Genetics of Dental Caries:

An Analysis of Genetic Susceptibility and Applied Gene Therapeutics in Scientific and Judaic Literature

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Abstract

Oftentimes dentists have patients who continuously have issues with dental caries. These patients follow the advice of dentists by properly brushing and flossing their teeth, as well as adjusting their diets to aid in the maintenance of their teeth. However, these precautions patients adhere to are not universally successful; many still find themselves susceptible to frequent dental caries. Therefore, it is important to understand the genetic underpinnings to explain why some are more prone to dental caries than others. This knowledge can further be applied in researching targeted gene therapies to prevent dental caries formation, while simultaneously altering the standard treatment plan. There has been promising therapeutic research to select genes for strong enamel growth and healthy saliva, as well as genes that attack and prevent bacteria in the mouth. The usage of gene therapy is subject to controversy in Jewish law. However, these specific therapies should escape this controversy, as there is a health motivation for its allowance. Both Judaism and science mutually agree that maintaining proper oral care is crucial to the health of the mouth. Without a healthy regimen, dental caries will certainly form irrespective of one's genetic makeup.

Introduction

Since dental caries is a multiple factorial disease, there are several potential genes within the oral cavity that likely contribute to their formation or resistance. Biologists and dentists have identified specific determinants that increase susceptibility to dental caries. These stimulants include the genes and physiological health of saliva, the inactivation or mutation of genes within the tooth bone, and varying immune responses. Each of these factors work alone or in tandem in forming dental caries. Moreover, epigenetic factors such as diet, acidic pH of the mouth, or a dry mouth play a key role in the development of dental caries.

Contemporary scientific knowledge and experimentation has allowed us to have a certain extent of control of genetic modifications. The ethics of manipulating and altering the genetic makeup of humans has been widely debated. According to Jewish law, employing gene therapeutics related to the health of the oral cavity may be permitted, as it aids in preventing disease. There has been promising research on the development of gene therapies that activate enamel growth or increase the health and fluidity of saliva. These applied therapies can be instrumental in the prevention of dental caries, while still within the confines of Jewish law.

Despite genetic explanations, most dental caries are ultimately caused by improper oral healthcare. Therefore, it is within the control of each person to take reasonable precautionary steps to prevent the formation of dental caries. Early Jewish sources record the importance of maintaining a healthy mouth and include different methodologies that were employed to alleviate dental related issues. Accordingly, this paper attempts to coalesce both the dental and Judaic approach to oral health.

Part I: Formation of Dental Caries

Dental biofilm; Dental plaque

A biofilm is a complex heterogeneous microbial community (Gurenlian, 2007). It systematically operates by utilizing the products and waste of other microorganisms within the biofilm for another bacteria’s nutritional needs. Bacteria within a biofilm flourish due to enhanced resistance to the host’s defense system and antimicrobials. Recently, dental plaque has been described as a biofilm that covers the surfaces of teeth (“Cavities/Tooth Decay”). Dental plaque is made of bacteria that form a clear and sticky film on the surfaces of the teeth. Its formation is due to the presence of sugar and starches within the saliva and mouth. This plaque can further harden along the gum line into tartar, which shields the underlying bacteria and makes it difficult to remove. Thus, it is imperative to prevent buildup of plaque on teeth, as its remainder allows for bacterial maturation and growth. Moreover, it allows pathogenic organisms that destroy teeth to flourish within the oral cavity. Once dental plaque forms on the teeth, it cannot be completely removed. Instead, it can only be maintained by proper oral hygiene and care. Accordingly, accumulated dental plaque initiates the growth of dental caries (Bowen, 2015).

Stages of Dental Caries Formation

The American Dental Association defines dental caries as a "biofilm-mediated, sugar-driven, multifactorial, dynamic disease that results in the phasic demineralization and remineralization of dental hard tissues" ("Caries Risk Assessment and Management"). Because of this demineralization and remineralization, cavities, or holes within the teeth form (Bowen, 2015).

The first stage of dental caries formation is caused by the erosion of dental enamel by the acids found within dental plaque (Gurenlian, 2007). After the enamel wears, the dentin of teeth becomes exposed to the various bacteria and acids found within the plaque and oral cavity. This results in a heightened sensitivity to the area of the tooth, since the dentin contains tubes that lead to the nerves of the tooth. Upon the continuation of decay, the pulp, or innermost part of the tooth is exposed. Bacteria and acids can irritate the nerves and blood vessels found within the pulp, which can lead to swelling against the nerve, causing pain and discomfort.

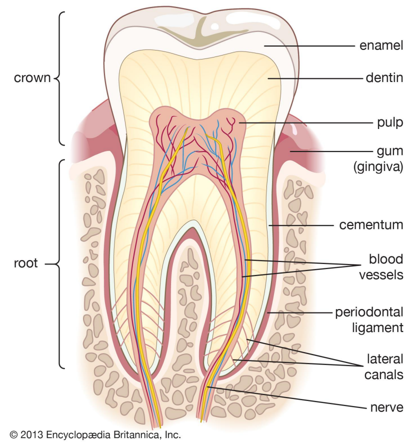

Figure 1. Diagram of the layers of a healthy tooth (Lane, 2017).

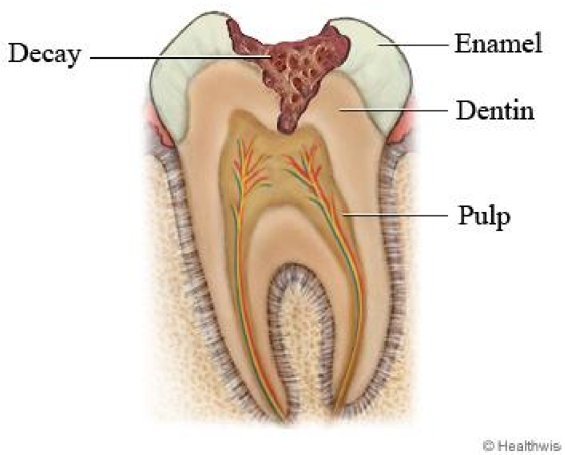

Figure 2. Diagram of a tooth in which there is decay to the enamel and dentin (Moore, 2015)

Relationship between Sugar and Dental Caries Formation

The remnants of sugar remaining within the biofilm, or dental plaque is probably the most classic explanation for the development of dental caries. Dentists often tell patients to stay away from consuming too much sugar and to clean their teeth thoroughly after consuming increased carbohydrates. This idea of increased intake of carbohydrates affecting the rate of dental caries was studied in the Vipeholm study in Sweden that began in 1945 (Gustafsson *et al,* 1954). The study tested 436 individuals and consisted of four stages over a five year period. The first stage was a preparatory period, in the second period vitamins A, C, or D or a combination were tested, and the last two periods were different carbohydrate periods. During the carbohydrate periods, participants were given increased carbohydrates, varying in the carbohydrates' levels of stickiness. Additionally, within the carbohydrate periods there were different groups, such as the sucrose group (which was given non-sticky carbohydrates), a bread group (for the purpose of studying the effects of carbohydrates remaining on the surfaces of teeth), and groups given sugar between meals in the form of chocolate, caramel, or two different degrees of toffee.

After five years, the study concluded that higher sugar consumption increases the likelihood for individuals to develop dental caries. Moreover, the rate was even higher when the participants ate foods that remained on their teeth, specifically sticky foods. Interestingly, the degree of dental caries formation varied greatly among participants within a single group. In addition, it was found that reduced sugar lowered the rate of dental caries formation. However, participants with restricted carbohydrate intake were still susceptible to carious lesions. Active caries lesions are observed when there is identified mineral loss based on both visual appearance and tactile perception (Fontana, 2017). These lesions likely develop into true dental caries, because of their ability to soften the dentin and potentially accumulate plaque. Therefore, it is clear from this study that although sugar consumption plays a role in the development of dental caries, there are factors that increase one's susceptibility.

Role of Saliva in the Health of the Oral Cavity

The salivary glands are extremely complex and play an instrumental role by producing saliva that serves as the first line of defense in preventing disease. After carbohydrate intake, bacteria attack and form acids within the mouth. The acid attacks the enamel and makes the teeth more prone to decay and dental caries formation ("Tooth"). Saliva helps maintain the strength of the enamel as it constantly showers the teeth with calcium and phosphate. Additionally, the salivary glands are highly organized protein producers, which allow them to easily release proteins into the bloodstream.

The knowledge of the health benefits of saliva is not a novel idea, rather there are clear indications in Talmudic literature that saliva proved to have healing effects (Katz, page 410). The Gemara in *Bava Basra 126b* notes the ability to identify if a boy is the firstborn son of the father and not his mother by his saliva because "there is a tradition that the saliva of the firstborn of a father is healing, but that of the firstborn of the mother is not healing." The Mishnah in *Niddah 61b-62a* also discusses the strength of saliva. In reference to removing an impure niddah stain, the Mishnah writes that "rok tafel" is defined as tasteless saliva (from someone who did not eat at all that day). According to the Mishnah, this saliva is so strong that it can be used as a detergent. Moreover, the Gemara in *Shabbos 108b* mentions that the Sages forbade using "rok tafel." According to Rashi, a prominent medieval Talmudic commentator, this saliva was prohibited because it has medicinal capabilities, which is not allowed on Shabbos. Dr. Julius Preuss noted that fasting saliva, as indicated by the Gemara, is curative because it contains large quantities of potassium sulphocyanide, which has many antibacterial properties (Katz, page 437).

Xerostomia, or dry mouth caused by salivary hypofunction, can be caused by salivary gland impairments, genetic mutations due to radiotherapy performed on the head and neck area, autoimmune diseases, or a side effect of certain medications (Siddique, 2016). According to the American Dental Association, Xerostomia can cause difficulty tasting and chewing, while also increasing the demineralization of teeth. This demineralization can lead to the development of dental caries, dental sensitivity, and oral infections. More specifically, patients with Xerostomia typically suffer from dental caries in the roots, incisal tips, or within the cervical portion of the tooth due to plaque accumulation. The standard treatment of Xerostomia is to use saliva substitutes and stimulants, such as sugar-free chewing gum. Another treatment option is to gently brush the teeth with a fluoridated toothpaste twice a day.

It is clear that saliva plays a key role in maintaining the health of the oral cavity. Poor saliva makes the oral cavity more prone to bacterial growth which can be detrimental to the health of the body and teeth. Thus, it is important to ensure proper saliva flow and health to maintain the strength and stability of the teeth.

Part II: Overview of the Genetic Relation to the Development of Dental Caries

Our genes play an instrumental role in the growth and maintenance of healthy teeth. They are found and controlled within the tooth itself, saliva, and our taste buds. Therefore, upon evaluating the origin of dental caries, it is imperative to explore the different genes within the respective areas of the body and understand their effects on the teeth.

There are thousands of genes that control and regulate different activities to maintain a homeostatic body. All genes are inherited from parents. Accordingly, the specific genes that cause a person to be more likely to develop dental caries should be similar amongst immediate family members. Furthermore, the studies of family members are helpful, because its effects can be analyzed without environmental differences. More specifically, it is best to study monozygotic twins who share 100% of their DNA. In doing so, studies can accurately pinpoint the genetic control in dental caries formation, aside from environmental influences and diet.

A study performed in 1988 measured the genetic variance of dental characteristics in twins and triplets that were separated for the six year long study (Boraas *et al*, 1988). Throughout the study the participants received regular clinical and radiographic assessments, questionnaires, and study models. The individuals were analyzed by their tooth size, tooth shape, malalignment occlusion, treatment, and dental caries status. Based on their evaluations, there were significant similarities amongst monozygotic twins, but less similarities between dizygotic twins in regards to the percentage of teeth with caries, teeth restored, tooth size, and malalignment. In a study performed in 1997, the WHO oral health assessment form was used to assess the oral health of nine monozygotic twins and twenty one dizygotic twins (Lovelina *et al*, 2012). A Pearson's correlation showed that there was a greater correlation in monozygotic twins compared to dizygotic twins for dental caries, periodontal disease, and malocclusion.

Another twin study was performed in 2018 at the Sathyabama Dental College and Hospital in India (Anu *et al,* 2018). They studied thirty sets of twins between the ages of fifteen and thirty. It was found that there was a higher positive correlation in monozygotic twins for decay, filling, decayed missing and filled teeth (DMFT), spacing, molar relation and diastema (gap between different types of teeth). Additionally, there was a 87.5% concordance rate, a 71.6% correlation, and a 87.8% heritability factor for dental caries.

From these highlighted studies, it is clear that there are genetic implications for the development of dental caries. This was observed by monozygotic twins showing strong similarity in dental caries prevalence. Therefore, an analysis on the genes that affect the oral cavity is essential to understanding their development and perhaps even understanding how to properly treat the caries.

Genetics of the Tooth that Affect Dental Caries Formation

All teeth are composed of three hard tissues: enamel, dentin, and cementin. They are also composed of pulp, which is a soft, uncalcified tissue that contains the nerves, blood vessels, and connective tissue of the pulp (“Tooth”). The enamel is the hardest part of the tooth and serves as the outermost protective layer of the tooth. Strong enamel is essential to the entirety of the tooth, because holes or cracks increase tooth fragility. In addition, enamel does not contain living cells, making the damage done to it irreversible (Wyatt, 2020).

A. Ameloblastin gene

There are several tooth specific genes that manage the process of mineralization and enamel development of teeth. Ameloblastin is one of the tooth-specific extracellular matrix proteins that genetically controls the enamel matrix. Its role is to aid in the crystal formation of enamel in developing teeth (Dhamija, 2001). A study was performed in 2001 on rats to better understand the ameloblastin gene. Upon their study, researchers found that the ameloblastin in rats contains a promoter that has cis-acting elements that control whether or not transcription is enhanced or suppressed. One of these transcription factors is Core-binding factor α1 (Cbfa1) and its active site, located at -248 base pairs, is called osteoblast-specific element 2 (OSE2). They found that a mutation to OSE2 led to a 50% decrease in ameloblastin promoter activity in ameloblast-like cells. This is because the unmutated active site showed specificity to the Cbfa1 protein. Therefore, it is clear from this study that Cbfa1 needs to bind properly to ensure ameloblastin growth. Perhaps the findings in this study can be similarly applied in understanding the human ameloblastin promoter.

B. AMELX gene

Another gene that regulates enamel formation is the AMELX gene. This gene is a part of the amelogenin family of the extracellular matrix proteins of teeth. Amelogenins make up 90% of the enamel organic matrix and are secreted by ameloblasts ("Amelogenin; AMELX"). Genetic mutations in the AMELX gene can affect the functions of its cellular proteins, leading to improper enamel growth and decay. The AMELX gene can be found on both X and Y homologous chromosomes. Its mutated form is phenotypically expressed differently depending on whether a mutation is found on the X chromosome or the corresponding Y homologous chromosome. Mutations in the AMELX gene can lead to X-linked forms of amelogenesis imperfecta and the Y-chromosomal location of the gene has been found to contribute to the size and shape of teeth.

A study was performed on 360 participants at the dental school of Shiraz University of Medical Sciences in Iran. The researchers wanted to see if there was a correlation between dental caries and single nucleotide polymorphisms in the AMELX gene (Koohpeima, 2019). They did so by analyzing the rs946252 SNP, located on the intron of the AMELX gene. The study found that there was a significant association between a T to C mutation in the rs946252 SNP and dental caries susceptibility for both recessive and dominant genetic models. Interestingly, there was no significant correlation within the results of the dominant genetic model. Nevertheless, it is clear that rs946252 polymorphisms play a distinct role in dental caries susceptibility. However, because of the narrowness of the study to the Iranian ethnicity, further studies are necessary to validate their findings.

C. Tuftelin Interacting Protein 11

Certain genes interfere with the interaction between the enamel surface and the oral cavity, thus increasing dental caries susceptibility. One example is Tuftelin interacting protein 11. This protein has been associated with the enamel surface's ability to uptake fluoride. Fluoride is important because it decreases the demineralization of teeth by forming fluorapatite with the calcium and phosphate in saliva. In addition to preventing demineralization, fluorapatite promotes the resistance to decay while simultaneously protecting teeth from dental caries ("The Superhero That Lives Inside Your Mouth"). Therefore, it is essential that Tuftelin interacting protein 11 functions properly to maintain the strength of enamel. If it does not function properly, it can form an enamel structure that is more susceptible to demineralization (Abbasoğlu, 2015). Additionally, the tuftelin gene is among several proteins that are associated with molar incisor hypomineralization (MIH), which can lead to the rapid progression of dental caries (Jeremias, 2013).

D. Genetically Controlled Conditions

A genetic mutation that alters the tooth structure can increase the presence of dental caries. One example is Epidermolysis Bullosa (EB), a condition that results in mechanical fragility of skin which affects the soft or hard tissue of the craniofacial complex (Wright, 2011). The abnormalities and phenotype of a patient are largely controlled by genetic mutations that alter normal proteins. Therefore, the effects on the tissues within the oral cavity are dependent on the specific EB type the patient presents. Patients with EB Simplex appear to have normal salivary functions, however, they are increasingly susceptible to dental caries due to their limited intra oral soft tissue. The biggest issue physicians have when studying EB is the multitude of variations and vastness of the disease. Its numerous phenotypic and genotypic variations make it difficult to pinpoint and trace its existence in patients.

Immunological Causes of Dental Caries

A. Beta-defensin Proteins

Scientists have recently shown a great interest in understanding the role of beta-defensins (hBD) towards the development of dental caries. There are three different beta-defensins (hBD-1, hBD-2, and hBD-3) that are predominantly expressed in human epithelial cells, including the cells of the oral cavity (Diamond and Ryan, 2011). The expression of these beta-defensin proteins have been observed in gingival epithelium, buccal epithelium, dental pulp and salivary gland tissues (Diamond and Ryan, 2011). Beta-defensins were originally characterized based on their ability to kill microbes; even with a minimal concentration, they are capable of acting against gram positive bacteria, gram negative bacteria, viruses, and fungi. These three beta-defensins are continuously produced within epithelial cells at low levels. The level produced in response to various stimuli or infections does not change for hBD-1. However, the levels produced of hBD-2 and hBD-3 can be further induced in response to microbial growth within the mouth.

Because beta-defensins play a crucial role in the innate immune response, it has been suggested that they act as the first line of defense against microbial growth within the oral cavity. When they interact with pathogens they exhibit a potent chemotactic activity for many innate immune cells. This is caused by pro-inflammatory cytokines, such as tumor necrosis factor and interleukin, which are able to induce the expression of beta-defensins. Because they are stimulated by cytokines in response to bacteria or infection, beta-defensins effectively prevent the growth of harmful microbes and are crucial in maintaining homeostasis within the mouth.

Mutations in the beta-defensin gene may result in a greater presence of bacteria within the mouth. The Department of Medical Sciences at the University of Trieste, Italy explored this hypothesis in a study on 654 adults (Navarra *et al*, 2016). The adults were examined and assessed for the prevalence of dental caries based on their DMFT index. The researchers studied five different polymorphisms of the DEFB1 gene that encodes for the hBD-1 protein on three functional SNPs at the 5' untranslated region of the protein. The results of this study indicated that there was a significant correlation between two of the DEFB1 SNPs and DMFT index. Therefore, these polymorphisms are potential markers for the development of dental caries, because the beta-defensin protein is not fully expressed and thus allows many bacteria to remain within the mouth.

B. Helicobacter pylori

A leading cause for the buildup of bacteria within the mouth is the result of Helicobacter pylori, which forms in patients with ulcers or erosive gastrointestinal systems (Moseeva *et al*, 2010). This is caused by the reduced activity of the antibacterial lysozyme within saliva when Helicobacter pylori protease combines with ammonia. The lysozymes in saliva play a nonspecific role in the host's immune system to allow for the maintenance of a homeostatic oral cavity. Accordingly, the saliva loses parts of its innate protective abilities when this activity is reduced. As a result, active pathogenic microflora are able to cause the depolymerization and demineralization of tooth enamel.

Because of the harm these bacteria causes to teeth, studies have been conducted on Helicobacter pylori and its effect on the formation of dental caries. To test the genetics of this bacteria, a study was performed in Sweden in 1994. (Malaty *et al*, 1994) The subjects of this study included 36 monozygotic twin pairs separated at birth, 64 monozygotic twin pairs together throughout life, 88 dizygotic twin pairs separated at birth, and 81 dizygotic twin pairs separated together throughout their life. They evaluated the mouths for the presence of dental caries in the different pairs of twins. Based on their evaluations of the oral cavity, they calculated that the correlation coefficient for monozygotic twins reared apart at birth was 0.66 and the heritability estimate from a model-fitting analysis was 0.57. Additionally, the concordance rate with a P-value of 0.001, was 81% in monozygotic twins and 63% in dizygotic twins.

The results of this study shed light on a genetic influence of dental caries due to the prevalence of Helicobacter pylori, since there were greater similarities demonstrated in monozygotic twins. Therefore, it is clear that there are specific genes that cause certain individuals to be more prone to dental caries, irrespective of environmental influences. Accordingly, to further understand the development of dental caries at every angle, it is important to understand the genes that potentially lead to their development.

Part III: Gene therapy

Introduction

In recent years, gene therapy has shown to have remarkable effects on healing the body. The idea behind gene therapy is to develop an analogue that can replace a defective gene. In doing so, the body will effectively produce the correct and functional protein to prevent disease and maintain the proper physiology of the body (Siddique *et al*, 2016). Gene therapy is a two step process. First, the genetic coding for the therapeutic protein is cleaned and inserted into a vector. After, the vector is introduced into the body, either *in vivo* (by direct injection) or *ex vivo* (by the injection of a vector that was genetically engineered into cultured tissue cells and then the altered tissues are transplanted in the body) to target the specific gene that needs repair. Then, DNA ligase operates to combine the newly synthesized DNA with the original to allow the gene to operate healthily and properly.

With continuous research and understanding of genes and how they can be applied for therapeutic treatments, scientists and doctors have been able to transform the conventional methods of treatment for diseased individuals. Particularly within the oral cavity, gene therapies can potentially be utilized to treat orofacial pain, carcinomas, bone repair, salivary glands, tooth movement, and tooth repair and regeneration. As many of these areas contain genes that affect the rate of dental caries development, understanding the specific therapies for each contributor can be key to preventing genetically induced dental caries. Additionally, understanding and targeting the genes that lead to the development of caries can limit the administration of drugs and surgeries performed on patients.

Approach of Judaism and Gene Repair

It is often true that ideas learned from the Torah and Judaic scholars emerge as prominent areas of study by the secular world thousands of years later. This idea holds true regarding the study of genetics. For example, the Gemara in *Yevamos 64b* describes hemophelia and its genetic transmission. Genetic understanding is also mentioned in *Ketuvos 10b*, in a discussion of a family in which the women were unable to menstruate and have children. Moreover, the Rambam in *Mishneh Torah, Hilchos Deos 4:1* discusses the proper lifestyle that should be kept that ensures a healthy wellbeing, except for those who were born with a hereditary or genetic defect (Rosner, 2016).

According to most, the origin of genetic testing occurred in the 1950s when scientists counted individual chromosomes and uncovered the additional chromosome 21 found in individuals with Down's syndrome (Molteni, 2019). Genetic testing refers to "the analysis of human DNA, RNA, chromosomes, proteins and certain metabolites in order to detect heritable disease-related genotypes, mutations, phenotypes or karyotypes for clinical purposes" (McPherson, 2006). Despite the immense knowledge gathered since about genetics, the studies are still relatively young. Because of the relatively modern phenomenon of genetic testing and therapy, it is not widely discussed in the Torah and Gemara. As a result, modern Rabbinic leaders have looked to similar cases in Judaic literature to infer if in fact the idea of genetic testing and repair would be permissible.

Although the idea of implementing gene therapeutics is not outright mentioned, there are areas of Judaic literature that can be used to support its usage. In *Bereishit 1:28*, God commanded Adam and Eve to "fill the earth and master it; and rule the fish of the sea, the birds of the sky, and all living things that creep on the earth." The Ramban interprets this command as God allowing mankind to control every living creature on the earth and derive any desired benefit from them. According to his view, perhaps there is a clear command from the Torah to uncover and develop the scientific knowledge that can be used to help mankind. Rav Samson Raphael Hirsch learns from this command that man is allowed to appropriately utilize and transform any part of the earth for human purposes.

From these sources, it is clear that Judaism emphasizes and permits scientific discovery. In addition though, the Torah mandates that individuals search for cures. In *Shemot 21:19*, the

Torah commands an assailant to pay for the medical treatment of the victim. Commenting on this idea the Gemara in *Bava Kama 85a* concludes that this command includes permitting the use of knowledge for healing or curing diseases. Additionally, the Rambam comments on the Mishnah in *Nedarim 4:4* that included in the Biblical obligation to restore a lost object is the necessity to restore a person's old health. He writes, "the requirement to heal is from the Torah" and a person should help heal another when he is in danger and he is able to save him, whether by means of his own body, with his money, or his wisdom.

According to these views, it would seem as though Judaism permits not only researching genetic therapies, but also utilizing them in order to save a life. It should be noted though that Torah law would restrict the allowance of gene therapy to situations when it is used for the treatment or cure of a specific disease. Thus, according to Torah law it would be forbidden to manipulate the genetic makeup to alter physical traits that would not be used to derive useful benefits to man (Rosner, 1988). Dental caries can have detrimental effects on one's health, therefore Torah law would seemingly permit utilizing different gene therapies in order to prevent and treat existing dental caries.

Gene Therapy for Tooth Repair and Regeneration

Gene therapy has been used to enhance osteoinduction (induce osteogenesis), osteoconduction (process in which a bone grows as directed by a surface), and the differentiation of osteoblasts for the production of the osteoid matrix (Karthikeyan and Pradeep, 2006). Gene therapy for bones is particularly of interest because it can be used as a precise and localized therapy to treat specific teeth that require new bone formation, such as in teeth that have dental caries (Prabhakar, 2010). Moreover, this treatment is advantageous because of its ability to last for extended periods of time.

Bone morphogenetic proteins (BMP) 2, 4, and 7, have been found to induce osteoinduction. These proteins are clinically valuable since they are the only signaling molecules within the body that can induce the formation of new bone in both orthotopic and heterotopic sites. In essence, these proteins could be applied to regrow tooth bone in teeth with dental caries. Different *in vivo* studies have been performed to deliver various BMP encoding genes by utilizing adenoviral vectors to tissues throughout the body. Utilizing this technique to introduce the BMP-2 gene led to the healing of mandibular osseous defects (Baum *et al*, 2002). The Center for Craniofacial Regeneration of the University of Michigan also used adenoviral vectors to test BMP-7 and its regenerative properties (Dunn, 2005). In the study, the maxillary first molars were extracted from 44 Sprague-Dawley rats. After a month, a titanium dental implant was inserted with severe osteotomy defects. Then using a collagen matrix, the adenoviral vector coding for either BMP-7 or the luciferase gene was delivered to the defected area. The results of this experiment showed that the genes were actively expressed for up to 10 days, but by 35 days their activity greatly diminished. It was also found that teeth with the expressed protein showed a greater ability in filling the alveolar portion of the bone, as well as new bone formation, and new bone-to-implant contact. Therefore, this shows that this form of therapy is potentially a great option for missing teeth, but also for teeth with cracks or caries.

Another genetic therapy that can be potentially used to treat dental caries is using the bone sialoprotein (BSP). This protein is a non-collagenous, highly glycosylated, and sulphated phosphoprotein that is found mostly within mineralized connective tissues and bone (Ganss *et al*, 1999). The expression of this gene is controlled by cytokines and hormones depending on whether there should be bone formation or breakdown within the body. A major gene involved in the BSP gene is Core-binding factor α1 (Cbfa1). This Cbfa1 gene is involved in bone formation and osteoblast differentiation (Komori and Tadamitsu, 2002). Because of its restorative and bone building properties, Cbfa1 is of particular clinical interest for its potential use in many different bone diseases. When introduced to defected teeth within the mouth, the *in vivo* transfer of the BSP gene helped regenerate periodontal alveolar bone.

Gene Therapy of Salivary Glands

Because the mouth serves as the portal to the rest of the body, there has been extensive research on human saliva and its healing capabilities. The complex protein system within saliva potentially can be used to correct genetic issues. Since the saliva is able to secrete proteins directly into the bloodstream, perhaps via intraductal cannulation, these proteins can be used as target sites for gene transfers. This technique is a more direct and effective way to transmit a drug or protein, because it immediately targets the gland of interest upon injection without entering the circulatory system (Kuriki *et al*, 2011). This method is also of particular interest because the proteins are introduced in a minimally invasive manner, since it all occurs within the mouth itself.

As mentioned earlier, one of the many potential causes of Xerostomia is radiotherapy. The reason for this is because ionizing radiation can irreversibly damage the acinar cells of the salivary glands. These cells play a crucial role in salivary function because they secrete a fluid that contains water, electrolytes, enzymes, and mucus (“Salivary Glands and Saliva”). Therefore, if these cells are damaged, there is reduced water found in the saliva, thus creating dry mouth.

A proposed genetic therapy has been to utilize the Aquaporin 1 gene. This gene encodes for a small integral membrane protein that functions as a water channel for the protein. It functions by an osmotic gradient as it passively transports water through the membrane of the protein (“AQP1 Gene (Protein Coding)”). By integrating this gene into the saliva of patients with Xerostomia, the Aquaporin 1 gene counteracts the previous damage performed by providing a new water channel that increases the permeability of the gland. The usage of this therapy has shown positive results in rats who had irradiated submandibular glands and in the parotid glands of adult rhesus monkeys.

Aquaporin gene therapy has also shown promising results in mice suffering from Sjögren’s syndrome, an autoimmune disease that causes oral and ocular dryness (Lai *et al*, 2017). Work from a study revealed that aquaporin 5 expression is regulated by bone morphogenetic protein 6. When there is an increased expression of the cytokine, there is a loss of salivary function which leads to the development of dry mouth. In the study they used aquaporin replacement therapy in the hope of treating patients with Sjögren’s syndrome by preventing the dryness that it causes. They found that this therapy restored the function of the salivary glands by increasing water flow. Moreover, this therapy helped treat the salivary gland and systemic inflammation that is caused by Sjögren’s syndrome. Perhaps the knowledge and mechanisms for these effective therapies on rats and mice could be further applied to fixing the human saliva as well.

Part IV: Maintaining Proper Oral Health

Basis of Oral Healthcare in Judaic Literature

Dental caries formation is not due solely to poor genetics. As discussed earlier, poor oral healthcare plays a crucial role in the development of caries. That being said, it is important to take cautious measures to ensure the wellbeing and health of teeth. This includes limiting sugar and carbohydrate intake to prevent the undigested sugars from sticking to the biofilm of teeth. Additionally, it is imperative to maintain the health of the teeth by taking the proactive steps to thoroughly clean the teeth.

Furthermore, a proper oral healthcare routine is necessary to prevent bad breath, or halitosis. Halitosis is caused by the overgrowth of odor-producing bacteria within the oral cavity. These bacteria secrete sulfur compounds, which results in a bad odor within the mouth ("What Causes Bad Breath? (for Teens) - Nemours KidsHealth"). Ordinarily these bacteria are found on or in between the teeth, gums, and tongue. In patients who have dental caries, there is a greater accumulation of bacteria that accumulates in the pockets of the teeth ("Do Cavities Cause Bad Breath?"). Accordingly, dental caries indirectly contribute to the overgrowth of odor-producing bacteria that may lead to bad breath.

Although the Gemara does not mention dental caries as a cause of bad breath, it does mention the importance of maintaining good breath. In *Brachot 44b*, a person who consumes vegetables before the fourth hour of the day should not be spoken to because the odor of his mouth is offensive to others. The Gemara in *Sanhedrin 11a* also recalls that anyone who ate garlic was asked to leave Rav Yehuda HaNasi's *Beit Midrash* (place of learning). Rabbi Hiyya left Rav Yehuda HaNasi's *Beit Midrash* because according to his view, it is improper to enter a place of learning with bad breath. Additionally, according to the Gemara in *Ketuvot 77a*, there are several activities or attributes that are considered potential issues in which a Jewish marriage certificate can be unquestionably annulled. One of the listed attributes is if one contains a “polyp”. Rav Yehuda, in the name of Rav Shmuel, quotes a *Braita* which explains that the polyp defined by the Gemara is referring to bad breath. This explanation would mean that if the husband was truly bothered by the foul odor of his wife’s breath after marriage, he can legitimately annul his marriage to her without paying the monetary obligations that are usually incumbent upon him. From these sources it is clear that Jewish law places an importance on having good breath and avoiding bad breath, because it is of serious concern and can even warrant an annulled marriage.

The Effects of Brushing Teeth

Brushing teeth regularly is perhaps the most commonly understood way of maintaining healthy teeth. Not only does it clean the surfaces of the teeth and remove harmful plaque and bacteria, but brushing also stops plaque from building up on the teeth. WebMD advises that teeth should be brushed at least twice a day for a two minute time period with a fluoridated toothpaste (Friedman, 2019). Additionally, it is advised that the teeth should be brushed in between meals if possible. The toothbrush should be held at a 45 degree angle to the tooth and should also have contact with the gums, to correctly remove debris and bacteria from the soft tissue and teeth. However, caution should be taken to prevent excessive or rough brushing that can cause gum recession.

The Importance of Dental Floss

Oftentimes, patients present dental caries in between their teeth because they have neglected to floss (Futch, 2020). Therefore, it is imperative to include flossing in one's oral healthcare routine to ensure the removal of sugars and food that often are trapped between the small crevices of the teeth. Moreover, according to Dr. Nigel Carter OBE, Chief Executive of the Oral Health Foundation, brushing alone fails to clean 40% of the surfaces of teeth ("Which Comes First, Brushing or Flossing? New Study Shows That We Should Clean between Our Teeth Before Brushing.") There are five surfaces of a tooth; (1) the distal surface which is towards the end of the mouth, (2) the mesial surface which is towards the front of the mouth, (3) the lingual surface which is closest to the tongue, (4) the buccal surface which is closest to the cheek, and (5) the occlusal surface which is the top surface of the tooth. Thus, brushing can only clean the buccal, occlusal, and lingual surfaces of the tooth. This is why it is essential to floss to prevent the formation of bacteria and plaque on the mesial and distal surfaces of the tooth.

The idea of flossing was alluded to by Jewish Sages in the Gemara in *Sota 4a*. The Gemara recounts how long a married woman must be witnessed secluded with another man to be categorized as adulterous. One of the proposed time intervals is the amount necessary for the woman to remove a splinter within her mouth. Rashi comments that the splinter in her mouth was really stuck in between her teeth, thus indicating a historical type of flossing (Katz, page 451).

Furthermore, the Gemara in *Shabbos 81b* discusses the idea of using wood as floss. The Gemara writes of a case where Rabbi Eliezer permitted a person to take a splinter from wood chips lying on the floor to clean his teeth. Rabbi Eliezer specifically mentioned that this was permitted, because normally moving a piece of wood violates the prohibition of *muktzeh*, moving something on Shabbos. The reason why he is allowed to forgo the decree of *muktzeh* in this situation is because it is important for him to clean between his teeth. In this Gemara the Sages disagree with Rabbi Eliezer and say that this man is not allowed to violate the decree of *muktzeh* by picking up a woodchip. However, the only reason why they say this is because they felt that the person was irresponsible in not preparing what was necessary for him prior to Shabbos (Katz, page 446). Ultimately from this Gemara it is clear though that not only was flossing performed in the times of the Gemara, but it was something that was encouraged by the Sages.

The Benefits of Utilizing a Mouthwash

Mouthwash is an oral rinse that usually contains zinc gluconate, quaternary ammonium compounds, or cetylpyridinium chloride to kill bacteria in the mouth (Friedman, 2019). Using an antiseptic mouthwash is an effective method of cleaning the mouth, because of its ability to reach all the surfaces of the mouth. Rinsing daily with an antiseptic mouthwash also prevents the buildup of plaque, by killing different bacteria within the mouth. Additionally, fluoridated mouthwashes can reduce tooth decay which prevents the formation or worsening of dental caries (Marinho, 2016).

Importance of Salt Rinses

Adding a saltwater rinse to an oral healthcare plan has been shown to be beneficial for the teeth, gums, and tongue ("How Saltwater Rinse Improves Oral Health: Guardian Direct"). A study published by the Journal of Indian Society of Pedodontics & Preventive Dentistry found that salt reduces plaque, since the alkaline environment neutralizes the acid released in the mouth ("How Salt Water Mouth Rinse Benefits Oral Health"). This alkaline environment also prevents bacteria in the mouth from growing spores, which inhibits bacterial growth and survival. Moreover, saltwater rinses can help remove food particles stuck in between teeth, which can prevent the formation of dental caries by inhibiting bacterial growth.

Proof of the curative effects of salt sources back to Judaic literature. The Gemara in *Shabbos 65a* in discussing items within the mouth that would not violate the prohibition of carrying on Shabbos writes:

> "...A peppercorn is used for mouth odor; a lump of salt is used for tooth disease. And a woman is permitted to go with anything that was placed (before Shabbos) in her mouth; (for example, one may place) ginger or cinnamon"

According to Rashi, the reason why there is no concern of her carrying on Shabbos is because all these food items were placed in her mouth with therapeutic intent. Therefore, it is clear that Jewish scholars were aware that salt aided in fighting infection, while also relieving pain in the mouth.

It should be noted though that people should only implement salt rinses in their oral health care routines three to four times a week. This is because the salt over time may adversely affect the enamel of teeth and can cause decay (Lin, 2020).

Part V: Conclusion

Understanding the genetic factors for dental caries susceptibility can be crucial to evaluating treatment plans for patients. This knowledge can be applied to prevent dental caries formation based on a potential predisposed genetic mutation. Furthermore, understanding the effects of different genetic mutations of teeth and saliva can be helpful in creating therapeutic treatments. If these therapies are mainstreamed in dental offices, it can revolutionize the standard treatment of care currently in place. Instead of using resin material to fill dental caries or replace teeth with implants, targeted gene therapy can be employed to regenerate one's own tooth. As a future dentist, the research and advancements thus far are extremely intriguing and exciting. I am eager to enter a career in dentistry where the technologies and techniques are constantly evolving to develop the greatest treatment and care. I look forward to seeing how dentistry progresses as I embark on my career as a dentist.

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