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Biochemical analysis of the porcine temporomandibular joint disc

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Abstract

Tissue engineering can be a boon in treating lesions of the disc in the temporomandibular joint (TMJ). Unfortunately, little is known about its biochemical content, so we analysed the discs of six slaughtered pigs. We measured the content and distribution of total DNA, glycosaminoglycan, and collagen. The mean (S.D.) content of DNA was 0.14% (0.08%) of the dry weight, of glycosaminoglycan 0.96% (0.39%), and of collagen 68.2% (14.5%). There were no significant differences from top to bottom, but from front to back the smallest concentration of glycosaminoglycan was in the posterior band, and the highest concentration of collagen was in the intermediate zone. The concentrations of DNA and glycosaminoglycan were higher in the medial than in the lateral area of the disc.

Keywords: Porcine; TMJ disc; DNA; Glycosaminoglycan; Collagen

Introduction

It is sometimes necessary to remove the disc of the temporomandibular joint (TMJ) to treat severe degenerative disorders of the joint.[1] It would improve the outcome if we could replace the disc with a tissue-engineered prosthesis, but a major hurdle in the design of current attempts at tissue-engineering on the disc is the lack of comprehensive understanding of the biochemical content of the disc. Knowledge of the distribution of cells, as measured by the amount of DNA present, and the distribution of extracellular matrix proteins, such as glycosaminoglycans and collagen, is of great importance to the structure function relation that is needed to be mimicked by the replacement prosthesis.

Some biochemical studies have been made on the TMJ disc, but no study to date has quantified comprehensively the total amount and distribution of DNA, or its cellularity. One of the few comparisons is a study that reported that human nasal cartilage contained about 25 million cells/g of wet weight.[2] The only studies of cellularity for the TMJ disc are histological, and these have shown that the tissue is fairly cellular.[3,4]

Glycosaminoglycans in the disc have been studied extensively, but there is still controversy about their total content and distribution. The total amount of glycosaminoglycans in the disc has been reported to range from 0.6% to 10% by dry weight.[5] There seems to be a general consensus that the centre of the disc has a higher content of glycosaminoglycan than the periphery, in both bovine and porcine models.[6,7] Other investigators have reported that the periphery of the human TMJ disc contains more glycosaminoglycan.[8]

In contrast, collagen, which is the major constituent of the disc, has not been as thoroughly investigated as glycosaminoglycan. Reports are at best sporadic and not consistent; collagen has been reported to make up about 37% by wet weight,[9] or 83% by dry weight.[10] A standard method of measurement must be agreed before any comparisons can be made.

The purpose of the present study was to measure the content and distribution of DNA, glycosaminoglycan, and colla-

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gen in the TMJ disc of pigs. Concentrations were measured along the superoinferior, anteroposterior, and mediolateral axes.

Material and methods

Preparation of specimens

Six TMJ discs were harvested from the right side of adult female pigs that weighed 70–80 kg, and were procured from a local slaughterhouse. Each disc was sectioned into nine portions and then separated into upper and lower surfaces to give a total of 18 portions (Fig. 1). The dry weight of each section was measured after the portions had been lyophilised for 2 days. Each sample was then placed in a papain solution, 125 μg/ml papain (Sigma, St. Louis, Missouri) in 50 mmol phosphate buffer (pH 6.5) containing 2 mmol *N*-acetyl cysteine (Sigma), overnight at 60 °C.[11]

Biochemical analysis

The content of DNA was measured by the reaction of DNA with picogreen (PicoGreen® dsDNA Quantitation Kit, Molecular Probes, Inc., Eugene, Oregon). The formula for conversion from concentration of DNA to number of cells was 7.7 pg DNA/cell, which was established in our laboratory for the cells of porcine TMJ discs. The total amount of glycosaminoglycan was measured by a dimethylmethylene blue colorimetric assay kit (Biocolor; Newtownabbey, UK). The total collagen content was assayed by first hydrolysing samples of each digest in 4N sodium hydroxide at 121 °C for 1 h. The samples were then neutralised and placed in buffer, and collagen content was measured by the modified protocol of reacting the samples with chloramine T and dimethylaminobenzaldehyde that allowed a colorimetric comparison.[12] Instead of using hydroxyproline standards, collagen standards (Accurate Chemical and Scientific Corporation, Westbury, NY) were chosen for a more direct comparison.

Statistical analysis

The total amounts of DNA, glycosaminoglycan, and collagen were calculated by averaging the content of the 18 portions for each of 6 discs. Variations of biochemical content between the regions were compared along the superoinferior direction at each surface (upper and lower) ($n = 54$), along the anteroposterior direction at each zone (anterior band, intermediate zone, posterior band) ($n = 36$), and along the mediolateral direction at each area (medial, central, lateral) ($n = 36$). A three-factor analysis of variance (ANOVA) for each direction was used to compare the regions. When the ANOVA showed a significant difference, a post hoc test was done (Fisher's protected least significance difference).

Results

Distribution of DNA

The total mean (S.D.) content of DNA in the TMJ disc was 0.14% (0.08) of dry weight. There are about 50 million cells/g in the porcine TMJ disc. There was no difference in the concentration of DNA between the superior and inferior surfaces (Fig. 2A). In the anteroposterior direction, there was a significantly lower concentration of DNA in the posterior band than in the anterior band ($p < 0.05$, Fig. 2B). In the mediolateral direction the medial area had a significantly higher content of DNA than the central ($p < 0.0005$), and lateral areas ($p < 0.001$) (Fig. 2C).

Distribution of glycosaminoglycan

The total mean (S.D.) dry weight of glycosaminoglycan was 0.96% (0.395). Again, there were no differences in concentration in the superoinferior direction (Fig. 3A). The posterior band had a significantly lower content of glycosaminoglycan than either the anterior band ($p < 0.0001$) or the intermediate zone ($p < 0.0001$), and though the intermediate zone had a larger concentration than the anterior band, the difference

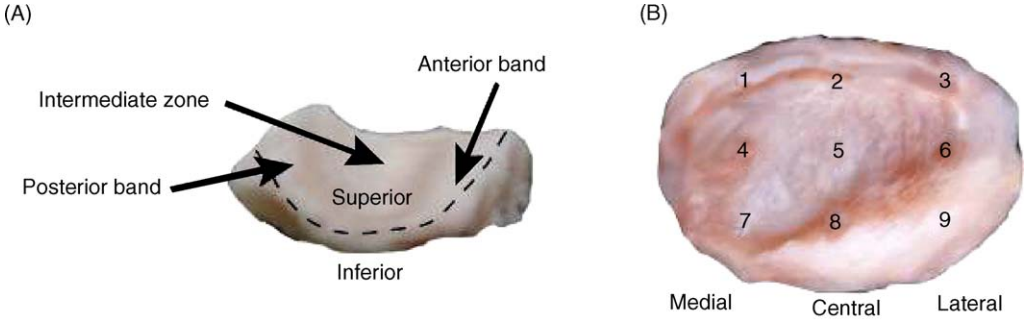

Fig. 1. (A) Lateral and superior view of the disc of the temporomandibular joint (TMJ) showing the upper and lower surfaces. (B) Superior view of the TMJ disc illustrating sites of portions taken.

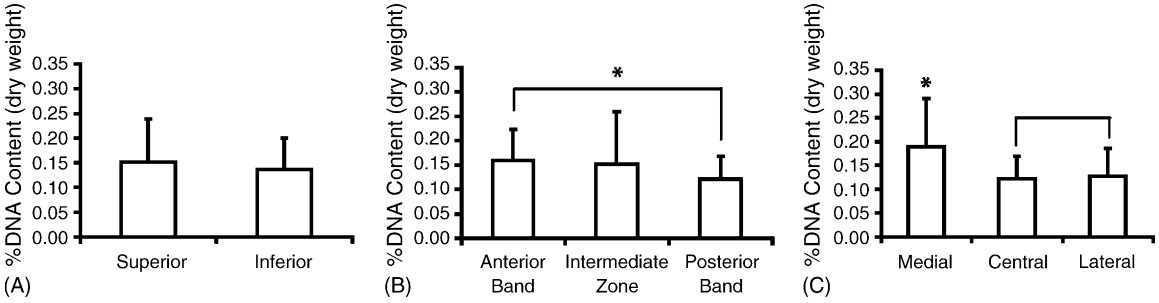

Fig. 2. Distribution of DNA. (A) Superoinferior direction ($n = 54$). (B) Anteroposterior direction ($n = 36$). (C) Mediolateral direction ($n = 36$). The symbol (*) indicates significance ($p < 0.05$). Error bars indicate S.D.

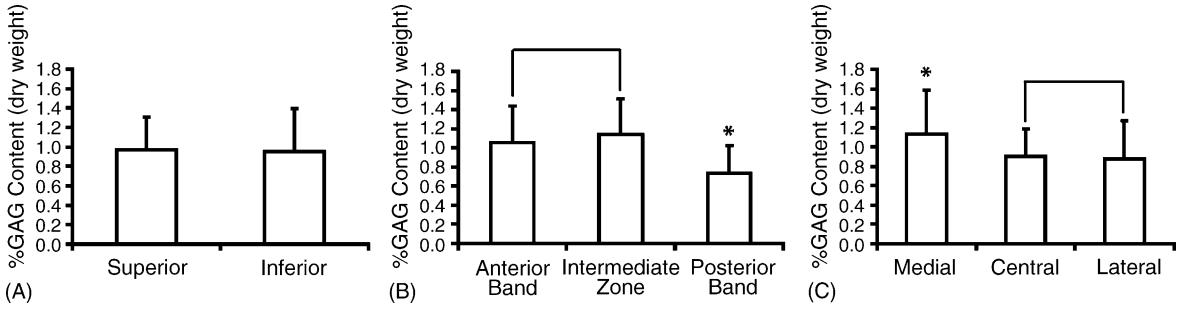

Fig. 3. Distribution of glycosaminoglycan. (A) Superoinferior direction ($n = 54$). (B) Anteroposterior direction ($n = 36$). (C) Mediolateral direction ($n = 36$). The symbol (*) indicates significance ($p < 0.05$). Error bars indicate S.D.

was not significant (Fig. 3B). In the mediolateral direction, the medial area had a significantly greater concentration of glycosaminoglycan than either the central ($p < 0.01$), or lateral areas ($p < 0.005$), and the central and lateral areas were not significantly different from each other (Fig. 3C).

Distribution of collagen

The total mean (S.D.) concentration of collagen in the porcine TMJ disc was 68.2% (14.5%) of dry weight. There were no significant differences in concentration of collagen between the superior and inferior surfaces (Fig. 4A). In the anteroposterior direction, the intermediate zone had a significantly higher content of collagen than the anterior band ($p < 0.005$) and posterior band ($p = 0.01$) (Fig. 4B). In the mediolateral direction, the concentration in the central area was significantly higher ($p < 0.0005$) than in the lateral area (Fig. 4C).

Discussion

As we embark on tissue engineering to effect successful regeneration of the TMJ disc, it is clear that scarcity of data about the characteristics of healthy tissue can hamper our efforts. The few studies that describe the tissue's biochemical content and regional distribution seem to provide only contradictory data. The objective of this study was therefore to establish the total amount and distribution of the constituents of the porcine TMJ disc. DNA, gly-

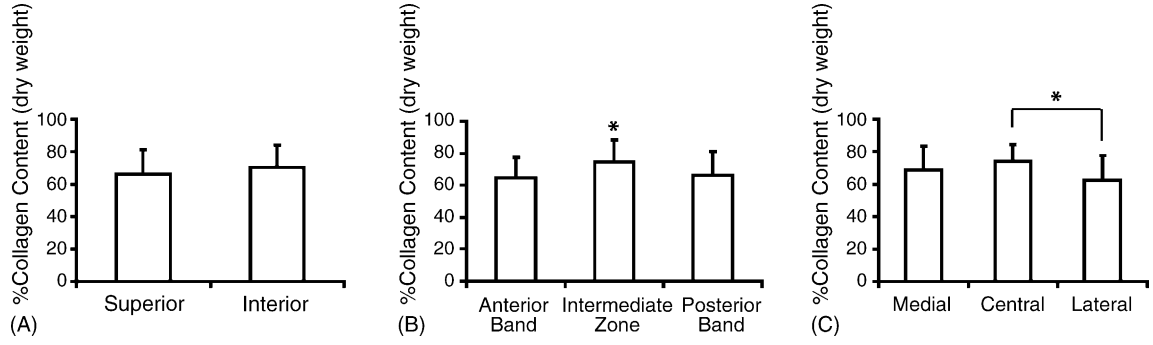

Fig. 4. Distribution of collagen. (A) Superoinferior direction ($n = 54$). (B) Anteroposterior direction ($n = 36$). (C) Mediolateral direction ($n = 36$). The symbol (*) indicates significance ($p < 0.05$). Error bars indicate S.D.

cosaminoglycan, and collagen were measured because the disc in this animal is regarded as a suitable model for the human TMJ disc, because of many matching characteristics, such as similarities in the size of articulating surfaces and the shape of the disc.[13] We showed that there were large regional differences in the porcine TMJ disc, suggesting different functional requirements in different zones and areas.

Direct inferences about the relation between the regional differences in distribution of glycosaminoglycan and the local compressive behaviour of the porcine TMJ disc can now be made.[14] The indentation aggregate modulus of the medial area of the porcine TMJ disc was found to be 36% and 44% higher than the central and lateral areas, respectively, which correlates well with the content of glycosaminoglycan of the medial area, that are also greater than the central and lateral areas, being 25% and 29%, respectively. This comparison alone tends to indicate an almost linear correlation between content of glycosaminoglycan and aggregate modulus, but the story is more complicated. The content of glycosaminoglycan does not necessarily indicate which type of proteoglycan is present, and it is the type of proteoglycan that truly dictates compressive behaviour.[5] An example of compressive behaviour that is not correlated with concentration of glycosaminoglycan can be seen along the anteroposterior axis of the porcine TMJ disc. There is an inverse correlation between lower content of glycosaminoglycan and higher aggregate modulus in the posterior band than in the anterior band and intermediate zone. So, although the concentration of glycosaminoglycan and the compressive response may correlate, a better predictor of aggregate modulus may be the content of proteoglycan.

Our data allow for the first time the elucidation of a connection between the tensile response of the porcine TMJ disc and its total concentration of collagen.[15] However, the orientation of collagen fibres may be the largest contributor to the tensile response of the tissue. Correlations between concentrations of collagen and tensile characteristics may therefore be sought when the direction of tensile tests is along predominant orientations of fibres. Comparisons between tensile stiffness and collagen cannot be made because the fibres of collagen in the intermediate zone do not align with the mediolateral direction of testing, whereas the fibres of the anterior and posterior bands do align.[15] However, comparisons can be made at the three locations studied in the mediolateral axis, because the collagen fibres are aligned in the same direction.[15] The relaxed moduli of the central and medial areas were 74% to 35% higher, respectively, than the modulus of the lateral area. Similarly, the collagen content of the medial and central areas was 10% to 19% higher, respectively, than the lateral area, so it seems that there is a correlation between content of collagen and tensile stiffness. Other factors contributing to the relation between collagen and tensile stiffness may be the radius of the collagen fibres and the total number of fibres present in the tissue.

Further experiments need to be made on the porcine TMJ disc to measure other biochemical constituents. We have shown that 10–20% of the dry weight of the TMJ disc was not accounted for. The statistical method that we used assumes that no differences existed, within a reasonable range of variation, between individual discs. In the future, this should be tested with replication. The amount of glycosaminoglycan that we measured was less than the amount of proteoglycan present. Other proteins such as elastin are potential candidates for being the next major component to be found in modest amounts in the porcine TMJ disc. The elastin content in the TMJ disc has been seen to range from 3% to 7% of dry weight in bovine TMJ disc.[16]

A recent study has identified the presence of collagen types I and II in porcine TMJ discs, collagen type I being more abundant.[17] That study also found chondroitin sulphate, dermatan sulphate, and keratan sulphate in porcine TMJ discs.[17] Minute amounts of types VI, IX, and XII collagen may also be present in the TMJ disc.[18,19]

Knowledge of the differences between the TMJ disc and hyaline cartilage is crucial to the future strategies of tissue engineering. Early attempts at tissue engineering of the TMJ disc have been made,[20–23] but these did not have access to more recent findings. As more data about the TMJ disc are obtained, and more appropriate criteria for design and validation are developed it will enable a more suitable tissue engineered replacement prosthesis to be made to correct lesions of the TMJ disc.

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References

1. Detamore MS, Athanasiou KA. Motivation, characterization, and strategy for tissue engineering the temporomandibular joint disc. *Tissue Eng* 2003;**9**:1065–87.
2. Homicz MR, McGowan KB, Lottman LM, Beh G, Sah RL, Watson D. A compositional analysis of human nasal septal cartilage. *Arch Facial Plast Surg* 2003;**5**:53–8.
3. Berkovitz BK, Pacy J. Age changes in the cells of the intra-articular disc of the temporomandibular joints of rats and marmosets. *Arch Oral Biol* 2000;**45**:987–95.
4. Milam SB, Klebe RJ, Triplett RG, Herbert D. Characterization of the extracellular matrix of the primate temporomandibular joint. *J Oral Maxillofac Surg* 1991;**49**:381–91.
5. Almarza AJ, Athanasiou KA. Design characteristics for the tissue engineering of cartilaginous tissues. *Ann Biomed Eng* 2004;**32**:2–17.
6. Nakano T, Scott PG. Changes in the chemical composition of the bovine temporomandibular joint disc with age. *Arch Oral Biol* 1996;**41**:845–53.
7. Sindelar BJ, Evanko SP, Alonzo T, Herring SW, Wight T. Effects of intraoral splint wear on proteoglycans in the temporomandibular joint disc. *Arch Biochem Biophys* 2000;**379**:64–70.

8. Axelsson S, Holmlund A, Hjerpe A. Glycosaminoglycans in normal and osteoarthrotic human temporomandibular joint disks. *Acta Odontol Scand* 1992;**50**:113–9.
9. Gage JP, Shaw RM, Moloney FB. Collagen type in dysfunctional temporomandibular joint disks. *J Prosthet Dent* 1995;**74**:517–20.
10. Nakano T, Scott PG. A quantitative chemical study of glycosaminoglycans in the articular disc of the bovine temporomandibular joint. *Arch Oral Biol* 1989;**34**:749–57.
11. Farndale RW, Sayers CA, Barrett AJ. A direct spectrophotometric microassay for sulfated glycosaminoglycans in cartilage cultures. *Connect Tissue Res* 1982;**9**:247–8.
12. Woessner JF Jr. The determination of hydroxyproline in tissue and protein samples containing small proportions of this amino acid. *Arch Biochem Biophys* 1961;**93**:440–7.
13. Bermejo A, Gonzalez O, Gonzalez JM. The pig as an animal model for experimentation on the temporomandibular articular complex. *Oral Surg Oral Med Oral Pathol* 1993;**75**:18–23.
14. Kim KW, Wong ME, Helfrick JF, Thomas JB, Athanasiou KA. Biomechanical characterization of the superior joint space of the porcine temporomandibular joint. *Ann Biomed Eng* 2003;**31**:924–30.
15. Detamore MS, Athanasiou KA. Tensile properties of the porcine temporomandibular joint disc. *J Biomech Eng* 2003;**125**:558–65.
16. Keith DA. Elastin in the bovine mandibular joint. *Arch Oral Biol* 1979;**24**:211–5.
17. Detamore MS, Orfanos JG, Almarza AJ, French MM, Wong ME, Athanasiou KA. Quantitative analysis and comparative regional investigation of the extracellular matrix of the porcine temporomandibular joint disc. *Matrix Biol* 2005;**24**:45–57.
18. Landesberg R, Takeuchi E, Puzas JE. Cellular, biochemical and molecular characterization of the bovine temporomandibular joint disc. *Arch Oral Biol* 1996;**41**:761–7.
19. Ali AM, Sharawy MM. An immunohistochemical study of collagen types III, VI and IX in rabbit craniomandibular joint tissues following surgical induction of anterior disk displacement. *J Oral Pathol Med* 1996;**25**:78–85.
20. Girdler NM. In vitro synthesis and characterization of a cartilaginous meniscus grown from isolated temporomandibular chondroprogenitor cells. *Scand J Rheumatol* 1998;**27**:446–53.
21. Puelacher WC, Wisser J, Vacanti CA, Ferraro NF, Jaramillo D, Vacanti JP. Temporomandibular joint disc replacement made by tissue-engineered growth of cartilage. *J Oral Maxillofac Surg* 1994;**52**:1172–8.
22. Springer IN, Fleiner B, Jepsen S, Acil Y. Culture of cells gained from temporomandibular joint cartilage on non-absorbable scaffolds. *Biomaterials* 2001;**22**:2569–77.
23. Thomas M, Grande D, Haug RH. Development of an in vitro temporomandibular joint cartilage analog. *J Oral Maxillofac Surg* 1991;**49**:854–7.