure 2. MTT assay to evaluate the effects of different concentration of TGF-β1 on survival and proliferation of chondrocytes seeded on fibrin biomaterial.

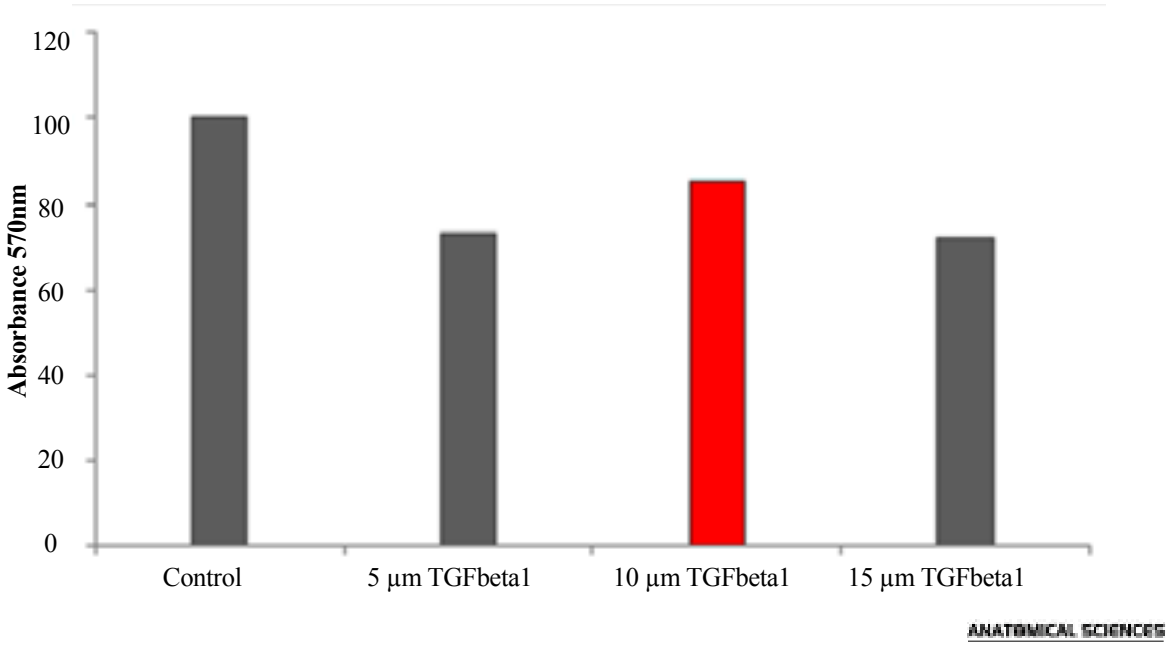

Figure 3. MTT assay to evaluate the effects of different concentration of curcumin on survival and proliferation of chondrocytes seeded on fibrin biomaterial.

on fibrin biomaterial containing the growth factor TGF-β1 showed significant difference compared to curcumin and control groups (Figure 4).

In this study, the effect of various concentrations of curcumin and TGF-β1 on the survival and proliferation of chondrocytes seeded on fibrin biomaterial was assessed

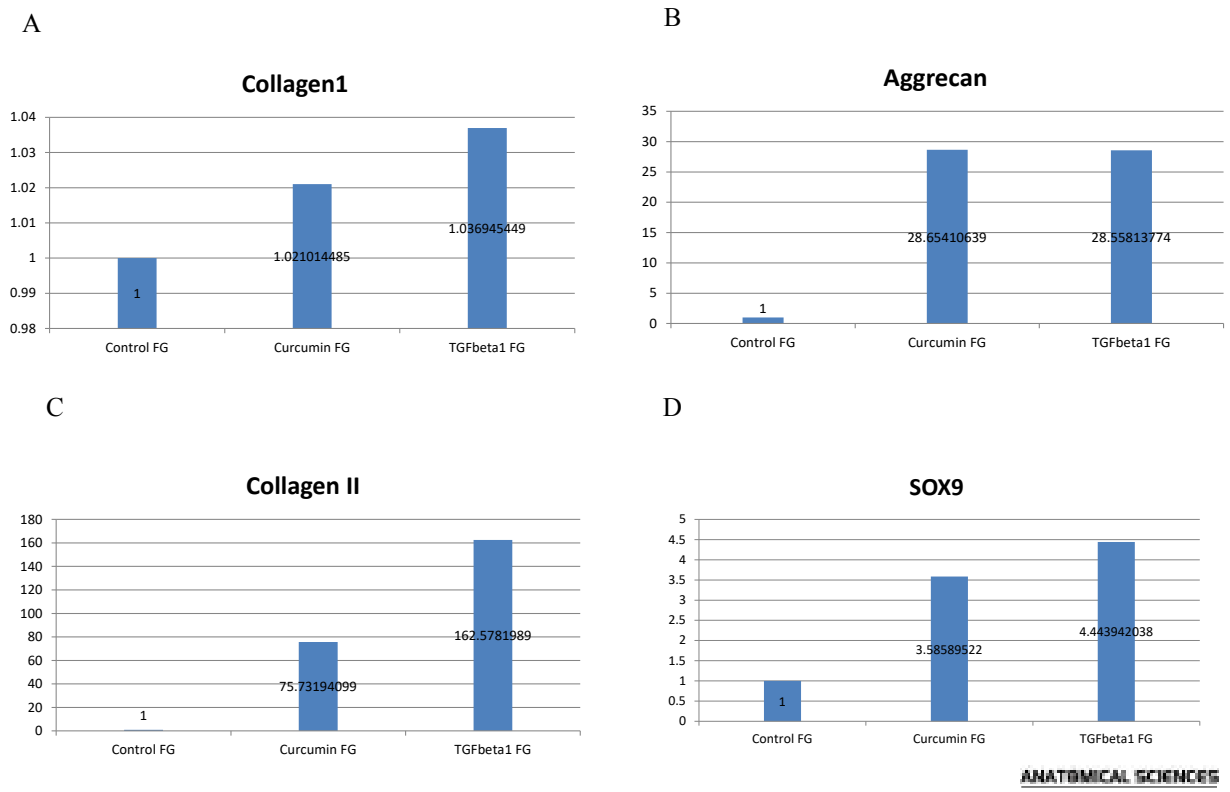

Figure 4. The mRNA expression of SOX9, collagen Type II, Aggrecan and collagen Type I in the human chondrocytes seeded on fibrin biomaterial supplemented with TGF-β1, curcumin (A-D respectively). *Statically significant (P<0.05).

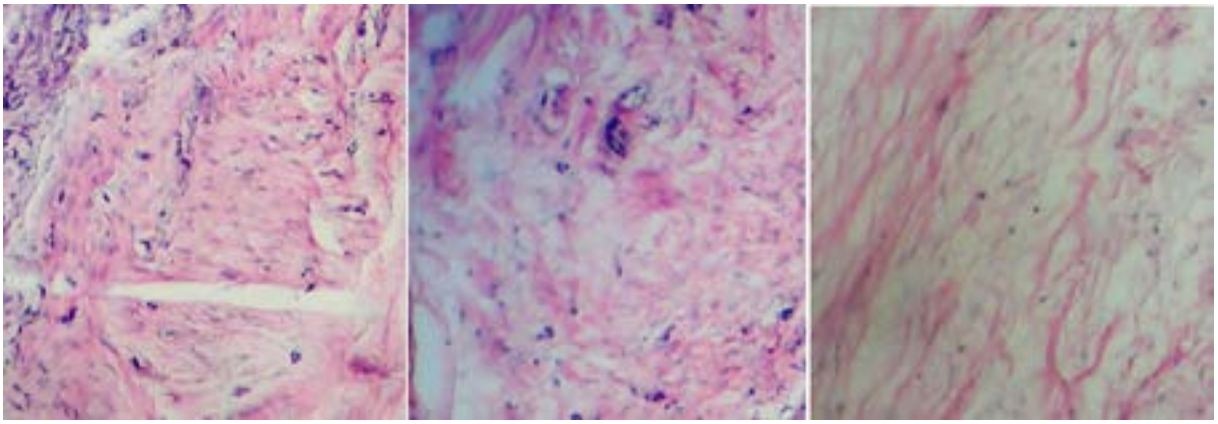

Figure 5. Histological staining (40x) with H&E in the chondrocytes seeded on fibrin biomaterial containing the growth factor TGF-β1 (A), in the chondrocytes seeded on fibrin biomaterial containing the growth factor curcumin (B), and the chondrocytes seeded on fibrin biomaterial as control (C).

by MTT assay. Chondrocytes were isolated from human nasal cavity. Histologically, chondrocytes seeded on fibrin glue contained matrix and many cells. There was histological evidence of more extensive cartilaginous matrix, also type II collagen content was significantly higher than in human chondrocyte/fibrin glue scaffold supplemented with TGF-β1 (Figure 5).

4. Discussion

According to our study, there were differences between 2 intervention groups and the control group. Moreover, based on developmental biology, the availability of novel scaffold-cell systems ex vivo with tight environmental control can provide valuable information about tissue formation processes as well as new insight into how tissues regenerate. This would optimize the approaches towards tissue repair, which is currently dominated by uncontrolled inflammatory wound healing pathways (that leave scars).

Articular cartilage possesses hyaline cartilage with low metabolic rate due to lack of vascular supply. The major limitation of adult articular cartilage is the inability to repair. Even the most minor injuries may leave damages and osteoarthritis joint degeneration, causing severe pain and disability [25]. With regard to repairing cartilage defects, there are multiple techniques, including drilling, abrasion arthroplasty, mosaicplasty, as well as autologous and allogeneic chondrocyte transplantation. The main problem of autografts and allografts is possibility of disease transmission, immune response, limitation of remodeling and performance of many surgeries, and morbidity of harvesting sites. TE has shown a promising approach in the repair of cartilage tissue [22, 26-29].

Chondrocytes have the regenerative capability to synthesize cartilage-like matrix in vitro and protecting chondrogenic phenotype while cultured in 3D construct with suitable agents. A 3D structure provides an environment to facilitate chondrocytes adhesion, expansion, and proliferation as well as maintenance of the cellular phenotype and function [25, 30-33]. Zheng et al. showed that using matrix-induced autologous chondrocyte implantation can support chondrocyte differentiation and after 6 months about 75% of biopsies from patients confirmed hyaline-like cartilage formation [34].

TGF-β can stimulate the production of proteoglycans and other components of cartilage matrix in the various stages of the cartilage regeneration process [35]. Since a long time ago, it has been known that curcumin has the anti-inflammatory property. Curcumin (50 μM concentration) has the ability to inhibit NF-KB activation. This agent can induce proinflammatory genes, Cox2, and VEGF [36].

Chowdhury et al. reported that curcumin (0.01-100 ng/mL) could inhibit activator protein (AP) -1 and consequently reverse the interleukin 1 beta stimulated production of nitric oxide and prostaglandin [37]. In vitro studies, human articular chondrocytes/alginate beads composite have been exposed to curcumin (1-20 μM). This study showed nontoxic properties of curcumin on cell viability [38].

In another investigation, Mathy et al. showed that curcumin suppressed Cox2 gene expression compared to non-steroidal anti-inflammatory drugs [39]. Barman et al. used curcumin (5 μM) and reported that it modulated inflammation in human tenocytes [40].

Huang et al. reported that curcumin was able to inhibit B-cell activating factor production in a mouse model [41]. In another study, Banji et al. showed that the combination of methotrexate and curcumin had anti-inflammatory effects and increased chondrocyte protective action [42]. The expression and secretion of various matrix metalloproteinase are regulated by the inflammatory and chondroprotective effects of curcumin [43-45]. In conclusion, curcumin can increase the expression of cartilage specific genes. Herbal extractions like curcumin have potential therapeutic effects on osteoarthritis.

Acknowledgements

The article is extracted from Ms. Mitra Narmashiri's thesis in the department of Oral and Maxillofacial Surgery, Babol University of Medical Sciences, Babol, Iran.

Conflict of Interest

The authors of this study declared no conflict of interests.

References

[1] Junqueira LC, Carneiro J. Basic histology: text & atlas. New York: McGraw-Hill Publication; 2005.

[2] Hardingham T, Tew S, Murdoch A. Tissue engineering: chondrocytes and cartilage. Arthritis Research. 2002; 4(3):63-68.

[3] Bronzino JD. Tissue Engineering and Artificial Organs. In: Bronzino JD, Editor. The Biomedical Engineering Handbook. New York: CRC Press; 2006, p. 1-8.

[4] Vunjak-Novakovic G, Freshney RI. Culture of cells for tissue engineering. New Jersey: John Wiley and Sons; 2006.

[5] Tabato Y. Recent progress in tissue engineering. Drug Discovery Today. 2001; 6(9):483-87.

[6] Tsuchiya K, Chen G, Ushida T, Matsuno T, Tateishi T. The effect of co-culture of chondrocytes with mesenchymal stem cells on their cartilaginous phenotype in vitro. Materials Science & Engineering. 2004; 24(3):391-96.

[7] Fautrel B, Hilliquin P, Rozenberg S, Allaert FA, Coste P, Leclerc A, et al. Impact of osteoarthritis: results of a nationwide survey of 10,000 patients consulting for OA. Joint Bone Spine. 2005; 72(3):235-40.

[8] Centers for Disease Control and Prevention. Prevalence and impact of arthritis by race and ethnicity-US, 1989-1991. Morbidity & Mortality Weekly Report. 1996; 45(18):373–78.

[9] Kuo CK, Li WJ, Mauck RL, Tuan RS. Cartilage tissue engineering: its potential and uses. Current Opinion in Rheumatology. 2006; 18(1):64-73.

[10] Casper D. Harrison's principles of internal medicine [M. Moosavi, M. Kheirkhahan, Persian trans]. Tehran: Teymurzadeh; 2014.

[11] Tew SR, Kwan AP, Hann A, Thomson BM, Archer CW. The reactions of articular cartilage to experimental wounding: Role of apoptosis. Arthritis & Rheumatoogy. 2000; 43(1):215-25

[12] Sheykhhasan M, Tabatabaei Qomi R, Kalhor N, Mehdizadeh M, Ghiasi M. Evaluation of the ability of natural and synthetic scaffolds in providing an appropriate environment for growth and chondrogenic differentiation of adipose-derived mesenchymal stem cells. Indian Journal of Orthopaedics. 2015; 49(5):8-15.

[13] Ghiasi M, Tabatabaei-Qomi R, Kalhor N, Fazaely H, Mehdizadeh M, Sheykhhasan M. The design of scaffolds for use in tissue engineering. Sikkim Manipal University Journal. 2014; 1(2):261-73.

[14] Sheykhhasan M, Tabatabaei Qomi R, Ghiasi M. Fibrin scaffolds designing in order to human adipose-derived mesenchymal stem cells differentiation to chondrocytes in the presence of TGF-β3. International Journal of Stem Cells. 2015; 8(2):1-9.

[15] Yamaguchi K, Shinohara C, Kojima S, Sodeoka M, Tsuji T. (2E,6R)-8-hydroxy- 2,6-dimethyl-2-octenoic acid; a novel anti-osteoporotic monoterpene, isolated from Cistanche salsa. Bioscience, Biotechnology & Biochemistry. 1999; 63(4):731–35.

[16] Masuda K, Ikeuchi M, Koyama T, Yamaguchi K, Woo JT, Nishimura T, et al. Suppressive effects of Anoectochilus formosanus extract on osteoclast formation in vitro and bone resorption in vivo. Journal of Bone & Mineral Metabolism. 2008; 26(2):123–29.

[17] Hwang YC, Jeong IK, Ahn KJ, Chung HY. The effects of Acanthopanax senticosus extract on bone turnover and bone mineral density in Korean postmenopausal women. Journal of Bone & Mineral Metabolism. 2009; 27(5):584–90

[18] Henrotin Y, Priem F, Mobasheri A. Curcumin: a new paradigm and therapeutic opportunity for the treatment of osteoarthritis: curcumin for osteoarthritis management. Springerplus. 2013; 2(1):56. doi: 10.1186/2193-1801-2-56

[19] Aggarwal BB, Kumar A, Bharti AC. Anticancer potential of curcumin: preclinical and clinical studies. Anticancer Research. 2003; 23(1):363-98.

[20] Bharti AC, Donato N, Singh S, Aggarwal BB. Curcumin (diferuloylmethane) down-regulates the constitutive activation of nuclear factor-kappa B and Ikappa Balpha kinase in human multiple myeloma cells, leading to suppression of proliferation and induction of apoptosis. Blood. 2003; 101(3):1053–062

[21] Shakibaei M, Csaki C, Nebrich S, Mobasheri A. Resveratrol suppresses interleukin-1beta-induced inflammatory signaling and apoptosis in human articular chondrocytes: potential for use as a novel nutraceutical for the treatment of osteoarthritis. Biochemical Pharmacology. 2008; 76(11):1426–439.

[22] Vinatier C, Mrugala D, Jorgensen C, Guicheux J, Noël D. Cartilage engineering: a crucial combination of cells, biomaterials and biofactors. Trends in Biotechnology. 2009; 27(5):307-14.

[23] Baghban Eslaminezhad M, Taghiyar L. Mesenchymal stem cell purification from the articular cartilage cell culture. Iranian Journal of Basic Medical Sciences. 2007; 10(3):146-53.

[24] Ghiasi M, Kalhor N, Tabatabaei Qomi R, Sheykhhasan M. The effects of synthetic and natural scaffolds on viability and proliferation of adipose-derived stem cells. Frontiers in Life Science. 2015; 8(4):1-12.

[25] Sandell LJ, Aigner T. Articular cartilage and changes in arthritis. An introduction: cell biology of osteoarthritis. Arthritis Research. 2001; 3(2):107-13.

[26] Shin H, Olsen BD, Khademhosseini A. The mechanical properties and cytotoxicity of cell-laden double-network hydrogels based on photocrosslinkable gelatin and gellan gum biomacromolecules. Biomaterials. 2012; 33(11):3143-152.

[27] Ahmed TA, Giulivi A, Griffith M, Hincke M. Fibrin glues in combination with mesenchymal stem cells to develop a tissue-engineered cartilage substitute. Tissue Engineering: Part A. 2011; 17(3-4):323-35.

[28] Jeong CG, Zhang H, Hollister SJ. Three-dimensional polycaprolactone scaffold-conjugated bone morphogenetic protein-2 promotes cartilage regeneration from primary chondrocytes in vitro and in vivo without accelerated endochondral ossification. Journal of Biomedical Material Research: Part A. 2012; 100(8):2088-096.

[29] Oshima Y, Harwood FL, Coutts RD, Kubo T, Amiel D. Variation of mesenchymal cells in polylactic acid scaffold in an osteochondral repair model. Tissue Engineering: Part C. 2009; 15(4):595-604.

[30] Domm C, Schunke M, Christesen K, Kurz B. Redifferentiation of dedifferentiated bovine articular chondrocytes in alginate culture under low oxygen tension. Osteoarthritis Cartilage. 2002; 10(1):13–22.

[31] Malda J, van Blitterswijk CA, Grojec M, Martens DE, Tramper J, Riesle J. Expansion of bovine chondrocytes on microcarriers enhances redifferentiation. Tissue Engineering. 2003; 9(5):939–48.

[32] Fukui N, Purple CR, Sandell LJ. Cell biology of osteoarthritis: the chondrocyte's response to injury. Current Rheumatology Reports. 2001; 3(6):496–505.

[33] Tallheden T, Bengtsson C, Brantsing C, Sjögren-Jansson E, Carlsson L, Peterson L, et al. Proliferation and differentiation potential of chondrocytes from osteoarthritic patients. Arthritis Research & Therapy. 2005; 7(3):560–68.

[34] Zheng MH, Willers C, Kirilak L, Yates P, Xu J, Wood D, Shimmin A. Matrix-induced autologous chondrocyte implantation (MACI): biological and histological assessment. Tissue Engineering. 2007; 13(4):737-46.

[35] Coutts RD, Sah RL, Amiel D. Effects of growth factors on cartilage repair. Instructional Course Lectures. 1997; 46:487-94.

[36] Csaki C, Mobasheri A, Shakibaei M. Synergistic chondroprotective effects of curcumin and resveratrol in human articular chondrocytes: inhibition of IL-1beta-induced NF-kappaB-mediated inflammation and apoptosis. Arthritis Research & Therapy. 2009; 11(6):165. doi: 10.1186/ar2850

[37] Chowdhury TT, Salter DM, Bader DL, Lee DA. Signal transduction pathways involving p38 MAPK, JNK, NFkappaB and AP-1 influences the response of chondrocytes cultured in agarose constructs to IL-1beta and dynamic compression. Inflammation Research. 2008; 57(7):306-13.

[38] Mathy-Hartert M, Jacquemond-Collet I, Priem F, Sanchez C, Lambert C, Henrotin Y. Curcumin inhibits pro-inflammatory mediators and metalloproteinase-3 production by chondrocytes. Inflammation Research. 2009; 58(12):899-908.

[39] Mathy M, Sanchez C, Priem F, Henrotin Y. Curcumin inhibits interleukin-6, -8, nitric oxide and prostaglandin E2 synthesis by bovine chondrocytes. Osteoarthritis and Cartilage. 2007; 15(Suppl C):115-17.

[40] Buhrmann C, Mobasheri A, Busch F, Aldinger C, Stahlmann R, Montaseri A, et al. Curcumin modulates nuclear factor kappaB (NF-kappaB)-mediated inflammation in human tenocytes in vitro: role of the phosphatidylinositol 3-kinase/Akt pathway. Journal of Biological Chemistry. 2011; 286(32):28556-8566.

[41] Huang G, Xu Z, Huang Y, Duan X, Gong W, Zhang Y, et al. Curcumin protects against collagen-induced arthritis via suppression of BAFF production. Journal of Clinical Immunology. 2013; 33(3):550-57.

[42] Banji D, Pinnapureddy J, Banji OJ, Kumar AR, Reddy KN. Evaluation of the concomitant use of methotrexate and curcumin on Freund's complete adjuvant-induced arthritis and hematological indices in rats. Indian Journal of Pharmacology. 2011; 43(5):546-50.

[43] Hong J, Bose M, Ju J, Ryu JH, Chen X, Sang S, et al. Modulation of arachidonic acid metabolism by curcumin and related beta-diketone derivatives: effects on cytosolic phospholipase A(2), cyclooxygenases and 5-lipoxygenase. Carcinogenesis. 2004; 25(9):1671-679.

[44] Schulze-Tanzil G, Mobasheri A, Sendzik J, John T, Shakibaei M. Effects of curcumin (diferuloylmethane) on nuclear factor kappaB signaling in interleukin-1beta-stimulated chondrocytes. Annals of the New York Academy of Sciences. 2004; 1030(1):578-86.

[45] Nanji AA, Jokelainen K, Tipoe GL, Rahemtulla A, Thomas P, Dannenberg AJ. Curcumin prevents alcohol-induced liver disease in rats by inhibiting the expression of NF-kappa B-dependent genes. Gastrointestinal& Liver Physiology. 2003; 284(2):321-27.