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Chondrocytes extract from patients with osteoarthritis induces chondrogenesis in infrapatellar fat pad-derived stem cells

E. López-Ruiz †[a], M. Perán †[a], J. Cobo-Molinos †, G. Jiménez ‡§, M. Picón ‡, M. Bustamante ||, F. Arrebola ¶, M.C. Hernández-Lamas #, A.D. Delgado-Martínez †, E. Montañez ††, J.A. Marchal ‡§*

† *Department of Health Sciences, University of Jaén, Jaén E-23071, Spain*
‡ *Biopathology and Regenerative Medicine Institute (IBIMER), Centre for Biomedical Research, University of Granada, Granada E-18100, Spain*
§ *Department of Human Anatomy and Embryology, Faculty of Medicine, University of Granada, Granada E-18012, Spain*
|| *Biosciences Institute, University College Cork, Cork, Ireland*
¶ *Department of Histology, Faculty of Medicine, University of Granada, Granada E-18071, Spain*
# *Málaga Tissue Bank, Centro Regional de Transfusión, Málaga, Spain*
†† *Department of Orthopedic Surgery and Traumatology, Virgen de la Victoria University Hospital, Málaga, Spain*

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SUMMARY

Objective: Infrapatellar fat pad of patients with osteoarthritis (OA) contains multipotent and highly clonogenic adipose-derived stem cells that can be isolated by low invasive methods. Moreover, nuclear and cytoplasmic cellular extracts have been showed to be effective in induction of cell differentiation and reprogramming. The aim of this study was to induce chondrogenic differentiation of autologous mesenchymal stem cells (MSCs) obtained from infrapatellar fat pad (IFPSCs) of patients with OA using cellular extracts-based transdifferentiation method.

Design: IFPSCs and chondrocytes were isolated and characterized by flow cytometry. IFPSCs were permeabilized with Streptolysin O and then exposed to a cell extract obtained from chondrocytes. Then, IFPSCs were cultured for 2 weeks and chondrogenesis was evaluated by morphologic and ultrastructural observations, immunologic detection, gene expression analysis and growth on 3-D poly (DL-lactic-co-glycolic acid) (PLGA) scaffolds.

Results: After isolation, both chondrocytes and IFPSCs displayed similar expression of MSCs surface makers. Collagen II was highly expressed in chondrocytes and showed a basal expression in IFPSCs. Cells exposed to chondrocyte extracts acquired a characteristic morphological and ultrastructural chondrocyte phenotype that was confirmed by the increased proteoglycan formation and enhanced collagen II immunostaining. Moreover, chondrocyte extracts induced an increase in mRNA expression of chondrogenic genes such as *Sox9*, *L-Sox5*, *Sox6* and *Col2a1*. Interestingly, chondrocytes, IFPSCs and transdifferentiated IFPSCs were able to grow, expand and produce extracellular matrix (ECM) on 3D PLGA scaffolds.

Conclusions: We demonstrate for the first time that extracts obtained from chondrocytes of osteoarthritic knees promote chondrogenic differentiation of autologous IFPSCs. Moreover, combination of transdifferentiated IFPSCs with biodegradable PLGA 3D scaffolds can serve as an efficient system for the maintenance and maturation of cartilage tissue. These findings suggest its usefulness to repair articular surface in OA.

Introduction

Chondral defects suppose a challenging clinical problem aggravated by the increase in elderly population in developed countries. Consequently, many strategies including arthroplasty, the stimulation of reparative tissues through arthroscopic, abrasion, drilling or microfracture[1] have been developed to treat the injured cartilage. However, these procedures cannot reproduce successfully the tissue characteristics of hyaline cartilage[2,3]. New biological based strategies such as the replacement of damaged

* Address correspondence and reprint requests to: J.A. Marchal, Department of Human Anatomy and Embryology, Faculty of Medicine, University of Granada, Avda de Madrid s/n, Granada E-18012, Spain. Tel: 34-958-249-321; Fax: 34-958-246-296.
E-mail addresses: elruiz@ujaen.es (E. López-Ruiz), mperan@ujaen.es (M. Perán), jcobos@ujaen.es (J. Cobo-Molinos), gemajg@correo.ugr.es (G. Jiménez), mpicon@ugr.es (M. Picón), 111224080@umail.ucc.ie (M. Bustamante), fav@ugr.es (F. Arrebola), carmen.hernandez.lamas.sspa@juntadeandalucia.es (M.C. Hernández-Lamas), adelgado@ujaen.es (A.D. Delgado-Martínez), elvira.montanez.sspa@juntadeandalucia.es (E. Montañez), jmarchal@ugr.es (J.A. Marchal).
[a] These authors contributed equally to this work.

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surface by healthy one (osteochondral grafts)[4] or the implantation of cultured expanded autologous chondrocytes have been used for cartilage repair[5]. Nevertheless, the difficulties to obtain sufficient amounts of autologous chondrocytes[6] or the dedifferentiation process that chondrocytes undergo after serial passages in monolayer culture[7] are the main disadvantages that limit their use.

MSCs represent an attractive cell source for cartilage regeneration cell therapy approach. These multipotent cells can be easily isolated from different sources such as bone marrow, fat tissue, umbilical cord or peripheral blood among others[8] and present a high proliferation capacity. The chondrogenic potential of MSCs derived from bone marrow have been demonstrated; [9] however, the extraction procedure is invasive and increases morbidity in patients with osteochondral disease. Therefore, it is necessary to search new sources of MSCs that could be isolated by less invasive methods. In this respect, MSCs obtained from adipose tissue represent a good alternative and recently, it has been shown that Hoffa's fat pad in patients with osteoarthritis (OA), contains multipotent and highly clonogenic adipose-derived stem cells[10,11]. These cells can be obtained from the articulation of the patient in a single surgical procedure[12,13].

Last years, many protocols for inducing adult stem cells to differentiate *in vitro* across germinal boundaries, a process referred to as transdifferentiation, has been described[14–16]. There are different strategies for achieving adult cell reprogramming or transdifferentiation: somatic nuclear transfer, cell fusion, ectopic expression of master switch genes and cell extract based methods[17]. The use of nuclear and cytoplasmic extracts has been showed to be effective in induction of cell differentiation and cell reprogramming[18,19]. Among potential benefits of the extract approach are the use of autologous material to direct differentiation and to avoid exogenous genes, recombinant proteins like exogenous growth factors or viral delivery methods.

In this study, we used cellular extracts-based transdifferentiation method to induce chondrogenic differentiation of autologous MSCs obtained from infrapatellar fat pad (IFPSCs) of patients with OA. Here, we demonstrate for the first time, that IFPSCs can undergo chondrogenesis when stimulated with an extract prepared from autologous chondrocytes obtained from patients' cartilage tissue.

Materials and methods

Patients

Both human IFPSCs and articular cartilage were obtained from 18 patients with knee OA during joint replacement surgery. Ethical approval for the study was obtained from the Ethics Committee of the Clinical University Hospital of Málaga, Spain. Informed patient consent was obtained for all samples used in this study. None of the patients had a history of inflammatory arthritis or crystal-induced arthritis. Human articular cartilage was obtained from the femoral side, selecting the non-overload compartment: lateral condyle in varus knees and medial condyle in the valgus cases. Only cartilage that macroscopically looked relatively normal was used for this study. Hoffa's fat pad was harvested from the interior of the capsule excluding vascular areas and synovial regions. Samples collected at joint arthroplasty were transported to the laboratory in Dulbecco's modified Eagle's medium (DMEM; Sigma, St. Louis, MO, USA) with 100 U/ml penicillin and 100 μg/ml streptomycin.

Isolation of human articular chondrocytes

Articular cartilage was minced and digested overnight in an overnight 0.08% collagenase IV (Sigma) digestion at 37°C with gentle agitation. Cells were centrifuged and rinsing to remove the collagenase. The remaining cells were then plated in cultured flasks with chondrocytes media:DMEM (Sigma) supplemented with 20% FBS (Lonza, Basel, Switzerland), 7 ml human insulin (Actrapid; Novo Nordisk, Bagsvaerd, Denmark), 6 μl DNase I (Sigma), 100 U/ml penicillin and 100 μg/ml streptomycin at 37°C in a humidified atmosphere of 5% CO_2. After 24 h the medium was replaced with fresh medium supplemented with 10% FBS. Chondrocytes were cultured for a maximum of 3 weeks before the experiments to avoid dedifferentiation phenomenon[7].

Isolation of MSCs from IFPSCs

Fat tissue finely minced was digested using enzymatic solution of 1 mg/ml collagenase type IA (Sigma) and incubated on a shaker at 37°C for 1 h. After digestion, collagenase was removed by a single wash in sterile phosphate-buffered saline (PBS), followed by two further washes in DMEM supplemented with 10% FBS. The cell pellet was resuspended in DMEM (Sigma) containing 10% FBS and 1% penicillin/streptomycin, added to tissue culture flasks, and cultured at 37°C in 5% CO_2. After 48 h the medium was removed to discard non-adherent cells. At 80% of confluency the cells were released with trypsin–ethylenediaminetetraacetic acid (EDTA) (Sigma) and subcultured.

Characterization of articular chondrocytes and IFPSCs

The immunophenotype of articular chondrocytes and cultured IFPSCs was analyzed by flow cytometry (FACS). MSC marker phenotyping was performed as previously described[20]. For the determination of collagen II expression cells were, first, fixed and permeabilized with Fix and Perm® reagent (Invitrogen, Carlsbad, CA, USA), then incubated with the primary monoclonal antibody (AbCam, Cambridge, UK) for 20 min, washed and finally incubated with a secondary FITC-conjugate monoclonal antibody (Sigma) for 30 min. All cells were washed in PBS and analyzed in a FACSCanto II cytometer (BD Biosciences).

Differentiation assays of IFPSCs

Human IFPSCs were plated at 2×10^3 cells/cm^2 in DMEM (Sigma) containing 10% FBS with penicillin and streptomycin at 100 μg/ml and allowed to adhere for 24 h. The culture medium was then replaced with specific inductive media to demonstrate adipogenic, osteogenic and chondrogenic differentiation potential as previously reported[20]. In brief, for adipogenic, osteogenic and chondrogenic differentiation, cells were cultured for 2 weeks in Adipogenic MSCs Differentiation BulletKit, Osteogenic MSCs Differentiation BulletKit (Lonza, Basel, Switzerland) and NH ChondroDiff Medium (Miltenyi Biotec, Auburn, CA, USA), respectively.

Cell permeabilization assay and exposure to cell extract of IFPSCs

Prior IFPSCs incubation with the nuclear and cytoplasmic extracts, the efficiency of Streptolysin O (SLO; Sigma) treatment was evaluated following a modification of the protocol described in[21]. Briefly, 3×10^5 IFPSCs were permeabilized with several concentration of SLO and exposed to Texas red-conjugated 70,000 *M*r dextran (Invitrogen). The uptake of the dye was observed by phase contrast and epifluorescence microscopy after 2 h and 24 h of SLO permeabilization. Chondrocytes extract preparation and exposure to IFPSCs were performed as described elsewhere[22] with slight modifications (See Supplementary data). 1,666 chondrocytes per 1,000 IFPSCs were used per reaction. After *in vitro* reprogramming in cellular extracts, cells were cultured for 2 weeks until use. We

used permeabilized cells not exposed to the extracts as control. Cell viability was assessed by phase contrast microscopy after 2 weeks of extract exposure by counting cells in four different regions of the dish and calculating the average.

Transmission electron microscopy (TEM)

Cells were assayed for by TEM as previously reported[17] and sections were examined with a LEO 906E transmission electron microscope.

Histological and immunohystochemical analysis

Cells seeded on glass coverslips and cell-seeded poly (DL-lactic-co-glycolic acid) (PLGA) sections were assayed for cartilaginous matrix production by toluidine blue and safranin O staining. Type II collagen expression was determined by immunofluorescence as previously described[17]. A primary monoclonal antibody anti-collagen type II (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used and photographs were taken with a Leica DM 5500B (Solms, Germany) fluorescent microscope, software Meta Systems Isis.

Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA from control SLO-permeabilized cells, SLO-permeabilized cells exposed to the extract and from cultured chondrocytes (positive control) was extracted using TriReagent (Sigma). RNA was reverse transcribed using the Reverse Transcription System kit (Promega, Madison, WI, USA) and the PCR reaction was performed with ReddyMix PCR Master Mix (Thermo, Waltham, MA, USA). After the initial denaturation (2 min at 94°C), 33 cycles were performed (30 s at 94°C, 50 s for annealing temperature and 1 min at 72°C) for all set of primers except for β-actin, which was 25 cycles. Primer sequences and annealing temperatures can be found in Table I. The PCR products were visualized on 1% agarose gels containing 0.1 mg/ml ethidium bromide using ultraviolet light.

In vitro primary culture on scaffold

We used a biodegradable cylindrical implant constructed of a porous cartilage phase and a porous bone phase (5 mm in diameter × 10 mm deep). The cartilage phase of the scaffold was cut to 3 mm in diameter and 3 mm in height under sterile conditions on a Petri dish and was plated in 96-well plates. The synthetic material is a blend of poly DL-lactide-co-glycolide, polyglycolide fibers and surfactant (TruFit CB Plug; Smith & Nephew, London, UK). Cells suspensions containing 6,000 cells in 50 μl of medium were slowly dropped onto the surface of each scaffold incubated in 96-well plates for 4 h at 37°C and then analyzed under the inverted microscope to check cell adhesion to the polymer surface. After that, 50 μl of fresh medium was added in each well plate. Cell culture medium was changed every 2 days.

Table I
Sequences of the primers used in the RT-PCR reactions

Target gene	Primers (forward and reverse)
Collagen type I	GAG AGC ATG ACC GAT GG
	GTG ACG CTG TAG GTG AA
Collagen type II	GAC AAT CTG GCT CCC AAC
	ACA GTC TTG CCC CAC TTA C
Collagen type X	GCC CAC TAC CCA ACA C
	TGG TTT CCC TAC AGC TGA
Aggrecan I	GTC TCA CTG CCC AAC TAC
	GGA ACA CGA TGC CTT TCA C
Sox-5	ATC CCA ACT ACC ATG GCA GCT
	GAT ACC TGC ATT GCA GCT
Sox-6	GCA GTG ATC AAC ATG TGG CCT
	TTC ATC ATG CGCTGC CAG TAG
Sox-9	GAG CAG ACG CAC ATC TC
	CCT GGG ATT GCC CCG A
Gapdh	GAA GGT GAA GGT CGG AGT C
	GAA GAT GGT GAT GGG ATT TC

Environmental scanning electron microscope (ESEM) analysis

After 3 days of culture, samples were fixed in 3% glutaraldehyde during 2 h at 4°C and then were rinsed several times with sodium cacodylate. The samples were kept refrigerated into PBS. The observations started at an initial water vapor pressure of 5.7 tors. At this pressure, a liquid water phase was present in the sample (100% RH). Then, vapor pressure was decreasing slowly until the surfaces of the samples were visible (4.5 and 5 tors). Accelerating voltages varied between 10 and 15 kV we obtained a good image resolution using small beam current (spot size 3–3.5) and a working distance of 5–6 mm. The ESEM used in this work was a Quanta 400 (FEI) located at the Centro de Instrumentación Científica of the University of Granada.

Results

Cell isolation and characterization of chondrocytes

FACS characterization showed that *ex-vivo* cultured chondrocytes expressed the surface markers CD73 (99.84%), CD90 (97.25%) CD105 (99.74%) and the intracellular protein collagen type II (60%), while lacked expression for both hematopoietic and endothelial cell markers CD45 (<1%), CD34 (1.4%), CD133 (1.82%), CXCR4 (2.27%), KDR (7.63%) [Fig. 1(A)].

Light microcopy observation showed that chondrocytes at day one were attached to the flask surface as cell-clusters of rounded shapes [Fig. 1(B)]. After a week in culture, cells displayed a polygonal shape appearing some of them with a star-like morphology [Fig. 1(C)]. These stellated-like cells occupied the entire surface and reached confluence after 2 weeks [Fig. 1(D)].

Cell isolation and characterization of IFPSCs

Isolated IFPSCs presented a spindle shape fibroblastic morphology. FACS characterization demonstrated a positive expression of the surface markers CD73 (>99.80%), CD90 (>99.42%), CD105 (>99.91%) and a negative expression for CD45 (1.16%), CD34 (8.16%), CD133 (1.41%), CXCR4 (1.97%) and KDR (1.49%) as shown previously[23]. In addition, IFPSCs showed a slightly expression of collagen type II (13%) [Fig. 2(A)]. IFPSCs treated with conditioned media displayed characteristics of adipogenic, osteogenic and chondrogenic differentiation after staining [Fig. 2(B)].

Cell permeabilization assay

Previous to perform the extract reprogramming experiment we optimize IFPSCs permeabilization procedure by testing different concentrations of the SLO toxin ranged from 0.230–305 ng/ml. Among the tested concentration, 185 ng/ml of SLO was the most efficient, permeabilizing 80–95% of the cells [Fig. 3]. Results showed that 20–25% of the cells survived to the extract exposure while 80% of control cells (permeabilized but not exposed to extract) were viable.

Phenotypic changes after exposition to chondrocyte extracts

Phase microscopy observation of cultured chondrocytes showed a typical polygonal morphology with a high rate of star-like cells [Fig. 4(A)]. On the other hand, permeabilized but not exposed to

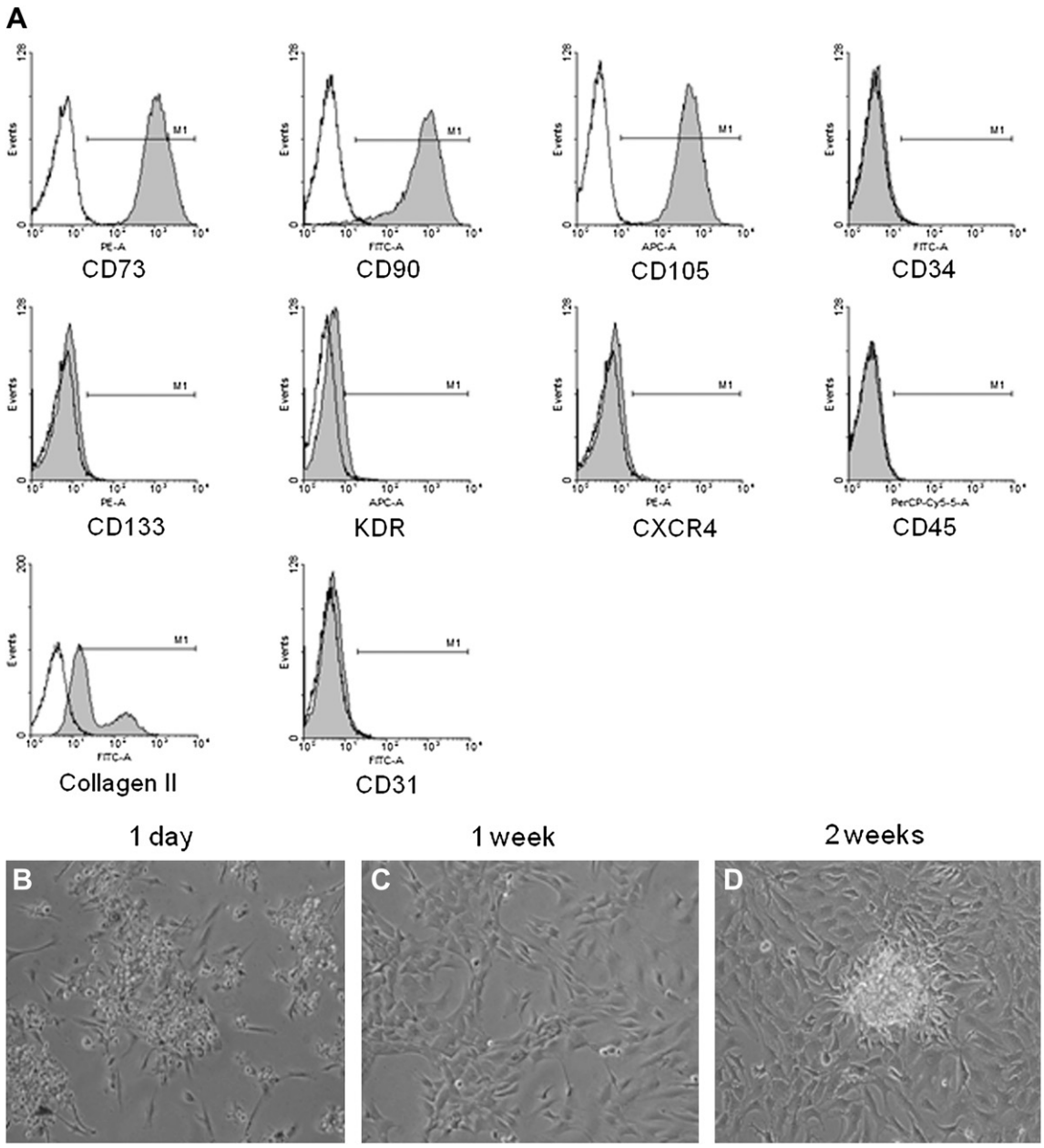


Fig. 1. Phenotypic characterization of chondrocytes. (A) Chondrocytes were cultured for 2 weeks and then tested for mesenchymal surface markers (CD105, CD73 and CD90), hematopoietic and endothelial markers (CD133, CD34, KDR, CD45 and CXCR4) and for collagen II by flow cytometry. White histograms identify the isotype controls (negative). (B–D) Phase-contrast light microscopy of cultured human articular chondrocytes for 1 (B), 7 (C) and 14 days (D). Original magnification: 10× for B and 20× for C and D.

extract cells (control IFPSCs) displayed a characteristic mesenchymal shape consisting in flatter cells with a spindle-like morphology [Fig. 4(B)]. It can be appreciated that IFPSCs acquired a chondrocyte-like phenotype after exposure to the autologous cell extract, showing an increased number of smaller polygonal and star-shaped cells [Fig. 4(C)].

Furthermore, toluidine blue assay demonstrated a characteristic positive metachromatic staining in both chondrocytes and IFPSCs treated with extracts which evidenced the synthesis of glycosaminoglycans (*GAGs*) [Fig. 4(D and F), respectively] when compared with control cells [Fig. 4(E)].

Ultrastructural analysis

TEM showed that cultured chondrocytes possessed an euchromatic nuclei, a cytoplasm rich in rough endoplasmic reticulum (RER), many transport vesicles, lipid droplets (LI) and proteoglycan granules. Moreover, ECM produced by cultured chondrocytes contained a homogeneous population of fibrillar collagens (FC) with typical striation [Fig. 4(G and J)]. Control IFPSCs exhibited morphological features typical of MSCs, including various mitochondrial profiles, small vacuoles and a nucleus with multiple nucleoli [Fig. 4(H and K)]. In contrast, extract exposed IFPSCs seemed to have undergone chondrogenic differentiation characterized by nuclei with prominent nucleoli, extended cistern of RER, Golgi apparatus (GA) and vacuoles and fine cytoplasmatic processes [Fig. 4(I and L)]. Moreover, a characteristic feature present in chondrocytes and found in extract exposed IFPSCs [Fig. 4(I and L)], but not in control [Fig. 4(H and K)], was the presence of proteoglycan granules in both cytoplasm and ECM. Round-shaped lipid vesicles of variable size were also present in differentiated cells.

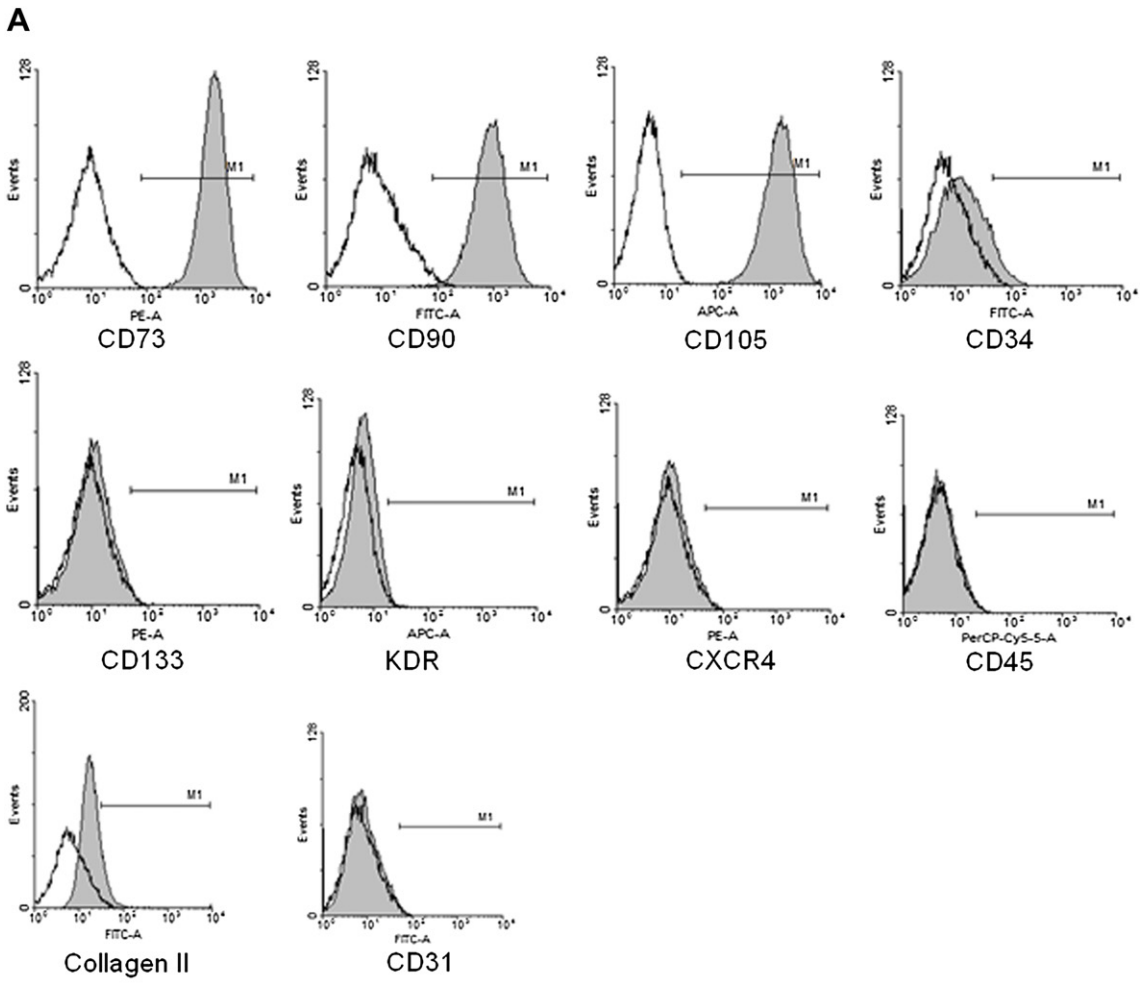


Fig. 2. Phenotypic characterization and differentiation potential of IFPSCs. (A) IFPSCs were cultured for 2 weeks and then tested for mesenchymal surface markers (CD105, CD73 and CD90), hematopoietic and endothelial markers (CD133, CD34, KDR, CD45 and CXCR4) and for collagen II by flow cytometry. (B) The differentiation potential of IFPSCs towards adipogenic, chondrogenic and osteogenic lineage was confirmed by Oil Red O, toluidine blue and Alizarin Red S staining, respectively. Upper pictures show negative controls, cells cultured in normal medium for 2 weeks and then histochemically stained. Original magnification: 10×.

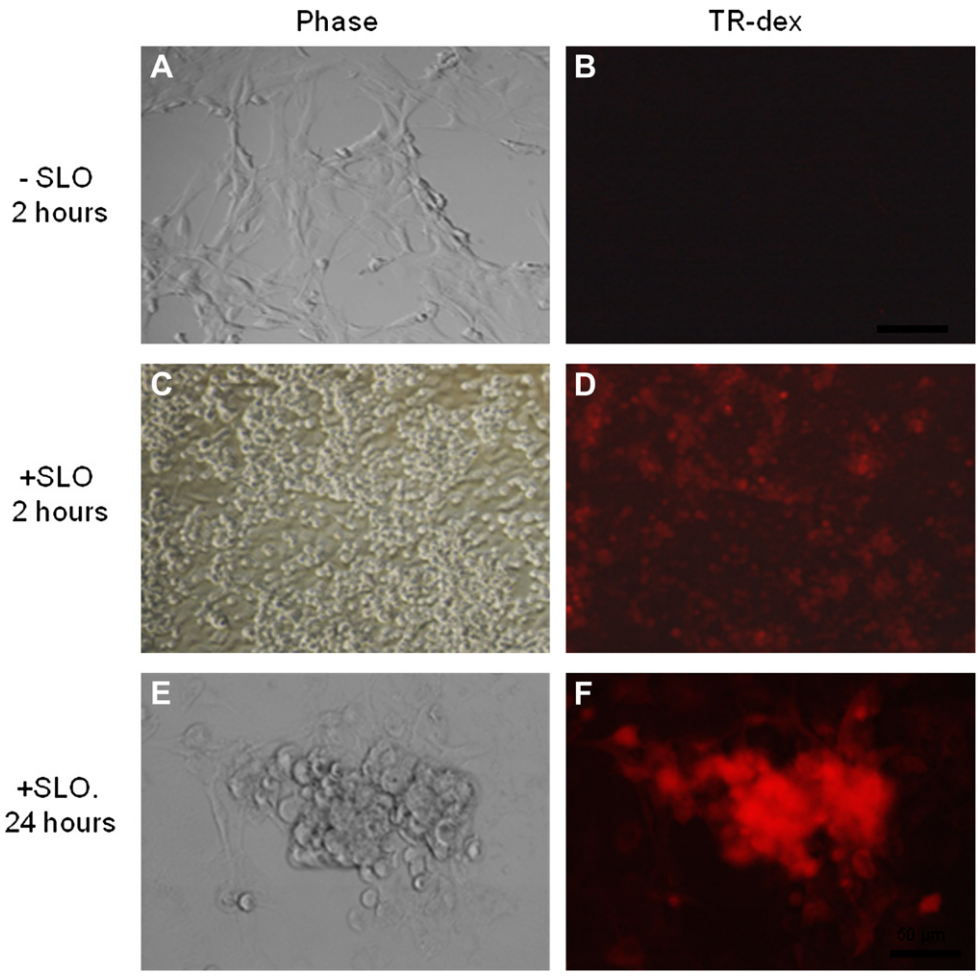


Fig. 3. Reversible cell permeabilization, uptake of a Texas red-conjugated dextran by IFPSCs. Intact IFPSC (−SLO) or SLO-permeabilized IFPSC (+SLO). Cells were incubated for 30 min in HBSS containing 50 µg/ml of Texas red-conjugated 70,000 *Mr* dextran, resealed with 2 mM $CaCl_2$ and cultured for 2 h and 24 h before observation by phase contrast and epifluorescence microscopy. Original magnification: 10× for A–D and 20× for E–F.

Transdifferentiated MSCs increased expression of chondrogenic markers

After 2 weeks of exposure to extracts, we analyzed the expression of collagen II by immunofluorescence. Extract exposed IFPSCs [Fig. 5(C, F and I)] showed a strong expression of collagen II protein with a similar pattern of cultured chondrocytes [Fig. 5(A, D and G)]. Collagen type II was confined in both cytoplasm and ECM. However, non-treated IFPSCs displayed a weak staining for the chondrogenic marker collagen II [Fig. 5(B, E and H)].

In addition, differential expression of selected chondrogenic key markers was evaluated by RT-PCR [Fig. 5(F)]. After 14 days, extract-treated cells showed increased gene expression for *Col2A1*, *Acan*, *Col10*, *L-Sox5*, *Sox6* and *Sox9*, in comparison with control IFPSCs, which showed a low expression for *Acan* and *L-Sox5* and a weak expression for *Col2A1*, *ColX*, and *Sox6*. Chondrocytes exhibited higher expression levels of these genes than exposed cells. No significant changes were observed in the expression of *Col1* in all samples. Interestingly, extract not exposed cells showed a basal expression for *Col2A1*, *Sox9*, *Acan*, *Sox6* and *ColX*.

Cell attachment and distribution in the scaffold

Chondrocytes, extract exposed cells and control cells were seeded in the cylindrical scaffold for 5 days and outside and inside layers of each scaffold were examined by ESEM [Fig. (6)]. All cells were able to attach on the polymer surface by filopodia and connected with each other. Chondrocytes acquired a predominantly spherical or sometimes fusiform and were surrounded with a dense matrix that extended between the cells to form a continuous network of cell and matrix [Fig. 6(A and B)]. In contrast, control non-exposed IFPSCs showed a flattened morphology with a high number of prolongations and secrete some dense material [Fig. 6(C)]. Moreover, the cross-section images clearly showed that these control cells were able to penetrate within scaffold [Fig. 6(D)]. Interestingly, extract exposed IFPSCs were able to grow covering the scaffold pores and to secrete a high dense extracellular matrix (ECM) that formed a homogeneously compact surface [Fig. 6(E and F)]. Furthermore, histological and immunohistochemical analyses of scaffold sections clearly showed cartilage-specific extracellular components produced by the IFPSCs exposed to the extract (Fig. 7).

Discussion

Mature hyaline cartilage has a very low self-repair potential due to its intrinsic properties. For this reason, researchers have focus in the search of methods to induce complete cartilage repair. In the last years, tissue engineering strategies combining cell therapy and scaffolds have emerged as a promising new approach for the treatment of articular cartilage defects[24,25].

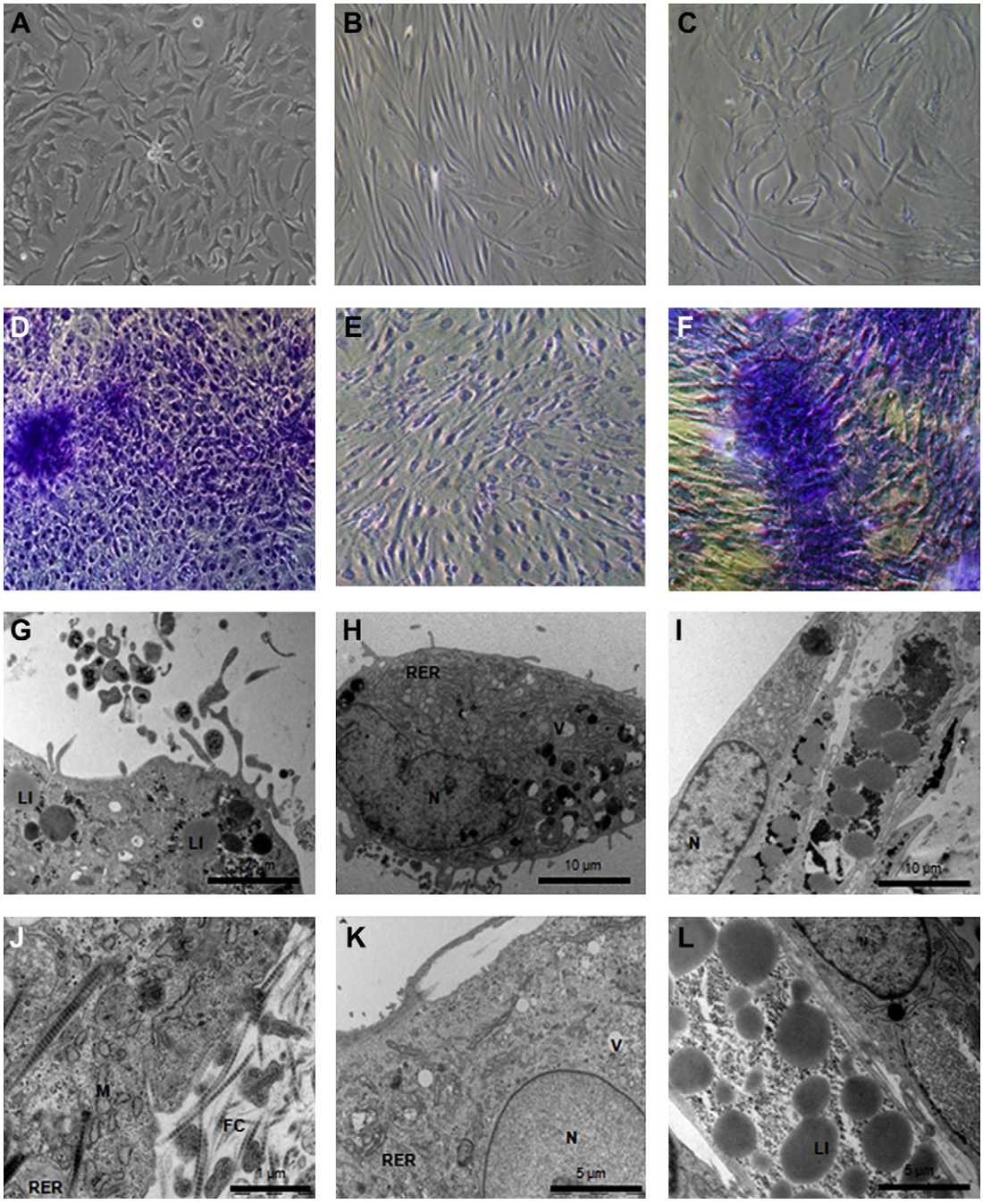
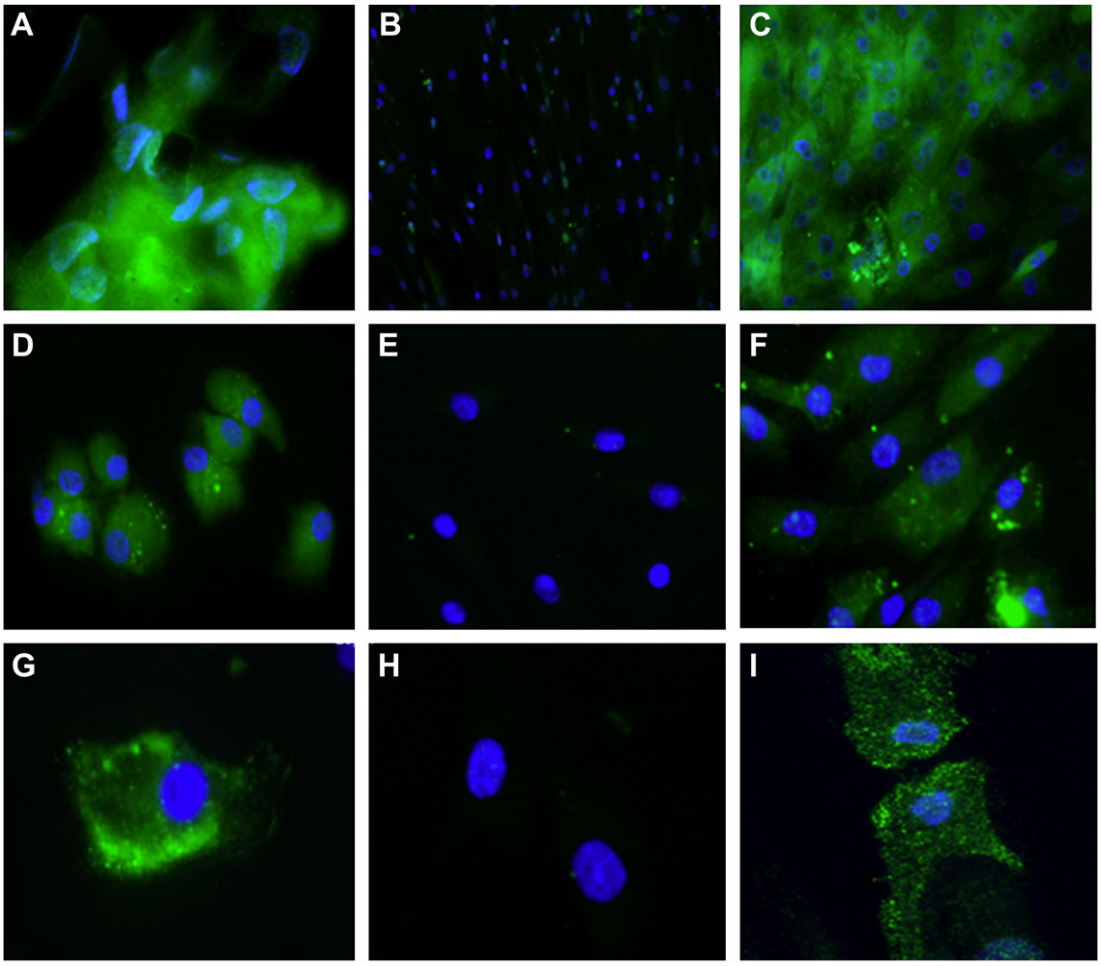


Fig. 4. Morphological analysis of control IFPSCs (permeabilized but not exposed to extract cells) and extract exposed cells. Phase-contrast light microscopy of chondrocytes (A), control IFPSCs (B) and extracts-treated IFPSCs (C). GAG synthesized was visualized by toluidine blue staining in chondrocytes (D) and in IFPSCs under chondrogenic differentiation (F), but not in control cells (E). TEM analysis of chondrocytes (G and J) showing a cytoplasm containing abundant RER, LI, proteoglycan granules and a homogeneous distribution of FC. Control IFPSCs with their complement of cytoplasmic organelles, such as RER, GA and vacuoles (H and K). Finally, IFPSCs exposed to the extract showed rounded-shaped lipid vesicles and proteoglycan granules in both cytoplasm and ECM (I and L). Original magnification 20× for A–F.

Cellular reprogramming based on cell extracts has shown that differentiated cells can be transdifferentiated in other differentiated cell types or dedifferentiated into pluripotent cells[17,19]. Moreover, we have demonstrated that using extracts from adult human heart tissue MSCs induced differentiation toward cardiomyocytes[26].

Recently, it has been shown that IFPSCs undergo chondrocyte differentiation, do not seem to have any age related decline in proliferative potential and are easily accessible with less discomfort to the patients[27,28]. In this work we analyze the chondrogenic potential of MSCs obtained from Hoffa's fat pad of patients with OA after the exposition to an extract, containing the intracellular components of chondrocytes.

We observed that human articular chondrocytes obtained from elderly patients maintained a chondrocyte phenotype in agreement with[29]. Chondrocytes and IFPSCs characterization by flow cytometry showed similar expression of MSCs surface makers CD90, CD105 and CD73. CD90 has been suggested as a new target to induce re-differentiation of dedifferentiated human chondrocytes in cartilage regeneration procedures and CD105 could be used as a molecular marker predictive of the capacity of cultured chondrocytes to form



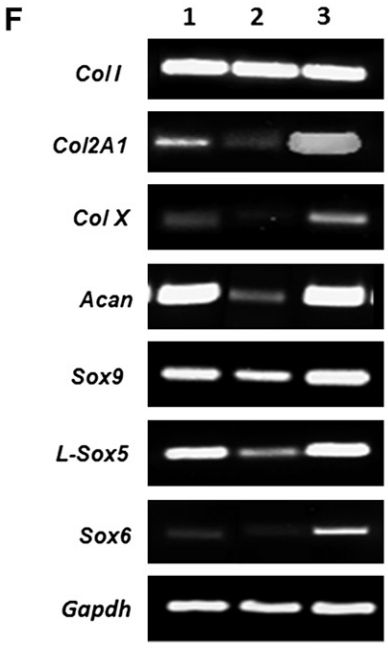


Fig. 5. Immunofluorescence of collagen II and gene expression of chondrogenic markers. Type II collagen indirect immunofluorescence of chondrocytes (A, D and G), control IFPSCs (B, E and H) and IFPSCs exposed to the extract (C, F and I). Expression of cartilage-specific collagen II protein with intense green staining can be appreciated on treated cells, showing the characteristic collagen fibers framework of cartilage. Original magnification 63× for A and G–I; 10× for B; 20× for C; 40× for D–F. RT-PCR analysis of chondrogenic markers (F). Two weeks in culture chondrocytes used as positive control (lane 3) and extracts-exposed IFPSCs (lane 1) showed increased gene expression for *Col2A1*, *Acan*, *Col10*, *L-Sox5*, *Sox6* and *Sox9*. Basal expression of these genes was seen in control IFPSCs (lane 2). Expression of *Gapdh* was used as an internal control. Experiments were performed in triplicate, were carried out at least twice and yielded similar results.

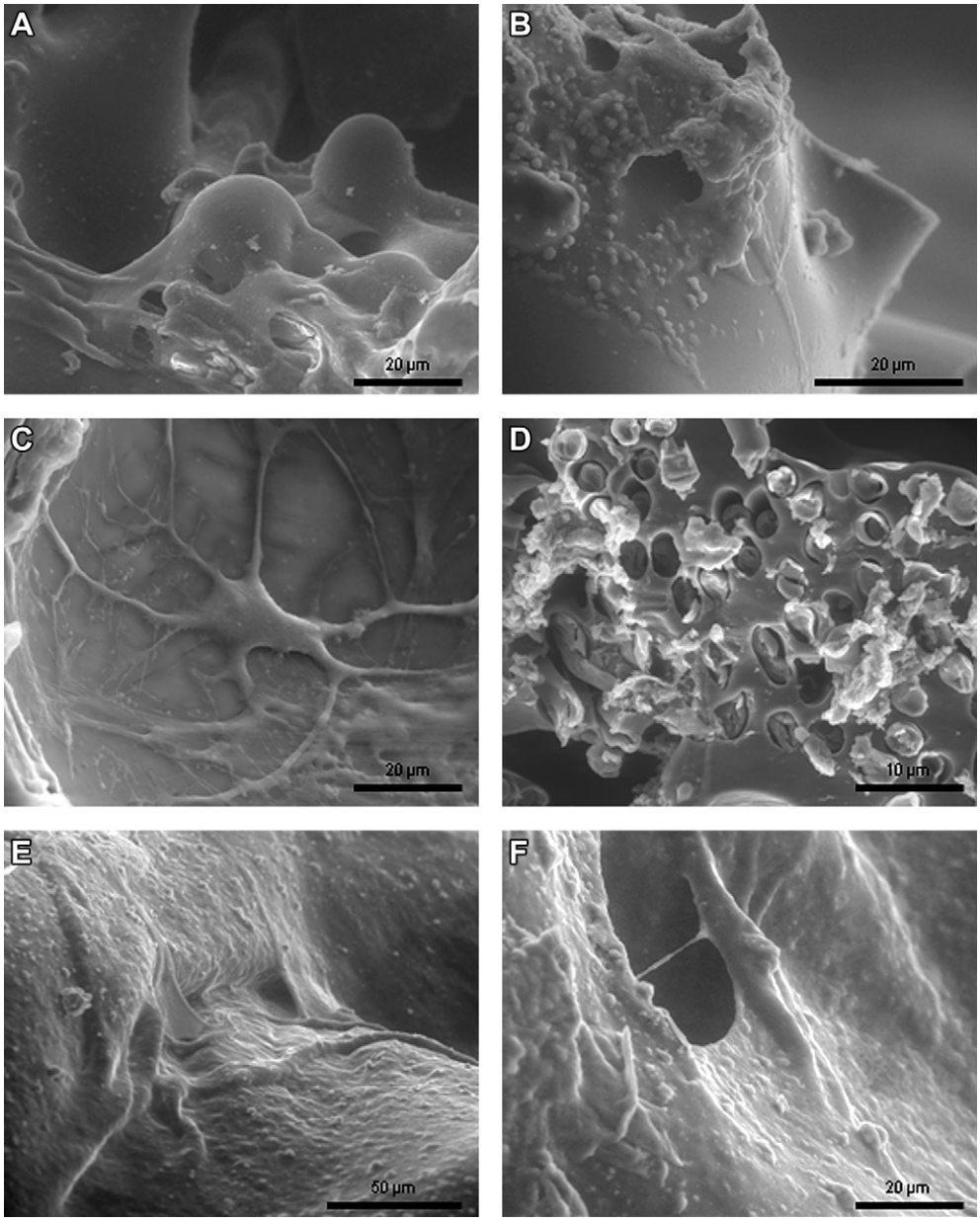


Fig. 6. Scanning electron micrograph of human of chondrocytes (A and B), control IFPSCs (C and D) and extracts-treated IFPSCs (E and F) after 5 days growing on the PLGA scaffold. Numerous cells firmly adhere to the scaffold and appeared to be suspended within the lumen or crawled around the walls. Chondrocytes show a round shaped morphology and a rough surface. Chondrocytes and extracts-treated IFPSCs appeared surrounded by matrix.

cartilage *in vivo*[30]. Also, collagen II was highly expressed in chondrocytes, and showed a basal expression in IFPSCs. In fact, mature articular joint develops from embryonic mesodermal precursors that differentiate into chondroprogenitors and ultimately into mature adult chondrocytes[31]. In addition, we found that IFPSCs were able to differentiate into specific lineages suggesting that fat pad cell population, as shown previously even in OA, contains a population of highly proliferative and multipotent MSCs[32].

IFPSCs exposed to chondrocyte extracts showed characteristic morphological changes suggesting the acquisition of a chondrocyte-like cell phenotype that was confirmed by the increased proteoglycan formation[33] in both toluidine staining and TEM observations, in agreement with chondrocytes typical features[34]. Further studies to demonstrate if the chondrocyte extract induce modifications in the expression of adipogenic and/or osteogenic markers will be of interest.

Collagen type II, a marker for hyaline cartilage together with aggrecan, are the predominant proteins in the ECM of cartilage[35]. Immunostaining of extracts-treated IFPSCs showed an increased expression of collagen II. Similarly, PCR analysis displayed an up-regulation of *Col2a1* and others chondrocyte-marker genes including *Sox9*, *L-Sox5* and *Acan*. *Sox9* is the master transcription factor for chondrogenesis, which acts in early stages of chondrocyte differentiation by directly induction of type II collagen[36] and is expressed in the mesenchymal condensations[37]. Moreover, it has been previously demonstrated that *Sox9* in concert with *L-Sox5* and *Sox6*, regulates cartilage formation and maintains the chondrocyte phenotype in the mature cartilage by activating expression of several cartilage-specific genes, including genes *Col2a1*, *Col9a1* and *Col11a1*, *Acan* and *Comp*[38]. In treated IFPSCs *Sox9* and its cofactor *L-sox5* expressions were elevated and a slightly induction of *Sox6* was found.

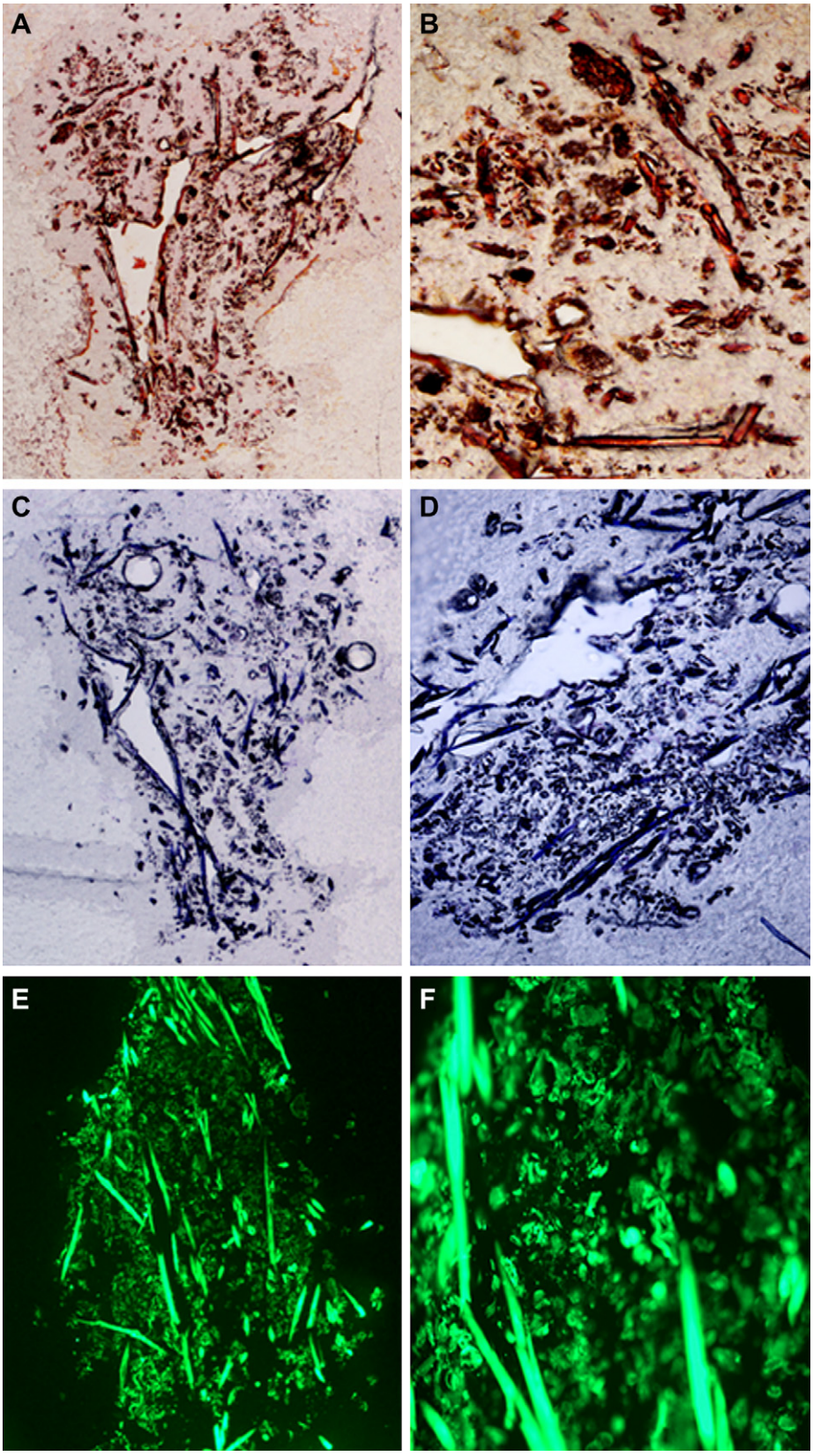


Fig. 7. Analysis for cartilage-specific extracellular components of PLGA scaffolds sections seeded with transdifferentiated IFPSCs. Safranin O staining (A and B) and toluidine blue staining (C and D) revealing *GAGs* production. Indirect immunofluorescence visualized in green for cartilage-specific collagen II protein (E and F). 5× original magnification for: A, C, E; 10× original magnification for: B and D; 20× F.

Some OA characteristics are the expression of hypertrophy markers such as collagen X with a concomitant decrease in the synthesis of type II collagen and aggrecan[39]. In our study chondrocytes isolated from patients with OA showed increased *Col10* expression, however IFPSCs exposed to the extract displayed a marked reduction. This fact together with the increased expression of *Sox9*, *L-Sox5*, *Sox6* and *Col2a1*, suggest the efficacy of this methodology to promote mature chondrogenesis. Nevertheless, the use of *Col10* mRNA as a marker of chondrogenic hypertrophy for *in vitro* studies has been questioned[40].

Type I collagen is categorized as a fibril-forming collagen and is usually produced when cells go into fibroblastic or osteoblastic differentiation[41]. In our experiment, *Col1a1* transcripts were constitutively expressed in chondrocytes and IFPSCs. After exposure to the extracts this expression was not affected. Type I collagen participates in regulating mesenchymal condensation and the onset of chondrogenic differentiation[42]. Moreover, *Col1* expression increase has been often closely related to chondrogenic differentiation *in vitro*[43]. The basal expression of characteristic chondrogenic markers in IFPSCs can be explained according to their micro-environmental niche. Recently, it has been demonstrated that non-cartilaginous knee joint tissues such as IFP possess significant chondrogenic potentials and this may be associated with the proximity to the niche they reside[31,44,45]. In addition, IFPSCs offer number of practical advantages such as they can be extracted from the same patient, avoiding rejection or side effects of immunosuppressive medication administration. Moreover, they are easily accessible, with less discomfort to the patient, as they can be obtained from osteoarthritic knee during knee arthroscopy. Furthermore, the fat pad has a greater yield of MSCs than bone marrow[46,47].

It has been shown that in comparison with monolayer 3-D matrices, which are developed to mimic the extracellular environment, could maintain better chondrocyte phenotypes and play a critical role in supporting chondrogenesis[24,48]. Therefore, we growth transdifferentiated IFPSCs in 3-D PLGA scaffolds, a cylindrical implant constructed with a porous cartilage phase which have been used before as cell-free for cartilage repair in surgery[49]. Nano-structured PLGA surfaces have been shown to accelerate chondrocyte adhesion and proliferation, as well as ECM production[50]. Our results showed that 3D PLGA scaffold was able to support growth and cell expansion and facilitate their free diffusion throughout the structure. These preliminary results demonstrated the affinity of IFPSCs for PLGA-based scaffolds and its ability to support chondrogenic differentiation.

Transdifferentiation studies have supported the notion that cell fate is controlled by master switch genes and that one or two factors can be sufficient to direct cells from one lineage to another[51]. Transdifferentation of the IFPSCs into chondrocytes can involve both the suppression and regulation of different genes in the cells, implying that genes from both cell types are co-expressed at some point. Further studies are necessaries to identify the factors and molecules presents in the extract that should enhance our understanding of the mechanisms involved in chondrocyte differentiation and development.

In conclusion, our results confirmed that extracts obtained from chondrocytes of osteoarthritic knees promote chondrogenic differentiation of IFPSCs. This chondrogenesis was not depended of exogenous growth factor induction, neither of the use of viral vectors. To our knowledge there have not been done experiments of IFPSCs programmed differentiation into chondrocytes using this methodology. Moreover, we show here, for the first time, that combination of transdifferentiated IFPSCs with biodegradable 3D PLGA scaffolds can serve as an efficient system for the maintenance and maturation of cartilage tissue. These findings encourage *in vivo* implantation studies to corroborate its usefulness to repair articular surface in OA.

Author contributions

ELR: design study, data acquisition, data analysis and interpretation, drafting the article, final approval of submitted manuscript. MP: design study, data acquisition, data analysis and interpretation, drafting the article, final approval of submitted manuscript. JCM: design study, data acquisition, drafting the article, final approval of submitted manuscript. GJ: data acquisition, data analysis and interpretation, drafting the article, final approval of submitted manuscript. MP: data acquisition, data analysis and interpretation, drafting the article, final approval of submitted manuscript. MB: data acquisition, data analysis, drafting the article, final approval of submitted manuscript. FA: data acquisition, data analysis, drafting the article, final approval of submitted manuscript. MCHL: data acquisition, data analysis, provision of study materials, drafting the article, final approval of submitted manuscript. ADDM: data analysis and interpretation, provision of patients, drafting the article, final approval of submitted manuscript. EM: design study, data acquisition, data interpretation, provision of patients, drafting the article, final approval of submitted manuscript. JAM: conception and design of the study, data acquisition, data analysis and interpretation, obtaining of funding, drafting the article, final approval of submitted manuscript.

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Conflict of interest

None of the authors have a conflict of interest to declare.

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Supplementary data

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