

Differences in collagen fibres in the capsule walls of parakeratinized and orthokeratinized odontogenic cysts

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Abstract. Epithelial–mesenchymal interactions are thought to play an important role in the pathogenesis of odontogenic lesions. Keratocystic odontogenic tumour (KCOT) is a benign cystic neoplasm with a characteristic parakeratinized epithelial lining, which differs histologically and behaviourally from the so-called orthokeratinized odontogenic cyst (OOC). The purpose of this study was to investigate the differences in collagen fibres within the fibrous tissue walls of KCOT and OOC. Formalin-fixed paraffin-embedded tissue samples from 15 cases of KCOT and 15 cases of OOC were collected. Paraffin sections were stained with picrosirius red and observed under a standard light microscope using optical polarization. Unicystic ameloblastoma (UA, 15 cases) and subcutaneous epidermoid cysts (EC, 15 cases) were included in the study for comparative purposes. Significant difference was detected between the polarization colours in the fibrous tissue walls of KCOT and OOC ($P < 0.05$), whilst no significant differences were found between KCOT and UA and between OOC and EC ($P > 0.05$). The stromal collagen fibres of KCOT were different from those of OOC, but similar to those of UA, which suggests that the stroma of KOCT may play an important role in determining the neoplastic behaviour of the lesion through epithelial–mesenchymal interaction.

Key words: collagen; odontogenic cysts; picrosirius red.

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Keratocystic odontogenic tumour (KCOT) is a benign, intraosseous tumour of odontogenic origin, with a characteristic lining of parakeratinized stratified squamous epithelium and locally aggressive behaviour. Some are associated with nevoid

basal cell carcinoma syndrome. The traditional designation is odontogenic keratocyst (OKC), which stresses the cystic nature of the lesion². Orthokeratinized odontogenic cyst (OOC) is a rare intraosseous cyst characterized by an orthokera-

tinized epithelium lining, and which shows little clinical aggressiveness^{4,5,7,14,23}. According to the new World Health Organization classification, cystic jaw lesions lined by orthokeratinized epithelium do not form part of the spec-

trum of KCOT and should thus be distinguished from the latter. Several studies have indicated that the lining epithelium of OOC lacked the typical features of KCOT and appeared to show lower proliferative activity^{4,5,14}. Reciprocal interaction between dental epithelium and mesenchyme is thought to be crucial for normal odontogenesis. Thus the mesenchymal influence of the fibrous capsules may play an important role in the maintenance of epithelial expression of KCOT and OOC^{1,11,19,21}.

Picrosirius red staining in combination with polarization microscopy has been used to study individual collagen fibres and to determine their content in the specific tissue. Collagen molecules, being rich in basic amino acids and disposed in a parallel orientation, can strongly react with acidic dyes. Sirius red is an elongated dye molecule which reacts with collagen and promotes an enhancement of its normal birefringence due to the fact that many dye molecules are aligned parallel with the long axis of each collagen molecule. The enhancement of birefringence promoted by the picrosirius polarization method is specific for collagenous structures composed of aggregates of orientated molecules^{12,15}.

Both fibre thickness and packing of collagen can cause differences in polarization colours⁶. Examination of collagen fibres by this method can serve as a procedure to differentiate precollagens, intermediate and pathological collagen fibres, which are not tightly packed, from normal packed fibres. This has been shown to be of value in human tooth germ papillae¹, skin lesions²⁰, odontogenic tumours¹⁶ and odontogenic cysts^{10,11,22}. Using picrosirius red staining, HIRSHBERG et al.¹¹ have shown that the polarization colour pattern of collagen fibres in the connective tissue stroma of KCOT differs from dentigerous and radicular cysts.

The aim of this study was to compare the collagen fibres within the fibrous tissue walls of KCOT and OOC using picrosirius red staining and polarizing microscopy. Unicystic ameloblastomas (UAs) and similar soft tissue epidermoid cysts (EC) were studied for comparison.

Materials and methods

583 cases coded as KCOT were reviewed from the files of the authors' department during the period 1985–2008. After reviewing the patient details, clinical information and histology, 61 OOC cases were identified based on previously established criteria^{5,14}. For inclusion in the

OCC group, all or a predominant portion of the lining epithelium had to exhibit orthokeratinization, in which the basal cells showed no tendency to palisade. A clinical and pathological study of these 61 cases has been previously reported⁷. For the present study, 15 more recent cases were selected from this OOC group. 15 cases of KCOT showing typical parakeratinized lining epithelium, 15 cases of UA (simple cyst type without intraluminal or mural tumour growth) and 15 cases of soft tissue EC were also included in the study for comparative purposes. Histologically, the lining epithelium of EC is almost identical to that of OOC although EC occurs in the soft tissues. It would be interesting to compare their fibrous capsules. All samples were formalin-fixed and paraffin wax-embedded for routine histological examination.

Picrosirius red staining

For visualization of the collagen fibres, 4- μ m thick sections were stained with picrosirius red. In brief, after deparaffinization in xylene and ethanol, sections were hydrated in distilled water, followed by incubation in sirius red (0.1% in saturated picric acid, Electron Microscopy Sciences, USA) for 1 h at room temperature, rinsed with distilled water, and counter stained with haematoxylin. Sections were examined by polarizing microscopy (BX51, Olympus, Japan). Collagen fibres were illustrated as orange-red and/or green colour.

Examination of the polarized colours of stained collagen fibres

To correct potential variability in the staining intensity of sections from different staining batches, all image-acquisition parameters were fixed during the process of image-capture, and the intensity of acquisition illumination was calibrated by adjusting only the microscope condenser aperture. The evaluation process was as follows. Mature and immature collagen fibres were differentiated by the polarization colours. Against a black background, thick yellowish-red fibres were mainly mature collagen (MC), whilst fine netlike greenish-yellow fibres were mainly immature collagen (IC)^{7,10,11,13,18,22}. The images were evaluated by an image analyzer (Image Pro Plus 6.0, Media Cybernetics Inc., Silver Spring, MD, USA), which can automatically calculate the area of the defined regions (yellow-red or green) in each section. Results were expressed as area of mature (SM) and

immature (SI) collagen fibres. The ratios of SM/SI were calculated to represent the content of mature and immature collagen fibres. In the preliminary experiment, the authors found that reproducible values, not affected by further counts, were achieved by observing eight non-inflammation and non-overlapping regions of capsule wall. Therefore, in each section, eight regions at 200 \times magnification were analysed.

For the image obtained by polarized microscopy, segmentation was done and the desired pseudo colour image was obtained by substitution with pure black colour. For MC, the orange-red part was obtained by segmentation and the rest of the image was substituted with pure black, which had a grey level of zero. Thus any pixel with a grey-scale level greater than zero represented MC. The image was converted to an eight-bit grey image, and the result of the conversion was an image consisting of grey collagen fibres on a black background. A histogram of the brightness of each pixel in the image was plotted. Thus, collagen content could be expressed as the mean fraction of pixels with a grey-scale level greater than zero. For the IC, a similar procedure was followed. After segmentation, substitution, and subtraction, the green part of the image was converted to an eight-bit grey level for quantification. The ratio of MC to IC was calculated²⁴. The authors also observed eight non-inflammation and non-overlapping regions of each section (at 200 \times magnification) for analysis.

Statistical analysis

To eliminate subjective bias, images were evaluated independently by two observers. Data were expressed as mean \pm standard error of the mean. Statistical differences between groups were analysed using SPSS 13.0 software (SPSS Inc, Chicago, Illinois, USA). One-way Analysis of Variance (AVONA) was also carried out. Differences were considered to be significant when $P < 0.05$.

Results

The tissue sections were observed with polarized microscopy after picrosirius red staining. In sections of KCOT and UA, a greenish-yellow polarization colour predominated in the subepithelial areas of the fibrous capsule. In OOC and EC, a yellowish-red polarization colour predominated in the subepithelial zone (Fig. 1a–h).

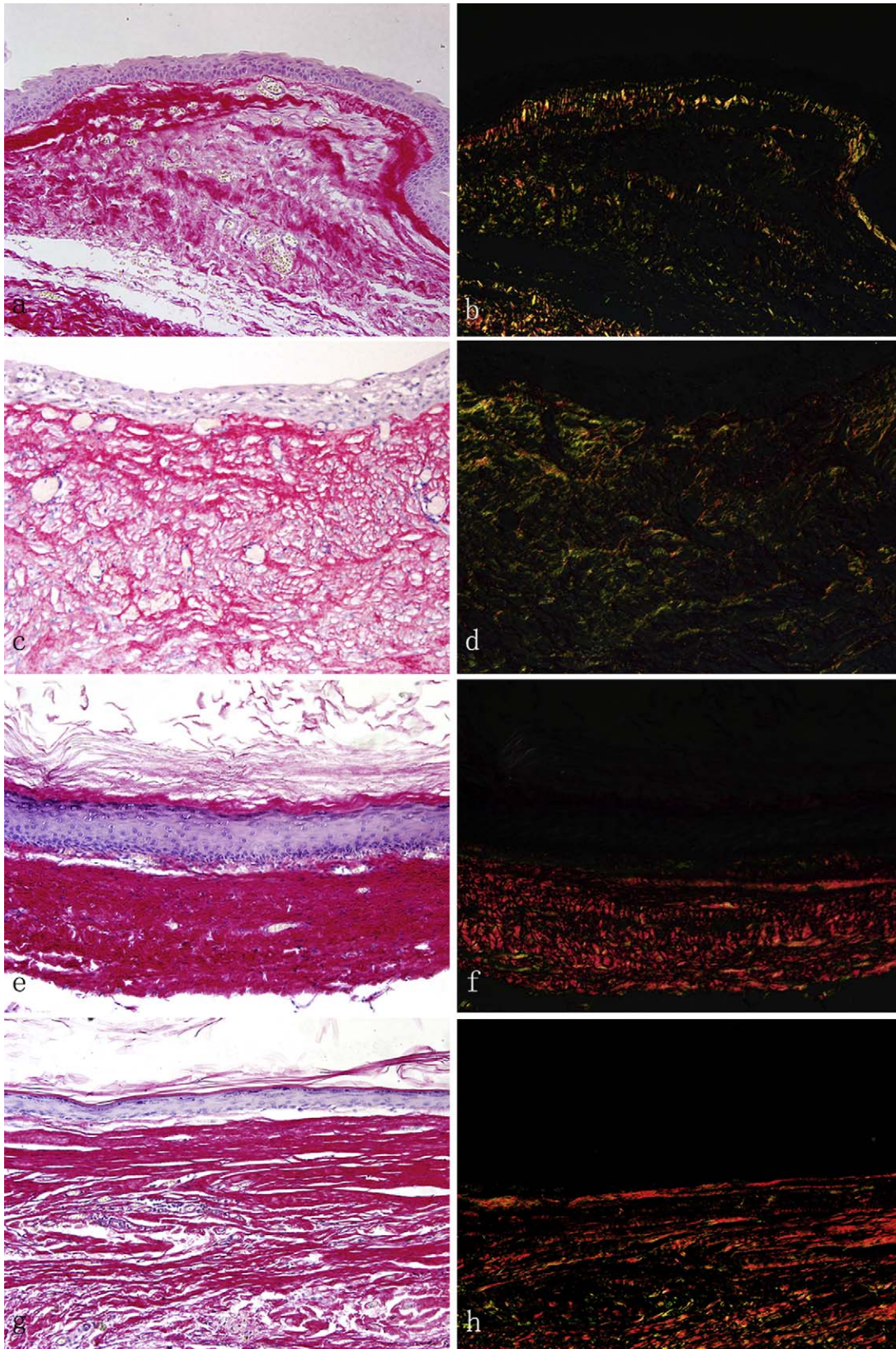


Fig. 1. (a) KCOT stained with picosirius red and photographed without polarization. Most of the fibrous capsule is fibrillar and amorphous. (b) The same field as shown in (a), illuminated with polarized light. KCOT with greenish-yellow polarization colour predominated in the subepithelial areas of the fibrous capsule. (c) UA stained and photographed without polarization. Similar to KCOT, the collagen fibres are predominantly loose and tender. (d) The same field as (c), illuminated with polarized light. UA with greenish-yellow polarization colour predominant in the subepithelial areas. (e) OOC stained and photographed without polarization. Most of the fibrous capsule consists of compact collagen fibres. (f)

Table 1. The ratio of SM/SI (mean \pm SE) and MC/IC (mean \pm SE) in different lesions.

Lesions	No. of cases	SM/SI (mean \pm SE)	MC/IC (mean \pm SE)
KCOT	15	0.67 \pm 0.52	0.54 \pm 0.32
OOC	15	2.62 \pm 1.26	3.21 \pm 1.88
EC	15	2.92 \pm 1.21	3.69 \pm 1.21
UA	15	0.76 \pm 0.30	0.69 \pm 0.45

IC, immature collagen; MC, mature collagen; SE, standard error of the mean; SI, area of immature collagen fibres; SM, area of mature collagen fibres.

Quantitative analysis confirmed that a predominance of greenish-yellow colour was found in the fibrous wall of KCOT, with its ratios of SM/SI and MC/IC being significantly lower than those of OOC and EC ($P < 0.01$), but similar to that of UA ($P > 0.05$; Table 1).

Discussion

In the developing tooth, morphogenesis and cell differentiation are controlled by epithelial–mesenchymal interaction^{17,25}. Development of odontogenic tumours and cysts is also dependent on these interactions^{3,21}. A recent study showed that CD105 (endoglin) is strongly expressed in microvessels of KCOT compared with dentigerous cysts and normal oral mucosa⁹. This suggests that angiogenesis may be associated with the locally aggressive biological behaviour of KCOT. These findings suggest that the stroma of KCOT could be regarded not just as a structural support of the cyst wall, but as playing a part in the neoplastic behaviour of the cyst.

Collagen is the major component of the extracellular matrix. In the pathological setting, collagen can show variations in the way individual fibrils are organized into fibres and in terms of diameter and cross-sectional profile. This should not be surprising given that varied pathological mechanisms are operating in these abnormal situations and that they are characterized by different biomolecular environments. Collagen fibril formation is complex and depends on numerous secondary or post-translational modifications. Defects in these modifications are associated with a number of diseases⁸.

Picrosirius red staining followed by polarizing microscopy is specific for collagen fibres. Both fibre thickness and packing of collagen can cause differences in polarization colours¹⁵. In the present study, a predominance of yellowish-red

colour, which originates from well-packed fibres, has been found in OOC. The major polarization colour of KCOT is greenish-yellow, which differs significantly from OOC, suggesting that the collagen found in these lesions is loosely packed and might be composed of precollagens, intermediates, or pathologic collagens¹¹ rather than the normal tightly packed fibres seen in OOC. Owing to different polarization colours, the composition of the mesenchymal component of KCOT appeared to differ from that of OOC. It has been demonstrated by the authors' group^{7,14} and others^{4,5} that the lining epithelium of OOC has less proliferative and self-renewal potential compared with that of KCOT, which appeared to reflect the contrasting differences in the biological behaviour of these two lesions. It is interesting to speculate that differences in collagen fibres within the fibrous capsules of KCOT and OOC may also be related to their behavioural differences. The authors also found that the polarization colour of KCOT was similar to that of UA, a cystic variant of ameloblastoma. This finding lends further support to the idea that KCOT should be regarded as a cystic neoplasm rather than a cyst^{2,11,19}. A similar polarization colour in the subepithelial zones of OOC and EC, together with the resemblance of the morphological appearances of their lining epithelium, suggest possible links in their histogenesis and/or pathogenesis.

In the present study, the authors evaluated collagen fibres within the capsule walls of KCOT, OOC, UA and EC using picrosirius red staining and polarizing microscopy. Significant differences in polarization colour were demonstrated in the subepithelial zones between KCOT and OOC, suggesting that different collagen fibres may exist in the two lesions and their role in pathogenesis requires further attention.

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Competing interests

The work described has not been submitted elsewhere for publication, in whole or in part, and no relevant conflict-of-interest in this manuscript exists. The authors have no relevant financial interest in the products or companies described in this article.

Ethical approval

The study protocol was approved by the Ethical Committee of Peking University Health Science Center.

References

1. ABRAHÃO IJ, MARTINS MD, KATAYAMA E, ANTONIAZZI JH, SEGMENTILLI A, MARQUES MM. Collagen analysis in human tooth germ papillae. *Braz Dent J* 2006; **17**: 208–212.
2. BARNES L, EVESON J, REICHART P, SIDRANSKY D. Pathology and Genetics of Head and Neck Tumours. Lyon, France: World Health Organization Classification of Tumours, IARC Press 2005.
3. CARNELIO S, VIJ H. Expression of tenascin and nucleolar organizer region in ameloblastoma and ameloblastic fibroma. *J Oral Pathol Med* 2010; **39**: 223–229.
4. CROWLEY TE, KAUGARS GE, GUNSOLLEY JC. Odontogenic keratocysts: a clinical and histologic comparison of the parakeratin and orthokeratin variants. *J Oral Maxillofac Surg* 1992; **50**: 22–26.
5. DA SILVA MJ, DE SOUSA SO, CORRÊA L, CARVALHOSA AA, DE ARAÚJO VC. Immunohistochemical study of the orthokeratinized odontogenic cyst: a comparison with the odontogenic keratocyst. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2002; **94**: 732–737.
6. DAYAN D, HISS Y, HIRSHBERG A, BUBIS JJ, WOLMAN M. Are the polarization colors of picrosirius red-stained collagen determined only by the diameter of the fibers? *Histochemistry* 1989; **93**: 27–29.
7. DONG Q, PAN S, SUN LS, LI TJ. Orthokeratinized odontogenic cyst: a clinicopathologic study of 61 cases. *Arch Pathol Lab Med* 2010; **134**: 271–275.

The same field as (e), illuminated with polarized light. OOC with yellowish-red polarization colour predominant in the subepithelial zone. (g) EC stained and photographed without polarization. Similar to OOC, most of the fibrous capsule consists of compact collagen fibres. (h) The same field as (g), illuminated with polarized light. EC with yellowish-red polarization colour predominates in the subepithelial zone of the fibrous capsule (a–h, picrosirius red staining, original magnification 200 \times).

8. EYDEN B, TZAPHLIDOU M. Structural variations of collagen in normal and pathological tissues: role of electron microscopy. *Micron* 2001; **32**: 287–300.
9. GADBAIL AR, HANDE A, CHAUDHARY M, NIKAM A, GAWANDE M, PATIL S, TEKADE S, GONDIVKAR S. Tumor angiogenesis in keratocystic odontogenic tumor assessed by using CD-105 antigen. *J Oral Pathol Med* 2010. [10.1111/j.1600-0714.2010.00962.x](https://doi.org/10.1111/j.1600-0714.2010.00962.x).
10. HIRSHBERG A, LIB M, KOZLOVSKY A, KAPLAN I. The influence of inflammation on the polarization colors of collagen fibers in the wall of odontogenic keratocyst. *Oral Oncol* 2007; **43**: 278–282.
11. HIRSHBERG A, SHERMAN S, BUCHNER A, DAYAN D. Collagen fibres in the wall of odontogenic keratocysts: a study with picrosirius red polarization microscopy. *J Oral Pathol Med* 1999; **28**: 410–412.
12. JUNQUEIRA LC, BIGNOLAS G, BRENTANI RR. Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. *Histochem J* 1979; **11**: 447–455.
13. KOREN R, YANIV E, KRISTT D, SHVERO J, VELTMAN V, GRUSHKO I, FEINMESSER R, SULKES J, GAL R. Capsular collagen staining of follicular thyroid neoplasms by picrosirius red: role in differential diagnosis. *Acta Histochem* 2001; **103**: 151–157.
14. LI TJ, KITANO M, CHEN XM, ITOH T, KAWASHIMA K, SUGIHARA K, NOZOE E, MIMURA T. Orthokeratinized odontogenic cyst: a clinicopathological and immunocytochemical study of 15 cases. *Histopathology* 1998; **32**: 242–251.
15. MONTES GS, JUNQUEIRA LC. The use of the Picrosirius-polarization method for the study of the biopathology of collagen. *Mem Inst Oswaldo Cruz* 1991; **86**(Suppl. 3):1–11.
16. NYSKA A, DAYAN D. Ameloblastic fibroma in a young cat. *J Oral Pathol Med* 1995; **24**: 233–236.
17. PISPA J, THESLEFF I. Mechanisms of ectodermal organogenesis. *Dev Biol* 2003; **262**: 195–205.
18. RETAMOSO LB, DA CUNHA TDE M, KNOP LA, SHINTCOVSK RL, TANAKA OM. Organization and quantification of the collagen fibers in bone formation during orthodontic tooth movement. *Micron* 2009; **40**: 827–830.
19. SHEAR M. The aggressive nature of the odontogenic keratocyst: is it a benign cystic neoplasm? Part 3. Immunocytochemistry of cytokeratin and other epithelial cell markers. *Oral Oncol* 2002; **38**: 407–415.
20. TRAU H, DAYAN D, HIRSCHBERG A, HISS Y, BUBIS JJ, WOLMAN M. Connective tissue nevi collagens: study with picrosirius red and polarizing microscopy. *Am J Dermatopathol* 1991; **13**: 374–377.
21. VEDTOFTE P, HOLMSTRUP P, DABELSTEEN E. Human odontogenic keratocyst transplants in nude mice. *Scand J Dent Res* 1982; **90**: 306–314.
22. VIJ R, VIJ H, RAO NN. Evaluation of collagen in connective tissue walls of odontogenic cysts—A histochemical study. *J Oral Pathol Med* 2010. [10.1111/j.1600-0714.2010.00961.x](https://doi.org/10.1111/j.1600-0714.2010.00961.x).
23. WRIGHT JM. The odontogenic keratocyst: orthokeratinized variant. *Oral Surg Oral Med Oral Pathol* 1981; **51**: 609–618.
24. ZHANG H, SUN L, WANG W, MA X. Quantitative analysis of fibrosis formation on the microcapsule surface with the use of picrosirius red staining, polarized light microscopy, and digital image analysis. *J Biomed Mater Res A* 2006; **76**: 120–125.
25. ZHANG YD, CHEN Z, SONG YQ, LIU C, CHEN YP. Making a tooth: growth factors, transcription factors, and stem cells. *Cell Res* 2005; **15**: 301–316.

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