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Ultrastructure of the human intra-articular disc of the temporomandibular joint

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SUMMARY The ultrastructural appearance of the human intra-articular disc (IAD) was investigated in three discs that had been surgically removed due to disease from three female patients aged 47, 50, and 54 years of age. Regions of the IAD were selected from central areas that appeared to be least affected by disease. Sections were fixed in 2.5 per cent gluteraldehyde in 0.1 M phosphate buffer, pH 7.3 immediately after surgery.

The regions examined showed no obvious signs of inflammation. The cells showed moderate amounts of the intracellular organelles associated with protein synthesis and secretion, and possessed considerable amounts of microfilamentous material, thus resembling those described in other mammals. Despite the large number of cells examined, only one cell showed evidence of a chondrocyte-like morphology in that it possessed an incomplete pericellular zone of microfilamentous material separating the cell membrane from the adjacent collagen bundles of the extracellular matrix (ECM). Thus, on morphological grounds, fibrocartilage was virtually non-existent in the specimens examined. The mean collagen fibril diameter was 43.9 nm and the fibril diameter distribution was not unimodal. Although the majority of fibrils had a relatively small diameter, two of the three specimens possessed many fibrils with diameters of over 100 nm, this being consistent with tissue subjected to tension. The mean area of a fibre bundle occupied by collagen (as opposed to the ground substance) was approximately 56 per cent.

Introduction

There is considerable literature concerning disorders of the temporomandibular joint (TMJ), especially internal derangement (for review see Haskin *et al*., 1995). The general light microscopic appearance of the intra-articular disc (IAD) has been well described, together with the orientation and crimping of the collagen fibre bundles (Minarelli and Liberti, 1997; Minarelli *et al*., 1997; Berkovitz, 2000) and the distribution of elastic fibres (Gross *et al*., 1999). Information also exists concerning the scanning electron microscopic (SEM) appearance of the IAD, especially its surface features (Jagger, 1980; Piacentini *et al*., 1994). However, whereas in animals accounts exist concerning the detailed ultrastructure of the cells (Silva, 1969; Berkovitz and Pacy, 1999, 2000) and quantification of collagen fibril diameters IAD (Berkovitz *et al*., 1992b; Berkovitz and Robertshaw, 1993; Kuc and Scott, 1994), no such studies appear to exist for the human IAD. Marchetti *et al*. (1997) have illustrated some flattened fibroblasts at the periphery of the human IAD.

Textbooks describe the human IAD of the TMJ as being comprised chiefly of fibrous tissue, but containing variable amounts of rounded, cartilage-like cells (Bhasker, 1980; DuBrul, 1988; McDevitt, 1989; Schroeder, 1991; Berkovitz *et al*., 1992a). The term fibrocartilage has been used to describe this latter tissue. Reports indicate that chondrocyte-like cells are present, particularly in the IAD of older persons (Rees, 1954; Dixon, 1962; Oberg *et al*., 1966; Boering, 1979; Takisawa *et al*., 1982; Minarelli and Liberti, 1997) and are characterized by lacunae, which stain differently to the rest of the extracellular matrix

(ECM) (Rees, 1954; Dixon, 1962). Kurito and Westesson (1989), however, denied the existence of chondrocytes in non-pathological human IAD. In this context, the IAD of certain animals (e.g. sheep, dog, rabbit, pig, Old world monkeys) has also been described as fibrocartilage from light microscopic examination (Gilbe, 1973; Christensen and Ziebert, 1986; Helmy *et al.*, 1988; Mills *et al.*, 1988, 1994a,b; Ali and Sharawy, 1995; Scapino *et al.*, 1996). Although Gilbe (1973) found no evidence of chondrocytes in the IAD of adult rats, Fujita and Hoshino (1989) reported the existence of chondrocytes that were surrounded by type II collagen, even in very young rats. The presence of type II collagen has been demonstrated around chondrocyte-like cells in the IAD of rabbits (Ali and Sharawy, 1995) and Old world monkeys (Mills *et al.*, 1994a).

Deciding from morphological criteria at the light microscope level whether a cell with a rounded outline is a chondrocyte is clearly difficult. However, one can answer this question with more certainty ultrastructurally, as chondrocytes in fibrocartilage have been described as being similar to those in hyaline cartilage (Merrilees and Flint, 1980; Okuda *et al.*, 1987), possessing a filamentous pericellular matrix, interposed between the cell membrane and the surrounding collagen fibrils. In hyaline cartilage, this pericellular matrix is itself surrounded by a felt-like pericellular capsule quite distinct and separate from the adjacent territorial matrix collagen fibres (Poole *et al.*, 1987). Such a capsule appears to be lacking around chondrocyte-like cells associated with fibrocartilage (Okuda *et al.*, 1987; Benjamin *et al.*, 1991; Lyon *et al.*, 1991; Rufai *et al.*, 1995). Recently, Berkovitz and Pacy (2000) demonstrated the presence of cartilage-like cells in an ultrastructural study of the cells of the IAD of rats and marmosets and related their presence to an age change.

The reason for the lack of information concerning the ultrastructure of the human IAD relates to the difficulty of obtaining normal material that can be placed immediately into fixative. As a possible alternative, certain patients suffering from TMJ disease have their IAD surgically removed to alleviate symptoms. From such material areas can be dissected out that appear to be least affected by the disease process. This report deals with the ultrastructure of this tissue in the light of previous studies using animal material. Particular reference has been paid to the ultrastructure of fibroblasts, to the presence of any fibrocartilage, and to collagen fibril diameters.

Material and methods

From female patients aged 47, 50, and 54 years, three human IADs that had been surgically removed due to symptoms associated with TMJ disease were placed in 2.5 per cent gluteraldehyde in 0.1 M phosphate buffer, pH 7.3 immediately on removal. From each IAD, two pieces of tissue from the central area were dissected out from regions that appeared to be relatively unaffected by the disease process and post-fixed for two hours in 1 per cent osmium tetroxide in distilled water. The tissues were then dehydrated through a graded series of alcohols and embedded in Spurr resin. Ultra-thin sections were cut on a Reichart Ultracut E ultramicrotome (Austria), stained with lead citrate and uranyl acetate, and viewed in a Jeol 100CX Mark II transmission electron microscope (Japan). For each disc, photomicrographs of collagen fibrils sectioned transversely were enlarged to a magnification of ×100,000 and mean collagen fibril diameters were measured using a digitizing planimeter. Six micrographs were taken for each disc and the diameter of 50 fibrils was measured in each, giving a total of 300 measurements for each disc. To ensure consistent magnification, a cross-grating replica was used for calibration. In addition, a circle with a diameter representing 0.4 μm and containing on average about 30 collagen fibrils was constructed in a collagen fibre bundle, and the area occupied by collagen (as opposed to ground substance) derived using the digitizing planimeter.

Results

The tissue of the IAD showed the features characteristic of a dense soft fibrous connective tissue, the cells being sparsely scattered throughout the dense matrix of collagen. Ultrastructurally,

the cells shared common features, possessing conspicuous amounts of cytoplasm in which could be seen moderate amounts of the intracellular organelles associated with protein synthesis and secretion (e.g. endoplasmic reticulum, Golgi complex, mitochondria, vesicles; Figures 1–3). A feature of many of the cells was the presence of considerable quantities of microfilamentous material dispersed throughout the cytoplasm, imparting on those regions a pale appearance (Figures 2–4). A number of the nuclei of the cells showed a thickened zone associated with the nuclear membrane (Figure 4). No intracellular collagen profiles were observed.

The collagen fibrils in the ECM were generally closely applied to the cell surface (Figures 1 and 2), although sometimes this was loosely organized (Figure 3). Of the large number of

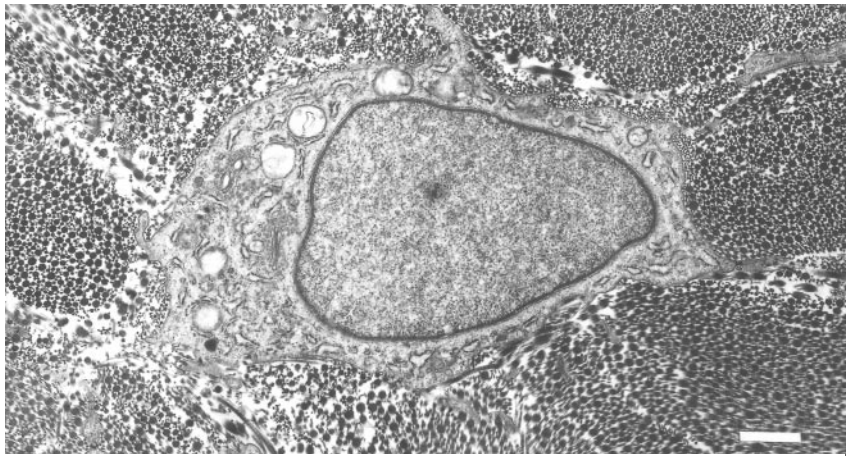

Figure 1 Electron micrograph of a fibroblast in the intra-articular disc of a female patient aged 50 years. The cell has an oval outline and the cytoplasm possesses moderate amounts of endoplasmic reticulum. It is closely surrounded by collagen fibrils of the extracellular matrix. Bar = 1 μm.

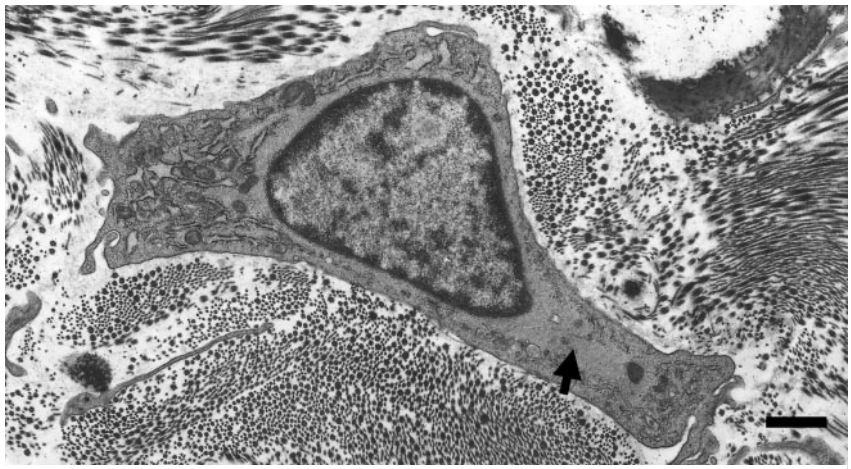

Figure 2 Electron micrograph of a fibroblast in the intra-articular disc of a female patient aged 47 years. The cell has an elongated outline. Endoplasmic reticulum can be seen on the left side of the cell, while paler areas (arrow) represent regions of microfilamentous material. The collagen fibrils of the extracellular matrix lie close to the cell membrane and there is no evidence of microfilamentous-containing pericellular space. Bar = 1 μm.

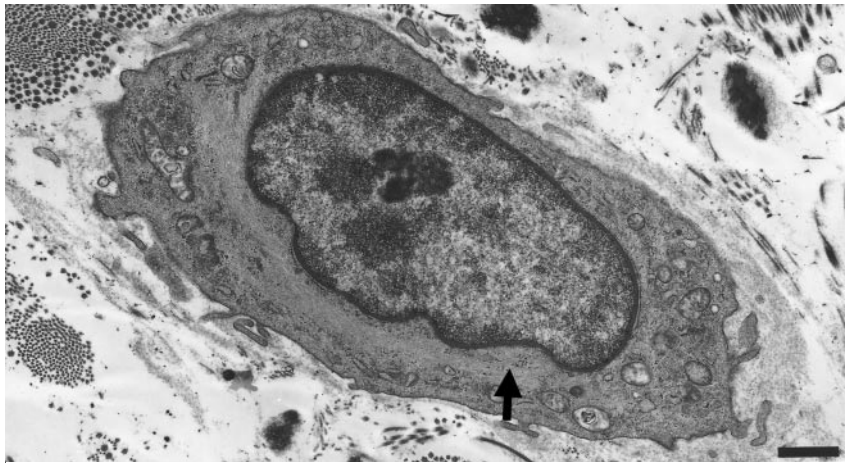

Figure 3 Electron micrograph of a fibroblast from the intra-articular disc of a female patient aged 47 years. The cell has an oval outline. The pale areas (arrow) represent zones with conspicuous amounts of microfilamentous material. The immediate surroundings of the cell contain only a loose arrangement of collagen fibrils, but no obvious surrounding pericellular zone is evident. Bar = 1 μm.

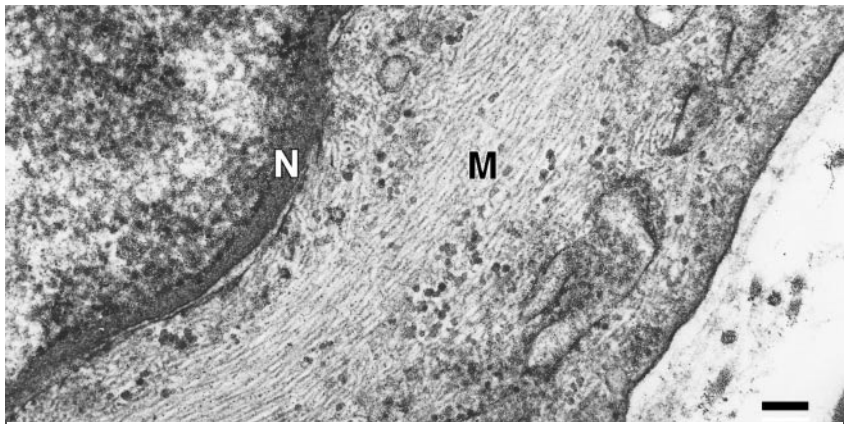

Figure 4 Higher power electron micrograph of fibroblast from a female patient aged 47 years showing microfilamentous material (M) and thickening of the nuclear membrane (N). Bar = 0.1 μm.

cells examined, only one cell showed evidence of possessing a pericellular zone of microfilamentous material. This microfilamentous material was distributed around part of the cell perimeter, separating the cell membrane from the adjacent collagen, giving the cell an appearance partly resembling that of a chondrocyte-like cell (Figure 5).

Data relating to collagen fibril diameters and the mean percentage of a fibre bundle occupied by collagen are shown in Table 1. The overall mean collagen fibril diameter for the three specimens was 43.9 nm. Although the majority of fibrils were small, in two of the three specimens they were interspersed with larger fibrils, ranging between 100 and 135 μm (Figure 6). The distribution therefore was not a sharp unimodal one (Figures 6 and 7). The overall mean area of a fibre bundle occupied by collagen was 56.1 per cent.

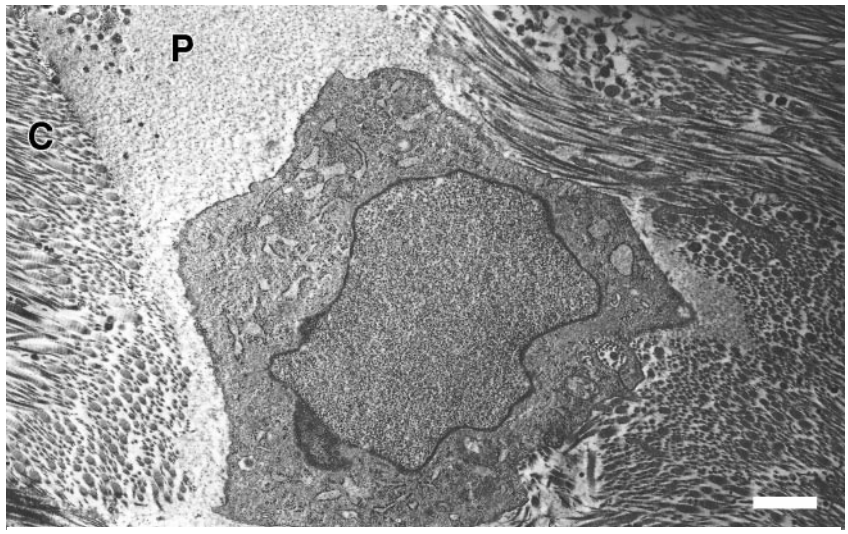

Figure 5 Electron micrograph from the intra-articular disc of a female patient aged 54 years showing a rounded cell separated in part from the adjacent collagen fibrils (C) of the extracellular matrix by a pericellular zone (P) comprised of microfilamentous material. Bar = 1 μm.

Table 1 Data related to mean collagen fibril diameters and percentage area of a fibre bundle occupied by collagen.

Patient	Mean collagen fibril diameter (±1 SD, n = 300)	% Bundle occupied collagen (±1 SD, n = 6)
47 years	38.1 ± 12.1	55.0 ± 7.0
50 years	51.4 ± 29.5	58.6 ± 8.2
54 years	42.3 ± 21.8	54.8 ± 8.2
Mean	43.9	56.1

Discussion

The first question that needs to be addressed is whether the findings related to the present study represent those associated with the appearance of a relatively normal human IAD disc, or whether they represent features more related to the onset of the disease process. Although it is not yet possible to answer this question with certainty, it could be argued that the former seems the more likely. First, there was an absence of any signs of inflammation in the material examined, particularly in regard to the absence of inflammatory cells and vascularization. Secondly, the detailed ultrastructure of the cells was similar to that previously described for the normal IAD of several mammalian groups, including marmosets (Berkovitz and Pacy, 1999, 2000).

That the cytoplasm of the cells in the human IAD contained moderate amounts of the intracellular organelles associated with the synthesis and secretion of the ECM implies a slow, but definite turnover of the ECM. Although the actual rate is not yet known, this could be determined in experimental animals by autoradiographic or biochemical techniques.

There is considerable evidence to indicate that collagen degradation in normal physiological situations is an intracellular process, as shown by the presence of intracellular collagen profiles in the cells of other connective tissues where collagen is turning over rapidly, such as the periodontal ligament (for review see Everts *et al*., 1996). Although there was an absence of intracellular collagen profiles in the cells of the IAD in the present study, this does not necessarily refute the idea that collagen degradation is principally an intracellular process. It may reflect a slower rate of collagen turnover, when the chance of encountering intracellular collagen profiles at the ultrastructural level is statistically

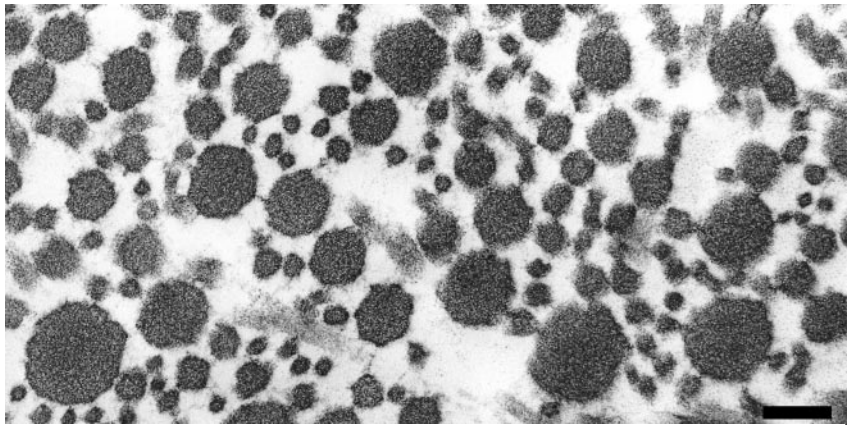

Figure 6 Electron micrograph of the intra-articular disc from a female patient aged 50 years showing collagen fibrils cut transversely. Note the lack of a sharp unimodal distribution of collagen fibril diameters and the presence of fibrils with a diameter exceeding 100 nm. Bar = 100 nm.

extremely small. That turnover of collagen is occurring raises the question as to whether the cells are to be regarded as fibroblasts or fibrocytes.

Although the outline of a cell can only be determined by three-dimensional reconstruction, the fact that many of the cells had an oval rather than flattened outline suggests that this may well be the shape of many of the cells. This outline is said to be typical of cells associated with tissues subjected to compression rather than tension (Merrilees and Flint, 1980). Many of the cells exhibited conspicuous amounts of concentrated microfilamentous material. Although fibroblasts would be expected to contain both actin and vimentin dispersed throughout the cell (Ralphs *et al*., 1991), these concentrations of microfilamentous material may represent vimentin. This could be determined in future studies when additional material becomes available by ultrastructural immunocytochemistry. Indeed, light microscopic immunocytochemical studies of fibrocartilage associated with the suprapatella suggest that such filamentous material is composed of vimentin (Ralphs *et al*., 1991). Although its precise function has yet to be determined, vimentin may play a role associated with the microskeleton.

As stated previously, a number of cells in the human IAD are rounded and have the appearance of cartilage-like cells when viewed at the light microscope level. However, when visualized ultrastructurally, only one cell was observed to be associated with a pericellular matrix characteristic of cartilage-like cells, and this matrix did not completely encircle the cell. Cells with a pericellular zone completely surrounding the cell membrane, thus giving it a distinct chondrocyte-like appearance, have been reported in the IAD of rats and marmosets in association with age (Berkovitz and Pacy, 2000). Assuming that the material investigated can be considered normal and that the average age of the subjects was approximately 50 years, then using the present criteria relating to the ultrastructural morphology of cartilage-like cells, the human IAD studied here should be considered fibrous rather than fibrocartilaginous. However, using other criteria, future studies might determine whether specific molecules, such as type II collagen and certain proteoglycans (such as aggrecan), are present at the periphery of human IAD cells. If such molecules were detected, but in the absence of a pericellular matrix around the cell, then the precise features of fibrocartilage might need to be redefined.

The mean collagen fibril diameter for human IAD is in line with data obtained for the guinea pig, cat, and the periphery of the rabbit disc (Berkovitz *et al*., 1992b; Berkovitz and

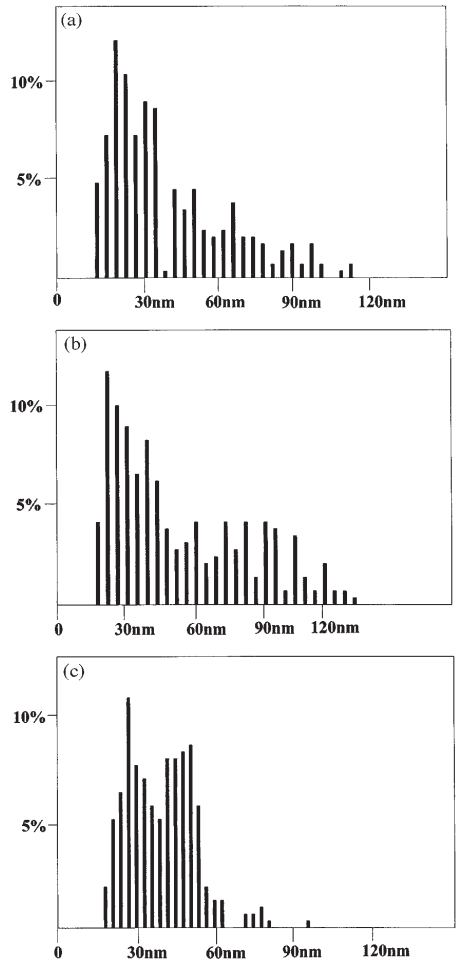

Figure 7 Histogram showing collagen fibre diameter distributions in the intra-radicular discs of three female patients aged 47 (A), 50 (B), and 54 years (C).

Robertshaw, 1993), being about 44 nm. However, a wider distribution of fibril diameters appeared evident in the human material and was more in line with that seen in the central region of the rabbit disc (Berkovitz and Robertshaw, 1993). Such variation is more consistent in tissues subjected to compression (Merrilees and Flint, 1980). Whether there is any significant regional variation in the human IAD, as is seen in the rabbit (Berkovitz and Robertshaw, 1993) and bovine IAD (Kuc and Scott, 1994), awaits further study. Concerning the area of a fibre comprised of collagen, the mean value for the human material (55.9 per cent) is also similar to that for animal data. Values of this size are more consistent with tissues subjected to compression (Merrilees and Flint, 1980).

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