

Age-related changes in the microarchitecture of collagen fibrils in the articular disc of the rat temporomandibular joint*

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Summary. The microarchitecture of collagen fibrils in the articular disc of the temporomandibular joint (TMJ) plays an important role in dissipating the mechanical load during jaw movement. However, little information is available on its adaptations to the biomechanical environment during development. To address this issue, we analyzed the diameter of collagen fibrils of the articular disc of the rat TMJ with quantitative ultrastructural analysis during postnatal development.

The mean diameter of the collagen fibrils significantly increased and the arrangement of the collagen fiber networks became compact during development. Articular discs of suckling rat pups were composed of thin, uniformly sized collagen fibrils (range: 30–60 nm, peak: 40–50 nm). At the age of 4 weeks, thicker collagen fibrils began to appear in articular discs, shortly after weaning (range: 20–70 nm, peak: 40–50 nm). In articular discs of adult rats, collagen fibrils varied widely in diameter, with thick fibrils predominating (range: 10–120 nm, peak: 40–70 nm).

These age-related changes in the microarchitecture of collagen fibrils in articular discs may reflect changes in their biomechanical environment during development.

Introduction

The articular disc of the temporomandibular joint (TMJ), whose disorders constitute the major cause of TMJ dysfunction, plays a critical role in absorbing mechanical stress and facilitating smooth condylar movement along the glenoid fossa during chewing (Scapino *et al.*, 1996; Kuboki *et al.*, 1997; Tanaka *et al.*, 1999, 2003). The biomechanical properties of the articular disc involved in stress absorption are largely dependent on its extracellular matrix containing proteoglycan and collagen. The biochemical and morphological changes in the articular disc during development, particularly during periods when mechanical load on the articular disc increases dramatically, have been studied extensively. For example, the amounts of proteoglycan and sulfated glycosaminoglycan increase in articular discs from fetal to adult stages (Carvalho *et al.*, 1993; Nakano and Scott, 1996). The articular disc in the early stages of development is composed of fibrous connective tissue and shows clusters of cartilage cells and type II collagen (Fujita and Hoshino, 1989; Landesberg *et al.*, 1996; Kondoh *et al.*, 2003) in adults. Moreover, vimentin filaments accumulate within the cells of articular discs during development (Bae *et al.*, 1998). These observations suggest that the biomechanical and structural features of the articular disc reflect adaptations to accommodate functional demands during development.

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Collagen fibers of articular discs also increase in number and become more compact and regularly arranged as animals age (Cunat *et al.*, 1956). To date, however, little information is available on developmental changes in the microarchitecture of collagen fibrils, which play an important role in the biomechanical properties of articular discs.

The thickness and distribution of collagen fibrils are closely related to the mechanical properties of tissues and correspond to tissue-specific functional demands (Zambrano *et al.*, 1982; Ottani *et al.*, 2001). We examined here the developmental changes in the diameters of collagen fibrils of articular discs of the TMJ by quantitative ultrastructural analysis.

Materials and Methods

Sprague-Dawley rats aged 1 day, 1, 2, 4, and 8 weeks, and 1 year (5 rats per age group) were used. After weaning, food (Purina Corporation, Seoul, Korea) and water were available *ad libitum*. The animals were deeply anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg) and perfused transcardially with a mixture of 1% paraformaldehyde and 2.5% glutaraldehyde in a 0.1 M phosphate buffer (PB). The left articular discs of the TMJs were dissected out, and the thin central regions, where large mechanical pressure is primarily applied during jaw movement (DeVocht *et al.*, 1996; Beek *et al.*, 2000), were cut transversely. The sections were kept in the same fixative for 2 h at 4°C, rinsed with PB, postfixed in 1% osmium tetroxide in PB for 2 h, dehydrated in a graded series of alcohol, and embedded in poly/bed 812 resins. Silver to gold thin sections were cut and mounted on formvar film-coated, single-slotted copper grids. The sections were stained with uranyl acetate and lead citrate and examined with a Hitachi H-7500 transmission electron microscope.

Areas of cross-sectioned, densely-packed collagen fibrils were photomicrographed at a magnification of $\times 20,000$ and printed to a final magnification of $\times 25,000$, which were then scanned into a Macintosh computer. Diameters of the collagen fibrils within boxes of 2×3 cm on each photomicrograph, corresponding to $0.62 \mu\text{m}^2$, were measured using NIH image software (version 1.60; NIH, Bethesda, MD, USA). Collagen fibrils with regular round outlines were chosen within areas of densely-packed fibril bundles. Diameters of 2,000 collagen fibrils from 5 animals in each age group were measured. Inter-animal variability in collagen fibrils diameters within the same age group was insignificant, and the data could be pooled per group.

Results

The mean diameter of the collagen fibrils in the articular disc significantly increased during development ($P < 0.05$, see Table 1).

The distribution of collagen fibril diameters in each age group are shown in Figure 1. Articular discs of young rats (1-day-, 1-, 2-, and 4-week-old) had loosely arranged collagen fiber networks (Fig. 2A). Collagen bundles in articular discs of 1-day-, 1-week-, and 2-week-old rats were composed of small, uniformly-sized fibrils (Fig 3A, B). The diameter of collagen fibrils was similar between the groups and varied from 30 to 60 nm, with a peak at 40–50 nm. Collagen fibrils of 40–50 nm diameters occupied 80–90% of the collagen fibrils analyzed in these young groups. In articular discs of 4-week-old rats, a small number of collagen fibrils were somewhat smaller or larger than those of the younger rats (Fig. 3C). At 8 weeks and 1 year, articular discs showed tightly-packed collagen fiber networks (Fig. 2B). Most collagen fibrils were considerably larger than those of younger rats and were organized in bundles, with small fibrils interspersed among the large ones (Fig. 3D). The collagen fibril diameters varied between 20 to 120 nm.

Discussion

The main finding of the present study is that the microarchitecture of collagen fibrils in the articular disc shows developmental changes: small, uniform-sized collagen fibrils in the articular disc at early (suckling) ages are replaced by bundles composed predominantly of large collagen fibrils and a few small collagen fibrils wedged between the large ones in the adult.

The microarchitecture of collagen fibril bundles is closely related to mechanical stress (Flint *et al.*, 1984; Parry, 1988) and shows tissue-specific differences that reflect functional demands. Tissues in the locomotor system, subjected to strong mechanical stress, are composed predominantly of large collagen fibrils with plurimodal diameter distribution. In contrast, those in blood vessels and interstitial connective tissues that are not subjected to mechanical stress are composed of smaller and uniformly-sized collagen fibrils (Merrilees and Flint, 1980; Zambrano *et al.*, 1982; Okuda *et al.*, 1987; Evanko and Vogel, 1990; Ottani *et al.*, 2001). In addition, skin subjected to repetitive compression (Sanders and Goldstein, 2001) or high tensile loads (Flint *et al.*, 1984) shows larger collagen fibrils than normal skin.

In this study, the collagen fibril diameter showed

Table 1. Collagen fibril diameters (mean \pm S.D., nm) of the articular disc of the rat temporomandibular joint with aging

Age	1 day	1 week	2 weeks	4 weeks	8 weeks	1 year
Diameter	44.9 \pm 3.7	44.6 \pm 2.9	45.0 \pm 3.0	44.3 \pm 6.4	*57.5 \pm 19.2	*59.0 \pm 21.7

Diameters of collagen fibrils were measured on 2,000 collagen fibrils from the articular discs of 5 animals in each age group. * indicates mean values of the collagen fibril diameter are significantly larger in 8-week- and 1-year-old groups than the younger groups of 1 day, 1 week, 2 weeks and 4 weeks of age ($p < 0.05$).

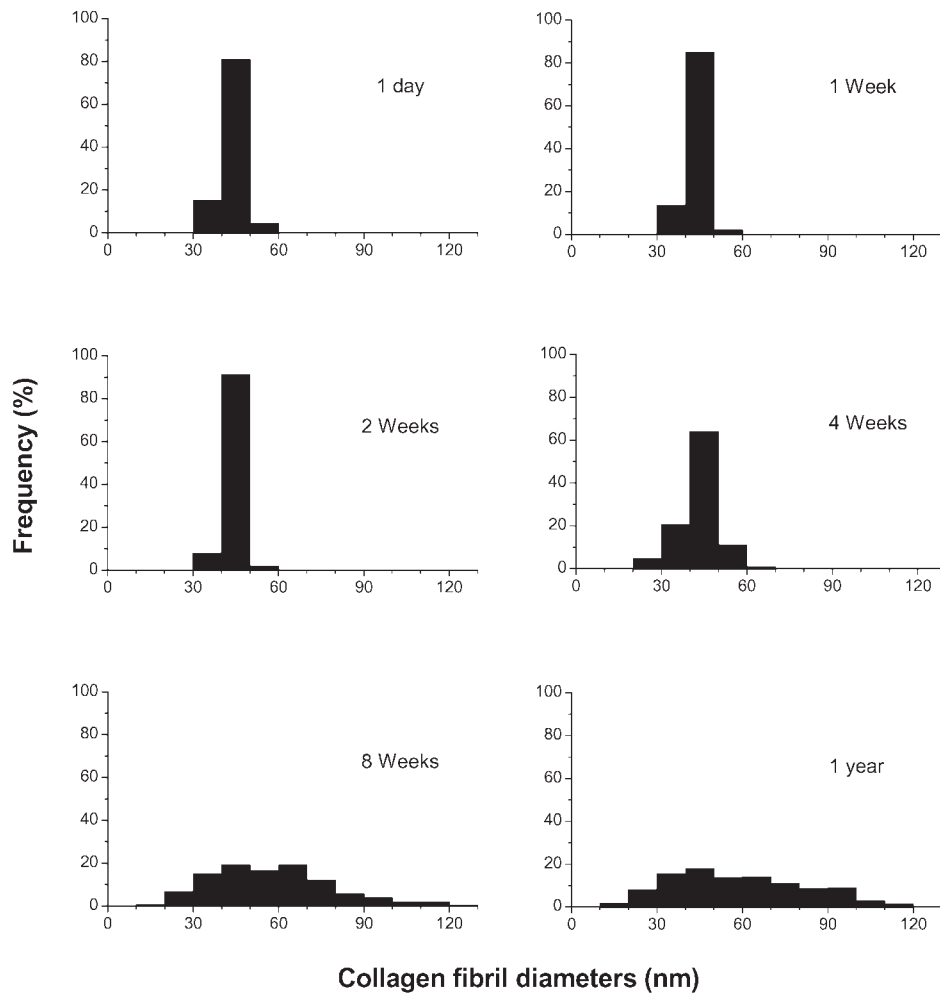


Fig. 1. Histograms of distribution of collagen fibril diameters (nm) in the central region of the articular disc of the rat temporomandibular joint at various age points.

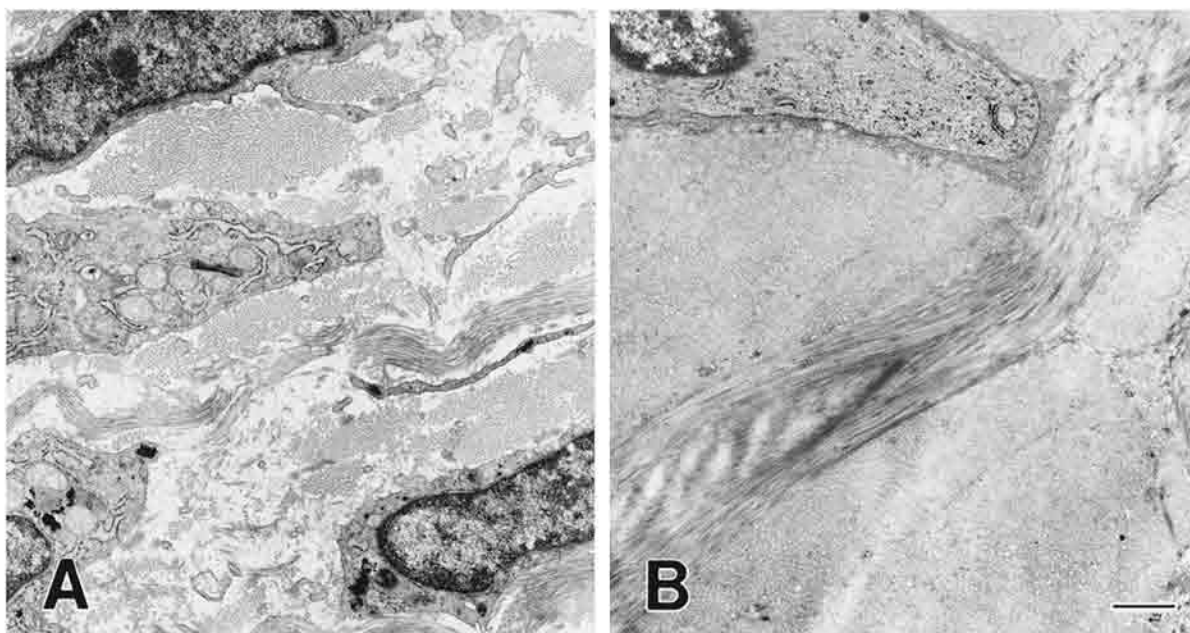


Fig. 2. Electron micrographs of articular disc of 1 week (A) and 8 weeks (B) old rat temporomandibular joints. Articular disc of 1 week old rat (A) shows loosely arranged collagen fibers and abundant extracellular amorphous ground substance. In contrast, that of 8 weeks old rat (B) shows tightly packed collagen fiber. Scale bar = 1 μm (A, B)

developmental changes in the distribution pattern. Articular discs of young (suckling) rats were composed of small uniformly-sized collagen fibrils. In this period, articular discs are subjected to only weak mechanical stress during suckling due to immature masticatory musculature. In articular discs of 4-week-old rats, a small number of larger collagen fibrils began to appear. This is the time when, shortly after weaning, the animals begin to chew solid food, thus applying somewhat stronger mechanical stress to the articular discs. At 8 weeks and 1 year, articular discs were composed predominantly of large collagen fibrils with bundles of small collagen fibrils wedged between the large ones, which was consistent with the previously reported microarchitecture of collagen fibrils in rabbits (Berkovitz and Robertshaw, 1993), cows (Kuc and Scott, 1994), cats, guinea pigs (Berkovitz *et al.*, 1992), and humans (Berkovitz and Pacy, 2002). It is the time when the masticatory musculature and TMJ are fully mature and apply strong mechanical stress to the articular disc during chewing. Therefore, the age-related changes in the microarchitecture of collagen fibrils observed in the present study may reflect changes in the biomechanical environment of the articular disc during development. This notion can be also supported by previous studies in the skin and tendon. The distribution of the collagen fibril

diameters becomes far wider in skin receiving repetitive compressive force compared with normal skin (Sander and Goldstein, 2001). This change is also observed in the tendon subjected to increasing mechanical stress during development (Evanko and Vogel, 1990; Strocchi *et al.*, 1991).

Collagen fibrils in connective tissue are mainly composed of collagen type I, III, and V at different ratios. In the adult articular disc, type II collagen also appears in addition to the collagen types. The direct interaction of type V collagen with type I forms hybrid fibrils and results in thicker collagen fibrils than those composed of only type I collagen (Adachi and Hayashi, 1986). In addition, type V collagen in the mouse endometrium predominates in the thick collagen fibrils whereas it is almost absent in the thin collagen fibrils (Spiess and Zorn, 2007). These findings support the notion that type V collagen may be related to the appearance of large collagen fibrils in the articular disc during development.

The specific organization of small collagen fibrils interspersed among larger ones may be particularly favorable for resisting strong mechanical loads applied to articular discs during chewing. During such incidences, small collagen fibrils may be wedged into the space between the larger ones, and extracellular fluid may be

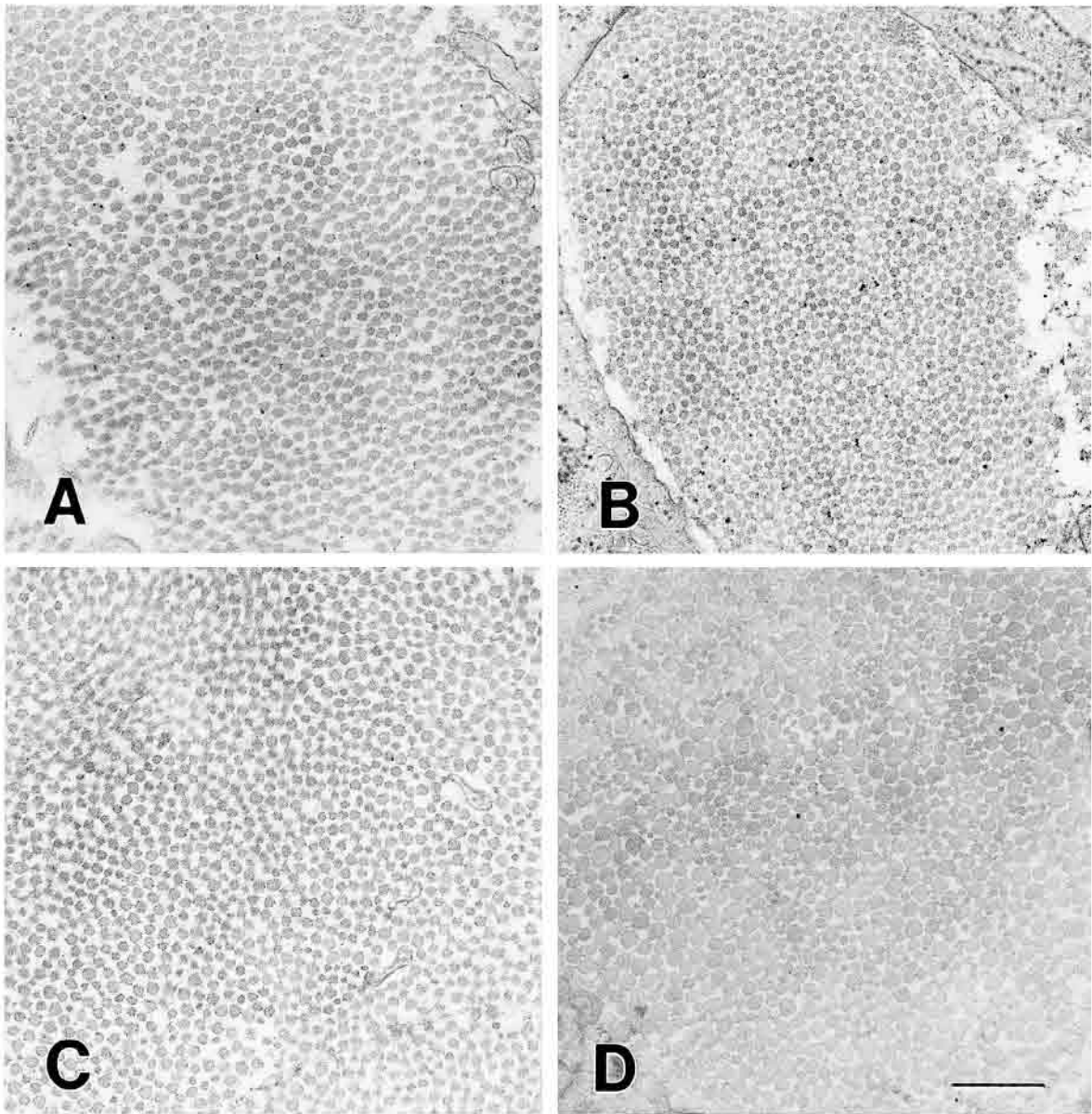


Fig. 3. Electron micrographs showing collagen fibrils in the articular disc of the rat temporomandibular joint at 1 week (**A**), 2 weeks (**B**), 4 weeks (**C**), and 1 year (**D**). Collagen fibrils in the articular disc of young rats (**A**, 1 week; **B**, 2 weeks) are small and uniform. In the articular disc of a 4 week-old rat (**C**), a few smaller collagen fibrils are visible among the uniform-sized collagen fibrils. The articular disc of a 1 year-old rat (**D**) shows collagen fibrils of various sizes, the majority of which are large. Small collagen fibrils are wedged in the spaces between large collagen fibrils. Scale bar = 500 nm (A–D)

squeezed into the small interstices among the packed collagen fibril network. In addition, the appearance of small-sized collagen fibrils among the larger ones would give the articular discs high compliance and render them more pliable. Articular discs showing this microarchitecture can be easily deformed and adapted to the articular surface of the mandibular fossa, thus spreading mechanical loads over a larger contact area to facilitate smooth jaw movement during chewing.

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