Iron triggers the early stages of cartilage degeneration in vitro: The role of articular chondrocytes

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ABSTRACT

Objective: Arthropathy is a major clinical problem in patients with hemochromatosis, the most common genetic disorder of iron overload. The pathological features of hemochromatosis arthropathy (HA) are heterogeneous and its specific nature remains unknown. One important drawback is the lack of proper in vitro models. The aim of the present study was to set up a model to investigate the biological response of cartilage to iron exposure.

Design: Bovine articular cartilage explants were incubated with ferric citrate for up to 9 days. We evaluated chondrocyte viability, iron deposition, and biomarkers of cartilage degradation in the conditioned medium.

Results: Iron accumulated within chondrocytes, which was associated with programmed cell death through chondroptosis. Iron treatment increased the release of sulfated glycosaminoglycans (sGAG), a component of the extracellular matrix, into the medium (p = 0.0189). This was dependent on the presence of viable chondrocytes and was associated with increased activity of matrix-degrading metalloproteinases (MMP) (pro/active MMP-9, p = 0.0317; pro MMP-2, p = 0.0092; active MMP-2, p = 0.0288). Co-treatment with the broad MMP/aggrecanase inhibitor prinomastat reduced iron-mediated sGAG release (0.02 μM, p = 0.0425; 2 μM, p = 0.0014), confirming that iron induces sGAG release via the activation of catabolic enzymes. Notably, iron-treated cartilage continued to release an increased amount of sGAG into the medium for 6 days after termination of the ferric citrate treatment (p = 0.0259).

Conclusions: Iron triggers the early stages of cartilage degeneration. Removal of iron exposure does not prevent further damage to the cartilage, thus providing a possible explanation why HA is not prevented after iron depletion by phlebotomy treatment.

1. Introduction

HFE related hemochromatosis (HFE-H) is a common genetic disorder of iron overload that is associated with homozigosity for the p.Cys282Tyr variant in the homeostatic iron regulator (HFE) [1]. The most severe manifestations of the disease, due to the accumulation of iron in parenchymal organs such as liver, pancreas and heart, can be prevented by iron depletion phlebotomy treatment [1,2]. In contrast, hemochromatosis arthropathy (HA), which occurs in up to two thirds of HFE-H patients, is not prevented or reverted by phlebotomy treatment and, in some cases, it is even aggravated [3]. Therefore, arthropathy is currently the main cause of morbidity and poor quality of life in HFE-H patients [4], which demands for a better understanding of its etiopathogenesis.

Clinically, HA is a chronic progressive condition that shows a preference for the second and third metacarpophalangeal joint, but large joints of the hip, knee, ankle, shoulder and elbow can also be affected [4].

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Symptoms are similar to those reported in idiopathic osteoarthritis (OA), including stiffness, pain on movement and minimal joint swelling. Likewise, the radiologic features include cartilage degeneration, sub-chondral sclerosis with joint space narrowing, chondrocalcinosis, deposition of calcium pyrophosphate dehydrate crystals, osteoporosis, and presence of osteophytes. Inflammatory reactions are not common and, when present, tend to be mild [4,5]. Histological features are also reminiscent of OA, but neutrophil infiltration is increased in HA and appears to be associated with iron deposition in joints [6].

Iron deposits are found in the synovium and cartilage of some patients [7-9]. However, whether and how iron deposition in different tissues contributes to articular damage remains elusive. Most of the information concerning joint pathology in HA originated from the observation of surgical specimens derived at the time of joint replacement surgery, and is thus representative of advanced disease [4]. There has been a lack of experimental in vitro or ex vivo models that allow the study of early osteoarticular modifications. The present study aimed at setting up a model to determine whether exposure to excess inorganic iron per se initiates cartilage degeneration. We incubated bovine articular cartilage explants in the presence of ferric citrate and investigated the effects of iron exposure on chondrocyte viability and on specific biomarkers of cartilage degradation. Hydroxyproline and sulfated glycosaminoglycans (sGAG) released from the cartilage to the conditioned medium were quantified and used as a measure of collagen type II and proteoglycan breakdown, respectively. We also measured cartilage oligomeric matrix protein (COMP), an extracellular matrix (ECM) protein that is released into synovial fluid upon cartilage erosion, and the activity of two matrix-degrading metalloproteinases (MMP).

2. Materials and methods

2.1. Reagents

All chemicals and reagents were purchased from Sigma-Aldrich, unless otherwise stated.

2.2. Preparation and culture of bovine articular cartilage explants

Bovine articular cartilage was obtained from the proximal interphalangeal joint of skeletally mature bovines from the local abattoir (Carnes Landeiro, Vila Nova de Famalicão, Portugal), following the directives of the national authority Direcção Geral de Alimentação e Veterinária (license number N.12.010.UDER). Joints were aseptically opened and exfoliated. Bovine articular cartilage explants were created using a 6 mm diameter biopsy punch (Kruuse) and a scalpel. Explants were weighed and randomly distributed into the wells of 24-well plates. In each well (representing the experimental unit), 3 explants were cultured with 1 mL Dulbecco’s modified Eagle’s medium (DMEM) of 24-well plates. In each well (representing the experimental unit), 3 explants were further incubated for 9 days at 37°C and explants were weighed and randomly distributed into the wells of 24-well plates. In each well (representing the experimental unit), 3 explants were cultured with 1 mL Dulbecco’s modified Eagle’s medium (DMEM) of 24-well plates.

2.3. Cell viability assay

Chondrocyte viability was determined using the live-dead assay kit (Invitrogen). Briefly, cartilage explants were cut in small fragments, washed with sterile phosphate buffered saline (PBS) and stained with calcien acetoxymethyl (AM)/ethidium homodimer-1. Fluorescence was recorded immediately with a Nikon Eclipse E400 microscope using fluorescein isothiocyanate (FITC)/Texas red filter.

2.4. Culture of SW982 synovial cell line

Human SW982 synovial cells were obtained from the American Type Culture Collection (ATCC HTB93) and cultured with DMEM, 10% (v/v) FBS and 1% penicillin-streptomycin at 37°C in a 5% CO₂ incubator. For the experiments, cells were seeded at 3 × 10⁴ cells/cm² onto cell culture inserts (polyethylene terephthalate track-etched membrane with 3 μm pore size, Corning). Cells were allowed to attach for 24h, and cell culture inserts were transferred to a 24-well culture plate containing bovine cartilage explants.

2.5. Quantification of sGAG

sGAG released from the cultured cartilage explants into the medium were quantified using the dimethylene blue (DMB)-binding assay. Briefly, 10 μL conditioned medium was applied to microtiter 96-well plates (Sarstedt). After addition of 200 μL DMB solution (16 mg/L DMB, 2.37 g/L NaCl, 3.04 g/L glycine, at pH=3), absorption was read in a microplate reader (BioTek), with baseline subtraction of the negative peak at OD 590 nm from the reading at OD 530 nm. A dilution series of chondroitin sulfate from shark cartilage was used to generate a standard curve. sGAG release was corrected for explant weight and cumulatively expressed as a fold increase relatively to the amount of sGAG released in the corresponding well during the 24h prior to treatment. Alternatively, we calculated the sGAG release rate for each sample as the slope of the linear regression of cumulative release vs. time, expressed as the amount of sGAG released (μg), per cartilage weight (mg), per day (μg mg⁻¹ day⁻¹).

2.6. Quantification of hydroxyproline

Levels of hydroxyproline released into the medium were quantified with the hydroxyproline assay kit. Medium samples were concentrated by speedvac (Savant) overnight and subsequently resuspended in ultrapure water. After addition of 12 M HCl, samples were incubated at 120°C for 3h. After cooling to room temperature, samples were centrifuged at 13,000×g for 10 min. The supernatant was transferred into a microtiter 96-well plate and evaporated through incubation at 60°C for 1h. A mixture of chloramine T/oxidation buffer and 4-(dimethylamino)benzaldehyde was added to the samples. Absorbance was read at 560 nm. Hydroxyproline release was corrected for explant weight and cumulatively expressed as a fold increase relatively to the amount released in the corresponding well during the 24h prior to treatment.

2.7. Quantification of COMP

The levels of COMP released into the culture medium were quantified with the bovine COMP enzyme-linked immunosorbent assay (ELISA) kit (MyBioSource), according to the manufacturer’s instructions.

2.8. Gelatine zymography

MMP activity was determined by zymography using 0.1% gelatine as a substrate in 10% sodium dodecyl sulfate (SDS) polyacrylamide gels.
Protein concentration in conditioned media was estimated with the protein quantification kit (BioRad). Samples of conditioned media (containing 16 μg of protein) were diluted with sterile PBS and mixed with 3 x 0.25 M Tris at pH=6.8 containing 10% (w/v) SDS, 4% (w/v) sucrose and 2% (w/v) bromophenol blue. The mixture was loaded on the zymography gels and run at 80 V for 3h. Gels were washed twice with 2% (v/v) triton X-100 and incubated in MMP buffer (10 mM CaCl2, 0.02% NaN3, 50 mM Tris-HCl, at pH=7.5) for 16 h at 37°C and 50 rpm. After staining with 0.1% coomassie blue R-250 (Thermo Scientific) in 40% (v/v) methanol and 10% (v/v) acetic acid, gels were recorded in a calibrated GS800 densitometer (BioRad). Band intensity was determined with Image J software freely available at https://imagej.nih.gov/ij/.

2.9. Histological analyses

Following fixation in 10% (v/v) buffered neutral formalin (Bio-optical), cartilage explants were embedded in paraffin. Following deparaffinization and hydration, 4 μm-thick sections were stained with Perls’ Prussian blue reaction for ferric iron and counterstained with nuclear fast red.

2.10. Terminal deoxynucleotidyltransferase-mediated dUTP nick end labelling (TUNEL) assay

Bovine cartilage explants were fixed in 10% (v/v) buffered neutral formalin. Following incubation with proteinase K (Ambion) at 60 °C for 30 min, 4-μm thick tissue sections were stained with fluorescein in situ cell death detection kit (Roche), according to the manufacturer’s instructions. Cells were counterstained with 4’,6-diamino-2-phenylindole (DAPI). Samples were analysed in a wide-field fluorescence microscope (Zeiss AxioImager Z1, Carl Zeiss). The % of TUNEL-positive cells was determined manually, in a blind manner, using Image J.

2.11. Transmission electron microscopy (TEM)

Cartilage explants were cut in small fragments and fixed by immersion in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH=7.4) solution. After post-fixation in 2% osmium tetroxide for 2h, tissues were incubated with 1% uranyl acetate overnight, dehydrated, and embedded in epon. Ultrathin sections (50 nm) stained with uranyl acetate and lead citrate were visualized with a JEM 1400 electron microscope (JEOL) operated at 80 kV. Electron micrographs were captured with an Orius CCD digital camera (Gatan).

2.12. Statistical analyses

Data were analysed using GraphPad Prism 6.07 (GraphPad Software) and the averaged values are presented as mean and standard deviation. All data sets passed the Shapiro-Wilk normality test with 0.05 significance level. To minimize variation between samples from different donors, differences among two group means were compared by paired Student’s t-test, and differences among multiple group means were compared by one-way or two-way repeated measures analysis of variance (ANOVA) with Sidak’s multiple comparisons test, unless stated otherwise. Differences were considered significant when p<0.05.

3. Results

To study the impact of iron loading on cartilage integrity, bovine articular cartilage explants were allowed 24h in culture medium to stabilize and subsequently incubated for up to 9 days with medium supplemented with 50 μM ferric citrate or sodium citrate (control). As outlined in Fig. 1, the medium was replaced every 3 days with fresh medium, and stored for quantification of biomarkers of cartilage degradation and MMP activity. At the end of the experiments, cartilage explants were collected to determine chondrocyte viability and iron accumulation. Preliminary experiments showed that, in the current experimental conditions, control chondrocytes remained mostly viable for at least 12 days in culture (Supplementary Fig. 1A). Also, after 9 days in culture, we observed no significant variation in the weight of cartilage explants that received either treatment when compared to untreated cartilage at 24h after collection (Supplementary Fig. 1B).

To determine whether ferric citrate was taken up by chondrocytes and whether these cells were able to accumulate iron intracellularly, cartilage sections were stained with Perls’ Prussian blue stain for ferric iron. As expected, control cartilage explants were devoid of iron deposits. In contrast, incubation with ferric citrate for 9 days led to marked intracellular iron loading of the chondrocytes present in the superficial zone of cartilage (Fig. 2A).

Since excess iron is suggested to cause chondrocyte death in vivo [5], we evaluated the impact of iron accumulation on chondrocyte DNA fragmentation with the TUNEL assay. A significant increase in dead (TUNEL-positive) cells was observed in cartilage explants incubated with ferric citrate for 9 days, when compared to control. TUNEL-positive cells were also found in the cartilage superficial zone, closely matching the distribution pattern of iron-loaded cells (Fig. 2B). This suggests that iron loading induced chondrocytes’ death.

In OA, chondrocytes have been reported to die by classical apoptosis and/or chondroptosis [12], a form of programmed chondrocytic cell death distinct from classical apoptosis that is associated with increased protein synthesis and ER stress [13]. But whether iron-laden chondrocytes die of apoptosis, chondroptosis or an alternative pathway of programmed cell death (e.g. ferroptosis, an iron-dependent form of programmed cell death associated with increased lipid peroxidation [14]) has not yet been determined. We have addressed this question using TEM, which is considered the ‘gold standard’ for detecting chondrocyte death [12]. As depicted in Fig. 3, after 9 days in culture, control chondrocytes retained the ultrastructural characteristics found in cells...
from freshly collected cartilage, including abundant rough ER and frequent cytoplasmic processes. In contrast, chondrocytes incubated with ferric citrate were characterized by the presence of membrane-bound cytoplasmic bodies containing hemosiderin (siderosomes), chromatin condensation (a common feature of apoptosis and chondroptosis), and a number of morphological changes consistent with death by chondroptosis, namely: expanded and dilated rough ER, autophagic vacuoles, presence of organelles in the extracellular space, and cell disintegration leading to empty lacunae.

Chondrocytes are responsible for maintaining cartilage homeostasis. Consequently, we have also investigated the impact of iron on cartilage integrity. To address this, we measured the release of specific constituents of the cartilage ECM into the culture medium, namely hydroxyproline, COMP and sGAG. Hydroxyproline and sGAG were measured every 3 days and results were expressed as the cumulative fold change compared to day 0. COMP levels were quantified at the end of the 9-day incubation period. Iron treatment did not significantly change hydroxyproline (p = 0.3488) (Fig. 4A) or COMP levels (p = 0.4657) (Supplementary Fig. 2). On the other hand, iron significantly increased the release of sGAG into the medium compared to control (p = 0.0189). The effect of iron was significant from day 6 in culture (Fig. 4B). Confirming a dependence on redox-active iron, the rate of sGAG release (between days 0 and 9) from cartilage explants treated with ferric citrate was significantly decreased by the specific iron chelator DFO (Fig. 4C).

There is increasing evidence associating cartilage degradation with chondrocyte death in OA [15]. However, it is unclear whether chondrocyte death is a cause of cartilage degeneration [12]. To investigate if the death of chondrocytes could trigger sGAG release in our experimental system, we have rendered cartilage explants metabolically inactive by repetitive freeze-thawing. In this condition, sGAG release was decreased when compared with the active cartilage. Importantly, no change in sGAG release was observed upon exposure of metabolically inactive explants to iron (Fig. 5A). This supports the hypothesis that the iron-dependent degradation observed in active explants depends on the presence of viable chondrocytes, and is neither the result of a direct chemical interaction between redox-active iron and the ECM, nor a direct consequence of chondrocyte death.

The putative contribution of cell death was further investigated by measuring sGAG release into the culture medium upon treatment of cartilage with known apoptosis/chondroptosis stimuli with an ER stress component, namely tunicamycin, a specific inhibitor of N-glycosylation in ER [16], and thapsigargin, which depletes ER calcium storage [17]. Both ER stress inducers were previously demonstrated to be effective in cartilage [18–20], but treatment of cartilage explants for 9 days failed to increase sGAG release (Suppl. Figure 3). Likewise, we incubated cartilage with different inducers of ferroptosis. Erastin and buthionine-sulfoximine (BSO) induce ferroptosis in a variety of cell types by inactivating glutathione peroxidase enzymes indirectly through glutathione depletion (via inhibition of the cystine glutamate antiporter or of γ-glutamyl cysteine synthetase, respectively), whereas RSL-3 binds to and directly inhibits
glutathione peroxidase 4 [21]. Ferroptosis inducers also failed to increase sGAG release (Suppl. Figure 3).

Conversely, we have incubated cartilage explants with ferric citrate in the presence or absence of ER stress/chondroptosis, apoptosis or ferroptosis inhibitors and evaluated sGAG release. The rate of sGAG release from cartilage explants treated with ferric citrate remained unaltered in the presence of the ER stress inhibitor TUDCA [22,23], the pan-caspase inhibitor Z-VAD-FMK [24,25] or the lipophilic antioxidant inhibitors of ferroptosis, Fer-1 [26] and α-tocopherol [27,28] (Suppl. Figure 4). It is thus unlikely that iron-induced sGAG release could be explained by the death of chondrocytes.

Instead, we hypothesized that iron loading could promote sGAG release by altering the catabolic profile of chondrocytes. To investigate this hypothesis, we measured the activity of two MMPs that are secreted into the extracellular space (MMP-2 and MMP-9) by gelatine zymography. In agreement with our hypothesis, we found significantly higher activity levels of both MMPs in the culture medium of cartilage explants incubated with ferric citrate (Fig. 5B). Our data thus suggest that iron-induced sGAG release is associated with increased metalloproteinase activity. To confirm this, we incubated cartilage explants with ferric citrate in the presence of increasing concentrations of the broad spectrum MMP/aggrecanase inhibitor prinomastat (AG-3340). Notably, iron-mediated sGAG released was significantly reduced in a concentration-dependent manner (Fig. 5C).

We also evaluated the reversibility of the effect of iron on cartilage degradation. After 9 days of incubation with ferric citrate, cartilage explants were exposed to sodium citrate for an additional 6-day period (Fig. 6A). Iron loading and sGAG release were assessed in comparison with cartilage that was further incubated with ferric citrate and with control cartilage (sodium citrate). As depicted in Fig. 6B, sustained
incubation with ferric citrate led to greater iron deposition within chondrocytes, which has extended to the transitional and radial zones of cartilage. The withdrawal of iron excess from the medium at day 9 of culture has apparently prevented the load of iron in chondrocytes at some extent. Nevertheless, chondrocytes remained iron-loaded, especially at the superficial zone of cartilage (Fig. 6B). Despite the iron removal, the rate of sGAG release into the medium between days 9 and 15 of culture remained significantly elevated when compared to control (Fig. 6C). We noted a trend for sustained iron exposure to increase the percentage of dead (TUNEL-positive) chondrocytes, when compared to what we had observed after just 9 days (Fig. 2B), which could not be prevented by iron withdrawal (Fig. 6D). Overall, these results suggest that chondrocyte iron loading leads to an irreversible onset of cartilage degeneration.

Finally, we hypothesized that iron-induced cartilage degradation may be further modulated in the presence of other cellular components of the joint. To explore this hypothesis, we have treated bovine articular cartilage explants with ferric citrate (or sodium citrate as control) for 9 days in the presence or absence of SW982 synovial cells previously seeded on cell culture inserts. Medium was refreshed every third day and conditioned medium was collected for the measurement of sGAG (Supplementary Fig. 5A). Interestingly, iron-induced sGAG release was exacerbated in the co-culture (Supplementary Fig. 5B). Furthermore, when cartilage explants were rendered metabolically inactive (by repetitive freeze-thawing) prior to incubation in the presence of SW982 cells, we observed no further increase in sGAG release into the medium (Supplementary Fig. 5C). This suggests that iron-induced cartilage degradation is augmented as a result of a crosstalk between chondrocytes and synovial cells, while confirming that the release of sGAG from articular cartilage is mediated by metabolically active chondrocytes.

4. Discussion

To study iron-induced cartilage degeneration, we incubated bovine articular cartilage explants with ferric citrate, which is predicted to be the dominant non-transferrin-bound iron (NTBI) species in plasma [29], increased in hemochromatosis patients [30]. We used ferric citrate at 50 μM, a concentration that is expected to saturate the transferrin present in culture medium containing 5% FBS [31]. Hence, we expect that iron was presented to cells in both transferrin-bound and non-transferrin-bound states.

We provide histological and ultrastructural evidence that the treatment of cartilage with ferric citrate causes iron accumulation within
chondrocytes, especially at the cartilage surface. The presence of intracellular iron deposits in chondrocytes has been previously reported in the articular cartilage from the affected joints of haemophilic patients [32] and in the cartilage of some hemochromatosis patients [7,8]. However, whether and how iron contributes to cartilage degradation has remained elusive.

In the present work, we showed that iron-treated chondrocytes present expansion and dilatation of ER, which is suggestive of increased protein synthesis and ER stress [33]. In OA, chondrocyte ER stress arises as a consequence of increased protein synthesis of both ECM molecules and matrix-degrading proteases [33]. Chronic ER stress, in turn, is known to induce programmed cell death of chondrocytes [34]. We showed here that iron loading causes programmed cell death (chondroptosis) of superficial chondrocytes.

Iron loading has also resulted in matrix degradation, observed as loss of sGAG. Loss of sGAG-containing aggrecans is a key pathophysiological event in joint diseases such as OA and rheumatoid arthritis (RA). It compromises both the functional and structural integrity of the cartilage matrix, leading to irreversible cartilage erosion [35]. As such, the presence of sGAG in the synovial fluid is a useful indicator for cartilage degeneration [36].

While the levels of sGAG released in our control cultures (0.5–0.6 μg mg⁻¹ day⁻¹) are similar to those reported in studies using bovine cartilage explants [37–39], the increase in sGAG release induced by iron exposure is of a much lower magnitude than that induced by IL-1β and/or TNF [39,40]. This is consistent with the chronic, late-onset nature of HA, when compared with typical inflammatory conditions such as RA.

There is evidence of an association between cartilage degradation and chondrocyte death in OA. Nonetheless, it remains to be established whether chondrocyte death is a cause or a result of cartilage degeneration [12]. We showed that, while iron-induced chondrocyte death is likely to contribute to the progression of joint disease in HA, it cannot explain the release of ECM components. This is supported by the fact that iron-induced sGAG release required a metabolically viable cartilage and was not inhibited by a variety of apoptosis, chondroptosis and ferroptosis inhibitors.

Instead, we showed that iron-induced loss of sGAG from articular cartilage is due to the activation of matrix-degrading proteases. In RA and OA cartilage, chondrocytes have an increased capacity to synthesize and secrete proteins that cleave membrane collagens and proteoglycans, including MMPs and aggrecanases [41]. We showed that iron loading promotes the production of matrix degrading metalloproteases, such as MMP-2 and MMP-9. While there is ample evidence that some MMPs (namely MMP-3 and MMP-13) are able to degrade aggrecans in vitro, it is now recognized that the main proteases that are responsible for in situ aggrecanolysis are aggrecanases [35]. We showed that prinomastat (AG-3340), a hydroxamate inhibitor with high specificity for MMP-2, MMP-9 and two MMPs known to be capable of degrading the interglobular domain of the aggrecan core protein, MMP-3 and MMP-13, prevented iron-induced sGAG release in a concentration-dependent manner. Notably, while far more potent against MMPs, prinomastat is also known to inhibit aggrecanase at concentrations around 10⁻⁶ M [42].

In summary, we showed that iron triggers the early stages of cartilage degeneration, which is associated with the activation of catabolic metabolism in chondrocytes leading to ECM degradation and programmed chondrocytic cell death. Moreover, our data suggest that the damage is irreversible and therefore removal of iron from the extracellular medium (mimicking the iron withdrawal achieved through therapeutic phlebotomy) is unlikely to prevent further damage to the cartilage. This may partly explain the progressive and irreversible nature of the
joint disease in HFE-H patients even after venesection regimens.

Our study represents a first attempt to generate an ex vivo model to study HA. We acknowledge that it has limitations. The use of young adult bovine cartilage in a highly controlled experimental setting does not mimic the full spectrum of physical insults to which human cartilage is exposed to over several decades of life. The amount of iron and how it is presented to cells (i.e. transferrin-bound and/or NTBI) in synovial fluid of hemochromatosis patients is unknown, which raises doubts on the physiological relevance of the iron concentration employed herein. Also, the oxygen levels present in standard cell culture conditions may increase the redox-cycling of iron, which could be avoided by culturing cartilage under hypoxic environment. Finally, we employed a simplified experimental model to study the impact of iron loading on cartilage stability, focusing on the role of chondrocytes. However, iron is known to accumulate also in the synovial cells of hemochromatosis patients [43]. Our preliminary co-culture experiments showed that iron-induced, chondrocyte-mediated cartilage degradation is further augmented as a result of a crosstalk with synovial cells. Future experiments should aim at characterizing the mechanisms governing this crosstalk. Likewise, the current experimental model could be modified to allow co-culture of cartilage explants with other cellular players that are also likely to contribute to cartilage degeneration, such as inflammatory cells (e.g. neutrophils), to better mimic the in vivo situation.

**Author’s credits**

Anaísa V Ferreira: Methodology, Investigation, Formal analysis, Writing – review & editing Tiago L Duarte: Conceptualization, Investigation, Formal analysis, Writing – original draft Sandra Marques: Investigation, Formal analysis Paula Costa: Investigation Sara C. Neves: Conceptualization, Methodology, Writing – review & editing. Tiago dos Santos: Methodology, Resources. Pedro L. Granja: Conceptualization, Methodology, Writing – review & editing. Graça Porto: Conceptualization, Methodology, Funding acquisition, Writing – review & editing.

**Declaration of competing interest**

The authors declare no conflicts of interest.
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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.joarto.2021.100145.

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