Morphological and Ultrastructural Collagen Defects: Impact and Implications in Dentinogenesis Imperfecta

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Declaration:

I, Lubabah Gadi confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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Funding

I am a Paediatric Dentistry DDent student funded by a scholarship from King Abdulaziz University Dental Hospital, Jeddah, Saudi Arabia. Grant Award number 40528.

Dedication

''All Praise is due to Allah''

This thesis is dedicated to my younger self (the 9-year-old Lubabah) and my family for their upmost love and support. To my mother (Azzah Hashim), all that I am or hope to be I owe to you, and to my beloved sisters, my pillars (Anwar & Asrar). Thank you all for the prayers and for giving me the strength to achieve my goals, for understanding and encouraging me to proceed in my path, and for teaching me to believe in God, myself, and in my dreams.

Project Summary and COVID-19 Impact

This project was evolving around a genetic disorder that is known to affect dentin (dentinogenesis imperfecta or DI), which has a basis pathophysiology of defective collagen thus our second main theme was collagen. The reason why this disorder was of interest goes back to the anomalies clinic run by the Paediatric Department at Eastman Dental Hospital, where we see a high flow of DI patients, yet have limited understanding of how teeth are affected on an ultrastructural level. The project aimed to examine collagen defects in DI teeth with an initial focus on dentinal collagen. However, the course of this project was changed due to the unavoidable impact of COVID-19.

The pandemic's effect can be divided into three phases. In phase one, due to the national lock-down, clinical services were restricted to emergency only and face to face services were reduced. This impacted subject recruitment and collection of study samples. In addition, access to university campus and labs was restricted, which hindered the continuation of laboratory training. At this stage, the Eastman Dental Institute lab was being moved from the old Eastman building to The Royal Free Hospital. At this stage and before conducting our own laboratory analysis, we sought the opportunity to conduct a systematic review to examine the literature for known dentinal collagen defects and where the gap of knowledge is (Figure A)

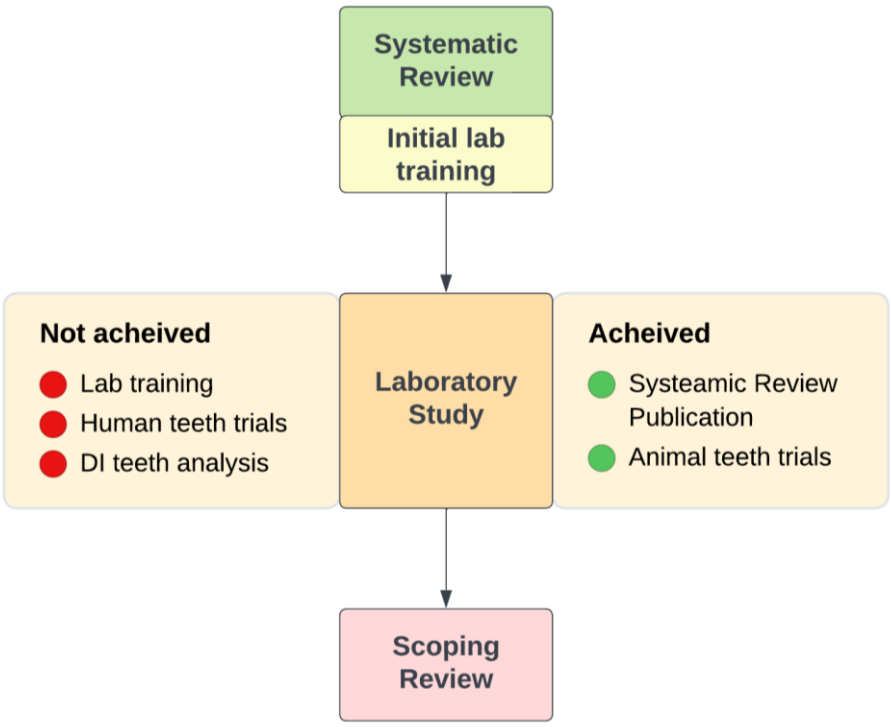

Figure A. Flow diagram of Project pathway

In the 2nd phase, the national restrictions were lifted, however because the Royal Free Hospital was designated for treatment of COVID patients, the labs remained closed. Which meant training completion and sample trials were not possible. At this stage we worked on formatting the systematic review paper to start the process of publication. Paper was submitted. In the 3rd and final phase, the labs were finally open, and laboratory testing on animal teeth was initiated. Nonetheless, due to staff sickness and periods of exposure and isolation, the project experienced severe delays, it was then decided that the study was not achievable, and an additional scoping review was added. The scoping review was targeting collagen defects in cementum, as the results of the systematic review revealed that no to little evidence existed on cemental defects in DI teeth.

Disruption was multisided, yet collectively they prevented this study from providing its own laboratory results. Although the quantity of the research was reduced, we ensured we maintain the quality of it. The protocol developed for the intended non-preformed study is added in the lab work sections, to answer the question proposed by the thesis.

Abstract of Systematic Review

Background: Collagen is the building block for extracellular matrix in bone, teeth and other fibrous tissues. Osteogenesis Imperfecta (OI), or brittle bone disease is a heritable disorder that results from defective collagen type I synthesis or metabolism. The disease manifests as bone fragility that leads to multiple fractures The dental manifestation of OI is Dentinogenesis Imperfecta (DI), a genetic disorder that affects tooth structure and clinical appearance with characteristic greyish-brown discolouration. Management of Dentinogenesis Imperfecta can be difficult. Therefore, understanding the ultrastructural defects in dentinal tissues is of clinical importance. Thus, the aim of the project was to answer the question of what the changes in dentinal collagen macro, micro and ultrastructure in Dentinogenesis Imperfecta.

Methods: Three data bases were searched for relevant articles: OVID Embase, OVID Medline, and PubMed Medline. Inclusion criteria were any study, written in English, published after 1990, that examined human dentinal collagen of teeth affected by DI. No exclusions were made by study design or examination protocol. A Cochrane data extraction form was modified to fit project aims and used for data collection.

Results: The final dataset of the systematic review included seventeen studies. The most prevalent findings on collagen in DI teeth were increased coarse collagen fibres, and decreased fibres quantity. Other findings included random to parallel fibres orientation and irregular organization. Ultrastructural defects were uncoiled collagen fibres and increased spread of D-banding periodicity.

Conclusion: Studies in collagen structure in DI reported changes to the surface topography, quantity, organisation, and orientation of the fibres. Moreover, ultrastructural defects such as the packing/coiling and D-banding of the fibrils, as well as differences in the presence of other collagens are also noted. Taken together, this study provides an understanding of the changes in collagen and its impact on clinical translation, paving the way for innovative treatments in dental management.

Abstract of Scoping Review

Background: Dentinogenesis imperfecta is known to affect dental tissues, which is mainly reported to be dentin since it is the most dental tissue abundant. Deformed collagen, abnormal dentinal tubules and reduced mechanical strength are criteria well reported on by studies. In less abundance, enamel has also been described as abnormal, defects as irregularly shaped enamel lamellae and reduced mechanical strength have been described. Cementum, nonetheless, is not a common tissue type to be examined when looking for the effects of this genetic disease. The aim of this scoping review is to identify the effect of dentinogenesis imperfecta on cementum, if present and clarify extent of periodontal involvement in DI patients.

Methods: The database of PubMed was searched. Search strategies developed, concepts were: collagen ultrastructure, DI and OI. Inclusion criteria were human or animal studies, of any primary or permanent teeth, affected with dentinogenesis imperfecta, isolated or syndromic DI type, that examined cementum defects. No limitations were applied on study type, date of publication, sample size, age or gender of subjects, nor species in case of animal studies. No restrictions applied on methodology of study as demineralization protocol or examination method.

Results: six studies were retrieved, four human and two animal studies. Isolated DI was examined in 3 of which, the observations found were hypomineralized cementum, that is generally hypoplastic but with an increase in cellular cementum in the certain regions. The two papers examining syndromic DI cementum had inconsistent results.

Conclusion: cementum defects of isolated DI were reported with consistent results, unlike syndromic DI, this can be because the function and defects of Dentin Sialophosphoprotein (DSPP) are further known than the defects in collagen of DI teeth. The evidence on cementum defects was scarce. The limited number of studies could reflect the limited periodontal involvement in DI patients hence the low clinical importance. From this scoping review we speculate the presence of cementum defects yet on a subclinical level.

Impact Statement

From 1882 when Dentinogenesis Imperfecta was first discovered, up until the twenty-first century, evidence on the exact pathophysiology of the disease is scarce. It is however known as a genetic disorder that affects dentin structure, yet the resultant histological defects are not fully discovered and modalities to manage this genetic disorder are very limited. Because of severe tooth wear, the treatment aims range from preventing loss of tooth structure by a form of coverage to restoration of the lost vertical dimension of occlusion. Dentin is known to be a mineralized tissue with collagen forming most of its organic compound. The defect in dentinal collagen defies the breakthroughs in adhesive dentistry. Discovering the damage in dentinal collagen and knowing the ultrastructural defects caused by the disorder, can be the first steps to preventing dental complications. Coronal symptoms as severe attrition or enamel breakdown are common in DI yet mobility, or periodontal involvement is less frequently seen, and it is unknown whether this is because cementum is unaffected by DI as dentin and enamel or if it's sub-clinically affected.

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List of abbreviations

α	Alpha
ALP	Alkaline Phosphatase
AAC	Acellular Afibrillar Cementum
AEFC	Acellular Extrinsic Fibre Cementum
AFM	Atomic Force Microscopy
CIFC	Cellular Intrinsic Fibre Cementum
CMSC	Cellular Mixed Stratified Cementum
COL1A1	Collagen Type 1, Alpha 1 Gene
COL1A2	Collagen Type 1, Alpha 2 Gene
DEJ	Dentino-Enamel Junction
DI	Dentinogenesis Imperfecta
DGP	Dentin glycoprotein
DPP	Dentin Phosphoprotein
DSPP	Dentin Sialophosphoprotein
DSP	Dentin Sialoprotein
EDTA	Ethylenediaminetetra-acetic Acid
ECM	Extracellular Matrix
EDS	Ehlers-Danlos Syndrome
GPa	Gigapascal
HMDS	Hexamethyldisilazane
mRNA	Messenger Ribonucleic Acid
OMIM	Online Mendelian Inheritance in Man
OI	Osteogenesis Imperfecta
PDL	Periodontal Ligament
PFA	Paraformaldehyde
SEM	Scanning Electron Microscopy
TC	Tropocollagen
TEM	Transmission Electron Microscopy
UHQ	Ultra-High Quality

1 Introduction

1.1.1 Collagen

Collagen accounts for one-third of the human body's protein. Specifically, collagen type I is the most abundant protein of the extracellular matrix in skin, vessels, heart, lungs and bone (Boskey, 2013). Bone is comprised of mineralised collagen (Figure 1.1) which is 90% collagen type I. In teeth, dentin is formed of about 70% inorganic components, 20% organic compounds and 10% water, 90% of the organic matter is collagen type I (Figure 1.1) (Breschi *et al.*, 2018).

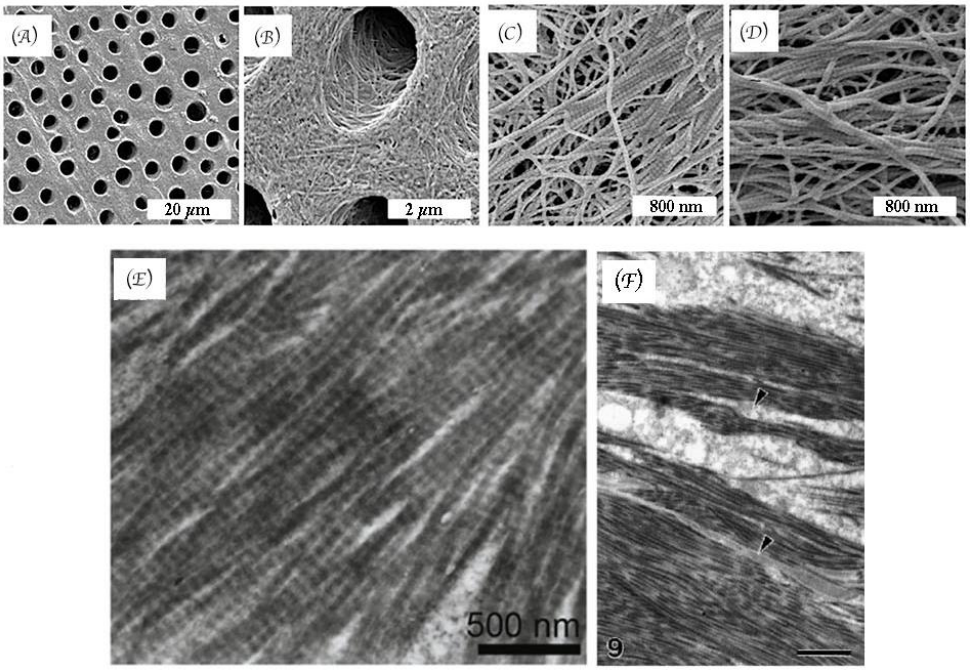

Figure 1.1: Similarity of collagen ultrastructural in dentin, bone and cementum (A) Dentinal tubules pre-demineralization. (B) Dentinal collagen scaffold post-demineralization (C and D) collagen fibres showing D-banding periodicity (Ibrahim *et al.*, 2019) (E) Collagen fibres in bone showing D-banding periodicity (Tzaphlidou, 2008) (F) collagen bundles in cementum, faintly showing D-banding periodicity (Yamamoto *et al.*, 1997)

Collagen Types

There are 28 different types of collagens. Overall, they can be described as classic fibril-forming collagens with D-banding cross-striations or non-fibril-forming. The fibril-forming collagens can be found in all connective tissues *(Myllyharju and Kivirikko, 2001, Kadler et al.*, 2007, Ricard-Blum, 2011). A few examples are teeth, bone, heart, lungs, blood vessels and skin (Gelse *et al.*, 2003). These are collagen types I-III, V and XI and they mostly coexist in tissues, therefore are denoted as heterotypic fibrils. In the cornea, studies found that extracellular matrix was formed by collagen types I and V, while skin was mostly made of

types I and III (Gelse *et al*., 2003, Bruckner, 2009). Non-fibril-forming collagen can be further divided into five categories: fibril-associated, network-forming, membrane-associated, anchoring-fibrils, and multi-triple helix fibrils (Table 1.1). Fibril-associated collagens are, as the name implies, found in association with collagen fibres (Kadler *et al*., 2007). A clear example of this is cartilage, where type II fibrils are found to be covered by collagen type IX, which is a fibril-associated collagen. When it comes to network-forming collagens, type IV collagen is known as the building block in all basement membranes (Knupp and Squire, 2005). In the skin, Anchoring collagens bind the dermis to epidermis, as the function of collagen type VII (Ricard-Blum, 2011).

Table 1.1 Collagen categories (Kadler et al., 2007, Ricard-Blum, 2011)

Categories	Fibres
Fibre-forming	I, II, III, V, XI, XXIV, XXVII
Fibre associated	IX, XII, XIV, XVI, XIX, XX, XXI, XXII, XXVI
Network-forming	IV, VI, VIII, X
Anchoring-fibrils	VII
Membrane associated	XIII, XVII, XXIII, XXV
Multiple triple helix fibres	XV, XVIII

Structure of Fibril-forming Collagens

Collagens are known to have a common unique conformation, the triple helix. Depending on the collagen type, the triple helix can be the configuration of the collagen almost entirely as collagen type I or form only 1 tenth of its structure (Ricard-Blum, 2011, Kadler *et al.*, 2007). The alpha (α) chains forming the helix can also vary based on the collagen type. In the fibril-forming family, the structure is formed of a right-handed triple helix, also known as a tropocollagen or TC (Figure 1.2). and helices are two identical α-1 chains and one α-2 chain and are held together by hydrogen bonds. Each chain or helix is made of peptides with a (X-Y-Gly) motif with Proline-Hydroxyproline-Glycine, the most common triplet. The reason mandating glycine to be the third residue is that it is the only amino acid with a side chain small enough to be contained in the tight structure of the helical conformation (Myllyharju and Kivirikko, 2001, Kadler *et al*., 2007, Shoulders and Raines, 2009, Ricard-Blum, 2011).

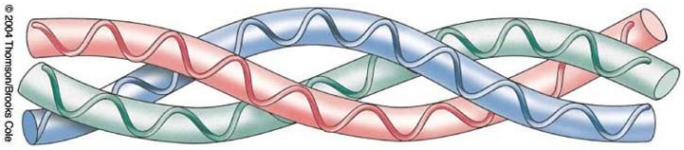

Figure 1.2: Collagen triple helix (Martin, 2005)

The Biosynthesis of Collagen

Collagens are a big family, however studies on collagen synthesis are almost exclusively on fibrillar collagens (Gelse *et al*., 2003, Shoulders and Raines, 2009, Ricard-Blum, 2011). Nonetheless, the basics of synthesis in all collagens are considered alike, with the exception of post-translation modification, regardless of the tissue to be made (Crane *et al*., 2020). As an example, in both bone and dentinal collagen, the synthesis process begins intracellularly and continues to the extracellular matrix. Overall, there are five main steps for collagen biosynthesis. These are mRNA transcription, pre-propeptide translation, post translation modification, propeptide cleavage, and finally collagen fibre assembly (Gelse *et al*., 2003, Crane *et al*., 2020). The former three steps are intracellular and the latter two are extracellular (Figure 1.3).

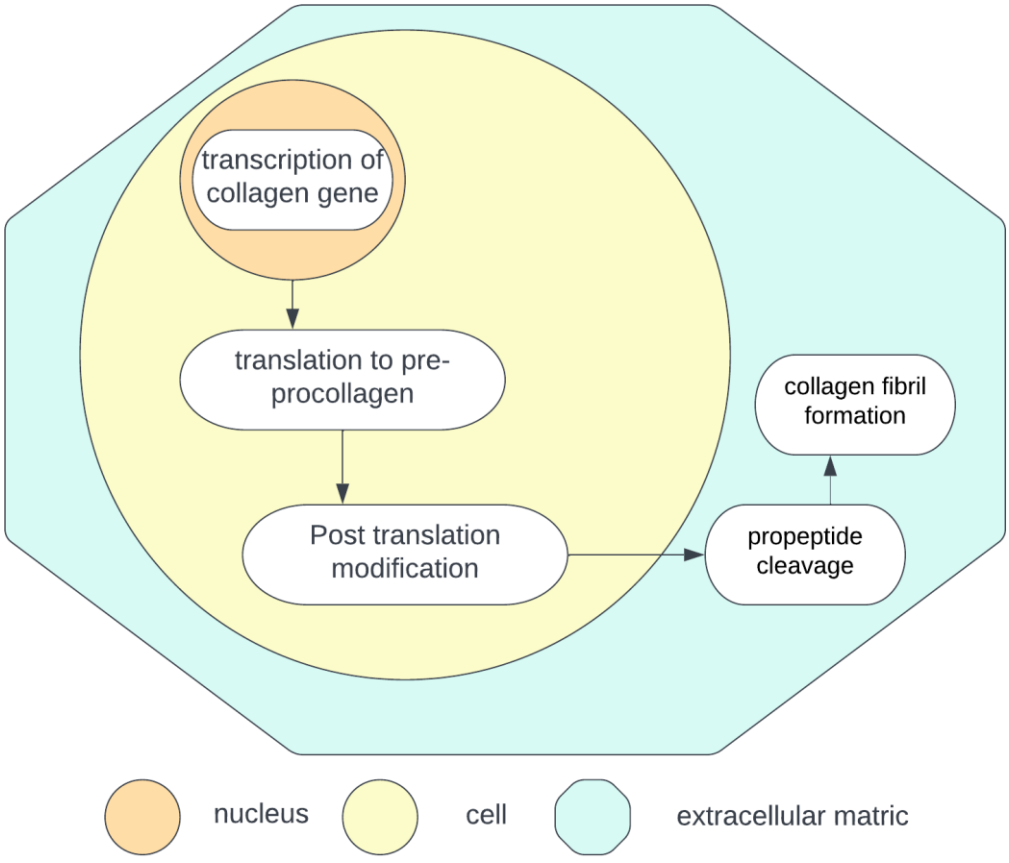

Figure 1.3 steps of collagen synthesis

In the cell's nucleus, as of fibroblasts, genes of collagen pro-α peptides are transcribed to give rise to mRNA. Depending on the type of collagen, the number and type of pro- α peptides change. For example, collagen type one has pro-α-1 and pro-α -2 peptides. The genes encoding them are COL1A1 and COL1A2, respectively. The next step is in the cell's cytoplasm, where ribosomes translate the mRNA into a pre-pro-polypeptide. The first unit at the beginning or at the N-terminal of the pre-pro-peptide is known as signal recognition peptide. This unit aids in transferring the pre-pro-peptide into the endoplasmic reticulum where post translation modification occurs (Crane *et al*., 2020). In fibril-forming collagens, there are three prime post translation modifications, one is the removal of the signal peptide forming the pro-peptide. Another modification is the hydroxylation of proline and lysine. This is done by the aid of hydroxylase enzyme and vitamin C. In this category of collagens, approximately half of the prolines are hydroxylated in position 4 to become 4-hydroxyproline. Presence of this residue is thought to have an important role in the thermal stability of the triple-helix as it facilitates the formation of hydrogen bonds (Gelse *et al*., 2003, Shoulders and Raines, 2009). In addition, hydroxylation of lysine residues adds on helix stability by the formation of intermolecular cross-linking (Gelse *et al.*, 2003). The third modification is the addition of glucose and galactose to hydroxyl groups of hydroxylysine molecules. Finally, the assembly of three pro-α chains into a pro-collagen molecule, after which the pro-collagen will be excreted extracellularly. The ends of pro-collagen, N-terminal and C-terminal, are removed by peptidases forming the basic building block for collagen fibrils, tropocollagen or TC (Gelse *et al.*, 2003, Shoulders and Raines, 2009, Crane *et al.*, 2020).

Tropocollagens in fibril-forming collagen such as I, II and III, auto-assemble by covalent bonding to form a fibril (Figure 1.4). In their assembly, they are aligned in parallel formation but staggered by approximately 234 residues, this gives the fibrils a characteristic appearance of D-banding or D-periodicity under the microscope. This phenomenon is defined by Shoulders and Raines as "the axial stagger of adjacent tropocollagen molecules by a distance, D, which is the sum of the gap and overlap regions. Studies estimated that D is equal to 67 nm (Figure 1.4). This means the length of a tropocollagen is about 4 times longer than the periodicity; Length = 4.46D, giving areas of gaps (0.54D) and overlaps (0.46D), that when combined they account for the 67nm D-periodicity. The exact role of this feature is not fully known, however it is thought that it serves an important role in collagen tensile strength, tissue mineralization, and regulation during tissue formation (Chen *et al.*, 2019). A fully formed fibril can be up to about 100 mm in length, and approximately 500 nm in diameter (Kadler *et al*., 2007, Shoulders and Raines, 2009, Ricard-Blum, 2011, Duan *et al.*, 2016, Marini and Cabral, 2018).

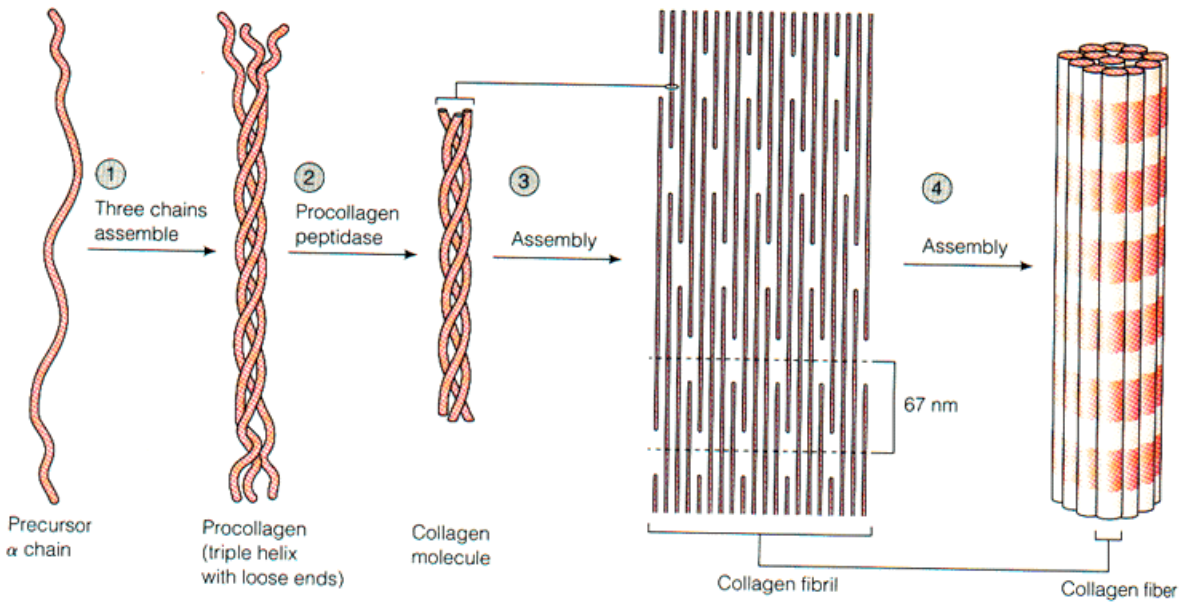

Figure 1.4: Collagen fibres formation (Friedrichs, 2009)

Although TC is a unit forming the collagen fibril, they possess dissimilar mechanical stability. An individual TC was found to have fracture strength of 11 GPa, while a collagen fibril has 0.5 GPa. It is believed that this is because individual TC are stabilized internally by covalent bonds (Buehler MJ 2006). However, when measuring the modulus of elasticity, they are known to have similar Young's modulus, ranging 6-7 GPa and approximately 5 GPa for a TC unit and fibril, respectively (Shoulders and Raines, 2009). Collagen synthesis is a highly regulated biochemical process that involves numerous enzymes, and cofactors. An error occurring in any step of collagen synthesis can lead to defective collagen formation, this can result from clinical nutritional deficiency as scurvy disease or gene mutations as Osteogenesis Imperfecta (OI), and Ehlers-Danlos syndrome or EDS (Myllyharju and Kivirikko, 2001, Gelse *et al*., 2003).

1.1.2 Gene Mutation

Defective collagen formation due to gene mutation is the basis of many skeletal disorders. There are approximately 50 genes that translate to polypeptide chains forming about 28 different types of collagens (Myllyharju and Kivirikko, 2001, Shoulders and Raines, 2009). Collagen genes start with “COL” and a number denoting the type of collagen then the number of α-chain, and so COL3A1 is the a gene that translates to chain α-1 of collagen type III. Studies found that at least a thousand mutations have been discovered in at least 22 of those genes. Furthermore, more than four-fifths of these mutations are exclusive to 5 of those genes, these are COL1A1, COL1A2, COL3A2, COL4A5 and COL7A2 (Myllyharju and Kivirikko, 2001). Gene mutations are variable, they can be minute affecting one base as a point mutation or substantial affecting up to thousands of pairs. A base substitution is an example of a point mutation, and it is where a base pair is replaced by another. Other types of mutations are gene insertion, deletion, duplication, and inversion as shown in Figure 1.5 *(Antonarakis et al*., 2000).

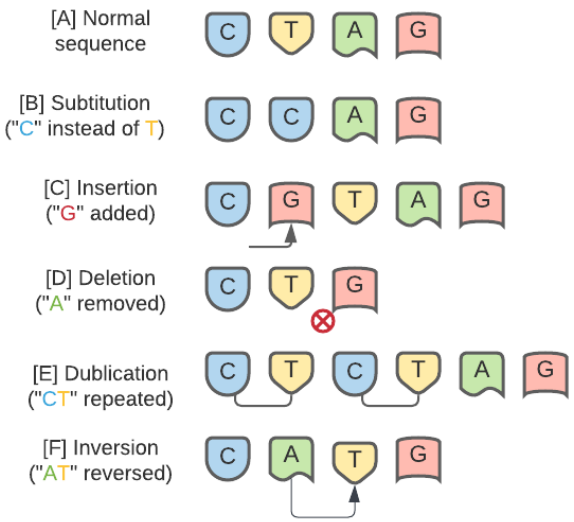

Figure 1.5: Common types of mutations

The resultant protein from the expression of the mutated gene can be entirely sound, and in that case the mutation is called silent mutation, or it can be defective of which is named a missense mutation. A more drastic effect results when the mutation causes a stop codon or a nonsense mutation, which prematurely terminates the protein synthesis (Clancy, 2008). The most damaging point mutation is Glycine substitution, and the severity of the expressed disease is thought to depend on the replacing amino acid and location of substitution. Because the folding of the triple helix is in a C-terminal →N-terminal propagation, mutations

near C-terminal are more severe due to interference and the resultant delay in helix folding. Another location factor is residues abundance in site. Scientists have found that mutations in proline rich zones are known to result in less severe form of disease than proline-poor portions (Hyde *et al.*, 2006). Therefore, a Glycine substitution in a proline-poor zone, near C-terminal in COL1A1 is known to result in a severe form of the disease (Myllyharju and Kivirikko, 2001, Shoulders and Raines, 2009). In fibril-forming collagens, the genes encoding the helices α-1 and α-2 chains, are COL1A1 and COL1A2, respectively. Mutations in these genes can result in multiple disorders such as Osteogenesis Imperfecta (OI), Ehlers-Danlos syndrome (EDS) and osteoporosis (Myllyharju and Kivirikko, 2001). However, the skeletal disorder most caused by these mutations is Osteogenesis Imperfecta (Willing *et al*., 1992, OMIM® and Man, 2020).

1.1.3 Osteogenesis Imperfecta

Osteogenesis Imperfecta (OI) is a genetically inherited connective tissue disease characterized by fragile bone, decreased bone mass, and increased fracture incidence rate, and is commonly known as Brittle Bone Disease (Rauch and Glorieux, 2004). The disease is mainly cause by a defect in collagen type I, meaning various systems of the body can be affected, as well as bone. This includes pulmonary involvement, cardiac defects and hearing impairment (Marini and Cabral, 2018). OI is considered a rare bone disorder, hence there are not up to date studies on its epidemiology. One historical study reported, in 1981 the disorder affected 6:100,000 individuals in the UK, and 21.8:100,000 in Denmark in 1989 (Martin and Shapiro, 2007). A more recent study dated 2015, found prevalence of OI to be 7.4:100,000 individuals in Sweden (Lindahl *et al*., 2015).

Classification of Osteogenesis Imperfecta

The most widely used classification of the disorder was established by Sillence *et al.* in 1979, which distinguished four phenotypes of OI: type I - characterized by mild deformity; a lethal perinatal disorder (type II); type III a moderate yet progressive form; and a varying type IV ranging from mild to severe phenotypes (Sillence *et al.*, 1979). However, this classification was mainly based on clinical and radiographic findings, in addition to the recognized genetic heterogeneity of the disease and the variability of the clinical representation, the need for an expanded version was required. An updated classification incorporated mutation mechanism as a factor in categorization (Rauch and Glorieux, 2004). The expanded classification proposed by Rauch has categorized OI into seven types, denoting type I with nonsense mutation in COL1A1; mis-sense mutation of Glycine in COL1A1 in types II, III and

IV; and unknown mutation in types V, VI and VII (Rauch and Glorieux, 2004, Van Dijk *et al.*, 2010). Furthermore, the most recent classification of OI lead to the identification of 18 subtypes. The classification was based on a holistic approach, accounting for the clinical presentation, radiographic features, genetic background, mode of inheritance and histological features (Forlino *et al.*, 2011, Marini and Cabral, 2018). The eighteen subtypes have been grouped into six categories based on the pathophysiology of the mutation. The 6 groups are: defects in collagen synthesis and structure; defects in bone mineralization; defects in collagen modification; defects in collagen processing and crosslinking; defects in osteoblasts differentiation and function; and unclassified Osteogenesis Imperfecta-like or collagen-based disorders as shown in table 1.2 (Marini and Cabral, 2018).

Table 1.2: Updated classification of osteogenesis imperfecta (Rauch and Glorieux, 2004, Marini and Cabral, 2018)

OI type	Gene mutation	Severity	Clinical presentation
Defects in collagen synthesis and structure			
I	COL1A1	Mild	- Dentinogenesis Imperfecta, uncommon but highly heritable - 50% of all OI - Blue sclera - Mild susceptibility to long bone fracture - Premature hearing loss
II	COL1A1 - COL1A2	Lethal	- High mortality rate - 80% die within the first week
III	COL1A1 - COL1A2	Progress with age	- Most severe but nonlethal - Skeletal deformity (scoliosis) - Blue sclera - Common presence of Dentinogenesis Imperfecta - Most cases non-ambulatory
IV	COL1A1 - COL1A2	Moderately severe	- Variable presence of Dentinogenesis Imperfecta - Hearing loss - Variable skeletal malformation - Blue sclera
Defects in bone mineralization			
V	IFITM5	Moderate to severe	- 5% of all OI patients - Similar to type IV yet with no DI nor blue sclera - High incidence of radial head dislocation & subluxation - Mesh-like bone trabeculation appearance in radiographs
VI	SERPINF1	Severe	- Worsening of cases after 12 months of age - Sclera, hearing, and teeth are not affected. - Fish-like bone trabeculation appearance in radiographs - Non-responsive to Bisphosphonate therapy
Defects in collagen modification			
VII	CRTAP	Severe to lethal	- Dentinogenesis Imperfecta - Clinically resembling types II and III - Low 5-year survival rate due to pulmonary disease
VIII	LEPRE1	Severe to lethal	Similar to Type VII

IX		Moderate to lethal	- Rare type - Similar to types VII and VIII
Defects in processing and crosslinking			
X	SERPINH1	Severe to lethal	- Similar to type III with increased severity
XI	FKBP10	Progress with age	- Joint laxity and scoliosis - Possibility of joint contracture - Normal teeth
XI	BMP1	Severe	- Osteoporosis - Kyphoscoliosis - Umbilical hernia - Normal teeth
Defects in osteoblasts differentiation and function			
XIII	SP7	Severe	- Very rare - Generalised osteoporosis - Normal teeth but delayed eruption
XIV	TMEM38B	Moderate to Severe	- Specific to Bedouin people - Severe bone deformity - Normal teeth and hearing
XV	WNT1	Mild to Severe	- Osteoporosis - Osteopenia - kyphoscoliosis
XVI	CREB3L1	Severe	- rare - multiple fractures during gestation period
XVII	SPARC	Moderate to Severe	- Born normal - Rapid development of kyphoscoliosis - muscle hypotonia - joint hyperlaxity
XVIII	MBTPS2	Moderate to Severe	- Prenatal fractures - Osteopenia - Kyphoscoliosis - Bowing of extremities

Unclassified disorders resembling OI

-	PLOD	Moderate to Severe	- Bruck syndrome
-	LRP5	Mild to Moderate	- Osteosclerosis - Osteopetrosis

Osteogenesis Imperfecta Clinical Presentation

As the name brittle bone implies, reduced bone density and fragile bones are clinical signs that define the disorder and are what the diagnosis usually is based on (Rauch and Glorieux, 2004, Goldberg, 2020). Other common skeletal features are osteoporosis, multiple fractures, bowing of long bones and shorter stature when compared to peers. In moderate to severe types of OI, morbid skeletal features are manifested. These include scoliosis, kyphosis (Figure 1.6), vertebral compressions, small head circumference, and chest wall pathology. As the disease progress in severity, immobilisation becomes eventually evident (Renaud *et al*., 2013, Marini and Cabral, 2018). Prevalent non-skeletal features include joint hypermobility, hearing impairment and blue sclera (Figure 1.6). In a few types of OI, teeth can also be affected, namely by Dentinogenesis Imperfecta (DI), as it is considered a mineralised tissue (Rauch and Glorieux, 2004, Marini and Cabral, 2018, Do and Marini, 2020).

Type I OI is known to be the most common type and patients of type I OI are known to exhibit the mildest form of the disorder (Marini and Cabral, 2018, Do and Marini, 2020). Commonly, symptoms develop later in life, with no prenatal or at birth manifestations. These patients present with osteoporosis, bowed legs and are known to be shorter than peers of the same age. Blue sclera is highly associated with type I OI. However, Dentinogenesis imperfecta is less common in type I OI (Rauch and Glorieux, 2004, Marini and Cabral, 2018, Do and Marini, 2020, Goldberg, 2020).

Type II OI is known to be the most severe or lethal type with high perinatal mortality rates. As for type III, it is considered the second most severe form of OI. Although type III is not associated with high mortality rates, it is considered to be extremely morbid (Do and Marini, 2020). The defects in type III OI extend beyond skeletal deformities to include respiratory abnormalities and neurological deficits. Patients of OI type III experience fractures from mild daily life activities, have skeletal deformities as scoliosis and tend to mobilise using a wheelchair from an early age. Craniofacial features commonly include blue sclera, flattening of the middle third of the face and Dentinogenesis Imperfecta. Type IV on the other hand, is thought to be less severe than type III yet both have common features. This includes scoliosis and multiple fractures. In this type, presence of Dentinogenesis Imperfecta is thought to be variable (Renaud *et al*., 2013, Do and Marini, 2020).

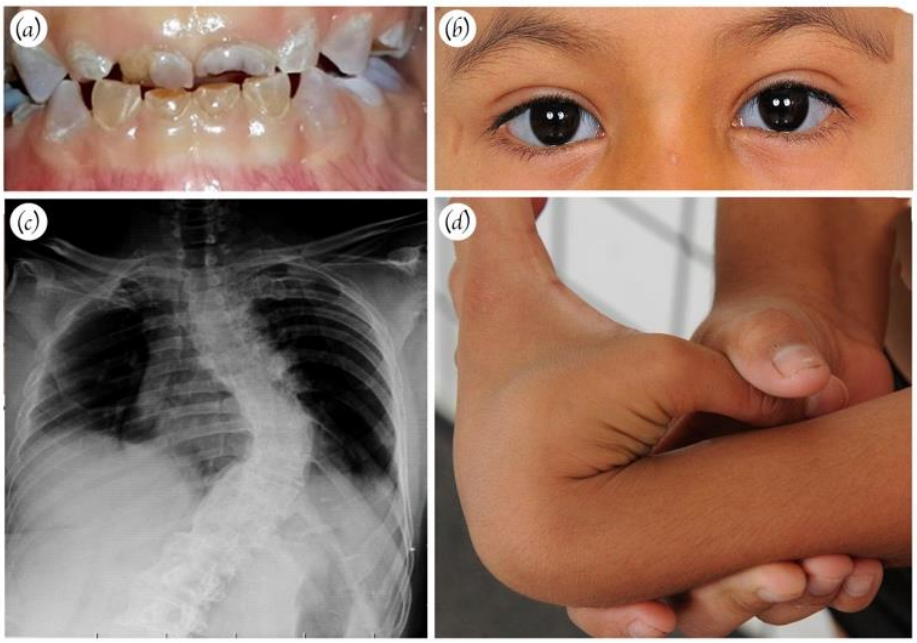

Figure 1.6: (a) DI in primary teeth (Akhlaghi et al., 2016). (b) OI bluish grey sclera (King C, 2018). (c) Bone deformity (Kyphoscoliosis) in an OI patient (T.R and L.J, 2005). (d) OI joint hypermobility (King C, 2018)

Diagnosis of Osteogenesis Imperfecta

The disorder can be diagnosed simply by patients exhibiting phenotypic traits, those as mentioned previously, especially in case of a positive family history. Presence of Dentinogenesis Imperfecta or blue sclera in conjugation to joint hypermobility or history of multiple fractures is another way to reach the diagnosis (Rauch and Glorieux, 2004). Unlike EDS, OI doesn't have an international diagnosis point system to set at least a provisional diagnosis (Rauch and Glorieux, 2004, Malfait *et al*., 2017). In addition, in cases that lack apparent skeletal deformities, investigation is required to reach a diagnosis. DNA sequencing is thought to be the most accurate way of diagnosing OI with 90% accuracy in sequencing COL1A1 and COL1A2 genes (Rauch and Glorieux, 2004, Bodian *et al*., 2009). Osteogenesis imperfecta and Dentinogenesis Imperfecta can be manifestations of the same disease, yet their diagnosis process is not the same.

1.1.4 Bone Versus Dentin

Bone and dentin have individual characteristics that they do not share. Most importantly is that bone exhibits continuous turnover and while dentinogenesis is a life-long process, the layers already secreted does not undergo remodelling. A second variation is the difference in tissue forming cell's location. Odontoblasts continue to lay dentin matrix while pushing themselves away from the dentino-enamel junction, extending their processes only, to

remain in the future dentin. The odontoblasts body resides in the pulp chamber through life. On the other hand, in bone formation, osteocytes are entrapped into the bone matrix, to be finally fossilized by the mineralization process (Opsahl Vital *et al*., 2012).

Bone and dentin also have many similarities, starting from formation to the final structural outcome. Both are formed by cells that secrete an extracellular matrix, which mainly consists of type I collagen, that then undergoes mineralisation. The inorganic component of both is hydroxyapatite in a crystalised form. The process is initiated by laying an organic matrix secreted by osteoblasts and odontoblasts in bone and dentin, respectively. The collagen fibres then form a highly organized fibre scaffold ready for mineralisation (Opsahl Vital *et al*., 2012). Given these similarities and because the basis of OI pathophysiology is collagen deformation, teeth can be affected in patients of OI, resulting in Dentinogenesis Imperfecta (Figure 1.6). In Osteogenesis Imperfecta where the mutation is in type I collagen, Dentinogenesis Imperfecta is considered to be a greatly penetrant trait where most carriers of collagen type one gene mutation have Dentinogenesis Imperfecta (Pallos *et al.*, 2001).

1.1.5 Dentinogenesis Imperfecta

Dentinogenesis

Dentinogenesis is the process of dentin formation by dentin forming cells or odontoblasts (Figure 1.7). Firstly, the process starts when odontoblasts are differentiated from precursor-mesenchymal cells, and they form a layer surrounding the pulp. The cells then secrete a layer of unmineralized matrix called pre-dentin (approximately 10-40 µm), this first deposition is dominated by thick type III collagen fibres. As the odontoblasts push themselves away from the dentino-enamel junction while extending their processes, they continue laying down a matrix yet mostly formed of organized collagen type I (Nanci, 2008).

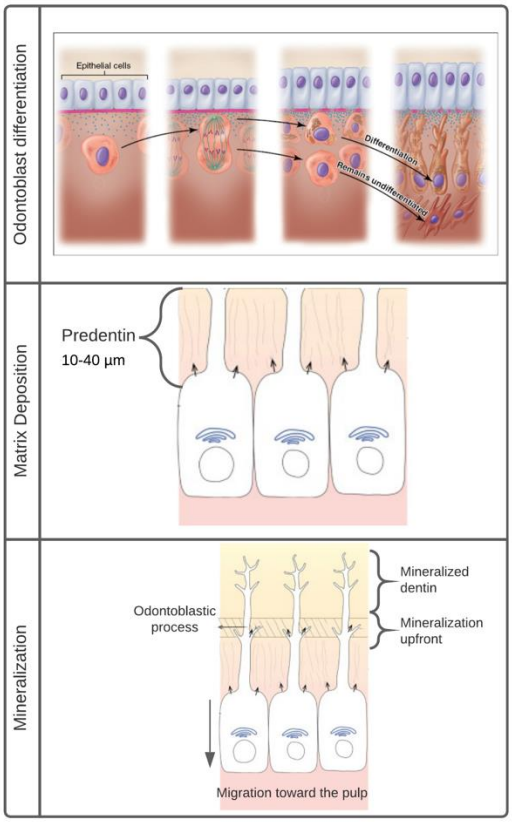

Figure 1.7:Process of Dentin formation (Giacaman et al., 2016)

The role of Dentin Sialophosphoprotein (DSPP) in dentinogenesis, is not yet confirmed. It is thought that the proteolytic event that results in the breaking of DSPP is an activation event to initiate mineralization of the dentin matrix as shown in Figure 1.8 (Zhu *et al.*, 2012). Furthermore, the proteolysis results in three molecules; Dentin Phosphoprotein (DPP), Dentin Sialoprotein (DSP),and Dentin Glycoprotein (DGP). The role of these molecules in dentin formation is not clear, however, it was found that DPP may have a role in controlling hydroxyapatite crystals nucleation and growth (George *et al.*, 1996, Barron *et al.*, 2008, Goldberg, 2020). DSP is found to be a dimer forming proteoglycan, but its function is yet to be studied. Lastly, DGP's role is also unknown however, it is suspected to resemble the function of DPP (Barron *et al.*, 2008). After mineralization, and the tooth Is fully formed, the dentin completed is named primary dentin. Other types of dentin are secondary dentin, which increases with aging and tertiary dentin, which forms in response to an insult (Weinstock and Leblond, 1973, Barron *et al.*, 2008).

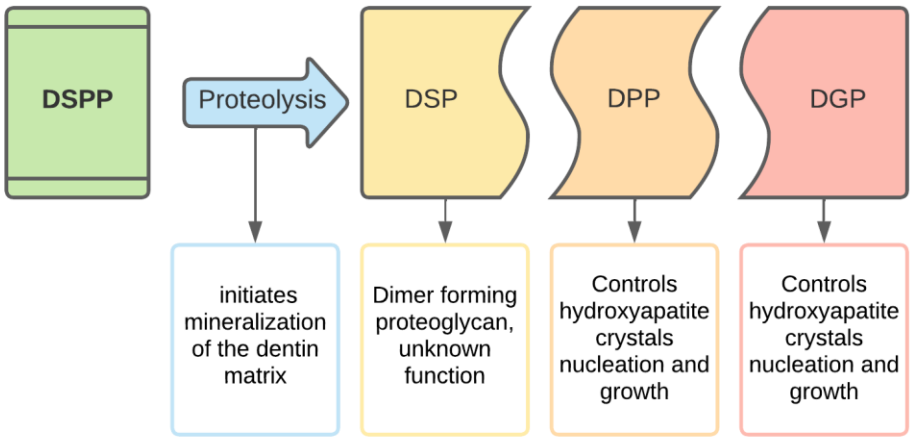

Figure 1.8 DSPP proteolytic products and their function (George et al., 1996, Goldberg, 2020)

In teeth affected by DI, the gene mutation results in defective collagen matrix. Type I DI is known to be caused by a missense mutation in the gene encoding collagen type I. Mutations are found to be specific to COL1A1 and COL1A2 genes encoding alpha-1 and alpha-2 chains, respectively. DI types II and III are caused by a mutation in the DSPP gene. Patients with DI caused by DSPP mutation do not appear to show clinical signs of bony defects (Barron *et al.*, 2008). This may be due to the low quantities of DSPP in bone or the presence of other matrix proteins with a similar role (D'souza *et al.*, 1997, Kim and Simmer, 2007).

Dentinogenesis Imperfecta (DI) or Hereditary Opalescent Dentin, is a genetic disease, in which the mutation affects the dentin structure of either one or both dentitions (Barron *et al.*, 2008). The disease presents with characteristic features of greyish-brown discolouration, pulpal obliteration, crown fractures and accelerated tooth wear (Shields *et al.*, 1973). DI is considered a rare genetic disorder as it is reported to affect 57/100,000 individuals in France, 90/100,000 in India and as formerly reported by Witkop affecting 13-17/100,000 of the total population (Witkop, 1975, Gupta *et al.*, 2011, Cassia *et al.*, 2017). Management of DI can be difficult. The American Academy for Paediatric Dentistry (AAPD) recommends early management of children affected by DI with preventive measures. Aspects of management include tooth structure preservation, and aesthetic improvement (Council, 2013). Studies recommend placement of stainless-steel crowns over primary molars and composite restoration build ups for anterior teeth as incisors and canines (Frassetto *et al.*, 2016).

Classification of Dentinogenesis Imperfecta

The disorder can present as part of a syndrome or isolated. The most well-known classification categorizes DI into three phenotypes: Syndromic DI or type I, typically associated with OI; non-syndromic isolated DI or type II, an isolated form of the disease; and type III is known to be specific to a triracial isolate from Maryland and Washington D.C. (Shields *et al.*, 1973). However, the classification was problematic, as the differentiation between Shields types I, II and III was unclear. In 1989, a different classification reported that Shields types II and III were different phenotypes caused by the same type of gene mutations. Therefore, they indicated type I Shields as DI, Type II Shields as Hereditary Opalescent Dentin and Type III Shields as Brandywine Isolate form of type II, as shown in Table 1.3 (Witkop Jr., 1988). In 2008, a study by Barron *et al*. reviewed classification and found that the classification adopted by the Online Mendelian Inheritance in Man (OMIM) abandoned Type I DI and only defined types II and III. Accordingly, it necessitated that although Shields' and Witkop's classifications were incomprehensive, they were the most valid classifications. In 2015, Dure-Mulla *et al.* revised the classification of isolated DI, and a similar disorder named Dentin Dysplasia. In their review, it was suggested that types II and III DI are the same condition with variable severity. They denoted type II as Moderate DI, and type III as Severe DI. As for the mild severity, they suggested that type II Dentin Dysplasia is not a separate condition but rather a mild form of isolated DI (de La Dure-Molla *et al.*, 2015). In this study type I DI is referred to as syndromic DI and type II and III DI are referred to as isolated DI.

Table 1.3: Dentinogenesis imperfecta classification, clinical representation, and associated gene mutation (Shields et al., 1973, Witkop Jr., 1988, Barron et al., 2008, de La Dure-Molla et al., 2015)

Shields 1973	**Witkop 1989**	**Barron *et al.* *2008***	**De La Dure-Molla M *et al.* 2015**	**Clinical presentation**	**Associated gene**
Type I DI / DI associated with OI / syndromic DI	Dentinogenesis Imperfecta	DGI-I	-	- Dentition discoloration - Progressive pulpal obliteration	COL1A1 COL1A2
Type II DI	Hereditary opalescent dentin	DGI-II	Moderate isolated DI	- Dentition discoloration - Crown constriction	DSPP
Type III DI	Brandywine isolate	DGI-III	Severe isolated DI	Shell teeth with enlarged pulps	DSPP

Clinical Presentation

Teeth affected by DI clinically can appear normal in shape with an opalescent amber hue (Figure 1.7) and frequently chipped enamel. This clinical presentation is common across all types of DI with variable degree of severity and expression (MacDougall *et al.*, 2006). The aetiology behind enamel wear in DI is thought to be weakness in the dentin body, resulting in separation between the mantle and circumpulpal dentin rather than abnormal DEJ (Sunderland and Smith, 1980, Levin *et al.*, 1980, Schwartz and Tsipouras, 1984). The exposed dentin then undergoes attrition that ranges from small wear facets to worn crowns to a gingival level (Figure 1.9).

In both isolated DI and syndromic DI, primary and permanent dentitions can be affected, with the latter exhibiting milder forms of the disease. Radiographically, teeth in syndromic DI are reported to have short and constricted roots. Obliteration of the pulp due to dentin hypertrophy is another pathognomonic trait, which can be seen early in developing teeth prior to eruption (MacDougall *et al.*, 2006, Barron *et al*., 2008). Similarly, in isolated DI teeth are characterized by conical roots and pulpal obliteration. However, in this type, bulbous crowns are frequently seen, which can be attributed to the marked constriction near the cervical line (Witkop and CJ JR, 1975, Barron *et al.*, 2008, de La Dure-Molla *et al*., 2015). “Shell teeth” is a term to describe teeth affected by the Brandywine isolated DI. In contrast with the previous two types, teeth of this racial isolate exhibit a marked reduction in dentin structure and pulpal enlargement, hence the prescription. This results in an increased incidence rate in pulp exposure, a characteristic finding in type Brandywine isolated DI (MacDougall *et al*., 2006, Barron *et al*., 2008, de La Dure-Molla *et al*., 2015).

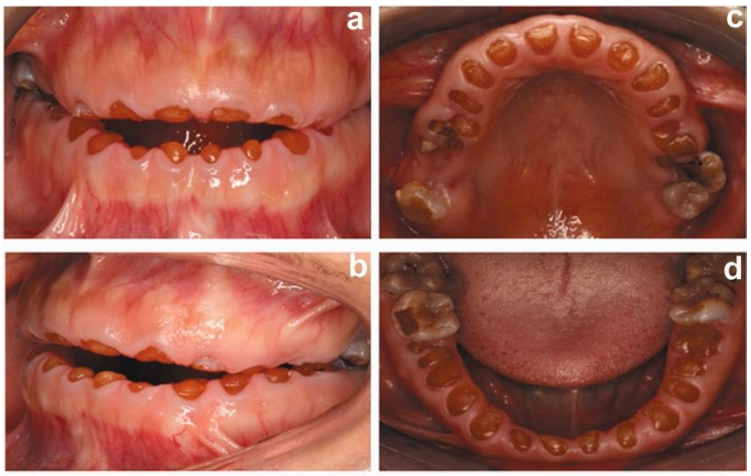

Figure 1.9: Dentinogenesis imperfecta phenotype. Yellowish brown hue of the teeth with severe wear (a-d). (Duan et al. 2016)

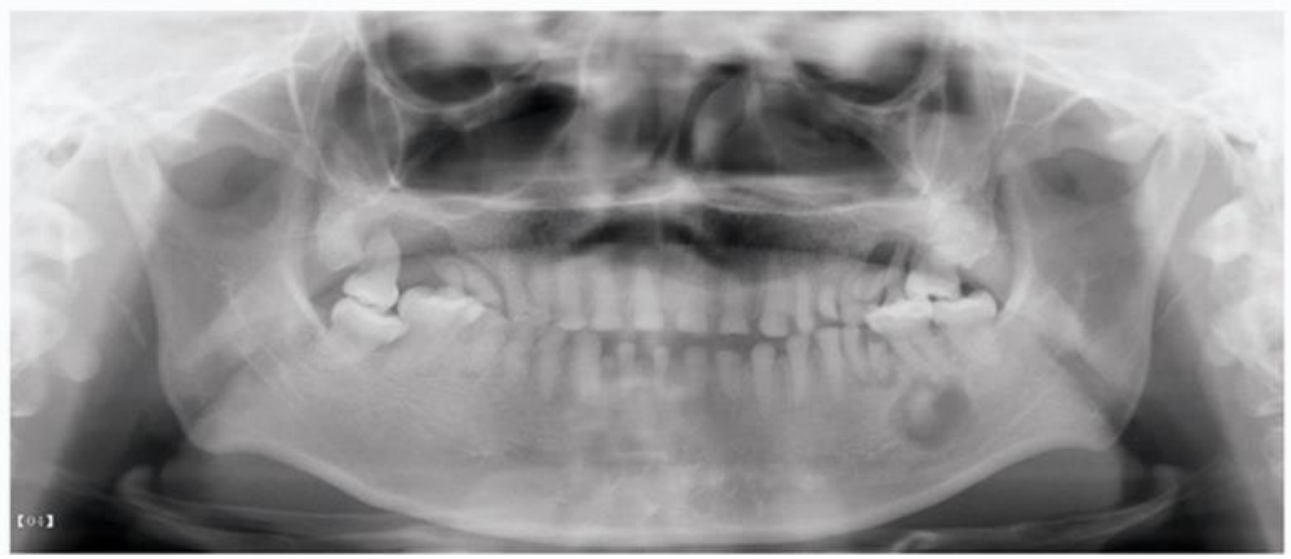

Figure 1.10:Panoramic radiograph of DI patient showing pulpal obliteration of all teeth (Duan et al. 2016).

The features observed in Dentinogenesis Imperfecta are because 90% of the organic components of dentin is made of collagen type I (Breschi *et al.*, 2018). It not only provides tensile strength to withstand forces but also forms a scaffold for mineral deposition. Non-collagenous components are also essential in the formation of a well-structured dentin. Dentin Sialophosphoprotein or DSPP constitutes about 90% of the non-collagneous components and is thought to have an important role in the biomineralization of the dentin matrix (Yamakoshi, 2009).

Aetiology of Dentinogenesis Imperfecta

Although the causative gene identity and type of mutation are known, the pathophysiology of DI is still poorly understood. It has been hypothesized that the aetiology of DI is due to is poorly differentiated odontoblasts, resulting in odontoblasts that are unable to produce fine collagen fibres (Herold, 1972). This theory was also supported by another study showing that odontoblastic cell final differentiation from mesenchymal cells is known to be signaled by collagen, collagenous proteins such as fibronectin and growth factors. Due to defective collagen metabolism, signaling is interrupted leading to differentiation of dysfunctional odontoblasts that secrete the defective dental matrix (De Coster *et al.*, 2007). Another theory suggested that defective collagen expression, results in overproduction of defective procollagen, which gets accumulated in the odontoblasts, changing their morphology into dilated dysfunctional cells. After the defective matrix is secreted and mineralized, the cells are entrapped, showing features of curved tubules and fossilized cells (Hall *et al.*, 2002).

Ultrastructural collagen defects in the bone of Osteogenesis Imperfecta have been extensively studied. The glycine substitution causes a mutation in the C-terminal region which has a destabilizing effect on the triple helix. The helix folding is interrupted, and the N-terminal cleavage site is damaged, which leads to the formation of uncleaved N-terminal procollagen (pN-collagen). The resultant fibrils were seen to have a reduced diameter and weakened integrity (Makareeva *et al.*, 2006). This also resembles a substitution mutation in pro-α1(I) and pro-α2 (I) near the C-terminal. The end product is incorporation of uncleaved C-terminal procollagen (pC-collagen), which leads to compromised fibril structure. Furthermore, substitution of arginine with a cystine base was found to cause increased intra-helical bisulfide bonds. These bonds lead to triple helix kinking and their fully formed fibrils were wider in diameter, however less dense than normal fibrils (Cabral *et al.*, 2007).

1.1.6 Extra-dentinal Dentinogenesis Imperfecta

DI was thought to mainly affect dentin for both genotypes. In the syndromic genotype, dentin is mostly affected since dentin is known to be 90% composed of collagen type 1 (Breschi *et al*., 2018). It was additionally thought that the dentino-enamel junction (DEJ) was unaffected, and therefore enamel detachment was due to a defect in the dentin matrix underlying the DEJ (Hall *et al*., 2002, Majorana *et al.*, 2010). However, studies found that the DEJ lacked the physiologic scalloping in DI affected teeth, reducing the surface area of interdigitation, weakening the junction, and subsequently leading to enamel loss (Devaraju *et al.*, 2014). Furthermore, studies reported that other dental tissues, as enamel, can demonstrate structural and mechanical defects in a way similar to dentin (Hall *et al*., 2002, Majorana *et al.*, 2010, Orsini *et al.,* 2014). It was found that enamel of diseased teeth exhibited reduced hardness and elasticity when compared to normal teeth (Budsamongkol *et al.*, 2019). Enamel structural defects were also reported. They included abnormal and smaller enamel rods (Budsamongkol *et al.*, 2019) and wider enamel lamellae (Hall *et al.*, 2002).

In the other genotype, the cause of defective dentin is abnormal DSPP. As previously mentioned, Dentin Sialophosphoprotein (DSPP) is a non-collagenous dentin matrix protein essential for dentin formation, which is where it is primarily expressed (Goldberg *et al*., 2011). DSPP is known to be secreted by odontoblasts, but it is also secreted by pre-ameloblasts during early development (Ritchie *et al*., 1994, Bègue-Kirn *et al.,* 1998). However more recently, the matrix protein was also detected in other dental tissues as enamel, cementum and periodontium, and non-dental tissues as bone (Baba *et al.*, 2004, Jing *et al.*, 2021). In dentin, DSPP function is not fully known but it is known to serve a regulatory function in dentin mineralization (Suzuki *et al*., 2009). Although this protein is not

necessary for the biological development of enamel, studies report that its presence was detected in enamel forming cells, more specifically precursor cells, namely pre-ameloblast cells (Jing *et al*., 2021). In addition, malformation in DSPP at these early stages, was found to cause enamel defects.

1.1.7 Cementum

Normal cementum is formed of both organic matrix and inorganic minerals, with the latter being hydroxyapatite crystals. The organic matrix is known to be entirely made of type I collagen in human teeth, and types I and III in bovine teeth (Yamamoto *et al*., 2016). The organic matrix additionally includes cementum proteins that are non-collagenous in nature. The two main proteins are bone sialoprotein, the cementum equivalent of dentin Sialophosphoprotein (DSPP) and osteopontin (Yamamoto *et al*., 2016). The previous reports demonstrate how DI can affect dental tissues other than dentin, and as demonstrated, enamel in DI affected teeth had abnormal enamel rods, less mechanical strength, and weaker dentino-enamel junction. However, the effect on cementum is less clear. Cementum is a vital part of the tooth support and anchoring system and understanding the morphological and ultrastructural defects caused by DI can lead to better understanding of the occasional periodontal involvement.

In DI, studies on the resultant dentin deformity have mainly focused on the macrostructure of collagen. Studies have reported an increased thickness of syndromic DI dentinal collagen diameter (Ranta *et al.*, 1993, Orsini *et al.*, 2014). Others reported random organization and disoriented of collagen fibres (Hall *et al.*, 2002, Majorana *et al.*, 2010). More recent studies found that the defect was in collagen quantity and reported a decrease in collagen fibres (Budsamongkol *et al.*, 2019, Intarak *et al.*, 2020, Nutchoey *et al.*, 2021). However, the literature is lacking papers that examine ultrastructural dentin collagen defects, especially when compared to studies on dermal or skeletal collagen. Thus, microscopic changes in collagen remain unknown, in addition to how these changes relate to differences in dentin mechanical properties. Dentin is known to be a mineralized tissue with collagen forming most of its organic compound. The defect in dentinal collagen defies the breakthroughs in adhesive dentistry. Discovering the damage in dentinal collagen and knowing the ultrastructural defects caused by the disorder, can be the first steps to prevent its morbid dental complications.

1.2 Aim

The overall project aims to examine and characterize collagen defects on ultrastructure levels, in teeth affected by isolated Dentinogenesis Imperfecta and syndromic DI. The aim of the systematic review is to report all known dentinal defects, with special interest in ultrastructural collagen defects. Lastly, the scoping review aims to assess the extent of the literature on the effect of dentinogenesis imperfecta of both isolated and syndromic DI, on cementum collagen if present and describe the topographic, compositional, and ultrastructural changes

1.3 Objectives

- Examine dentinal and cemental collagen quantity, orientation, and organization
- Examine dentinal and cemental collagen ultrastructural defects including triple helix, D-banding periodicity, and fibrils deformation
- Study the effect of structural abnormality on mechanical strength
- Study the effect of abnormal cemental matrix formation on mineralization defects as hyper or hypo-mineralization

2 A Systematic Review of Morphological and Ultrastructural Collagen Defects: Impact and Implications in Dentinogenesis Imperfecta

2.1 Methodology

2.1.1 Search Strategy

The chosen databases were OVID Embase, Cochrane Library, and Medline. In addition to, Google Scholar search engine for free hand search.

In June 2020 the databases were searched for ultrastructural collagen defects in teeth of Dentinogenesis Imperfecta and Osteogenesis Imperfecta. However, terms were divided into four main categories: Dentinogenesis Imperfecta, Teeth, Collagen and Osteogenesis Imperfecta.

- **Level one:** The former two categories were fixed in every search, and they accounted for level 1 in every search phrase construction.
- **Level two:** adding either one of the subcategories, subdomain or Osteogenesis Imperfecta terms. The subdomain group contains search words that are either related to the search question or can fit in all groups, words as genotype, disorder, and mutant.
- **Level three:** was collagen terms (Figure 2.1).

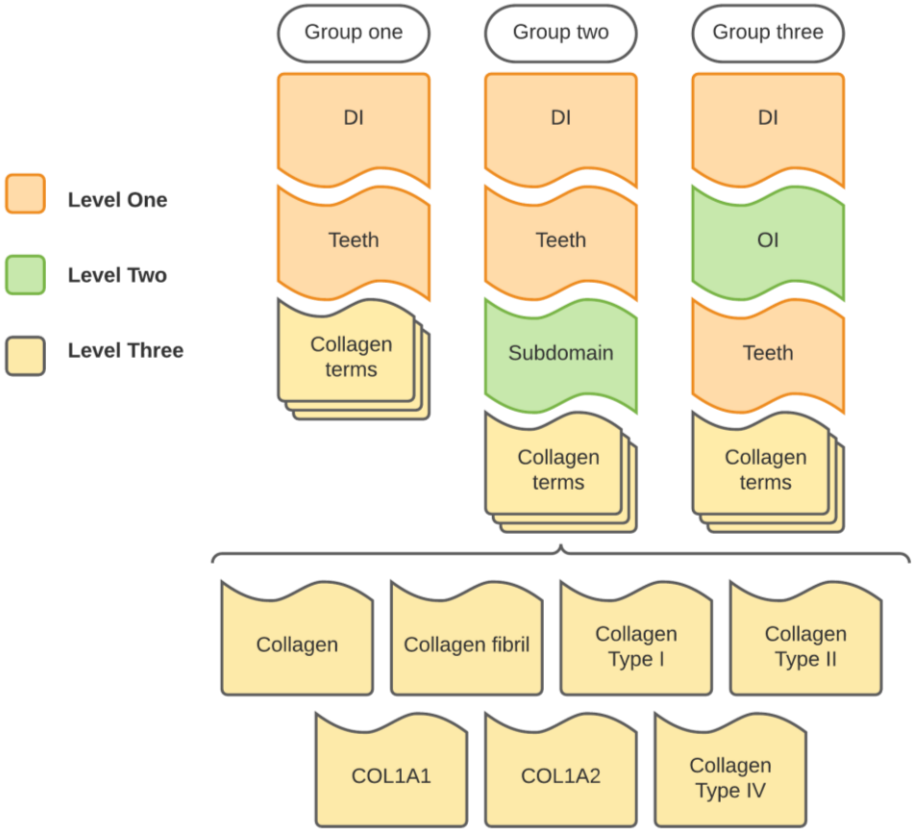

Figure 2.1:Search terms levels and grouping

2.1.2 Search Terms

Search words were obtained by multiple methods. Firstly, controlled vocabulary in PubMed were found by using the Medical Subject Headings (MeSH) search engine. The terms Dentinogenesis Imperfecta, Osteogenesis Imperfecta, collagen and tooth were used. In all four MeSH searches, no subheadings were chosen to refine the search. DI MeSH search terms yielded thirteen other wordings, all of which were added to the free text terms of DI. Osteogenesis Imperfecta also had thirteen phrases in its MeSH Search terms, these were added to the OI terms for free text search. In regard to collagen, no other phrases were selected from the entry terms as they were unrelated to our topic. However, four expressions were taken from the collagen MeSH tree that were thought to be related to our search. These were collagen fibril, collagen type I, collagen type II and collagen type IV. In the last term to search tooth, no other terms were selected as all were likely to limit the search to certain teeth rather than the entire dentition.

Similarly, in OVID Embase, controlled vocabulary was selected by mapping the term to subject heading. The terms Dentinogenesis Imperfecta, Osteogenesis Imperfecta, collagen and tooth were used. In the first two, neither broader nor narrower terms were selected as they were unlikely to refine the search. DI mapping resulted in a subject heading named Tooth Malformation, two out of twenty-eight terms, Capdepont tooth and opalescent dentin, were chosen and found to be related to our search. OI headings search resulted in an OI subject heading. All terms used for OI were added to its free text search terms (Table 2.1). After that, collagen mapping had forty-two subject headings. The subject heading with “collagen” only was chosen. Four narrower terms were selected: collagen fibril, collagen type I, collagen type II and collagen type IV. Lastly, tooth search resulted in more than fifty Subject headings, only “tooth” heading was chosen, and the narrower term dentin. OVID Medline subject heading search was comparable to OVID Embase.
After search terms collection, each group’s terms were refined according to the data base used in. That was done by using the term to conduct a search and whenever a term yielded zero results, it was excluded from search terms in that data base. Lastly, to include alternative forms of words, an asterisk was utilized (Table 2.1).

Subject headings and free text words were further refined for use in the search concepts by project team members. Further terms were identified and tested from known relevant papers. Two phrases were added to DI, Dentin Sialophosphoprotein and dentin dysplasia.

Similarly, two search words were added to collagen group, COL1A1 and COL1A2, while OI terms had no further additions. The search was reviewed by an Information Specialist with the aid of UCL library services. Please see Appendix [A] for full search strategies.

Table 2.1 Categories of search terms and their data base.

	Database	DI	OI	Collagen
PubMed	Controlled vocabulary (MeSH terms)	Capdepont Teeth Dentinogenesis Imperfecta Opalescent Dentin	Brittle Bone Fragilitas Ossium Lobstein* Disease Osteogenesis Imperfecta	Collagen
	Free text terms	Capdepont teeth Capdepont tooth Dentinogenesis Imperfecta Dentin* Dysplasia Dentin* Sialophosphoprotein Opalescent Dentin* Opalescent teeth/ tooth	Brittle bone Bruck syndrome* Fragilitas Ossium Fibrogenesis imperfecta ossium Lobstein* Disease Osteogenesis Imperfecta Osteopsathyrosis*	Collagen Collagen fib* COL1A1 COL1A2 Collagen type Ⅰ Collagen type Ⅱ Collagen type Ⅳ
OVID	Controlled vocabulary (MeSH terms)	Dentin* Dysplasia Dentinogenesis Imperfecta	Osteogenesis Imperfecta	Collagen Collagen type Ⅰ Collagen type Ⅱ Collagen type Ⅳ
	Free text terms	Dentinogenesis Imperfecta Dentin* Dysplasia Dentin* Sialophosphoprotein Opalescent Dentin* Opalescent teeth Opalescent tooth	Brittle bone Bruck syndrome* Fragilitas Ossium Fibrogenesis imperfecta ossium Lobstein* Disease Lobstein* syndrome Osteogenesis Imperfecta Osteopsathyrosis*	Collagen Collagen fib* COL1A1 COL1A2 Collagen type Ⅰ Collagen type Ⅱ Collagen type Ⅳ
Teeth	MeSH teeth abnormality	Teeth tooth dentin*		
Subdomain	bond, mutant, disorder, genotype			

2.1.3 Search Terms Grouping

When conducting the search, three word-groupings were formulated for all data bases while accounting for their own search terms (Figure 2.1).

Group one search had DI and teeth as a base then these were combined to add in seven individual queries with the seven collagen search terms, collagen, collagen fibril, collagen type I, collagen type II, collagen type IV, COL1A1 and COL1A2 (Figure 2.1).

Group two search, after DI and teeth, had an additional query added, subdomain. These are: bond, mutant, disorder, and genotype, in an attempt to refine the search result. Then, similarly to group one, seven individual queries were done with each term in the collagen group.

Group three, OI terms were added firstly to DI then teeth. After that, the seven collagen queries were done as previously.

As a base, when terms were searched, both free text and MeSH were combined by Boolean operator OR. For example, the base of Dentinogenesis Imperfecta search was

(((((((Dentinogenesis Imperfecta[Text Word]) OR Opalescent Dentin*[Text Word]) OR Opalescent tooth[Text Word]) OR Opalescent teeth[Text Word]) OR Capdepont Teeth[Text Word]) OR Capdepont Tooth[Text Word]) OR Dentin* dysplasia[Text Word]) OR Dentin Sialophosphoprotein[Text Word]) OR Dentinogenesis Imperfecta[MeSH Terms]

The reason behind that is that in group one, across all data bases, each term was searched with and without subject headings, and the outcome was that using MeSH terms resulted in a higher number of results thus, it was decided to continue using free text term with corresponding MeSH heading joined by a Boolean operator “or”.

The results of the database searches were stored, and duplicates removed in an EndNote library. Further relevant studies were sought by citation searching (forwards and backwards) of the included studies. Search engines used for citation screening were Google scholar, ResearchGate and PubMed search engines. Numbers of citation for a study varied between engines thus, articles were screened and cross-referenced for relevance. Finally, Hand searches of Google scholar and UCL Explore search engine were carried out, yet new relevant studies were not found.

2.1.4 Selection

Inclusion criteria

The population included were human teeth, both of primary and permanent dentition, of patients with DI ± OI (Table 2.2). The aim of the study necessitates the inclusion of collagen defect examination and description. Although this study focused on the ultrastructural defects in dental collagen, as cross-banding, other defects of size, shape, and density were included. Patients' age was not limited to a range. At the first stages of articles selection, the date of publication was not limited to an interval, as well as articles that examined animal tissues. This was intended to look for and include studies as much as possible from the literature and was changed when the number of articles obtained was not affected by these criteria. Therefore, the publication date was changed to 1990 to present and only human teeth were accepted. Our review was interested in studies based on collagen defects in isolated DI and syndromic DI teeth examined with any assessment method. This includes, Light Microscopy (LM), Scanning electron microscopy (SEM), Atomic Force Microscopy (AFM), Transmission Electron Microscopy (TEM), Micro-computerized tomography, high-resolution synchrotron radiation tomography (SRCT) and confocal laser-scanning microscopy (CLSM). No studies were excluded by study design. Therefore, studies as case reports, review, systematic review or laboratory studies, published or conference paper were all included. Finally, only papers written in English were included or translated to English.

Table 2.2:Inclusion and exclusion criteria

	Inclusion Criteria	Exclusion criteria
Population	Human Primary and permanent dentitions of patients of all ages and both genders with DI ± OI	Animal teeth studies
Outcome	Dentinal Collagen defects	Generalized dentin studies with no specification on collagen Non-dental collagen
Study design	All including reviews, case reports, and laboratory studies.	None.
Publication date	From 1990 to 2021	Earlier than 1990
Study language	English or translated to English	Non- English studies

Exclusion Criteria

Reasons for exclusion were mainly, studies unrelated to teeth, unrelated to collagen, of dentin pathology other than DI, regenerative studies and dentinogenesis studies (Table 2.2). Examples of papers unrelated to teeth were, articles that only reported genetic background of DI, articles about OI only, and papers that tested dermal or skeletal collagen. Furthermore, unrelated to collagen Dentinogenesis Imperfecta studies means clinical and radiographic descriptive studies, genetic mutations of DI, treatment trials and studies of generalised dentin defects or dentinal tubules. Dentin disorders unrelated to DI were excluded, as dentin dysplasia and amelogenesis imperfecta. Lastly, regenerative studies on dental tissues, dental pulp studies and dentin formation papers were all excluded. At the end stages of article selection, papers older than 1990 were excluded to limit the results to a period of 30 years, in addition to the exclusion of animal studies or papers that examined animal tissues as rat teeth or bovine dentin.

2.1.5 Data extraction

A data extraction form was developed from the Cochrane Library form, *Effective Practice and Organisation of Care (EPOC) Data collection form* (Cochrane, 2013).

Tables of unrelated content, i.e. intervention groups, were deleted. Furthermore, necessary tabs were added for detailed methodology recording (Appendix B)

2.2 Results

2.2.1 Article Selection

The database searches identified 1,689 records. Once duplicates were removed there were 376 records (Figure 2.2).

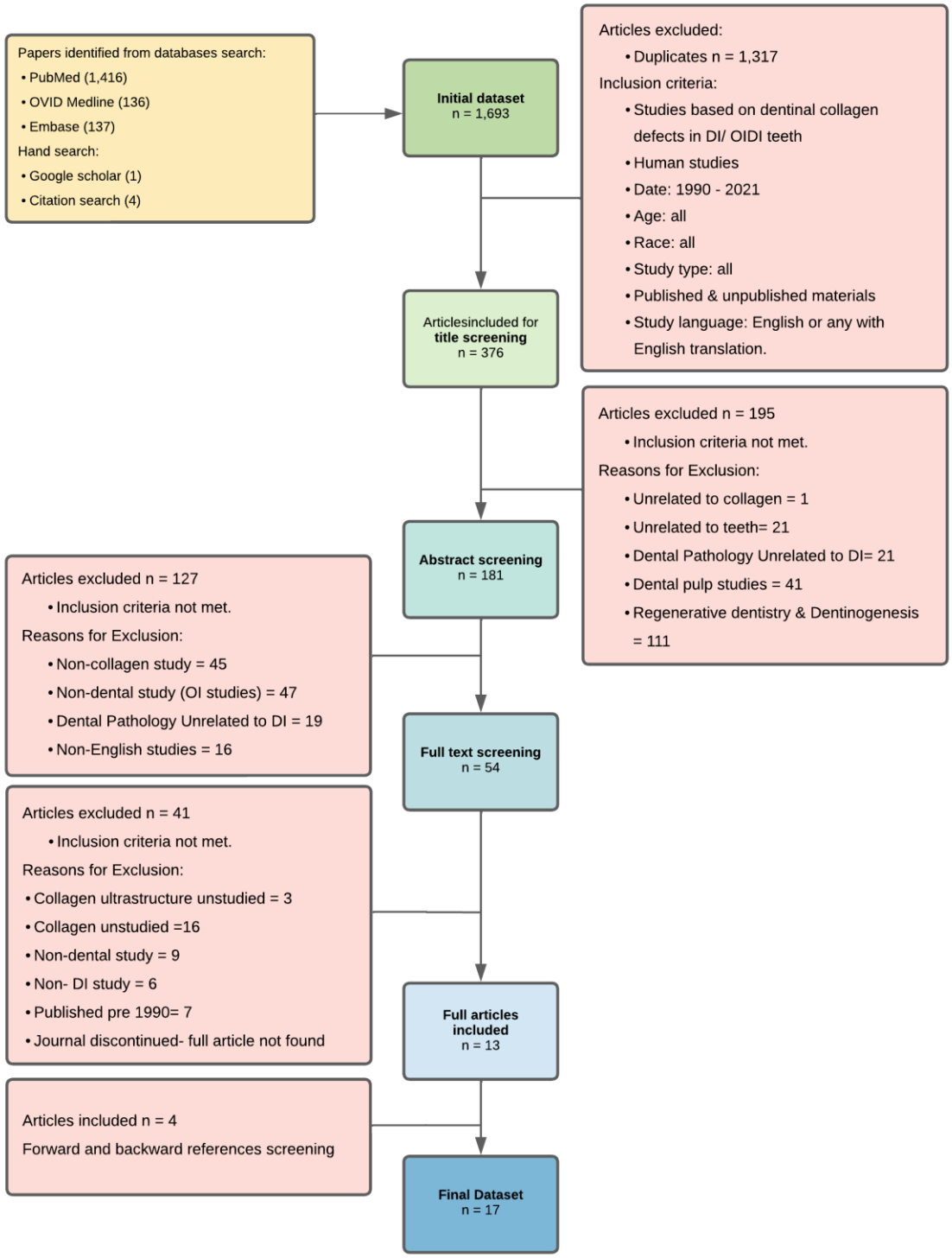

Figure 2.2:CONSORT (Consolidated Standards of Reporting Trials) flow diagram of article selection process

The identified 376 papers were screened for exclusion by title. 195 studies were excluded for the reasons mentioned in Box 1. Then, 181 papers were screened by abstract, of which 54 were included. Reasons for exclusion are shown in (Table 2.3) Non-English articles that did not have a translated version were excluded at this stage.

Full articles of fifty-four studies were retrieved for the final stage of screening. Three exclusion criteria were common to abstract screening level (Table 2.3). Other reasons for exclusion were studies that examined DI affected teeth solely on radiographical basis and genetic background-basis. At this stage, publication date, was added as a new exclusion criterion. All studies published before the 1990's were excluded and the included studies were 13. Finally, using forward and backward citation screening, four studies were found to fulfil the inclusion criteria and were added to the final dataset, reaching 17 articles.

Box 1. Reasons for Article Exclusion at Tile Level:

- Studies unrelated to collagen
- Studies unrelated to teeth
- Dental Pathology unrelated to DI.
- Dental pulp studies
- Regenerative dentistry & Dentinogenesis

Table 2.3:Numbers and reasons of articles excluded by common reasons

Number of papers Excluded by abstract	Reasons for exclusion	Number of papers Excluded by full article
45	Studies unrelated to collagen	16
47	Non-dental studies (OI related)	9
19	Dental pathology unrelated to DI studies	6
16	Articles not translated to English	0

Overview

The 17 included articles (Table 2.4) were divided based on the type of DI (isolated and syndromic DI), then subdivided based on type of Osteogenesis Imperfecta. Five papers reported on isolated DI. Nine papers studied DI affected teeth in patients of type I OI, eight papers examined type IV OI and DI and five studies on type III OI and DI. Four papers did not classify the type of OI for samples. As there was an overlap between studies, a Venn diagram was used to clarify the duplicate number of articles as shown in Figure 2.3.

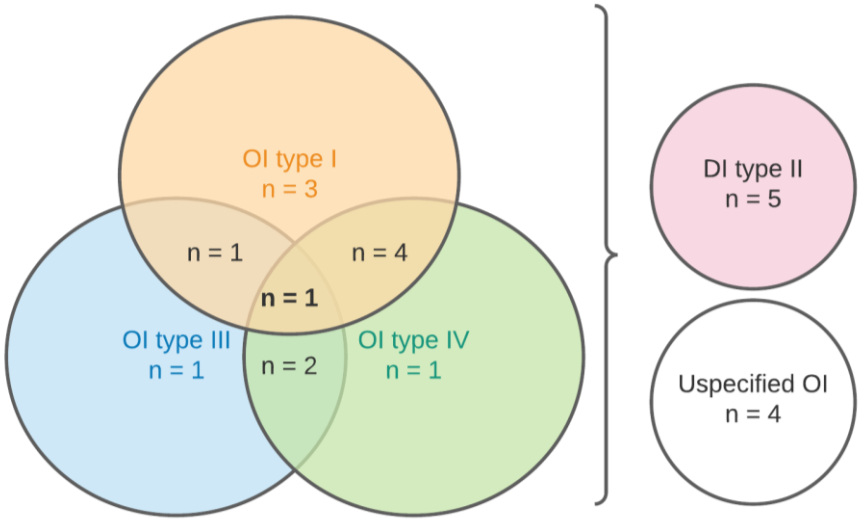

Figure 2.3:Distribution of studies based on types of Osteogenesis Imperfecta where n= number of papers

The most frequent criteria used for assessment were fibrils organization and collagen diameter, accounting for approximately 70%(12/17) and 65% (11/17) of the studies, respectively. Fibre shape, structure, and orientation was described by 35% (6/17) of the papers, followed by 29% of the studies (5/17) covered fibre quantity, and presence of collagen type III. Other less commonly used parameters were collagen density, cross-banding, physical properties such as dentin hardness and elasticity, and presence of other types of collagen. Second to collagen type I, collagen type III was more commonly tested in studies than types VI and IV, accounting for 29% vs 11% and 5%, respectively (Figure 2.4).

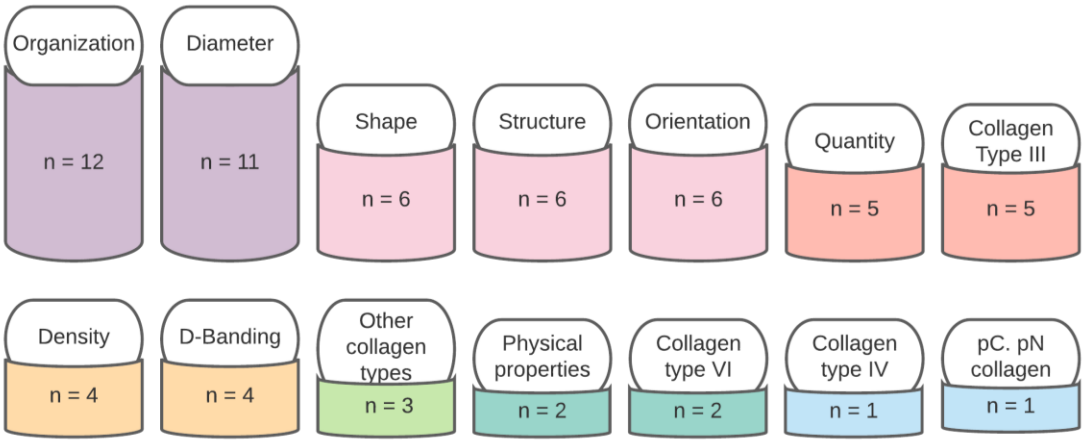

Figure 2.4: Number of studies covering each parameter

Table 2.4: Included studies, date of publication, type of OI and collagen parameter studies

Author(s)	Date	Study design	Dentition	DI type	OI ype	Parameter studied
Budsamongkol *et al*	2019	Cross sectional	1 primary tooth	Type I	Type III	• Collagen quantity • Structural deformation • Dentin hardness
De Coster *et al*	2006	Cross sectional	Primary and permanent unspecified number	Type I	Types III and IV	• Collagen shape • Collagen density • Type III presence and density
Hall *et al*	2002	Cross sectional	Primary and permanent unspecified number	Types I and II	Types I, III and IV	• Collagen Quantity • Collagen diameter • Collagen orientation • Collagen organization • Structural deformation
Kinney *et al*	2001	Cross sectional	3 permanent teeth	Type II	unclear	• Structural deformation • Collagen organization
Waltimo *et al*	1996	Cross sectional	Primary and permanent unspecified number	Type I	Type I	• Collagen diameter • Collagen shape • Collagen organization • Collagen density • Structural deformation
Waltimo *et al*	1995	Cross sectional	Primary unspecified number	Types I and II	Types I and IV	• Collagen diameter • Collagen shape • Collagen organization • Presence of types III and VI and pC-collagen and pN-collagen
Waltimo	1994	Case series	8 primary teeth	Type I	Types I and IV	• Collagen diameter • Collagen shape • Collagen orientation • Hyperfibres

						• D-banding
Waltimo	1996	Cross sectional	2 primary teeth	Type I	Types I and IV	• Collagen D-banding • SCS (symmetrical collagen segments) • FLS (fibrous long-spacing collagen)
Waltimo, Ranta, Lukimna	1995	Review	Primary and permanent unspecified number	Types I and II	Types I and IV	• Collagen diameter • Collagen orientation • Collagen organization • Types III and IV presence • Structural deformation • Hyperfibres
Ranta *et al*	1993	Review	Unspecified	Types I and II	Types I and III	• Collagen orientation • Collagen diameter • Collagen organization • Type III presence
Majorana *et al*	2010	Cross sectional	7 Primary teeth	Type I	Types I, III and IV	• Collagen orientation • Presence of type III collagen
Ibrahim *et al*	2019	Cross sectional	8 Primary teeth	Type I	Type I	• Collagen D-banding • Collagen organization • Collagen diameter • Physical properties
Orsini *et al*	2014	Cross sectional	Primary unspecified number	Type I	Type I	• Collagen quantity • Collagen diameter • Collagen organization • Presence of type VI
Josic *et al*	2020	Cross sectional	Primary unspecified number	Type I	Unspecified	• Collagen diameter • Collagen organization • Collagen density
Intarak *et al*	2020	Cross sectional	1 primary and 1	Type I	Type IV	• Collagen quantity

			permanent teeth			• Collagen organization
Nutchoey *et al*	2021	Cross sectional	3 primary teeth	Type I	unspecified	• Collagen quantity • Collagen diameter • Collagen organization • Collagen orientation
Duan *et al*	2016	Cross sectional	28 Permanent teeth	Type I	unspecified	• Collagen diameter • Collagen shape • Collagen organization • Collagen density • D-banding • Structural deformation

2.2.2 Collagen Organization

Twelve out of 17 studies (70%) reported on fibres organization with a total of 14 primary teeth and 4 permanent teeth. Seven studies reported on primary teeth, and about half of which declared the number of teeth, n=13 primary teeth *(Waltimo et al., 1994, Waltimo et al.*, 1996, Hall *et al*., 2002, Orsini *et al*., 2014, Ibrahim *et al.*, 2019, Josic *et al*., 2020, Nutchoey *et al*., 2021). Two studies reported on both primary and permanent teeth (Waltimo *et al.,* 1995), and only one specified the number of teeth examined (Intarak *et al*., 2020). They were one primary and one permanent teeth. One study had a sample size of three permanent teeth only (Kinney *et al*., 2001). And two studies did not declare the number of teeth examined (Ranta *et al*., 1993, Duan *et al*., 2016).

The studies only reported on collagen of syndromic DI and the majority described collagen fibres as either haphazardly organized or in a form of abnormal circular bundles (Figure 2.5). In OI type I, fibrils were described in three studies as encircling the dentinal tubules in a transverse, cross-striated pattern (Ranta *et al.*, 1993, Waltimo *et al.*, 1996, Hall *et al.*, 2002). An additional study reported a heterogenous organization of the fibrils giving a completely altered and disorganized meshwork (Ibrahim *et al.*, 2019).

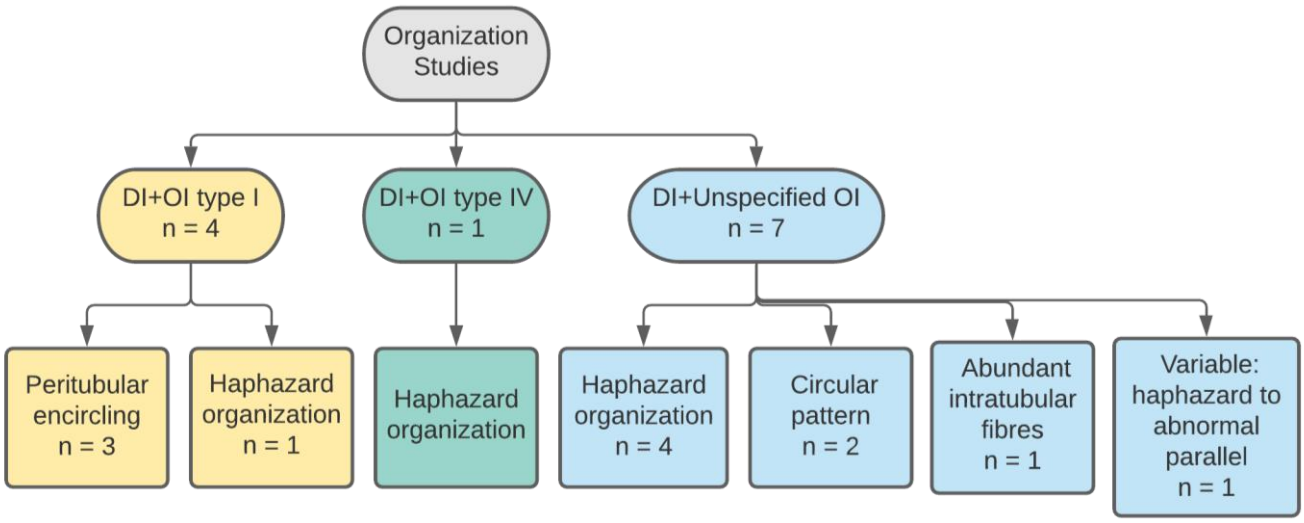

Figure 2.5 Categorization of studies reporting on collagen organization by DI and OI types

Similarly, in unspecified types of OI, DI teeth were found to have 4 patterns. One is altered organization and malalignment of collagen fibres (Figure 2.6-7) (Kinney *et al.*, 2001, Duan *et al.*, 2016, Nutchoey *et al.*, 2021). Another is a characteristic circular pattern. These characteristic fibre bundles were found to be unorganized, and unevenly distributed with large gaps and spaces (Orsini *et al*., 2014, Josic *et al*., 2020). Another study reported lack of bundle formation and instead fibres were either haphazardly arranged or forming an

abnormal parallel pattern (Waltimo *et al.*, 1995). Finally, two distinctive features were also reported where fibres found lacking clear cross-striated pattern and abundant intratubular fibres (Waltimo *et al.*, 1994, Waltimo *et al.*, 1996). In OI type III, one study found an occasional occurrence of parallelly aligned collagen fibres in defective atubular dentin. While in type IV OI on the other hand, fibres were only described as inconsistently arranged (Ranta *et al.*, 1993, Intarak *et al.*, 2020).

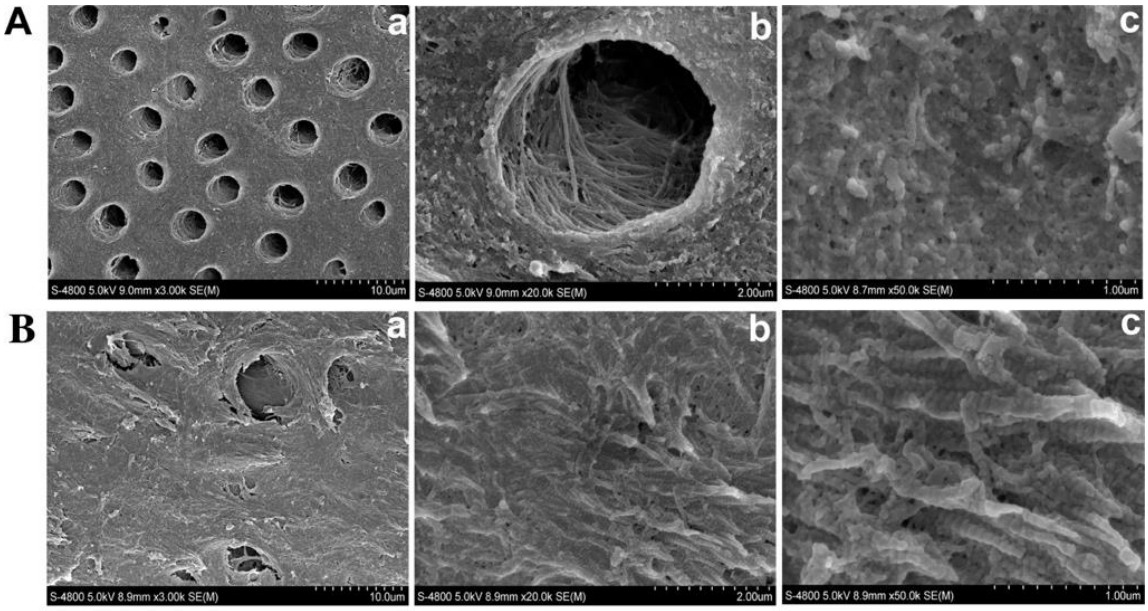

Figure 2.6 SEM image of normal dentin (A) and syndromic DI dentin (B). (A) (a-b) control dentin has regular dentinal tubules and (c) highly mineralized and organized collagen fibres. (B) Defective dentin occluded dentinal tubules (a) irregular organization of exposed collagen (b-c). Duan et al. 2016.

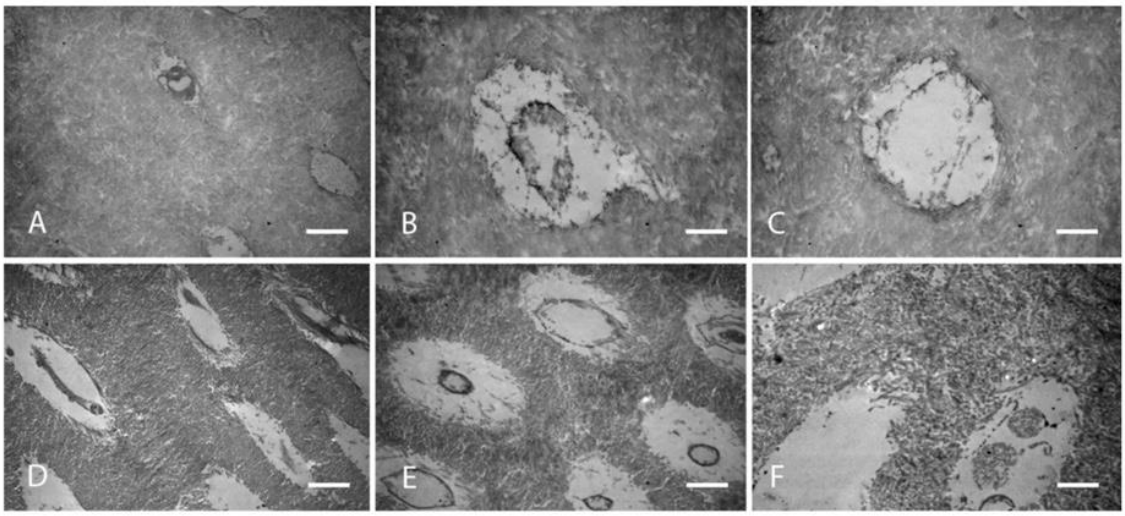

Figure 2.7 TEM images of normal and syndromic DI dentin. Normal condensed collagen matrix (A-C). Irregular organization of circular collagen fibres in a loose abnormal matrix (D-F). Josic U et al. 2020.

2.2.3 Collagen Diameter

The second most frequent criterion was collagen diameter. Eleven of the 17 studies (65%) described defects in collagen diameter of DI teeth either isolated or in syndromic DI (Figure 2.8). Eight papers studied primary teeth (Waltimo *et al.*, 1994, Waltimo, 1994, Waltimo *et al.*, 1996, Hall *et al.*, 2002, Orsini *et al*., 2014, Ibrahim *et al*., 2019, Josic *et al.*, 2020, Nutchoey *et al*., 2021), and only four of which declared the number of teeth examined, (n = 21 teeth). One paper examined permanent teeth (Duan *et al.*, 2016), and another examined both primary and permanent teeth (Waltimo *et al*., 1995), both of which without specifying the number of teeth examined. The eleventh study did not declare details about the type nor number of teeth (Ranta *et al*., 1993).

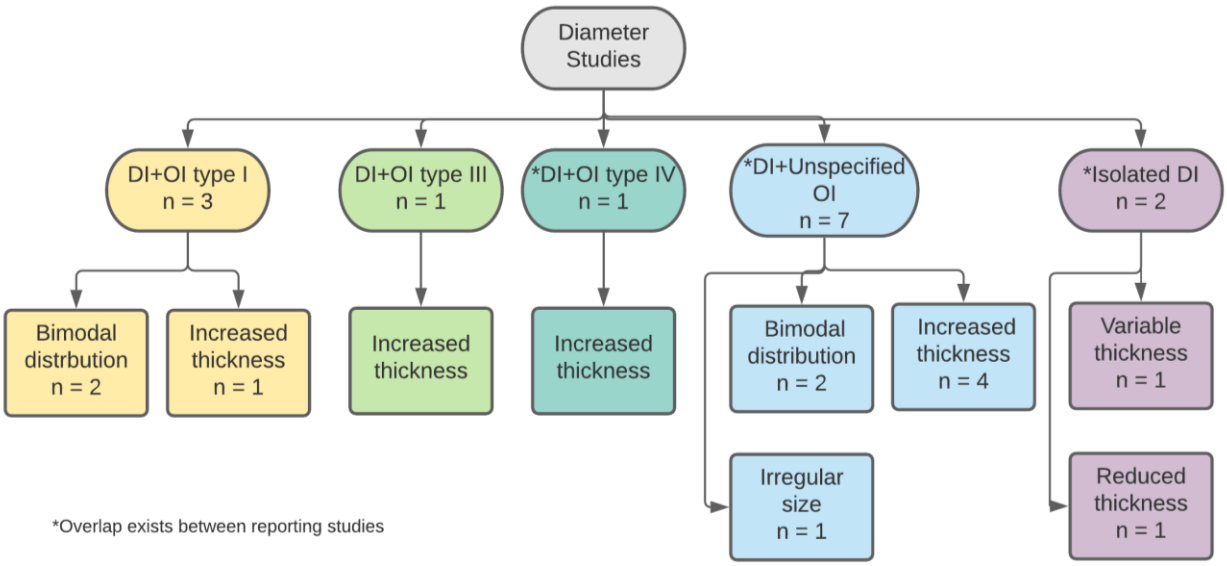

Figure 2.8 Categorization of studies reporting on collagen diameter by DI and OI types

Overall, studies described the defective dentin as to have thick, coarse, abnormally enlarged collagen fibres when compared to the dentin of normal teeth. This was mostly in syndromic DI teeth while in isolated DI two studies reported that fibres had reduced thickness. Normal dentin collagen fibres were found to have a diameter of 50 – 75nm (Ibrahim *et al.*, 2019). In OI type I, studies reported that collagen fibres did not have a uniform diameter (Waltimo *et al.*, 1996, Ibrahim *et al.*, 2019). In fact, they are thought to have a bimodal distribution (Ibrahim *et al.*, 2019). The upper level of the abnormal thickness can reach up to 300 nm, with a median of 62.1 nm, while the lower limit median was approximately 30 nm. This was also reported by studies that examined DI teeth with unspecified OI types. The smaller fibres diameter range between (40-60 nm), while the larger population of fibres range was (80-100 nm) (Waltimo *et al.*, 1994, Waltimo *et al.*, 1995).

In a more recent study, enlarged fibres were found to range between 81-124 nm (Duan *et al.*, 2016). However, most studies, from the earliest paper included to the most recently published, described defective dentin as to have thick, coarse, abnormally enlarged collagen fibres that are variably increased in diameter when compared to dentin of normal teeth (Ranta *et al.*, 1993, Orsini *et al.*, 2014, Duan *et al.*, 2016, Josic *et al.*, 2020, Nutchoey *et al.*, 2021). Similarly, in OI type III and type IV studies, defective dentin had abnormally thickened and coarse collagen fibres (Waltimo, 1994, Hall *et al.*, 2002). In isolated Dentinogenesis Imperfecta teeth, collagen diameter was also found to have a bimodal distribution (Waltimo *et al.*, 1995). However, the range was more divergent than in other types of DI or OI affected teeth. Thin fibrillar structures had a thickness of 10-20 nm and increased length reaching up to 1700 nm (Waltimo *et al.*, 1994).

2.2.4 Shape

Altered shape of collagen fibres was described by 6 papers (Figure 2.9). Four studies reported results on primary teeth and only two declared the number of teeth, with a total sample size of 10 primary teeth (Waltimo, 1994, Waltimo *et al*., 1995, Waltimo *et al.*, 1996, Orsini *et al.*, 2014). A study examined 1 primary tooth and an unknown number of permanent teeth (De Coster *et al*., 2007). Another examined permanent teeth only (Duan *et al*., 2016). Three studies reported the following features in an unspecified type of OI, atypical recognizable collagen fibres, irregular threads and wavy formations, and curved groups of cross-striated fibres (Waltimo *et al.*, 1996, Orsini *et al.*, 2014, Duan *et al.*, 2016). In types III and IV OI, collagen fibres were found having an extension of irregular branches(De Coster *et al.*, 2007). While in types I and IV OI, they were described as thick and wavey shapes (Waltimo, 1994). The previous studies examined teeth with syndromic DI and various types of OI. One study examined teeth of isolated DI and found a distinctive shape of collagen fibres described as “rope or needle-like” structures (Waltimo *et al.*, 1994).

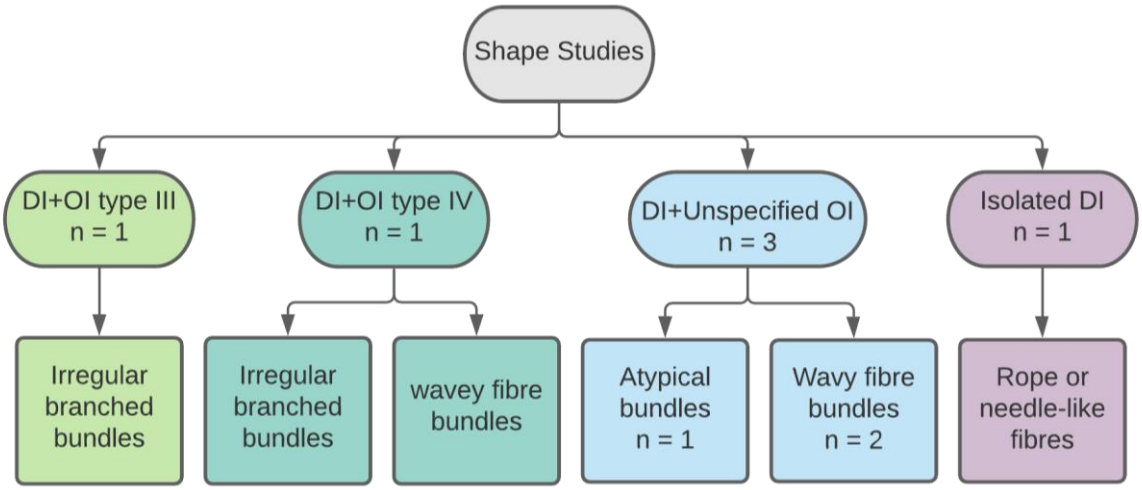

Figure 2.9 Categorization of studies reporting on collagen shape by DI and OI types

2.2.5 Quantity

Five studies examined the quantity of collagen fibres in syndromic DI affected teeth. None of which specified a description for DI collagen defects in type I OI. All studies reported results on primary teeth; three of which declared the number of teeth, with a total sample size of 5 primary teeth. One paper also studied an additional permanent tooth (Intarak *et al*., 2020). Three papers did not specify the exact type of OI affecting the subjects (Orsini *et al.*, 2014, Hall *et al.*, 2002, Nutchoey *et al.*, 2021). While two studies specified their results for OI types III and IV (Budsamongkol *et al.*, 2019, Intarak *et al.*, 2020). Despite the variation of the type of disorder affecting the subjects, all samples of DI defective dentin showed similar findings. Collagen fibres quantity was reduced in all study groups when compared to controls (Hall *et al.*, 2002, Orsini *et al.*, 2014, Budsamongkol *et al*., 2019, Intarak *et al*., 2020, Nutchoey *et al*., 2021).

2.2.6 Orientation

Six studies reported on collagen fibres orientation in DI affected teeth (Figure 2.10). Four papers reported on primary teeth yet only three specified the number of teeth, n= 18 (Waltimo, 1994, Hall *et al*., 2002, Majorana *et al*., 2010, Nutchoey *et a*l., 2021). A study reported on both dentitions without sample size specifications, and another did not declare any information on study sample. One paper examined DI teeth in subjects affected by types I, III and IV OI. All of which had comparable fibres orientation. The direction of orientation differed according to proximity to the dentinal tubules. Intratubular fibres found mostly parallel to the long axis of the tubule, while in sections away from dentinal tubules fibres were in random orientations (Majorana *et al.*, 2010). Occasional parallel orientation was also

found in DI teeth affected by OI type III (Ranta *et al*., 1993). This was also reported by a study that examined syndromic DI affected teeth, with the addition of 45◦ orientation of some intratubular fibres (Hall *et al.*, 2002). One study reported a unidirectional parallel fibres orientation in types I and IV OI and syndromic DI (Waltimo, 1994). In contrast, a more recent study found that the orientation of collagen fibres is mostly disoriented and haphazard (Nutchoey *et al.*, 2021). This was also the finding of a study that examined teeth of isolated DI, collagen fibres did not follow a specific orientation and were mostly aberrant (Waltimo *et al.*, 1995).

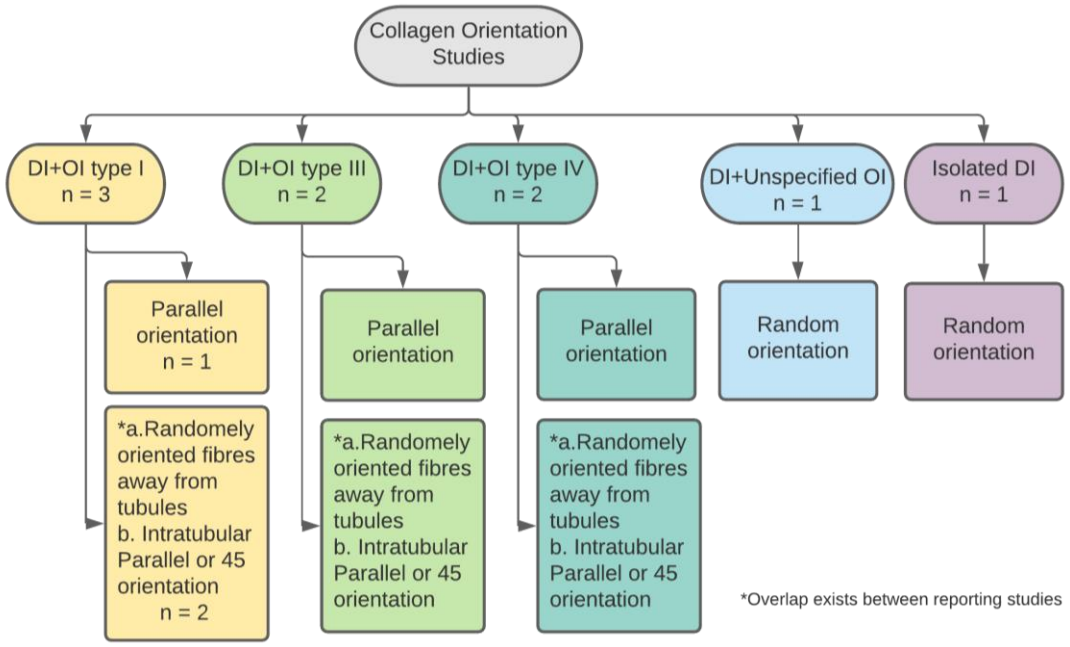

Figure 2.10 Categorization of studies reporting on collagen orientation by DI and OI types

2.2.7 Density

Collagen fibres density was described by four studies. The known sample size n=3 primary teeth, reported by two studies. Other papers did not report on sample size. In general, three papers examined primary teeth, and one additionally examined permanent teeth. The fourth study examined permanent teeth only. In type I OI, a study reported increased density in collagen bundles to a degree that single fibres forming the bundle were undistinguishable (Waltimo *et al.*, 1996). However, this finding was contrary with other studies results. Reduced fibres density and loose collagen bundles were common findings in multiple studies, one of which examined OI types III and IV affected DI teeth (Duan *et al.*, 2016, De Coster *et al.*, 2007, Josic *et al.*, 2020).

2.2.8 Structural Deformation

Six studies described collagen in DI teeth as structurally deformed or abnormal (Figure 2.11). The known sample size n=6, three primary teeth and three permanent, reported by three studies. Other papers did not report on sample size. A study of syndromic DI, reported the finding of unraveled helices of collagen fibres (Waltimo *et al.*, 1996). Similarly, a study of unspecified OI type reported uncoiled collagen fibres in defective dentin of syndromic DI teeth (Waltimo *et al.*, 1995). Another study with similar subjects, found high presence of abnormally formed collagen that was uncoated with minerals (Duan *et al.*, 2016). One study examined DI affected collagen in OI type III affected subjects, and reported presence of altered "pulsed" formations of abnormal collagen fibrils (Hall *et al.*, 2002).

Another study that also examined OI type III affected subjects, found abnormal quality, and structured of collagen fibres (Budsamongkol *et al.*, 2019). The last paper studied isolated DI teeth and reported abnormal collagen structure that led to buckling of the fibres when dried (Kinney *et al.*, 2001).

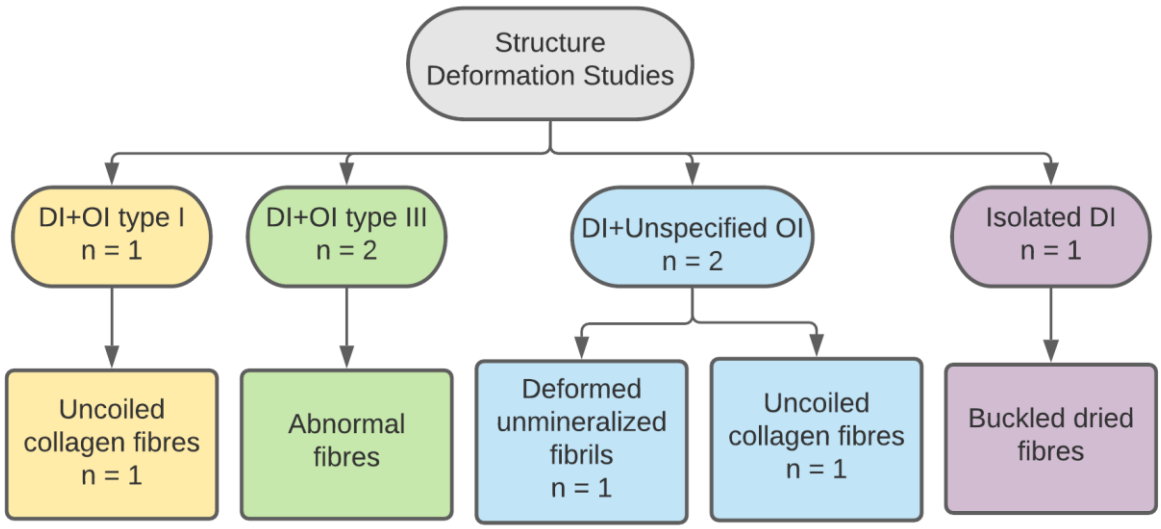

Figure 2.11 Categorization of studies reporting on collagen structural deformation by DI and OI types

2.2.9 Physical Properties

Two studies only examined physical properties of defective dentin in DI teeth and both papers tested dentin hardness and elasticity. The total sample size n=9 primary teeth. One study reported, In type III OI, DI teeth exhibited significantly lower dentin hardness and elasticity than normal dentin (Budsamongkol *et al.*, 2019). The other study examined type I OI, DI teeth and reported 50% reduction in dentin hardness when compared to control teeth. This study also found that modulus of elasticity had a bimodal distribution, because defective

collagen fibres were divided into large and small diameters. The higher end fibres had an elastic modulus of approximately 4 GPa, while the fibres in the lower end had an elastic modulus of approximately 10 GPa (Ibrahim *et al.*, 2019).

2.2.10 Collagen Type III

In healthy dentin, collagen type III is known to be exclusively present in reactionary dentin. Presence of type III collagen in dentin of DI teeth was tested by five studies (Figure 2.12). Four studies reported on primary teeth, with a total sample size 8 primary teeth, declared by two papers (De Coster *et al*., 2007, Majorana *et al.*, 2010). Two papers additionally examined permanent teeth without declaring the number of teeth and one study did not declare the dentition examined nor the sample size. High staining for these fibres was evident in all DI type I affected teeth, including OI types I, III and IV (De Coster *et al.*, 2007, Majorana *et al.*, 2010, Ranta *et al.*, 1993, Waltimo *et al.*, 1994). One of these studies found an association between evidence of type III collagen and poor mineralization (Majorana *et al.*, 2010). Another paper found an increased availability for type III collagen fibres in isolated DI than syndromic DI (Waltimo *et al.*, 1994). A description of the fibres was provided as having a “fan-like” layered structure that was more frequently dense in the periphery of the defective dentin. This was found in DI teeth of subjects affected by type III and IV OI (De Coster *et al.*, 2007).

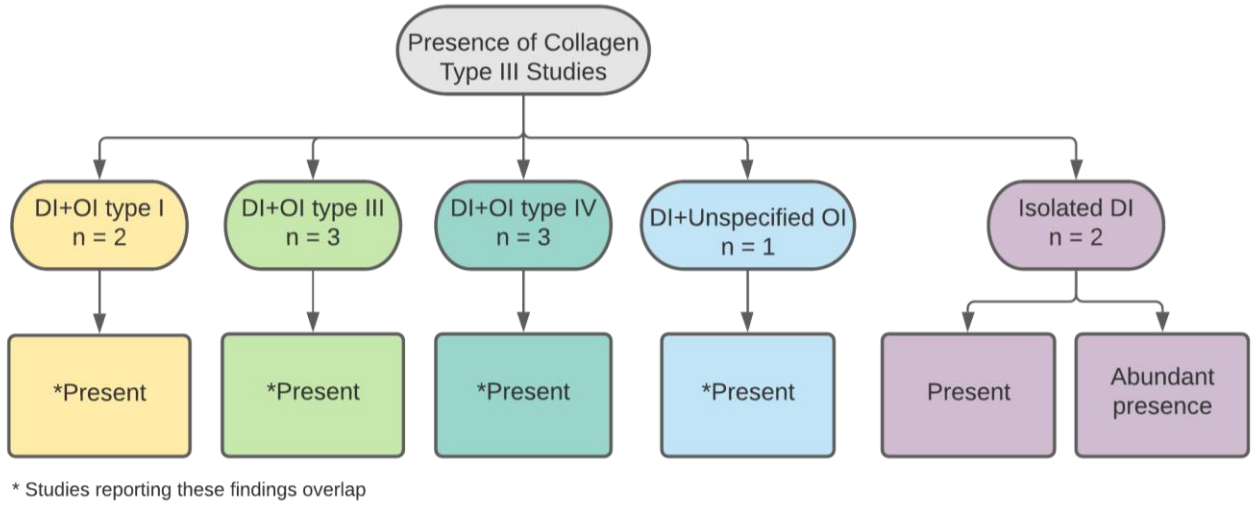

Figure 2.12 Categorization of studies reporting on collagen type III by DI and OI types

2.2.11 Collagen types IV and VI

Presence of other types of collagens as types IV and VI was documented by three papers. The sample size of all papers is unknown. In general all examined primary teeth and one additionally examined permanent teeth. In types I and IV syndromic DI teeth, intense

staining for collagen type VI was evident yet more significantly in type IV OI subjects (Waltimo *et al.*, 1994). This finding was also supported by another study, the results state that collagen type VI was detected in all syndromic DI affected teeth (Orsini *et al.*, 2014). In isolated DI on the other hand, type IV collagen was found as thin fibrillar structures in the dentin matrix (Waltimo *et al.*, 1995).

2.2.12 Other types of Collagen

Two other studies reported unusual types of collagen found in syndromic DI affected teeth. Both studies examined primary teeth; one declared n=2 primary teeth (Waltimo, 1996). A study of Type I OI, DI teeth found excessively large diameter fibres or hyperfibres that reach about 300 nm in thickness (Waltimo, 1994). These extraordinarily coarse hyperfibres were also found in another study of syndromic DI teeth with unspecified OI type (Waltimo *et al.*, 1995). Other forms of collagen as symmetrical collagen segments (SCS) and fibrous long-spacing collagen (FLS) were found in syndromic DI teeth of unspecified OI type. Their dimensions are at least double in size than native collagen fibres, about 265 nm length x 950 nm thickness. Although they have similar dimension, their periodicity varied. SCS have a periodicity of approximately 55 nm, while of FLS is about 125 nm. This study however specified that type IV OI-DI teeth exhibited more FLS than other types (Waltimo, 1996).

2.2.13 Ultrastructural defects: D-Banding Periodicity

A total of four studies described the ultrastructural collagen characteristic D-banding (Figure 2.13). Three papers examined primary teeth, n=18 primary teeth (Waltimo, 1994, Waltimo, 1996, Ibrahim *et al*., 2019). One examined permanent teeth without specification of sample size. Two studies of type I OI affected teeth, one of which reported presence of a few sporadic D-banding periodicity. However, the majority of the fibres exhibited normal D-banding periodicity but of wider distance. It was found that the spacing was larger than in normal teeth, measuring between 50-80 nm versus 52-75 in healthy dentin, with however no significant difference (Ibrahim *et al.*, 2019). The other study reporting in type I OI, found that although the periodicity of the fibres was as the control teeth, the D-banding could not be seen. Instead, only larger dark areas and narrower light areas were observed (Waltimo, 1994). The findings of the first study were also reported by the remaining two papers. In unspecified OI types, wider periodicity of defective collagen was found, ranging between 58.4-70.2 versus 55.9-67.9 in normal collagen as shown in Figure 2.14 (Waltimo, 1996, Duan *et al.*, 2016).

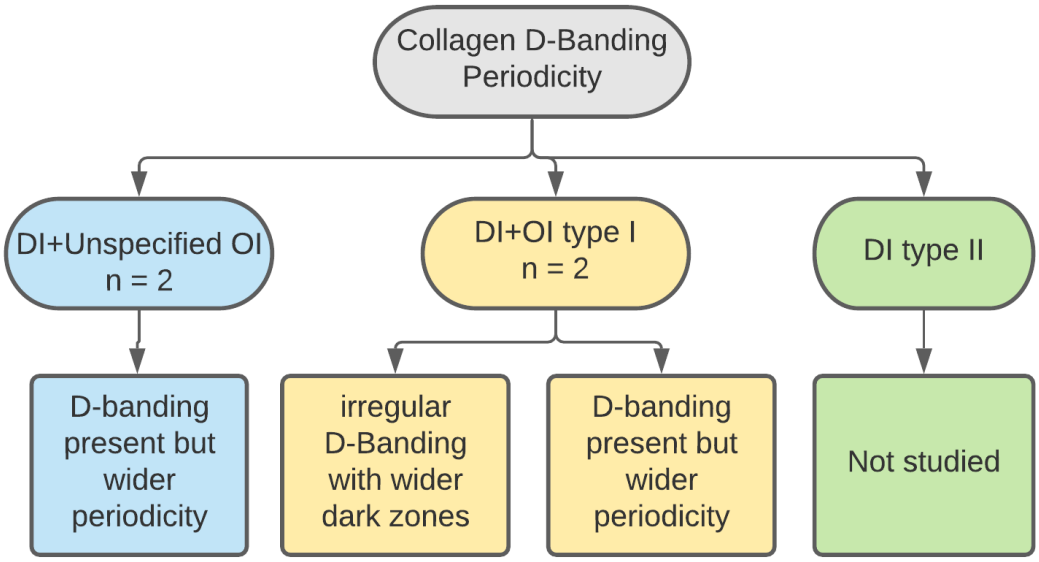

Figure 2.13 Categorization of studies reporting on collagen D-banding by DI and OI types

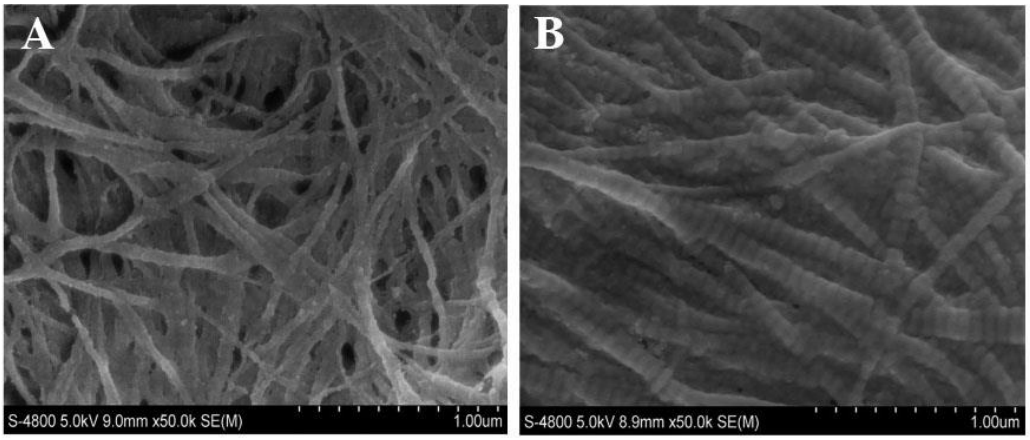

Figure 2.14:SEM of collagen fibres. Normal and intact fibres with normal banding periodicity in control dentin (A). DI dentin collagen fibres have variable and wide banding periodicity (B). Duan et al 2016.

2.2.14 Ultrastructural defects: pC-collagen and pN-collagen

One paper found that dentin of isolated DI affected teeth, stained heavily for C- terminal peptide region of type I fibres but much less for N-terminal peptide region. The study was conducted on primary teeth of unknown number. Their interpretation was that the heavy staining for C-terminal peptide and the lack of staining for the N-terminal peptide meant proper formation of mature collagen fibres, since the latter region is mostly cleaved away during collagen formation (Waltimo *et al.*, 1994). Therefore, the reduction in fibres diameter in isolated DI reported by other studies is not a result of immature fibre formation, rather abnormal fibre formation or presence of other fibre types.

2.2.15 Isolated DI Versus syndromic DI

Five studies reported on isolated Dentinogenesis Imperfecta; among those, six criteria can be described and compared with syndromic DI (Table 2.5). The criteria were collagen diameter, shape, orientation, ultrastructure, presence of collagen type III and other types of collagen. In isolated DI, fibres had a range of variable diameter, which expands increasingly, below and above the normal range, but fibres were generally described as large and coarse (Waltimo et al., 1995, Duan *et al.*, 2016, Josic *et al*., 2020). This was also reported in syndromic DI teeth. However, in isolated DI, an additional description was found, the presence of thin fibrillar structures (Waltimo *et al*., 1994, Waltimo *et al*., 1995). With regards to shape observations, in isolated DI, thin needle-like fibrils were found, which was not seen in the dentin of syndromic DI teeth (Waltimo *et al*., 1995).

The presence of these needle-like structures can be correlated with the decrease in fibril diameter and presence of the thin fibrillar structures. The orientation of collagen fibres in isolated DI teeth was described as abnormal, with no further clarification on whether the direction was different intra- and intertubular as in syndromic DI teeth (Waltimo *et al.*, 1995). In the latter, studies found that parallel fibrils were occasionally found in the intratubular dentin (Hall *et al.*, 2002, Majorana *et al.,* 2010). Presence of collagen type III was tested in both isolated DI teeth and syndromic DI teeth. Reactivity to this type of collagen was found in all teeth specimens (Waltimo *et al*., 1995, De Coster *et al*., 2007, Majorana *et al*., 2010). However, one study reported higher reactivity in isolated DI teeth when compared to syndromic DI teeth and control teeth (Waltimo *et al.*, 1994, Waltimo *et al.*, 1995). Finally, in both types of DI, studies reported reduced mineralisation of collagen fibrils. More specifically, in isolated DI intrafibrillar mineralisation was absent (Kinney *et al.*, 2001, Duan *et al*., 2016).

Table 2.5. Criteria Describing Isolated versus Syndromic DI

Criteria	Syndromic	Isolated
Organization	Haphazardly organized or in a form of abnormal circular unorganized bundles	--
Diameter	thick, coarse, abnormally enlarged collagen fibres	Bimodal – extremely thin fibres to thick
Shape	Wavey, curvy and branched	Thin, needle-like
Quantity	reduced	--
Orientation	Parallel near tubules - haphazard away	haphazard
Density	Reduced w loose fibre bundles*	--
Structure	Uncoiling, unravelling, uncoated	Abnormal reversible buckling
Mechanical	Reduced elasticity and hardness esp intratubular dentin	--
Type III	Present	Abundant
Type IV + VI	Type VI found in all types of OI	Type IV present as thin structures
Other types	Hyperfibers, FLS, SCS	--
D-banding	Present– wider spread	--
P-collagen	--	Normal cleavage of N-terminal

2.3 Discussion

Dentinogenesis Imperfecta is genetic disorder that renders affected teeth with aesthetic and functional defects. The disease presents with a range of features of greyish-brown discolouration, pulpal obliteration, crown fractures and severe attrition (Shields *et al.*, 1973). Clinically it can affect both primary and permanent dentitions. Management of Dentinogenesis Imperfecta is thought to be challenging. The American Academy for Paediatric Dentistry (AAPD) recommends early management of children affected by DI with preventive measures. Aspects of management include tooth structure preservation, and aesthetic improvement (Council, 2013).

Studies of dental defects in Dentinogenesis Imperfecta are numerous however, the literature is limited on the defects of collagen in DI teeth. This systematic review identified 14 abnormal changes, to clarify the deformity in collagen of DI teeth and examine its ultrastructural defects. Increased collagen diameter and disorganized fibres were the most common defects found in studies. A qualitative examination of collagen fibres showed that, although smaller sized fibres were occasionally found, thick coarse collagen fibres were a common finding in defective dentin of DI teeth of both isolated DI and syndromic DI. The organization of collagen fibres in both DI types was found to be irregular, haphazard and with abnormal circular pattern. Other commonly described changes in isolated DI and syndromic DI, were reduced collagen quantity, abnormal fibres' shape and having a parallel to random orientation (Nutchoey *et al.*, 2021, Orsini *et al.*, 2014).

Among the 12 studies that described changes in collagen diameter, five studies explained the presence of both smaller sized fibres and thick coarse collagen fibres (Ibrahim *et al.*, 2019, Waltimo *et al.*, 1996, Waltimo *et al.*, 1995, Waltimo *et al.*, 1994). Two of these studies also examined the presence of collagen type III fibres (Waltimo *et al.*, 1995, Waltimo *et al.*, 1994). Collagen type III is similar to type I as both are fibril forming collagens rather than hexagonal or network forming types. However, type III is much less abundant than type I and is rarely found in normal dentin. All studies that investigated collagen type III found high presence of these fibres in all DI types. However, higher abundance was found related to isolated DI teeth. Presence of collagen type III and its role in the disease pathophysiology is not entirely known but it is thought that these fibres may cause abnormal mineralization of the dentin matrix. Furthermore, correlation was found between the presence of type III fibres and the small fibrillar structures Another study suggested that these fine fibrils could be immature collagen type I fibres (Ibrahim *et al.*, 2019). In dentin of normal teeth, collagen type III fibres are covered by those of type I. Therefore, the presence of type III collagen

fibres could be due to defective formation of type I fibres exposing those of type III to detective stains and antibodies.

The disorganization of collagen fibres was described by twelve studies (Ranta et al., 1993, Waltimo et al., 1995, Waltimo et al., 1996, Kinney et al., 2001, Hall et al., 2002, Orsini et al., 2014, Duan et al., 2016, Ibrahim et al., 2019, Josic et al., 2020, Intarak et al., 2020, Nutchoey et al., 2021). Fibres were described as irregularly organized, assembling in abnormal circular patterns and in lax derangements with large gaps (Kinney et al., 2001, Hall et al., 2002, Josic et al., 2020). In normal dentin, collagen acts as a scaffold for mineral deposition and crystal growth. Accordingly, one study suggested a negative effect on crystals growth, given that these large spaces offset the geometrical limitation set by the scaffold allowing overgrowth of hydroxyapatite crystals. A finding also reported by this study (Kinney *et al.*, 2001).

In normal dentin, collagen fibres in dentin are mostly found and arranged in intertubular dentin. Peritubular dentin on the other hand, is mainly formed of non-collagenous matrix proteins and naturally inside the tubules lay the odontoblastic process (Goldberg *et al.*, 2011). In syndromic DI dentin, as fibres organization varies, lateral intratubular fibres were abundant (Waltimo, 1994, Waltimo *et al.*, 1996). This can also explain how parts of the defective dentin lack odontoblastic processes and the dentinal tubules were obliterated by collagen fibres (Josic *et al.*, 2020).

Other deformities as, low collagen density, reduced fibre quantity, and irregular shapes of collagen fibres, can be considered factors in the reduced dentin hardness found and reduced modulus of elasticity (Budsamongkol *et al.*, 2019, Ibrahim *et al.*, 2019). The reduced elasticity of peritubular collagen fibres is thought to be the first evidence in understanding the structural defects in syndromic DI teeth (Ibrahim *et al.*, 2019). In isolated DI, studies did not report on the mechanical strength of dentin nor collagen, although it can be inferred from studies on syndromic DI teeth that both are reduced for the same reasons. However, one study correlated the reduced intratubular mineralization with decreased resistance to bending stresses, which is observed with excessive drying (Kinney *et al*., 2001).

Ultrastructural defects were reported by seven studies (Waltimo, 1994, Waltimo et al., 1995, Waltimo, 1996, Waltimo et al., 1996, Duan et al., 2016, Ibrahim et al., 2019, Budsamongkol et al., 2019). Three of which described structural defects while the remaining examined D-banding periodicity (Waltimo, 1994, Waltimo, 1996, Duan et al., 2016, Ibrahim et al., 2019). The deformities found were loss of triple helix structure and uncoiled collagen fibres

(Waltimo et al., 1994, Waltimo et al., 1995). The fiber uncoiling is normally seen at the end of the collagen fibres (Holbrook and Byers, 1987), in this case the uncoiling was seen throughout the entire length of the fiber, suggesting abnormal structure due to defective fibrillogenesis (Waltimo *et al*., 1996).

Increased D-banding periodicity was a common finding in four different studies. D- banding periodicity is equal to 67 nm in normal dentin. It exists because the length of tropocollagens is about 4 times longer than their periodicity. Meaning that, L= 4.46D, giving areas of gaps (0.54D) and overlaps (0.46D), that when combined they account for the 67nm D-periodicity. In normal dentin, these gaps and overlaps are nucleating sites for mineral deposition and crystals growth (Nudelman *et al*., 2010). However, fewer mineral depositions were found in DI teeth when compared to normal teeth, suggesting that normal D-banding periodicity is a prerequisite for mineral deposition (Duan *et al*., 2016). This theory is supported by the finding that D-banding sites contain positively charged amino acids, making these sites to have a high potential to interact with the negatively charged mineral phases as amphoras calcium phosphate to initiate intrafibrillar mineralization. Nonetheless, it is unknown how applicable these studies are (Nudelman *et al.*, 2010, Xu e*t al*., 2015) to naturally occurring biomineralization as they were conducted using simplified models in vitro (Yu and Wei, 2021).

Abnormal characteristic collagen types have been reported by multiple studies, these include SCS, FLS and hyperfibres (Waltimo, 1994, Waltimo et al., 1995, Waltimo, 1996). The significantly thick hyperfibres were found away from the dentinal tubules in the loose dentinal matrix of syndromic DI teeth (Waltimo, 1994, Waltimo *et al*., 1995). While FLS and SCS collagen forms were found inside the dentinal tubules. Both forms were evident in teeth of syndromic DI with an unspecified type OI. However, the FLS collagen was specifically related to type IV OI. It was also reported by the study that the SCS collagen is unrelated to the formation of defective dentin matrix, and that SCS does exist in normal dentin. FLS relation to syndromic DI on the other hand is still unconfirmed (Waltimo, 1996).

The systematic review demonstrates variable defects in collagen which may also affect mineralization and tooth mechanical strength. Abnormal collagen formation, organization and behaviour impose a clinical challenge in restoration and maintenance of these teeth. Studies on DI affected teeth recommend placement of stainless-steel crowns over primary molars and composite restoration build ups for anterior teeth as incisors and canines (Frassetto *et al.*, 2016). However, the success of composite restorations is affected by the

hybrid layer formed by the interdigitation of the composite restoration, adhesive resin, and collagen fibres. Due to the presence of abnormal collagen fibres in the dentin of DI teeth, the adhesive system will not be able to infiltrate the collagen layer exposed after demineralization. This will lead to incomplete immersion of collagen fibres in the adhesive resin system (Breschi *et al.*, 2018).The hybrid layer formed is of questionable quality as the exposed collagen fibres are prone to hydrolysis by endogenous enzymes, leading to degradation of the hybrid layer and subsequent failure of adhesive restoration (Josic *et al.*, 2020). Therefore, the use of adhesive systems that have the capability to form chemical bonds with collagen fibres is an area of research for the future of restorative dentistry in DI patients. For this reason, understanding the microscopic changes in collagen is of clinical importance. Furthermore more, future studies should not be exclusive to dentin morphological and collagen defects as collagen defects or topographic abnormalities in cementum represent a gap of knowledge. Study limitations are small sample size of available literature and that this study was conducted by a single reviewer.

2.4 Conclusion

This systematic review reports changes in collagen of teeth affected by Dentinogenesis Imperfecta. The most frequently found macro-deformities were coarse collagen fibres, decreased fibres quantity, random to parallel fibres orientation and irregular organization. Ultrastructural defects were uncoiled collagen fibres and increased D-banding periodicity. In addition to the presence of types III, IV and VI collagen fibres, hyperfibres, SCS and FLS collagen forms were found. Understanding the ultrastructural changes in collagen is of clinical importance as it will enable us to clarify the extent of collagen damage to decide whether the innovation of adhesive systems that form chemical bonds with collagen fibres is the future of restorative dentistry in DI patients or avoiding collagen as a whole, would be a better option.

3 The Effect Of Dentinogenesis Imperfecta On Cementum: A Scoping Review

3.1 Introduction

Results of the previous systematic review report on the defects in dentinal collagen of DI teeth and how this may affect teeth clinically. However, none of the studies reported on defects in cementum of DI teeth, and the ramifications of such abnormalities on tooth anchorage or support. The pathophysiology in isolated Dentinogenesis Imperfect originates from a mutation resulting in a defect in the Dentin Sialophosphoprotein (DSPP). This protein was primarily reported to exist in dentin, yet few other studies examined its presence in enamel and cementum (Chen *et al*., 2016, Jing *et al.*, 2021). Syndromic DI on the other hand, is a connective tissue disorder, specifically of the most abundant component in all tissues, namely collagen type 1 (Barron *et al.*, 2008).

3.1.1 Classification of Cementum

The biologic composition of cementum is variable according to the cementum type (Schroeder, 1992). In general cementum can either be cellular or acellular, based on the entrapment of cementocytes. The first widely used classification was developed in 1981 (Jones, 1981). However soon afterwards, a modified version was was updated by Schroeder in 1986, it divided cementum into four principal types, all of which are based on inclusion or exclusion of cells and the presence and origin of collagen fibres (table 3.1) (Schroeder, 1986, Schroeder, 1992, Yamamoto *et al.*, 2016). The classifications are acellular extrinsic fibre cementum also known as AEFC (Figure 3.1) (Yamamoto *et al.*, 2016); acellular afibrillar cementum (AAC); cellular intrinsic fibre cementum (CIFC) and cellular mixed stratified cementum (CMSC). Where extrinsic denotes to fibres originating from the periodontal ligament (PDL), namely Sharpey's fibres and intrinsic for collagen fibres of cementum. A second variation between them is the secreting cells, extrinsic fibres are majorly secreted by fibroblasts and secondarily by cementoblasts while intrinsic fibres are solely secreted by cementoblasts (Schroeder, 1992, Yamamoto *et al.*, 2016).

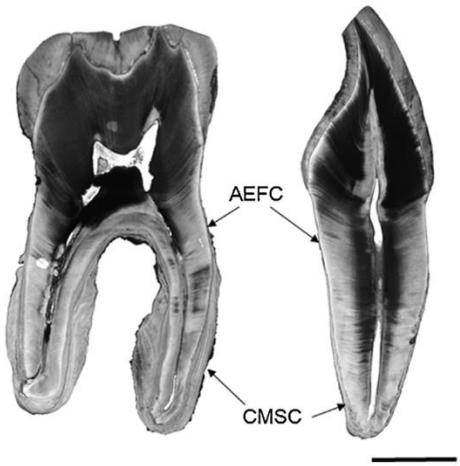

Figure 3.1. Ground section of (left) mandibular molar and (right) maxillary incisor, demonstrating locations of different types of cementum AEFC and CMSC respectively

Table 3.1. Types of cementum, collagen content, location, and function (Schroeder, 1992, Schroeder, 1986, Yamamoto et al., 2016)

Type	Collagen composition	location	function
Acellular afibrillar cementum (ACC)	none	On cemento-enamel junction (CEJ)	unknown
Acellular extrinsic fibre cementum (AEFC)	Extrinsic (PDL) collagen fibres	Single rooted: covers up to 90% of the root Multi-rooted: cervical up to 1/3 of roots	Provides anchorage
Cellular intrinsic fibre cementum (CIFC)	Intrinsic fibres	Interradicular and apical 2/3 of root surface	Adaptation to forces and repair
Cellular mixed stratified cementum (CMSC)	Both intrinsic and either - Extrinsic rich - Extrinsic poor - Extrinsic free	Interradicular and apical 2/3 of root surface	Miscellaneous

3.1.2 Cementum in Dentinogenesis Imperfecta

Cementum is known as a bone-like tissue, it is a mineralised connective tissue with similar chemical composition (Jing *et al.*, 2021). However, unlike bone, cementum does not contain blood vessels. In addition, although cementum has continuous deposition, it does not possess a remodelling capacity (Yamamoto *et al*., 2016). As dentin and bone can be affected by a few Osteogenesis Imperfecta genotypes, or dentin in isolated Dentinogenesis Imperfecta, it is hypothesized that cementum might also be affected given the similarities in

structure and composition (Soni *et al.*, 1967). Bone, dentin and even enamel have been well reported on yet studies on cementum are scarce. This is probably because periodontal signs and symptoms are not commonly a direct effect of DI, and the experience of such clinical signs is probably related to poor oral hygiene leading to inflammation and eventually the breakdown of the junctional epithelium, which is not collagen dependent thus, the defects in cementum and periodontium of DI patients could be present yet in a subclinical form.

Given the limited number of studies, a scoping review was chosen to find and assess the information in the literature regarding defects in cementum of DI teeth. A preliminary search of MEDLINE, the Cochrane Database of Systematic Reviews and JBI Evidence Synthesis was conducted and no current or underway systematic reviews or scoping reviews on the topic were identified.

3.2 Methodology

Eligibility criteria

Human or animal studies, of any primary or permanent teeth, affected with dentinogenesis imperfecta, isolated (defective DSPP) or syndromic DI type (collagen type 1 mutation), that examined cementum defects. This includes but not exclusive to irregular collagen, defective mineralisation, or new abnormal findings. Subjects had no other comorbidities other than OI, if present. No limitations applied on age or gender of subjects, nor species in case of animal studies.

Types of Sources

This scoping review considered both experimental and quasi-experimental study designs including randomized controlled trials, non-randomized controlled trials, before and after studies and interrupted time-series studies. In addition, analytical observational studies including prospective and retrospective cohort studies, case-control studies and analytical cross-sectional studies were considered for inclusion. This review also considered descriptive observational study designs including case series, individual case reports and descriptive cross-sectional studies for inclusion. Qualitative studies were also considered that focus on qualitative data including, but not limited to, designs such as phenomenology, grounded theory, and qualitative description. In addition, systematic reviews that meet the inclusion criteria were also considered, depending on the research question. Text and opinion papers were also considered for inclusion in this scoping review. No restrictions applied on date of publication, sample size, nor methodology of study as demineralization protocol or examination method. In the search trial phase, language limitations were not set to include a larger number of studies. At the final stage of article selection it was applied when English abstracts were found but not translated full articles.

Search strategy

The search strategy aimed to locate both published and unpublished studies. An initial limited search of PubMed was undertaken to identify articles on the topic. Search of text words contained in the titles and abstracts of relevant articles, and the index terms used to describe the articles was done to develop a full search strategy. Terms included: Dentinogenesis Imperfecta, Hereditary Opalescent Dentin, Cementum, Osteogenesis Imperfecta, Brittle bone disease and cementum. The search strategy included all identified keywords and index terms and was adapted for each included database and/or information

source, PubMed and OVID Embase. The reference list of all included sources of evidence was screened for additional studies. Studies published in any language will be included. Studies published since 1970s will be included given the limited number of studies done on cementum affected by Dentinogenesis Imperfecta.

Following the search, all identified citations were collated and uploaded into EndNote X9.3.3 (Clarivate Analytics, PA, USA) and duplicates removed. Following the pilot test, titles and abstracts were then screened for assessment against the inclusion criteria for the review. Potentially relevant sources were retrieved. The full text of selected citations was assessed in detail against the inclusion criteria. Reasons for exclusion of sources of evidence at full text that did not meet the inclusion criteria were recorded and reported. The results of the search and the study inclusion process is reported and presented in the Preferred Reporting Items for scoping review (PRISMA-ScR) flow diagram.

Data extraction

Data was extracted from papers included in the scoping review by using a data extraction tool developed by Cochrane library and edited by the reviewer (Cochrane, 2013). The data extracted included specific details about the type of study subjects, (either human, animal study or both) participants and type of dentition (primary, permanent or both), concept, and context. Study methods were extracted including method of examination and finally key findings relevant to the review questions.

An extraction form is provided (see Appendix B). The draft data extraction tool was modified and revised during the process of extracting data for each included evidence source. The addition of presence of control teeth was done.

3.3 Results

Initially, 57 papers were found across three databases, Ovid Medline (22), Ovid Embase (17) and PubMed (18) as demonstrated in Figure 3.2. Then duplicate studies were removed leaving 37 papers. Inclusion and exclusion criteria were applied for article selection by title screening. Reasons for exclusions were, dental pathology unrelated to Dentinogenesis imperfecta and studies that examined dentin, enamel or PDL without cementum. Dental anomalies excluded were dens in dente, dentin dysplasia, amelogenesis imperfecta, and hypercementosis. Experimental studies that examined cementum repair post traumatic dental injuries were also excluded, in addition to regenerative studies of both human or animal tissues. Finally, any periodontal disease due to a systemic disorder as

hypophosphatasia was excluded. 11 studies were included to be screened by abstract and only one was excluded as the study did not report on cementum defects. Initially 10 papers were planned to be retrieved. However, 3 of which had English abstracts, but full articles were in French or German languages and English translations were not found and one study did not examine cementum. The final data set included 6 papers, 4 human and 2 animal studies (Table 3.2).

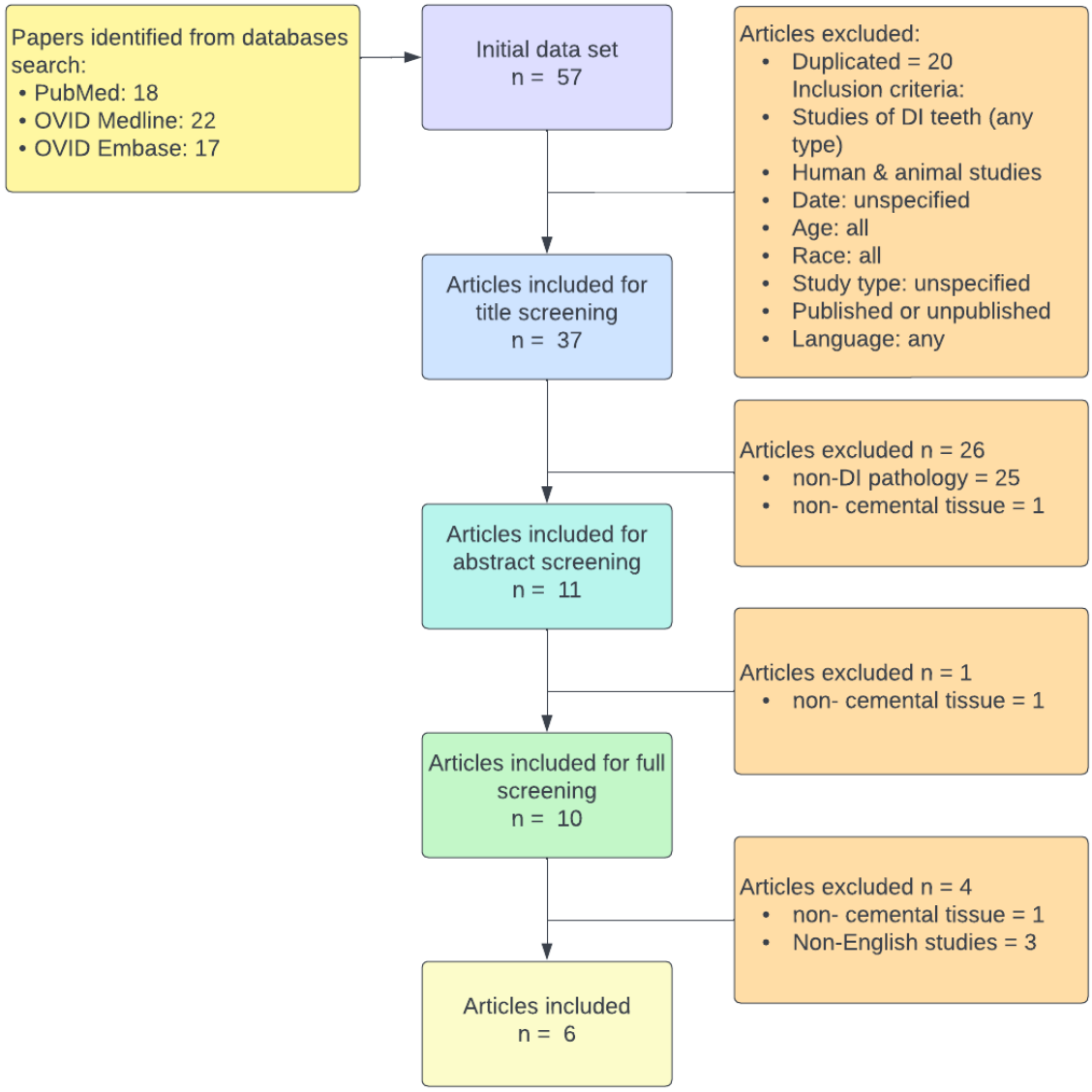

Figure 3.2. Flowchart of article selection process and inclusion and exclusion criteria

Table 3.2. Articles included in final data set

Author	Date	Study design	Sample size	Dentition	DI	Cementum criteria
Soni *et al.*	1976	Cross sectional	n = 30	Human unspecified	Unspecified type	• Cellular cementum hyperplasia
Clergeau-Guerithault, and Jean R	1985	Case report	n = 2	Human Primary canine and primary molar	Isolated DI	• Thin cementum with areas of hyperplasia • Numerous cell inclusions
Kantaputra *et al.*	2017	Case report	unreported	Human primary incisor	Syndromic DI	• Ectopic mineralisation on cementum
Turkkahraman *et al.*	2020	Case report	n = 3	Human permanent molars and incisor	Isolated DI	• Hypomineralized cementum • Increased cellular cementum
Xu *et al.*	2020	Experimental study	unreported	Animal teeth	Syndromic DI	• Increased cellular cementum
Jing *et al*.	2021	Review	unreported	Animal teeth	Isolated DI (DSPP dental defects)	• Loss of cementum • Defective mineralisation

Three studies reported on isolated DI, one of which is an animal study, as shown in Table 3.2. Overall, the observations found in this type of DI were hypomineralized cementum, areas of hypoplastic cementum and sites of increased cellular cementum (Clergeau-Guerithault, 1985, Turkkahraman *et al.*, 2020, Jing *et al*., 2021). The first study which was done in 1985, found that acellular cementum was increased in the furcation area. The study also reported middle and apical root cementum was thin and abnormal. The cementum in the furcation area however was found hyperplastic (Clergeau-Guerithault, 1985). The second study was conducted recently, and authors confirmed, these teeth had an abnormal increase in cellular cementum, in the apical region, which had a lamellar pattern. They have also discovered that the defective cementum was hypomineralized (Turkkahraman *et al.*, 2020). It is unknown however if this was a single or multirooted tooth. The most recent study

was an animal study. The authors also found defective cementum to be hypomineralized, with the thickness being reduced overall. This study nonetheless was conducted via complete deletion of DSPP in mice, resulting in severe bony defects, which is not seen in patients of isolated DI and need to be considered (Jing *et al.*, 2021).

Two papers examined syndromic DI however the findings were inconsistent (Kantaputra *et al.*, 2018, Xu *et al*., 2020). One study examined human teeth and found abnormal mineral deposits on cementum of which they defined as “ectopic” (Kantaputra *et al.*, 2018). The second paper examined animal teeth and discovered that cellular cementum was in fact decreased, while acellular cementum was increased, unlike the reports on isolated DI (Xu *et al*., 2020).

Lastly, one article had examined DI teeth of unreported type, they found that acellular cementum was reduced, in a way an increased cellular cementum. This study additionally found a lamellar pattern of cementum, suggesting areas of damage and repair (Soni *et al.*, 1967). This “lamellar organization” was also later described by Turkkahraman *et al.* however in isolated DI cementum *(*Turkkahraman *et al.,* 2020*).*

3.4 Discussion

Dentin Sialophosphoprotein is non-collagenous protein that is found in dentin, yet an experimental study done in 2004 reported that DSPP is additionally secreted by cementocytes, cementoblasts and detected in PDL tissues including cementum. Although when compared, levels of secretion were substantially lower than that of odontoblasts (Baba *et al.*, 2004). What is noteworthy is that DSPP was found exclusively in cellular cementum and not acellular cementum. This distribution could be related to the functionality and dynamics of associated cementum. Cellular cementum is generally found apically in the root where it provides the capability for continuous adaptation to forces and tissue repair, where acellular cementum is found cervically, partly providing tooth anchorage (Yamamoto *et al*., 2016). The 2004 study also found this protein in tissues with active remodelling, i.e alveolar bone and root, which supports the previous explanation. These findings consistently demonstrate the unknown but important role of DSPP in maintenance of PDL tissues (Baba *et al*., 2004).

Short abnormal roots is a finding known in DI affected teeth. This study aimed to examine defects in cementum of both isolated DI and syndromic DI teeth. The three studies found reporting on isolated DI had consistent results, cementum was reported to have decreased

mineralization, decreased width, and increased cellular cementum (Clergeau-Guerithault, 1985, Turkkahraman *et al.*, 2020, Jing *et al.*, 2021). Reduced mineralisation was a defect repeatedly found in cementum of DI teeth (Turkkahraman *et al*., 2020, Jing *et al.*, 2021), which is a trait also observed in enamel and dentin of isolated DI teeth (de La Dure-Molla *et al.*, 2015, Taleb *et al.*, 2018) which can be directly related to the malfunction of the mineralization regulator protein DSPP (Suzuki *et al*., 2009). The abnormal increase in cellular cementum was found to have a lamellar pattern, and given that this was observed in the apical part of the root, it can be speculated that the pattern is following alternating periods of rest and function representing repeated trauma and compensation (Turkkahraman *et al.*, 2020). However the overall increase in cellular cementum can be due to the expedited cementogensis possibly to compensate loss of coronal tissue, which resulted in entrapment of cementum producing cells (Clergeau-Guerithault, 1985). However, the validity of such assumption is questioned until the occlusion of the patient is considered and the functional load. It is also noteworthy to compare teeth of the same location in the arch and whether the tooth examined is an anterior tooth or posterior, to be able to decide if the increase in cellular cementum or cementum hyperplasia is disease related.

In syndromic DI teeth results were inconsistent. One study reported that abnormal mineralisation was also a trait observed in this type of DI, in a form of ectopic mineral deposits, which could be attributed to defective collagen formation (Kantaputra *et al*., 2018) as in dentin of syndromic DI teeth (Majorana *et al*., 2010, Duan *et a*l., 2016). However, Xu *et al.* reported no abnormality found in mineralization between DI and control teeth (Xu *et al*., 2020). This study simulated type VII OI which results from a mutation in CRTAP gene and is also known to be associated with Cole-Carpenter Syndrome in humans (Balasubramanian *et al.*, 2015). Therefore, whether the resultant disease is OI and if the dental manifestation were DI need to be considered. In addition, it is unknown if the mutation will cause the same defects in human and animal teeth.

Cementum mineralization is thought to be an important factor in the final ratio between cellular and acellular cementum, thus functional tissues of cementum (Zweifler *et al*., 2014). Cementum with abnormal alkaline phosphatase (ALP) was found to be associated with hypomineralisation and increased cellular cementum. This will lead to the next finding in syndromic DI teeth, thickness of cellular cementum. Xu *et al.* found conflicting results to previous studies, their study reported increased acellular cementum where cellular cementum was in fact reduced (Xu *et al*., 2020). This was also the study that found syndromic DI cementum had normal mineralization, which proves the hypothesis of their

correlation. Finally, It can be hypothesized that the variable defects in syndromic DI teeth are representing variable unidentified abnormalities in collagen ultrastructure, corresponding to variable forms of mutations. However, one of the two conducted studies was an animal study and may not be comparable to human diseased teeth.

3.5 Conclusion

The number of studies is limited to develop a conclusion even though cementum defects of isolated DI were more commonly reported with consistent results, which can be because the function and defects of DSPP are further known than the defects in collagen of DI teeth. Although cementum defects could be present, their effect is only subclinical and may not be clinically relevant to DI patients.

4 Laboratory Work

The results clearly demonstrate the lack of evidence in understanding the ultrastructural changes in collagen of teeth affected with Dentinogenesis Imperfecta. We aimed to study the affected dentin and collagen macro, micro and ultrastructural changes to achieve a better understanding and therefore management of disorder, specifically, collagen triple helix deformity, cross-banding changes, and their effect on mineralization and tissue strength. DI and control teeth were collected from Eastman Dental Hospital Paediatric Department, they were either exfoliated teeth or extracted as required in the patients' treatment plan. Then, teeth were kept and tested in the Royal Free Hospital Laboratory. The examination of collagen fibrils was meant to be through using Scanning electron microscopy (SEM), and atomic force microscopy (AFM). The latter is known to give a high-resolution topographic image with no required staining. SEM on the other hand gives a higher magnification at the micron scale but requires extensive sample preparation. In the development of this protocol, animal teeth samples were trial prepared and examined under SEM.

Samples preparation

Samples have been previously prepared using an existing protocol developed as a part of a DDent dissertation at EDI (Ibrahim, 2015). Fresh teeth, following excision, were initially stored in a 70% ethanol solution for 3 days at room temperature (~19°C), before any residual tissue removed by manual abrasion. Cleaned samples were stored in 70% Ethanol Alcohol for an additional 2 days before being washed with distilled water and then stored in a 0.1% thymol solution at 4°C (Ritter, 2001), in the dark/foiled container, until required. Storage did not exceed 3 months. The teeth were then sectioned longitudinally with approximately thickness of 1.5mm using an Accutom-50 diamond microtome (Struers Ltd., Birmingham, UK) before being sonicated in Ultra-High-Quality (UHQ) water for 30 seconds. Samples were then transferred for demineralisation in a solution of 37% phosphoric acid placed in an ultrasonic bath for a time-controlled acid exposure. Thereafter, sections were washed with UHQ water being placed in 6.5% sodium hypochlorite for 5 seconds to remove any acid-etched derived smearing. Sections were thereafter washed again in UHQ water before being air-dried for 24h at room temperature. Prior to SEM, samples were fixed in 4% paraformaldehyde (PFA) in 0.1 M sodium cacodylate buffer overnight.

A parallel protocol using Ethylenediaminetetraacetic acid (EDTA), a more milder digestion approach, was also used. Trials on animal teeth with an aim to optimise the study protocol to ensure that adequate exposure of collagen fibres is achievable with retaining

intact collagen architecture for the SEM to confirm disease presence, which requires a high-quality sample to visualise. As we know, the disease-infected samples are very hard to obtain, so in protocol development trials, sheep teeth were used to compare the classic “acid-etch” protocol to an EDTA-based one. When examining the samples and looking at the images, it was found that the EDTA samples even though they took longer to prepare, were superior at the higher magnifications than acid-etched samples, the 10K EDTA chelating samples being better than the equivalent 15K acid. However, COVID hindered moving on to trials of EDTA-human samples.

SEM

Following fixing using 4% PFA, samples were washed in distilled water for 10 minutes before being soaked in 1% (w/v) tannic acid in 0.05 M sodium cacodylate, for 1h, to enhance the stabilization of collagen fibrils. The samples were then dehydrated in a graded ethanol series (i.e. 25%, 50%, 70%, 90% and 100% dry, 15 min each step, 2 × 100%) before the use of hexamethyldisilazane (HMDS) for further drying. The dried samples were mounted on aluminium stubs and then sputter coated with gold palladium alloy before being examined under a scanning electron microscope (JOEL JSM-5500LV) with an accelerating voltage of 15 kV.

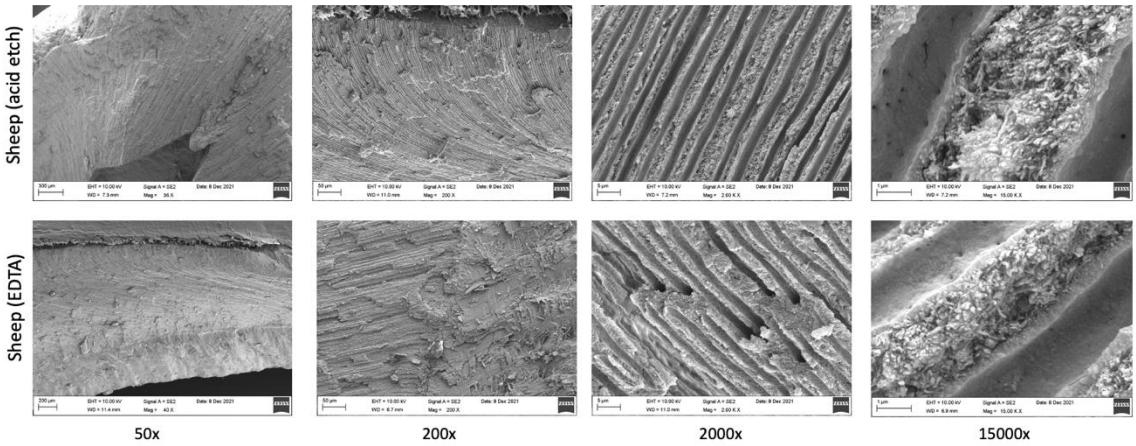

Figure 4.1. SEM images showing sections of sheep teeth prepared in acid etch (top) and EDTA chelating solution (bottom) in 4 powers

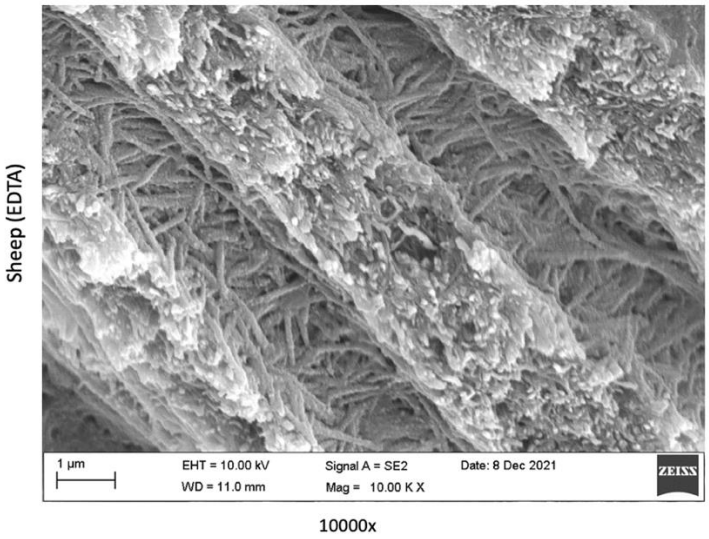

Figure 4.2. SEM image of sectioned sheep teeth prepared in EDTA chelating solution, showing tubular dentin with exposed collagen fibres

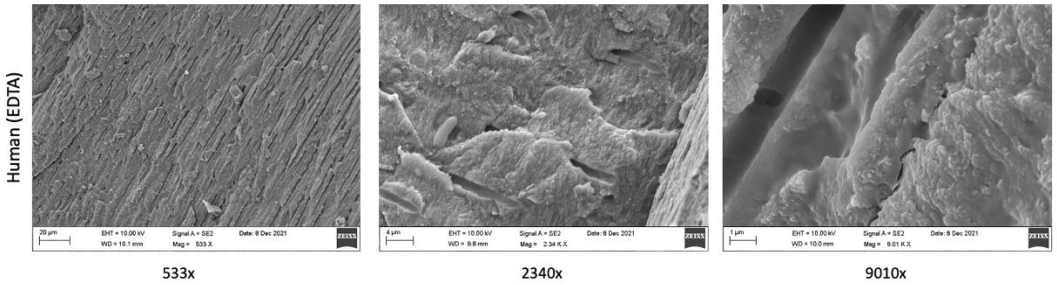

Figure 4.3. SEM image of human teeth prepared in EDTA chelating solution in three powers

However, due to the impact of COVID, the laboratory studies were terminated, and the previous scoping review was conducted.

5 Project conclusion

This study provides an understanding of the changes in collagen and its impact on clinical translation, more specifically in dentinal collagen. What can also be inferred from this project is that in DI, all tooth layers, enamel, dentin and cementum can exhibit somewhat similar deformations, e.g. abnormal mineralisation and deformed collagen matrix (de La Dure-Molla *et al*., 2015, Taleb *et al*., 2018, Turkkahraman *et al*., 2020, Jing *et al*., 2021). It is unknown yet if this is more characteristic in one type of DI (isolated or syndromic) than the other.

The systematic review revealed that the majority of studies examined syndromic DI (16 studies vs. 5, overlap exists), this finding has two considerations. Firstly, the classification used for DI in this study is adapted from Shields, syndromic DI being type I DI which is associated with OI and isolated DI being types II and III (Shields et al., 1973). However, when considering the 2008 OMIM classification of DI, Dentin Dysplasia is added to the equation, as it was classified as a mild form of isolated DI and Shields types II and III, moderate and severe forms, respectively. Therefore, a definite diagnosis of what was examined in the studies may not be possible and whether or not this form of disease is isolated DI or DD could not be decided. In addition, in isolated DI studies, the presence of subclinical OI was not ruled out, thus a clear cut between isolated and syndromic DI was also not achievable.

The second consideration is that, if in this study syndromic DI was a definitive diagnosis, the increase in syndromic DI studies could be because it was part of a systemic disorder, making it arguably easier to classify as DI. Nonetheless, OI is currently classified into at least 18 subtypes (Rauch and Glorieux, 2004, Marini and Cabral, 2018) with a wide range of genetic heterogenicity that also overlaps with other known syndromes as Ehlers Danlos syndrome and Cole-Carpenter Syndrome, with reports of the latter having dental manifestations resembling DI (Balasubramanian *et al*., 2015). In conclusion, OI and DI are complex genetic disorders and it is highly possible that a complete understanding of the disorders has not yet been reached. Understanding the deformation, however, may explain the morphological and mechanical abnormalities found in DI affected teeth and help develop a scoring system to predict prognosis and future complications, this in combination with genetic testing can lead the way to better understanding of these genetic disorder.

Both reviews demonstrated although doubtful but present variable deformation when comparing isolated and syndromic DI teeth. Further understanding of the degree of variation may help clarify why some cases exhibit severe attrition, and post eruptive breakdown while

others have a mild amber hue as the only clinical sign of DI (Gama *et al*., 2017, Kaur *et al*., 2019).

Results of the scoping review on cementum defects were limited and partly inconsistent. The review presented two animal and three human studies. Because the animal studies were an in vitro simulation of the disorder, the resultant mutations (DSPP or CRTAP) were extreme and possibly incomparable to the human manifestation, however, a relation between these mutations and dental defects is confirmed as previously discovered by the literature (Rauch and Glorieux, 2004, Barron et al., 2008). Future assessment of cementum defects should also consider location of tooth and functional load, as this would indicate if any cementum hyper or hypoplasia is caused by the disease or is a compensatory mechanism to the disease complications. Furthermore, clinical assessment and correlation of such symptoms is crucial to understand whether or not DI patients have masked periodontal involvement and if not, could this be due to the fact that cementum fibres are extracted by both cementoblasts and fibroblasts, which may not be affected by the disease similarly.

Finally, before conducting this study, the authors believed that examining the ultrastructural defects might facilitate the innovation of an adhesive system that binds to collagen instead of mechanically interlocking with it. However, even with the limited results, the direction of future work has shifted to questioning if the incorporation of this severely abnormal collagen is of use, or if it will enhance the binding to DI affected teeth at all. Alternatively, even though mineral content is reduced, chemically bonding to the inorganic content is a valid option, as glass ionomer restorations.

6 Future work

- Conduct a multi-center study to maximize sample size and teeth variation
- Examine the effect of abnormal collagen organization e.g. lack of bundle formation, on matrix deformation, mechanical strength, and mineralisation
- Study presence of collagen types III, IV and VI and its correlation to mineralization
- Define underlying ultrastructural defect in abnormal d-banding and the effect of abnormal d-banding distance on mineralization
- Examine the defects in cementum of DI teeth and correlate with patients' clinical presentation with consideration to occlusion
- Define the consequences of abnormal dentinal and cementum mineralisation
- Compare previous findings in isolated and syndromic DI
- Trials to develop durable adhesives that override collagen interlocking and bind chemically to inorganic tooth structure

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8 Appendices

Appendix A. Systematic Review Search Strategy

Date: 26/06/2020

Database: Embase <1974 to 2020 June 30>

1 ((Dentinogenesis adj3 Imperfecta) or dent* dysplasia or opalescent dentin* or opalescent teeth or opalescent tooth or dentin* sialophosphoprotein).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (1571)

2 tooth/ or dentin*/ (38074)

3 Osteogenesis Imperfecta.tw. (5600)

4 ((Osteogenesis adj3 imperfecta) or Brittle bone or Bruck syndrome* or Fibrogenesis imperfecta ossium or osteopsathyrosis or Lobstein* disease or Lobstein* syndrome or fragilitas Ossium).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (7496)

5 collagen.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (292263)

6 collagen fib*.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (30454)

7 COL1A1.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (5884)

8 COL1A2.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (2209)

9 (((((Collagen adj3 type II) or collagen) adj3 type two) or collagen) adj3 type 2).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (16378)

10 (((((collagen adj3 type I) or collagen) adj3 type one) or collagen) adj3 type 1).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (50457)

11 (((((collagen adj3 type IV) or collagen) adj3 type 4) or collagen) adj3 type four).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (0)

12 (Bond or Mutant or Disorder or genotype).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (2954792)

13 Dentinogenesis Imperfecta/ or dent* dysplasia.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading word, candidate term word] (7386)

14 collagen type 2/ or nonfibrillar collagen/ or collagen disease/ or collagen fiber/ or collagen synthesis/ or fibrillar collagen/ or collagen defect/ or collagen type 1/ or collagen/ or collagen type 4/ or fibril associated collagen/ or collagen fibril/ (209137)

15 1 or 13 (8310)

16 3 or 4 (7496)

17 2 and 15 (825)

18 5 or 14 (292263)

19 17 and 18 (24)

20 6 or 14 (216680)

21 17 and 20 (18)

22 7 or 14 (211504)

23 17 and 22 (17)

24 8 or 14 (209942)

25 17 and 24 (18)

26 10 and 17 (8)

27 9 and 17 (0)

28 11 and 17 (0)

29 12 and 17 (221)

Annotation: ASAS the subdomain

29 = 17 (DI & teeth) + subdomains

30 18 and 29 (7)

31 7 and 29 (1)

32 22 and 29 (6)

33 8 and 29 (2)

34 24 and 29 (7)

35 9 and 29 (0)

36 10 and 29 (3)

37 11 and 29 (0)

38 6 and 29 (1)

39 15 and 16 (366)

40 2 and 39 (26)

41 18 and 40 (9)

42 6 and 40 (1)

43 7 and 40 (2)

44 22 and 40 (7)

45 8 and 40 (2)

46 24 and 40 (8)

47 9 and 40 (0)

48 10 and 40 (3)

49 11 and 40 (0)

50 7 and 17 (3)

51 8 and 17 (2)

52 6 and 17 (3)

Date: 26/06/2020

Database: Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations and Daily <1946 to June 26, 2020>

1 dentin dysplasia/ or Dentinogenesis Imperfecta/ (706)

2 ((Dentinogenesis adj3 Imperfecta) or dent* dysplasia* or Hereditary Dentinogenesis Imperfecta or opalescent dentin* or opalescent teeth or opalescent tooth or dentin* sialophosphoprotein).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms] (1852)

3 1 or 2 (1852)

4 Osteogenesis Imperfecta.tw. (4728)

5 ((Osteogenesis adj3 imperfecta) or Brittle bone or Bruck syndrome* or Fibrogenesis imperfecta ossium or Osteopsathyrosis or Lobstein* disease or Fragilitas Ossium or Lobstein* syndrome).mp. (5897)

6 4 or 5 (5897)

7 tooth/ or dentin*/ (23597)

8 Collagen Type I/ or Collagen Type IV/ or Collagen/ or Collagen Type II/ (112224)

9 collagen.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms] (220548)

10 collagen fib*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms] (21407)

11 COL1A1.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms] (3301)

12 COL1A2.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms] (1374)

13 (((((collagen adj3 type I) or collagen) adj3 type one) or collagen) adj3 type 1).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms] (2412)

14 (collagen type four or collagen type 4 or collagen type IV).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms] (5538)

15 (collagen type two or collagen type 2 or collagen type II).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms] (6741)

16 3 and 7 (107)

17 8 or 9 (220548)

18 16 and 17 (21)
19 8 or 10 (124109)
20 16 and 19 (12)
21 8 or 11 (114017)
22 16 and 21 (12)
23 8 or 12 (112817)
24 16 and 23 (12)
25 8 or 13 (113576)
26 16 and 25 (12)
27 8 or 15 (113541)
28 16 and 27 (12)
29 8 or 14 (113253)
30 16 and 29 (12)
31 2 and 6 (283)
32 (Bond or Mutant or Disorder or genotype).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms] (1457933)
33 16 and 32 (15)
34 17 and 33 (3)
35 19 and 33 (3)
36 21 and 33 (3)
37 23 and 33 (3)
38 25 and 33 (3)
39 27 and 33 (3)
40 29 and 33 (3)
41 3 and 6 and 7 (13)
42 17 and 41 (4)
43 19 and 41 (3)
44 21 and 41 (3)
45 23 and 41 (3)
46 25 and 41 (3)
47 27 and 41 (3)
48 29 and 41 (3)

Date: Done on 26/06/2020 and re-run on 30/06/2020.

Database: PubMed Medline

1 (((((((Dentinogenesis Imperfecta[Text Word]) OR Opalescent Dentin*[Text Word]) OR Opalescent tooth[Text Word]) OR Opalescent teeth[Text Word]) OR Capdepont Teeth[Text Word]) OR Capdepont Tooth[Text Word]) OR Dentin* dysplasia[Text Word]) OR Dentin Sialophosphoprotein[Text Word]) OR Dentinogenesis Imperfecta[MeSH Terms] (2062)
2(((((((Osteogenesis Imperfecta[Text Word]) OR Brittle bone[Text Word]) OR Lobstein* Disease[Text Word]) OR Bruck syndrome*[Text Word]) OR fibrogenesis imperfecta ossium[Text Word]) OR osteopsathyrosis[Text Word]) OR Fragilitas Ossium[Text Word]) OR Osteogenesis Imperfecta[MeSH Terms] (5937)
3 (((teeth[Text Word]) OR tooth[Text Word]) OR dentin*[Text Word]) OR abnormality, teeth[MeSH Terms] (241641)
4 (((bond[Text Word]) OR mutant[Text Word]) OR disorder[Text Word]) OR genotype[Text Word] (1454295)
5 1 and 3 (2061)
6 1 and 3 and 4 (255)
7 collagen[Text Word] (220087)
8 COL1A1[Text Word] (3308)
9 COL1A2[Text Word] (1365)
10 collagen fib*[Text Word] (21298)
11 ((collagen type 1[Text Word]) OR (collagen type one[Text Word])) OR (collagen type I[Text Word]) (21149)
12 ((collagen type 2[Text Word]) OR (collagen type two[Text Word])) OR (collagen type II[Text Word]) (6739)
13 ((collagen type 4[Text Word]) OR (collagen type four[Text Word])) OR (collagen type IV[Text Word]) (5534)
14 5 and 7 (356)
15 5 and 10 (33)
16 5 and 11 (158)
17 5 and 12 (6)
18 5 and 13 (1)
19 5 and 8 (72)
20 5 and 9 (56)
21 6 and 7 (76)
22 6 and 10 (7)
23 6 and 11 (36)
24 6 and 12 (1)
25 6 and 13 (0)
26 6 and 8 (32)
27 6 and 9 (32)
28 1 and 2 (278)
29 28 and 3 (78)
30 29 and 7 (126)
31 29 and 10 (12)
32 29 and 11 (53)
33 29 and 12 (1)
34 29 and 13 (0)

35 29 and 8 (50)

36 29 and 9 (53)

Appendix B. Systematic Review Data Extraction Form

Review title or ID

Study ID *(surname of first author and year first full report of study was published e.g. Smith 2001)*

1.General Information

1. **Date form completed** *(dd/mm/yyyy)*	
2. **Name/ID of person extracting data**	Lubabah Gadi
3. **Report author contact details**	
4. **Publication type** *(e.g. full report, abstract, letter)*	Full report
5. **Study funding source** *(including role of funders)*	
Possible conflicts of interest *(for study authors)*	declared as non present
6. **Notes:**	

2.Reference Details:

1. **Authors**		
2. **Year of Publication**		
3. **Journal details**	**Name**	
	Volume	
	Issue	
	Pages	

3.Eligibility

Study Characteristics	Review Inclusion Criteria	Location in text
7. **Type of study**	rev	--
	Case report	
	Cross sectional /Laboratory	
8. **Dental collagen tested:**	Not specified	
9. **Types of lab test**	•	
10. **Types of statistical test**	None	
11. **Types of outcome measures**	•	
12. **Decision:**	Included.	
13. **Reason for exclusion**	--	
14. **Notes:**	collagen ultrastructure not studied. Implication on collagen defect only	

DO NOT PROCEED IF STUDY EXCLUDED FROM REVIEW

4.Methods

	Descriptions as stated in report/paper	Location in text
15. **Aim of study**		
16. **Demineralization protocol**		
17. **Examination tool**		
18. **Control teeth present?**		
19. **Notes:**		

5.Participants Population and setting

	Description	Location in text
20. **Population description**		Pg.
21. **Inclusion criteria**	collagen study of teeth with DI	Pg.
22. **Exclusion criteria**	-	
23. **Method/s of recruitment of participants**	Undeclared (assume convenient sample?)	
24. **Age**		Pg.
25. **Sex**		Pg
26. **DI type?**		Pg.
27. **OI presence (if yes, type?)**		Pg.
28. **Co-morbidities**		Pg.
29. **Primary or permanent teeth:**		Pg.
30. **Control teeth**		
31. **Type of collagen examined**		Pg.
32. **Notes:**		

6.Results

	Description as stated in report/paper		Location in text
33. **Outcome name**			Pg.
34. **Outcome**			Pg.
35. **Is outcome/tool validated?**	Unclear *Yes/No/Unclear*		
36. **Notes:**			

7.Applicability

37. **Does the study directly address the review question?**	No *Yes/No/Unclear*	
38. **Notes:**		

Appendix C: Scoping Review Search strategy

Database: Ovid MEDLINE(R) ALL <1946 to June 17, 2022>

1 hereditary opalescent dentin.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms] (30)

2 cementum.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms] (5842)

3 Dentinogenesis imperfecta.mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms] (774)

4 1 or 3 (774)

5 2 and 4 (13)

Database: Embase <1974 to 2022 June 17>

1 dentinogenesis imperfecta.mp. or tooth malformation/ (8880)

2 tooth cementum/ (710)

3 Dentinogenesis Imperfecta.mp. (629)

4 osteogenesis imperfecta/co, cn, si [Complication, Congenital Disorder, Side Effect] (853)

5 hereditary opalescent dentin.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word] (28)

6 dentinogenesis imperfecta.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word] (629)

7 osteogenesis imperfecta.mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword heading word, floating subheading word, candidate term word] (8224)

8 4 or 7 (8224)

9 1 or 3 or 5 or 6 (8881)

10 2 and 8 (5)

11 2 and 9 (12)

Appendix D: Scoping Review Data collection form

Review title or ID

Study ID

1.Reference Details:

1. **Authors**		
2. **Year of Publication**		
3. **Journal details**	**Name**	
	Volume	
	Issue	
	Pages	

2.Eligibility

Study Characteristics	**Review Inclusion Criteria**	**Location in text**
39. **Type of study**	*Review / case report*	
40. **Human or Animal study**		
41. **Criteria reported**		
42. **Decision:**		
43. **Reason for exclusion**		

3.Methods

	Descriptions as stated in report/paper	**Location in text**
44. **Aim of study**		
45. **Dentition**		
46. **Dental collagen tested**		
47. **Examination method**		
48. **Demineralization protocol**		
49. **Control teeth present?**		

4. Participants Population and setting

	Description	**Location in text**
50. **Age**		Pg.

	Description	Location in text
51. **Sex**		Pg
52. **DI type**		Pg.
53. **OI presence (if yes, type?)**		Pg.
54. **Co-morbidities**		Pg.

5. Results

	Description as stated in report/paper	Location in text
55. **Outcome**		Pg.
56. **Notes:**		

6.Applicability

57. **Does the study directly address the review question?**	*Yes/No/Unclear*	
58. **Notes:**		