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Analysis of collagen fibres in human dental pulp using picrosirius red stain and polarised microscopy



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ABSTRACT

Background: The extracellular matrix (ECM) performs a very important function in growth regulation, tissue differentiation and organization. Diseases of pulp are seen in all ages. Collagen, the main macromolecule of dental pulp, varies in its number, thickness and distribution with age. Hence a study was designed to identify and analyze the collagen fibers in human dental pulp on the basis of polarizing colors in various age groups.

Material and methods: A study was conducted using the pulp of 30 non-carious teeth from patients of various ages and one tooth germ. Picrosirius red stain was used to stain the collagen present in dental papilla, dental follicle and dental pulp and the thickness of the fibers was assessed on the basis of the polarizing colors.

Results: Collagen fibers in dental follicle stained red while they were not stained in papilla. Collagen fibers in the pulp of teeth of younger individuals stained green to greenish yellow, in middle aged individuals, it stained orange–orange red while in older individuals, it stained red.

Conclusion: The results from the study show that with aging, thickness of collagen fibers increases and there is change in their arrangement and packaging indicating fibrosis.

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1. Introduction

Human dental pulp is a connective tissue with unique organization and location. It is surrounded by hard, inelastic dentin.[1] The dental pulp comprises of cells like fibroblasts, odontoblasts and undifferentiated mesenchymal cells in contact with a complex chain of macromolecules secreted extracellularly, which forms the extracellular matrix (ECM).[2] This matrix provides a structure for tissues and plays a bioactive role in regulation of cell behavior and influences its development, migration, proliferation, form and function.[3] The role of ECM in the pathogenesis and behavior of epithelial odontogenic lesions has been demonstrated by some authors who studied collagen fibers and suggested a complex relationship between epithelium and mesenchyme.[4]

The main macromolecules in the dental pulp are collagenous proteins (especially types I and III collagen), non-collagenous proteins (fibronectin, tenascin, osteonectin, sialoprotein and osteocalcin) and proteoglycans, including

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hyaluronic acid, chondroitin sulphate, heparin sulphate and phospholipids.[2,3,5,6]

Nearly 34% of total ECM proteins is collagen (Types I and III predominates). This collagen functions to maintain the structural integrity and determination of tissue function. Type I collagen is commonly seen in dense connective tissues and is necessary for tissue architecture stabilization. Type III collagen is seen in loose connective tissues and helps in tissue elasticity.[7,8]

Diseases of the pulp are seen in all ages and their prevalence rate is high. Collagen is the main macromolecule of the human dental pulp ECM and variations in its number, thickness and distribution are seen as pulp matures. Therefore to understand the role of collagen in pulpal diseases the methods to detect and analyze collagen fibers in human dental pulpal tissue are valuable.

Few studies have been done on collagen fibers of the ECM in human dental pulp, dental papilla or dental follicle of developing tooth germ. Hence a study was designed to identify and analyze the collagen fibers in dental pulp of various age groups on the basis of polarizing colors seen under polarizing microscope.

Traditionally Van Gieson and the various forms of trichrome have been used to detect collagen fibers in tissue sections.[9] These stains which combine two or more anionic dyes, rely on differential binding by tissue components. Such differentiation is determined by a combination of differences in the relative size of dye molecules, differences in the physical structure of tissue (for e.g., tightly packed v/s loosely packed), and differences in the amino acid composition of tissue elements.[10] However, they have a disadvantage that they have poor specificity for thin fibers which under certain circumstances lead to low sensitivity of collagen content.[10,11]

To overcome this disadvantage, Picrosirius red stain which detects thin fibers and is frequently used in studies of collagen fibers in precancerous and cancerous lesions, odontogenic cysts and gingival lesions was chosen to study the collagen present in human dental papilla, dental follicle and dental pulp in various age groups.

2. Material and methods

Thirty freshly extracted, non-carious teeth from patients of different ages were selected for the study. One tooth germ in the late bell stage of tooth development from the lower central incisor of a 23 week human fetus (forensic post mortem) was also included in the study as given in Table 1. The project was approved by the ethical committee. The study was carried out over a period of one month. The teeth taken for study were extracted either prior to orthodontic treatment or were periodontally compromised. Care was taken to see that the teeth were caries free.

Table 1 – Thirty non-carious teeth and one human fetus showing the color of collagen fibers stained.

Sr. No.	Age/sex	Tooth number	Reason for extraction	Color of collagen fiber stained
1	Tooth germ in Bell stage of 23 week fetus	71		Follicle-Red Papilla-Not stained
2	29/F	35	Orthodontic treatment	Orange–Orange red
3	43/F	17	Periodontally compromised	Orange–Orange red
4	35/M	36	Periodontally compromised	Orange–Orange red
5	38/M	12	Periodontally compromised	Orange–Orange red
6	17/F	14	Orthodontic treatment	Green–Greenish yellow
7	14/M	24	Orthodontic treatment	Green
8	21/F	44	Orthodontic treatment	Green–Greenish yellow
9	63/F	36	Periodontally compromised	Red
10	56/F	17	Periodontally compromised	Red
11	19/M	34	Orthodontic treatment	Green–Greenish yellow
12	12/M	24	Orthodontic treatment	Green
13	33/M	37	Periodontally compromised	Orange–Orange red
14	48/M	15	Periodontally compromised	Red
15	42/M	11	Periodontally compromised	Orange–Orange Red
16	32/F	14	Periodontally compromised	Orange–Orange red
17	16/M	44	Orthodontic treatment	Green–Greenish yellow
18	41/F	23	Periodontally compromised	Orange–Orange red
19	23/F	45	Orthodontic treatment	Orange–Orange red
20	51/F	26	Periodontally compromised	Red
21	17/M	14	Orthodontic treatment	Green–Greenish yellow
22	22/M	35	Orthodontic treatment	Orange–Orange red
23	53/M	12	Periodontally compromised	Red
24	11/F	14	Orthodontic treatment	Green
25	59/M	46	Periodontally compromised	Red
26	31/F	45	Orthodontic treatment	Orange–Orange red
27	54/M	41	Periodontally compromised	Red
28	17/M	24	Orthodontic treatment	Green–Greenish yellow
29	26/M	34	Orthodontic treatment	Orange–Orange red
30	57/F	32	Periodontally compromised	Red
31	16/F	44	Orthodontic treatment	Green–Greenish yellow

The teeth were vertically cut and the pulp tissue was removed. The tissues were fixed in 10% neutral buffered formalin, dehydrated, embedded in paraffin and serially sectioned longitudinally. Five micrometer thick cut sections were obtained and stained with picrosirius stain using Picrosirius histological methods for staining.[12] The stained sections were viewed under polarizing microscope, and the thickness of collagen fibers was analyzed depending on the color of collagen fibers.

Picrosirius red is a strong anionic dye. Collagen fibers are stained when the sulphonic acid groups of the dye react with basic groups present in the collagen molecule. The elongated dye molecules get attached to collagen fibers such that their long axes are parallel. This parallelism between dye and collagen fibers results in enhanced birefringency.[13]

3. Results

On staining the collagen fibers of human dental follicle and papilla (lower central incisor) in a 23 week old fetus and human dental pulp using picrosirius red stain, the following polarizing colors were observed:

Collagen fibers in the dental follicle of the tooth germ of the human fetus (23 weeks) in late bell stage, on staining with picrosirius red, showed red color when viewed under polarized light while those in the papilla were not stained. In younger individuals, the collagen fibers of the pulp demonstrated green to greenish yellow color. In adult individuals, the fibers presented orange–orange red color while in older individuals, the fibers showed red color.

Table 1 given below summarizes the results.

4. Discussion

Human dental pulp, like all body tissues, is constantly changing throughout life. One of the most evident changes is the decreasing size of the pulp chamber and root canals caused by continued dentin deposition. This reduction in pulp volume causes a decrease in the vascular supply to the pulp and initiates many of the other changes found in the tissue. From about the age of 20 years, there is a gradual decrease in the number of cells until age of 70 years and the cell density also decreases by half. The distribution of the collagen fibrils may change with age, leading to the appearance of fibrous bundles.[14]

As pulp ages, there is accumulation of diffuse fibrillar components as well as bundles of collagen fibers. Arrangement of these fiber bundles differ in coronal as well as radicular pulp. In radicular pulp the fiber bundles may appear longitudinal while in coronal pulp, a more diffuse arrangement is seen. The increase in number of fibers in the pulp organ is gradual and generalized throughout the organ.[15]

Very thin collagen fibers in human dental pulp are not stained by the Van Gieson and trichrome stains which are the most commonly used stains for collagen fibers. This lead to substantial underestimation of the collagen content.[10,11] This perplexing issue of poor and variable staining of thin collagen fibers by Van Gieson along with the stains tendency to fade prompted Sweat and colleagues to find a better method.[16] Their study on connective tissue showed that thin collagen fibers were stained consistently with Sirius red F3BA (color index 35780) when dissolved in saturated picric acid solution. The staining did not fade and was suitable for use with polarized light microscopy. Vidal and Junqueira also studied the combination of picrosirius red and polarized light for the recognition and analysis of collagen fibers in the tendons.[13,17] However, this method became popular only after the work of Pick and Weber in the1980's.[18,19]

Diverse polarizing colors have been observed when the collagen fibers are stained with picrosirius red stain and viewed under polarized light. The polarizing color depends on the thickness of the collagen fibers. Thin fibers show green to yellowish green polarization colors and thick fibers show longer wavelength colors mainly yellow to yellowish-orange, orange to red.[20,21] Packing of collagen molecules in fibers also play an important role in pattern of polarization colors. Densely packed and better-aligned collagen molecules have polarization colors of longer wavelengths.[22]

In the present study, collagen fibers in dental follicle were demonstrated and stained red (Fig. 1) in the late bell stage of tooth development. This result was expected because this collagen protein has a well established function. Numerous studies have shown that collagen works as a guide in mineralization progression throughout its long axis.[23,24]

Dental papilla of tooth germ in bell stage of tooth development showed few, fine and scattered collagen fibrils occupying the extracellular spaces.[25] Hence the fibers in the dental papilla of the tooth germ were not stained in the present study.

In the younger subjects, collagen fibers stained green to greenish yellow (Fig. 2) showing that the fibers were thin and loosely arranged.

In adult individuals, collagen fibers stained orange to orange red (Fig. 3) showing that there is shift towards densely packed collagen. Also the thickness of the fibers had increased.

In the older patients collagen fibers stained red (Fig. 4) indicating there was increased fibrosis and dense packing of collagen bundles.

Hillmann and Greutsen investigated the distribution of ECM molecules in human dental pulp of various age groups using polarization and indirect immunofluorescence microscopy by using a conventional fluorescence microscope and a

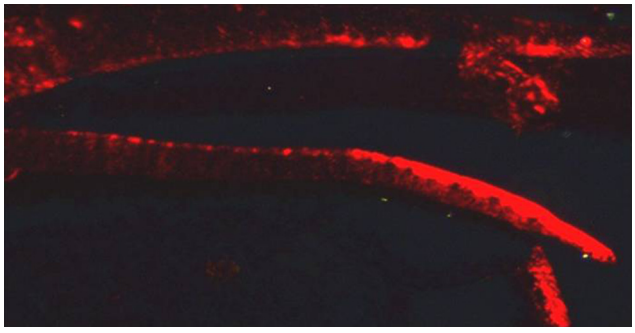


Fig. 1 – Collagen fibers in follicle of developing tooth germ in late bell stage of tooth development stained red.

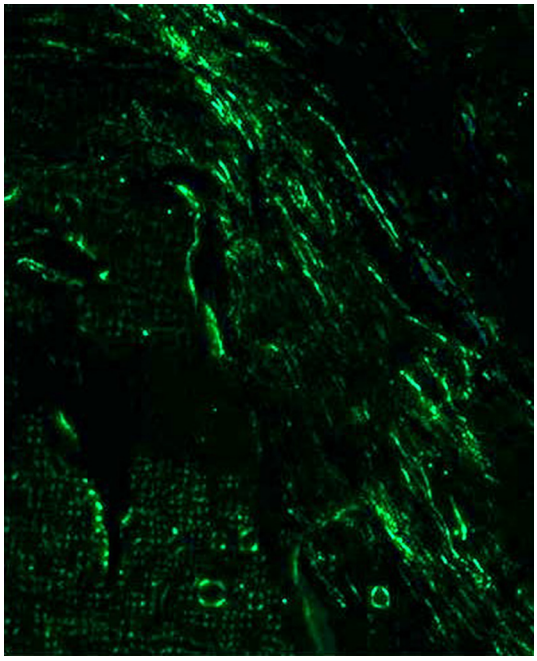


Fig. 2 – Collagen fibers stained green–greenish yellow in young pulp.

confocal laser scanning microscope. They found that with advancing age, the connective tissue matrix appears to be condensed and aggregates of thick fiber bundles could be observed.[23] This was in accordance with the observations seen in the present study.

Magloire et al studied the distribution of type III collagen in pulp parenchyma of human developing teeth using light and electron microscope immunotyping and found that type III

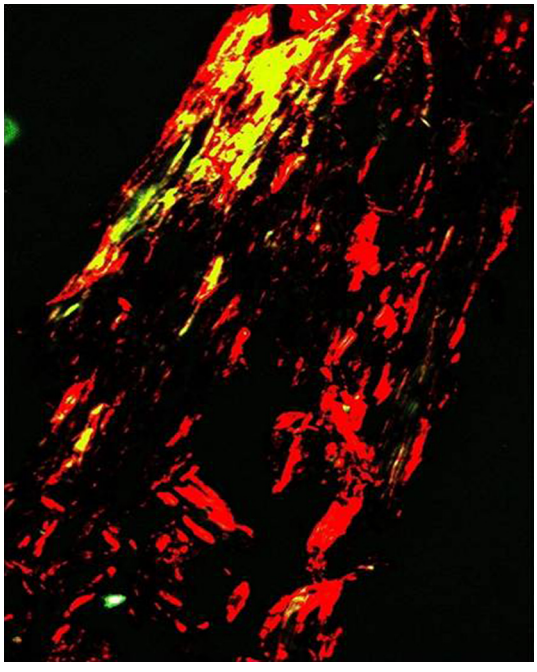


Fig. 3 – Collagen fibers stained orange–orange red in adult pulp.

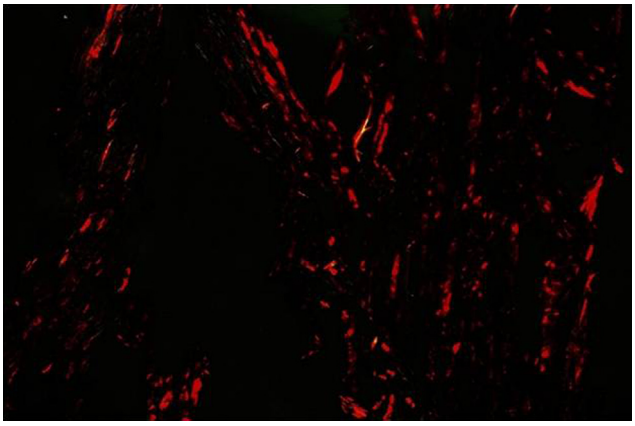


Fig. 4 – Collagen fibers stained red in matured adult pulp.

and type I collagen are present in pulp and the staining intensity of type III collagen seemed to correlate with its relative high proportion.[26]

Moure S et al have studied collagen and elastic fibers in odontogenic entities using light and confocal laser microscopic methods. They compared the collagen in dentigerous cyst and keratocystic odontogenic tumor. Comparison revealed similar collagen organization, showing distinct layers: thin collagen fibers with loose arrangement near the epithelium and thick fibers with dense arrangement in distant areas.[4] Zhang et al studied the differences in collagen fibers in the capsule walls of parakeratinized and orthokeratinized odontogenic cysts using picrosirius red stain and polarization microscopy and observed that the stroma of keratocystic odontogenic tumor may play an important role in determining the neoplastic behavior of the lesion through epithelial–mesenchymal interactions.[27] J Hangelbroek et al studied collagen in 19 odontogenic tumors using picrosirius red stain under high intensity polarized light and distinguished types I, II and III collagen on basis of color of birefringence. Type I collagen, yellow to red with strong birefringence, type II red with weak birefringence and type III green with weak birefringence. They found that type I collagen is mainly seen in the capsule.[28]

5. Conclusion

The present study shows that picrosirius red stain and polarizing microscopy is useful in studying collagen fibers in ECM of dental pulp and in assessing the collagen present in dental follicle or papilla of tooth germ at various stages of tooth development. The results from the present study are in accordance with our assumptions and knowledge of collagen fibers in human dental pulp that with aging, there is increase in thickness of collagen fibers along with change in their arrangement and packaging indicating fibrosis. This increase in fibers in pulp organ is gradual and is generalized throughout the organ. This study acts as a nidus to evaluate collagen fibers in pulp and hopefully will spawn further investigations in understanding the nature and role of collagen fibers in pulpal and periapical diseases.

Conflicts of interest

All authors have none to declare.

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