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Оценка хондрогенного потенциала дермальных фибробластов человека после модификации дифференцировочными средами и цитокином TGF-β3

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АННОТАЦИЯ

Обоснование. Гиалиновый хрящ представляет собой аваскулярную ткань, покрывающую крупные суставы. Данная ткань с малым количеством клеток и большим содержанием белков внеклеточного матрикса обладает ограниченной способностью к регенерации. Восстановление поверхности суставного хряща на сегодняшний момент является актуальной и не до конца решённой задачей. Перспективным направлением можно считать применение биомедицинских продуктов, содержащих модифицированные клетки.

Цель исследования — выявление влияния различных питательных сред на хондрогенную модификацию клеток и сравнение результатов между собой.

Материалы и методы. Дермальные фибробласты человека и мультипотентные мезенхимальные стромальные клетки кролика модифицировали при помощи среды для хондрогенной дифференцировки StemPro или рекомбинантного белка TGF-β3. Экспрессию генов, ответственных за хондрогенез (*Acan*, *Tgfβ3*, *Col2α1*, *Comp*), измеряли с помощью полимеразной цепной реакции в реальном времени (real-time PCR) методом ΔΔCt.

Результаты. Показано, что дермальные фибробласты человека способны к хондрогенной модификации с помощью белковых сред. Данный тип клеток легко доступен для забора, не требует особых условий при культивировании, легко масштабируется, а также может быть использован для аллогенной трансплантации.

Заключение. Полученные данные можно использовать при конструировании тканеинженерных продуктов для регенерации гиалинового хряща на основе аллогенных дермальных фибробластов.

Ключевые слова: тканевая инженерия; real-time PCR; мультипотентные мезенхимальные стромальные клетки; ММСК; TGF-β3.

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Evaluation of chondrogenic potential of human dermal fibroblasts after modification with differentiation media and cytokine TGF-β3

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ABSTRACT

BACKGROUND: Hyaline cartilage is an avascular tissue that envelopes the surface of major joints. The regenerative capacity of this tissue is restricted due to its high content of extracellular matrix proteins and its modest number of cells. Articular cartilage recovery remains relevant and yet unresolved to date. Utilizing biomedical products that contain modified cells is a promising approach.

AIM: To examine the effects of various culture media on the chondrogenic modification of cells and to compare the results.

MATERIALS AND METHODS: Human dermal fibroblasts and rabbit multipotent mesenchymal stromal cells were modified using the chondrogenic differentiation medium StemPro or recombinant TGF-β3 protein. Alterations in the expression of genes involved in chondrogenesis (*Acan*, *Tgfβ3*, *Col2α1*, *Comp*) were assessed using the real-time polymerase chain reaction method ΔΔCt.

RESULTS: It was demonstrated that human dermal fibroblasts can induce chondrogenic modification when used in protein media. This cell type is easy to harvest, does not necessitate special cultivation conditions, is readily scalable, and is suitable for allogeneic transplantation.

CONCLUSION: The obtained data can be employed to develop tissue engineering products for the regeneration of hyaline cartilage using allogeneic dermal fibroblasts.

Keywords: tissue engineering; real-time PCR; multipotent mesenchymal stromal cells; MMSCs; TGF-β3.

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INTRODUCTION

Hyaline cartilage, a tissue devoid of blood vessels and nerves, typically covers large joints. Joint movement is facilitated by the reversible deformation and minimal friction that occur as the tissue's layers slide. Hyaline cartilage is primarily composed of extracellular matrix proteins (predominantly type II collagen and aggrecan), a significant quantity of water, and a few terminally differentiated and specialized cells [1]. Hyaline cartilage's regenerative capacity is restricted by its unique structure, and its defects exacerbate over time, potentially resulting in complete tissue degradation and, consequently, joint dysfunction [2]. Multiple factors influence hyaline cartilage surface defect development and joint function impairment: age [3, 4], active participation in sports [5], genetic predisposition [6], excess weight [7], inflammatory joint diseases, and ligamental injuries. The structure of the hyaline cartilage itself [8] and the substantial biomechanical stresses it endures also play a crucial role.

Hyaline cartilage damage is a significant concern for many individuals [9–14]. Despite the availability of diverse techniques for local surgical restoration of hyaline cartilage, a complete resolution of joint surface defects remains a challenge [11, 15–18]. The final stage of joint defect progression involves the complete destruction of the hyaline cartilage, requiring a highly invasive and expensive endoprosthetic procedure [19, 20].

Cell engineering is one of the promising and rapidly developing approaches to repairing localized injury to hyaline cartilage [21–24]. The procedure entails the implantation of a cell-engineered construct consisting of a biodegradable scaffold with living cells in the damaged joint surface area [25–27].

In our opinion, it is logical that the initial cell culture modification to increase the synthesis of extracellular matrix proteins of hyaline cartilage results in a more effective replacement of the cartilage surface defect, as evidenced by our own research [2, 28, 29].

Typically, researchers employ commercially available differentiation media or create their own to promote cell differentiation toward chondrogenesis [2, 30–32]. In experimental and clinical research, a variety of cell cultures, including both autologous and allogeneic, are employed [33]. Autologous cell culture, initially utilized for hyaline cartilage cell engineering, has the benefit of a greater body of statistical and experimental evidence. However, it is characterized by challenges in harvesting, standardization, and increased economic costs. We believe that allogeneic cell product implantation exhibits advantages over autologous implantation because it enables the preparation of a standardized cell product in advance, which eliminates the need for autologous material harvesting, pure fibroblast culture isolation, and subsequent cultivation. Thus, the highlights of an allogeneic non-immunogenic cell culture include its availability (which can be derived from an individual or a specialized cell bank), the potential for reprogramming [34], and the lack of an immune response [35].

There are relatively few experimental and clinical studies on the use of allogeneic cell cultures, despite the evident advantages they offer. Allogenic cell cultures are primarily employed for healing superficial wounds [36–38] with fibroblasts being the primary component [39, 40]. However, there are emerging studies that examine the trans-differentiation of fibroblasts, which have the potential to be applied in tissue engineering across various fields [41]. To date, we have identified only a few studies focused on the trans-differentiation of fibroblasts toward chondrogenesis using chondrogenic differentiation media, in addition to our own preliminary experiments. The trans-differentiation of human dermal fibroblasts toward chondrogenesis appears to be lacking in both theoretical foundation and practical methodological justification.

This study aimed to compare and assess the chondrogenic potential and the fundamental possibility of modifying the culture of human dermal fibroblasts with the known chondrogenic modification capability of rabbit multipotent mesenchymal stromal cells using two differentiation media: the StemPro™ Chondrogenesis Differentiation Kit (Thermo Fisher Scientific, USA) or a self-made medium that utilizes the crucial chondrogenesis cytokine TGF-β3.

MATERIALS AND METHODS

Cell culture

The study was employed using two cell cultures: 1) the DF2 cell line of dermal fibroblast from a 45-year-old female donor [42], acquired from the Center for Collective Use "Collection of Vertebrate Cell Cultures" (Institute of Cytology of the Russian Academy of Sciences), and 2) MMSCs from the bone marrow of a newborn rabbit of the "Soviet Chinchilla" breed. The rabbit was euthanized, and the femur bones were harvested immediately to obtain MMSCs. A portion of the femur bone was extracted in sterile conditions without the need to expose the bone marrow. The bone fragment was then placed in DMEM nutrient medium containing a mixture of antibiotics, penicillin (200,000 IU/mL) and streptomycin (200 μg/mL) (Gibco, USA), where the soft tissues were removed. Subsequently, the bone was incised with a sterile scalpel, and the bone marrow suspension was completely flushed out into PBS buffer using a 5G needle. The cell fraction was sedimented by centrifuging the suspension for 5 minutes at 1000 rpm. The cell fraction was subsequently isolated from the supernatant and seeded onto sterile adhesive plastic. The cytofluorometric status of cells (surface markers) was confirmed in keeping with the method described by Khorolskaya et al., 2021 [43]. Cells were cultured to the third passage in DMEM (Gibco,

USA) supplemented with 10% fetal bovine serum (Gibco, USA), and a mixture of antibiotics, penicillin (50,000 IU/mL) and streptomycin (50 μg/mL) (Gibco, USA) under standard conditions (37 °C, 5% CO_2).

Cell modification

Two distinct protocols were implemented for modifying rabbit MMSCs and human dermal fibroblasts. The first protocol entailed the development of a self-made medium for chondrogenic differentiation that was based on the TGF-β3 protein. In the second protocol, a ready-made StemPro™ Chondrogenesis Differentiation Kit medium was employed.

The TGF-β3 protein-based mixture was prepared promptly prior to each medium change. It comprised TGF-β3 growth factor at a concentration of 10^{-2} ng/μl (Sigma-Aldrich, USA), L-proline at a concentration of 50 μg/ml (Sigma, USA), dexamethasone at 10^{-4} mmol/μl (Sigma, USA), and L-ascorbic acid at 50 μg/ml (Sigma, USA). Cells were seeded in 48-well culture plates. After the third passage, the transition from standard nutrient medium to differentiation medium was implemented. Then, the medium was replaced every three days. The cultivation volume for each sample was 2 ml. Differentiation efficiency was monitored through gene expression on the 7th, 14th, and 21st days. The experiment was repeated a total of ten times in order to acquire statistically significant results.

RNA isolation

The phenol-containing Extract RNA reagent (Evrogen, Russia) was employed to extract total RNA from the modified cells in accordance with the manufacturer's protocol. The solution was subsequently collected into tubes. The samples were stored at –80 °C, if required.

The concentration of total RNA was evaluated employing the Implen NanoPhotometer NP80 spectrophotometer (Germany). The fragmentation of the RNA was analyzed following its separation by electrophoresis to verify its integrity. To minimize RNA loss because of degradation, reverse transcription was performed immediately following extraction to obtain complementary DNA (cDNA).

Complementary DNA synthesis

Following RNA extraction, cDNA was synthesized through a reverse transcription reaction employing the commercial MMLV RT kit (Evrogen, Russia), in keeping with the manufacturer's protocol. The reaction was conducted under standard conditions using the T100 Thermal Cycler (BioRad, USA). The concentration of the synthesized cDNA was determined using the Implen NanoPhotometer NP80 spectrophotometer (Germany) and standardized for subsequent experiments (diluted to a standard concentration in DNase-free water). After that, the cDNA was frozen and stored in DNase-free water at a temperature of –80 °C for a maximum of one month.

Selection of primers and probes for real-time polymerase chain reaction

Real-time polymerase chain reaction (real-time PCR) was implemented to evaluate the change in relative gene expression responsible for chondrogenesis. Modifying this procedure was necessary for sensitivity. FAM (carboxyfluorescein) probes with Taq-Man technology were introduced to the region being analyzed. Gene sequences were obtained from the Ensembl database (https://www.ensembl.org/index.html). Genomics DNA amplification was prevented by selecting primer pairs (Table 1) that captured various exon-exon regions and followed general design characteristics. The annealing temperature and the absence of potential primer-dimer formation were determined using the online oligonucleotide calculator OligoCalc (http://biotools.nubic.northwestern.edu/OligoCalc.html). The specificity of the amplicon and primer pairs was verified using BLAST alignment tools (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Primers were synthesized by Biagle (Russia). To ascertain the optimal annealing temperature at which simultaneous and specific annealing of all primers would occur, preliminary experiments to optimize real-time polymerase chain reaction conditions were conducted. The primer and zond triplets were selected as alternatives for the rabbit Acan gene after being tested against databases to determine their specificity for various organisms.

Real-time polymerase chain reaction

The commercial BioMaster HS-qPCR (2×) kit (Biolabmix, Russia) and previously selected and synthesized primers (see Table 1) were employed to conduct real-time polymerase chain reaction. A final volume of 20 μl was used, which contained 10 μl of master mix, 20 pmol of each forward/reverse primer and probe, and 1 μl of cDNA solution.

The reaction was established on a CFX96 Touch device (BioRad, USA) in accordance with the following protocol: one cycle at 95 °C for ten minutes, 40 cycles at 95 °C for 3 minutes, 64 °C for 20 s, and 72 °C for 20 s.

Gene expression levels were evaluated in terms of fold induction relative to the untreated cell population (control) using the ΔΔCt method, which demonstrates the amount of amplification product relative to the calibrator sample of the "housekeeping" gene *Gapdh* [44]. A substantial increase (more than 10 times) in the quantity of target gene transcripts compared to transcript levels in the untreated sample suggested a change in expression.

Statistical analysis

Statistical analysis of the acquired data was conducted using the Statistica 10.0 program. To compare two independent groups, the nonparametric Mann–Whitney criterion (U) with an approximation by a normal distribution (Z) was employed. The differences were considered statistically significant at $p < 0.05$.

Таблица 1. Перечень анализируемых генов и используемых для проведения полимеразной цепной реакции в реальном времени праймеров и зондов

Table 1. List of analyzed genes, primers and probes employed for real-time polymerase chain reaction

Gene	Type of primer	Sequence	CAT (°C)	Length of primer (b.p.)	Function of gene
	Forward	TGACTTCAACAGCGACACCCA	54	21	Household gene (catalyst of the sixth stage of glycolysis)
	Reverse	CACCCTGTTGCTGTAGCCAAA	54	21	
	Probe	CCAGCCCCAGCGTCAAAGGT	58	20	
Acan	Forward (human)	CGTGATCCTTACCGTAAAGC	52	20	Participation in chondrogenesis, extracellular matrix protein
	Reverse (human)	CTATTTCAGGGGCAAACGTG	52	20	
	Probe (human)	CAGTCCCCTGGAACCCGAG	58	19	
	Forward (rabbit)	ATGACGTCCCCTGCAATTACCAC	57	23	
	Reverse (rabbit)	TACCGAACCAGGGAGTTGATCTCG	59	24	
	Probe (rabbit)	CTGTCCAAAGGTCCTGGCGTGCTC	62	24	
Comp	Forward	AACGCTGAAGTCACGCTCACCGA	59	23	Participation in chondrogenesis, extracellular matrix protein
	Reverse	CCTCGAAGTCCACGCCATTGAAGG	61	24	
	Probe	CCTCCGGGTCCAGCACGACTGTCT	63	24	
Tgfβ3	Forward	GCCAAAGAAATCCATAAATTC	48	21	Participation in chondrogenesis, the main inducer of chondrogenesis
	Reverse	GAATTCTGCTCGGAATAGGTT	50	21	
	Probe	ACAGCCAGTTCGTTGTGCTCC	56	21	
Col2a1	Forward	GGGCAAGACTGTTATCGAGTACC	57	23	Participation in chondrogenesis, extracellular matrix protein
	Reverse	AGACCGGCCCTATGTCCAC	55	19	
	Probe	CCCCATCATTGACATTGCACCCA	57	23	

Note: CAT — calculated annealing temperature (°C).
Примечание: CAT — рассчитанная температура отжига, °C.

RESULTS

The concentration of all the obtained cDNA varied from 391 to 426 ng/μL. Based on the preliminary experiments, the optimal annealing temperature for the primers was determined to be 64 °C (Table 2). The gel electrophoresis contained only one amplification product, and no non-specific primer binding was observed at this temperature.

After optimizing the polymerase chain reaction conditions, data on the relative expression of the genes *Col2a1*, *Acan*, *Comp*, and *Tgfβ3* were collected. There was a relative increase in the expression of genes responsible for chondrogenesis in both cell lines at all-time points following the addition of both variants of differentiation medium. Figures 1 and 2 illustrate the graphs.

Statistical analysis of gene expression in DF2 on the studied differentiation mediums revealed the following results (Tables 3 and 4): Almost all of them, except for *Tgfβ3* on the 21st day, were significantly distinct (p <0.05).

Таблица 2. Параметры используемых праймеров и зондов

Table 2. Parameters related to the primers and probes employed in the study

Parameters	Values
AT of primers (°C)	Minimum — 48
	Maximum — 63
	Optimal — 58
	Maximum difference — 16
GC composition of primers (%)	Minimum — 33
	Maximum — 68
Length of primers (b.p.)	Minimum — 19
	Maximum — 24
The length of the received amplicons (b.p.)	Minimum — 149
	Maximum — 202

Note: AT — annealing temperature (°C).
Примечание: AT — температура отжига, °C.

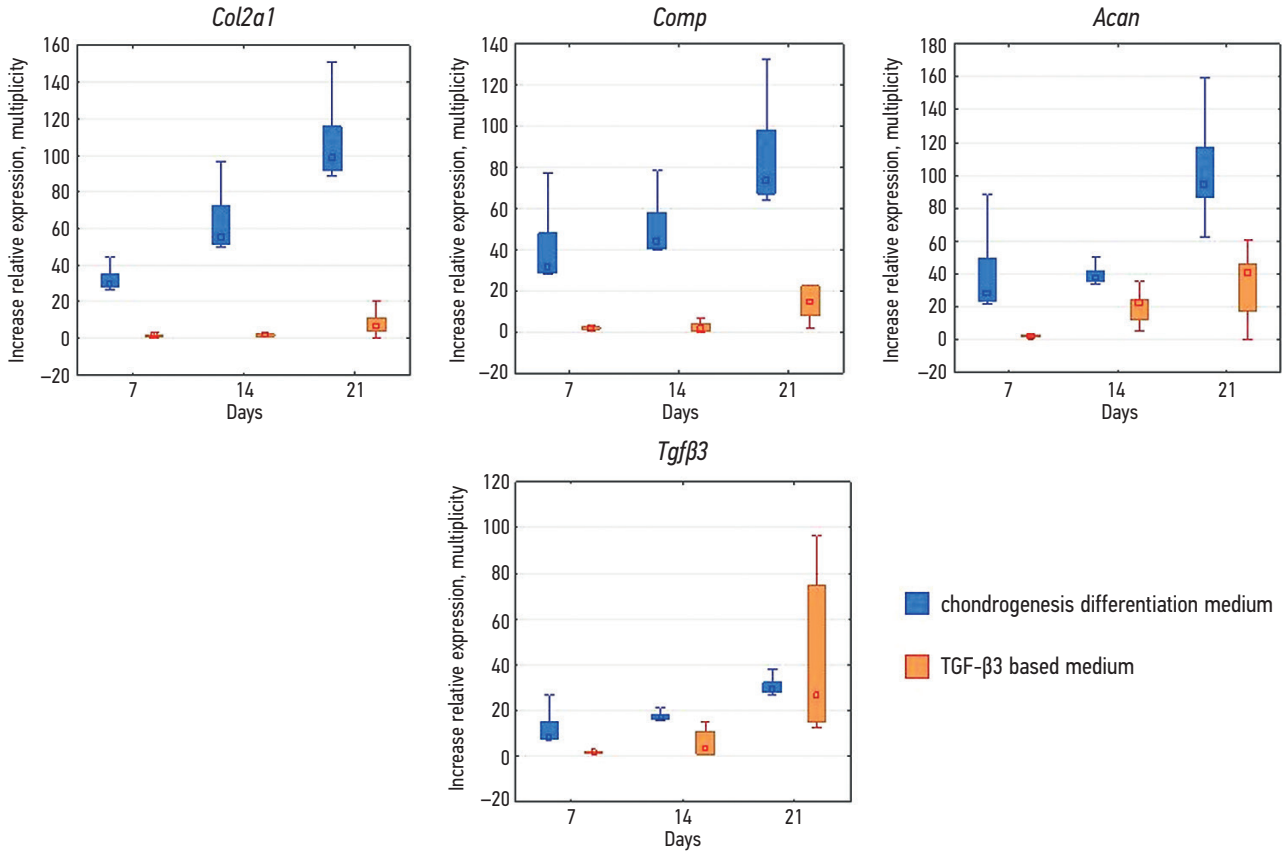

Рис. 1. Изменения относительных значений экспрессии генов, рассчитанных методом –ΔΔCt для дермальных фибробластов человека.
Fig. 1. Alterations in the relative gene expression values calculated using the –ΔΔCt method for human dermal fibroblasts.

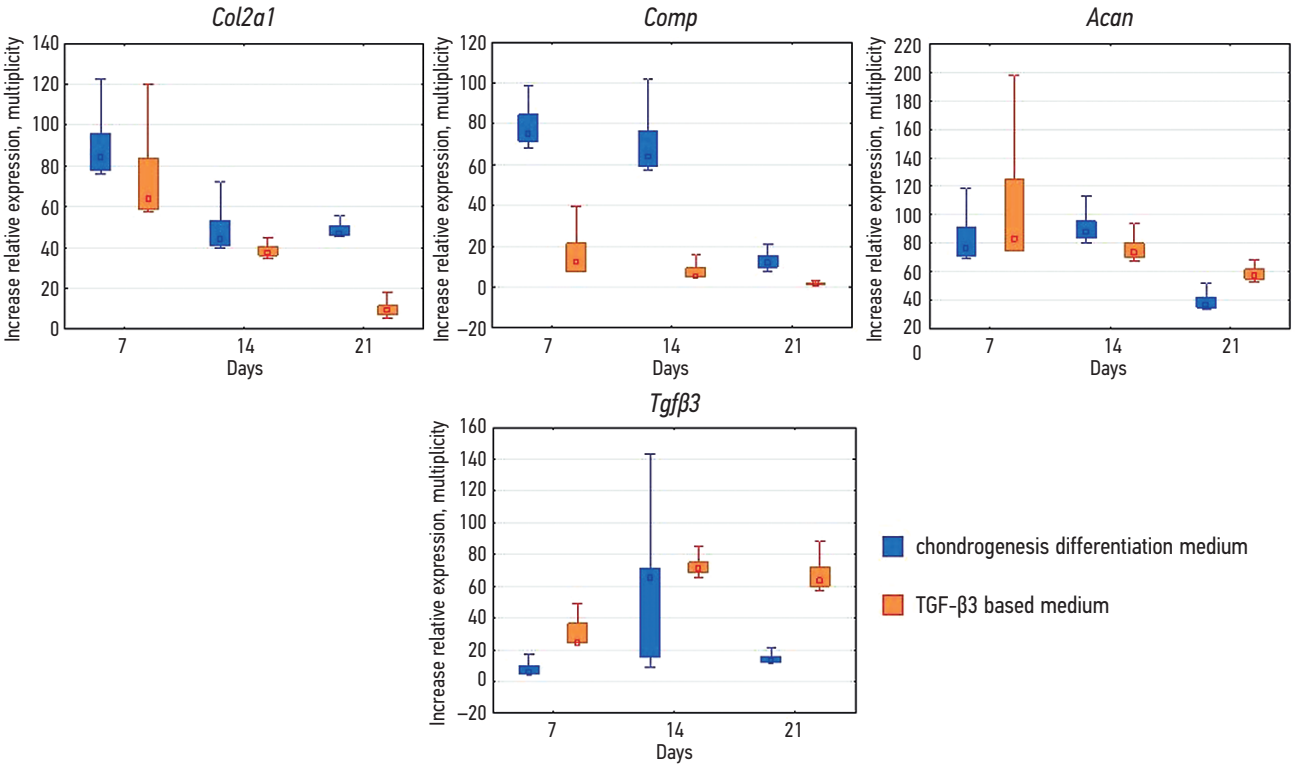

Рис. 2. Изменения относительных значений экспрессии генов, рассчитанных методом –ΔΔCt для мезенхимальных стромальных клеток костного мозга.
Fig. 2. Alterations in the relative gene expression values calculated using the –ΔΔCt method for bone marrow mesenchymal stromal cells.

Таблица 3. Сравнение экспрессии исследуемых генов в дермальных фибробластах человека в зависимости от среды дифференцировки (U-тест Манна–Уитни с приближением к нормальному распределению, Z)

Table 3. Comparison of the investigates genes expression in human dermal fibroblasts depending on differentiation medium (Mann–Whitney U-test with normal distribution approximation, Z)

Days	Me [25; 75] Chondrogenic medium	Me [25; 75] Homemade medium	Sum Rank Chondrogenic medium	Sum Rank Homemade medium	Z	*p*
			Col2α1			
7	29,70 [28,02; 34,97]	1,78 [1,36; 2,0]	1005900	171	7,29	<0.01
14	55,40 [51,17; 72,06]	1,63 [1,13; 2,25]	1005900	171	7,29	<0.01
21	98,40 [91,72; 115,73]	6,60 [4,08; 9,83]	54750	528	9,29	<0.01
			Comp			
7	31,47 [28,70; 48,27]	1,70 [1,45; 2,61]	2669184	21	4,23	<0.01
14	44,32 [40,74; 57,63]	1,76 [0,64; 3,87]	46950	21	4,19	<0.01
21	73,45 [67,30; 98,28]	14,74 [8,32; 22,09]	21100	15	3,81	<0.01
			Acan			
7	28,18 [23,22; 49,52]	1,70 [1,45; 2,59]	1569078	28	4,57	<0.01
14	37,40 [35,22; 41,42]	22,33 [12,51; 24,31]	4725	28	4,38	<0.01
21	94,35 [86,83; 117;17]	40,78 [18,81; 44,68]	76072	36	4,88	<0.01
			Tgfβ3			
7	8,0 [7,21; 15,0]	1,67 [1,35, 1,91]	2781240	21	4,23	<0.01
14	16,90 [16,09; 18,02]	3,45 [1,22; 7,99]	3132	28	4,33	<0.01
21	29,20 [27,95; 32,16]	26,53 [18,70; 64,18]	5040	316	0,62	0.53

Таблица 4. Сравнение экспрессии исследуемых генов в мультипотентных мезенхимальных стромальных клетках в зависимости от среды дифференцировки (U-критерий Манна–Уитни с приближением к нормальному распределению, Z)

Table 4. Comparison of the investigated genes expression in mesenchymal stem cells depending on differentiation medium (Mann–Whitney U-test with normal distribution approximation, Z)

Days	Me [25; 75] Chondrogenic medium	Me [25; 75] Homemade medium	Sum Rank Chondrogenic medium	Sum Rank Homemade medium	Z	*p*
			Col2α1			
7	84,2 [77,67; 95,72]	64,0 [58,59; 89,91]	917765,0	30988,00	7,14	<0.01
14	44,3 [40,74; 53,0]	37,40 [36,26; 40,43]	54468,00	2148,000	7,11	<0.01
21	47,0 [46,73; 53,0]	9,30 [7,21; 11,22]	24900,00	300,0000	7,99	<0.01
			Comp			
7	75,10 [71,60; 84,61]	12,20 [7,58; 21,38]	9261,000	10440,00	10,82	<0.01
14	63,90 [59,40; 79,66]	5,03 [5,07; 9,39]	134080,0	18548,00	19,35	<0.01
21	12,0 [9,70; 14,78]	1,60 [1,19; 2,20]	46764,00	5886,000	14,67	<0.01
			Acan			
7	76,6 [71,57; 91,03]	83,1 [75,45; 114,00]	65536,00	40034,00	–5,24	<0.01
14	87,7 [83,80; 95,91]	73,8 [70,60; 80,06]	26336,00	3554,000	8,83	<0.01
21	37,1 [33,17; 42,43]	57,7 [55,24; 61,97]	5944,000	4496,000	–8,69	<0.01
			Tgfβ3			
7	5,5 [5,05; 9,90]	24,4 [24,19; 36,13]	3685920	649120,0	–20,94	<0.01
14	65,4 [15,45; 71,40]	71,3 [68,59; 75,12]	182083,0	135123,0	–10,92	<0.01
21	13,1 [12,39; 15,84]	63,6 [59,88; 72,42]	9180,000	20466,00	–13,38	<0.01

The chondrogenic media expression of numerous genes was much higher than self-made medium on all examined criteria. Expression of *Tgfβ3* on 21st day did not vary significantly based on the protein medium.

The expression of all mesenchymal stem cell genes was significantly higher on chondrogenic medium, except for *Acan* expression on 7th and 21st days, and *Tgfβ3* for all times. The expression of *Acan* and Tgfβ3 was higher on self-made medium.

DISCUSSION

Currently, experimental tissue engineering of hyaline cartilage is actively advancing [45], and there are clinical examples of applying this technique for joint surface restoration [46, 47]. Several researchers are developing cell-engineered constructs based on diverse autologous cells — mesenchymal stem cells or chondrocytes, different cell cultures, such as autologous chondrocytes [48], peripheral blood cells [49], and peripheral blood stem cells [50]. In our opinion, this significantly complicates the clinical application of this method, as it necessitates testing and standardization of each cell culture employed on every occasion. Allogeneic cell cultures, such as chondrocyte cultures and allogeneic MMSC cultures, are already being utilized in clinical practice [51]. Although we believe that allogeneic cultures are the future of tissue engineering, there are presently only a few studies on the applications of allogeneic cell cultures in traumatology. Of particular interest is the use of allogeneic, commonly used, easily accessible, and standardized cell cultures, such as human dermal fibroblasts.

This study employed the common cell model — MMSCs (as a positive control) and human dermal fibroblasts — as the cell culture under investigation. Allogeneic human fibroblasts, in our opinion, are a promising source for hyaline cartilage tissue engineering, as this culture is readily and minimally invasive to isolate, and amenable to modification for forming articular cartilage regenerates, as demonstrated by Yu et al. [52]. Tissue fibroblasts generate extracellular matrix. Hyaline cartilage disorders are caused primarily by the insufficiency of ECM synthesis. Diverse data regarding the immunogenicity of fibroblasts exist; however, we are of the opinion that they can persist in avascular hyaline cartilage for an extended period and, in accordance with our hypothesis, can execute their therapeutic functions.

Although individual studies exist in the scientific literature on modifying and reprogramming dermal fibroblasts to alter their proliferative status, they usually involve the use of lentiviral transduction. Hiramatsu et al. [53] demonstrated the effective differentiation of fibroblasts into chondrocyte-like cells employing a set of transduced transcription factors, including the reprogramming factors c-Myc and Klf4 and the chondrogenic factor Sox9; however, the presence of the first two transgenes contributes to the potential tumorigenicity of the transduced fibroblasts [53]. In another study, it was also demonstrated that the cytokine TGF-β3 as an additive lead to an increased expression of chondrogenic markers in dermal fibroblasts while maintaining type I collagen expression and fibrous cartilage characteristics [54, 55]. A study from 2015 also reveals the divergent and dose-dependent effect of the cytokine TGF-β3 on the culture of dermal fibroblasts [56]. Table 5 illustrates instances of directed differentiation in the osteogenic and chondrogenic orientations of dermal fibroblasts [53, 55–71].

Таблица 5. Примеры хондрогенной и остеогенной дифференцировки дермальных фибробластов различными способами с указанием источника литературы

Table 5. Examples of chondrogenic and osteogenic differentiation of dermal fibroblasts utilizing different methods with reference to the literature source

Direction	Protein	Genetic	Other type of modification
Chondrogenic	[53, 55–60]	[53]	[61–66]
Osteogenic	[64–66]	[67–69]	[70, 71]

Currently, different methods are being utilized for modifying cell culture, ranging from simple physical interventions such as alterations in temperature and gas composition inside the incubator to the application of genetic methods employing diverse approaches [24, 29, 72–77]. One particular class of chondrogenic modification of cell culture, in our view, involves the use of protein mediums (both self-made and commercial) due to numerous advantages such as accessibility, ease of use, and effectiveness. Even though research demonstrates that multifactorial modification of cells using various growth factors provides a better effect than using a single factor, we opted to focus on a self-made medium that was solely based on the cytokine TGF-b3, since we aimed to demonstrate the fundamental potential of such modification.

The scientific objective was to assess the possibility and impact of diverse protein modifications on human dermal fibroblast cells to facilitate their chondrogenic modification. Two cell cultures and two differentiation media were employed to accomplish this task, in addition to three observation periods (7th, 14th, and 21st days). For statistically significant results, each culture with each form of modification at each time point was analyzed in ten replicates (2·2·3·10), and gene expression analysis was conducted on five genes (4+1). A testing system utilizing FAM probes was created to address the issue of false positive results, which arose due to the restricted modification media and the high number of replicates.

The study compared the chondrogenic potential of two distinct protein modification systems on two separate cell cultures and validated the functionality of the developed FAM probe system. It was shown that human dermal fibroblasts

were capable of undergoing chondrogenic differentiation, as indicated by the elevated relative expression of key genes responsible for chondrogenesis. Additionally, the experiment demonstrated that commercial media is more efficient for modifying cells (both fibroblasts and MMSCs). However, the prohibitive cost of such products and the logistical challenges of conducting the experiment on a large scale in a nutrient medium should be considered. It was hypothesized that there is a constant increase in the expression of chondrogenesis genes in fibroblasts as the observation period increases and the reverse dynamics in MMSCs due to the initially differing expression levels of chondrogenesis genes in MMSCs and fibroblasts. This hypothesis, however, requires additional validation.

This screening study is a necessary step for a quantitative analysis of alterations in protein composition. Further research is needed to determine which cell type, with various alterations, produces more specific extracellular matrix proteins in hyaline cartilage under in vivo settings. However, performing such an experiment would be expensive as it would need a significantly larger amount of protein and/or commercial medium for chondrogenic differentiation. Additionally, it is important to acknowledge that the selection of allogeneic or autologous cell culture for the purpose of tissue engineering in hyaline cartilage has not been entirely resolved, and this scientific and applied issue has not yet been completely resolved.

CONCLUSION

Chondrogenic differentiation of dermal fibroblasts results in a relative elevation in the expression of chondrogenesis-related genes. This cell model is promising for allogeneic tissue engineering of hyaline cartilage. Additional research is required to investigate the quantitative variations in protein synthesis of the extracellular matrix in hyaline cartilage under comparable circumstances.

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Authors' contribution. All authors confirm that their authorship meets the international ICMJE criteria (all authors have made a significant contribution to the development of the concept, research and preparation of the article, read and approved the final version before publication). All authors have made an equal contribution to the work. M.S. Bozhokin — development of FAM probes methodology, cell cultivation, protein modification, conducting real-time PCR, editing of the article; D.M. Marchenko — development of FAM probes methodology, cell cultivation, protein modification, conducting real-time PCR; E.R. Mikhaylova — development of FAM probes methodology, cell cultivation, protein modification, conducting real-time PCR; B.R. Rakhimov — protein modification, RNA extraction, cDNA synthesis, conducting real-time PCR, editing and submission of the article to the editor; S.A. Bozhkova — editing of the article; Yu.S. Korneva — statistical processing of results, editing of the article; S.A. Aleksandrova — cell cultivation, protein modification, editing of the article; M.G. Khotin — editing of the article, payment for publication.

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