Cell-associated type I collagen in nondegenerate and degenerate human articular cartilage

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Abstract
Chondrocytes with abnormal morphology are present in nondegenerate human cartilage suggesting dedifferentiation to a fibroblastic phenotype and production of a mechanically-weakened matrix of unknown composition. We determined the relationship between in situ chondrocyte morphology, chondrocyte clusters, and levels of cell-associated collagen type I. Chondrocyte morphology in fresh femoral head cartilage from 19 patients with femoral neck fracture and collagen type I labelling was identified with Cell Tracker™ fluorescence and immunofluorescence, respectively, in axial/coronal orientations using confocal microscopy with images analysed by Imaris™. In axial images of grade 0 cartilage, 87 ± 8% were normal chondrocytes with a small (10 ± 6%) abnormal population possessing ≥1 cytoplasmic process. More normal chondrocytes (78 ± 11%) were collagen type I negative than those labelling positively (p < 0.001). For abnormal chondrocytes, 81 ± 14% labelled negatively for collagen type I compared to those labelling positively (19 ± 3%; p = 0.007; N(n)=11(3)). Overall, approximately 9% of the cells in normal cartilage labelled for collagen type I. With degeneration, the percentage of normal chondrocytes decreased (p < 0.001) but increased for abnormal cells (p = 0.036) and clusters (p = 0.003). A larger percentage of normal, abnormal and clustered chondrocytes now demonstrated collagen type I labelling (p = 0.004; p = 0.009; p = 0.001 respectively). Coronal images exhibited increased (p = 0.001) collagen type I labelling in the superficial zone of mildly degenerate cartilage with none in the mid or deep zones. These results show that collagen type I was identified around normal and abnormal chondrocytes in nondegenerate cartilage, which increased with degeneration. This suggested the presence of mechanically weak fibro-cartilaginous repair tissue in otherwise macroscopically nondegenerate human cartilage which progressed with degeneration as occurs in osteoarthritis.

KEYWORDS
articular cartilage, chondrocytes, femoral head, osteoarthritis, phenotype, Type I collagen
1 | INTRODUCTION

Osteoarthritis (OA) is a complex and painful whole joint disorder in which the increasing mechanical weakness of the degenerating cartilage plays a central role. The failure of the extracellular matrix (ECM) to bear load is pivotal in OA progression. However, the role of chondrocytes, the metabolism of matrix components (collagens, proteoglycans, and other minor components), and the sequence of the changes is not fully understood. This is of paramount importance to identify targets to slow cartilage loss and protect the pain-sensitive underlying bone. Although it is known that there are substantial changes to the collagen type and content with primary OA (e.g., Hollander et al., 1995), there are gaps in our knowledge particularly in relation to collagen type I. While collagen type I has an essential role in many types of connective tissues, its presence in hyaline cartilage in preference to type II collagen, leads to a mechanically-weakened fibro-cartilaginous “repair” tissue (Huey et al., 2012) and thus potentially accelerates the degenerative process.

There has been some controversy and contradictory evidence about the presence of collagen type I in human cartilage and the concept of a “phenotypic switch” for the synthesis of type II to type I collagen during cartilage degeneration. Early studies by Adam and Deyl (1983) using immunofluorescence suggested that only collagen type II was present in normal human femoral head cartilage obtained from necropsy samples. However, in freshly-resected OA cartilage from arthroplasty procedures, collagen types I and III were also reported to be present. Studies by Aigner et al. (1993) and Aigner et al. (1997) on the other hand, using in situ hybridisation could not detect collagen type I messenger RNA (mRNA) signal in normal or OA cartilage. Nevertheless, they reported “hardly any” collagen type I protein, but in agreement with others (Wotton & Duance, 1994) have described the presence of type III collagen (a characteristic of dedifferentiated chondrocytes; Charlier et al., 2019) increasing with cartilage degeneration (Hosseininia et al., 2016).

More recently, Gebhard et al. (2003) and Miosge et al. (2004) reported increased levels of collagen type I expression with degeneration but because the ratio of type I to type II did not increase in OA, this suggested that there was no general shift to a dedifferentiated phenotype. Using laser capture microdissection, Fukui et al. (2008) reported that the expression of collagen type I, alpha-2 (COL1A2), was most enhanced in the superficial zone of OA cartilage. Brew et al. (2010) studying late-stage OA from knee arthroplasty operations described a marked increase in gene expression (but not protein levels) for collagen type I compared to age-matched controls. They commented that this probably did not reflect a generalised change in chondrocytes to a proliferative or hypertrophic phenotype as seen, for example, in the growth plate. These and other studies (Eyre et al., 2006; Roberts et al., 2009) suggest that there is negligible collagen type I present in normal nondegenerate human cartilage, however, there is a marked increase in mRNA and protein levels with OA progression.

The increased production of collagen type I during cartilage degeneration strongly suggests a change in the differentiation status of some chondrocytes towards a fibroblastic phenotype (Charlier et al., 2019; Hall, 2019). The process may also be coupled with other events, for example, the development of a fibroblastic morphology, with associated increased polymerisation of G to F-actin, with reduced levels of aggrecan and Sox9 (SRY sex determining region Y)-box 9 (Sox9) production (Martinez-Sanchez & Murphy, 2013). This phenotypic modification of chondrocytes leading to the production of a mechanically-incompetent ECM is recognised as an important mechanism that contributes to the loss of cartilage homeostasis in OA (Fukui et al., 2001).

During studies on the in situ morphology of fluorescently-labelled chondrocytes within macroscopically normal (grade 0) human femoral head cartilage, a significant proportion of cells demonstrated a fibroblastic-like morphology with fine cytoplasmic processes (Karim et al., 2018). These morphological changes suggested that even in apparently nondegenerate cartilage, chondrocytes were undergoing dedifferentiation potentially producing collagen type I with the implication that microscopic areas of cartilage were becoming mechanically weakened. To clarify the situation, in the present work, the relationship between in situ human chondrocyte morphology within nondegenerate and degenerate human femoral head cartilage and levels of collagen type I has been investigated using fluorescent labelling of chondrocytes, immunofluorescence and confocal laser scanning microscopy (CLSM). The results demonstrated that cell-associated collagen type I was present in nondegenerate cartilage and increased with degeneration. Importantly, chondrocytes of both normal and abnormal morphology produced this protein, suggesting an “uncoupling” between chondrocyte morphology and collagen type I production.

2 | MATERIALS AND METHODS

2.1 | Human femoral heads

Femoral heads were obtained with Ethical permission (Ref, 20/ES/0061; Tissue Governance, National Health Service, Lothian) and patient consent from 19 patients (12 females, 7 males, mean age 75.8 years [range: 55–90 years]) undergoing hip replacement or hemiarthroplasty for a femoral neck fracture. Femoral heads removed intraoperatively were immediately placed in sterile saline (0.9% wt/vol; 21°C) to prevent chondrocyte death from drying. They were then transferred to a sterile container with Dulbecco’s modified Eagle’s medium including D-glucose (25 mM), L-glutamine (4 mM), pyruvate (1 mM), 100 U/ml penicillin, 100 µg/ml streptomycin, 2.5 µg/ml amphotericin B (Sigma-Aldrich), and 10 µg/ml Fungin™ (InvivoGen) ready for transportation to the laboratory with the femoral heads being cultured and available for experiments within 24 h.
2.2 Cartilage grading, sampling, fluorescent labelling of in situ chondrocytes, and CLSM

Grading of the articular cartilage was determined by visual assessment of the macroscopic appearance, using the Osteoarthritis Research Society International criteria (Pritzker et al., 2006). Cartilage was assessed as grade 0 (nondegenerate) or grade 1 (mildly degenerate). In some cases, it was difficult to tell precisely if the cartilage was grade 1 or 2, and thus it was described as grade 1–2 (see Figure 1). Full-depth cartilage explants were harvested using 3-mm diameter biopsy punches (Kai Medical) (Styczynska-Soczka et al., 2020). Cartilage samples were then incubated (1.5 h; 21°C) with CMFDA (5-chloromethylfluorescein diacetate) Cell Tracker green (12.5 µM; Invitrogen) to label living cells. Explants were incubated in bovine testicular hyaluronidase (H3506; 1000 U/ml; Merck) for 30 min at room temperature to facilitate the primary antibody penetration. Following this, sections were incubated with the primary antibody (Alexa Fluor 568, A11031, Thermo Fisher Scientific) and imaged by CLSM using established methods (Karim et al., 2018).

2.3 Chondrocyte morphology and collagen type I analysis

Axial and coronal view images were analysed using Imaris™ (Version 8.3.1; Bitplane). Chondrocyte morphology (green) and fluorescent labelling of collagen type I (red) were analysed in both grade 0 and grade 1 femoral head cartilage using a standardised image size (1024 × 1024). Chondrocyte morphology was quantified by manually counting the number of chondrocytes with “normal” morphology, chondrocytes forming clusters and chondrocytes with cytoplasmic processes, using previously published criteria (Karim et al., 2018). The data are expressed as a percentage of the total number of chondrocytes viewed on the standardised image. Chondrocytes with “normal” morphology were considered to be round/elliptical, with no observable cytoplasmic processes. “Abnormal” chondrocyte morphology was defined as cells exhibiting ≥1 cytoplasmic process(es) of at least 2 µm in length. A cluster was considered to be 5+ chondrocytes within one lacuna (Karim et al., 2018). An estimate of the lacunar region surrounding chondrocytes could be visualised because of the secondary antibody labelling in immunofluorescence images as a darker area around chondrocytes. Fluorescent-labelling of collagen type I was determined on a per-cell basis. This allowed the categorisation of the cells as being collagen type I “positive” (+) or “negative” (−) and the cells were considered to be collagen type I “positive” if the fluorescence was cell-associated, that is, strong specific staining was visible in the direct vicinity and surrounding the cell (see Figure 2). Cells were classified into six morphological and collagen-staining categories ([i] normal morphology collagen type I [+], [ii] normal morphology collagen type I [−], [iii] abnormal morphology collagen type I [+], [iv] abnormal morphology collagen type I [−], [v] clustered morphology collagen type I [+], and [vi] clustered morphology collagen type I [−]). For coronal views, cartilage explants (typically 2.5 mm thick) were divided into the superficial zone (SZ) = 15%, middle zone = 50%, deep zone (DZ) = 35% of the total cartilage thickness (Venn, 1978).

2.4 Statistical analysis

Data are presented as mean ± SD for [N(n)], with N representing the number of femoral heads from individual donors and n representing the total number of standardised images (1024 × 1024 pixels) obtained from individual cartilage explants from the same femoral head with typically 243 cells assessed per image (range: 28–595). Graphs and statistical tests were performed using GraphPad Prism-8 (GraphPad Software). To assess chondrocyte morphology or collagen labelling within a cartilage grade (nondegenerate [grade 0], or mildly degenerate, [grade 1 or 1–2]), one-way analysis of variance (one-way ANOVA) tests, followed by Tukey’s post hoc multiple comparison test, were used with significant differences indicated with an asterisk symbol (*). Two-tailed Student’s t tests were used to compare data between the cartilage grades. Significant differences were accepted when p < 0.05. The single, double and triple symbols show the level of significance for p < 0.05, p < 0.01 and p < 0.001, respectively.

**FIGURE 1** Examples of fresh human femoral heads obtained from femoral neck fracture. (a) Shows a femoral head with grade 0 cartilage and (b) a femoral head with an area of grade 1–2 cartilage. F identifies the fovea and the scale bar represents 1 cm.
3 RESULTS

3.1 Human chondrocyte morphology and cell-associated collagen type I labelling in normal (grade 0) femoral head cartilage

Collagen type I was identified by fluorescence immunohistochemistry and related to the morphology of SZ chondrocytes in nondegenerate and mildly degenerate cartilage at low and high magnification (Figure 2). At low magnification (Figure 2a,b), the heterogeneity of collagen type I labelling was clearly evident with some cells labelling strongly around the cell body (cell-associated), whereas others did not demonstrate labelling. There was also collagen type I labelling in the ECM of the mildly-degenerate cartilage in regions distant from chondrocytes (Figure 2b). However, it was difficult to observe the fine details of chondrocyte morphology (as reflected by the presence of cytoplasmic processes) in these images, and thus high magnification was required (Figure 2c–h).

Strong cell-associated labelling was present around the three categories of chondrocytes, which were (a) those of normal morphology, (b) those with cytoplasmic processes, and (c) those in clusters (Figures 2c, 2e, 2g, respectively). However, there were also cells in each of these categories and on the same tissue sections where no collagen type I was detected (Figures 2d, 2f, 2h, respectively). The distribution of collagen type I labelling which did not appear associated with chondrocytes and instead was in the interterritorial matrix, was also noted in mildly degenerate cartilage explants (Figure 2e).

Fluorescently-labelled in situ human chondrocytes were imaged by CLSM and their morphology classified as described (see Section 2; Karim et al., 2018). The majority (87 ± 8%) of chondrocytes within grade 0 femoral head cartilage demonstrated a rounded/elliptical morphology. There was a small (10 ± 6%) but distinct population of chondrocytes possessing one or more cytoplasmic processes (Figure 2) but only very rarely clusters of chondrocytes (>5 chondrocytes/lacuna) were observed (Figure 3a). There were significant differences between the three groups of chondrocyte morphologies (Figure 3a).

The semiquantitative assessment of collagen type I fluorescence labelling was performed using imaging software (see Section 2) for individual CMFDA-labelled chondrocytes in the three categories. The percentage of chondrocytes in the different categories labelling positively (+) or negatively (−) for collagen type I immunofluorescence in the whole in situ cell population was then determined with pooled data for grade 0 cartilage presented in Figure 3b. Of the cells with normal morphology, 78 ± 11% labelled negatively for collagen type I which was greater ($p < 0.001$) than for the percentage of cells labelling positively (6 ± 5%). For the cells in the population with one or more cytoplasmic process, the majority (81 ± 14%) labelled negatively for collagen type I which was also greater than for the percentage of cells labelling positively (19 ± 3%; $p < 0.001$; data from N(n)=11(3)). In the whole chondrocyte population in grade 0 cartilage, approx. 9% of the cells labelled positively for collagen type I. A negligible proportion (<4%) of the cells were in clusters and there was no difference between those that were positive or negative for collagen type I ($p = 0.13$). Thus, for the whole chondrocyte population in nondegenerate cartilage (i.e., both normal and abnormal chondrocytes) a
small, but noticeable proportion (~10%) of the cells labelled positively for collagen type I (Figure 2a). The observation that for some cells, cytoplasmic processes were evident but there was no collagen type I identified, whereas there was labelling around some of normally shaped chondrocytes, suggests that the morphological change for these cells was not a necessary prerequisite for the presence of collagen type I.

3.2 | Human chondrocyte morphology and cell-associated collagen type I labelling in mildly-degenerate (grade 1–2) femoral head cartilage

With mild cartilage degeneration to grade 1 or 1–2 (see Section 2), the morphological characteristics of the fluorescently-labelled in situ superficial zone chondrocytes were markedly different (Figure 3a). Although there were still more chondrocytes with normal morphology compared to cells with processes (p < 0.05), a large proportion (~40%) of the cells were present in clusters and the percentage was greater than for abnormal chondrocytes (p < 0.05). Compared to grade 0 cartilage, there was a significant decrease (to 39 ± 23%) in cells of normal morphology (p < 0.001) but there were significant increases (to 25 ± 21%) in the percentage of the cell population with one or more cytoplasmic process (p = 0.036) and the percentage of cells in clusters increased markedly (to 35 ± 28%; p = 0.003; data are means ± SD from N(n)=11(3)) (Figure 4a). The relatively large scatter in the data was notable and this may be because of the difficulty in precise cartilage grading of individual explants and the heterogeneity between the locations of individual cartilage samples obtained from the femoral heads of different patients.

The immunofluorescent labelling of collagen I was present around almost all of the cells (Figure 4b) with 91 ± 20% being collagen positive, and 8.7 ± 3.3% collagen negative (data are means ± SD from N(n)=9(3)). Of the cells with normal morphology, or with one or more cytoplasmic process, the majority (85 ± 10% and 93 ± 10%, respectively) now labelled positively for collagen type I which was significantly greater than the percentage of cells labelling negatively (p = 0.004 and p = 0.002, respectively). Of the cells in clusters, virtually all (98 ± 4%) labelled positively for collagen type I. Thus, for the whole cell population in mildly degenerate cartilage, it was unusual to observe cells that were not fluorescently tagged with collagen type I antibody compared to the situation in grade 0 cartilage. Thus, comparing the data for chondrocytes in grade 0 to those in mildly degenerate cartilage, there was an increase in the percentage of normal chondrocytes labelling positively (p = 0.004), however, there was a decrease in the proportion of cells labelling negatively (p < 0.001). Similarly, for chondrocytes with one or more cytoplasmic process(es), there was an increase in the cells labelling positively (p = 0.029) but a decrease in the proportion of cells labelling negatively (p = 0.002). Finally, there was a marked increase in the percentage of chondrocytes in clusters labelling positively for collagen type I (p = 0.001) but no significant difference for these cells labelling negatively (p = 0.07) (Figure 4b).

3.3 | Distribution of cell-associated collagen type I with cartilage depth and degeneration

Full thickness coronal images of femoral head cartilage with chondrocytes labelled fluorescently and collagen I identified by immunofluorescence were studied by CLSM (Figure 5). Cartilage thickness was divided into zones as described (see Section 2) and in grade 0 cartilage there was no evidence of cell-associated collagen type I labelling in any zones. In mildly degenerate cartilage, there was significant (p < 0.001) collagen type I labelling in situ superficial zone chondrocytes were markedly different (Figure 3a). Although there were still more chondrocytes with normal morphology compared to cells with processes (p < 0.05), a large proportion (~40%) of the cells were present in clusters and the percentage was greater than for abnormal chondrocytes (p < 0.05). Compared to grade 0 cartilage, there was a significant decrease (to 39 ± 23%) in cells of normal morphology (p < 0.001) but there were significant increases (to 25 ± 21%) in the percentage of the cell population with one or more cytoplasmic process (p = 0.036) and the percentage of cells in clusters increased markedly (to 35 ± 28%; p = 0.003; data are means ± SD from N(n)=11(3)) (Figure 4a). The relatively large scatter in the data was notable and this may be because of the difficulty in precise cartilage grading of individual explants and the heterogeneity between the locations of individual cartilage samples obtained from the femoral heads of different patients.

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labelling identified around chondrocytes in the SZ but not in the MZ or DZ (Figure 5c). High magnification images indicated that there was labelling around SZ chondrocytes but there was also labelling more distant from the immediate vicinity of the cells (Figure 5b).

**FIGURE 4** Chondrocyte morphology and collagen type I labelling in grade 1–2 femoral head cartilage. (a) Shows the distribution of chondrocytes into the classified categories of morphology as described (see Section 2). (b) Summarises the percentage of cells labelled with or without cell-associated collagen type I labelling in each of the three categories. Each symbol represents the averaged data from one femoral head (N = 9 and 7 for [a] and [b], respectively) with at least three images analysed for each femoral head. Data were compared between the categories using ANOVA followed by Tukey’s post hoc multiple comparison test with ns indicating no significant difference (p > 0.05). For statistical comparisons between the data shown here and that for Grade 0 cartilage see text. ANOVA, analysis of variance

**FIGURE 5** Collagen type I labelling in (a) grade 0 and (b) grade 1–2 femoral head cartilage visualised in coronal sections. Low magnification images showed the absence of cell-associated collagen type I labelling in grade 0 cartilage, but extensive labelling only in the SZ of mildly degenerate cartilage. Areas (labelled 1, 2) in the SZ of grade 1–2 cartilage are shown at high magnification. (c) Shows the percentage of cells exhibiting cell-associated collagen type I labelling. Pooled data were obtained from (N=5(3)) with each symbol representing averaged data from separate femoral heads. The difference between number of collagen type I positive cells in SZ in grade 0 and grade 1–2 was significant (p = 0.001). DZ, deep zone; MZ, mid zone; SZ, superficial zone

**4 | DISCUSSION**

Although the majority of fluorescently-labelled in situ chondrocytes visualised in the axial plane in nondegenerate grade 0 human femoral head cartilage demonstrated a rounded/elliptical morphology, a small, but potentially important population of cells possessed one or more cytoplasmic process. The majority of chondrocytes labelled negatively for collagen type I. However, a small population labelled positively (Figure 1a) and appeared to be mainly with cells of normal morphology. Mild degeneration of cartilage to grade 1–2 significantly reduced the proportion of chondrocytes with normal morphology and increased those with one or more cytoplasmic processes. There was also a large increase in the presence of cell-associated collagen type I for both chondrocytes of normal and abnormal morphology,
particularly in the superficial zone. Although no chondrocyte clusters were observed in grade 0 cartilage, mild cartilage degeneration clusters were evident which labelled strongly for type I collagen. These observations are important for identifying microscopic properties of individual chondrocytes within otherwise normal, healthy human cartilage, and some of the changes which occur with cartilage degeneration as occurs in osteoarthritis.

A particular strength of the current study was the routine, ready and rapid access to fresh living ex vivo human femoral head cartilage obtained from femoral neck fractures. This required effective co-ordination between orthopaedic surgeons, theatre staff, and the research laboratory, and the collaboration allowed joints to be delivered quickly from the operating theatre to the research labs. Preventing injury during removal of femoral heads and maintaining joint hydration throughout was essential as articular cartilage chondrocytes are very sensitive to even mild mechanical (Howard et al., 2020) and drying trauma (Paterson et al., 2015). Many previous studies have utilised tibial plateau or femoral condyle cartilage removed from joints of cadavers or during joint replacement surgery for osteoarthritis (e.g., Bush & Hall, 2003). However, as osteoarthritis is recognised as a whole joint disorder (Loeser et al., 2012) there is a relationship between levels of cell architecture and collagen type I production followed by collagen type II production as the cells differentiate into chondrocytes. This raises the interesting possibility that some of the collagen types at the cartilage surface has been described during the synthesis of type I collagen is usually considered an indicator of fibrocartilage, the cartilage progenitors appear to follow a developmental process in matrix synthesis initially with collagen type I production followed by collagen type II production as the cells mature (Craig et al., 1987). Indeed, evidence of the presence of both collagen types at the cartilage surface has been described during cartilage development in the chick diarthrodial joint (Archer et al., 1994). This raises the interesting possibility that some of the cells we observed which have cytoplasmic processes and label positively for collagen type I, might in fact be cartilage progenitor cells. However, the proportion of cells with one or more cytoplasmic process was approx. 10% (Figures 2 and 3a) and so considerably higher than that expected solely for the progenitor cell population. A potential explanation is that there are two cell types comprising the SZ of cartilage, a tiny but potentially important population of progenitor cells has been identified. Using cell surface markers, Dowthwaite et al. (2004) reported that in normal calf cartilage 1%-2% of the cell population were progenitor-like cells and could differentiate into chondrocytes. The group also identified chondrogenic progenitor cells (CPCs) in normal human femoral condylar cartilage, which accounted for 0.7% of the total cell population (Williams et al., 2010). Of particular interest in the present context is that the cartilage progenitor cell population expresses chondrogenic markers and matrix components including collagen type I and II. Although the synthesis of type I collagen is usually considered an indicator of fibrocartilage, the cartilage progenitors appear to follow a developmental process in matrix synthesis initially with collagen type I production followed by collagen type II production as the cells mature (Craig et al., 1987). Indeed, evidence of the presence of both collagen types at the cartilage surface has been described during cartilage development in the chick diarthrodial joint (Archer et al., 1994). This raises the interesting possibility that some of the cells we observed which have cytoplasmic processes and label positively for collagen type I, might in fact be cartilage progenitor cells. However, the proportion of cells with one or more cytoplasmic process was approx. 10% (Figures 2 and 3a) and so considerably higher than that expected solely for the progenitor cell population. A potential explanation is that there are two cell types comprising this small population, (a) CPCs and (b) dedifferentiated chondrocytes. At the present time, their relative contributions are unknown and thus further studies investigating the properties of cell types within the SZ of nondegenerate human cartilage are warranted.

It was perhaps unexpected that there was not a closer relationship between levels of cell-associated collagen type I and chondrocyte morphology. The hypothesis was that chondrocytes with abnormal morphology would demonstrate increased labelling, whereas those of normal morphology would not, but this was clearly not the case (Figures 2 and 3). This suggests an “uncoupling” between these two processes as a significant proportion of chondrocytes possessed “normal” morphology (i.e., no cytoplasmic processes were observed) however, they exhibited strong collagen type I labelling (Figure 2). Thus, while changes to chondrocyte shape might suggest a shift to a fibroblastic tissue “repair” phenotype, it is unlikely that cell
shape, per se, controls chondrogenesis, but rather the organisational status of the chondrocyte cytoskeleton and its interaction with second messenger pathways. Although modification of the actin cytoskeleton does not appear to control the full chondrogenic phenotype (Benya et al., 1988; Watt & Dudhia, 1988; Mallein-Gerin et al., 1991; Parreno et al., 2017), the manipulation of other cytoskeletal proteins which bring about the morphological changes can influence matrix metabolism. For example, tubulin polymerisation reduced the production of interleukin (IL)-1β and protease gene expression in primary chondrocytes (Hui et al., 1998) whereas disruption of vimentin intermediate filaments (reportedly in the absence of changes to F-actin labelling) has been observed in chondrocytes from OA cartilage possibly leading to downstream regulation of protease activity (Lambrech et al., 2008). Although there might not be a strict relationship between chondrocyte morphology and levels of collagen type I, there is a correlation between the number and length of cytoplasmic processes per chondrocyte and levels of cell-associated IL-1β (Murray et al., 2010). The presence of this potent proinflammatory cytokine will likely have profound effects on the local synthesis/release of degradative enzymes and matrix constituents. We should also be alert to the fact that the present results are only a single “snap shot” in time of the events occurring in cartilage. For example, it is possible that increases in collagen type I levels precede the development of cytoplasmic processes possibly as a result of changes to the lacunar/pericellular structure surrounding chondrocytes (Guilak et al., 2018). In any event, it seems probable that dedifferentiation and any phenotypic changes leading to alterations to matrix metabolism are progressive and complex time-dependent processes. These appear to occur initially in only a relatively small subset of SZ chondrocytes and deserve further study to better understand early degenerative changes in cartilage.

Although there seems to be general agreement that levels of type I collagen are increased as cartilage degenerates (e.g., Fukui et al. 2008; Brew et al. 2010), this begs the question of whether there is evidence of any collagen type I in normal nondegenerate cartilage. This is not a trivial point because as it might be assumed that there was none, the method of analysis needs to be carefully considered in addition to whether the human cartilage was truly grade 0 or, for example, grade 0.5 where a more detailed microscopic analysis of the cartilage might be required. This could potentially also include chondrocyte morphology, the presence of collagen type I, and the cartilage surface (Figure 5). Thus, it is conceivable that while macroscopically consistent with a grade 0 assessment, the relatively aged cartilage used in the present study might have small areas of surface cartilage in the very early stages of degeneration.

The present investigation utilising CLSM analysis of fluorescently-labelled in situ human chondrocytes to define their morphology associated with the immunofluorescent labelling of collagen type I labelling, clarifies the literature regarding the presence of collagen type I in nondegenerate and degenerate human cartilage. The presence of cell-associated collagen type I was identified in chondrocytes of normal and abnormal morphology within the superficial zone of grade 0 cartilage. With cartilage degeneration, there was a marked increase in the presence of cell-associated type I collagen labelling for both of these cell types and chondrocytes present in clusters. The presence of collagen type I in macroscopically normal cartilage indicates early microscopic changes to the properties of the cartilage matrix potentially leading to a reduced capacity for load-bearing.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHORS CONTRIBUTIONS

Study concept and design: Katarzyna Styczynska-Soczka, Anish K. Amin, and Andrew C. Hall. Acquisition of data, analysis, and interpretation of data: Katarzyna Styczynska-Soczka, Anish K. Amin, and Andrew C. Hall. Manuscript preparation: Katarzyna Styczynska-Soczka, Anish K. Amin, and Andrew C. Hall. Approval of submission: Katarzyna Styczynska-Soczka, Anish K. Amin, and Andrew C. Hall.
DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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