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Research Article

Natural Cartilage-Derived Scaffolds for 3D Mesenchymal Stem Cell Culture: Promoting Chondrogenesis and Modulating Secretome Composition

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ABSTRACT

Introduction: The regenerative potential of stem cells lies in their secretome, a collection of signaling molecules whose composition is shaped by the cellular microenvironment and external stimuli. To enhance cartilage regeneration, a 3D culture system using a natural cartilage scaffold has been developed to create a more chondrogenic secretome. This study investigates the chondrogenic differentiation of mesenchymal stem cells (MSCs) cultured in a decellularized bovine cartilage scaffold and analyzes the resulting secretome's composition. **Methods:** This study employed a randomized time series design to examine MSCs chondrogenic differentiation. A control group was cultured in standard 2D conditions, while two experimental groups were cultured in either 2D medium supplemented with chondrocyte differentiation medium (positive control) or a 3D decellularized bovine cartilage scaffold. The study hypothesized that the 3D culture would promote chondrogenesis at least as effectively as the positive control. Key chondrogenic markers were evaluated at various time points. **Results:** Statistical analysis revealed significant differences in marker expression between the experimental and control groups. SOX-9 and aggrecan were elevated in both experimental groups. The 3D group showed higher RUNX-2 expression and the highest Coll-2 expression at later time points. Additionally, growth factor analysis showed the 3D group had the highest levels of IGF-1 and FGF-2 towards the end of the study. **Conclusion:** 3D culture of MSCs in a bovine cartilage scaffold enhances chondrogenic differentiation and produces a secretome with comparable chondrogenic potential to traditional 2D culture with differentiation medium, suggesting its promise for cartilage regeneration.

Keywords: Stem Cells, Three-Dimensional, Culture Technique, Secretome, Cartilage.

INTRODUCTION

Osteoarthritis (OA) is a progressive degenerative joint disease involving synovium, cartilage, and subchondral bone disorders. Conservative treatments for mild to moderate OA, which include stem cell therapy, aim to slow down disease progression. Mesenchymal stem cells (MSCs) vital for promoting tissue regeneration and healing.[1] They possess a unique self-renewal capacity, adaptability, and differentiation into specialized tissue types, including chondrocytes, making them promising candidates for cartilage regenerative medicine. While stem cells offer significant potential for tissue repair, their use is complicated by factors such as immune response, tumor formation risks, cost-inefficiency, and infection transmission risks.[2, 3] Contrary to previous beliefs that stem cells' therapeutic benefits come from their integration and transformation at injury sites, recent research indicates their secreted paracrine factors, collectively known as the secretome, are primarily responsible. These individual factors can be isolated and used for therapeutic purposes.[4, 5]

The factors that shape secretome composition include the type of cells used, the isolation protocol, and the environmental conditions, along with any chemical and physical stimuli the cells experience. To enhance cartilage regeneration, the secretome should be optimized to be more chondrogenic, meaning it should contain increased levels of specific proteins essential for cartilage formation, such as bone morphogenetic protein-2,7 (BMP), fibroblast growth factor-2 (FGF), insulin-like growth factor-1 (IGF), transforming growth factor beta-1,3 (TGF-β), type II collagen (Coll-2), and aggrecan.[6] MSCs culture medium is crucial in producing secretome with the desired properties. For example, stem cells cultured for chondrogenic differentiation require a specific chondrocyte differentiation medium, which can be expensive. With regard to culture medium, secretomes are usually obtained from stem cells grown in flat, two-dimensional (2D) environments. However, 2D cultures do not accurately reflect the complex 3D environment stem cells naturally inhabit within the human body. Consequently, many of their beneficial properties may be diminished or lost. In 2D cultures, cell growth is restricted, leading to slower proliferation and altered morphology and function due to contact inhibition.[7, 8] In contrast, three-dimensional (3D) cultures provide a more accurate representation of the function and structure of living tissues, providing a more accurate simulation of the stem cell microenvironment. This allows for improved cell growth and proliferation, encourages self-renewal, and inhibits premature differentiation. Additionally, 3D cultures facilitate better molecular exchange between cells and their surroundings, nutrient uptake, gas exchange, and balanced waste removal, similar to how cells function in vivo.[9]

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To better preserve the natural chondrogenic characteristics of cells and realistically simulate their in vivo state, a 3D culture system utilizing a natural cartilage scaffold has been developed. This system aims to produce secretomes with a more potent chondrogenic composition. A decellularized bovine cartilage scaffold acts as an ideal 3D cartilage medium, fostering a natural environment for MSC proliferation and differentiation. Compared to synthetic and other natural 3D culture media, the bovine scaffold is natural, has an abundant supply of raw materials, is easily processed, affordable, and safe. Its porosity allows for cell growth and differentiation.[10, 11] Research evaluating the chondrogenic capabilities of MSCs and the resulting secretome in natural 3D culture media are still limited. This study investigates MSCs chondrogenic differentiation cultured in a decellularized bovine cartilage scaffold as a 3D medium and analyzes the composition of the resulting secretome.

MATERIAL AND METHODS

Study Design

This experimental study utilized a randomized time series design with a control group (C) of MSCs grown in a standard 2D monolayer medium. Two experimental groups were included: P1, where MSCs were grown in the same 2D medium supplemented with chondrocyte differentiation medium (CDM), and P2, where MSCs were cultured in a 3D medium using a decellularized bovine cartilage scaffold (DBCS). The P1 group, utilizing CDM commonly used for chondrocyte culture, served as a positive control. This research hypothesized that the P2 group, cultured in a 3D DBCS medium, would perform equally to or better than the P1 group in terms of chondrogenic differentiation. All groups were maintained under identical conditions. The expression of key markers for chondrogenesis, including transcription factors SOX-9 and RUNX-2, matrix components type-II collagen and aggrecan, and secreted proteins TGF-β1, TGF-β3, IGF-1, FGF-2, BMP-2, and BMP-7, were evaluated at specific time points (days 2, 7, 14, and 21) throughout the culture period.

Mesenchymal Stem Cells

Human adipose-derived mesenchymal stem cells (MSCs) were harvested from the infrapatellar fat pad (IFP) of eight eligible donors. Adipose-derived MSCs from IFP were chosen due to the ease of tissue collection, their high proliferation capacity, and superior chondrogenic potential compared to MSCs derived from other sources.[12, 13] Donors aged 20-45 years, free from infectious diseases (TORCH, hepatitis B/C, HIV, COVID-19), and providing informed consent were included. Donors with infections, inflammation, or degenerative tissue conditions were excluded. MSC cultures were expanded to reach a minimum of 2 x 10^7 cells. Cells were identified using the International Society for Cellular Therapy criteria, requiring the presence of CD73, CD90, and CD105 surface markers, and the absence of CD14, CD34, CD45, and HLA-DR.[14]

Culture Media

This study employed several types of media. First, conventional cell culture media, consisting of Dulbecco's Modified Eagle medium (DMEM), penicillin, and streptomycin, provided essential nutrients for cell growth (Gibco, UK). Second, a chondrocyte differentiation medium (CDM) containing nutrients, proteins, and growth factors was used to create a chondrogenic environment, stimulating MSCs to differentiate into cartilage-like cells. This CDM consisted of 15% fetal bovine serum (FBS), dexamethasone (Cyagen, USA), transforming ascorbic acid, fibroblast growth factor 2, and growth factor beta 1.[12, 13] Lastly, a decellularized bovine cartilage scaffold (DBCS) derived from certified slaughterhouses served as a 3D culture medium. The DBCS was processed according to the international tissue bank standards as described in a previous study.[10, 11] The sponge-form DBCS used had a 2 cm diameter, 1 cm thickness, and 100-130 micrometer porosity. The entire experiment was conducted under standard cell culture conditions with 21% oxygen concentration at atmospheric pressure.[10, 11] To confirm that the MSCs successfully integrated into the 3D cartilage scaffold, occupying its pores and filling the entire medium, a scanning electron microscope analysis was performed (Figure 1a & b). Alcian blue staining is to visualize and identify acidic polysaccharides, particularly glycosaminoglycans (GAGs as these are critical components of the extracellular matrix in cartilage (Figure 1c).

Secretome Processing and Extraction

Once cell confluence exceeded 80%, the secretome was processed under controlled conditions. It was placed in a 50 ml dialysis tubing membrane, sealed tightly, and submerged in a beaker containing cold PBS solution. This beaker was stirred overnight using a magnetic stirrer until the secretome's color faded. The dialysis tube was then opened, and the secretome was filtered using a 0.22-micron filter. For further analysis, the filtered secretome was collected in 50 ml conical tubes, sealed, and stored at -20 °C.

Immunohistochemistry Evaluation

Immunohistochemical analysis was performed to assess the expression of transcription factors SOX-9 and RUNX-2, as well as extracellular matrix components type-II collagen and aggrecan. This process began by removing cells from paraffin blocks, drying them at 37°C, and heating them on a slide warmer at 60 °C. Deparaffination was achieved using xylol, followed by rehydration with decreasing alcohol concentrations. After washing, endogenous peroxide activity was blocked, and antigen retrieval was performed in a decloaking chamber. Following washes with PBS and aquabidest, slides were treated with hydrogen peroxide, incubated, and washed again. A trypsin solution was applied for further antigen retrieval, and after washing, diluted monoclonal antibodies were applied to the slides and incubated. A final series of washes concluded the preparation.

Samples were blindly assessed by two independent observers. Immunostaining was conducted utilizing monoclonal antibodies against aggrecan, type-II collagen, RUNX-2, and SOX-9 (Sigma, USA). Expression was visualized using a light microscope (Nikon H600L with digital camera DS Fi2 200 mp and Nikon Image System software), where positive cells exhibited brown-colored cytosol (Figure 2). The expression level was quantified using a modified immunoreactive score (IRS) based on Remmele and Stegner's method. This modification considered not only the intensity of color staining but also the proportion of cells within each intensity category. The final score was calculated by multiplying the percentage of positive cells by the corresponding color intensity (see Table 1).[15]

Enzyme-linked Immunoassay Evaluation

An enzyme-linked immunosorbent assay (ELISA) was employed to quantify specific proteins within the secretome, namely TGF-β1, TGF-β3, IGF-1, FGF-2, BMP-2, and BMP-7. The ELISA protocol involved coating wells with diluted capture antibodies, followed by overnight incubation. After washing, samples were added to the wells and incubated, allowing for antigen binding (Sigma, USA). Streptavidin-HRP conjugate, diluted in blocking buffer, was then added and incubated to bind to the captured antigens. Finally, substrate solution was added, generating a color signal proportional to the protein concentration. After the reaction was stopped, absorbance at 450 nm was measured within a 30-minute window, and the results were calculated using either a log-log plot or a 4-parameter curve fit.

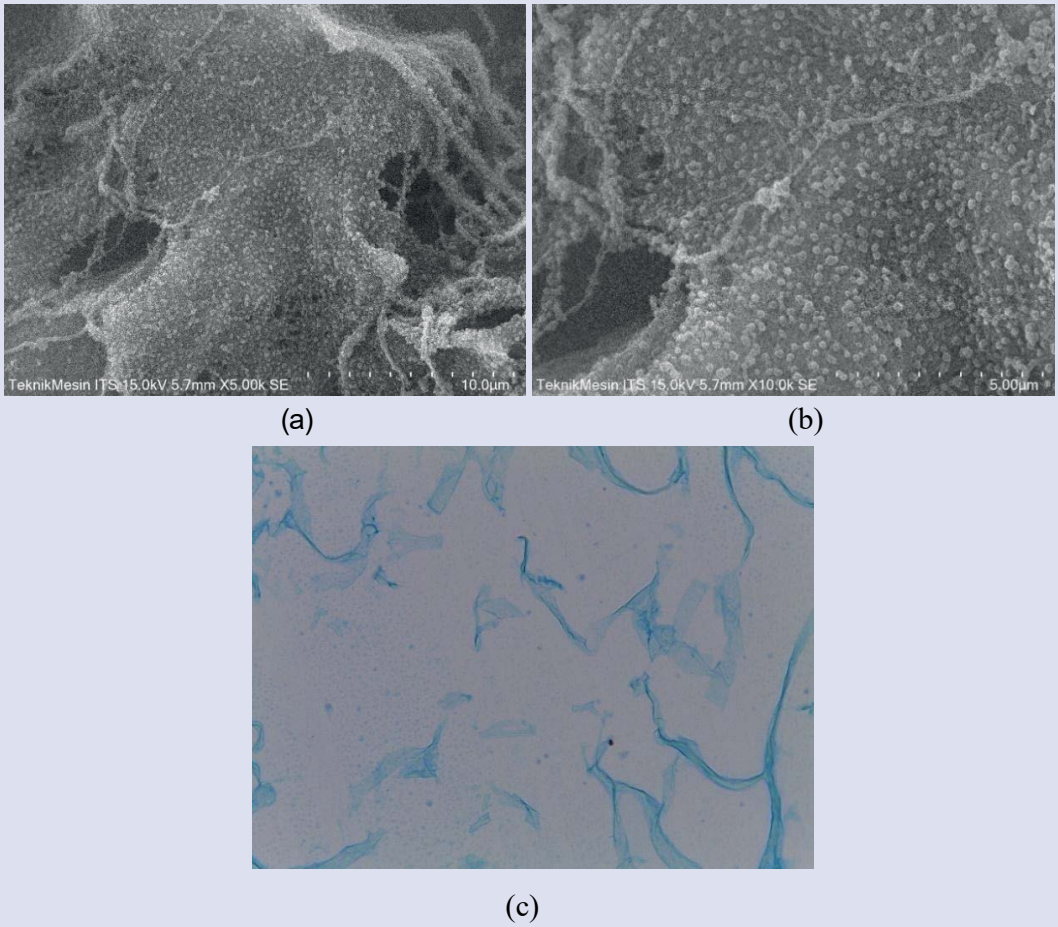

Figure 1. Scanning electron microscope images of 5,000x (a) and 10,000x (b) magnification showed that the cells not only adhered to the surface but also penetrated the porous structure of the 3D medium. Alcian blue staining is to visualize and identify glycosaminoglycans (GAGs), as these are critical components of the extracellular matrix in cartilage (c).

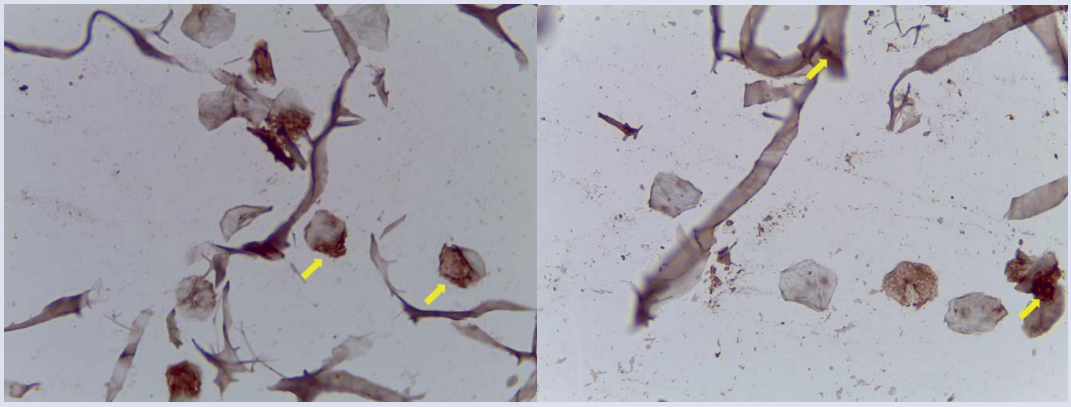

Figure 2. Positive expression of monoclonal antibody shown by brown-colored cytosol (arrow).

Table 1. Semi-quantitative immunoreactive score (IRS) is the result of multiplication between percentage of positive cells (A) and color intensity (B).

A	B
Score 0: No positive cell	Score 0: No color observed
Score 1: Positive cells <10%	Score 1: Low color intensity
Score 2: Positive cells 11-50%	Score 2: Intermediate color intensity
Score 3: Positive cells 51-80%	Score 3: Strong color intensity
Score 4: Positive cells >80%	

Data Collection and Statistical Analysis

All results were collected and statistically analyzed for normality and homogeneity. Two-way parametric ANOVA was employed to assess the significant effects of each variable in this study. Post hoc tests were used to determine significant relationships between variables within the ANOVA analysis. For nonparametric, Kruskal-Wallis test was used. An alpha value of 5% was considered significant. To ensure reliability and validity, this study was conducted in a standardized laboratory with extensive experience in scaffold manufacturing and stem cell processing. Observations were performed by experts proficient in immunohistochemistry and ELISA measurement. A double-blind approach was utilized to minimize bias between researchers and examiners, and data collection occurred in a controlled environment.

RESULT

Immunohistochemistry Evaluation for Transcription Factors and Extracellular Matrix Components

Statistical analysis revealed that only RUNX-2 expression followed a normal distribution across all groups (Kolmogorov-Smirnov test, p>0.05), while RUNX-2 and Coll-2 showed homogeneous variances (Levene test, p = 0.074 and p = 0.161, respectively). ANOVA indicated substantial variations in RUNX-2 expression between groups (p=0.000), and Kruskal-Wallis test confirmed significant differences in SOX-9, Coll-2, and aggrecan expression (p=0.000 for all markers) (Table 2).

SOX-9 expression peaked in the P2 group on day 21 (8.23) and was lowest in the same group on day 2 (5.56). The P1 group exhibited the lowest SOX-9 expression on day 7 (7.23) and the highest on day 14 (7.85). Both study groups (P1 and P2) showed significantly different SOX-9 expression compared to the control group (Bonferroni post hoc test). RUNX-2 expression remained relatively stable across groups (3.5-3.64), with the P2 group exhibiting a significant difference compared to the control group (Tukey test) (Table 3).

Coll-2 expression was highest in the P2 group on days 14 and 21 (9.56 and 9.85, respectively). Aggrecan expression was lowest in the P2 group on day 2 (7.89) and in the P1 group on day 7 (8.87), while peaking in the P2 group on days 14 and 21 (9.83 and 10.34, respectively). Both study groups demonstrated significantly higher aggrecan expression compared to the control group.

ELISA Evaluation of Secretome Composition

None of the markers analyzed via ELISA exhibited normal distribution or homogeneity. BMP-7 levels fluctuated significantly across the observations (Kruskal-Wallis, p = 0.000), decreasing in both study groups in the final observation (Table 4). Only BMP-7 in the P1 group (4.068 ± 0.085) was significantly different from the control group (3.824 ± 0.068) according to the Bonferroni test (Table 5).

BMP-2 levels fluctuated only in the P1 group, decreasing slightly in the last two observations. In contrast, BMP-2 levels gradually increased in the P2 group. Both study groups showed significantly different mean BMP-2 levels compared to the control group (Bonferroni test). TGF-β1 and TGF-β3 levels remained below 1 across all groups and time points. However, both study groups exhibited significantly different levels of these growth factors as opposed to the control group (Bonferroni test). The lowest IGF-1 level was observed in the P2 group at the initial time point (6.892). The highest IGF-1 levels were also found in the P2 group on days 14 and 21 (9.288 and 9.926, respectively). Both study groups showed significantly different IGF-1 levels compared to the control group. FGF-2 levels ranged from 3.094 to 4.891 throughout the observations, with the highest level occurring in the P2 group on day 14 (4.891). Both study groups demonstrated significantly higher FGF-2 levels compared to the control group.

Table 2. Immunohistochemistry evaluation of transcription factors and extracellular matrix components.

		Control (C)	2D Medium + CDM (P1)	3D Medium (P2)	p
SOX-9	**Day 2**	5.61	6.88	5.56	0.000
	Day 7	6.69	7.23	7.85	
	Day 14	6.64	7.85	7.79	
	Day 21	6.94	7.88	8.23	
RUNX-2	**Day 2**	3.46	3.52	3.61	0.000*
	Day 7	3.54	3.51	3.64	
	Day 14	3.47	3.56	3.53	
	Day 21	3.57	3.48	3.52	
Coll-2	**Day 2**	5.61	6.69	6.64	0.000
	Day 7	7.62	8.97	8.99	
	Day 14	7.83	9.13	9.56	
	Day 21	7.86	9.35	9.85	
Aggrecan	**Day 2**	7.07	8.01	7.89	0.001
	Day 7	7.88	8.87	9.24	
	Day 14	9.07	9.79	9.83	
	Day 21	9.89	10.12	10.34	

**Parametric ANOVA*

Table 3. Post-hoc Analysis for Immunohistochemistry Evaluation.

	Control (C) (Mean ± SD)	2D Medium + CDM (P1) (Mean ± SD)	3D Medium (P2) (Mean ± SD)
SOX-9	6.467 ± 0.516[a]	7.463 ± 0.432[b]	7.359 ± 1.066[b]
RUNX-2*	3.510 ± 0.060[a]	3.516 ± 0.046[a]	3.573 ± 0.064[b]
Coll-2	7.231 ± 0.951[a]	8.533 ± 1.088[b]	8.759 ± 1.280[b]
Aggrecan	8.479 ± 1.099[a]	9.198 ± 0.835[b]	9.324 ± 0.925[b]

**post-hoc Tukey;*
[a,b,c] Different letter labels assigned to groups in a statistical analysis indicate that those groups have significantly different results from each other

Table 4. ELISA evaluation of secretome composition.

		Control (C)	2D Medium + CDM (P1)	3D Medium (P2)	P
BMP-2	**Day 2**	2.173	3.156	2.496	0.000
	Day 7	2.426	3.086	2.712	
	Day 14	2.465	3.096	2.998	
	Day 21	2.572	2.955	3.083	
BMP-7	**Day 2**	3.775	4.186	3.087	0.000
	Day 7	3.877	4.031	3.987	
	Day 14	3.888	4.093	4.276	
	Day 21	3.756	3.975	4.161	
TGF-β1	**Day 2**	0.652	0.789	0.692	0.000
	Day 7	0.688	0.883	0.923	
	Day 14	0.701	0.894	0.946	
	Day 21	0.721	0.851	0.945	
TGF-β3	**Day 2**	0.458	0.513	0.378	0.000
	Day 7	0.473	0.516	0.498	
	Day 14	0.466	0.517	0.554	
	Day 21	0.461	0.513	0.544	
IGF-1	**Day 2**	7.325	7.996	6.892	0.000
	Day 7	7.218	8.808	8.759	
	Day 14	7.211	9.139	9.288	
	Day 21	7.198	9.029	9.926	
FGF-2	**Day 2**	3.448	4.513	3.094	0.000
	Day 7	3.922	4.333	4.115	
	Day 14	3.892	4.079	4.891	
	Day 21	3.876	4.071	4.875	

Table 5. Post-hoc Analysis for ELISA Evaluation.

	Control (C) (Mean ± SD)	2D Medium + CDM (P1) (Mean ± SD)	3D Medium (P2) (Mean ± SD)
BMP-2	2.408 ± 0.148^{a}	3.073 ± 0.074^{b}	2.822 ± 0.236^{c}
BMP-7	3.824 ± 0.068^{a}	4.068 ± 0.085^{b}	3.877 ± 0.474^{a}
TGF-β1	0.690 ± 0.026^{a}	0.854 ± 0.041^{b}	0.876 ± 0.108^{b}
TGF-β3	0.464 ± 0.006^{a}	0.514 ± 0.003^{b}	0.493 ± 0.070^{b}
IGF-1	7.237 ± 0.051^{a}	8.743 ± 0.452^{b}	8.715 ± 1.145^{b}
FGF-2	3.784 ± 0.197^{a}	4.248 ± 0.187^{b}	4.243 ± 0.743^{b}

^{a,b,c} Different letter labels assigned to groups in a statistical analysis indicate that those groups have significantly different results from each other

DISCUSSION

Reduced chondrocyte synthesis of cartilage tissue and increased cartilage tissue degradation result in osteoarthritis. Osteoarthritis worsens when aggravating factors arise in a low-grade inflammation of the joint. Joint inflammation disrupts chondrocytes' ability to synthesize and degrade the extracellular matrix, which results in cartilage destruction. Numerous strategies have been tried to prevent osteoarthritis from progressing, but none have been shown to be clinically beneficial.[16, 17] Biological therapies that employ stem cells or their secretome are thus considered highly promising in this context.[18] As a less invasive option to bone marrow-derived MSCs, adipose-derived stem cells exhibit remarkable proliferation and differentiation potential into several mesenchymal lineages.[19, 20] Some studies suggested that bone marrow-derived MSCs have a greater osteogenic capacity than adipose-derived stem cells[21, 22], others suggested that adipose-derived stem cells have an equal or greater osteogenic potential than bone marrow-derived MSCs[23, 24], which makes adipose-derived stem cells suitable for osteogenic regenerative medicine and bone tissue engineering.[20] Most of these investigations contrasted bone marrow-derived MSCs and adipose-derived stem cells derived from various individuals. We used infrapatellar fat pad-derived MSCs since the cells offer promising chondrogenic potential as shown by various in vivo and in vitro studies.[25]

The primary goal of tissue engineering is to create a scaffold that allows cells to grow in three dimensions while retaining their differentiation abilities.[26] A wide range of scaffolds, whether natural or synthetic, has been applied in clinical and experimental settings. Numerous investigations have shown the functional superiority of organically produced decellularized tissue and organ scaffolds over synthetic polymers.[27, 28] Their biocompatibility is typically sustained, as the removal of xenogenic or allogenic antigens enables them to evade immune system activation. Additionally, due to the preservation of a large portion of the scaffold's structural and functional proteins, decellularized tissue and organ scaffolds facilitate signals that support cell adhesion.[29] We used 3D DBCS as one of the study groups that generally exhibited more transcription factors and secretome markers and maintained all markers at the highest rate until the last observation.

SOX-9, a marker of adipose-derived MSCs, regulates the expression and proliferation of osteogenic markers. In terms of influencing cell survival, SOX-9 helps MSCs commit to either the osteogenic or adipogenic development pathways.[30] Nonetheless, several investigations have demonstrated a correlation between SOX9 expression and tumor aggressiveness and carcinogenesis in several forms of cancer.[31, 32] Further investigation is necessary to find out if MSCs that overexpress SOX9 will cause tumors. Research on RUNX2 has predominantly focused on its function in triggering chondrocyte hypertrophy during

endochondral bone formation, but various studies have indicated SOX9's critical role in cartilage development and chondrogenic differentiation.[33, 34] Further support for the hypothesis that RUNX2 positively regulates pre-hypertrophic chondrogenesis independently of SOX9 indicates that RUNX2 is already involved in early stages of development.[35] The effects of RUNX2 and SOX9 on each other are not clearly understood, but one study reported that RUNX2 deletion caused phosphorylation of SOX9 activity[36], and another explained that RUNX-2 provoked translocation of SOX-9.[37] Our study revealed that both study groups had substantially greater SOX-9 levels than the control group, with the first study group slightly higher than the second one (7.463 ± 0.432 vs 7.359 ± 1.066). The rate of RUNX-2 was relatively similar across the groups (3.5–3.64). Compared to the control group, the group with DBCS contained significantly more RUNX-2 and had the highest rate of RUNX-2 (3.573 ± 0.064) than other groups.

Cells generally necessitate a three-dimensional environment, such as an aggregate culture, to undergo chondrogenic differentiation. Precartilage condensation, which occurs during embryonic development and enhances cell-to-cell contact and produces a matrix that resembles cartilage, is mimicked by the micromass pellet culture.[38] When chondrocytes are fully differentiated, they produce substantial proteoglycans and Coll-2 with a spheroidal form. On the other hand, chondrocytes will dedifferentiate, disperse throughout the culture surface, and primarily create collagen type I (Coll-1) and a bit of proteoglycans when separated and cultured in a traditional 2D format.[39, 40] The extensive availability of collagen type I makes it a popular choice for scaffolding in tissue engineering applications.[41, 42] However, studies have demonstrated that Coll-2 selectively improves chondrocyte-mediated wound healing, ECM deposition, and cell proliferation.[43, 44] Aggrecan, an immense proteoglycan, contains chains of chondroitin and keratan sulfate, which provide articular cartilage its resistance to compressive stresses. It can be found in the extracellular matrix as proteoglycan aggregates, where a link protein stabilizes each contact between many aggrecan molecules and hyaluronan.[45] This study showed that Coll-2 was at the highest rate in the second study group (8.759 ± 1.280), significantly different from the control group ($p<0.05$). Aggrecan was observed at the highest rate in the second study group (10.34) in the last observation, with the highest average of all groups (9.324 ± 0.925).

A study explained that the co-culture model determines the biological characteristics of 3D structures. In the direct model, MSCs and chondrocytes are co-cultured on a scaffold or in a culture dish; in the indirect model, MSCs and chondrocytes are separated by a transmembrane.[46] Because primary chondrocytes gradually release a range of protein molecules such as FGF-2, BMP-2, IGF-1, and TGF-β, chondrogenesis of MSCs in direct co-culture is effective.[47] In the absence of exogenous inducers, this method may more effectively impact MSC-assisted cartilage regeneration. The growth factors and cytokines released by MSCs are crucial in reducing inflammation.[46, 47] Furthermore, BMP-7, or osteogenic protein (OP-1), induces the synthesis of collagen and proteoglycan, two components of the chondrocyte matrix.[48] Initially, BMP-7 was isolated and found to be a protein in bone that could stimulate ectopic endochondral bone growth. However, recent findings indicate that this expression is present in several tissues, particularly in the articular cartilage of adults.[49] This suggests that a recombinant bone morphogenetic protein promotes mesenchymal cell ingrowth into chondral defects, whereupon the cells undergo transformation into newly formed tissue that resembles articular cartilage. In this direct co-culture model, BMP-7 was the only marker in the first study group (4.068 ± 0.085) that differed significantly compared to the control group (3.824 ± 0.068). This marker remained at the highest peak (4.161) in the second group on the last observation, along with BMP-2 (3.083), TGF-β1 (0.945), TGF-β3 (0.544), IGF-1 (9.926), and FGF-2 (4.875).

CONCLUSION

Culturing mesenchymal stem cells within a three-dimensional bovine cartilage scaffold promotes enhanced chondrogenic differentiation, as evidenced by the expression of specific extracellular matrix components (aggrecan, type-II collagen) and transcription factors (SOX-9, RUNX-2). The secretome derived from these 3D cultures also demonstrates a favorable chondrogenic profile, with comparable levels of FGF-2, IGF-1, TGF-β3, TGF-β1, BMP-7, and BMP-2 to cells grown in traditional 2D culture with chondrogenic differentiation medium. These findings suggest that the 3D culture system provides a more conducive environment for chondrogenic differentiation, resulting in a secretome with favorable chondrogenic properties.

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DISCOLUSRE

The authors declared no conflicts of interest.

AUTHORS' CONTRIBUTIONS

KD Hernugrahanto: Conceived and designed the study, performed experiments, analyzed data, and wrote the manuscript

NR Noerda: Performed experiments, analyzed data, wrote the manuscript

JAMD Sedar: Performed experiments, analyzed data, wrote the manuscript

L Widhiyanto: Contributed to study design, analyzed data, reviewed and edited the manuscript.

DN Utomo: Conceived and designed the study, supervised the project, analyzed data, reviewed and edited the manuscript

D Santoso: Conceived and designed the study, supervised the project, analyzed data, reviewed and edited the manuscript

ETHICAL CONSIDERATION

Approval by the Medical Research Ethical Committee, Dr Soetomo General Academic Hospital, Surabaya, Indonesia (Certificate number: 0076/KEPK/IX/2020).

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