Republic of Iraq Ministry of
Higher Education and
Scientific Research
University of Baghdad
College of Dentistry

Biomimetic Dentistry

A Project Submitted to The College of Dentistry, University of Baghdad, Department of Conservative Dentistry in Partial Fulfillment for the Bachelor of Dental Surgery

By:
Noor Alhuda Sabeeh Flayyih

Supervised By:
Dr. Bashaer Abd elsahib
B.D.S,M.Sc. (conservative dentistry)

May, 2023

Certification of the Supervisor

I certify that this project entitled "Biomimetic Dentistry" was prepared by the fifth-year student Noor alhuda sabeeh flayyih under my supervision at the College of Dentistry/University of Baghdad in partial fulfilment of the graduation requirements for the Bachelor Degree in Dentistry.

Supervisor’s name: **Bashaer Abd elsahib**

Dedication

I would like to dedicate my work and effort to my precious **family**, my mother, father and brother. Their love , assistance, reassurance and prayers have helped me through all the hard times and I couldn’t have done it without their support. So, I would like to thank them for making me the person I am today.

Noor Alhuda Sabeeh

Acknowledgment

All praise and thanks to Allah the exalted for his never-ending blessings and mercy upon us. I pray for Him to continue guiding me to the right path.

Deep thanks to Assist. Prof. Dr. Raghad Abdulrazzaq AL Hashimi, Dean of the College of Dentistry- University of Baghdad for his support to accomplish this review and Dr. Anas Head of the Department of conservative at Baghdad university-college of Baghdad.

I would like to express my appreciation to my supervisor Dr.Bashaer Abd elsahib (B.D.S ,M.Sc.) for her valuable and constructive suggestions.

Finally, I would like to thank my family and friends, whose love and guidance are with me wherever I pursue. I have no valuable word to express my thanks, but my heart is still full of the favours received from every person.

Table of Content

List of figures

INTRODUCTION

Biomimetics is defined as the study of the formation, structure, or function of biologically produced substances and materials and biological mechanisms and processes especially for the purpose of synthesizing similar products by artificial mechanisms which mimic natural ones. A material fabricated by biomimetic technique based on natural process found in biological systems is called a biomimetic material. **(Kottoor, 2013) (Viswanath and reddy, 2014)**

Biomimetic is derived from Latin word "bio" meaning life, and "mimetic" is related to the imitation or mimicking biochemical process with inspiration from nature. **(Zafar et al, 2020) (Sharma et al, 2021)**

The main objectives of biomimetic dentistry are to return the tooth to its function, esthetics, and strength. In dentistry there is no one biomaterial that has the same, mechanical, physical and optical properties as tooth structure (i.e., dentin, enamel, and cementum) and possesses the physiological characteristics of intact teeth in function.**(Goswami, 2018) (Viswanath and reddy, 2014)**

In 2006 Magne said "The goal of Biomimetics in restorative dentistry is to return all of the prepared dental tissues to full function by the creation of a hard tissue bond that allows functional stresses to pass through the tooth, making the entire crown into the final functional biologic and esthetic unit. The intact tooth in its ideal hues and shades, and more importantly in its intracoronal anatomy, location and mechanics in the arch, is the guide to reconstruction that determines success"**(Viswanath and reddy, 2014)(Magne, 2006)**

The term biomimetic was officially listed for the first time in the Webster's Dictionary in 1974. Although biomimetic history goes back to the first century, it did not become popularized among scientists and researchers except after publishing a ground breaking book about it by author Janine

Benyus, 1997 namely ‘Biomimicry: Innovation Inspired by Nature.’**(Singer et al, 2023)**

The word “bionics” was first used by Jack Steele in 1960. Although the concept was very old, its real implementation is possible only recently due to the enormous research in the fields of biochemistry and molecular biology. It is believed that the attempts to replace body parts started at least 2500 years ago when artificial teeth were carved from the bones of oxen.The middle of 20 th century was important in the history of biomimetic medicine due to the sophisticated inventions of the cardiac pacemaker, artificial heart valves, and knee joint replacement. Accidental organ and tissue loss have been treated by surgical reconstruction and the use of mechanical devices such as kidney dialyzers and organ transplant from one individual to other increases in recent years.**(Goswami, 2018)(Vanishree, 2011)(Srinivasan and Chitra, 2015)**

In clinical dentistry, biomimetics refers to the repair of affected dentition mimicking the characteristics of a natural tooth in terms of appearance, biomechanical, and functional competences. For example, adhesive restorative materials have demonstrated tooth morphology and esthetics mimicking natural teeth. Similarly, biomimetic dental implant coatings of calcium phosphate (CaP) and HA have been investigated to improve osseointegration of dental implants to achieve therapeutic benefits. In addition, tissue-engineering approaches have reported promising results in regeneration of oral tissues. Biomimetic endodontic regeneration includes the formation of dentin barrier by pulp-capping agents, root formation during apexogenesis and apexification, apical healing by root-end fillings, and pulp regeneration by cell-homing strategies. **(Zafar et al, 2020)(Bottino et al, 2013)(Yang et al, 2016)**

(Chapter One)

1- Biomemetic Approaches In Restorative Dentistry

The main objective of biomimetic restorative dentistry is to return the hard tissues (enamel, dentin, cementum) to attain full function through a restorative material that can mimic or restore the biomechanics of the natural tooth. This allows the tooth to function as one unit against functional forces and provides near normal biology, and aesthetics.**(Singer et al, 2023)(Goswami, 2018)**

a. In conventional extension for prevention approach, not only the diseased but also sound tooth structure are removed and replaced with rigid, non-responsive materials. This treatment plan usually weakens the remaining tooth structure and yields a short life span restoration**(Fig.1)**.**(Singer et al, 2023)(Burke FJ, 2003)**

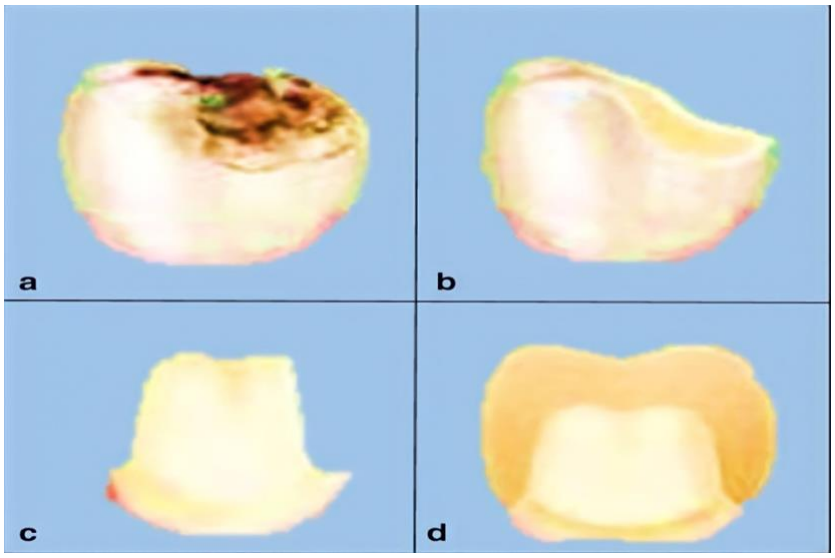

Fig. 1: conventional approach **a** Decayed tooth, **b** the decay is completely removed, **c** more tooth structure is removed to allow space for the placement of rigid restoration, **d** restoration is placed over the weakened tooth structure.**(Singer et al, 2023)**

b. In Biomimetic approach, the concept of less or no dentistry is the best dentistry has been adopted. It is conservative and only focuses on restoring the teeth and simulating the natural dentition as much as possible. The biomimetic

restorative protocols aim to achieve these results by stress-reducing protocols and bond-maximizing protocols. Cavities and other lesions are carefully repaired using advanced materials and adhesives so the tooth retains its inherent natural properties **(fig.2)**. **(Singer et al, 2023)(Burke FJ, 2003)**

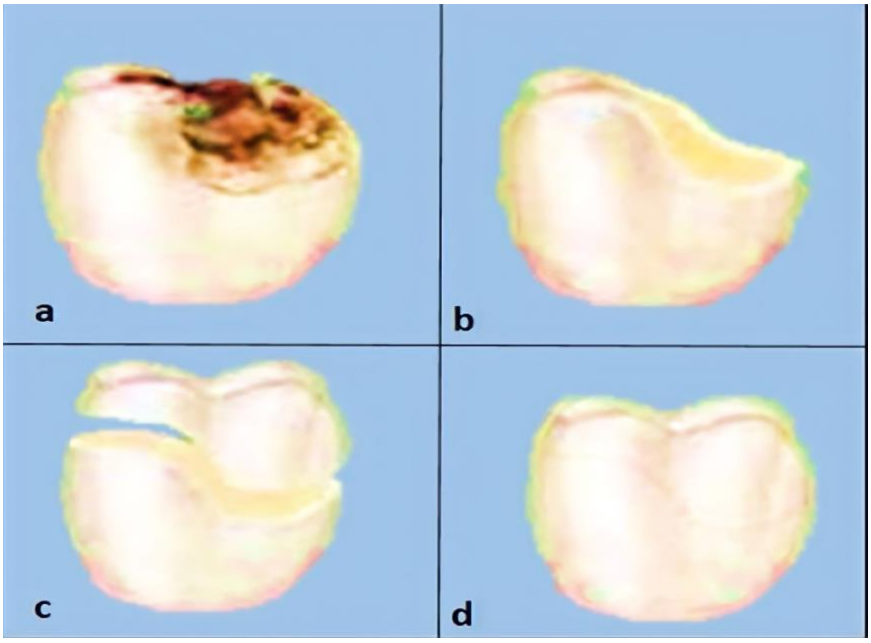

Fig. 2: Biomimetic approach **a** Decayed tooth, **b** the decay is removed completely, **c** limited tooth structure is removed to maintain tooth strength, **d** restoration is bonded to the tooth restoring the structural integrity and strength. **(Singer et al, 2023)**

1.2. Enamel Biomimetics

- Tooth enamel is a highly unique tissue-specific biomaterial characterized by exceptional structural and mechanical properties as well as esthetic beauty. high content in hydroxyapatite, the parallel arrangement of individual elongated apatite crystals into enamel prisms, and the interwoven alignment of perpendicular prisms in a picket fence resembling three dimensional order. Together, these characteristics result

in a biomaterial of great hardness and physical resilience. Due to its toughness and relative fracture resistance, enamel-like biomaterials hold great promise as structural components for future biomedical and engineering applications, including tooth enamel repair, orthopedic defect restoration, and as functional components of insulators, brakes, and exhaust pollutant filters.**(Pandya and Diekwisch, 2019)(Szczes et al, 2017)(Nasr-Esfahani and Fekri, 2012)**

- As desirable as the regeneration or fabrication of tooth enamel may seem, de novo enamel tissue engineering and its potential future clinical implementation remain a daunting task.In biological organisms, enamel is manufactured only once prior to tooth eruption, and the capacity to form new enamel in each individual tooth organ is lost forever, once the tooth is fully erupted.The high ion concentrations and dramatic pH changes involved in initial amelogenesis pose a formidable hurdle in cell based approaches toward tooth enamel regeneration. And even though the synthesis of hydroxyapatite blocks may appear straight forward from a manufacturing perspective, the faithful fabrication of true enamel with its parallel-aligned filigree apatite crystals and decussating prism bundles has rarely been accomplished so far.**(Pandya and Diekwisch, 2019)(Simmer et al,2012)(Ryu et al, 2009)(Bronckers et al, 2016)(Lacruz et al, 2010)(Line, 2005)**

- The cells at the core of nature's ability to manufacture tooth enamel are called ameloblasts. Ameloblasts are highly specialized epithelial cells originally derived from the enamel organ.**(Pandya and Diekwisch, 2019)**

- Although enamel has a hard, dense crystalline structure, it is still permeable to certain ions and molecules which may pass through rod sheaths

due to crack formation and hypo-mineralized enamel structure. The organic contents of enamel and water have a significant role in transporting ions through inter-crystalline enamel rod space. However, due to enamel maturation with age, this permeability decreases with time. It is well established that the enamel solubility decreases whereas hardness is increased with the adsorption of fluoride ions on the enamel surface. Fluoride has a significant role in reducing tooth decay by enhancing remineralization In addition, fluoride influences the chemical and physical properties of enamel, by altering the rate of demineralization, improving remineralization, and preserving apatite structures.**(Zafar et al, 2020)(Perdigão et al, 2019)(Ullah and Zafar, 2015)(Zafar, 2015)**

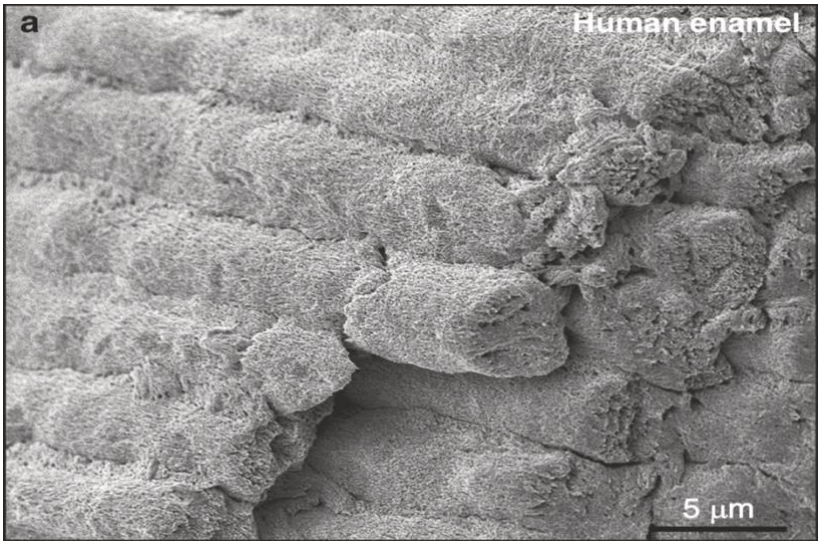

Fig. 3: Scanning electron micrographs of mammalian enamel topography of Human enamel. **Note:** the densely packed apatite crystal network organized into cylindrical enamel prisms (rods). **(Pandya and Diekwisch, 2019)**

1.1.1.PHYSICAL SYNTHESIS APPROACH:

- In nature, the conversion of inorganic calcium phosphates into crystalline apatites requires extreme conditions such high temperature, high pressure, or unusual pH.Any synthetic process seeking to manufacture hydroxyapatite as the principal component of biosynthetic tooth enamel would need to mimic the biological conditions required for apatite biomineralization and generate an environment that resembles some of the extreme environments that occur during physiological hydroxyapatite crystallization. Therefore, physical approaches toward enamel synthesis rely on extreme conditions in terms of temperature, pressure, or isoelectric point, or a combination thereof. **(Pandya and Diekwisch, 2019)**

- The first synthetic generation of apatite nanorods was based on an aqueous solution of hydroxyapatite titrated to pH 2 in conjunction with surfactant docusate sodium salt as a colloidal suspension solution. Adjusting this solution to only slightly acidic conditions (pH 5.8) resulted in the precipitation of 200–400 nm long apatite crystals with a Ca/P (Calcium/Phosphate) ratio of 1.6, fairly close to atomic Ca/P ratio of hydroxyapatite at 1.67.This study represented the first successful approach toward the synthetic generation of parallel-aligned and elongated enamel like apatite crystals.**(Pandya and Diekwisch, 2019)(Chen et al,2005)**

- To generate apatite nanorods that more closely matched the size of natural enamel crystals, the hydroxyapatite solution from the previous study was replaced with a fluorapatite solution, and the atmospheric conditions were altered to include intense hydrothermal pressure by autoclaving the crystallization solution for about 10 h on an iron plate substrate.**(Pandya and Diekwisch, 2019)(Chen et al, 2006)**

- A third synthetic approach was devised to avoid some of the extreme conditions employed as part of the previous two approaches, namely high pressure, high acidity, and the use of toxic nucleation or emulsification conditions. Instead, this third approach relied on high temperature (150–200 °C for up to 72 h), a sodium bicarbonate buffer to regulate the pH during crystal formation, and a crystallization solution consisting of calcium nitrate tetrahydrate, di-sodium hydrogen phosphate, sodium bicarbonate, and octacalcium phosphate at a pH of 6.6. The apatite rods that resulted from these experiments were substantially smaller than human enamel crystals, measuring between 200 nm and 500 nm in length, 100 nm and 200 nm in width.**(Pandya and Diekwisch, 2019)(Ren et al, 2012)**
- Recently, a three-step synthetic process was conceived to mimic key aspects of initial enamel formation, including:

(i) conjugation of carboxymethyl chitosan (CMC) with alendronate (ALN) to stabilize amorphous calcium phosphate (ACP) and form CMC/ACP nanoparticles.

(ii) application of sodium hypochlorite (NaClO) to degrade the CMC-ALN matrix generated in step (i).

(iii) use of 10 nmol•L −1 glycine (Gly) to guide HAP/ACP (hydroxyapatite/ amorphous calcium phosphate) nanoparticles to organize into well-ordered rod-like apatite crystals.

This process is based on a polysaccharide/bisphosphonate matrix (chitosan/alendronate) and mimics key steps of initial amelogenesis, including:

(i) formation of a Ca/P rich amelogenin protein matrix.

(ii) enzymatic degradation and continued crystal growth.

(iii) crystal elongation as facilitated by elongated amelogenin fragments.

(Pandya and Diekwisch, 2019)(Wang et al, 2017)(Pandya et al, 2017)

- Together, the four studies summarized above have made substantial progress toward the goal of synthesizing enamel like structures in vitro. However, current approaches still rely heavily on synthetic conditions such as high temperature, pressure, or extreme pH, or employ toxic chemicals such as surfactant, bisphosphonate, or sodium hypochlorite, preventing such synthetic approaches from immediate application in the oral cavity. Moreover, soft intermediate materials as they would be generated during the three-step chitosan/glycine-based synthesis would not withstand the masticatory pressures on occlusal surfaces during mastication. **(Pandya and Diekwisch, 2019)**
- Nevertheless, the simple feat of synthesizing enamel like apatite materials en block will avail a future generation of dental practitioners with highly biomimetic materials that may replace parts of the enamel layer or the entire enamel layer when used in combination with digital grinding and milling technologies. **(Pandya and Diekwisch, 2019)**

1.1.2.BIOCHEMICAL ENAMEL ENGINEERING:

Tissue engineering has often been described as a means to copy developmental biology for regenerative purposes. Mimicking natural tooth enamel formation for tissue engineering purposes would involve the manufacture of an amelogenin-rich protein matrix and enriching this matrix with calcium phosphate ions. As it turns out, enamel development is exponentially more complex in nature, and the simplified approach mentioned above has not yet been successful in the laboratory. Challenges encountered when using a simple developmental approach toward enamel tissue engineering include mimicking the coordinated movement of ameloblast cells as the formative units associated with the secretion of each individual prism, adjusting the pH value of the mineralization solution in a dynamic fashion as it occurs in

vivo, counteracting the inhibitory effects of the amelogenin protein on crystal growth and selectively applying individual amelogenin fragments in a biomimetic fashion to control apatite crystal growth. **(Pandya and Diekwisch, 2019)**

Developmental biology has informed us that the initial enamel matrix consists to 60–70% of water, 20–30% of proteins, and 15–20% of mineral ions. Three unique matrix proteins have been associated with the developing enamel matrix, amelogenin, ameloblastin, and enamelin, which have therefore been coined enamel proteins.Among these, amelogenin is by far the most abundant protein component in the developing enamel layer, contributing to more than 90% of its overall volume. major functions of the developing enamel matrix, i.e., (i) enamel hydroxyapatite crystal nucleation, (ii) enamel apatite c-axis crystal growth, and (iii) the spacing between individual apatite crystals during crystal nucleation and growth.Only upon completion of mineralization, enamel proteins and water are resorbed from the developing enamel layer, resulting in a 1% organic matter content in the mature enamel, while the remaining 99% volume contains inorganic material, mostly apatite. **(Pandya and Diekwisch, 2019)**

(Atsawasuwan et al, 2013)

Based on the interaction between majority components, combinations of enamel proteins and calcium phosphate growth solutions would be a logical first step toward the biological synthesis of tooth enamel. In support of this approach, we have been able to grow elongated and parallel apatite crystals within decussating enamel prisms using an enamel protein matrix. It has also been demonstrated that a cooperation between amelogenin and another enamel protein, enamelin, resulted in the stabilization of the amorphous calcium phosphate precursor phase and an increase in the length to width ratio of resulting octacalcium phosphate crystals, likely due to a coassembly between amelogenin and enamelin. While combinations of octacalcium phosphate

crystal growth solutions and amelogenins succeeded in the growth of calcium rich parallel oriented crystal bundles, this approach did not result in enamel like biomaterials in terms of size, hardness, and structure. **(Pandya and Diekwisch, 2019)**

So far, studies using a biochemical approach have only mimicked individual aspects of apatite and calcium phosphate crystal growth. Additional improvements would result in biomaterials that more closely resemble natural enamel which then could be applied to the patient's mouth. **(Pandya and Diekwisch, 2019)**

1.1.3.IN SITU ENAMEL ENGINEERING (ENAMEL SURFACE REMINERALIZATION):

- Once teeth are erupted, the cells and tissues involved in enamel formation, i.e., the ameloblasts and the enamel organ, are no longer present on the tooth surface. The lack of natural means for enamel regeneration has created an opportunity for restorative dentistry and for the replacement of enamel tissues with synthetic substitutes such as amalgams, gold, porcelain, and polymer composites. **(Pandya and Diekwisch, 2019)**
- The advent of biomimetics has led to an exploitation of natural mechanisms that either alter tooth enamel apatite mechanical and chemical surface properties or to grow layers of enamel-like apatite material onto the surface of already existing tooth enamel, using enamel protein-like substrates together with apatite growth solutions. **(Pandya and Diekwisch, 2019)**
- The benefits of fluorides for dental health and resistance of tooth enamel against tooth decay. Fluoride affects enamel properties through the exchange of the hydroxyl group (-OH) in the hydroxyapatite $Ca_5(PO_4)_3$

OH with a fluoride ion to form either fluorapatite or fluor-hydroxyapatites. **(Pandya and Diekwisch, 2019)**

- Other approaches have focused on enamel remineralization strategies through toothpastes.An in vivo study based on a gelatin gel in conjunction with calcium and fluoride ions to treat enamel surface defects demonstrated formation of a smooth enamel-like layer, but little is known about the long-term success rates, hardness or structural integrity of the newly formed enamel layer. **(Pandya and Diekwisch, 2019)**
- A third biomimetic approach toward enamel surface remineralization has focused on the natural ability of tooth enamel proteins to nucleate and guide the growth of enamel apatite crystals. Earlier studies have demonstrated that full-length tooth enamel proteins such as amelogenins and enamelins inhibit apatite crystal growth in vitro. Thus, it has long been assumed that alternative splicing and post translational cleavage of enamel proteins are essential for the ability of the enamel protein matrix to promote enamel crystal growth. In addition to natural proteins, synthetic self-assembling peptides have been developed as agents to promote the remineralization of the white lesions associated with initial caries in humans. **(Pandya and Diekwisch, 2019)**

In summary: recent strategies to achieve enamel tissue regeneration through surface remineralization have shown promising data, suggesting that further studies are likely to improve the integration of the newly synthesized apatite layer with the already existing enamel and enhance the thickness and mechanical properties of the regenerated enamel. **(Pandya and Diekwisch, 2019)**

1.1.4.WHOLE-TOOTH REGENERATION APPROACH:

- Regenerating whole-tooth organs has long been considered the penultimate dream of dental regenerative medicine. During initial tooth development, epithelial and mesenchymal tissues interact, form bud-stage tooth organs, and continue to develop and differentiate into odontogenic tissues, including ameloblasts, odontoblasts, and cervical loop cells. From a conceptual point of view, mimicking those signaling cascades to induce de novo tooth formation at any less differentiated epithelial mesenchymal interface appears to be a logical next step. However, the progression of bud-stage epithelial mesenchymal interfaces into fully differentiated teeth has proven to require unique structural and inductive environments. One such example for successful whole tooth restoration has recently been accomplished by bioengineered tooth germ transplantation into a donor model.However, this approach relies on the utilization of reconstituted canine tooth germ cells. **(Pandya and Diekwisch, 2019)**
- The ability of cap stage tooth organs to form fully differentiated dentin and enamel mineralized tissues when explanted onto Trowel organ culture dishes has been known for decades.This Trowel organ culture model represents a viable model to grow thin layers of fully developed prismatic enamel in vitro.Cap stage tooth organs may also be transplanted into the kidney capsule or into the anterior chamber of the eye, yielding further advanced stages of enamel deposition. With rapid advances in three-dimensional cell culture technology, application of ameloblast-specific factors to further the growth and differentiation of enamel-like tissues will harness the natural ability of the enamel organ to manufacture prismatic enamel with mechanical properties similar to human enamel.
- In addition, computer aided design/computer aided manufacture preparations of biomimetic enamel grown in bioreactors may evolve into

enamel repair materials for caries lesions. **(Pandya and Diekwisch, 2019)**

- From a clinical perspective, it is not clear whether synthetic or regenerated enamel will ever become a mainstream biotechnology product used in future dental offices. Rather, enamel-like biomaterials are likely to find use in many other biomedical or engineering applications because of their enormous strength, resilience, and biocompatibility. **(Pandya and Diekwisch, 2019)**

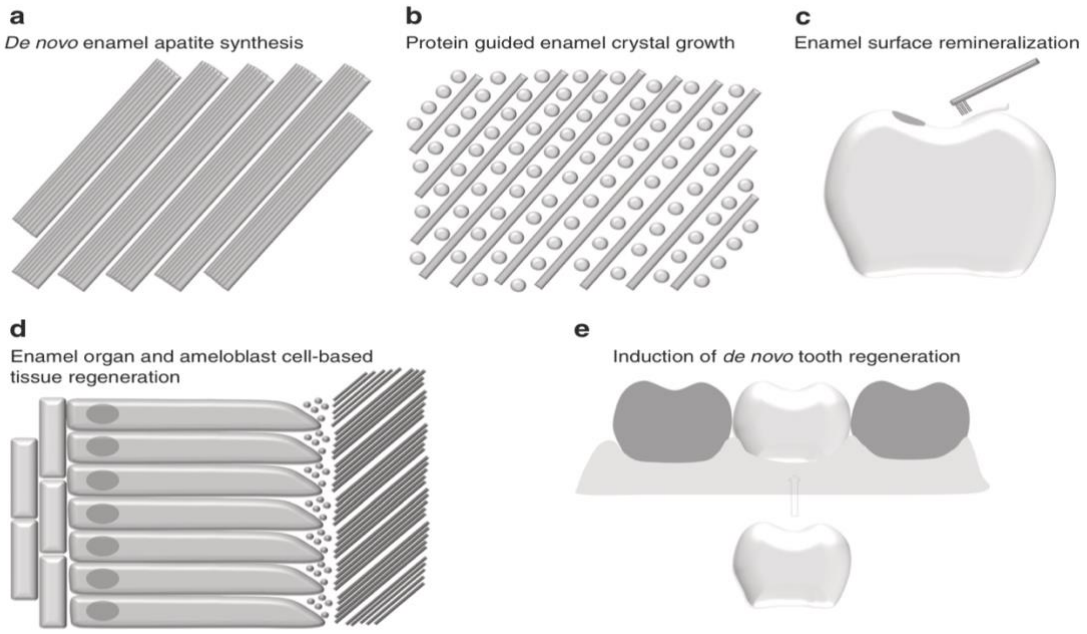

Fig. 4: Summary of the five enamel engineering strategies including **a** de novo enamel synthesis, **b** protein matrix-guided enamel crystal growth, **c** surface remineralization of white lesions and fluoride applications, **d** enamel organ and ameloblast cell-based tissue regeneration, and **e** induction of de novo tooth regeneration. **(Pandya and Diekwisch, 2019)**

1.2. Approaches for dentin remineralization:

Dentin is a mineralized tissue consisting of apatite (the mineral phase), collagen and other proteins, and water. Remineralization of dentin can occur either by simple precipitation of calcium phosphates into the loose demineralized dentin matrix between collagen fibrils (net remineralization), or

by the chemical tight association of mineral to the dentin matrix structure (functional remineralization). Phosphate ions of the apatite surface should be attracted to the positively charged N-terminal end of the peptides. The peptides originating from the gelatin of BIMIN may orient perpendicular to the substrate and parallel to each other. Polar regions on the molecules attract ions, which mineralize to apatite, template by the ordered gelatin. This leads to the growth of fluorapatite crystals perpendicular to the surface. The long axis of the apatite crystals and gelatin peptides preferentially orient themselves parallel to each other. Thus the introduced experimental biomaterial may lead to (at least superficial) functional remineralization in existing dentin structures, with an additional mineralization of an enamel-like fluorapatite layer. **(Viswanath and reddy, 2014)**

Biomimetic remineralization of dentin is possible using some ion-containing solutions or ion-leaching silicon-containing materials. Recent studies reported the use of bioactive "smart" composites containing reactive calcium silicate. Many researchers used "agarose" gel containing sodium hypophosphate that covered an acid-etched dentin material. **(Goswami, 2018)**

a. Crystallite's growth conventional remineralisation approach:

Conventional remineralisation protocols of the carious dentin often involve using formulations with calcium and phosphate ions of different concentrations. In this case, remineralisation occurs by epitaxial growth of residual apatite crystals in partially demineralized dentin rather than new crystals nucleation If there are no or very few residual crystals, there will be no remineralisation. The mineral content of the surface layer of the lesion affects the quality of the resulting remineralisation, including its location and mineral deposition density. **(Singer et al, 2023)**

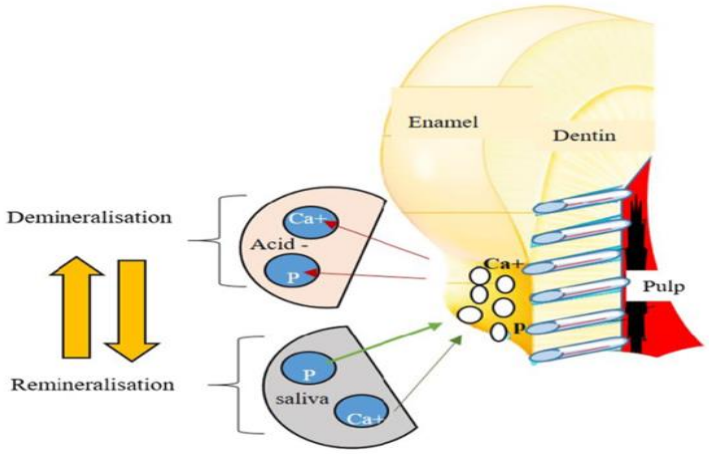

Fig. 5: Demineralisation and remineralisation processes. **(Singer et al, 2023)**

b. Bottom-up biomimetic remineralisation approaches:

The biomimetic remineralisation process is a bottom-up approach used to create nanocrystals that are small enough to fit in the gaps between adjacent collagen molecules in order to backfill the demineralized dentin collagen. These nano precursor particles (amorphous calcium phosphate (ACP) nano-precursors) are stabilized by biomimetic analogs of non-collagenous proteins (dentin matrix protein (DMP1) and dentin phosphophoryn (DPP, DMP2) that regulate the HA crystal nucleation growth. In this direction, several bioactive materials and non-collagenous proteins (NCPs) analogs have been used to promote remineralisation.**(Singer et al, 2023**

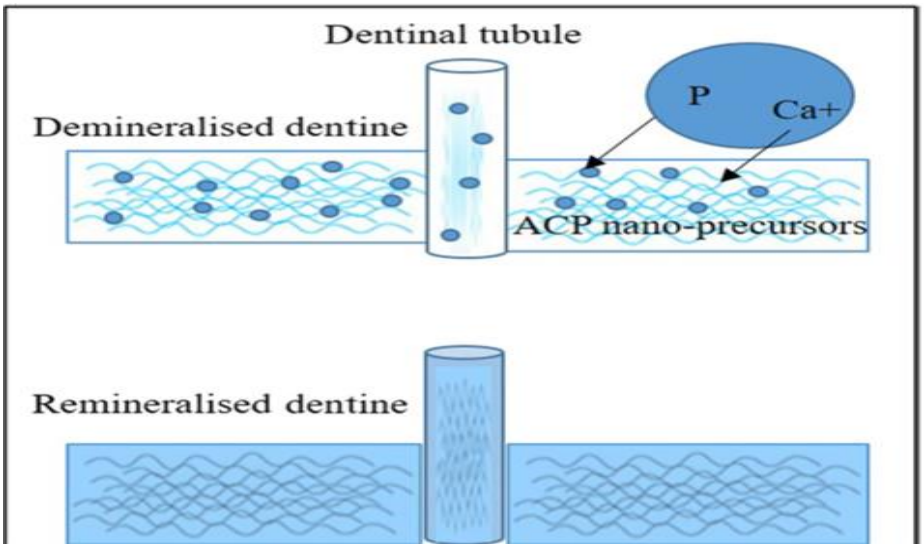

Fig. 6: Bottom up biomimetic approach.**(Singer et al, 2023)**

1.3.Biomimetic materials used in restorative dentistry:

The material used to restore the function of the tooth should exhibit properties such as modulus of elasticity, tensile strength, and compressive strength for the replaced tooth structure. **(Paryani et al, 2023)**

- **Glass ionomer cement**

Glass ionomer cement is regarded as a biomimetic material because it exhibits characteristics of dentin, such as adhesiveness to tooth structures and fluoride release. It is a restorative material that is bioactive and has a wide range of uses, such as bonding, lining, luting, sealing, or restoring teeth. It has a coefficient of thermal expansion identical to that of a natural tooth. **(Paryani et al, 2023)**

in spite of these advantages, conventional glass ionomers suffer from disadvantages such as short working times, long setting times, brittleness, low fracture toughness, and susceptibility to moisture contamination or dehydration during the early stages of the setting reaction. Various modifications in the powder and liquid of glass ionomer material have been done to improve its performance. **(Singer et al, 2023)**

- **Dental composite resin**

Dental composite resin (DCR) represents an important category of hybrid biomaterials, which are composed of a resin matrix and inorganic fillers. DCR has been widely applied in dentistry to restore diseased and defective teeth since the 1960s because of their excellent aesthetics, good biocompatibility, and ease of use. Self-healing/Bleeding material is a resin composite system, which employs a biomimetic approach to perform a self-repairing function. Many natural materials are themselves self-healing composites as the natural bone, which has the ability to self-heal even with major fracture. Bilayered resin composite structure is considered a new biomimetic restoration system, which mimics the fibrous structure of dentin-enamel complex.**(Singer et al, 2023)**

❖ **Ceramics**

Dental ceramics are able to mimic the natural appearance of the tooth. The popularity of dental ceramics has been widely extended in the last three decades after the evolution in computer based dental technologies and the introduction of the "digital workflow" concept in dentistry. The ambition of researchers to develop biomimetic dental restorative ceramics existed long time ago. Biomimetic dental ceramics should be able to establish gap free adhesion to the restored dental substance and to promote natural regeneration of surrounding tissues. **(Singer et al, 2023)**

Biomimetic application of dental ceramics also includes bioactive coated ceramic implant. Several bioactive glass–ceramics are commercially available and were used to coat titanium and zirconia dental implants. The coating enhanced the osseo integration and tissue bonding around ceramic implants. **(Singer et al, 2023)**

Biomimetic dental ceramics are another example of biomimetic ceramic materials. They were introduced in a trial to combine the advantages of ceramics and composites in order to get physical properties similar to that of enamel and dentin. **(Singer et al, 2023)**

Since the tooth is made up of different structures (enamel and dentin) which have different optical and physical properties, the possibility of mimicking both tissues with one layer of material is not an easy task. Recently manufacturers began to implement the concept of gradient shade and translucency in their ceramic products. Furthermore, the concept of biomimetics was recently extended to include gradients in strength within the same ceramic block. **(Singer et al, 2023)**

2. <u>Biomimetic regenerative endodontics</u>

The definition of regenerative endodontic procedures has been given by Murray and Gracia as "events based on biological design to substitute missing,

diseased, underdeveloped or damaged components of the tooth structures including root and dentine structures to restore physiological functions of pulp dentine complex".**(kumar et al, 2022)**

The complete assembly involved in the regenerative endodontics procedures are stem cells, signalling molecules, and scaffolds harvested on the extracellular matrix (ECM).**(kumar et al, 2022)**

The essential goal in REP is to promote pulp-tissue regeneration, development of roots, and proliferation of the progenitor-stem cells from the bone/tooth region.In the apical papilla, these osteo/odonto-progenitor-stem cells prevent the infection and necrosis of the root that is caused due to the proximity of the periodontal-blood supply. In addition, REP may influence angiogenesis, cell survival, differentiation migration, and proliferation. **(kumar et al, 2022)**

2.1.Appraoches:

A. Revascularization or Revitalization:

Teeth with apical periodontitis and immature root apex having periapical infection underwent the revascularization process in 1971. However, due to limitations in materials, instrumentation, and techniques, this attempt failed. The process of revascularization technique is different from both apexification and apexogenesis.Apexification is defined as 'an apical barrier to avert the route of toxins and bacteria into periapical tissues from root canal". Apexogenesis is a technique that discourses the inadequacies involved with capping the inflamed dental pulp. **(kumar et al, 2022)**

Revascularization is the terminology that is used to describe the treatment of immature-necrotic teeth which involves the proliferation of the tissues in the pulp space of the involved tooth. When canal space is induced with bleeding, undifferentiated mesenchymal-stem cells accumulate significantly.
In the case of necrotic pulp, an endodontic procedure was carried out to rejuvenate tooth vitality known as "revitalization", while the replacement of lost

or damaged pulp-dentin tissue complex is known as “regeneration. However, the underlying mechanism for regeneration of the dentine-pulp complex is poorly understood. Instead, root-canal therapy may undergo a repair/healing process. **(kumar et al, 2022)**

Advantages of the Revascularization Approach:

1. Technically simple approach.
2. There is no need of using expensive biotechnology due to currently available instruments and medicament techniques.
3. There are almost negligible chances of immune rejection as this approach relies on the patient’s own blood.
4. Bacterial microleakage can be eliminated through the induction of stem cells into the root canal space, followed by the intra-canal barrier, inducing a blood clot.
5. The concerns of restoration retention need to be overcome.
6. When this approach is applied to immature teeth, it reinforces their root walls.
7. As the avulsed immature tooth has necrotic-pulp tissue along with an open apex, and short and intact roots; therefore, the newly formed tissue will easily reach the coronal-pulp horn because proliferation in a short distance is required. Therefore, the strategy behind the development of new tissue is to maintain the balance between the pulp-space infection and the proliferation of new tissue.
8. Additional growth of open-apex root takes place due to minimum instrumentation that will preserve viable pulp tissue.
9. The potential to regenerate more stem cells and the rapid capacity to heal the tissue in young patients needs to be recognised.

(kumar et al, 2022)

Disadvantages of the Revascularization Approach:

1. The origin of where the tissue has been regenerated from is yet to be known.
2. According to researchers, effective composition and concentration of cells are mandatory for tissue engineering. However, these cells are entombed in fibrin clots; therefore, researchers do not rely on blood-clot formation for tissue engineering function.
3. Treatment outcomes will be variable by the variations in the composition and concentration of the cells.

(kumar et al, 2022)

Prerequisites for Revascularization Approach:

Revascularization studies have established the following prerequisites:

• There should be open apices and necrotic pulp secondary to trauma.

• In addition, open apex should be less than 1.5 mm.

• The following agents can be incorporated to remove microorganisms from the canal.

- Antibiotic paste
- Calcium hydroxide paste
- Formocresol

• The coronal seal should be effective.

• There should be a matrix or the growth of new tissues.

• There When should trying be to a induce matrix or bleeding, the growth anaesthesia of new tissues. should be used without a vasoconstrictor.

• When trying to induce bleeding, anaesthesia should be used without a vasoconstrictor.

• Canals should not be instrumented.

• Sodium hypochlorite should be used as the irrigant.

• There should be blood clot formation. **(kumar et al, 2022)**

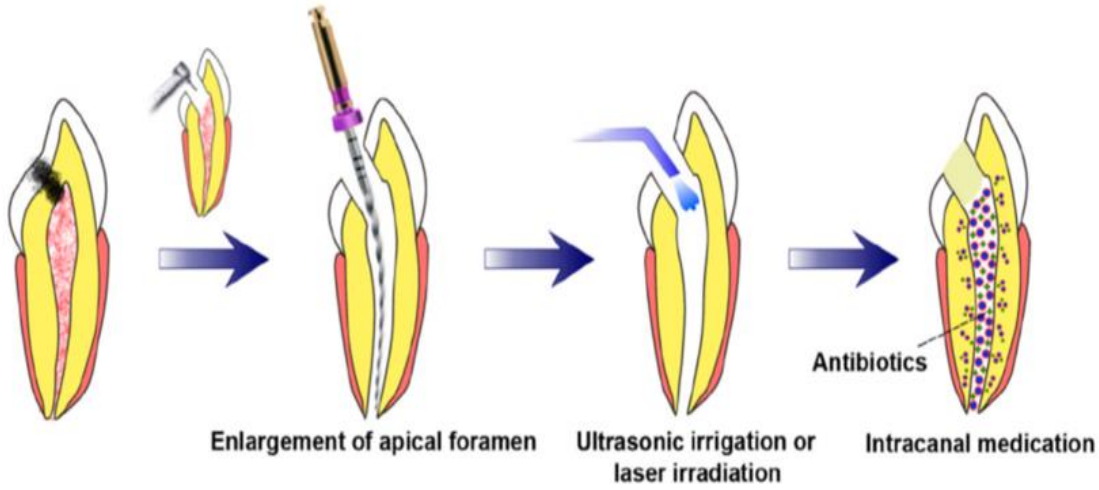

Fig.7: Requisite preconditions for pulp regeneration (root canal disinfection and enlargement of the apical foramen). **(kumar et al, 2022)**

B. Stem cell therapy:

Stem cell therapy is an upgraded procedure that can be used for the treatment of degenerated tissues. Stem cells are multipotent cells that can be differentiated into any other forms of cells. The easiest method of stem cell therapy is to administer cells of definite regenerative potential into the disinfected root canal system. **(Goswami, 2018)**

However, low survival rates are one of the advantages major disadvantages of this technique. Moreover, cells can migrate into different locations of the body, which presents peculiar of mineralization. For the development of dental tissues by the differentiation of stem cells, bioactive-signalling molecules, growth factors and scaffolds are required. **(kumar et al, 2022)**

Pulp Implantation:

In this procedure, after cleaning and shaping the root canal, the substituted pulp tissue is transplanted. Purified pulp-stem-cells line is among one the sources of the pulp tissue. This pulp tissue can also grow in the laboratory by cell biopsy. For this invitro technique, pulp tissues can be cultured by biodegradable-polymer nanofibers. It is a restriction of this technique that the apical portion of the root canal should be harvested with pulp cells. The reason

behind this concept is that the sheets of the extracellular matrix are very thin, fragile, and they lack vascularity. Further, in vivo investigation and controlled clinical trials are needed to explore the success rates and outcomes of functioning pulp tissue and concerns over immune responses, although this technique presents a low possibility of health risks to patients. **(kumar et al, 2022)**

Scaffold Implantation:

In this procedure, pulp tissue is obtained by tissue engineering process and then it is administered in a soft three-dimensional scaffold matrix. **(Goswami, 2018)** Distribution of therapeutic medicines to precise tissues can successfully be accomplished by these nano scaffolds. Moreover, the biological and mechanical properties needed for proper functioning are also provided by these scaffolds. **(kumar et al, 2022)**

Among all the injectable biomaterials, hydrogels are more favorable in the field of tissue engineering. Hydrogels are injectable scaffolds that can be delivered by a syringe, are noninvasive in nature, and easy to deliver into the root canal systems. Theoretically, hydrogel promotes pulp regeneration by providing a substrate for cell proliferation and differentiates into an organized tissue structure. **(Goswami, 2018)**

Three-Dimensional Cell Printing:

The three-dimensional cell printing technique is considered the final approach for the replacement of pulp tissues. This approach can be used to position cells precisely. This technique mimics the natural pulp-tissue structure. In tissue-engineering technique, to maintain and repair dentine, odontoblastoid cells should be positioned around the periphery of the pulp. Moreover, the fibroblasts support the vascular and nerve cells and should be positioned inside the pulp core. This technique required great expertise and careful orientation as

during this procedure apical and coronal asymmetry is the prerequisite during the placement of the pulp tissue into the shaped and cleaned root-canal system. However, currently, this technique is not available clinically and there is a dearth of literature regarding the functionality of the three-dimensional cell-printing technique. **(kumar et al, 2022)**

Gene Therapy:

In regenerative endodontics, gene delivery has been discussed in a recent review. To promote tissue mineralization, mineralizing genes would be delivered into the pulp tissues. **(kumar et al, 2022)**

It is a method of delivering genes with viral or non viral vectors. Viral vectors are genetically altered to eliminate ability of causing disease without losing the capacity of infecting a cell. **(Goswami, 2018)**

Platelet-Rich Plasma (PRP):

Special challenges are faced by clinicians for the treatment of an immature tooth with necrotic pulp and open apex. One of the strategies for its treatment is the traditional apexification procedure. This treatment process requires the formation of the apical barrier by multiple applications of calcium hydroxide. This apical barrier can also be formed by placing mineral trioxide aggregate (MTA) into the canal, which is followed by the conventional root-canal procedure.Due to incomplete root formation with these procedures, the chances of root fracture are very common. PRP can be utilized as a scaffold as it can form a three-dimensional fibrin matrix. It is easily prepared from the patient's autologous whole blood. Human dental pulp stem cells (DPSCs), when treated with PRP, resulted in an increase in the differentiation and proliferation of these cells. Moreover, in regenerative-endodontic procedures, the optimal level of MTA placement is mandatory which can be done by the collagen matrix present in the PRP. PRP can be employed in clinical cases when little or

no bleeding is observed from apical tissues. The source of stem cells, their interaction, and the role of inflammatory cells in the root canal need further exploration for the advancement of regenerative-endodontic treatment. **(kumar et al, 2022)**

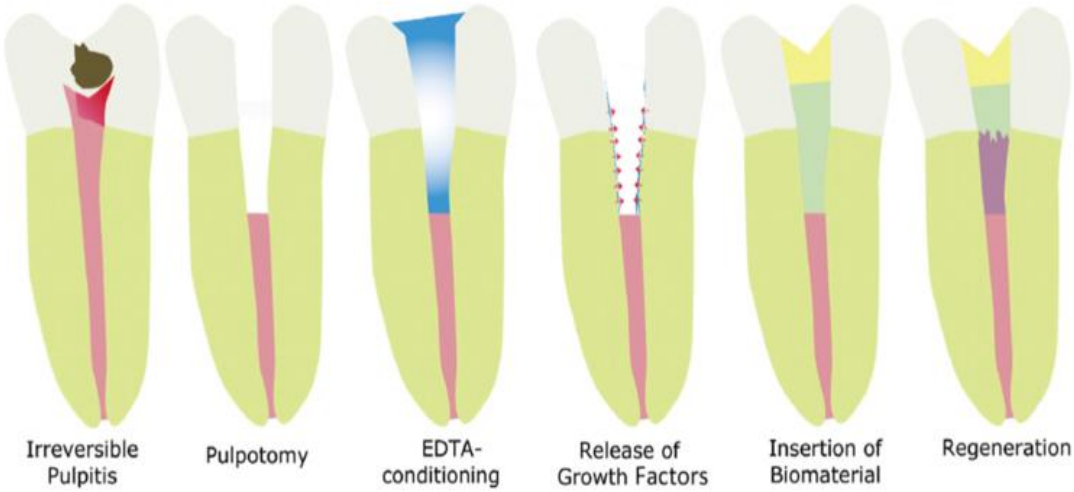

Fig.9: Clinical procedure for dental-pulp regeneration using a cell-free approach. **(kumar et al, 2022)**

Bioengineered tooth:

Research on whole tooth regeneration is also advancing using a strategy of transplanting artificial tooth germ and allowing it to develop in the adult oral environment. Tooth regeneration is an important stepping stone in the establishment of engineered organ transplantation, which is one of the eventual goals of regenerative therapy. **(Viswanath and reddy, 2014)**

2.3 Biomimetic Materials in Endodontics:

- Biointeractive Materials: is the one that elicits a specific response by releasing biologically relevant ions. **(kumar et al, 2022)**

I. Calcium Hydroxide

Calcium hydroxide was introduced by Hermann in dentistry. It contains calcium ions and hydroxyl ions. It is used as a cavity liner, as an interim root

canal dressing to induce hard tissue formation, in the treatment of root fracture and root resorption, and as a permanent root canal sealer. It has antibacterial properties due to the alkaline pH, and it may aid in dissolving necrotic tissue remnants, bacteria, and their byproducts. It also has the ability to induce tertiary dentin formation. **(Paryani et al, 2023)**

II. Calcium Sulfate

Calcium sulfate, a natural mineral, has been widely used in orthopedics and dentistry to repair bony defects. It is biocompatible, bioresorbable, and osteoconductive. It undergoes complete and rapid resorption without eliciting any inflammatory-tissue response. studies have proved that calcium sulfate can induce new-bone formation when placed in bony defects. However, it lacks osteoinductivity, and the proteins present in blood and tissue fluids prolong its setting time. Rapid resorption sometimes hinders its use in large-bone defects, and thus, limiting its use in endodontics. To overcome these constraints, biphasic calcium sulfate and composites of calcium sulfate with other bio-materials are also available. **(Paryani et al, 2023)**

- Bioactive Materials: is one that elicits a specific biological response at the interface of the material which results in the formation of a bond between the tissues and the material. **(kumar et al, 2022)**

    i. **Mineral trioxide aggregate (MTA)** is a hydrophilic substance made of calcium silicate. This is a preferred substance for pulp capping, root-end filling in apicoectomy procedures, vital pulp therapy, apexogenesis, and apexification. When implanted, it stimulates the growth and development of odontoblast-like cells, which result in the production of a collagen matrix. The matrix is

thus first mineralized by osteodentin and later by the development of tertiary dentin. It shows good adhesion to dentin. When employed in vital pulp therapy, it has low solubility, and no tunnel defects are seen as compared to calcium hydroxide. **(Paryani et al, 2023)**

ii. **Biodentine:** was introduced in 2009 as a dentine-replacement material. It is formulated using MTA-based technology to improve the physical, setting, and handling properties while providing the same range of clinical applications of MTA. biodentine increases the proliferation, migration, and adhesion of human-dental-pulp-stem cells, promoting remineralization. Biodentine can also be used as a barrier material in regenerative endodontics. **(Paryani et al, 2023)**

iii. **Theracal:** TheraCal is made of light-cured silicate resin that has been modified with resin. It serves as a protective layer underneath base materials including cement, amalgam, and composite. Compared to Dycal and MTA, TheraCal has low calcium solubility and high calcium release. **(Paryani et al, 2023)**

iv. **Hydroxyapatite:** Hydroxyapatite is a nonrestorable calcium phosphate material. It is composed similarly to bone and has an osteoconductive characteristic. Due to its poor mechanical properties, it is not employed in areas that sustain loads. It serves as a filler in composite resin and is used in bone grafting. For endodontic therapy, hydroxyapatite has been used for perforation repair, formation of the apical barrier, pulp capping, and periapical defect repair. When compared to calcium hydroxide-produced reparative dentin, Malik et al. claimed that tricalcium phosphate hydroxyapatite produces dentin mineralization that is more extensive and thicker. **(Paryani et al, 2023)**

v. **Bioactive glass:** They have the ability to react in liquid or water. The formation of silica gel polycondensed coating on glass bulk serves as a template for the development of calcium phosphate due to its characteristics and high biocompatibility. Bioactive glasses have also been used in bioregeneration. These can be utilized for implant coating, dentin hypersensitivity treatment, and bone grafting. **(Paryani et al, 2023)**

vi. **Emdogain:** Emdogain is made from enamel matrix protein from the tooth germ of swine and propylene glycol alginate as a matrix. Hertwig epithelial root sheath secretes an enamel matrix-derived protein that induces the formation of periodontal tissue. Emdogain imitates these tooth-developmental mechanisms. Ameloblastin, enamelin, growth factor, tuftelin, and bone morphogenic protein are examples of non-collagen proteins that are also present in Emdogain. It has been used in the treatment of vital pulp therapy and pulpotomy because it causes reparative dentin formation. It is used to reduce external root resorption in replantation situations. **(Paryani et al, 2023)**

Clinical Outcomes of Regenerative Endodontics Procedure:

The clinical success of regenerative endodontic procedures, as defined by The American Association of Endodontists, is evaluated by the following three outcomes.

• Primary goal: This is an essential goal. It consists of elimination of signs, symptoms, and bony healing.

• Secondary goal: This is a desirable goal. In this, there will be increased root length and root-wall thickness.

• Tertiary goal: Vitality testing is positive.

(kumar et al, 2022)

(Chapter Two)

Discussion

In order to develop biomimetic restorative biomaterials, plenty of research has been conducted conducted either In modifying order to develop the existing biomimetic materials restorative or developing biomaterials, new plenty materials. A variety of processing nanotechnology, fabrication methods, and functionalization of biomaterials technologies including nanotechnology, fabrication methods, and functionalization of biomaterials has been explored Considering the major challenges faced by researchers and clinicians, perhaps it may take a more than a decade for biomimetic materials to be implemented on a bigger scale to treat dental lesions. New alternative treatment modalities are likely to be available for clinical applications after innovative discoveries in genetics, molecular biology, cell biology, and materials science. Through these treatment modalities, regeneration of dentin, enamel, pulp, restorative procedures, and management of soft tissues of periodontium can possibly be carried out. In the near forthcoming years the reinforcement and completion of the tooth structure by biological regeneration will be evident through these modalities. The development and translation of smart biomimetically driven dental restoratives from lab to clinical dentistry also have a tremendous potential. Although much more scientific development and technical research are required, a good prognosis, high biocompatibility, and excellent success rate have been observed with the regeneration of lost dental tissues.

(Chapter Three)

Conclusions

Biomimetics in dentistry is a very useful concept and further and firmer multidisciplinary scientific and technical research is needed in this field. Regeneration of the lost dental tissue rather than mild replacement with dental materials ensures better prognosis, excellent biocompatibility, and high success rate. Biomimetic dentistry would successfully replace lost dentin, enamel, cementum, and pulp and open a new era of dentistry.Restorative dentistry in the future would no longer be using inert materials that only fill the prepared cavity, but instead, it will rely mainly on bioactive materials with the capability of dental tissues regeneration. New mechanisms for tissue engineering and regeneration of the dentin pulp complex using biomimetic technologies and concepts can emerge as a major turnover in the dental field. The last few decades have seen tremendous growth in the field of dentistry. But each procedure has its own drawbacks and limitation due to the complex natural tooth structure. Therefore, the utilization of such biomimetic materials that could successfully restore the destroyed enamel, dentine, dentinoenamel junction, cementum, and even the pulp tissue will be required in the future of dentistry. The development of a substitute that restores or mimics the natural dental tissue is in progress. Moreover, the role of various biomimetic molecules and materials requires further study.Despite the aforementioned advantages, future developments in pulp-dentin tissue regeneration are needed to demonstrate the functional tissue regeneration and the ultimate favorable clinical benefits.

References:

-A-

- Atsawasuwan, P. et al. Expression and function of enamel-related gene products in calvarial development. J. Dent. Res. 92, 622–628 (2013).

-B-

- Bottino, M.C.; Kamocki, K.; Yassen, G.H.; Platt, J.A.; Vail, M.M.; Ehrlich, Y.; Spolnik, K.J.; Gregory, R.L.Bioactive nanofibrous scaffolds for regenerative endodontics. J. Dent. Res. 2013, 92, 963–969.
- Bronckers, A. L., Lyaruu, D. M., Jalali, R. & DenBesten, P. K. Buffering of protons released by mineral formation during amelogenesis in mice. Eur. J. Oral. Sci. 124, 415–425 (2016).
- Burke FJ. From extension for prevention to prevention of extension: (minimal intervention dentistry). Dent Update. 2003;30(9):492–8.

-C-

- Chen, H. et al. Acellular synthesis of a human enamel-like microstructure. Adv.Mater. 18, 1846–1851 (2006).
- Chen, H., Clarkson, B. H., Sun, K. & Mansfield, J. F. Self-assembly of synthetic hydroxyapatite nanorods into an enamel prism-like structure. J. Colloid Interface Sci. 288, 97–103 (2005).

-G-

- Goswami, S., 2018. Biomimetic dentistry. Journal of Oral Research and Review, 10(1), p.28.

-K-

- Kottoor J, Biomimetic endodontics: Barriers and Stratergies, Health Sciences, 2(1), 2013, JS007.
- Kumar, Naresh, Maher, Nazrah, Amin, Faiza, Ghabbani, Hani, Zafar, Muhammad Sohail, Rodríguez-Lozano, Francisco Javier & Oñate-

Sánchez, Ricardo E. 2022. Biomimetic Approaches in Clinical Endodontics. Biomimetics 7: 229.

-L-

- Lacruz, R. S., Nanci, A., Kurtz, I., Wright, J. T. & Paine, M. L. Regulation of pH during amelogenesis. Calcif. Tissue Int. 86, 91–103 (2010).
- Line, S. R. P. The development and evolution of mammalian enamel: structural and functional aspects. Braz. J. Morphol. Sci. 22, 67–72 (2005).

-M-

- Magne P, Composite Resins and Bonded Porcelain: The Post amalgam Era? CDA. JOURNAL, 34(2), 2006.

-N-

- Nasr-Esfahani, M. & Fekri, S. Alumina/TiO2/hydroxyapatite interface nanostructure composite filters as efficient photocatalysts for the purification of air. React. Kinet., Mech. Catal. 107, 89–103 (2012).

-P-

- Pandya, M. and Diekwisch, T.G., 2019. Enamel biomimetics—fiction or future of dentistry. International journal of oral science, 11(1), p.8.
- Pandya, M. et al. Post-translational amelogenin processing and changes in matrix assembly during enamel development. Front. Physiol. 8, 790 (2017).
- Paryani, Meghna, Bhojwani, Priyanka R, Ikhar, Anuja, Reach, Amit & Paul, Priyanka. 2023. Evolution of Biomimetic Approaches for Regenerative and Restorative Dentistry. Cureus 15.
- Perdigão, J.; Walter, R.; Miguez, P.A.; Swift, E.J. Sturdevant's Art and Science of Operative Dentistry.In 5–Fundamental Concepts of Enamel and Dentin Adhesion; Ritter, A.V., Boushell, L.W., Walter, R., Eds.; Elsevier: St. Louis, MO, USA, 2019; pp. 136–169.

-R-

- Ren, F. et al. Growth of one-dimensional single-crystalline hydroxyapatite nanorods. J. Cryst. Growth 349, 75–82 (2012).
- Ryu, S.-C. et al. Regeneration of a tooth enamel layer using hydroxyapatite regeneration of a micro-scratched tooth enamel layer by nanoscale hydroxyapatite solution. Bull. Korean Chem. Soc. 30, 887–890 (2009).

-S-

- Sharma, V.; Srinivasan, A.; Nikolajeff, F.; Kumar, S. Biomineralization process in hard tissues: The interaction complexity within protein and inorganic counterparts. Acta Biomater. 2020.
- Simmer, J. P., Richardson, A. S., Hu, Y. Y., Smith, C. E. & Ching-Chun, Hu,J. A postclassical theory of enamel biomineralization… and why we need one. Int. J. Oral. Sci. 4, 129–134 (2012).
- Singer, L., Fouda, A. and Bourauel, C., 2023. Biomimetic approaches and materials in restorative and regenerative dentistry. BMC Oral Health, 23(1), pp.1-14.
- Srinivasan K, Chitra S. Emerging trends in oral health profession: The biomimetic – A review. Arch Dent Med Res 2015;1:40-7.
- Szczes, A., Holysz, L. & Chibowski, E. Synthesis of hydroxyapatite for biomedical applications. Adv. Colloid Interface Sci. 249, 321–330 (2017).

-U-

- Ullah, R.; Zafar, M.S. Oral and dental delivery of fluoride: A review. Fluoride 2015, 48, 195–204.

-V-

- Vanishree N, Naganandini S, Chaithra V. Biomimetics: A new paradigm in dentistry. J Indian Assoc Public Health Dent 2011;1:712-7.

- Viswanath, D. and Reddy, A.V.K., 2014. Biomimetics in dentistry-a review. Indian Journal of Research in Pharmacy and Biotechnology, 2(5), p.1384.

-W-

- Wang, H. et al. Oriented and ordered biomimetic remineralization of the surface of demineralized dental enamel using HAP@ACP nanoparticles guided by glycine. Sci. Rep. 7, 40701 (2017).

-Y-

- Yang, J.; Yuan, G.; Chen, Z. Pulp regeneration: Current approaches and future challenges. Front. Physiol.2016, 7, 58.
- Zafar, M.S., Amin, F., Fareed, M.A., Ghabbani, H., Riaz, S., Khurshid, Z. and Kumar, N., 2020. Biomimetic aspects of restorative dentistry biomaterials. Biomimetics, 5(3), p.34.

-Z-

- Zafar, M.S.; Ahmed, N. Therapeutic roles of fluoride released from restorative dental materials. Fluoride 2015, 48, 184–194.
- Zafar, M.S.; Fareed, M.A.; Riaz, S.; Latif, M.; Habib, S.R.; Khurshid, Z. Customized therapeutic surface coatings for dental implants. Coatings 2020, 10, 568.