Collagen in Odontogenic Tumours: A Histochemical- and Immunohistochemical Study of 19 Cases

J Hangelbroek (NHD Med Tech), EJ Raubenheimer (MChD, PhD, DSc), R Vorster (NHD Med Tech), SP Ngwenya (MDent)

INTRODUCTION
Odontogenic tumours arise from the epithelial- and/or ectomesenchyme from which teeth and their supporting structures originate. The epithelial component gives rise to dental enamel and the ectomesenchyme to the remainder of the tooth, periodontal ligament and alveolar bone. During embryogenesis complex interactions between these tissues result in the orderly formation of a tooth and its supporting structures. Pathological proliferations with varying levels of induction between the remnants of these primordial tissues may lead to the development of complex tumours which differ in microscopic appearance and biological behaviour. The World Health Organization (WHO) classification, which has recently been refined, categorizes odontogenic tumours into those of odontogenic epithelial origin, odontogenic ectomesenchymal origin and a third group comprised of a mixture of odontogenic epithelium and odontogenic ectomesenchyme [1]. In several odontogenic tumours the deposition of basement membrane material, dental hard tissue (enamel, dentin and cementum), bone and fibrous connective tissue occur and an accurate diagnosis depends on identification of these tissue types. The organic component of most of the mineralized odontogenic tissues is collagen. In pathological proliferations, microscopical distinction between cementum, bone and dentin may be difficult and distinction often depends on the associations- and spatial arrangement of these mineralized deposits.

Collagen is the most important structural component of human connective tissue. At least 10 distinct collagen types have been described [2]. Collagen types I, II and III consists of a helix of 3 coiled polypeptide chains and represent the most important structural collagens. Collagen type I is typically found in the dermis of the skin and type II in bone and cartilage where their most important function is to provide strength to the tissue. Collagen type III is found in reticulin fibres around blood vessels and the viscera where their flexibility allows movement, expansion and contraction. Collagen types I-III are deposited by fibroblasts and other cells of mesenchymal origin. Collagen type IV is synthesized by cells of epithelial- or endothelial origin, often as a component of basement membranes. Their reticular distribution facilitates a supportive function [3].

Studies on collagen distribution and typing in odontogenic tumours are infrequent in the literature. The purpose of this study was to record the distribution of collagen in odontogenic tumours of epithelial-, ectomesenchymal- and mixed epithelial and ectomesenchymal origin (keratocystic odontogenic tumours were excluded from this study).

MATERIALS AND METHODS
Wax blocks of 19 cases representative of the epithelial-, ectomesenchymal- and mixed epithelial ectomesenchymal categories of odontogenic tumours were retrieved from the archives of our laboratory (Table 1). Although odontogenic keratocysts are classified as keratocystic odontogenic tumours in the recent WHO classification [4], we did not include examples in this study. Five-3 micron sections were prepared and stained with haematoxylin and eosin, Picrosirius-4, Gomori’s reticulin- and the Masson Trichrome techniques and the Pasqual modification of the immunoperoxidase technique for Collagen Type IV (Dako monoclonal anti-human collagen IV M0785). All sections were viewed by conventional light microscopy and the Picrosirius stained sections by employing high intensity polarized light. The distribution of the collagen types was recorded. Areas of inflammation were not analyzed. Collagen type I is reported...
to appear yellow and red with strong birefringence, collagen type II red with weak birefringence and collagen type III, green with weak birefringence in sections stained by the Picrosirius technique and examined by high intensity polarized light [4].

Table 1: Tumour type and numbers examined.

<table>
<thead>
<tr>
<th>TUMOUR TYPE</th>
<th>NO. OF CASES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odontogenic Tumours of Epithelial Origin</td>
<td></td>
</tr>
<tr>
<td>Ameloblastoma</td>
<td>3</td>
</tr>
<tr>
<td>Adenomatoid odontogenic tumour</td>
<td>3</td>
</tr>
<tr>
<td>Mixed Odontogenic Tumours</td>
<td></td>
</tr>
<tr>
<td>Ameloblastic fibroma</td>
<td>3</td>
</tr>
<tr>
<td>Ameloblastic fibro odontoma</td>
<td>2</td>
</tr>
<tr>
<td>Ameloblastic fibro dentinoma</td>
<td>2</td>
</tr>
<tr>
<td>Odontogenic Tumours of Ectomesenchymal Origin</td>
<td></td>
</tr>
<tr>
<td>Odontogenic myxoma</td>
<td>3</td>
</tr>
<tr>
<td>Odontogenic fibroma</td>
<td>3</td>
</tr>
</tbody>
</table>

Reticulin stains are reported to be indiscriminately positive for collagen types I, II and III [5].

RESULTS

In all odontogenic tumours the capsule consisted of Type I collagen. No collagen Type IV was found as a product of any tumour in the study, except for positive staining of the basement membranes of blood vessels within and around the tumours. As expected, collagen deposits in the ectomesenchymal- and mixed categories of odontogenic tumours were more extensive than in those of epithelial origin. In ameloblastomas, argyrophilic collagen type I fibres radiated from the basal area of the ameloblasts perpendicular through the epithelial basement membrane zone into the surrounding coarse stromal collagen. Delicate type III fibres were present between the type I fibres (Figures 1A and 1B). Areas of plexiform change in ameloblastomas showed dense collagen type I fibres between and linear to the plexiform strands of ameloblastic epithelium. The shape of the plexiform strands generally correlated with the direction and volume of the collagen bundles in the connective tissue stroma.

At the periphery of the solid epithelial nodules in adenomatoid odontogenic tumours, foci showing accumulations of delicate wavy collagen fibres correlated with basement membrane- like deposits surrounding the anastomosing chords of epithelium seen in reticulin stained sections (Figure 2). Calcifications had a tendency of occurring in areas where these delicate fibres were found. The central spaces of the rosette- and duct like structures in the solid epithelial nodules contained varying densities of entangled collagen type I fibres.

In the ameloblastic fibromas, irregularly distributed volumes of collagen were present around the epithelial follicles. In the zones characterized by dense woven collagen, thick fibres radiated into the ectomesenchymal stroma and anastomosed with a more delicate fibre network around individual plump mesenchymal cells. Further away from the epithelial follicle, fibres associated with less plump mesenchymal cells, followed a course parallel to the layer of ameloblasts of the follicle (Figure 3A). The density of the fibres around the epithelial follicles was generally found to impact on the direction of enlargement- and shape of the epithelial follicle (Figure 3B).
The biopsy samples of the mixed epithelial and ectomesenchymal category with induction of dental hard tissue formation (ameloblastic fibro-odontoma and ameloblastic fibro-dentinoma) were decalcified due to their mineralized content. The earliest sign of induction of mineralized tissue was demonstrable by employing the reticulin staining method which showed homogenization of fibres which enveloped plump ectomesenchymal cells in lacunae close to the epithelial follicle. In these areas the mineralized tissue resembled bone. In other foci, a linear arrangement of plump ectomesenchymal cells on the surface of the deposit (delicate white arrows) (Reticulin stain, X200).

The outlines of enamel rods (which remain after demineralization) stain red with the Masson Trichrome technique. The transition between the red and blue areas was sharp (Figure 4B). The biopsies showed the presence of odontoblastic tubules in the latter (Masson trichrome stain, tissue decalcified, X200).
Masson Trichrome technique which stains course collagen and in particular the delicate reticulin fibers and the colourful stains which exploit the argyrophilic characteristic of collagen several odontogenic tumours. The techniques included Reticulin highlight- and type collagen in routine embedded wax blocks of methods and immuno-histochemistry for collagen type IV to of 3 microns. Our study exploited several routine histochemical meticulous care was taken to prepare all sections at a thickness of the connective tissue walls of odontogenic cysts, employing only the Picrosirius staining technique, it was demonstrated that differences exist in the birefringent nature of collagen in the walls of inflamed- (dentigerous- and radicular cysts) versus non inflamed cysts (dentigerous- and odontogenic keratocysts). For this reason, areas of inflammation were not examined in our study. In order to avoid the shift in colour during polarization when viewing sections of different thicknesses, meticulous care was taken to prepare all sections at a thickness of 3 microns. Our study exploited several routine histochemical methods and immuno-histochemistry for collagen type IV to highlight- and type collagen in routine embedded wax blocks of several odontogenic tumours. The techniques included Reticulin stains which exploit the argyrophilic characteristic of collagen and in particular the delicate reticulin fibers and the colourful Masson Trichrome technique which stains course collagen fibres blue. The argyrophilia of the delicate reticulin fibres makes the Reticulin technique appropriate for their identification unlike the Masson Trichrome technique which stains the coarser fibres blue and fails to demonstrate the delicate reticulin fibres. Sections stained with the Picrosirius technique are viewed with high intensity polarized light making differentiation between the three most common collagen types (I, II and III) possible. At least six tumours in each of the major categories of odontogenic tumours (epithelial, mixed and ectomesenchymal) were included in the study.

The capsules of all odontogenic tumours contained mainly type I collagen. Type IV collagen was not found in any tumour except for positivity in the basement membranes of blood vessels. The collagen fibres in the basement membranes of ameloblastomas were found to be spatially organized with thick type I collagen passing perpendicularly through the basement membrane zone and merging with the collagen in the capsule and fibrous septa between the epithelial follicles. The Picrosirius staining technique showed delicate fibres with the staining characteristics for collagen type III between the coarse type I fibres. The organized arrangement of the collagen in the basement membranes of ameloblastomas may be helpful in distinguishing ameloblastomas from other odontogenic lesions with inactive epithelium. The lack of incorporation of plump ectomesenchymal cells adjacent to the basement membrane zone distinguishes ameloblastomas from ameloblastic fibromas in which induction of ectomesenchymal cells are present. The volume and direction of the collagen fibres deposited between the ameloblastic follicles had a bearing on the shape of the epithelial follicle, with plexiform growth patterns exhibiting denser collagen between the epithelium. Although the study did not include a case of desmoplastic ameloblastoma, we propose that extreme collagenization may be the reason for the flattened and atrophic morphology of the chords of odontogenic epithelium characteristic of this variant of ameloblastoma. Further research is indicated in order to determine whether the structure of the basement membrane zone of the desmoplastic ameloblastoma follows the description of the ameloblastomas reported in this study.
In a paper on the collagen fibre component of adenomatoid odontogenic tumours, El Labban (1992) describe a layer of fine filaments running perpendicular to the epithelial basal lamina, similar to that seen during induction of dentin formation. Stromal calcifications in these tumours mainly involve fine fibrils resembling amyloid, an observation supported by our study. The absence of an organized collagen fibre arrangement (as in ameloblastomas) in the basement membrane zones close to the epithelium in adenomatoid odontogenic tumours was noteworthy. The shape of the narrow anastomosing epithelial strands (which are reported to be present mainly at the periphery of adenomatoid odontogenic tumors) was found to be the result of accumulation of collagen which appeared to restrict spatial enlargement of the epithelium.

The fibres in the basement membrane zone of ameloblastic fibromas resembled those described in ameloblastomas. Adjacent to the basement membrane zone further differentiation of the ectomesenchyme was present. Collagen fibres surrounded individual plump ectomesenchymal cells, mimicking the first step in induction of mineralized tissue deposition. Due to the formation of collagen and subsequent expansion of the extracellular space, the cells in this zone were plump and it appeared less cellular than in the surrounding connective tissue. Collagen in the surrounding connective tissue was oriented in parallel bundles and the ectomesenchymal cells were inactive-appearing and compressed in greater density. Early signs of mineralized tissue induction, as described in ameloblastic fibro-odontomas, were not present. The role of collagen in determining the shape of the epithelial follicles in ameloblastic fibromas was noteworthy. Growth and enlargement of the epithelial follicles were generally found to be restricted in areas of dense collagen deposits, leading to enlargement of the epithelial follicle along ectomesenchymal planes of lesser resistance. The situation is analogous to the inflation of a balloon (epithelial follicle) in a closed hand, where the balloon will enlarge through the spaces between the fingers. This study suggests that although differences in the mitotic rate of the epithelium may play a role as a determinant of the shape of the follicle, the varying density of surrounding collagen undoubtedly determine the planes of enlargement of the follicle. This study is the first to propose that follicular, plexiform or multi-lobular shapes of the odontogenic epithelial follicles in odontogenic tumours are influenced by the density and spatial arrangement of collagen in the ectomesenchymal tissue around the follicles.

With the aid of ultrastructural investigations, Josephsen, Larsson and Fejerskov (1980) demonstrated a rim of finely filamentous meshwork separating the epithelium from the connective tissue in ameloblastic fibro-odontomas. This meshwork was demonstrated in this study to consist of two layers. The centrifugal fibres close to the odontoblasts showed the spatial arrangement described in the basement membrane zone of ameloblastomas and ameloblastic fibromas. The second layer showed the same morphology as in ameloblastic fibromas (plump ectomesenchymal cells surrounded by collagen), with foci of hard tissue deposits. The earliest sign of dental hard tissue deposition was seen adjacent to the epithelial basement membrane zone as an area of homogenization of fibers around plump ectomesenchymal cells. In situations where ectomesenchymal cells are located within lacunae in a mineralized matrix and formation of odontoblastic tubules cannot be demonstrated, the term “osseodentin” is recommended. Although structurally similar, the term “cementum” should be reserved for mineralized deposits on the surface of a tooth root only. In our study odontoblastic tubules were apparent only in areas where formation of hard tissue was at a more advanced stage. Linear arrangement of plump ectomesenchymal cells on the surface of homogenized collagen, as demonstrated in our study, may be the first indication of tubular dentin formation as the arrangement of the cells mimics those of odontoblasts in the pulp of a tooth. The distinct difference in the colour- and sharp transition between recently formed- and more mature tubular dentin as demonstrated by the Masson Trichrome staining technique cannot be explained satisfactorily, except that it may reflect a chemical difference between the two dentin types.

Odontogenic fibromas are described microscopically as proliferations of varying cellularity with dispersed delicate collagen fibers in a fibromyxoid background and scattered groups of inactive appearing strands of odontogenic epithelium. In our study the epithelium lacked the delicate arrangement of fibres described for ameloblastomas and this feature may be helpful in distinguishing these two tumour types. Metaplastic dysplastic dentin, cementum or osteoid may be present. The thick collagen fibres were suggested to distinguish central odontogenic fibromas from the connective tissue of dilated tooth follicles. In our study odontogenic fibromas showed thick and wavy type I collagen bundles which were oriented roughly parallel to the inactive odontogenic epithelial strands. The epithelial basement membrane zones lacked the organized spatial arrangement of collagen observed in ameloblastomas. Foci of mineralization were surrounded by concentric collagen bundles in an unstructured way. These mineralized deposits, unlike those in odontogenic myxomas, could therefore be regarded as dystrophic in nature.

Odontogenic myxomas are characterized microscopically by randomly arranged stellate, spindle shaped and round cells with centrally placed nuclei with long pale cytoplasmic extensions dispersed in abundant mucoid or myxoid stroma. Only a few collagen fibres are present and unlike odontogenic fibromas, odontogenic epithelial remnants are scarce. In a study by Simes (1975) the stroma of odontogenic myxomas showed collagen bundles and smaller fibres similar to myxomas elsewhere in the body. Our study showed a varying quantity of collagen in odontogenic myxomas with thick type I collagen fibres that run roughly parallel. Tumours with an increase in collagen are generally referred to as fibromyxomas (or myxofibromas). There are no clear criteria reported in the literature for applying the latter terminologies and for this reason we recommend the use thereof to be suspended. More delicate collagen type III fibres intersect at obtuse angles to the main fibre type. This feature may be helpful in distinguishing peripheral odontogenic myxomas from oedematous irritation fibromas, a diagnostic pitfall which probably contributes to the low number of peripheral odontogenic myxomas recorded in the literature. Unlike the dystrophic calcifications identified in odontogenic fibromas, the structured arrangement of the collagen around the acellular calcifications in the stroma of odontogenic myxomas resembles Sharpey’s fibres radiating from acellular dental cementum. As cementum is defined by its location on the root surface of a tooth, the term “cementoid” is suggested for these deposits.
ACKNOWLEDGEMENT
The study was performed with the aid of a grant provided by the Department of Education and Training in 2010.

REFERENCES