European Journal of Orthodontics 26 (2004) 359–365

European Journal of Orthodontics vol. 26 no. 4

Histochemistry of the foetal human temporomandibular joint articular disc

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SUMMARY The human temporomandibular joint (TMJ) develops from mesenchymal cells that form condensations appearing as condylar and temporal blastema which give rise to the respective anterior and posterior regions of the TMJ articular disc. Previous reports have shown the foetal disc to be avascular, with a high content of organized collagen fibres and a lesser content of elastic fibres. In this study, the articular discs from TMJs of a human foetus at age 22 weeks were evaluated. At this stage of intrauterine (iu) development, the disc was found to be a highly cellular, biconcave structure with a dense arrangement of collagen fibres. Cell density was not uniform, with increased density in the intermediate band relative to the anterior and posterior bands. In contrast to earlier reports, capillaries containing red blood cells were observed along the inferior surface of the disc. Immunohistochemical staining for proteoglycans and glycosaminoglycans (GAGS) revealed abundant chondroitin sulphate proteoglycan (CSPG) and hyaluronic acid in the disc while relatively little amounts of dermatan sulphate proteoglycan II (DSPGII) were found. No keratin sulphate proteoglycan (KSPG) was detectable. Foetal human TMJ articular discs at this age were found to have morphology and regional characteristics similar to adult discs.

Introduction

The temporomandibular joint (TMJ) develops from two cellular condensations—the condylar and temporal blastema. The anterior portion of the articular disc is a derivative of the condylar blastema, while the posterior portion is derived from the temporal blastema (Baume, 1962, 1970). The articular disc begins its formation as undifferentiated mesenchymal cells that develop and form fibrous connective tissue (Symons, 1952; Furstman, 1963; Youdelis, 1966; Keith, 1982). Although the development of the TMJ articular disc has been described as early as 7–7.5 weeks intrauterine (iu) (Van der Linden *et al.*, 1987), it is not until the 14th week iu that the disc is easily recognized as a structure interposing between the superior and inferior joint cavities (Furstman, 1963).

During foetal development, the composition of the disc becomes more fibrous while it is increasing in thickness and density (Morimoto *et al.*, 1987). Collagen fibre formation is not seen until 10–10.5 weeks iu (Van der Linden *et al.*, 1987). The foetal human disc has been reported to be avascular with a thinner central region relative to its periphery (Wong *et al.*, 1985). Elastic fibres have been reported in the anterior and posterior bands of the disc as early as 21 weeks iu (Morimoto *et al.*, 1987). The proteoglycan composition of the human foetal disc has not been previously reported, although studies on adult discs show a concentration of sulphated glycosaminoglycans (GAGs) (Oberg *et al.*, 1966; Kopp, 1976) in the central region, which is thought to be related to a load-bearing function. Such highly negatively charged and strongly hydrophilic GAGs are a feature of weight-bearing tissues (McDevitt *et al.*, 1981). They create a swelling pressure or turgor that allows the matrix to withstand compressive forces (Hascall and Hascall, 1981; Evered and Whelan, 1986).

The microscopic and ultrastructural (Strauss *et al.*, 1960; Jagger, 1980; Piacentini *et al.*, 1994) appearance of the adult human TMJ articular disc has been described along with its collagen fibre bundles (Minarelli *et al.*, 1997; Berkovitz and Pacy, 2002) and elastic fibres (Gross *et al.*, 1999). The disc is described as being of fibrocartilage as it also contains variable amounts of cartilage-like cells (Berkovitz *et al.*, 1992). However, some studies have not been able to find chondrocytes in non-pathological human specimens (Kurita *et al.*, 1989). This variability may be due to the difficulty in determining whether a cell is a chondrocyte or another cell type at the light microscopic level.

The lack of information on the human TMJ articular disc is undoubtedly related to the difficulty in obtaining normal material that can be processed immediately for scientific study. Alternatives have been the study of animal material, as well as discs that have been removed from patients for clinical indications. However, the latter is probably subject to the disease process and not indicative of the normal situation.

One of the most poorly understood areas of clinical dentistry is that of TMJ disorders. Within this category are discal derangements of the TMJ. It is thought that one of the early events of disc derangement are alterations in the extracellular matrix (ECM) composition of the disc, rendering it unable to withstand applied forces,

resulting in its deformation and subsequent deterioration. To understand better the pathophysiology of TMJ disc derangements, it is necessary to have a fundamental knowledge of the changes in the ECM in normal growth, development, and function. A considerable limitation to these studies is the lack of normal material available for scientific study, particularly developing discs. Given this limitation, descriptive studies at various time points can assist in furthering the understanding of age-related changes. Therefore, the aim of this study was to describe the structure and proteoglycan content of the TMJ articular disc at 22 weeks iu.

Materials and methods

Histological sections of the TMJ articular disc of a foetal Caucasian male aged 22 weeks were studied. The foetus had no known pathology or genetic disorder and was normally developed. The discs were previously fixed in 4 per cent formalin containing 0.5 per cent cetylpyridinium chloride and embedded in paraffin wax. Sections, 5 μm thick, were cut and mounted on glass slides. The sections used in this study were cut antero-posteriorly from the middle of the disc. Standard procedures were followed for histochemical staining. Enzymatic digestion of sections with *Streptomyces* hyaluronidase (Calbiochem, San Diego, California, USA) and chondroitinase ACII (Seikagaku America, Ijamsville, USA) was performed according to the manufacturers' directions. Spectral absorbance of sections stained with Alcian blue was read at 525 nm on a Gilford 250 spectrophotometer by placing the glass slide in the cuvette holder. Calculations were performed after subtracting the blank value (non-stained section) from the reading. Immunohistochemical staining was carried out with monoclonal antibodies to chondroitin sulphate [CS; CS56 (Avnur and Geiger, 1985), diluted 1:1000 in phosphate-buffered saline (PBS)], dermatan sulphate proteoglycan [DSPG II; 6D6 (Pringle *et al.*, 1985), diluted 1:5 in Tris-buffered saline (TBS)], keratan sulphate [KS; 5D4 (Caterson *et al.*, 1983), diluted 1:1000 in TBS], and hyaluronic acid [HA; NDOG1 (Sunderland *et al.*, 1981, 1985), diluted 1:4 in PBS]. Human and bovine skin, and bovine nasal cartilage were used as respective positive controls. In all trials using antibodies, skin, nasal cartilage, and fetal disc sections were incubated with non-immune serum in place of the antibody to serve as controls and to detect non-specific binding. No staining was seen using non-immune serum (results not shown). The specificity of the antibodies was evaluated by using degradative enzymes for the various GAGs prior to immunostaining. Staining intensity was judged qualitatively: –, no detectable staining; +, staining just visible; ++, mild staining; +++, moderate staining; ++++, intense staining.

This study was performed at the University of Alberta, Canada and approval was granted by the university's Health Research Ethics Board.

Results

The foetal human TMJ articular disc at 22 weeks was found to be a biconcave structure of approximately 4–5 mm in length with a very thin central region approximately 0.25 mm thick compared with the anterior and posterior bands approximately 0.75 mm thick (Figure 1). At low magnification, the TMJ articular disc was found to be highly cellular with an abundance of collagen fibres distributed throughout the disc. At higher magnification, the concentration of cells and collagen fibres was found to be denser in the intermediate band of the disc (Figure 2). Cell counts in the anterior, intermediate, and posterior bands produced an approximate ratio of 1:1.5:1. A zone of relative acellularity was observed between the inferior surface of the intermediate band and the remainder of the disc. Below the acellular layer were capillaries containing red blood cells (Figure 3). Elastic fibres were found in the anterior and posterior bands of the disc. Relative to the collagen fibres, the elastic fibres appeared to be very small without any type of regular arrangement (Figure 4).

Stains such as Alcian blue and Safranin-O were used to illustrate the rich content of GAGs in the articular disc (Figure 5). The staining pattern with Alcian blue

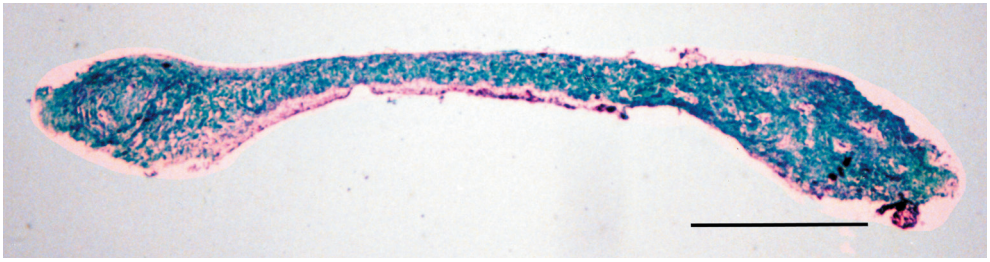

Figure 1 Collagen fibres in the foetal temporomandibular joint articular disc. Collagen fibres are seen as greenish-blue staining, while cytoplasm, nuclei, and other intercellular fibres stain red (Masson's trichrome stain). The left side of the image is the anterior band and the right side the posterior band of the disc. Bar = 1 mm.

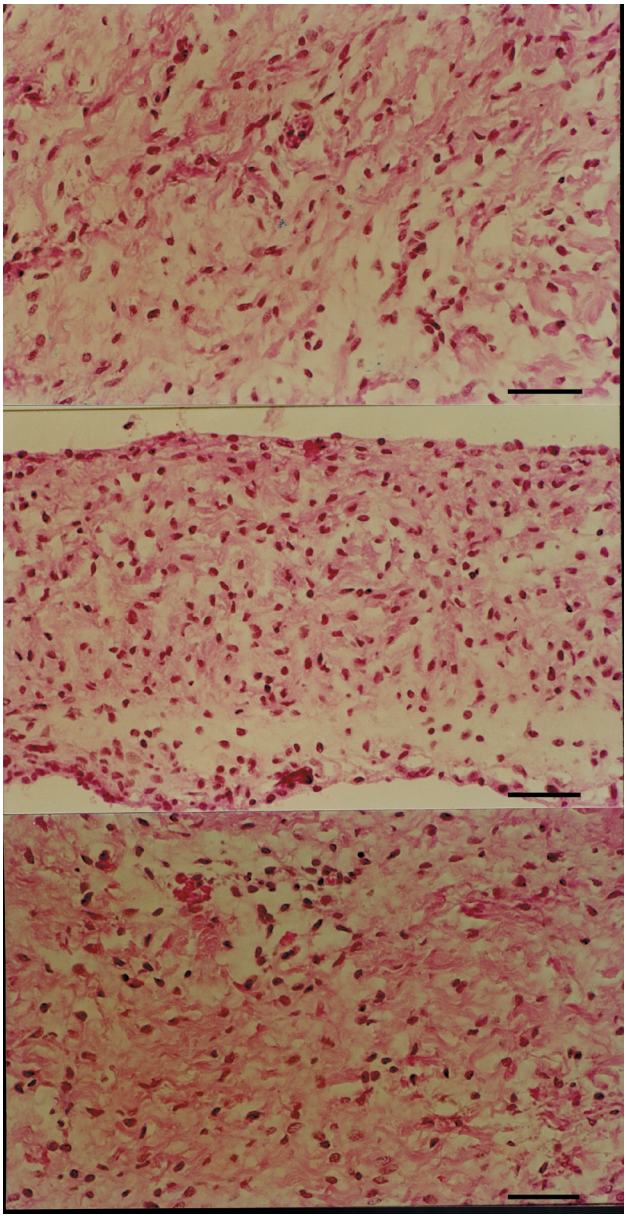

Figure 2 Cell distribution in the foetal temporomandibular joint articular disc. The disc is highly cellular with a higher concentration of cells in the intermediate band (central panel) compared with the anterior (top panel) and posterior (bottom panel) bands. A count of cells in these regions produced an approximate ratio of 1:1.5:1. Haematoxylin and eosin stain. Bar = 50 μm.

was relatively uniform, while Safranin-O showed more intense staining in the central area of the disc. The absorbance of the Alcian blue-stained sections was 0.448 and decreased to 0.415 following enzymatic digestion with *Streptomyces* hyaluronidase, a reduction of 7.4 per cent. Following digestion with chondroitinase ACII, the staining intensity decreased to 0.200, a reduction of 55 per cent. Because this enzyme exhibits activity for both CS and HA, hyaluronidase activity of 7.4 per cent was subtracted from 55 per cent indicating

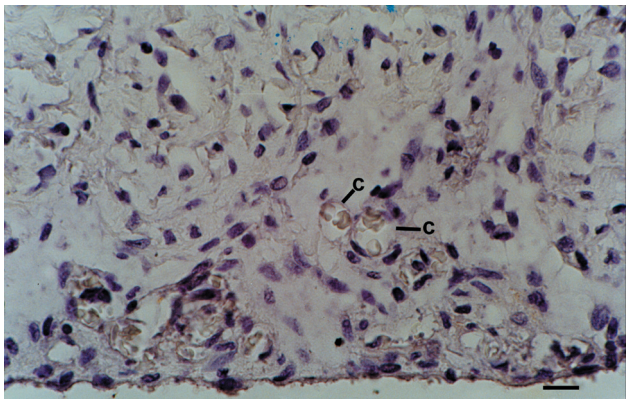

Figure 3 Capillaries in the inferior aspect of the temporomandibular joint articular disc. Capillaries (c) containing red blood cells are clearly seen. In addition, an acellular layer may be seen above and surrounding the vessels. Orcein stain. Bar = 10 μm.

that the vast majority (47.6%) of this reduction in absorbance was due to digestion of CS.

Immunological staining of articular disc sections with monoclonal antibodies for CS (CS56), DSPG II (6D6), KS (5D4), and HA (NDOG 1) demonstrated heavy staining for CS, moderate staining for HA, minimal staining for DSPG II, and no apparent staining for KS (Table 1). The specificity of the antibodies was verified by using degradative enzymes for the various GAGs prior to immunostaining. Following treatment with chondroitinase ACII much of the staining in the foetal disc, and other CS-containing tissues, was greatly reduced. As anticipated, treatment with chondroitinase ACII had no effect on immunostaining for DSPG II in either the disc or human skin. Treatment with *Streptomyces* hyaluronidase reduced immunostaining for HA only by a minimal amount. As chondroitinase ACII also has activity towards HA, it also reduced immunostaining for HA. Interestingly, treatment with both enzymes further reduced staining for HA.

Discussion

The general morphology of the foetal human TMJ articular disc was found to be consistent with earlier reports in the literature. At 14 weeks iu and later in pre-natal development it was shown to have a thin central region that became wider towards the periphery (Furstman, 1963). In addition, the disc was also found to be highly cellular with a dense arrangement of collagen fibres. However, in contrast to earlier reports (Wong *et al.*, 1985; Van der Linden *et al.*, 1987) which described the central region of the foetal human TMJ articular disc to be devoid of blood vessels, capillaries containing red blood cells were observed in the inferior region of the intermediate band of the disc. This suggests that nutrients and growth factors from the general circulation may have an important role in the development of the disc.

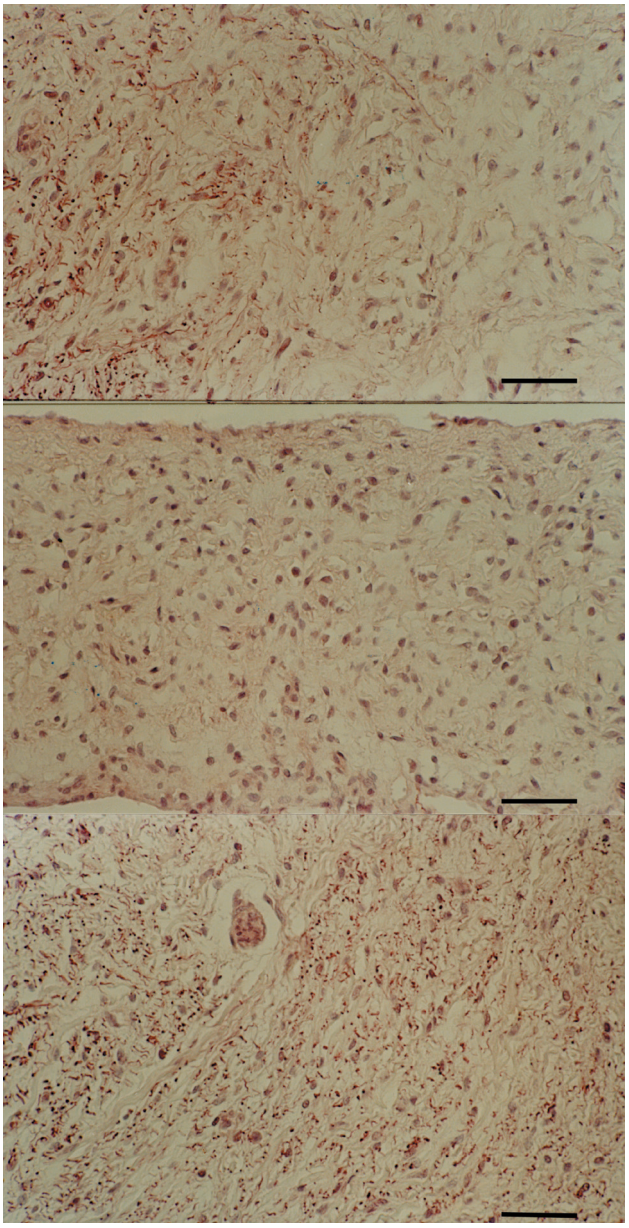

Figure 4 Elastic fibres in the temporomandibular joint articular disc. Elastic fibres can be seen in the anterior (top panel) and posterior (bottom panel) bands of the articular disc as small dense spots interspersed among cells and the relatively larger collagen fibres. No elastic fibres are seen in the intermediate band (central panel). Taenzer-Unna Orcein stain. Bar = 50 μm.

In addition, decreased oxygen tension is widely assumed to encourage the differentiation of chondrocytes, while higher levels of oxygen allow for the development of fibroblasts. Capillaries in this location that provide a readily available oxygen supply could have a role in the development of fibrocartilage in the disc.

Cellular distribution in the articular disc was not uniform. Overall it was found to be highly cellular with cell counts of approximately ×1.5 more in the central region of the disc compared with the anterior and posterior bands. A possible explanation for this increase in cellularity may be related to an increase in proteoglycan production in this area to prepare for the forces of loading or an increase in the number of cells over which forces may be distributed. As joint forces at this stage of development are probably very minimal, this suggests a predominantly genetic determination of the morphology of the TMJ articular disc. In addition, an acellular layer was seen between the inferior surface of the intermediate band and the remainder of the disc, giving the impression of cellular invasion in this region. This possibly suggests that the external morphology of the disc forms initially, followed by cellular invasion to form its internal composition.

As in earlier reports (Morimoto *et al.*, 1987), elastic fibres were seen in the anterior and posterior bands of the disc. Similar findings have been reported in animals (Nagy and Daniel, 1991). Previous investigators have suggested that the absence of elastin in the central portion of the disc indicates that this area may not be particularly stress bearing (Frommer and Monroe, 1966). Their perspective is that tensile forces are countered by elastic forces, therefore if elastic fibres are not found, there must be limited stresses in this area. However, this view does not account for stresses from compressive and shear forces, which may be acting in this location. In fact, it is more likely that compressive forces exist on the disc, especially in the central region, which are resisted by the proteoglycans. Support for this view comes from articular cartilage which features an extremely high CS proteoglycan content (Miles and Dawson, 1962). The elastic fibres in the disc may have dual roles of imparting a proteoglycan-like resilience to the structure as well as in resisting shear and tensile forces. This latter role may be shared with the organized arrangement of collagen fibres.

The results of the present investigation show that the disc contains large quantities of CS with comparatively lesser amounts of HA and DSPG II and no detectable KS. In foetal bovine discs, the GAG content was found to consist of approximately 79 per cent CS, 5 per cent HA, 14 per cent dermatan sulphate (DS), and 2 per cent KS (Nakano and Scott, 1989). In young rodents, large quantities of chondroitin-6-sulphate (C_6S), HA, and KS/chondroitin-4-sulphate (KS/C_4S) have been reported (Carvalho *et al.*, 1993). In another rodent study, both CS and DSPG were found, although DSPG was the predominant proteoglycan with lesser amounts of CS that decreased with age (Okazaki *et al.*, 1996). In the present study, intense staining with Alcian blue and Safranin-O indicated a significant content of sulphated GAGs and HA. Enzymatic digestion with chondroitinase ACII and SH caused a reduction in staining intensity with Alcian blue of 55 and 7.4 per cent, respectively. Because the latter enzyme has activity for both HA and CS, 7.4 per cent was subtracted leaving 47.6 per cent

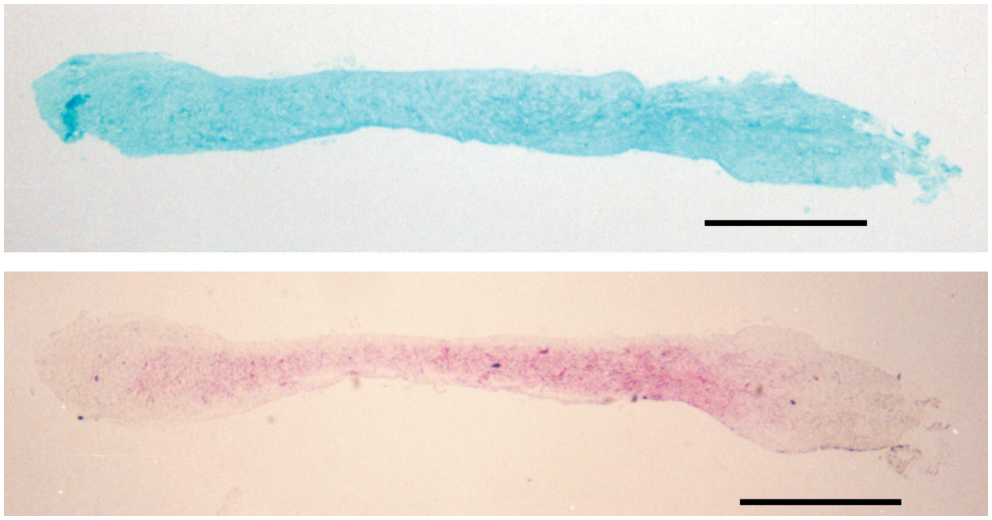

Figure 5 Glycosaminoglycans (GAGS) of the articular disc. Abundant GAGS can be seen throughout the disc. The staining pattern of Alcian blue is uniform (top panel) while Safranin-O shows more intense staining in the central portions of the disc, particularly in the intermediate band (bottom panel). The left side of the image is the anterior and the right side the posterior band of the disc. Bar = 1 mm.

Table 1 Summary of the immunohistochemistry results.

Tissue	Enzyme treatment	Antibody for	Relative staining
Foetal disc	None	Chondroitin sulphate	++++
Bovine skin	None	Chondroitin sulphate	+++
Bovine nasal cartilage	None	Chondroitin sulphate	+++
Foetal disc	Chondroitinase ACII	Chondroitin sulphate	+
Bovine skin	Chondroitinase ACII	Chondroitin sulphate	+
Bovine nasal cartilage	Chondroitinase ACII	Chondroitin sulphate	+
Foetal disc	None	Dermatan sulphate proteoglycan II	+
Human skin	None	Dermatan sulphate proteoglycan II	++++
Bovine skin	None	Dermatan sulphate proteoglycan II	++++
Foetal disc	Chondroitinase ACII	Dermatan sulphate proteoglycan II	+
Human skin	Chondroitinase ACII	Dermatan sulphate proteoglycan II	++++
Foetal disc	None	Keratan suphate	–
Bovine skin	None	Keratan suphate	+
Bovine nasal cartilage	None	Keratan suphate	++
Foetal disc	None	Hyaluronic acid	+++
Human skin	None	Hyaluronic acid	+++
Foetal disc	*Streptomyces* hyaluronidase	Hyaluronic acid	++
Human skin	*Streptomyces* hyaluronidase	Hyaluronic acid	++
Foetal disc	Chondroitinase ACII	Hyaluronic acid	++
Human skin	Chondroitinase ACII	Hyaluronic acid	++
Foetal disc	*Streptomyces* hyaluronidase and chondroitinase ACII	Hyaluronic acid	+
Human skin	*Streptomyces* hyaluronidase and chondroitinase ACII	Hyaluronic acid	+

–, no detectable staining; +, staining just visible; ++, mild staining; +++, moderate staining; ++++, intense staining.

of the reduction due to digestion of CS. Treatment with chondroitinase ACII also significantly reduced immunostaining for CS. Together these results suggest that large quantities of CS exist in the disc at this age. The presence of HA was not at all surprising as it is present in abundant quantities in proliferating tissues (Trelstad, 1984). HA is thought to play an important role in cell proliferation, migration, and invasion by producing and maintaining an open, loose ECM structure (Comper and Laurent, 1978; Toole, 1981). The presence of DSPG II in a highly collagenous structure was anticipated as it has been reported to have an important role in determining the size and tensile properties of collagen fibrils (Scott *et al.*, 1981; Poole *et al.*, 1986). The presence of DSPG II in the disc would attest to its highly collagenous structure. KS was not

detected with immunological staining. Studies of other foetal tissues such as the notochord (Salisbury, 1988) and the flexor tendon (Evanko and Vogel, 1990) indicate that there are very minute or undetectable amounts of KS in foetal tissues. KS appears to be associated with ageing as it is found in higher amounts in maturing tissues (Webber *et al.*, 1987; Dziewiatkowski *et al.*, 1989).

There appears to be variations in GAG species between the different regions of the disc. Although Alcian blue and Safranin-O both stain for GAGs and HA, Alcian blue stained the disc uniformly while Safranin-O stained the central region more intensely. This observation was also found in the bovine articular disc (Nakano and Scott, 1989). The pattern of intense staining corresponded with the area of increased cellularity. Curiously, immunostaining for CS revealed its distribution to be uniform. A possible explanation for this differential staining with Safranin-O is that it is more selective for certain highly sulphated GAGs in the disc. It is likely that variations in chemical composition of GAGs occur in the disc, such as hybridization between C_4S, C_6S, and DS, and variation in the degree of sulphation and molecular weight polydispersity (Granstrom and Linde, 1973). Indeed, a higher concentration of sulphated GAGs was found in the central region of the adult TMJ articular disc (Kopp, 1976). Sulphated GAGs seem to have an essential role in the function of load-bearing tissues as they comprise a significant portion (10%) of the dry weight of adult weight-bearing articular cartilage (McDevitt, 1973). It is remarkable that foetal TMJ discs would exhibit staining patterns similar to mature TMJ discs. This further suggests that the disc at this stage of development seems to be designed for a load-bearing role.

A study of pathological TMJ articular discs removed from patients diagnosed as having reducible or non-reducible disc displacements indicated that a higher amount of sulphated GAGs may be detected in the posterior band and bilaminar zone of the disc (Blaustein and Scapino, 1986). This may be indicative of the ability of tissues to remodel and increase their compressive stiffness in order to cope with load bearing. It remains unexplained as to why this response is manifest in some individuals and not in others.

Conclusions

The TMJ articular disc at 22 weeks iu is a biconcave structure of 4–5 mm in length, and 0.25 mm thick in its central region and 0.75 mm at its periphery. Its content is highly cellular with a dense arrangement of collagen fibres with capillaries along the inferior surface of the disc. CSPG and HA are abundant with relatively little amounts of DSPG II.

Knowledge of the biochemical and cellular changes that occur in the TMJ articular disc in normal growth, development, and function is essential in providing insights into the accompanying biochemical and cellular changes of TMJ dysfunction. Further studies of the disc during other periods of foetal development and at various times post-natally are required. An understanding of TMJ pathophysiology is important in developing diagnostic tests such as magnetic resonance imaging which is sensitive to the hydrodynamic nature of proteoglycans. Current thinking is that pathological changes in the TMJ articular disc begin at the cellular and biochemical levels which are manifest in alterations in proteoglycan structure and production. Accompanying these alterations are changes in the nature, species, and characteristics of proteoglycans with accompanying changes in their hydrodynamic volume and response to biological loads. These changes lead to susceptibility and contribute to disc derangements in TMJ dysfunction.

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Acknowledgements

Takuo Nakano and Carol Dodd for their assistance with the histology and Paul Scott and Geoffrey Sperber for their guidance and support.

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