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Optimisation of Chondrocyte Propagation from Human Articular Cartilage to Preserve the Chondrocyte Characteristics

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Abstract

Introduction: Osteoarthritis (OA) is a major health burden and affects millions of people worldwide. This occurs when the cartilage degenerates over a period of time and OA commonly affects the joints in the hand, hips, and knees. Autologous chondrocyte implantation (ACI) and Matrix, matrix-associated autologous chondrocyte implantation (MACI) is a procedure used to treat isolated full-thickness articular cartilage defects of the knee. The cornerstone of these procedures is the ability to cultivate and grow a large number of chondrocytes for a successful outcome.

Materials and Methods: Cartilage specimens of patients undergoing total knee arthroplasty (TKA) were digested using standard collagenase treatment. The resulting cells isolated were evaluated for viability. The cells at equal seeding density were plated on different media for evaluation. Cells from each passage were counted, assessed for viability, immunophenotyping utilizing surface markers used for the characterization of chondrocytes and RT-PCR was performed to confirm expression of chondrocyte genes.

Results: Primary cells plated in DMEM F12 attained faster confluency, the addition of ITS supplement was not essential. Cells maintained their chondrocyte morphology and surface marker expression over passages and expressed genes for chondrocytes. Additional 5 samples were cultured on DMEM F12 without ITS and found to maintain chondrocyte characteristics.

Clinical Significance: Chondrocytes are crucial not only for the development of therapeutic approaches in cartilage repair but are necessary in cartilage tissue engineering to allow the development of functional cell models and novel scaffolds. As a result, an optimized, proven method for chondrocyte isolation must be developed and shared with other groups.

Conclusion: In this study, we describe a simple and affordable procedure of isolation and cultivation of human articular chondrocytes that demonstrated a high chondrogenic potential to the third passage, which is sufficient to grow cells for an ACI procedure.

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Chondrocyte, Immunophenotyping, Osteoarthritis, Primary research, RT-PCR

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INTRODUCTION

Osteoarthritis is the most common form of arthritis, affecting millions of people worldwide. It occurs when the protective cartilage that cushions the ends of the bones wears down over time. Although osteoarthritis can damage any joint, the disorder most commonly affects joints of the hands, knees, hips and spine.[1]

Osteoarthritic cartilage degradation primarily targets the extracellular matrix of articular cartilage. However, cartilage cells play a crucial role throughout osteoarthritis because they are primarily in charge of the anabolic-catabolic balance necessary for matrix maintenance and tissue function. The cells exhibit abnormalities during osteoarthritic cartilage degeneration in addition to the severe changes in the extracellular matrix, including inappropriate activation of anabolic and catabolic activities, changes in cell number due to processes like proliferation and (apoptotic) cell

death, and abnormal activation of anabolic and catabolic activities. The cells are also exposed to extra stimuli such as aberrant cytokine and growth factor levels, non-physiologic loading circumstances, and matrix breakdown by-products. Matrix-assisted autologous chondrocyte implantation (MACI), an upgraded version of the original generation, of autologous chondrocyte implantation (ACI), which has demonstrated promising outcomes, is one important therapeutic method for articular cartilage repair.[2] The process comprises isolating, culturing and implanting autologous chondrocytes on a membrane or scaffold.[1] In 1987, ACI was initially employed to treat human cartilage abnormalities.[3] Cell isolation, a crucial step in ACI/MACI, is often accomplished by mechanically or enzymatically digesting tissue samples, followed by the growth of the cells in a sterile culture medium. Collagenase has been used to isolate chondrocytes from cartilage in the past.[3-5] Only 5 to 10% of cartilage volume comprises chondrocytes.[6] The need for large populations of viable cartilage cells has been a major challenge for tissue engineering that uses primary cells to repair cartilage damage. The yield of cells from cartilage digestion is usually low (less than 20% of the total number of cells available) and the result of the procedure varies due to factors such as donors and user competency.[7] Despite this limitation, a high cell density is critical for the maintenance of chondrocyte phenotype and matrix synthesis[8] and remains a major challenge in cartilage repair. In order to improve on the cell yield required for cartilage repair, *in vitro* cell expansion has been employed. This process may be futile because chondrocyte expansion in a monolayer or passaged chondrocytes can undergo dedifferentiation, thereby exhibiting a more fibroblastic phenotype. A report indicated that total knee replacement surgeries contributed large amounts of biological waste to operating room waste production.[9] Cartilage biopsies that are removed during total knee arthroplasties are biological waste that can be harnessed to isolate viable primary chondrocytes for cartilage tissue engineering. The purpose of this study was to standardise the culture conditions for the isolation and propagation of articular chondrocytes and to evaluate the surface marker expression in various culture media compositions over several passages.

MATERIALS AND METHODS

Cartilage Biopsies

The protocol was approved by the Wockhardt Hospitals Institutional Review Board (registration number - ECR/624/Inst/MH/2014/RR-20) India with study number – WH/IRB/STUDY/2019-07. Written informed consent was obtained from the patients. Cartilage specimens of osteoarthritic patients ($n = 10$) undergoing total knee arthroplasty (TKA) in Wockhardt Hospitals, Mumbai Central, Mumbai, India were obtained from femoral condyles and tibial plateau of the knee joint under aseptic conditions and immersed in sterile bottle containing an appropriate amount of 1x phosphate-buffered saline (PBS) (GIBCO) with 1% antibiotic / antimycotic solution (MP Biomedical) and transported immediately to the laboratory for further processing.

Cartilage Preparation for Digestion

The macroscopically healthy part of the cartilage was washed 3 times with 1x PBS (GIBCO) containing 1% antibiotic / antimycotic solution (MP Biomedical). The soft articular cartilage was scraped and then minced with a sterile scalpel blade into small pieces in a petri dish and weighed.

Cartilage Digestion Using Collagenase

In this protocol, collagenase Type I in PBS was used for the dissociation of cells from primary tissue. 10 mL solution of 0.2% of collagenase Type I (275 U/mg; GIBCO) in 1xPBS per gram of cartilage was added to the petri dish containing minced cartilage. The cartilage pieces were then digested overnight (16 hours) at 37°C and 5% CO_2. After overnight incubation, the cell suspension was neutralized using 0.2% v/v of FBS and incubated at 37°C for 10 minutes. Once neutralized, the cell suspension was strained through 100 and 70 µm cell strainer (BD Falcon) sequentially and washed 2 times by centrifugation in 1xPBS without collagenase. Post the spin, the cells were resuspended in 1-mL 1xPBS and an aliquote was taken for cell count and cell viability. The remaining cells were divided into 4 parts and plated onto 2xT25s with its corresponding media mentioned below.

Cell Count and Cell Viability

10 µL of sample was taken after mixing well and was loaded onto a TC-20 cell counter (BIO-RAD) and total cells were counted and calculated. Cell viability was analyzed by flow cytometry using the DNA dye, 7-amino-actinomycin D (7-AAD). The 7AAD dye is excluded by intact cells, but enters dead cells to form fluorescent DNA complexes, thereby allowing discrimination of dead and live cells.[10] Aliquots of 0.2×10^6 were incubated with the dye 7AAD (BD Pharmingen) for 20 minutes in dark. The cells were analysed on the FACS Calibur flow cytometer (Becton Dickinson) using CellQuest Pro software (Becton Dickinson). Data analysis was performed using the FlowJo software (Becton Dickinson).

Chondrocytes obtained from five different cartilage samples plated with their respective media compositions were cultured in 2xT25s per composition at 37°C with 5% CO_2 and 95% humidity. A media change was given every third day till a confluency of 90% was achieved and were sub-cultured till P3. At every passage, cell count, cell viability and immunophenotyping of cell surface markers were performed for all four media formulations.

Chondrocyte Expansion in four different culture media formulations

Four media compositions were employed for the culture of primary chondrocytes: - a) FORMULATION 1 (F1) - only

DMEM-HG (GIBCO); b) FORMULATION 2 (F2) - DMEM-HG (GIBCO) supplemented with ITS (MERCK); c) FORMULATION 3 (F3) - only DMEM-F12 (GIBCO); d) FORMULATION 4 (F4) - DMEM-F12 (GIBCO) supplemented with ITS (MERCK). All formulations were made with respective media and 10% FBS (GIBCO) with 1% antibiotic / antimycotic solution (MP Biomedical).

Characterization of chondrocytes by immunophenotyping of cell surface markers

The immunoprofile of chondrocytes grown in the four different media formulations was analyzed at passage 0 (P0) and passage 3 (P3) by flow cytometry. The cells were analyzed for two negative markers – CD45 and HLA-DR and seven positive markers – CD44, CD49c, CD151, CD73, CD90, CD105 and HLA-ABC (BD Pharmingen).[11,12] Cells collected after trypsinization from culture flasks were washed twice with 1X PBS (GIBCO). Aliquots of 0.2×10^6 in 100 µL 1X PBS were incubated with monoclonal antibodies conjugated to fluorochromes for 60 minutes in dark. The cells were washed with 1X PBS to remove the unbound antibody and were analysed on the FACS Calibur flow cytometer (Becton Dickinson) using CellQuest Pro software (Becton Dickinson). Data analysis was performed using the FlowJo software (Becton Dickinson).

Chondrocyte Isolation with Optimized Media Formulation

Once the media formulation was standardized with 5 samples, a new set of 5 samples were used for proving the proof-of-concept study for media optimization. These 5 samples were processed in the same manner and sub-cultured till P3 with media which gave best results and was cost effective. Cell counts were taken at every passage till P3 using TC-20. Cell viability and Immunophenotyping of cell surface markers were performed at P3 with same procedure as mentioned before. Safranin O staining along with RT-PCR were done for all chondrocyte passages till P3 - P0, P1, P2 and P3 as mentioned below.

Chondrocyte Staining

At every passage, a micro-mass culture was performed by plating 0.25 million cells reconstituted in their respective media in a drop on 30mm dishes and allowing it to sit at 37°C with 5% CO_2 and 95% humidity for 20 mins and then adding 1-mL media and allowing it to grow. After 7 days, the micro-mass was stained with Safranin O staining.[13]

Reverse Transcriptase Polymerase Chain Reaction

At every passage, 0.25 million cells were taken and RT-PCR was performed on the chondrocytes. The total RNA was extracted and purified using the GeneJET RNA Purification Kit (Thermo) as per the manufacturer instructions. Single-stranded cDNA was synthesized from 0.5 ug of RNA using random hexamers from RevertAid First Strand cDNA Synthesis Kit (Thermo). The isolated RNA quality was estimated using Thermo Scientific™ µDrop™ plate. Genes were assessed (Eurofins Genomics LLC) using the primers given in Table 1.[14] Reaction mixture with nuclease-free water instead of cDNA template prepared parellely was used as PCR negative control for all the lineage genes and the PCR cycling was performed as reported earlier. GAPDH was used as housekeeping gene. The PCR products were separated on 2% agarose gel stained with Nex-Gen Green Stain (Puregene) and visualized using LED book transilluminator.

RESULTS

Cell Count and Cell Viability

Upon calculation, cell counts of all different formulations through different passages were similar. The formulations with ITS had slight higher cell count when compared with formulations without ITS, but not enough to have a drastic impact. But when formulation containing DMEM-F12 was compared with DMEM-HG (with or without ITS), DMEM-F12 showed a slightly higher cell count at P0. This is due to cells growing in colonies during the start of culturing of chondrocytes from a tissue sample. When the cells are seeded for the first time in passage 0, they attach sparingly over a large area of the tissue culture plate and not evenly, allowing cells to grow in colonies and overpopulating the area around the attachment. But once the cells are trypsinized and plated, the attachment occurs evenly, resulting in a monolayer. This is the reason for higher cell counts in Passage 0 in both DMEM-HG and DMEM-F12. However, the cell counts were higher for DMEM-F12 than DMEM-HG (with or without ITS) proving DMEM-F12 was better culture media.

The use of ITS in DMEM-F12 or DMEM-HG didn't make a difference in the cell counts when compared to only DMEM-F12 or DMEM-HG. Hence its use can be avoided.

Similarly, the cell viability over the passages remained similar and comparable within the media formulations, and the presence of ITS didn't have much of an effect on the viability of cells.

Immunophenotyping of Cell Surface Markers

Flow cytometry was used to investigate whether changes in culture conditions caused any alteration in the cell surface markers of chondrocytes. Analysis was performed at P0 and P3 stages of culture in each of the media formulation.

Chondrocytes grown in all four different culture conditions were positive for the markers CD44, CD49c, CD151, CD73, CD90, CD105, and HLA-ABC, with more than 90% cells positive for each of the markers. Cells in all four different culture conditions were negative for the markers CD45 and HLA-DR, with less than 5% of cells expressing each of the markers. As seen in Figure 3 and 4, no difference was observed in marker expression among the media formulations. Also, no difference was seen in the expression at P0 and P3 (Figures 3 and 4).

Days to Confluency

As at P0, DMEM-F12 (with and without ITS) showed more cell count, and the days to achieve confluency were less when compared to DMEM-HG (with and without ITS). Supplementing the media with ITS also had comparable results. Hence, using DMEM-F12 reduced the need for additional media changes.

Post optimization of media formulation, 5 samples were processed as per the procedure and their results show that proof of concept worked for isolation and expansion of chondrocytes. The representative cell count, cell viability, immunophenotyping of surface marker, Safranin O staining of Chondrocytes as Micromass and RT-PCR results are as follows –

Cell Morphology and Cell Viability

Cell morphology and viability are presented in figures 7 and 8.

Immunophenotyping of Surface Markers

Immunophenotyping is presented in figure 9.

Safranin O Staining of the Chondrocytes as Micromass

Safranin O is a cationic dye that binds to the glucosaminoglycans (GAGs) in chondrocytes, staining them pink to red in color. The safranin O staining of the micromass cultures of chondrocytes of all five samples at different passages showed the GAGs in the chondrocytes stained pink in color and demonstrated the typical structure of chondrocytes. This staining confirmed the morphology and characteristics of the isolated cells. The staining was consistently observed at all passages from P0 to P3 (Figure 10).

Figure 10: Safranin O staining of human primary chondrocytes cultured in DMEM F12 post standardization in micromass culture of a representative sample showing consistent staining over passages from P0 to P3; A – Micromass culture of Passage 0; B – Micromass culture of Passage 1; C – Micromass culture of Passage 2; D – Micromass culture of Passage 3.

Reverse Transcriptase Polymerase Chain Reaction

Molecular analysis of chondrocytes, showed presence of genes associated with chondrocytes, clearly proving that cells isolated are chondrocytes from all four different formulations. The RT-PCR analysis shows presence of Col1A1, Col2A1, Aggrecan, Sox 9 and ColX and housekeeping gene – GAPDH, thus proving that isolated and sub-cultured cells at every passage were chondrocytes.

CLINICAL SIGNIFICANCE

Cartilage tissue engineering is slowly progressing toward *in vivo* application; several scaffolds have been transplanted into humans, and chondrocytes are employed in the treatment of osteoarthritis. As a result, an optimized, proven method for chondrocyte isolation must be developed and shared with other groups. This would be an important step toward quality assurance. Furthermore, because much of the available material focuses solely on harvesting cartilage from small volumes of cartilage, techniques rarely perform well when scaled up. The goal of chondrocyte modification for autologous implantation is to create viable and phenotypically stable cells capable of enhancing repair processes in the area of cartilage damage. Cells are responsible for the balanced turnover of the extracellular matrix, which is required for the extracellular cartilage's integrity. Monolayer culture allows for in vitro chondrocyte expansion, in which cells change their shape and metabolism in a process known as dedifferentiation.[15] The significance of cell monolayer expansion from tiny biopsies is based on its practical application in repair procedures such as autologous chondrocyte transplantation.[4] The purpose of this study was to evaluate if chondrocytes isolated from human cartilage of 10 patients undergoing a TKR with osteoarthritis maintained their proliferation and chondrogenic potential on different media compositions. We found that the use of F12 media without the addition of the ITS supplement gave similar results to media supplemented with ITS. The addition of ITS would significantly raise the cost of culturing chondrocytes, hence can be done without. Here we demonstrated that using F12 media not only resulted in similar growth and characteristics of chondrocytes, but

Table 1: Primers and amplicon size of genes used in this study

Human Gene	Forward primer (5'-3')	Reverse primer (5'-3')	Amplicon size
Chondrocyte lineage			
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	CCATGAGAAGTATGACAACAGCC	CCTTCCACGATACCAAAGTTG	498
Collagen type I alpha 1 chain (COL1A1)	AGAGTGGAGAGTACTGGATTGA	GTTGGGATGGAGGGAGTTTAC	604
Collagen type II alpha 1 chain (COL2A1)	GTCCTCTGCGACGACATAATC	CATCAAATCCTCCAGCCATCT	382
Aggrecan Core Protein, (ACP)	CAGAATGGGAACCAGCCTATAC	TCAAGGTGTCCTGAAACATCTC	424
Human Transcription Factor SOX-9, (SOX-9)	ACCTATCCAAGCGCATTACC	AAGGCAGCTCCTCCTTAAATC	536
Collagen Type X alpha 1 Chain , (COL X) (COL10A1)	GACCCAAGGACTGGAATCTTTA	CTGAGAAAGAGGAGTGGACATAC	274

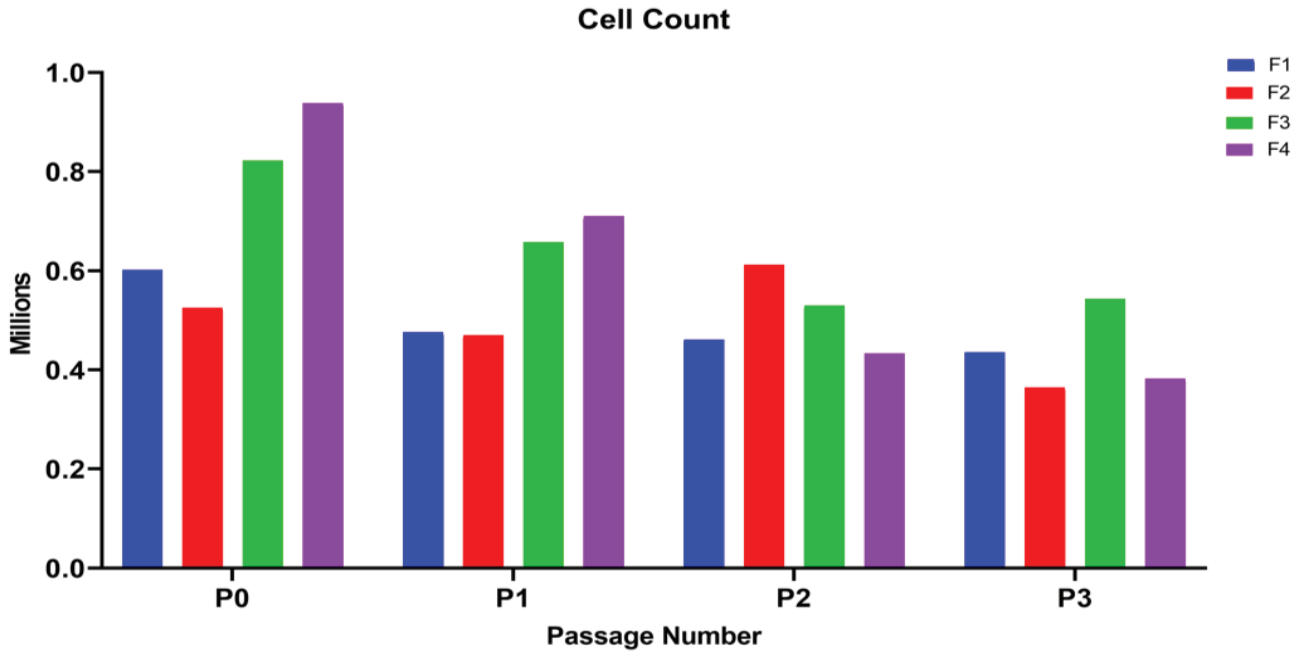

Figure 1: Cell Count – Cell counts obtained from four formulations F1-F4, at passages P0 to P3; Cell counts of primary cells plated in the four different media formulations yielded higher number of cells in F3 and F4 compared to F1 and F2 (P0). Among F3 and F4, there was no appreciable difference. The cell yield at P1 showed the same trend as observed in P0. There was no difference in cell counts at P2 and P3 among the four media formulations; where F1 is only DMEM-HG, F2 is DMEM-HG with ITS supplement, F3 is only DMEM-F12, and F4 is DMEM-F12 with ITS supplement.

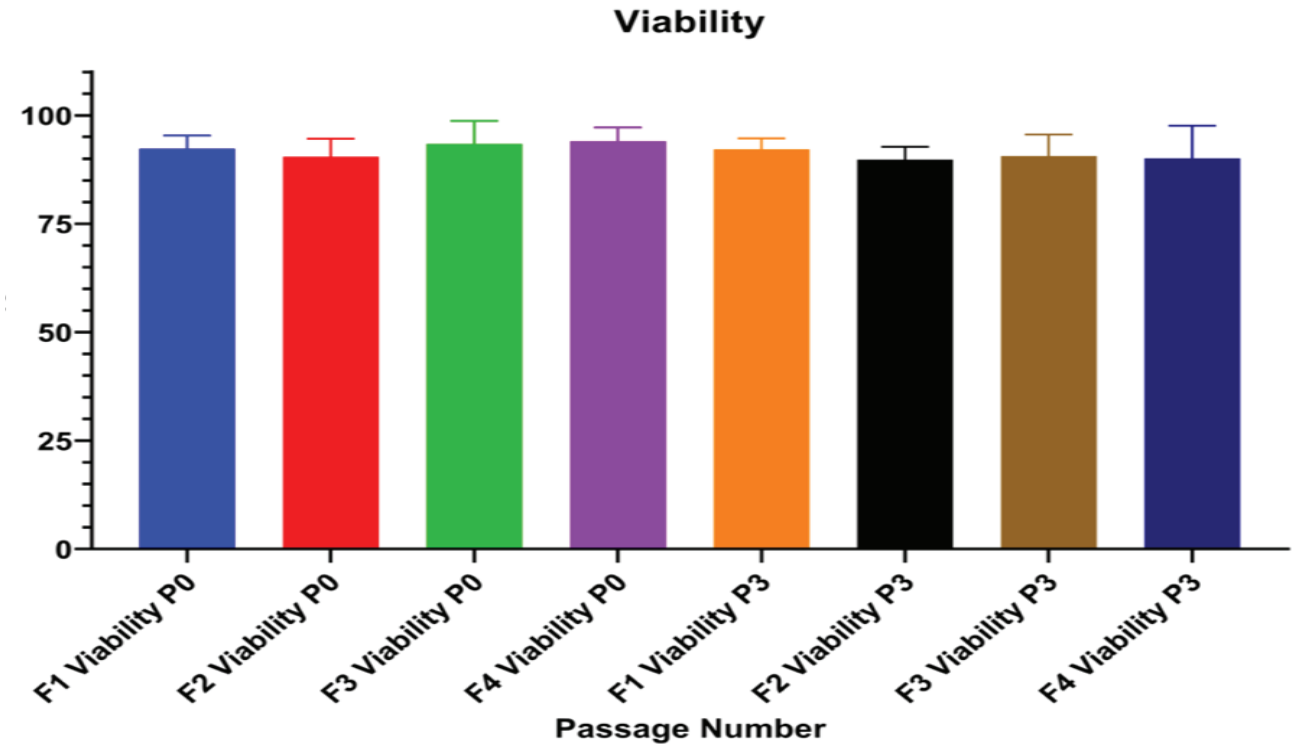

Figure 2: Cell Viability –Viability of chondrocytes cultured in the four formulations F1-F4 remains above 90% over passages P0 to P3; where F1 is only DMEM-HG, F2 is DMEM-HG with ITS supplement, F3 is only DMEM-F12, and F4 is DMEM-F12 with ITS supplement.

we also observed that these cells grew faster and achieve confluency faster. Chondrocytes re-enter the cell cycle and multiply after digestion of the ECM from a cartilage biopsy and subsequent attachment to the cultured surface. In the last two decades, several chondrocyte isolation protocols were developed and reported in other studies.[6,7,16-19] For example, an important recent study was conducted,[17] which isolated chondrocytes from 211 osteoarthritic (OA) patients undergoing total joint replacement. The authors of this study analysed specific features of chondrocytes such as cellular yield, cell doubling rate and the dependence between these parameters and patient-related data (e.g., joint type, age and gender). They concluded that such a systematic characterization of important cell source parameters could be useful in view of possible autologous cell therapy for osteoarthritis since the cell source quality is known to greatly influence the outcome of engineered tissue.[18] Another crucial study that we studied in detail prior to our experimental design, was performed.[6] In this study, the authors focused on a very important factor related to the possible clinical use of cartilage tissue-engineered products, namely the optimization of the culture protocol to allow for large-scale production. The result of their study was an optimized culture protocol with exactly defined isolation parameters (e.g., enzyme and concentration to be used, time of digestion and the seeding density for tissue culturing). Two other studies have to be mentioned in this context as well.[7,16] One of these publications focused on the research of possible chondrocyte isolation yield improvement by using various combinations of enzymes and reagents. Their results indicated that chondrocyte yields and capacity to attach and proliferate are not highly sensitive to the specific isolation protocol used.[7] Finally, a study was conducted,[16] in which they tested combinations of three different enzymes and variable incubation/digestion times. A very important discussion point raised by the authors of this study was that different isolation protocols are to be used, if the focus is only on the yield or the goal is to produce preferentially "native" chondrocytes.[16] The protocol of chondrocyte isolation described in this article led to successful growth and proliferation of cells with a proven chondrogenic potential up to the second passage as shown using molecular and immunocytochemical analysis.

Flow cytometry was used to assess and quantify a high number of epitopes on single cells in a short amount of time, allowing for the immunophenotypic characterisation of chondrocytes. Immunophenotypic examination of cells isolated from solid tissues using enzymatic digestions may be hampered as a

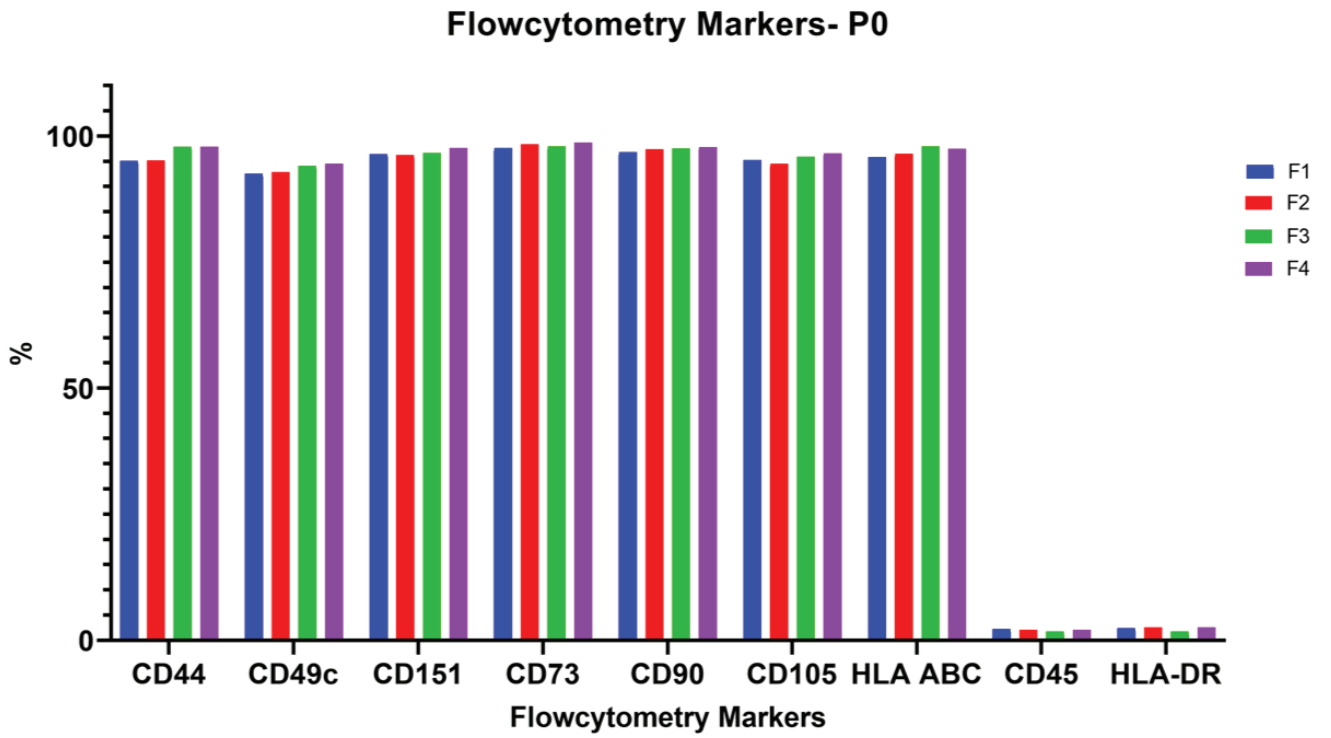

Figure 3: Flow cytometry analysis of chondrocytes at P0 demonstrates positive expression of surface markers - CD44, CD49c, CD151, CD73, CD90, CD105, and HLA-ABC and negative expression of surface markers - CD45 and HLA-DR in chondrocytes grown in all four media formulations F1 to F4. The marker expression is not affected by the culture medium formulation; F1 is DMEM-HG without ITS supplement, F2 is DMEM-HG with ITS supplement, F3 is DMEM-F12 without ITS supplement, and F4 is DMEM-F12 with ITS supplement.

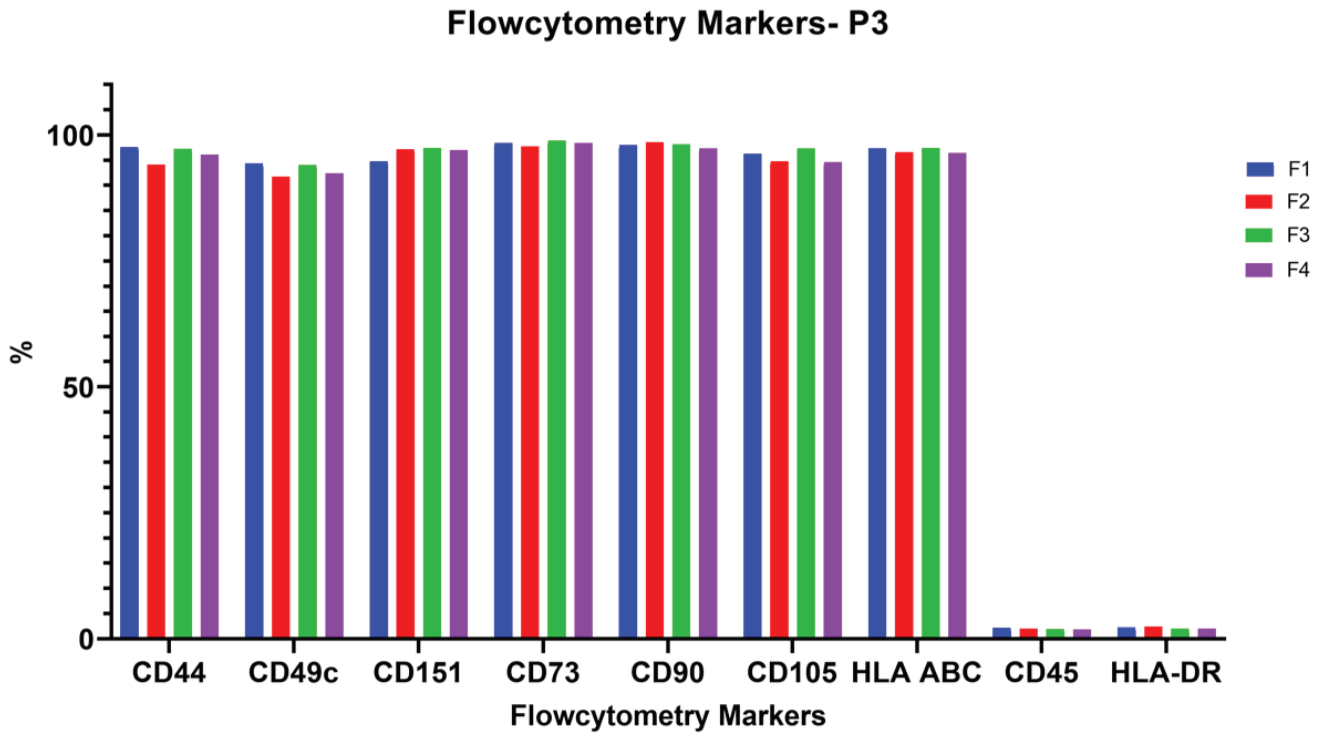

Figure 4: Flow cytometry analysis of chondrocytes at P3 demonstrates positive expression of surface markers - CD44, CD49c, CD151, CD73, CD90, CD105, and HLA-ABC and negative expression of surface markers - CD45 and HLA-DR in chondrocytes grown in all four media formulations F1 to F4. The marker expression is not affected by the culture medium formulation; F1 is DMEM-HG without ITS supplement, F2 is DMEM-HG with ITS supplement, F3 is DMEM-F12 without ITS supplement, and F4 is DMEM-F12 with ITS supplement.

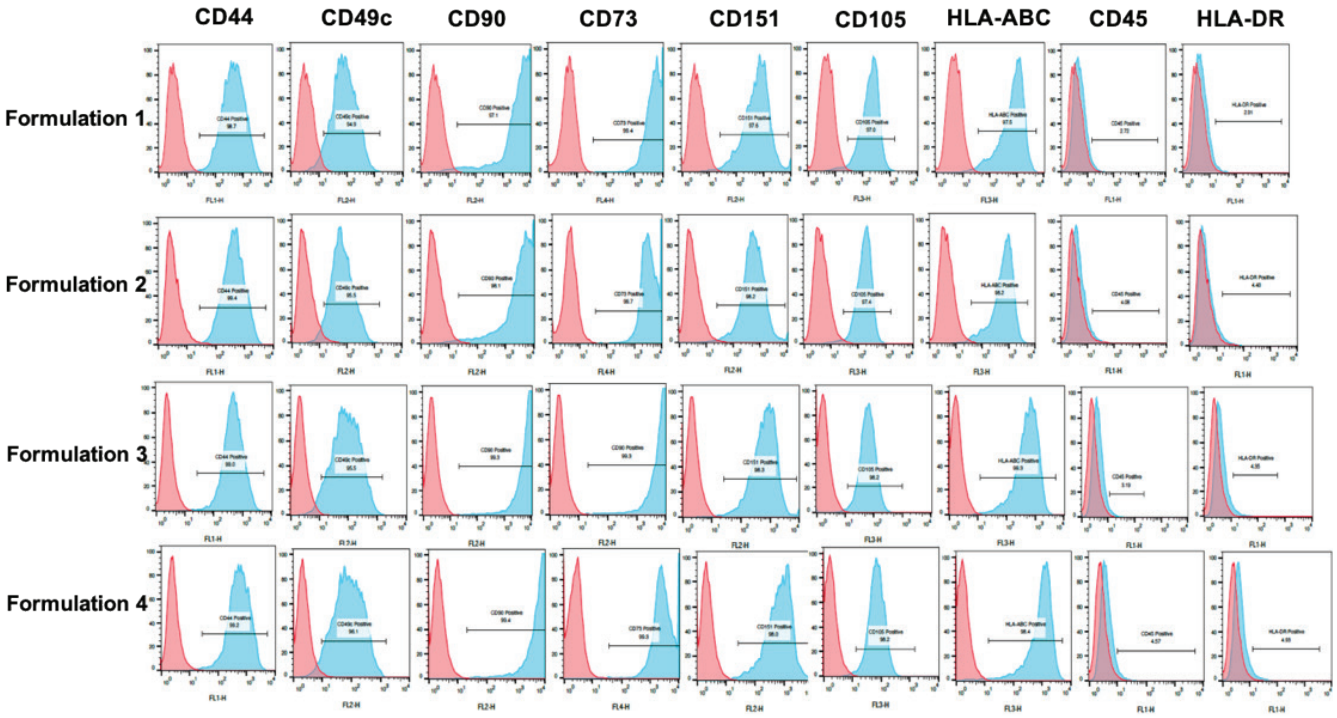

Figure 5a: *Representative histograms of one sample showing positive expression of markers - CD44, CD49c, CD151, CD73, CD90, CD105, and HLA-ABC and negative expression of markers - CD45 and HLA-DR in chondrocytes grown in all four media formulations F1 to F4 at P0; where F1 is DMEM-HG without ITS supplement, F2 is DMEM-HG with ITS supplement, F3 is DMEM-F12 without ITS supplement, and F4 is DMEM-F12 with ITS supplement.*

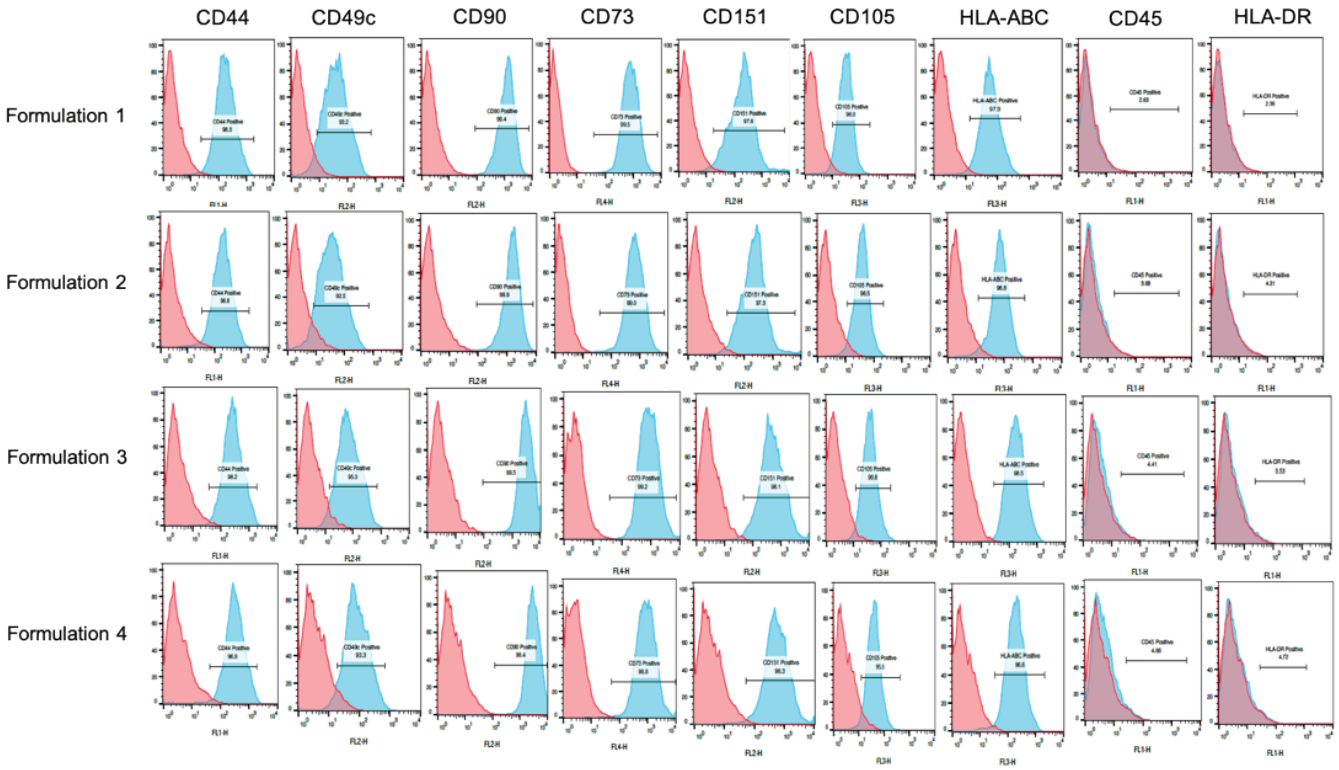

Figure 5b: *Representative histograms of one sample showing positive expression of markers - CD44, CD49c, CD151, CD73, CD90, CD105, and HLA-ABC and negative expression of markers - CD45 and HLA-DR in chondrocytes grown in all four media formulations F1 to F4 at P3; where F1 is DMEM-HG without ITS supplement, F2 is DMEM-HG with ITS supplement, F3 is DMEM-F12 without ITS supplement, and F4 is DMEM-F12 with ITS supplement.*

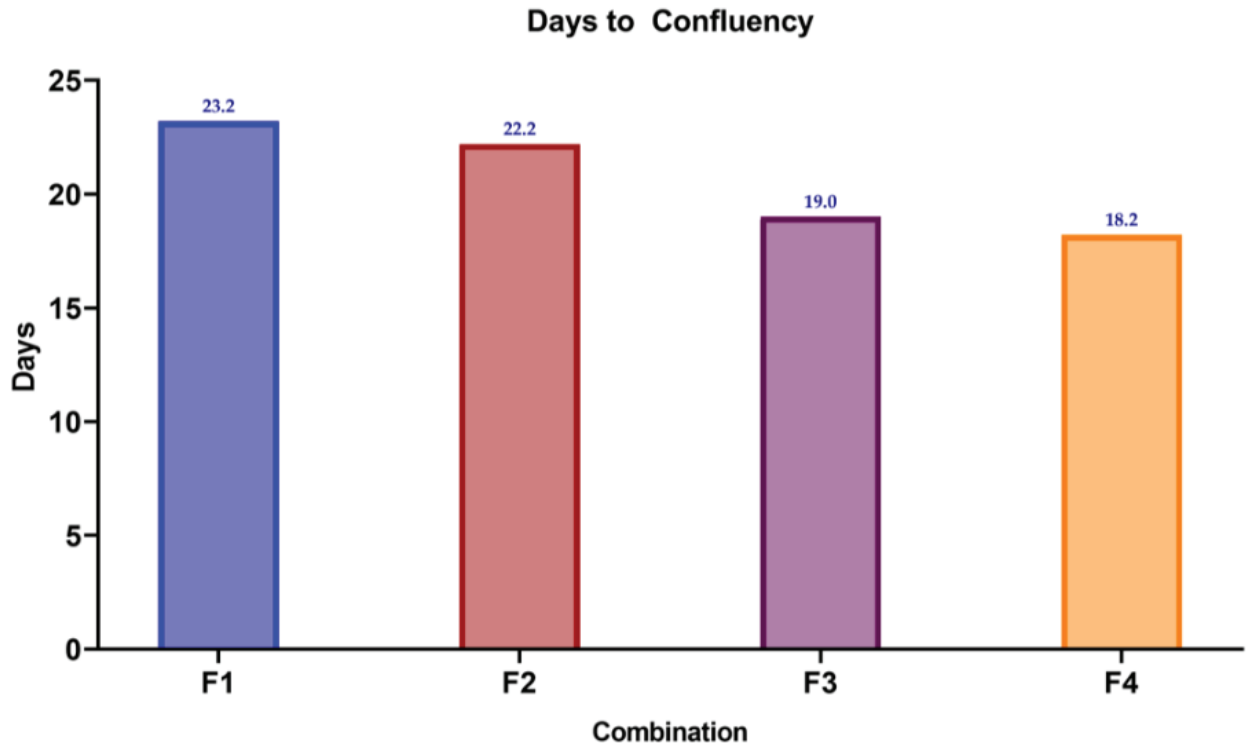

Figure 6: Days to confluency; Primary cell plated in F3 and F4 achieved faster confluency compared to F1 and F2; where F1 is only DMEM-HG, F2 is DMEM-HG with ITS supplement, F3 is only DMEM-F12, and F4 is DMEM-F12 with ITS supplement

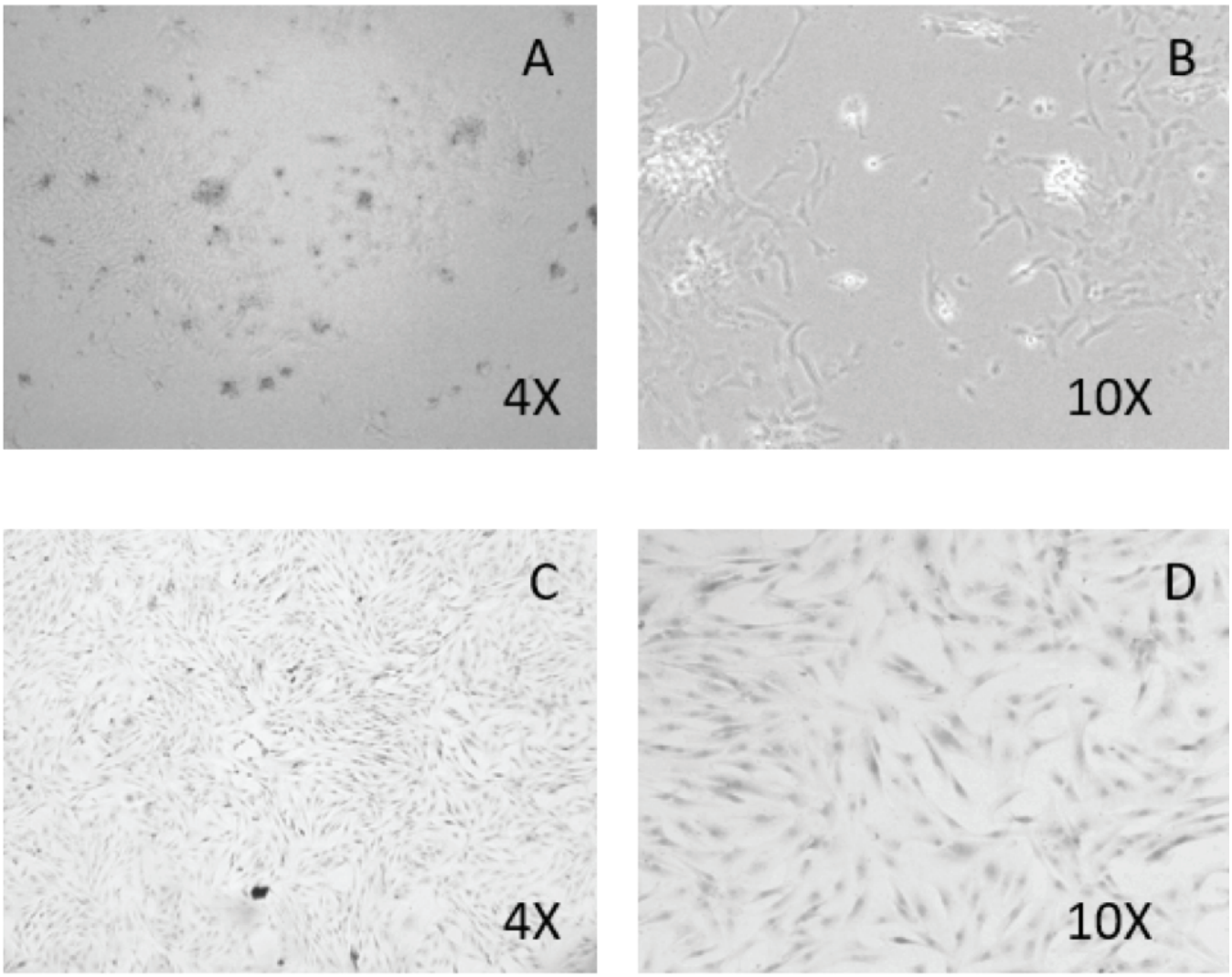

Figure 7: Representative Picture of Chondrocytes growing at P0. Photomicrographs of chondrocytes isolated from representative TKA sample post standardization and cultured in DMEM F12. Cells (at P0) can be seen growing in colonies on day 3 of culture at 4X (a) and 10X (b). On day 10 of culture, cells (at P0) are seen covering the entire surface of the culture flask at 4X (c) and 10X (d).

result of reduced or even complete loss of surface molecules susceptible to enzyme treatment. The Collagenase used in our study did not impair the detection of the CD90, CD44 and CD45 markers. We have confirmed the expression of the hyaluronan receptor CD44 and CD 90 (Thy-1) on chondrocytes.[20-22] The hyaluronan receptor CD44 is a member of the polymorphic family of CD44 glycoproteins, which are engaged in a variety of cellular processes such as adhesion to hyaluronan and collagen and are found in normal chondrocytes.[20] In addition to CD44, CD49c and CD151, known to be the markers of chondrogenic potency that aid in cell-cell and cell-matrix interactions[11,12] were also positively expressed in the isolated cells. CD90 has previously been shown to be expressed in a subset of chondrocytes in normal articular cartilage.[21] During monolayer culture, upregulation of markers on chondrocytes thought to be exclusive to mesenchymal stem cells (CD90, among others) revealed that dedifferentiation leads to reversion to a primitive phenotype.[22] Chondrocytes did not express the hematopoietic marker CD45, also known as the leukocyte common antigen. The CD45 marker should not be present in normal chondrocytes but can appear in dedifferentiated ones.[20]

It is important to enhance that cells used in this study were not applied in clinical practice and the standardisation of a cost-effective culture protocol and basic characterization of chondrocytes was the only aim of this study.

In this study, we used a standardised technique to digest the articular cartilage with collagenase as previously described. The cells obtained from the digestion were plated on a tissue culture plate with a uniform seeding density onto 4 different tissue culture media. The cells were passaged and at every passage, the cell number was determined. The time taken for the cells to become confluent was noted. At Passage P0 and Passage P3,

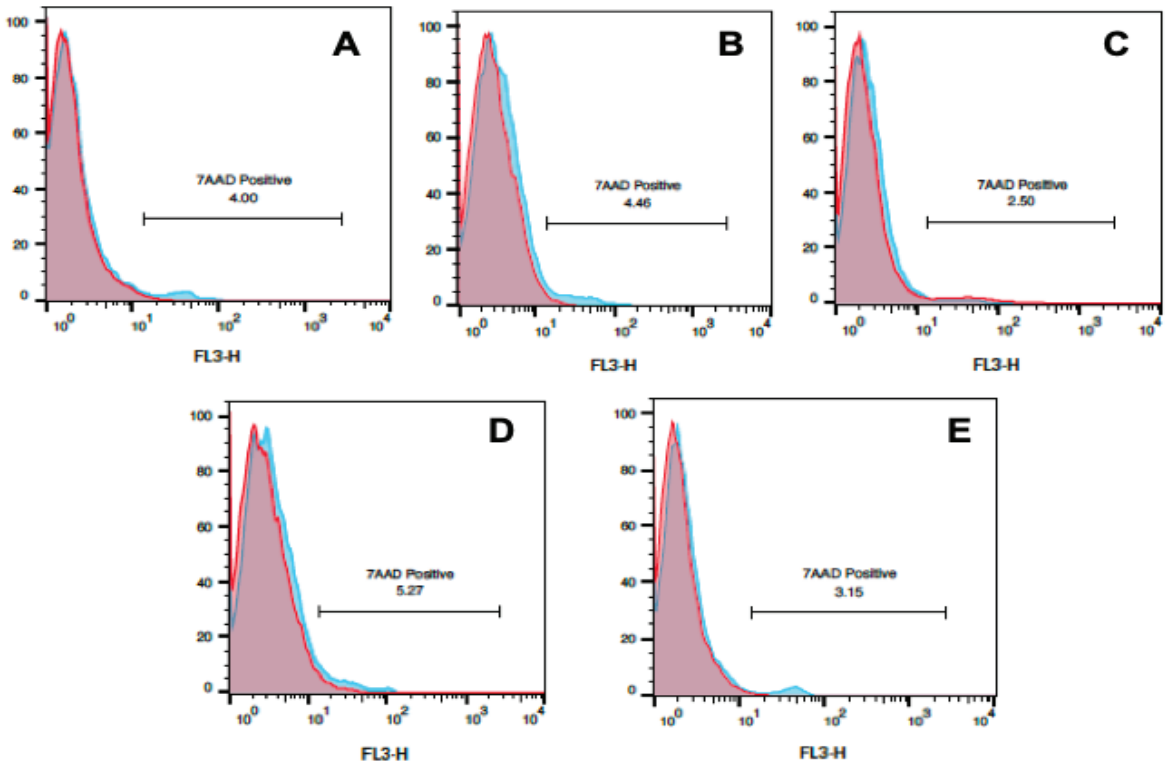

Figure 8: Viability of five Chondrocyte Samples (A to E) at P3 Post standardization. Post standardization, additional five TKA samples were cultured in DMEM F12 and were assessed for their viability at P3 using DNA dye 7AAD. All samples showed viability of greater than 95%.

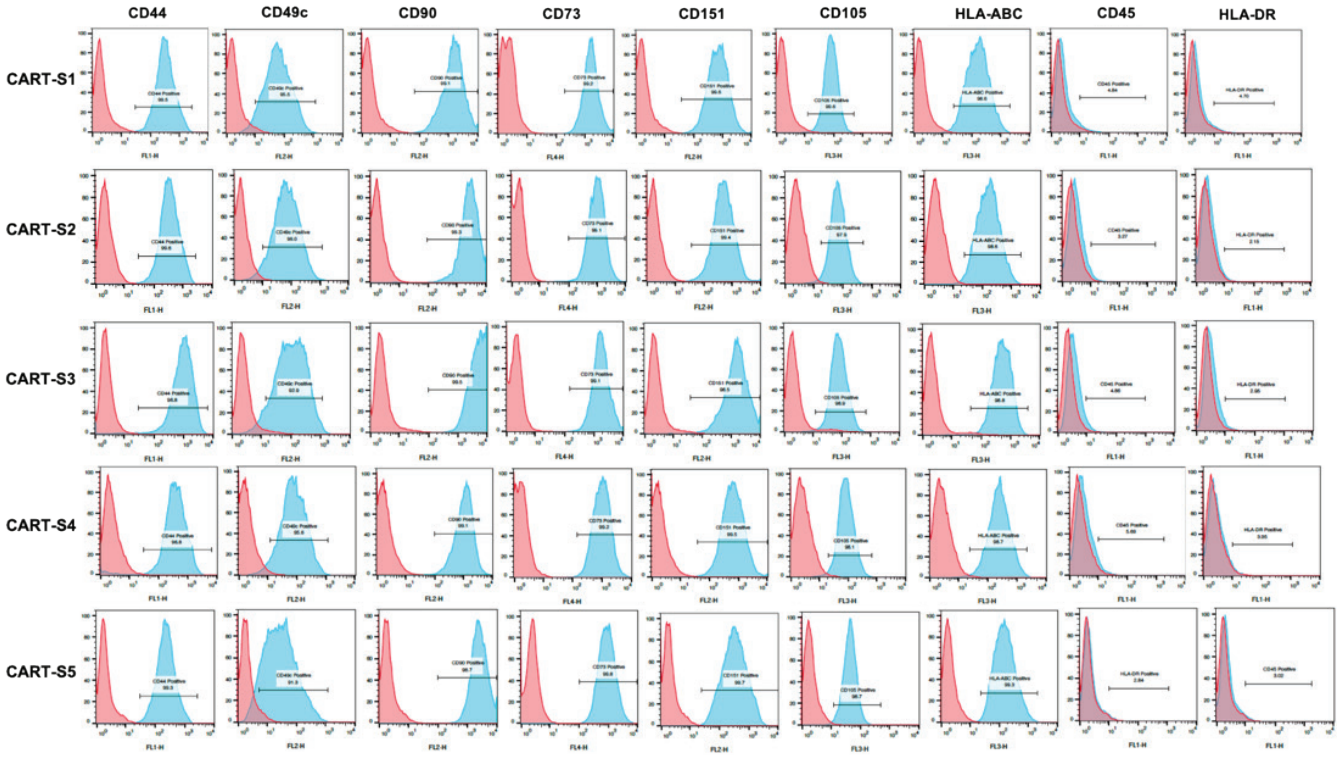

Figure 9: The immunoprofile of five samples used to isolate Chondrocytes grown in the standardized media was analysed at passage 3 (P3) by flow cytometry. The cells were analysed for two negative markers – CD45 and HLA-DR and seven positive markers – CD44, CD49c, CD151, CD73, CD90, CD105 and HLA-ABC.

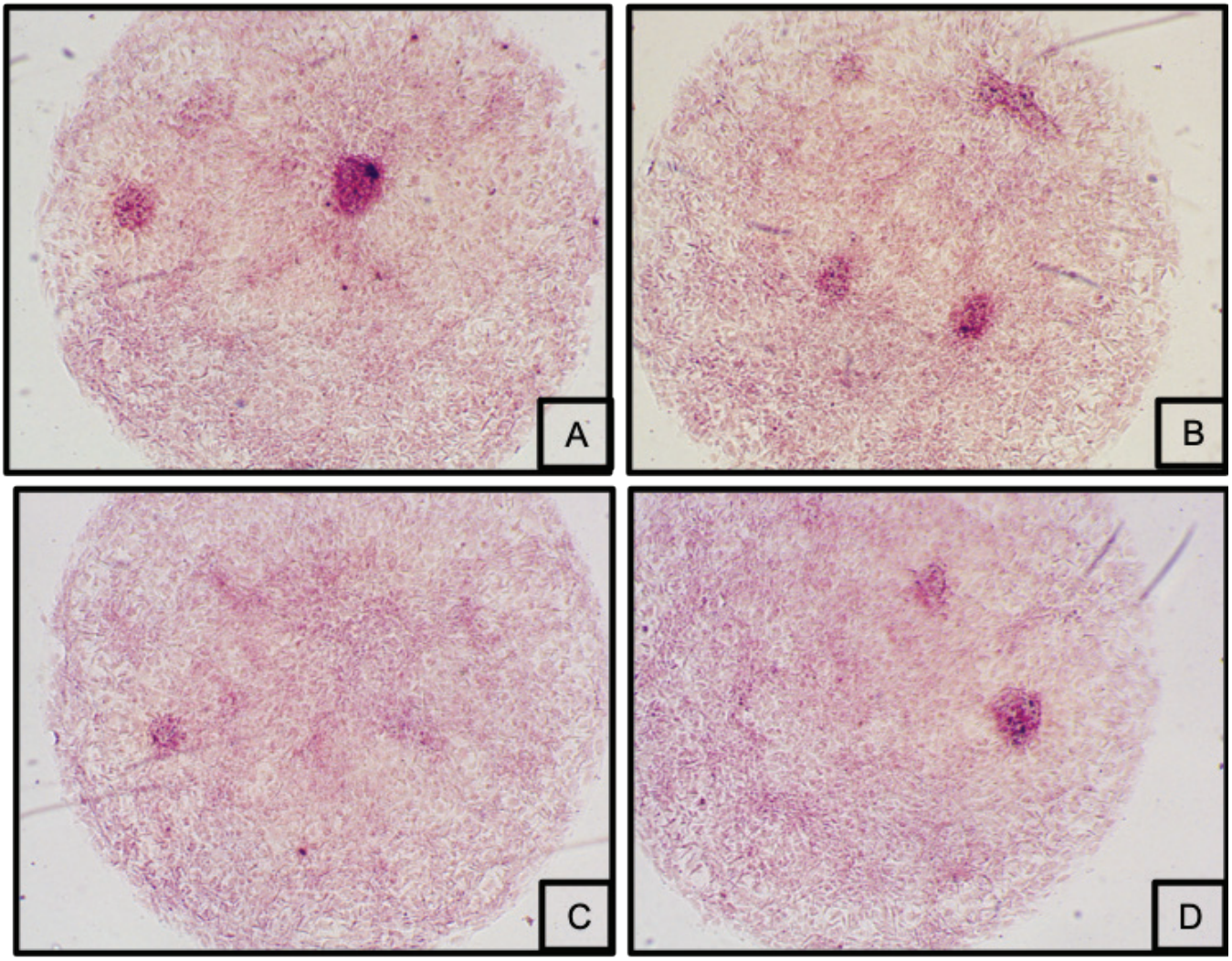

Figure 10: Safranin O staining of human primary chondrocytes cultured in DMEM F12 post standardization in micromass culture of a representative sample showing consistent staining over passages from P0 to P3; A – Micromass culture of Passage 0; B – Micromass culture of Passage 1; C – Micromass culture of Passage 2; D – Micromass culture of Passage 3.

immunophenotyping was performed. We observed that the cell numbers were higher in DMEM F12 and DMEM F12 with ITS. The difference in cell number between these two media was not appreciably different. Keeping in mind the additional expense and availability of ITS supplement we decided to choose DMEM F12 as the media for culturing chondrocytes. Characterization by immunophenotyping was found to be similar in all the media compared, however, we noted that using DMEM F12 with or without ITS, the cells became confluent faster compared to DMEM-HG with and without ITS, 18 days as compared to 22 days. As the time for confluency was found to be the same with or without addition of supplement, the use of normal DMEM F12 without the addition of ITS significantly lowers the cost to culture.

CONCLUSION

In this study, we describe a simple and affordable procedure of isolation and cultivation of human articular chondrocytes that demonstrated a high chondrogenic potential to the second passage. As the source material, we propose the surgical waste tissue occurring during total knee arthroplasty (TKA). Chondrocyte cells are crucial not only for the development of therapeutic approaches in cartilage repair (e.g., autologous chondrocyte implantation- ACI) but are necessary in cartilage tissue engineering to allow the development of functional cell models and novel scaffolds. For this purpose, chondrocytes have to be isolated in sufficient quantities and their phenotype should be preserved.

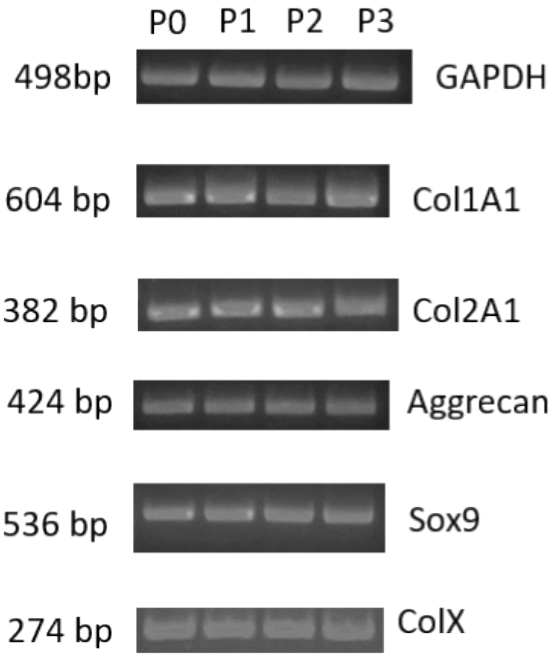

Figure 11: Gene expression of Chondrocytes by RT-PCR. Gene expression analysis of representative sample of chondrocytes grown in DMEM F12 post standardization demonstrates positivity for chondrocyte specific genes. Sample was positive for GAPDH, Col1A1, Col2A1, Aggrecan, Sox 9 and ColX over passages P0 to P3. Amplicon size is on the left side of the image and gene is mentioned on the right side.

Conflict of Interest

The author declares no conflict of interest.

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