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The Superior Chondrogenic Potential of Bone Marrow-Derived MSCs: A Comparative Study with Adipose Tissue and Wharton´s Jelly Sources

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Abstract

Background

Thanks to their proven multipotency, independent of source tissue, human mesenchymal stromal cells now attract attention in tissue engineering applications. In our study, we hypothesized that these cells may possess different innate responsiveness to extracellular chondrogenic signals.

Methods

We selected the three most common sources of mesenchymal stromal cells – adipose tissue, bone marrow and Wharton´s jelly. The harvested cells were embedded in collagen type I hydrogel and cultured either in chondrogenic medium (containing ITS, ascorbate and TGF-β3) or in growth medium. To investigate the effect of human platelet lysate, we supplemented the chondrogenic medium with either 1% or 5% human platelet lysate. Growth medium supplemented with 5% human platelet lysate served as the control.

Results

A significant improvement of collagen type II and aggrecan mRNA expression and collagen type II synthesis was observed in the chondrogenic medium, with a reduced platelet lysate content in adipose tissue and bone marrow mesenchymal stromal cells. A strong reaction of bone marrow mesenchymal stromal cells was subsequently confirmed by collagen type II deposition in the extracellular matrix.

Conclusions

Our results suggest using bone marrow-derived mesenchymal stromal cells for cartilage tissue engineering research and applications.

Introduction

Due to the native characteristics of cartilage, its intrinsic ability to heal is poor. Cartilage lacks vascularization, the cells represent only 1–5% of the total tissue volume[1], have low proliferative activity and tend to dedifferentiate. Therefore, most injuries result in gradual tissue degeneration, osteoarthritis, pain and disability of the patient[1,2].

The main function of cartilage is to reduce joint friction, protect bones from biomechanical stress associated with high loads and to work as a key connective element in various tissues. When loaded, hyaline cartilage in joints has to withstand multiaxial compressive and shear forces while also experiencing hydrostatic and osmotic pressure[3]. The most abundant component of its extracellular

matrix is water entrapped in between collagen fibrils (mainly type II), large aggregating proteoglycan aggrecan and hyaluronic acid. Thus, cartilage is considered as a two-phase material which performs like a sponge. The negative charges of aggrecan functional groups repel each other while being approximated during compression. Alternatively, the collagen network spatially limits the movement of proteoglycans and reduces the fluid flow. This altogether gives the tissue its basic features, such as mechanical stiffness and a Young´s modulus ranging from 5 to 25 MPa[4,5].

All components of cartilage extracellular matrix are produced by terminally differentiated chondrocytes trapped in lacunae. Chondrocytes can be localized in three main zones of cartilage: the superficial zone, the middle zone and the deep zone. The zones differ in collagen and aggrecan content, arrangement of the fibers and, subsequently, also mechanical properties and chondrocyte morphology[6]. Chondrocytes in any layer of cartilage are adapted to hypoxia and nutrient deficiency. They are round in shape and express specific markers. The most abundant are collagen type II, aggrecan and superficial zone protein, and in smaller quantities also other collagen types, chondroitin sulphates, glycoproteins and proteoglycans[7]. When cultured in a monolayer and planar environment, they tend to de-differentiate and lose their characteristics. As there is no blood supply in the cartilage tissue, the only way of nutrition is diffusion from synovial fluid. This is the cause of the very limited cartilage repair, and usually, any lesion ends in fibrocartilage formation associated with its suboptimal characteristics.

Defects may be caused by trauma or articular disease. The gold standard in chondral defect treatment is cartilage microfracture, which involves disruption of the cartilage to expose the subchondral bone in order to promote bleeding accompanied by progenitor cell migration and nutrient supply[8]. Since this approach may fail and lead to fibrocartilage formation, efforts are being made to find more effective and safer treatment options.

Cell therapy could help to overcome the many limitations of cell-free protocols. Since the maintenance of chondrocyte culture is very challenging in terms of phenotype preservation and reduced proliferative capacity, using mesenchymal stromal cells (MSCs) would be more favorable. MSCs are relatively easy to harvest, expand and handle. To mimic the nature of cartilage tissue, the chondrogenesis of MSCs can be supported by reduced nutrient supplementation[9]. FBS is commonly used as a source of proteins in cell culture. However, its bovine origin represents a potential risk of disease transmission and an immune system reaction[10]. Therefore, for human medicine applications, only human platelet lysate (hPL) is acceptable.

Chondrogenic differentiation can be further supported by using a proper scaffold with biomechanical features such as the original healthy tissue. Soft and flexible biomaterials like hydrogels are the most suitable option for this purpose. One of the most common materials is collagen. A cell-free collagen scaffold improved the spontaneous healing of an osteochondral defect in a rabbit model[11], proving that the material is suitable for cartilage formation. This positive effect was further supported by implantation of the collagen matrix with embedded MSCs; 7 months after the surgery, hyaline-like tissue

was observed in the chondral defect[12]. However, evidence regarding the source of the MSCs most suitable for both chondrogenesis induction and potential tissue engineering applications is still missing.

In our study, we aimed to compare the chondrogenic potential of human mesenchymal stromal cells of three origins cultured in rat collagen type I gel. The MSCs were isolated from bone marrow (BM-MSCs), adipose tissue (AT-MSCs) and Wharton´s jelly (WJ-MSCs). Furthermore, we investigated the effect of reduced platelet lysate supplementation on cell metabolic activity and chondrogenesis.

Materials and Methods

Cell culture

MSCs were supplemented with growth medium consisting of Alpha MEM (BE02-002F, Lonza), 5% human platelet lysate (Bioinova, a.s.) and 1% penicillin/streptomycin (BM-MSCs), 1% penicillin/streptomycin/amphotericin (AT-MSCs), 50 µg/mL gentamicin (WJ-MSCs). The MSCs were cultured at 5% CO_2 and 37°C. The culture medium was refreshed every three days. Cells were harvested when 80% confluence was reached, and they were embedded in the collagen type I scaffold.

Scaffold preparation

Collagen type I (08-115, Sigma Aldrich) gels with cell suspension were prepared according to Gangatirkar et al.[13] Briefly, a pre-mix solution containing Alpha MEM, human platelet lysate, L-glutamine, gentamicin, and NaHCO3 was prepared. The pre-mix solution was mixed with rat tail collagen on ice, followed by the gentle addition of the MSC cell suspension. The final collagen concentration in the gel was 3.73 mg/mL. The density of the cell suspension in the gel scaffold was 1,000 cells/1 µl of collagen gel. 50 µL of gel with cells were added per well of a 96-well plate and 200 µL of gel with cells were added per well of a 48-well plate. The plates were placed in an incubator for the gel to polymerize, and after one hour, 200 µL, 400 µL of chondrogenic cell culture medium was added, respectively. The chondrogenic medium consisted of Alpha MEM (BE02002F, Lonza), 5% or 1% human platelet lysate, an appropriate antibiotic/antimycotic solution according to the cell type, 10 ng/mL TGF-β3 (100-36E PeproTech®, ThermoFisher Scientific), 1% ITS (I3146, Sigma Aldrich), and 50 µg/mL L-ascorbic acid 2-phosphate (A8960, Sigma Aldrich). The composition of the growth medium is stated above. During the experiment, the cell culture medium was refreshed every three days. A brief overview of the experimental groups is given in Table 1.

Table 1
Tabulated overview of experimental groups and the composition of the cell culture medium. Abbreviations: CH = Chondrogenic; G = Growth; hPL = human Platelet Lysate.

Group name	Type of medium	hPL supplementation
1hPL CH	Chondrogenic	1% hPL
5hPL CH	Chondrogenic	5% hPL
5hPL G	Growth	5% hPL

Viability test

To evaluate the level of cellular metabolic activity and viability, the CellTiter 96® AQ_{ueous} One Solution Reagent system (G358C, Promega) was used. The substrate was added to the culture medium according to the manufacturer's instructions and the cells were incubated for 2 hours at 37°C. Absorbance was measured using spectrophotometer InfiniteM200Pro (Tecan) at λ = 490 nm, reference wavelength λ = 690 nm. Samples were carried out in pentaplicates.

qPCR

For relative quantification of chondrogenic marker mRNA, qPCR was used on Day 1, Day 14 and Day 21. The total mRNA was isolated using RNeasy Mini Kit (74106, Qiagen) according to the manufacturer's instructions with slight modification. The samples underwent a freeze/thaw cycle in the RLT buffer to increase the efficiency of the process. The concentration and quality of the isolated nucleic acid was measured on InfiniteM200Pro (Tecan) using NanoQuant plate (Tecan). The obtained concentrations were in the range of 6.68 ng/μL to 86.24 ng/μL. For cDNA synthesis, qScript™ cDNA Synthesis Kit (95047, Quantabio) was used. The quantification of chondrogenic marker mRNA was relative; thus, EEF1 was used as a housekeeping gene. To verify the chondrogenic differentiation, the following probes were used: Sox9 (Hs00165814_m1), COL2A1 (Hs00264051_m1), ACAN (Hs00153936_m1), all of them TaqMan Gene Expression Assays (4331182, Life Technologies). The qPCR was performed in LightCycler480 (Roche) with parameters set as follows: activation – 95°C, 10 min; amplification – 95°C for 10 s, 60°C for 10 s (50 cycles); termination – 40°C for 1 min. For quantification of mRNA expression, the $2^{-\Delta cp}$ method was used. Each sample was carried out in pentaplicates.

Immunofluorescence staining

Samples were rinsed with PBS, fixed with frozen methanol for 20 minutes at room temperature and stored at -20°C until analysis. Then, the samples were rinsed with PBS and 0.1% Triton X-100 (T8787, Sigma Aldrich) with 1% BSA (SH30574.02, HY Clone) in PBS was added. After 30-minute incubation, the liquid was aspirated and 1% Tween20 (P9416, Sigma Aldrich) in PBS was added for 20 minutes. Then, the scaffolds were rinsed with PBS and primary mouse anti-collagen type II (II6B3-c, DSHB) antibody diluted in PBS 1:200 was added, and the samples were incubated overnight at 4°C. Following the

incubation, the samples underwent three washing cycles with 0.05% Tween20 – for 3, 5 and 10 minutes. After the final rinse with PBS, secondary anti-mouse rabbit Alexa Fluor 488 (4408S, Molecular Probes) antibody diluted 1:400 in PBS was added and the samples were incubated for 60 minutes at room temperature in dark. The secondary antibody was aspirated and propidium iodide (P4864, Sigma Aldrich) diluted 1:200 in PBS was added for 15 minutes. Finally, the samples were rinsed twice with PBS and scanned using confocal microscope Zeiss LSM 880 Airyscan with the following excitation and emission wavelength maxima: propidium iodide λ_{ex} = 561 nm, λ_{em} = 575 nm; AF488 λ_{ex} = 488 nm, λ_{em} = 515 nm. Propidium iodide was used to visualize cell nuclei. The anti-collagen II primary antibody specifically binds to collagen type II as the main protein of cartilage extracellular matrix and is further visualized using AF488. Representative images were captured from each scaffold.

Statistical analysis

The acquired data were analyzed using SigmaStat® 3.5 (Systat software Inc., Middlesex, Hounslow TW4 6JQ, UK) software. Statistical significance between a pair of groups was determined using One-Way ANOVA with Tukey's test for post-hoc analysis. The results are displayed as the mean ± SD. Significant differences were considered at $p < 0.05$ and $p < 0.001$.

Results

Cell viability

To evaluate the cell viability on Days 1, 7, and 21, the MTS assay was performed using the human mesenchymal stromal cells isolated from adipose tissue, bone marrow and Wharton´s jelly embedded in collagen type I gel (Fig. 1).

In the case of AT-MSCs, there were no statistically significant differences among the three different culture conditions. The reduced human platelet lysate supplementation (1%) did not lead to a metabolic activity decrease when compared with both the chondrogenic and growth medium supplemented with 5% hPL. Throughout the experiment, the AT-MSCs metabolic activity was maintained at the baseline level. In the group 5hPL G (control group), a mild increase in viability was observed.

The behavior of BM-MSCs was different. On Day 1, the metabolic activity in all tested groups was comparable, independent of the platelet lysate content and chondrogenic supplementation. However, on day 7, the statistically significant highest absorbance values were measured in the group 5hPL CH, containing chondrogenic medium supplemented with 5% human platelet lysate. The significantly lowest values were measured in the group 1hPL CH. On Day 21, no significant difference was observed. During the 21-day experiment, the detected absorbance gradually declined.

In the WJ-MSCs, no significant differences were observed during the experiment. In the tested groups, a moderate decrease in the absorbance values was observed from Day 1 to Day 21.

Although the gels were seeded with equal numbers of MSCs, the levels of metabolic activity differed greatly among the tested types of MSCs. This difference is especially visible on Day 1; three-fold absorbance values were observed in BM-MSCs when compared to AT-MSCs and WJ-MSCs.

qPCR

Transcription of the genes of interest (transcription factor Sox9, collagen type II and proteoglycan aggrecan) was analyzed using qPCR and the $2^{-\Delta cp}$ method. All the above-mentioned genes are markers of ongoing chondrogenic differentiation. The results obtained are shown in Fig. 2.

AT-MSC

No statistically significant differences in relative expression of chondrogenic transcription factor Sox9 in AT-MSCs were observed on Day 1 or Day 14. On Day 21, the significantly highest transcription rate was observed in the 1hPL CH group. On Day 1, no collagen type II (ColII) mRNA expression was detected in any of the tested groups. On Day 14, there was some level of ColII mRNA expression; however, only duplicate (from pentaplicate) was positive in the 5hPL CH group, there was no statistical significance detected. On both Day 1 and Day 14, expression of the late phase chondrogenesis marker aggrecan was negative in all tested groups. On Day 21, aggrecan mRNA was present only in the 5hPL G group; however, without statistically significant difference.

BM-MSC

On Day 1, the significantly highest Sox9 mRNA expression in BM-MSCs was observed in the 5hPL CH group. On Day 14, there was no significant difference among the three experimental groups. On Day 21, the significantly lowest levels of Sox9 mRNA expression were reached in the 5hPL G control group. The significantly highest level was measured in the 1hPL CH group. Extensive collagen type II mRNA expression was observed in BM-MSCs cultured in the reduced platelet lysate content in chondrogenic medium on Day 14 and especially Day 21. These two values were significantly higher among all experimental groups. Aggrecan mRNA transcription exhibited a similar trend, as in the case of collagen type II on both Day 14 and Day 21. On Day 21 in the growth medium, the aggrecan mRNA expression was almost negligible when compared to the two groups cultured in the chondrogenic medium (1hPL CH and 5hPL CH groups). The significantly highest extent of aggrecan mRNA expression was observed on Day 21 in the BM-MSCs cultured in chondrogenic medium supplemented with 1% platelet lysate (1hPL CH).

WJ-MSC

The different compositions of culture medium also significantly affected the expression of transcription factor Sox9 in WJ-MSCs. In the 5hPL CH group, there was increased transcription by Day 14 in comparison with the 5hPL CH group. On Day 21 there was a statistically significant difference between

the 1hPL CH and 5hPL CH groups. There was no statistically significant difference in collagen II mRNA expression. The expression of mRNA of late chondrogenic marker aggrecan was present on Day 1 independent of culture conditions and with no statistically significant difference. On Day 14, there was no aggrecan mRNA expression. On Day 21, the aggrecan mRNA expression remained negative.

Although the data derived from various cell types were not compared among each other and statistically evaluated, it is clear that the expression of certain genes was several orders of magnitude stronger. Pronounced transcription of collagen type II and aggrecan mRNA was present in BM-MSCs and AT-MSCs. Generally, WJ-MSCs exhibited little to no expression of chondrogenic marker mRNA and there was high sample variability.

Immunofluorescence staining

A weak green signal indicating deposition of cartilage extracellular protein collagen type II was detected in AT-MSCs cultured in chondrogenic medium with reduced platelet lysate content (1hPL CH group). The cell nuclei were not oriented in any direction. Cultivation in 5% platelet lysate in chondrogenic medium (5hPL CH) also led to a weak collagen type II deposition in extracellular matrix. There was no green signal present in the 5hPL G group (control group); suggesting there was no collagen type II synthesized. The synthesis of collagen type II was abundant and strong in BM-MSCs cultured in 1% platelet lysate and chondrogenic medium (1hPL CH). The collagen type II was also present in the 5hPL CH group. However, it was not observed as a confluent layer as in the 1hPL CH group. In the 5hPL G group, there was no synthesis of collagen type II. Reduced platelet lysate content in the chondrogenic medium (1hPL CH) induced collagen type II synthesis in WJ-MSCs. In the two remaining groups (5hPL CH and 5hPL G), no signs of collagen type II deposition were observed.

Discussion

The majority of chondrogenesis studies with MSCs have been performed in pellets or spheroid cultures[14,15]. This is due to the well-known agreement in literature that the chondrogenic process is triggered by cell condensation and cell-to-cell contacts. Providing a sufficient number of cells for creating appropriate cell-to-cell interactions is a crucial determinant for the formation of pre-chondroblasts and the deposition of extracellular matrix[14,16]. However, there is no agreement on the optimal cell density. A number of studies have been published with cell density ranging from 2.5×10^5 MSC/mL up to 50×10^6 MSC/mL; the higher cell density did not translate into more effective differentiation[14]. Any density below 12×10^6 MSC/mL was reported as suboptimal for chondrogenesis in the work of Stüdle and colleagues[17]. On the other hand, Nöth and colleagues observed signs of pre-differentiation, collagen type II and proteoglycans synthesis with 2.5×10^5 BM-MSC per mL of collagen type I gel[18]. In our study, we decided to use a cell density of 1×10^6 cells per mL of collagen type I gel. Even though the 3D cultures like spheroids and pellets support chondrogenesis on their own, they are not suitable for implantation into the defect site; they do not fill up the defect, and it would be difficult to

immobilize them in the loadbearing areas of the joint. Scaffolds are associated with easier manipulation and more resilient mechanical properties and may further promote chondrogenesis.

Collagen type II scaffold would appear to be the first choice as it is the major protein of cartilage extracellular matrix (ECM). It has been shown that embedding MSCs into collagen type II hydrogels enhances cartilage ECM deposition and promotes a rounded cell shape, an important factor for chondrogenic differentiation[16,19]. However, due to strong glycosylation, collagen type II gels exhibit poor mechanical properties and higher immunogenicity in comparison to collagen type I gels[20,21]. Collagen type I gel has been shown to induce chondrogenesis both *in vitro*[22,23] and *in vivo*[24,25] in defect sites. In our study, we observed chondrogenesis to some extent in all AT-MSCs, BM-MSCs and WJ-MSCs embedded in collagen type I gel. During the experiment, a clear scaffold contraction occurred, most likely leading to cell condensation, further cell-to-cell contact formation and thus induction of chondrogenesis. However, because of this, our collagen type I gel itself is not suitable for filling a cartilage defect alone. Therefore, further adjustments of the carrier must be considered carefully to prevent contraction while maintaining the chondrogenic potential of the scaffold. Decreased contraction of the reconstructed rat tail collagen gel was observed following its maturation after 35 days compared to 11 days, and was accompanied with higher thermal stability[26]. We observed only a mild contraction in a composite fibrin/collagen/hyaluronate scaffold *in vivo*[25], which can be explained by the stabilization of the collagen fibers with mucopolysaccharides via electrostatic interaction, water binding and mechanical restraints[26].

Mesenchymal stromal cells' characteristics make them attractive for regenerative medicine in general. In particular their self-renewal potential and ability to differentiate into the cells of connective tissues are very beneficial. Thus, all three tested MSC types—AT-MSCs, BM-MSCs, and WJ-MSCs—are potential candidates for cartilage tissue engineering. Given the native cartilage environment in our study, we decided to investigate the effect of reduced nutrients supply on chondrogenic differentiation. Cells in culture are usually supplemented by fetal bovine serum (FBS), however, due to its xenogeneic origin, FBS is completely unacceptable for human medicine and, furthermore, MSCs cultured with FBS showed low expression of chondrogenic marker genes[14]. Human platelet lysate is a practical alternative for both cell expansion and the applications of MSCs in clinical practice. Human platelet lysate has been shown to promote proliferation and chondrogenesis of human AT-MSCs[27] and WJ-MSCs[28]. Differentiation of umbilical cord-derived MSCs into a chondrogenic phenotype was reported even without the usual TGF-β3 supplementation when 5% platelet lysate in culture medium was used[29]. On the other hand, it was observed that human platelet lysate led to limited chondrogenic capacity of primary chondrocytes *in vitro* when compared to FBS, while promoting proliferation of chondrocytes[30]. The discrepancy between proliferation and differentiation is characteristic for cartilage ontogenesis. In the early phase of differentiation, a high cell density with low extracellular matrix volume is present. During differentiation, the cells become hypertrophic, apoptotically active and senescent[31,32]. If the metabolic activity correlates with the cell proliferation and number of cells present, we can assume that the most effective process of chondrogenesis was promoted in BM-MSCs, considering the steep decline of the values observed (Fig. 1). Another factor which could possibly be involved in the reduction of the absorbance

values is the scaffold contraction. The longer the cells were in culture, the smaller the scaffold diameter was. Furthermore, the stiffness of the scaffolds seemed to increase as well. It was clear that the MTS product (formazan) was partially bound to the scaffold with an increasing tendency as the experiment continued. Thus, we consider that the differences in absorbance might have also been caused by interaction of the dye with the shrunken scaffold.

Chondrogenesis is most explored in BM-MSCs, firstly described by Friedenstein and colleagues[33]. Previously, the superior chondrogenic and osteogenic potential of BM-MSCs was noted when compared to MSCs from adipose tissue and Wharton´s jelly [34,35]. Moreover, to achieve comparable results in chondrogenic differentiation, greater doses of growth factors were necessary for AT-MSCs than for BM-MSCs[36]. To the best of our knowledge, no study has been published comparing the chondrogenic potential of MSCs derived from adipose tissue, bone marrow and Wharton´s jelly in three different cell culture media. A study by Fabre and colleagues, conducted in serum-free conditions, emphasized that given the low variability of age and sex of the donors, AT-MSCs and WJ-MSCs cannot simply be rejected as donor tissues, even though BM-MSCs appeared to be the most reliable candidates for cartilage tissue engineering[37]. Significantly elevated levels of both ColII and Agg mRNA expression are in strong agreement with our data and thus we consider it as further confirmation of our premise. We assumed that BM-MSCs could be the most suitable for cartilage tissue engineering due to their residency in bone and natural developmental proximity to chondrocytes. In addition, chondrocytes residing in cartilage do not have a high supply of nutrients. This fact is advantageous for growth factor supplementation. It is possible to use lower platelet lysate concentration, which means less proteins, e.g. growth factors and other cytokines non-specifically interacting with TGF-β3, BMP-2 and other receptors; and thus a more predictable and consistent effect of the present growth factor TGF-β3 can be expected. In BM-MSCs, all chondrogenic markers were to some extent expressed in both chondrogenic media (supplemented with 1% hPL or 5% hPL) which makes them the most responsive cell type to chondrogenic stimulation among all the tested cell types. A multiple-fold increase of ColII and aggrecan mRNA expression was observed, particularly in the reduced platelet lysate culture conditions (1% hPL). Sox9 mRNA transcription was stronger in the groups supplemented with 5% hPL; however, only in the 1% hPL group was it maintained without a decrease throughout the experiment, indicating that chondrogenesis was strongly induced and stable in the 1hPL CH group. The gene expression quantification method used enables us to compare the levels of gene expression within the three cell types. mRNA expression of both ColII and Agg was up to six orders of magnitude higher in BM-MSCs than in AT-MSCs or WJ-MSCs which is also in accordance with the aforementioned studies[34,35]. In both AT-MSCs and BM-MSCs, a significant positive effect of TGF-β3, a well-known chondrogenic biomolecule[38], on ColII and Agg mRNA expression was observed. The reduction of concentration of human platelet lysate to 1% hPL led to the promotion of chondrogenic transcription factor Sox9 mRNA synthesis, with its levels significantly elevated on Day 21. The weaker effect of TGF-β3 in 5% hPL could possibly be caused by the higher content of human platelet lysate in culture medium which contain a broad spectrum of cytokines, which can interact with different growth factor receptors and lead to a more complex effect. Moreover, the different composition of hPL and FBS significantly influences their effects on cells[39]. hPL was found to contain a significantly higher BMP-2

amount than FBS[40]. Potential antagonist mechanisms between TGF-β and BMP, described by Junya Ning et al, are more complex. TGF-β suppresses BMP signaling via upregulation of CTGF, miR-155, Gremlin, Tmeff1; BMP suppresses TGF-β signaling via upregulation of CTGF and Smad-6, and both growth factors compete for Smad4. They also regulate the transcription of target genes[41].

The superior chondrogenic potential of BM-MSCs over AT-MSCs and WJ-MSCs was further confirmed by the deposition of cartilage extracellular matrix protein collagen type II. The immunofluorescence staining revealed that collagen type II was present in both groups with chondrogenic culture medium (1hPL CH and 5hPL CH). Cultivation in the growth medium did not result in collagen II production in any of the studied cell types. This was probably due to the absence of ascorbate, a crucial co-factor of collagen type II synthesis.

We would like to highlight the discrepancy between collagen type II gene expression and collagen type II synthesis in AT-MSCs. The mRNA expression levels were significantly elevated in both groups with chondrogenic medium (1hPL CH and 5hPL CH). In the 1hPL CH group, the gene expression resulted in a weak formation of the collagen type II layer covering part of the scaffold surface. In the 5hPL CH group, the amount of the synthesized collagen type II was even lower. In our opinion, this data also indicates that higher platelet lysate content can partially suppress the effect of TGF-β3. The deposition of collagen type II in the WJ-MSC ECM was very mild; collagen type II signal was detected in the 1hPL CH group. In groups containing 5% hPL (5hPL CH and 5hPL G), no sign of collagen type II synthesis was detected. Our results contradict the data from Reppel and colleagues who supplemented the culture medium with 10% FBS and 100nM dexamethasone, and declared Wharton´s jelly as a real alternative source of MSCs for cartilage tissue engineering[42]. However, the high age variability of patients BM-MSCs (25–60 years) used in the study may have also negatively influenced the more limited chondrogenic potential of the BM-MSCs. Based on our data, we believe that the suitability of WJ-MSCs for this purpose should be considered carefully. Interestingly, Lim et al. found that WJ-MSCs isolated from different anatomical segments of the umbilical cord have different differentiation potential. The study reported that cells isolated from the maternal and fetal ends of the umbilical cord have better osteogenic, adipogenic and chondrogenic differentiation potential compared to those isolated from the middle segment[43]. Chen and colleagues published a study with WJ-MSCs embedded in a collagen type I gel with the conclusion that the construct may have potential for cartilage tissue engineering[44]. Even though the experimental design was similar to our work, the outcomes are different. Our head-to-head comparison of WJ-MSCs with BM-MSCs and AT-MSCs clearly showed that despite some extent of chondrogenesis in WJ-MSCs and AT-MSCs, the chondrogenic potential of BM-MSCs is distinctly superior.

Conclusion

In our study, we experimentally verified and proved that MSCs derived from bone marrow have superior chondrogenic potential over adipose tissue-derived MSCs and Wharton´s jelly-derived MSCs. Regardless of the cell type, the chondrogenic process was the most effectively induced in the chondrogenic medium

containing 1% human platelet lysate. Embedding cells into collagen type I gel appeared to be a functional construct enabling tight cell-to-cell interaction and thus further promoting differentiation. The significant contraction of the collagen hydrogel scaffold was a limitation of this study. For the purposes of a potential *in vivo* study, the hydrogel composition needs to be optimized to prevent its contraction and eventual dislocation from the defect site.

Abbreviations

AT-MSC
Adipose Tissue-Derived Mesenchymal Stromal Cell
BM-MSC
Bone Marrow-Derived Mesenchymal Stromal Cell
BMP-2
Bone Morphogenetic Protein 2
BSA
Bovine Serum Albumin
CTGF
Connective Tissue Growth Factor
ECM
Extracellular Matrix
FBS
Fetal Bovine Serum
hPL
human Platelet Lysate
ITS
Insulin-Transferrin-Selenium
MSC
Mesenchymal Stromal Cell
PBS
Phosphate Buffer Saline
qPCR
real-time Polymerase Chain Reaction
TGF-β3
Transforming Growth Factor Beta 3
WJ-MSC
Wharton's Jelly-Derived Mesenchymal Stromal Cell

Declarations

The authors declare that they have not use AI-generated work in this manuscript.

Ethics approval and consent to participate

We declare that the patient(s) or their guardian(s)/legally authorised representative(s) provided written informed consent for participation in the study and/or the use of samples.

Title of the approved project: “Regenerace osteochondrálních defektů a hojení ran” project, registration number CZ.02.01.01/00/22_008/0004562 of the European Union Programme entitled Johannes Amos Comenius in the call “Excellent Research”. Name of the institutional approval committee: Ethical committee of the Institute of Experimental Medicine of the Czech Academy of Sciences. Approval number: 2024/04, date of approval: 21. 03. 2024.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

VHB and KV performed conceptualization, methodology, data curation, experimental work, writing and original draft preparation. LW performed reviewing and editing. EF performed supervision, reviewing and editing. All authors contributed to the article and approved the submitted version.

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Figures

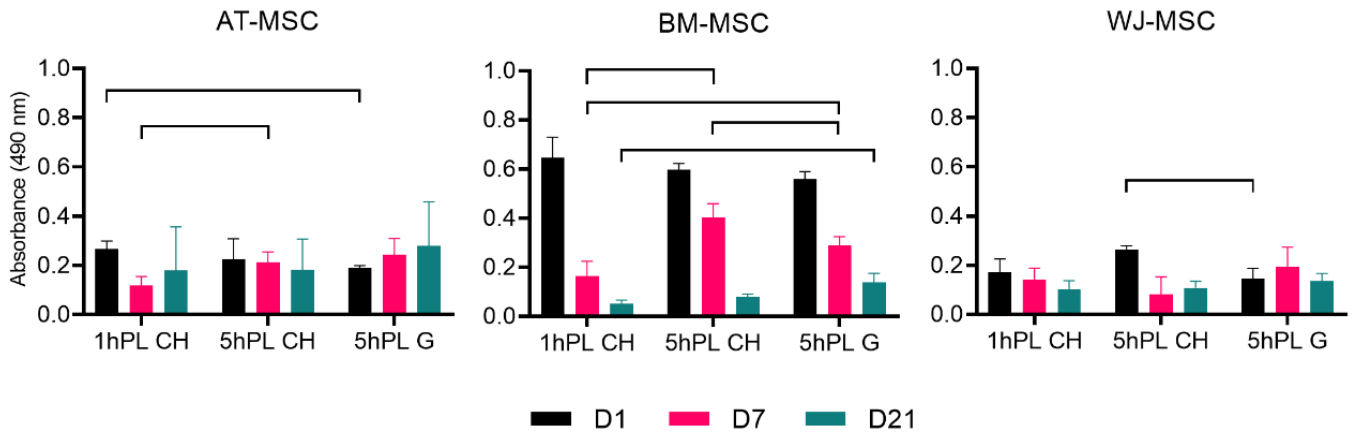


Figure 1

Metabolic activity of mesenchymal stromal cells derived from human adipose tissue, bone marrow and Wharton´s jelly embedded in collagen type I gel. Absorbance was measured at 490 nm using a spectrophotometer, the reference wavelength was 690 nm. Level of statistical significance p<0.01 is marked with a line above the respective experimental groups. Abbreviations: CH = Chondrogenic; G = Growth; hPL = human Platelet Lysate.

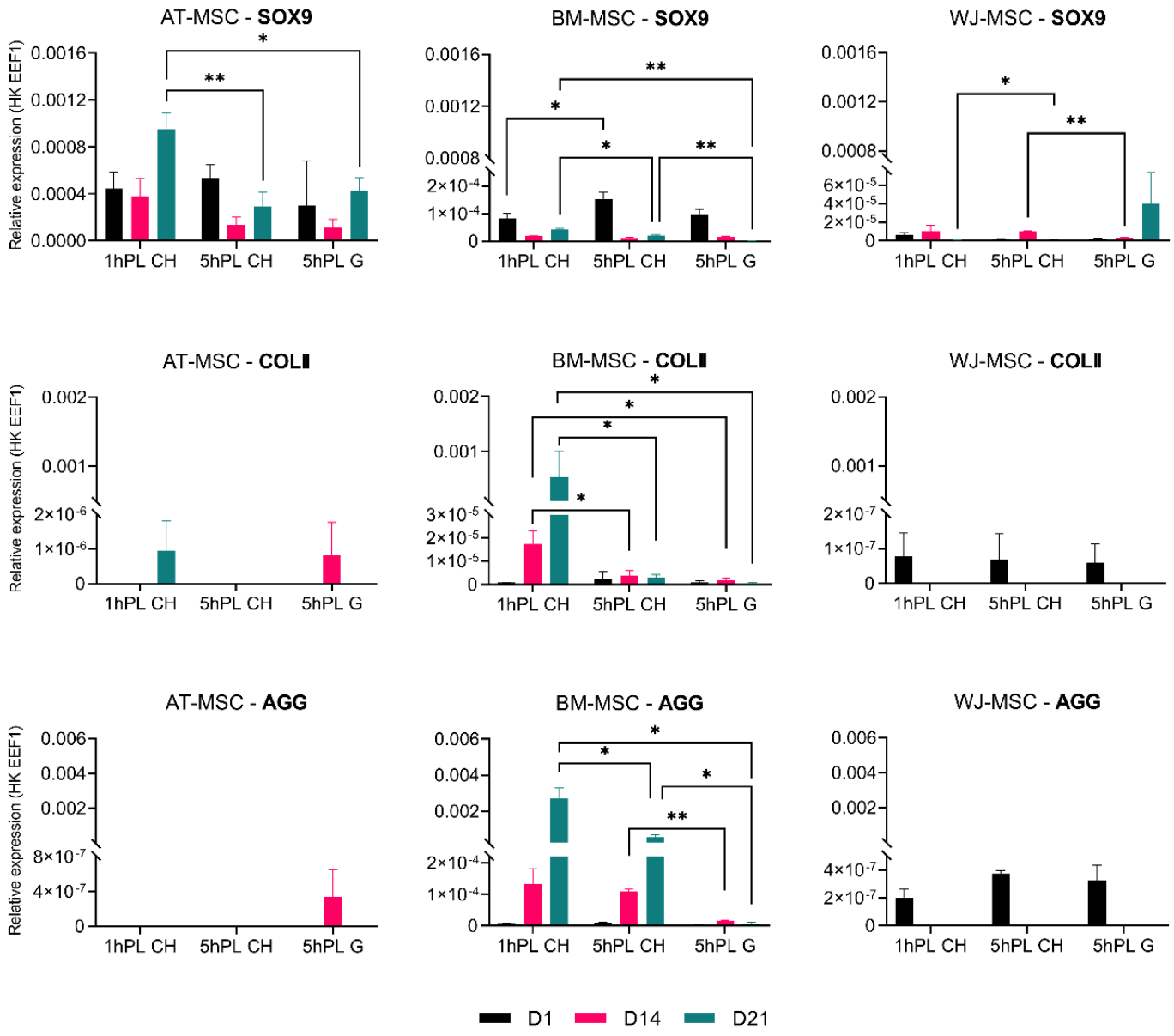


Figure 2

Relative expression of mRNA chondrogenic markers in human adipose tissue, bone marrow and Wharton´s jelly derived mesenchymal stromal cells embedded in collagen type I gel. EEF1 was used as the housekeeping gene. Sox9 was considered as an early marker of chondrogenic differentiation, collagen type II a middle phase marker and aggrecan as a late marker. Datasets were analyzed using the

$2^{-\Delta cp}$ method. The level of statistical significance p<0.01 is marked with two asterisks, the level of statistical significance p<0.05 is marked with one asterisk. Abbreviations: CH = chondrogenic; G = growth, hPL = human platelet lysate.

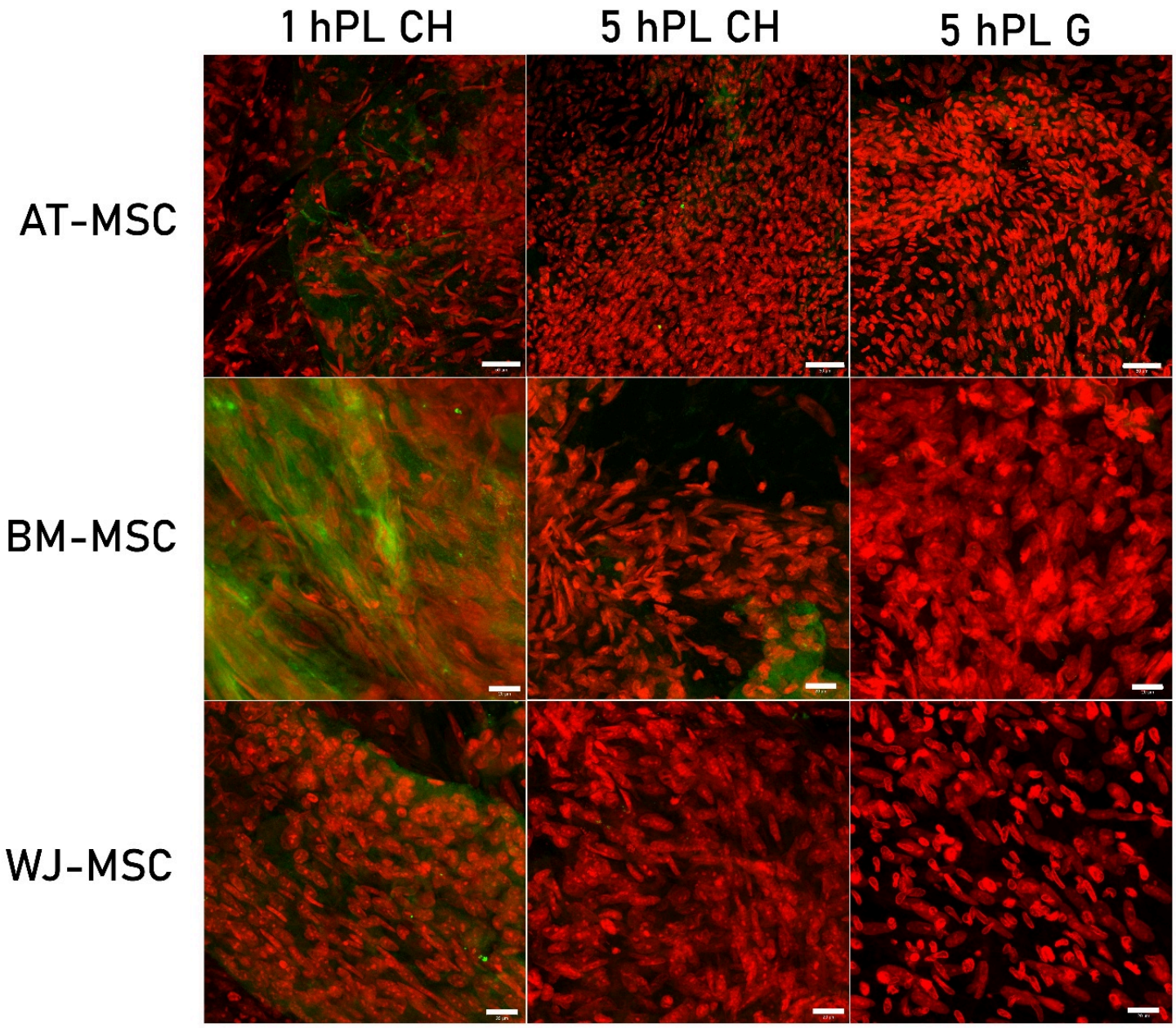


Figure 3

Immunofluorescence staining of cartilage extracellular protein collagen type II deposition using human adipose tissue (AT-MSC), bone marrow (BM-MSC) and Wharton´s jelly (WJ-MSC) mesenchymal stromal cells embedded in collagen type I gel on Day 21. Collagen type II was stained with mouse primary antibody II-II6-B3 and AlexaFluor488 (λ_{ex} = 488 nm, λ_{em} = 515–535 nm), green signal. Cell nuclei were stained with propidium iodide (λ_{ex} = 561 nm, λ_{em} ≥ 575 nm), red signal. Magnification AT-MSCs 40×, BM-MSCs and WJ-MSCs 20×. Scale bar AT-MSCs 50 µm, BM-MSCs and WJ-MSCs 20 µm.