EFFECT OF CELL RATIO ON CHONDROGENESIS OF CO-CULTURED HUMAN ADIPOSE-DERIVED STEM CELLS AND NASAL CHONDROCYTES IN ALGINATE HYDROGEL

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Cell sourcing, expansion and differentiation of chondrogenesis remain a challenge in cell-based tissue engineering. A co-culture strategy represents a feasible approach to address these limitations. The efficacy of co-culture ratio is known to play a pivotal role in cell cross-talk for successful cartilaginous regeneration. In this study, we investigated the effect of cell ratio on chondrogenic differentiation by co-culturing human adipose-derived stem cells (ADSCs) and human nasal chondrocytes (NCs) at a cell ratio of 1:1, 2:1 or 1:2 for chondrogenesis efficacy. The mixed cells were encapsulated in alginate hydrogel and cultured in basal medium supplemented with Insulin-Transferring-Selenium-X (ITS) for 7 days, followed by examinations on cell viability, cell proliferation, immunohistochemistry, glycosaminoglycans (GAG) synthesis and gene expression. Cell viability and cell proliferation rate showed no different between co-cultured groups. Densely packed collagen type II was detected in the 2:1 group in addition to the increase of GAG, aggrecan core protein (ACP) and transcription factor SOX-9 (SOX-9). Therefore, the co-culture ratio of 2:1 would be potential in driving cartilage-like phenotype and chondrogenic differentiation of ADSCs. The developed strategy is promising for improvement of cartilaginous tissue engineering efficacy.
The lack of available cells in articular cartilage and the problem of rapid dedifferentiation of cultured chondrocytes has raised interest in investigating the capability of mesenchymal stem cells (MSCs) for cartilage regeneration strategies. Bone marrow-derived MSCs have been extensively studied in clinical and pre-clinical settings (8-10). However, extracting such bone marrow-derived cells involved a painful and invasive procedure with a certain level of anaesthesia and a low cell yield (11-13). More recently, adipose-derived stem cells (ADSCs) have been proposed as an alternative source (14, 15). Recent evidence shows the multilineage potential of ADSCs to differentiate into the specific mesoderm tissues, including chondrogenic lineage, osteogenic lineage, and adipogenic lineage, making ADSCs a promising candidate for cartilaginous regeneration (14, 16, 17). After a substantial amount of chondrogenic induction, the cartilaginous progenitor cells tends to move towards hypertrophy (18). This is a major limiting factor of ADSCs.

One approach to overcome these challenges is by co-culturing human nasal chondrocytes (NCs) with multilineage ADSCs (21-24). Several studies have shown that molecular signals play a significant role in the chondrogenic differentiation process (19). These studies report that a co-culture system enables cell cross-talk through the secretion of paracrine factors and also maintains chondrogenic phenotype (20). By co-culturing MSCs and chondrocytes, efficient transduction of chondrogenic differentiation molecular signals between the surface receptors of different cells is facilitated (20-22). However, two-dimensional (2D) co-culture strategy does not mimic the spatial aspects of the physiological microenvironment in vivo (23). The issues regarding availability of primary chondrocytes, large degree expansion of cells, and maintenance of cartilage phenotype remains a challenge (3, 24). A three-dimensional (3D) culture environment is crucial to maintain the cartilaginous phenotype and to provide a supportive matrix for early chondrogenic progenitor cell formation (25). Few studies investigate the efficacy of a co-culture system for chondrogenic induction of MSCs in a 3D system, whether using a porous (24) or a hydrogel scaffold (21, 26). Aung et al. (2011) showed that a higher ratio of chondrocytes to MSCs tends to promote chondrogenesis. The authors demonstrated the presence of cell-cell communication and the effects of cell-secreted morphogens in a co-culture system on chondrogenic phenotype during the induction of chondrogenic differentiation.

Previous studies have demonstrated the ability of MSCs encapsulated in alginate hydrogel to induce chondrogenesis efficiently and maintain chondrocytic phenotype in vitro (27-31). In this study, alginate hydrogels were used to create a 3D microenvironment to enhance chondrogenesis. Alginate is naturally bioinert material, which allows cell-cell interaction due to the lack of native ligands, and its ability to release encapsulated cells enabling cultured cells to be harvested for clinical injection (32-34).

Although there is a similar study on variation of co-culture ratio which evaluated the feasibility of MSCs and articular chondrocytes for promoting chondrogenic and osteogenic differentiation of MSCs (35), the preclinical data relating to this optimal co-culture ratio is inconclusive and no previous studies on similar cell sources (human ADSCs and human nasal septum chondrocytes) exist. The aim of this study is to contribute data on the effect of co-culturing ADSCs with NCs in various cell ratios on chondrogenesis in beads of alginate hydrogel. It was hypothesized that a co-culture of ADSCs and NCs encapsulated in alginate hydrogel would lead to enhanced chondrogenesis compared to ADSCs or NCs alone. To test this hypothesis, ADSCs were co-cultured with NCs in alginate hydrogel at the ADSCs:NCs ratios of 1:1, 2:1, and 1:2, which were maintained in basal medium supplemented with ITS. The effect on chondrogenesis was quantified with a variety of assays.

2.0 Materials and methods

2.1 Human Adipose-Derived Stem Cells (ADSCs) Isolation and Culture

Human abdominal subcutaneous adipose tissues were collected from patients (n = 6, where n is sample size) with written informed consent after caesarean section performed at Universiti Kebangsaan Malaysia Medical Centre (Approved by Research Ethics Committee Universiti Kebangsaan Malaysia with approval code, FF-2015-220). The adipose tissues were cut into small segments and digested with 0.3 % collagenase type I (Worthington Biochemical Corporation, NJ, USA) in an orbital shaker incubator at 37°C for 1 hour. The digested tissues were then centrifuged at 1200 r.p.m. for 10 minutes and the cell pellet was washed twice with phosphate-buffered saline (PBS; pH 7.22, Gibco, NY, USA). The isolated ADSCs were suspended in complete culture medium, which consists of Ham's F12 and Dulbecco's modified Eagle medium (DMEM/F-12; Gibco) supplemented with 10 % fetal bovine serum (FBS; Gibco), 1 % antibiotic-antimycotic (Gibco), 1 % glutamix (Gibco), and 1 % of 50 ug/ml ascorbic acid (Sigma-Aldrich, St. Louis, USA) and maintained in an incubator with 5% CO2 at 37 °C. The medium was changed every other day. ADSCs at 80-90 % confluence in the
culture were trypsinized with 0.125 % trypsin-ethylene diamine tetra acetic acid (EDTA; Gibco) and expanded until passage 3-5 at an expansion degree of 1:4 (14).

2.2 Human Nasal Chondrocytes (NCs) Isolation and Culture

Cartilage specimens were harvested from the human nasal septum of six patients (n = 6) who underwent an elective septoplasty procedure with written informed consent (approved by Research Ethics Committee Universiti Kebangsaan Malaysia with approval ethic code, FF-2015-220). The isolation and culture of NCs was performed as previously described (36). Briefly, the cartilage was separated from bone tissues and washed with PBS. The cartilage tissues were minced into fine pieces and digested with 0.6% collagenase type II (Worthington Biochemical Corporation, NJ, USA) in an orbital shaker incubator at 37 °C for 4 hours. The digested tissues were then centrifuged at 1200 r.p.m. for 10 minutes, and the cell pellet was washed with PBS. The isolated NCs were cultured in complete culture medium as described in the section of ADSCs isolation and culture and maintained at 5 % CO₂ in an incubator at 37 °C. The medium was changed every 48 hours. The cells from passage 1 were harvested with 0.125 % trypsin-EDTA upon 80-90 % confluence for microencapsulation.

2.3 ADSCs and NCs Co-culture in Alginate Beads

ADSCs and NCs were trypsinized and harvested and the cell numbers were determined by a trypan blue (Life Technologies, USA) exclusion test. Five experimental groups were prepared: 1). Pure ADSCs, as negative control, 2). Pure NCs, as positive control, ADSCs:NCs co-culture groups with 3). 1:1, 4). 2:1 and 5). 1:2 ratios. The cell pellet was resuspended at a final concentration of 3 × 10⁶ cells/ml by adding 1 ml of 1.2 % alginate beads each (Sigma-Aldrich, USA). The suspension was aspirated into a syringe with a 27-gauge needle and gently expressed dropwise into the 102 mM calcium chloride (CaCl₂) polymerization solution (37). Calcium divalent ions, Ca²⁺, involved in the polymerization is crucial for gelling by forming alginate bead droplets and encapsulated the cells from the CaCl₂ solution. The alginate beads were polymerized completely for an additional of 8 minutes. The solution containing the beads was poured on the cell strainer and the beads were rinsed twice with 0.9 % sodium chloride (NaCl) solution. The beads were then rinsed with basal culture medium, comprising Ham's F12 and Dulbecco's modified Eagle medium (DMEM/F-12; Gibco) supplemented with 1 % Insulin-Transferring-Selenium-X Supplement (ITS; Gibco), 1 % antibiotic-antimycotic, 1 % glutamax, and 1 % ascorbic acid, and cultured in 6-well culture plates (SPL Life Sciences, Korea) with 2 ml of basal culture medium with ITS. Culture medium of all study groups were changed every two days continuously for a week and incubated with 5 % CO₂ in the temperature of 37 °C.

2.4 Cell Recovery

Alginate beads encapsulated with cells were recovered by adding 10 ml dissolution solution (55 mM EDTA) to remove calcium ions from the gel and incubated for 8 minutes at 37°C. The beads of alginate gel lost its gel structure and dissolved after calcium ions bonded to EDTA, allowing cells to be released. Suspended cells were centrifuged at 1200 r.p.m. for 10 minutes subsequently the cell pellet was rinsed with PBS. The cells were then centrifuged for second time at 1200 r.p.m. for 10 minutes. The supernatant was discarded and the cell pellet was then collected for further quantitative analysis.

2.5 Viability Assessment

The viability of the encapsulated cells in alginate beads was assessed by the percentage of viable cells at day 7 measured with trypan blue exclusion test using haemocytometer.

2.6 Immunohistochemical Assessment

The intact alginate beads, after one week of cultivation, were fixed in 10% formalin at 4°C for 24 hours. Fixed beads were processed through dehydration in an ethanol gradient, followed by xylene, and embedded in paraffin. The beads in paraffin blocks were sectioned at 5µm for immunohistochemistry staining of Collagen type II (Col II) by indirect immunoperoxidase procedure. The deparaffinized sections were treated with 3% hydrogen peroxide for 6 minutes, followed by a washing step in running tap water. The sections were placed in Tris-Borate-EDTA (TBE; BioBasic, USA) buffer solutions and heated in water bath with a temperature of 90 °C for 20 minutes. The slides were then cooled in tap water for 10 minutes and rinsed with TBS 1x buffer solutions (Sigma-Aldrich, USA). A circle was drawn on each slide with a Dako pen and rinsed in TBS 1x buffer. All sections were incubated with Col II primary antibody (Thermo Scientific, USA) at room temperature for 30 minutes then rinsed with TBS 1x buffer. The slide thereafter was incubated with the horseradish peroxidase secondary antibody (HRP; DAKO, Denmark) for 30 minutes. Col II was subsequently detected by a rabbit/mouse peroxidase/DAB+ kit (DAKO) with a protocol.
of 8 minutes incubation of 3,3'-Diaminobenzidine (DAB) followed with 1 minute of hematoxylin for nucleus staining. All sections were rehydrated then xylene and mounted with DPX mountant (Sigma-Aldrich, USA). Sections were examined and photographed with a microscopy imaging system (Q550IW; Leica, Germany).

2.7 Biochemical Assays

Cells cultured in alginate beads at day 7 were analyzed for synthesis of GAG and Deoxyribonucleic acid (DNA) content. The recovered cells were digested for 3 hours at 65°C with 1 ml of papain lysis buffer (Sigma-Aldrich, USA). The amount of DNA from each sample was quantified by PureLink Genomic DNA mini kit (Life Technologies, USA) in accordance with the manufacturer's instructions. A UV-Vis spectrophotometer (NanoDrop 2000; Thermo Scientific, USA) was used to measure the concentration of DNA. Using a microplate spectrophotometer (Thermo Scientific, USA) at a wavelength of 595 nm, 1,9-dimethylmethylen blue assay (DMMB; Biocolor, UK) was used to measure the amount of GAG against the standard curve derived from bovine tracheal chondroitin-4-sulfate (Biocolor, UK). The amount of GAG was normalized to DNA content of respective samples to further evaluate biosynthetic activity of the cells encapsulated in alginate beads.

2.8 Real-time polymerase chain reaction (RT-PCR)

All samples were prepared for total RNA isolation using TRizol reagent (Invitrogen, USA). Total RNA was reverse transcribed with a SuperScript III First-strand synthesis kit (Life technologies, USA) based on the manufacturer's protocol. There are three set of genes: chondrogenic genes, (A) COL I, (B) COL II, (C) ACP, (D) SOX-9, stemness genes, (E) Nanog, (F) SOX-2, (G) REX-1, osteogenic genes, (I) OSC (J) OSP, and hypertrophy chondrogenic gene (K) COL X for NCs group, 0:1, ADSCs groups, 1:0, and co-cultured groups of 2:1, 1:1, and 1:2 ratio. Gene expression was analyzed using SYBR Select Master Mix (Life technologies, USA) on an iCycler (Bio-Rad, USA). All genes were relative to a housekeeping gene (GAPDH). Chondrogenic genes and stemness genes were used to regulate the chondrogenesis of the samples as shown in Table 1 (38) respectively. In addition, osteogenic genes were used to determine exclusion of osteogenesis in the experiment model (Table 1). Data of COL I and COL II levels were analyzed by calculating the relative ratio for the efficacy of restoration of specific cartilaginous phenotype. COL II is the most abundant gene in chondrocytes, whereas COL I acts as a negative marker for chondrogenic differentiation.

2.9 Statistical analysis

Data were expressed as mean ± standard deviation and analyzed by paired student's t test in all study groups. P <0.05 was considered significant.

3.0 Results

3.1 Cell viability during co-culture

![Cell viability graph](image)

Fig 1. The cell viability of three co-cultured groups in alginate hydrogel was significantly higher at day 7 relative to NCs group.
ADSCs
NCs
1:1
2:1
1:2

Fig. 2. Immunohistochemical staining evaluation of cartilaginous phenotype for ADSCs in co-cultured with NCs.

The control groups (single cell type) and co-culture groups (ADSCs and NCs) cell viability at day 7 culture was showed in Fig. 1. The results show that both the ADSC-alone group and the co-culture groups maintained a relatively high viability with no significant difference. The cell viability of all co-cultured groups showed a significant higher compared to the NC-alone group.

Figure 1 demonstrated the significant differences between NCs group and study groups respectively (*p<0.05). (Δ) Demonstrates the significant differences between ADSCs group and study groups respectively (Δp <0.05). (#) Demonstrated the significant differences among all co-culture groups (#p <0.05).

3.2 Immunohistochemical evaluation of cartilaginous phenotype

Fig. 2 presents a microscopic view of immunohistochemical staining for COL II in all experimental groups. The cells exhibited round and plump shape at day 7. Intense staining with COL II was observed in both the NCs group (Fig. 2B) and all co-cultured groups (Figs. 2C-2E). A very minimal level of positive staining was seen in the ADSCs group (Fig. 2A). The presence of lacunae and cell aggregation forming large cluster was frequently detected in the matrix of 2:1 group in comparison to other groups (Fig. 2D). Comparatively 1:1 group (Fig. 2C) showed more positive staining of cartilage-specific extracellular matrix (ECM) than 1:2 group (Fig. 2E).

3.3 Quantitative analysis of GAG production
The DNA content was higher among the co-cultured groups (2:1 and 1:2) at day 7 compared to pure NCs and pure ADSCs groups (Fig. 3A). However, there was no difference between 2:1 and 1:1 groups. The pure ADSCs group produced the lowest DNA content after 7 days of culture in alginate beads.

The normalized GAG/DNA ratio demonstrates a significantly higher value in all co-cultured groups compared to NCs and ADSCs groups (Fig. 3C). Further, the GAG/DNA ratio of 2:1 group was significant higher compared to 1:2 group, but no significant difference between 2:1 group and 1:1 group.

Fig. 3. Quantitative analysis of GAG production. (A) DNA content, (B) GAG synthesis and (C) GAG content normalized with DNA content shown higher ratios in all co-cultured groups. (*) significant difference between NCs group and study groups respectively (*p<0.05). (Δ) significant differences between ADSCs group and study groups respectively (Δp <0.05). (#) significant differences among all co-culture groups (#p <0.05).

3.4 Gene Expression Analysis

The mRNA samples of each group on day 7 were analysed to detect three sets of specific gene expressions; chondrogenic genes, stemness genes, and hypertrophy genes, relative to the housekeeping gene (GADPH). The 2:1 ratio co-cultured group showed an overall significant upregulation in cartilaginous gene expression, COL II, ACP and SOX-9; comparable to the other co-cultured groups and ADSCs groups (Figs. 4A-4D). The gene expression levels for COL I was significantly lower in the 2:1 group in relative to all other groups. The cartilaginous markers for COL II, ACP and SOX-9 were statistically significant and most highly expressed in NCs group, followed by the 2:1 group, whereas in ADSCs group the expression was relatively low compared to all the other groups. The 2:1 co-cultured group showed a significantly lower gene expression of the stemness markers; SOX-2 and REX-1 compared to ADSCs group (Figs. 4F and 4G). Stemness gene expression was higher in the ADSCs group than in the co-cultured groups. The NCs group demonstrated a lower expression of all stemness genes (Figs. 4E-4H). The expression of hypertrophy gene, COL X, was significantly lower in the 2:1 group, whereas the 1:1 and 1:2 group highly expressed the hypertrophy markers especially COL X (Figs. 4I-K). The 2:1 group expressed the osteogenic genes, OSC and OSP, significantly lower after 7 days of culture.

The cartilaginous phenotype restoration index by normalizing COL II and COL I showed that pure NCs was significant higher than co-cultured groups, whereas pure ADSCs showed the lowest restoration index (Fig. 5). There was a significant difference in the ratio of COL II-to-COL I between the 2:1 group and both pure NCs and pure ADSCs groups. The 2:1 ADSCs ratio co-cultured group has the highest index among all co-cultured groups.
4.0 Discussion

In this study, we aim to investigate the effect of co-culture ratio using ADSCs with NCs to explore the chondrogenic differentiation in alginate beads. The current study used basal medium with minimal supplementary growth factors to create a controlled in vitro culture environment for different cell types to achieve cellular cross-talk while maintaining efficiency of production of cartilaginous ECM (39, 40). The co-cultured groups at all cell ratios retained a moderately high viability throughout the 7 days of culture. The percentage of viable cells in pure ADSCs group did not show significant differences with other co-cultured groups. This indicates that variation of co-culture ratio does not contribute to cell viability, this finding is consistent with previous report (35).

The synthesis of the most abundant matrix in articular cartilage, Collagen type 2 was demonstrated by immunohistochemistry. All co-cultured groups in alginate beads exhibited similar chondrocytes phenotype with spherical morphology and relatively uniform distributed throughout the 7 days of culture. The ECM of co-cultured cells encapsulated in alginate beads strongly stained for COL II at day 7. The cell cluster was observed largely at the lacunae, especially in the 2:1 group, which showed strong positive Col 2 staining. The amount of newly synthesized Col II increased at higher ADSCs ratio of 2:1 group, suggesting either that ADSCs might differentiate into chondrogenic progenitor cells under the influence of NCs with gradual cartilaginous matrix production, or that NCs induced by the stemness properties of ADSCs preserve their immunophenotype and continue to produce ECM, or even both.

### Fig. 4. Analysis of quantitative gene expressions.
There are three set of genes: chondrogenic genes, (A) COL I, (B) COL II, (C) ACP, (D) SOX-9, stemness genes, (E) Nanog, (F) SOX-2, (G) REX-1, (H) OCT-4, and osteogenic genes, (I) OSC and (J) OSP, (K) COL X for control groups and co-cultured groups of 2:1, 1:1, and 1:2 ratio.

### Fig. 5. Ratio of total COL II to total COL I quantitative analysis of cartilaginous phenotype restoration index.

The co-cultured groups demonstrated an increased in amount of DNA, which surpassed both NCs and ADSCs groups. The proliferation of co-cultured 2:1 and 1:1 groups was significantly higher, even after culture for 7 days. This suggests that co-culture with ADSCs had increased the self-renewal ability of both cell types and lead to high proliferation as well as a high viability rate (14, 41, 42). The concentration of both GAG and DNA were normalized and the co-cultured group at 2:1 ratio showed a higher GAG/DNA ratio than other groups. This indicates that the increased synthesis of GAG and DNA at higher ADSCs co-culture ratio of 2:1 was able to maintain a high viability and enhance chondrogenic differentiation, which was possibly due to the induction by ADSCs (as opposed to the lower ratio of GAG/DNA in the high NCs ratio of 1:2 group). These data are supported by the recent publication by Bian.
et al. (2011) demonstrating that engineered constructs with higher chondrocyte ratios showed a lower GAG and collagen content, and demonstrated that higher MSCs ratio are likely to contribute to the increased of biosynthesis activities.

The quantitative mRNA expression profile further supported the contribution of ADSCs to chondrogenesis in alginate beads as shown by the upregulation of chondrogenic gene expressions. The expression of COL II, one of the crucial markers in chondrogenesis, was significantly increased in the higher ADSCs ratio group (2:1 ratio) over the higher NCs ratio group (1:2 ratio). The result was paralleled after normalizing the expression of COL II to COL I and revealed a low level of COL I in the 2:1 group. The 3D environment in the alginate bead is able to maintain the plump shape of the cells to increase chondrogenesis by inhibiting the production of COL I, which is consistent with other recent finding (43). Early chondrogenic markers, especially SOX-9 which is important for regulating the expression of other genes involved in chondrogenesis (44), were highly expressed in the 2:1 and 1:2 groups indicates the upregulation of chondrocytic differentiation even co-cultured of ADSCs. 2:1 and 1:2 groups showed the expression of the mature chondrogenic markers specifically COL II and ACP, which suggests newly differentiated chondrogenic progenitor cells were formed in the initial stage, as the genes represent the major components of cartilaginous tissues, as demonstrated by previous studies (41, 45, 46). The authors showed gene expression of COL II and SOX-9 as early as on day 7.

The high expression of chondrogenic genes in the 2:1 group was partly explained by the decline in the stemness genes. The expression of stemness genes, NANOG, SOX-2 and REX-1 was at its lowest in the 2:1 group, which suggests that the cells actively underwent chondrogenic differentiation. Also, this reveals that ADSCs may induce the other co-culture cell type, NCs, to proliferate continuously and differentiate to chondrogenic progenitor cells (1, 38). The osteogenesis markers, OSC and OSP, were studied to confirm that the differentiation process promotes chondrogenesis in ADSCs instead of osteogenesis. The results demonstrated the expression of osteogenic markers in 2:1 group was lowest relative to the high expression of 1:1 and 1:2 group, and this could imply positive regulation of chondrogenesis, as seen in the downregulation of osteoblast specific genes (47).

The co-cultured group, especially at a ratio of 2:1, demonstrated cartilaginous matrix formation between both the alternative sources, ADSCs and NCs, both of which are easily harvested with their respective phenotypes well preserved. This is supported by a previous study Mo at al. (2009) demonstrating that the presence of both human MSCs and rabbit articular cartilage are crucial for providing insight into cell-cell interaction to drive differentiation (48, 49). The significant difference between co-culture and single cell type culture is important for research into cartilaginous tissue regeneration in order to replace the required chondrocyte number with ADSCs, while still maintaining the cartilage phenotype upon implantation. 1:2 and 2:1 groups showed similar viability, chondrogenic and stemness genes expression. However, 1:2 ratio exhibited high tendency to influence ADSCs towards osteogenesis which is undesirable for chondrogenis. On the other hand, 2:1 ratio group, which was capable to maintain the chondrogenic phenotype and achieved the similar effect of chondrogenesis without showing tendency towards osteogenesis compared to higher NCs ratio, is favourable for chondrogenic differentiation.

Furthermore, ADSCs might serve as an allogenic cell source in the future, due to the immuneinhibitory properties and capability for in vitro expansion to a larger degree (50). Nonetheless, the microenvironment is important for the facilitation of the biosynthesis of cartilage-specific molecules. In summary, the expression of cartilaginous phenotype was significantly higher at the 2:1 ratio of co-culturing ADSCs and NCs in alginate beads. This suggests that the 2:1 group is the most favourable co-cultured ratio for the regulation of chondrocytic differentiation.

5.0 Conclusion

In conclusion, it is clear that variation of cell ratio plays a crucial role in the regulation of chondrogenesis in a co-culture system of ADSCs and NCs in alginate beads. The higher ADSCs ratio (2:1) demonstrated distinct chondrogenic differentiation as evidenced by the positive outcome of high viability associated with high proliferation, the synthesis of cartilaginous matrix content, and the expression of cartilage-specific markers. This study may provide an effective co-culture system from alternative cell sources to mitigate the shortcomings of current clinical applications of cell-based tissue engineering for cartilage regeneration.

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References


