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Genetic Engineering in Pediatric Dentistry

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Abstract: ***Advances in biotechnology have brought gene therapy to the forefront of medical research. The concept of transferring genes to tissues for clinical applications has been discussed nearly half a century, but the ability to manipulate genetic material via recombinant DNA technology has brought this goal to reality. Applications of gene therapy to dental and oral problems illustrate the potential impact of this technology on dentistry. In the past years, remarkable progress has been made in the field of gene therapy, including various areas relevant to pediatric dental practice like prevention of dental caries, stem cells in tooth tissue regeneration, rapid orthodontic movement.***

Keywords: Caries vaccines, genetically modified food, replacement therapy, stem cells in tooth tissue regeneration, rapid orthodontic movement

1. Introduction

James Wartson quoted as saying that "We used to think that our fate was in our stars, but now we know, in large measures, our fate is in our genes". Genes are the functional unit of heredity, specific sequences of bases that encode instructions to make proteins. Although genes get a lot of attentions, it is the proteins that perform almost all life functions[1].

Different genes are responsible for the different characteristics and properties of a living organism. To change part of an organism's genome to create some desired trait we use genetic engineering. The basic concept of it is to introduce a gene with the capacity to cure or prevent the progression of a disease. Gene therapy introduces a normal, functional copy of a gene into a cell in which that gene is defective. Cells, tissue, or even whole individuals modified by gene therapy are considered to be transgenic or genetically modified[2]. It could eventually target the rectification of genetic defects, eliminate cancerous cells, prevent cardiovascular diseases, block neurological disorders, and even eliminate infectious pathogens.

Typically, therapeutic genes are identified, isolated and cloned and then introduced into a vector. There are two general approaches for introducing genes into a cell: viral and nonviral. Viral vectors have been used in 70% of the clinical trials to date and are extremely efficient at transferring genes, but can create some safety risks[3]. Nonviral vectors are considered to be much safer than viral vectors, but at present, they are fairly ineffectual at transferring genes

Depending on the delivery method of the vector, gene transfer can be achieved via two techniques in vivo gene transfer that engages direct injection of genetically engineered vectors into the patient, or ex vivo gene transfer that involves injection of the genetically engineered vector into cultured tissue cells followed by transplantation of the altered tissues into the body[4].

Currently gene therapy has emerged as a major medical breakthrough and a number of researchers have focused on using modern advancements in genetic therapy to treat life threatening and refractory diseases such as prevention of HIV, management of hematological disorders, metabolic disorders, cancer treatment and stem cell approach. But the major problems hindering gene transfer applications are biological, resulting from limitations in our knowledge of the essential components involved in the process. These include inadequate understanding of virus biology, recombinant vector interactions with different cell types and the targeted diseases.

Remarkable progress has been made in the field of genetic therapy for a range of applications in dentistry. It has variety of applications in the field of dentistry like in periodontal disease, cancerous and precancerous condition, salivary gland disorders, autoimmune diseases, bone repair, DNA vaccination, rapid orthodontic movement, replacement therapy etc. Minor salivary glands and keratinocytes present in the oral mucosa are excellent target sites for gene therapy since it can be readily accomplished with minimal invasive manner. As a new aspect scientists investigating for tooth regrown using shark gene network activation in human cells[5]. It has made remarkable progress in the field of pediatric dentistry by introduced caries prevention methods, tooth tissue regeneration by stem cells and rapid orthodontic tooth movement.

1.1 Implications of Genetic Engineering in Pediatric Dentistry

In 1995, the Bruice J and a colleague described the potential impact of gene therapy on dentistry, on the basis of initial studies of gene transfer applications to salivary glands, keratinocytes and cancer cells[6]. Their conclusion was that gene therapy would have a significant impact on the nature of dental practice within 20 years. In the past years, remarkable progress has been made in the field of gene therapy, including various areas relevant to pediatric dental practice like

1) Prevention of dental caries
   - Caries vaccine
   - Genetically modified food
   - Replacement therapy
2) Stem cells I tooth tissue regeneration
3) Rapid orthodontic tooth movement

Prevention of dental caries

Dental caries is one of the most common preventable childhood diseases; people are susceptible to the disease throughout their life time. It is the primary cause of oral pain and tooth loss. It can be arrested and potentially reversed in its early stages, but is often not self-limiting and without proper care, caries can progress until the tooth is destroyed. Soits always better to take prevent the occurrence of dental caries than suffering pain and other discomforts, especially for children. There are several conventional method to prevent dental caries but some are offering only short term effect. To overcome these pitfall researchers thought about caries prevention in genetic level. For the sustained prevention of dental caries genetically modified cariogenic bacterias and foods were introduced these include caries vaccine, genetically modified food and replacement therapy.

1.2 Caries Vaccine

Dental caries is one of the most common diseases in humans. In modern times, it has reached epidemic proportions. It is a multifactorial disease, which is caused by host, agent and environmental factors. The time factor is important for the development and progression of dental caries. A wide group of microorganisms are identified from carious lesion of which S.mutans and S.sorbinus exclusively isolated from humans and S.mutans is the most prevalent species[7].

The traditional way of managing dental caries was by a surgical approach of "drill and fill". This approach has slowly evolved into a more conservative mode. Various preventive measures have been implicated for the prevention of dental caries, among which is immunization of the population against the disease.

A vaccine against dental caries, for so long a subject of purely academic research, is currently undergoing phase 2 clinical trials and could be available commercially within next few years[8]. The approach, which is safe, effective and provides long term protection for up to a year, is based on a topical application and does not require any injections. The development of this vaccine has been made possible by recent advances in molecular biology and genetic engineering[8].

Mechanism of action of vaccine

Saliva contains approximately 1-3% of immunoglobulin concentration, a majority of which is secretary IgA. However, saliva also contains the humoral immunoglobulin IgG and IgM from the gingival sulcular fluid[9]. In addition, cellular components of the immune system such as lymphocytes, macrophages, and neutrophils are also present in gingival sulcus. Some of the possible ways antibodies might control bacterial growth are listed below (Diagram 1):[9]

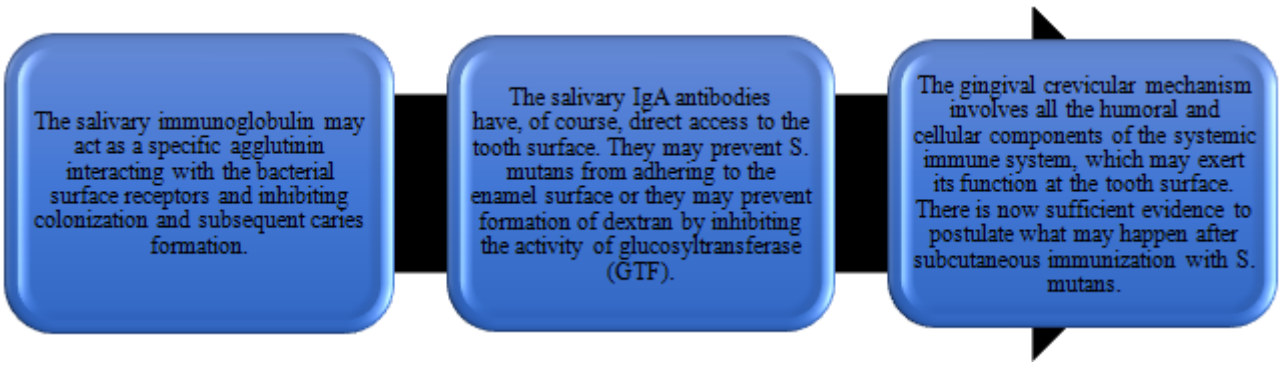


Diagram 1: Mechanism of action of vaccine

1.3 Studies

a) Animal studies

Rapid decay of rodents teeth can be induced by S. mutans when present during the provision of a sugar containing diet. Immunization experiments with cells of S. mutans both in rats and monkeys have consistently resulted in a significant decrease in dental caries. Purified components of S. mutans have been used only to a limited extent. Protection has been induced by immunization with GTF in rats[10]. Recently, immunization with purified protein antigen I/II, which resides in the cell wall of S. mutans, has induced protection against caries. The latter utilizes only one subcutaneous injection of the antigen with adjuvant, unlike all other experiments, which have not been performed in rhesus monkeys and have used from 5 to 15 injections. Immunization with whole cells of S. mutans or with purified I/II antigen produces a reduction of about 70% in both smooth surface and fissure caries when compared with controls[9]. In gnotobiotic rats, ingestion of whole S. mutans selectively produces S-IgA. The appearance of S-IgA correlated with a reduced incidence of the caries vaccine.

b) Human studies

As dental caries fulfills the criteria of an infectious disease, the possibility of preventing it by vaccination has been pursued. Currently, clinical trials are underway to test the use of a pill of S. mutans for control of caries. There have been some conflicting results thus far in

human studies. Some workers have actually reported a negative correlation between S-IgA and caries prevalence[10]. However, this result could be the result of the experimental design. It has been shown that ingestion of capsules containing S. mutans stimulates the production of S-IgA. Stimulation of serum immunoglobulin in humans has also produced mixed results and no correlation could be made between caries experience and serum immunoglobulin stimulation[10].

Antigenic Components of Streptococcus Mutans

S. mutans posses various cell surface substances including adhesins, GTFs, and glucan binding proteins (GBP). These substances are used for vaccine preparation. Most of the recent experimental efforts have been directed toward these compounds[11] .

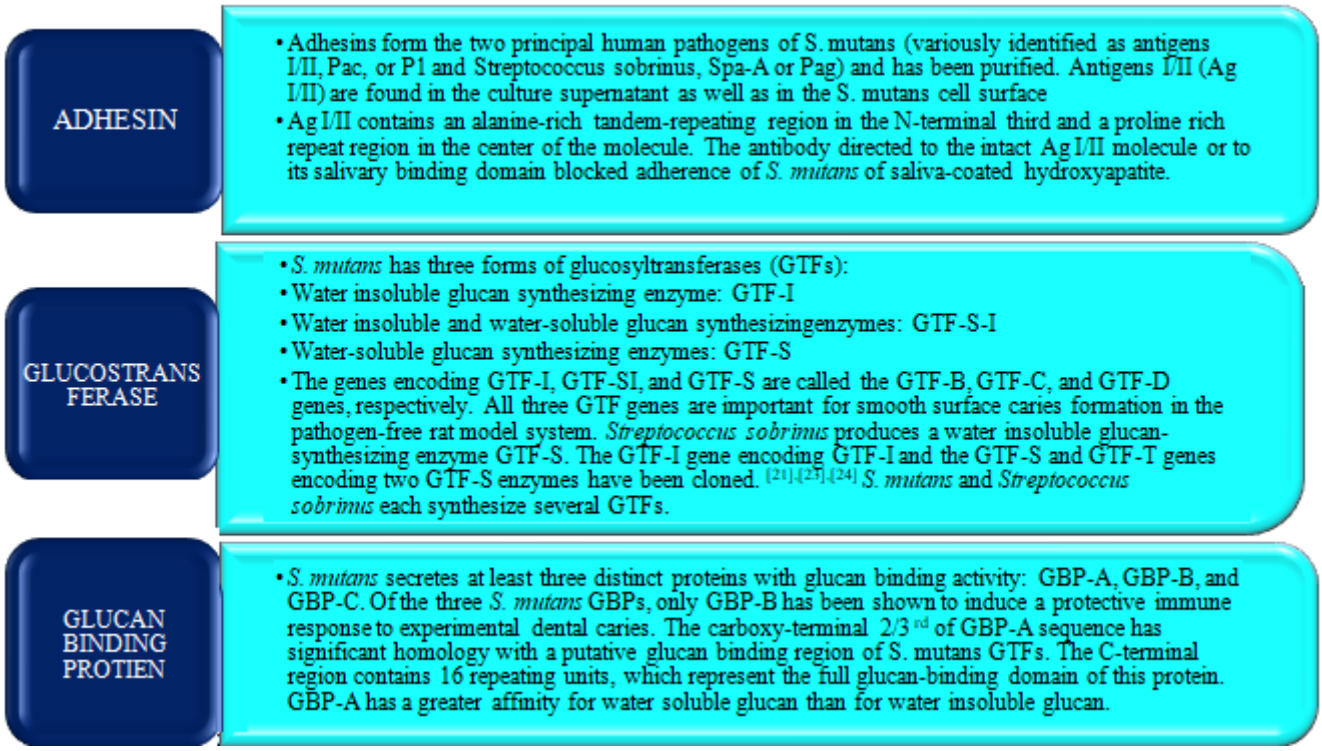


Diagram 2: Antigenic Components of Streptococcus Mutans

Routes of immunization

In general, 4 routes of immunization

1) Oral
2) Systemic (subcutaneous)
3) Active gingiva-salivary
4) Passive dental immunization

1) Common mucosal immune system

Mucosal applications of dental caries vaccines are generally preferred for the induction of secretory IgA antibodies in the salivary compartment, since this immunoglobulin constitutes the major immune component of major and minor salivary gland secretions. Many investigators have shown that exposure of an antigen to a mucosally associated lymphoid tissue in the gut, nasal, bronchial, or rectal site can give rise to immune responses not only in the region of induction but also in remote locations. This has given rise to the notion of a "common mucosal immune system". Consequently, several mucosal routes have been used to induce protective immune responses to dental caries vaccine antigens (table 5)[9].

Table 5: Common mucosal immune system

Oral	Intranasal	Tonsillar	Minor salivary gland	Rectal
The oral route failed to reduce caries significantly, as compared with subcutaneous immunization. The rise in secretory antibodies produced was small and of short duration, even after secondary immunization. Experiments in humans of the	Intranasal installation of the antigen, the nasal associated lymphoid tissue (NALT), has been used to induce immunity to many bacterial antigens including those associated with mutans Streptococcal colonization and accumulation. Protective immunity after	The tonsillar tissue contains the required elements of immune induction of secretory IgA responses although IgG, rather than IgA, response characteristics are dominant in this tissue. Nonetheless, the palatine tonsils, and especially the nasopharyngeal tonsils,	The minor salivary glands populate the lips, cheeks, and soft palate. These glands have been suggested as potential routes for mucosal induction of salivary immune responses, given their short, broad secretory ducts that facilitate retrograde access of bacteria and	rectal immunization with non oral bacterial antigens such as Helicobacter pylori or Streptococcus pneumoniae, presented in the context of toxin-based adjuvant, can result in the appearance of secretory IgA antibodies in distant salivary sites. The colo-rectal region as an inductive location for mucosal immune responses in humans is

ingestion of S. mutans in gelatins capsules resulted in an increase in secretory IgA antibodies in saliva, although for a limited time only	infection with cariogenic mutans streptococci could be induced in rats by the intranasal route with many *S. mutans* antigens or functional domains associated with these components.	have been suggested to contribute precursor cells to mucosal effector sites, such as the salivary glands.	their products and give the lymphatic tissue aggregates that are often found to be associated with these ducts.	suggested from the fact that this site has the highest concentration of lymphoid follicles in the lower intestinal tract.

2) Systemic route of immunization

Subcutaneous administration of S. mutans was used successfully in monkeys and elicited predominantly serum IgG, IgM, and IgA antibodies. The antibodies find their way into the oral cavity via gingival crevicular fluid and are protective against dental caries. A subcutaneous injection of killed cells of S. mutans in Freund's incomplete adjuvant or aluminium hydroxide elicits IgG, IgM, and IgA classes of antibodies. Studies have shown that IgG antibodies are well maintained at a high titer, IgM antibodies progressively fall and IgA antibodies increase slowly in titer. The development of serum IgG antibodies takes place within months of immunization, reaching a tire of up to 1:1280 with no change in antibodies being found in the corresponding sham-immunized monkeys. Protection against caries was associated predominantly with increased serum IgG antibodies.

Active gingivo-salivary route

There has been some concern expressed regarding the side effects of using these vaccines with the other routes. In order to limit these potential side effects, and to localize the immune response, gingival crevicular fluid has been used as the route of administration. Apart from the IgG, it is also associated with increased IgA levels.

The various modalities tried were as follows[10]:

1) Injecting lysozyme into rabbit gingival, which elicited local antibodies from cell response
2) Brushing live *S. mutans* onto the gingiva of rhesus monkeys, which failed to induce antibody formation
3) Using smaller molecular weight Streptococci antigen, which resulted in better performance probably due to better penetration.

Passive immunization

As the name suggests, passive immunization involves passive or external supplementation of the antibodies. This carries the disadvantage of repeated applications, as the immunity conferred is temporary. Several approaches tried were[9]:

- Monoclonal antibodies
  Antibodies to S. mutans cell surface antigen I/II have been investigated. The topical application in human subjects brought a marked reduction in the implanted S. mutans. Thus, by bypassing the system, less concern exists about the potential side effects.
- Bovine milk and whey
  Systemic immunization of cows with a vaccine using whole S. mutans led to the bovine milk and whey containing polyclonal IgG antibodies. This was then added to the diet of a rat model. The immune whey brought a reduction in the caries level. This whey was also used in a mouth rinse, which resulted in a lower percentage of S. mutans in the plaque.
- Egg-yolk antibodies
  The novel concept of using hen egg-yolk antibodies against the cell-associated glucosyltransferase of S.mutans was introduced by Hamada. Vaccines used were formalin killed whole cells and cell associated GTFs. Caries reduction has been found with both these treatments.
- Transgenic plants
  The latest in these developments in passive immunization is the use of transgenic plants to give the antibodies. The researchers have developed a caries vaccine from a genetically modified (GM) tobacco plant. The vaccine, which is colorless and tasteless, can be painted onto the teeth rather than injected and is the first plant derived vaccine from GM plants.

Risks of Using Caries Vaccine

ll vaccines, even if properly manufactured and administered, seem to have risks. The most serious is that sera of some patients with rheumatic fever who show serological cross-reactivity between heart tissue antigens and certain antigens from hemolytic Streptococci. Experiments from antisera from rabbits immunized with whole cells of S. mutans and with a high molecular weight protein antigen of S. mutans were reported to cross react with normal rabbit and human heart tissues[10]. Polypeptides (62-67 KDA) immunologically cross-reactive with human heart tissue and rabbit skeleton muscles myosin are found in the cell membrane of S. mutans and Streptococcus ratti[9]

Replacement Therapy for Prevention of Dental Caries

Dental caries is a major public health concern worldwide, affecting more than 80% of the population[12].Its impact on individuals and communities are as pain and suffering, impairment of function, and reduced quality of lifeis considerable. Dental caries initiation and progression is dependent upon four independent factors like host, diet, microflora and time. So its incidence can be prevented by modification of one of these four factors.

However, because of limited access to professional care, oral hygiene education, health status, huge population and economic limitations the total preventive programs for dental carries cannot be fully successful. Little effort has been taken to addressing this actual pathogen that causes the infection, because the oral cavity is a complex ecosystem in which a rich and diverse microbiota has evolved. Clearly, additional caries prevention approaches

that can augment existing ones (e.g., fluoride, sealants, brushing etc) are highly desirable. One such potential approach depends on the observation that the indigenous microflora can benefit its host by inhibiting the colonization or proliferation of many potential cariogenic pathogens. In recent years, this observation has developed into a therapeutic approach called 'replacement therapy'. This lead to explore thealternate promising concept bio-engineering modified S. mutansto combat this complex situation[12].

The concept of bacteria replacement therapy against dental caries has been put up early in the middle of last century by J. D. Hillman, related to none or less virulent strains of tooth-cloning bacteria pre-empting the tooth surface and replacing the more cariogenic counter parts[13].Manyrelatively non-pathogenic oral commensals found in high numbers at oral cavity have been suggested as a replacement or interference organism, such as Streptococcus sanguis, Streptococcus gordonii, and Streptococcus salivarius. Unfortunately, those substitute cannot compete with Streptococcusmutans and failed in replacement therapy. The attempts to search an appropriate strain to prevent caries became focused on gene-modulation for the main pathogen S. mutans[14].

Majority of effort in the field of replacement therapy to prevent of dental caries has focused on isolating effector strains with decreased acidogenic potential. This is a microorganism that does not cause the disease itself, but rather persistently colonizes host tissues, which are susceptible to infection by a particular microorganism. By virtue of its presence, it must almost be able to prevent infection by that pathogen whenever the host is exposed to it. A "recombinant Streptococcus mutans strain" is a non-naturally occurring strain of S. mutans that has been generated using any of a variety of recombinant nucleic acid techniques[15].

However, to prevent dental caries, an effector strain must have following prerequisites[16]:

1. It must have a significantly reduced pathogenic potential to promote caries.
2. It must persistently colonize S. mutans sites, thereby preventing colonization by disease causing strains whenever host comes in contact with them.
3. It must aggressively displace indigenous strains of S. mutans and allow previously infected subjects to be treated with replacement therapy.
4. It must be safe and not make the host susceptible to other disease conditions.

Types of Replacement Therapy

There are two types of strain replacement therapy[16]:

1. Pre-emptive colonization
2. Competitive displacement

1.P**re-emptive colonization**:

S. mutans, which were unable to produce caries either due to their inability to produce lactic acid (lactate dehydrogenase mutants) or to synthesize intracellular polysaccharides (ICP mutants) were implanted into the oral microflora of experimental animals prior to the introduction of potentially pathogenic strains of S. mutans[16]. The concept is that the nonvirulent S. mutans will have an ecological niche similar to that of virulent S. mutans, thus will be capable of interfering with colonization by the cariogenic bacteria.

2.**Competitive displacement:**

A noncariogenic microorganism is introduced that is capable of competing with and displacing the indigenous cariogenic Mutan streptococci. The ideal effector strain would be a noncariogenic bacterium, which is continuously present in the mouth and competes successfully with MS[16]. It should accumulate preferentially on the tooth surfaces, be able to grow rapidly and withstand sudden and wide changes of pH.

Strategies of Replacement Therapy

1. One of the physiological properties, which might provide a selective advantage in colonization is bacteriocin production, known for some time to be a common feature of S. mutans. Bacteriocins are proteins that kill representatives of same species as the producer organism or related species. In a preliminary screening of S. mutans strains for bacteriocin production, one strain, JH1001 inhibited the growth of virtually every other strain of this organism.[17]
2. Colonization of the human oral cavity by the mutant of JH1001 that produces three-fold elevated inhibitor activity has also been tested. The results of this study indicated that a practical and effective regimen for the implantation of an effector strain could be developed for the replacement therapy of dental caries in humans.[18]
3. Mutants of S. mutans defective in intracellular polysaccharide metabolism have also received attention. Studies by Tanzer et al have indicated the ability of IPS mutants of S. mutans to colonize the teeth of experimental animals pre-emptively.[19]
4. A natural variant of S. salivarius called TOVE-R has been studied. Like typical S. salivarius strains, TOVE-R is noncariogenic. Atypically, it preferentially colonizes tooth surfaces and produces plaque much like S. mutans.[20]
5. Lactic acid is the strongest of the metabolic acid end products of oral microorganisms, and its production is catalyzed by lactate dehydrogenase (LDH). Hillman hypothesized that LDH– mutans streptococci would have reduced cariogenic potential and that they could be useful effector strains for replacement therapy of dental caries.[21] However, at high sugar concentrations, the levels of activity of these enzymes are apparently insufficient to compensate for the absence of LDH. A supplemental alcohol

dehydrogenase (ADH) activity can complement the LDH deficiency when expressed in the temperature sensitive LDH mutant. LDH-deficient mutants of S. mutans produced approximately half as much titrable acid as did their parent when grown in a broth containing excess glucose. The mutant was found to colonize the teeth of animals to the same extent as did its parent. The difference in cariogenic potential between parent and mutant, therefore, could be ascribed solely to differences in acid production.

6. The most extensive research in using genetically modified bacteria for preventing dental caries used a wild-type S. mutans strain that naturally produces an antibiotic called mutacin 1140 capable of killing all other strains of S. mutans [Hillman, 2002][22]. This strain was genetically modified by deleting the open reading frame for lactate dehydrogenase, to yield a viable strain called BCS3-L1 that still produced wild-type levels of mutacin 1140, but notably produced no lactic acid.
7. Idone generated a mutant with gene gcrR knock out, a gene regulated with sucrose dependent adherence of S. mutans, called GMS900. The studyshowedanenhancementingeneexpressionrelatedt osucrose-dependent adherence in vivo, as well as significantly fewer cavities on it, which suggest the capacity of replacement therapy.[23]
8. J.D. Hillman and others studied the ability of daily applications of Streptococcus rattus strain JH145 to affect the numbers of an implanted Streptococcus mutans strain in a rat model. The results showed that daily application of JH145, a naturally occurring LDH-deficient variant of S. rattus, can compete with S. mutans for its habitat on the tooth surface and S. rattus JH145 has potential as a probiotic for use in the prevention of dental caries.[24]
9. Genetically modified probiotics with enhanced properties can be developed (‘designer probiotics’). For example, a recombinant strain of Lactobacillus that expressed antibodies targeting one of the major adhesions of S. mutans (antigen I/II) was able to reduce both the viable counts of S. mutans and the caries score in a rat model.[25]
10. A variety of bacteria including S. sanguis is able to utilize arginine catabolically via argnine deaminase system. In this pathway, AD catalyses the hydrolysis of arginine to citrulline and ammonia. Though S. sanguis is inherently less acid tolerant than other organisms like S. mutans, it can be protected against lethal acidification by catabolism of arginine by AD pathway. Protection probably occurs through production of ammonia and associated rise in environmental pH. This protection may be critical to the survival of S. sanguis in dental plaque in which pH value can drop below 4.0 and in which cycles of acidification-alkalinization occur frequently. Thus, with the isolation of genes from S. sanguis, it should be possible to introduce the AD system into cariogenic bacteria S. mutans.[26]

- **S. mutans strain BCS3-L1**

According to the acidogenic theory of dental caries, lactic acid production by S. mutans has long been considered to be the main pathogenic mechanism for production of caries lesions. Consequently, lactate dehydrogenase (LDH) deficiency was chosen as the approach or reducing acidogenicity in construction of BCS3-L1. Earlier work (Johnson et al. 1980) with a closely related S. rattus strain had provided convincing evidence for the effectiveness of this approach. LDH-deficient mutants were virtually non-cariogenic in gnotobiotic rats and did not contribute significantly to the cariogenic potential of the indigenous flora in conventional pathogen-free rats.[22] Attempts to transfer these findings directly to S. mutans proved to be difficult.

Evolution

LDH-deficient mutants of various strains of S. mutans were not found using the same screening methods used to isolate mutants of S. rattus. Cloning the structural gene encoding the S. mutans LDH (Hillman et al. 1990) provided the basis for solving this puzzle.[27]Standard insertional mutagenesis methods failed to yield LDH-deficient clones, suggesting that LDH-deficiency was a lethal mutation in most S. mutans strains. Chemostat studies indicated that some aspect of glucose metabolism was toxic during growth under the non-permissive condition. The toxic effect could be overcome by limiting the amount of environmental glucose. This and other data accorded with studies of S. mutans central intermediary metabolism indicating that this organism has enzymatic activities.

However, at high sugar concentrations, the levels of activity of the seen zymes are apparently insufficient to compensate for the absence of LDH. It was found (Hillman et al. 1996) that a supplemental alcohol dehydrogenase (ADH) activity, when expressed in the temperature sensitive LDH mutant, could complement LDH deficiency.[28]
With this background of information, BCS3-L1 construction started with the LDH gene cloned into an appropriate suicide vector for S. mutans. Essentially the entire gene except for transcription and translation signal sequences was deleted and replaced with the Zymomonas mobilis open reading frame for alcohol dehydrogenase (ADH) II. Transformation of the recombinant molecule into the S. mutans starting strain, JH1140, and allelic exchange resulted in the isogenic mutant, BCS3-L1.

This effector strain had no measurable LDH activity and Ca. 10-fold elevated levels of ADH activity relative to its parent. Fermentation end-product analysis showed that BCS3-L1 made no detectable lactic acid. As predicted from earlier work with S. rattus, much of the metabolized carbon was converted to the neutral end-products, ethanol and acetoin. Under various cultivation conditions, including growth on a variety of sugars and polyols, such as sucrose, fructose, lactose, mannitol, and sorbitol, BCS3-L1 yielded final pH values that were 0.4 to 1.2 pH units higher than those of its parent, JH1140.

Mechanism of action

From the standpoint of replacement therapy of caries, these results suggest that implantation of an effector strain would best be accomplished in children immediately after the onset of tooth eruption and before their acquisition of a wild-type strain. In order to prevent supercolonization by wild-type strains when the host comes in contact with them, an effector strain should have some significant selective advantage to colonization. This would also enable subjects who have already been infected with wild-type S. mutans to be treated by replacement therapy. The ability of an effector strain to preemptively colonize the human oral cavity and aggressively displace indigenous wild-type strains was initially thought to be a complex phenomenon dependent on a large number of phenotypic properties. However, it was discovered that a single phenotypic property could provide the necessary selective advantage.[10] A naturally occurring strain of S. mutans was isolated from a human subject that produces a bacteriocincalledmutacin1140thatis capable of killing virtually all other strains of mutans streptococci against which it was tested. Mutants were isolated that produced no detectable mutacin 1140 or that produced approximately three-fold elevated amounts. A correlation was also made between mutacin 1140productionandtheabilityofS.mutanstopersistently colonize the oral cavities of human subjects and aggressively displace indigenous mutans streptococci.[22]

Taken together all these studies the reduced acidogenic potential of BCS3-L1 was reflected in its dramatically decreased cariogenic potential as shown in several animal models (Hillman et al. 2000).[29] The results of these studies provided strong evidence that an LDH-deficient S. mutans strain such as BCS3-L1 has significantly reduced pathogenic potential, and thus satisfies the first prerequisite for use as an effector strain in replacement therapy for dental caries.

Safety and Stability

To serve as an effector strain in replacement therapy of dental caries, BCS3-L1 must be safe in several important regards.

- It must be genetically stable, BCS3L1 has a low transformation frequency due to a natural mutation in its gene for competence stimulating peptide, allelic exchange has been used to delete the comE gene to provide added assurance that exogenous DNA will not transform this strain.[22]
- Mutacin 1140 has been shown to be a member of a small class of antibiotics called lantibiotics. Sufficient mutacin 1140 has not been purified to directly test its toxicity and found that its have extremely low toxicity.[22]
- Mutacin 1140 up-production clearly provides a selective advantage to BCS3-L1 colonization, but the minimal infectious dose has not been determined for this strain or any S. mutans strain in humans.

➢ **GCRR-deficient S. mutans**

S. mutans is recognized as the main pathogen of dental caries in humans. In the process ion development to fcaries, the paramount main virulence factor involves the ability to produce acid and adhere to the tooth surface. In particular, the sucrose-dependent adherence of S. mutans to the tooth surface prevents the bacteria from being washed away by chewing or by the flow of saliva.The rationale of the sucrose dependent adherence can be used by the low acid-producing strains to enhance their adherence ability. Thus, these strains occupy the same ecological niche in plaque similar to their more cariogenic progenitor. Idone generated a mutant with gene gcrR knock out, a gene regulated with sucrose dependent adherence of S. mutans, called GMS900.[23] The study showed an enhancement in gene expression related to sucrose-dependent adherence in vivo, as well as significantly fewer cavities on it, which suggest the capacity of replacement therapy.

Glucan-binding lectin (GBL), which enables bacteria to aggregate by binding the α-1, 6-glycosidic linkages, is ubiquitous on the S. mutans surface.[30] GBL is an adhes in and can be isolated from many S. mutans subtypes. With the help of adhesions such as GBL, S.mutan scan synthesize many kinds of glucant of or mawrapofmucosal fluid, which promotes its tight adherence to the tooth surface. Although different opinions on the mechanism by which GBL functions have been presented, GBL has a very important function in the formation of dental plaque and adherence of S. mutans. The gcrR gene functions as a negative transcriptional regulator of the gbpC gene, which encodes the S. mutans GBL.

Some researchers revealed that the gcrR expression product shares a high homology with the signaling molecule of the density inductance system, which may be involved in the regulation of the GBL expression. For instance, Sato et al. revealed the down regulation of gbpC gene by gcrR gene. On the contrary, the gcrR gene knockout results in the over expression of GBL.

Taken together all these studies, MS-gcrR-def showed reduced acid production and significantly enhanced adhesion ability at the early stage, which might confer it an absolute advantage in the competition with wild type. These findings suggest that MS-gcrR-def is a promising effect strain in place of UA159. In the replacement therapy, even if the wild-type strain interference cannot be avoided, the mutant, with its strong early colonization ability, is still able to become the dominant bacteria on the surface of tooth and reduce the incidenceofdentalcaries.[14]

Benefits of Replacement Therapy

- Increased desire of consumers to use natural methods for health maintenance. Parenterally administered broad spectrum antibiotics indiscriminately kill a wide variety of bacterial species associated with the host microflora resulting in formation of an ecological vacuum and encouraging super infection and resistance development.

- Modulation of the microflora composition by specific introduction of strains of 'naturally occurring' species that are capable of excluding colonization and/or infection by target pathogens could be viewed as the controlled manipulation of a process that otherwise occurs haphazardly in nature.
- Directed implantation of relatively harmless effector bacteria known to be strongly competitive with potential pathogens offers a cost-effective, long-term means of achieving tailor made protection for the host against specific bacterial infections.
- It might also foster increased herd protection through natural transmission of the effector strain to close contacts of the host.

Difficulties and Possible Risks

- The equilibrium is regularly upset by various events, most dramatically by exposure to broad-spectrum antibiotics or antiseptics but also possibly following substantial nutritional, hormonal or physical changes to the microenvironment.
- Significant reductions in the numbers of individual components of the balanced microflora could result in overgrowth (super infection) by previously suppressed minority members of the population.
- Long-term retention of antibiotic producing effector strains might not be easily achieved.
- The selection of pathogens resistant to the effector strain remains a problem, particularly if microbial interference is largely mediated by antibiosis.

Conclusion

For prevention of dental caries, a single colonization regimen that leads to persistent colonization by the effector strain should provide lifelong protection. If ultimately successful, the use of genetic engineering to tailor an effector strain for replacement therapy for dental caries will encourage similar efforts to prevent other infectious diseases as well.

Genetically Modified Food

Genetic engineering and biotechnology are very complex and dynamically developing technologies in the present world. The organisms produced as a result of these technologies are called genetically modified organisms (GMOs). WHO defined it as "organisms (i.e., plants, animals or microorganisms) in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination".[31]

The term genetically modified foods or genetically modified organisms (GMOs) is most commonly used to refer to crop plants created for human or animal consumption using genetic engineering technology. These plants have been modified in the laboratory to enhance desired traits such as increased resistance to herbicides, improved nutritional content, Drought tolerance/salinity tolerance etc. Genetic engineering can create plants with the exact desired trait very rapidly and with great accuracy. Not only genes be transferred from one plant to another, but genes from non-plant organisms also can be used, to produce GM foods. GM foods are offering a vast usage in medical and dental fields.

In dentistry GM foods plays a promising role in the prevention of dental caries. Certain modifications by means of genetic engineering can lead to production of food material containing inherent property of anticariogenicity without altering the oral microflora. It is a transgenic crop that contains genes known for their desirable qualities.[32] Some researches are going on to produce foods with anticariogenic properties like genetically modified yogurt, genetically modified milk, genetically modified apple, transgenic plant antibody containing egg yolk etc. We are going to discuss in detail about these foods with anticariogenic property below:

1) Genetically Modified Apple

Recently antagonist peptides have been searched to work against the specific enzyme system (GTF) of S.mutans. These 'antagonist peptides' can be successfully incorporated to various genetically modified crops e.g.: Apple, Strawberries etc. Thus without altering oral ecology, caries prevention can be possible. The peptide works by controlling the growth of Streptococcus mutans, the bacteria that cause tooth decay. It stops the microbes from binding to the tooth, preventing dental caries for upto 80 days at a time without using antibiotics which also kill 200 other species of mouth bacteria that cause no harm and which promote the development of resistant bacteria's. Clinical & Biological research is still going on & only time will tell that whether by genetically modifying the oral micro organisms in one way or other will we be able to safely tackle the problems that has crippled our oral health for generations.[33]

2) Transgenic Plant Antibody

Progress over the past 10 years demonstrates that plants will be a facile and economic bioreactor for large-scale production of industrial and pharmaceutical recombinant proteins. Genetically engineered, transgenic plants have many advantages as sources of proteins compared with human or animal fluids/tissues, recombinant microbes, transfected animal cell lines, or transgenic animals.

The most clinically advanced secretary IgA plantibody, called CaroRx™, recognizes and inhibits the binding of the major oral pathogen, Streptoccocus mutans, to teeth. CaroRx™ has been produced and purified from tobacco under GMP conditions for clinical testing in the UK and USA. CaroRx™ was engineered with an additional IgG CH2 domain to facilitate purification of the antibody by protein G affinity chromatography. A Poros™ Protein G affinity purification was used to obtain 95% pure CaroRx™ from green plant tissue.[34]

These initial clinical studies demonstrate that topically applied anti-S. mutans SIgA plantibody (CaroRx™) is safe and prevents colonization by S. mutans, the major cause of human dental caries.

3) **Genetically Modified Yogurt**

Bacteria can prevent tooth decay, as well as can be the cause of it. Probiotic bacteria are live microbial food supplements that beneficially affect the host by improving intestinal balance. That micro organisms are usually part of the normal flora and this approach in therapy and prevention was first applied in the treatment of intestinal diseases. The specific actions of the probiotic micro organisms involved are competition with pathogenic bacteria, influence on mucosa permeability and restitution of gut micro-ecology and influence on inflammation process. Generally speaking, the oral cavity is also part of the gastrointestinal system. The same mechanisms of action of probiotic bacteria can be adopted for caries prevention. In the oral cavity direct and indirect actions of probiotics can be observed: they directly influence binding of bacteria to proteins and other bacteria, influence bacterial metabolism and produce substances that inhibit cariogenic bacteria. Genetic engineering is strongly involved in the fabrication of new species of probiotics.

Lactobacillus rhamnosus (LGG) is today one of the most popular bacterial species that is used as probiotic. It was isolated in 1985 by Gorbach and Goldin from the human intestine. Laboratory research showed that it has inhibitory action on Streptococcus mutans and Candida albicans, it also has good adhesion to mucosal and dental tissues and it does not metabolise sucrose. So these genetically modified probiotics were incorporated into yogurt. Daily consumption of LGG yoghurt can have an inhibitory effect on oral pathogenic microflora, so it can be recommended as a beneficial procedure in caries prevention.[35]

Bifidobacteria is another commonly used bacterial species used as probiotic. Bifidobacteria are the predominant anaerobic bacteria naturally occurring within the intestinal lumen and play a critical role in maintaining the equilibrium among normal intestinal flora. probiotic yogurt contained genetically modified Bifidobacterium DN-173 diminishes the caries-associated microorganisms like streptococci strains in the oral cavity.

Lennart Hammarstorm and his colleagues engineered Lactobacillus zeae to carry an antibody against streptococcus mutans on their surface. The antibody sticks to the molecule on S.mutans that normally sticks to the teeth. The two species clump together & slide harmlessly down the throat. This is the first time that such bacteria have been used to deliver passive immunity – antibodies from a source other than the immunized subject.

So these genetically engineered bacterium were incorporated into Yoghurt, and consumption of this will kill the cariogenic streptococcus mutans by passive immunity.[36]

4) Genetically Modified Milk

Technologies can make milk better for tooth health. A team of European researchers has genetically engineered lactobacillus often found in milk to make it capable of neutralizing the bacteria that cause tooth decay in rats. The genetically engineered lactobacillus surface was studded with antibodies targeting the cariogenic bacteria, the souped up milk microbes could neutralize tooth decay.

Milk supplemented with *L. rhamnosus* (LB21 or GG), are used for caries prevention. Around three randomized control trials were done based on this and All the studies drag into the promising conclusion that, no significant reductions in the levels of *S. mutans* or lactobacilli were seen yet all showed an obvious effect on caries.[37]

Safety Aspects Of Genetically Modified Food

- All DNA, including DNA from GMOs are composed of the same 4 nucleotides. Genetic modification results in the re-assortment of sequences of nucleotides leaving their chemical structures unchanged. Therefore, DNA from GMOs is chemically equivalent to any other DNA.
- There is no difference in the susceptibility of recombinant DNA and other DNA to degradation by chemical or enzymatic hydrolysis.
- There are effective mechanisms to avoid genomic insertion of foreign DNA. There is no evidence that DNA from dietary sources has ever been incorporated into the mammalian genome.

Criticisms of Genetically M odified Foods

- **Allergenicity**

There is a possibility that introducing a gene into a plant may create a new allergen or cause an allergic reaction in susceptible individuals.

- **Toxic reactions in the digestive tract**

The digestive tract, which is the first and largest point of contact with foods, can reveal various reactions to toxins and should be the first target of GM food risk assessment.

- **Inherently unsafe**

There are several reasons why GM plants present unique dangers. The process of genetic engineering itself creates unpredicted alterations, irrespective of which gene is transferred. This creates mutations in and around the insertion site and elsewhere.

- **Economic Concerns**

Bringing a GM food to market is a lengthy and costly process. Yet consumer advocates are worried that patenting these new food varieties will raise the price.

Stem Cells in Tooth Tissue Regeneration

In the last decade, tissue engineering flourish a lot in the fields of regenerative medicine and dentistry. The regenerative medicine aims to repair or re-grow parts or tissues which are lost due to disease or injury. One of the building blocks of this therapy is stem cells. Stem cells are cells found in most of the multi-cellular organisms. They are characterized by self renewal and potency i.e. - the ability to renew themselves through mitotic cell division and differentiating into a variety of specialized cell types.[38] They are essential for the development, growth,

maintenance, and repair of our brains, bones, muscles, nerves, blood, skin, and other organs

Classification of stem cell

Stem cells can be broadly classified into two.

1) Based on their potency
2) Based on their tissue of origin

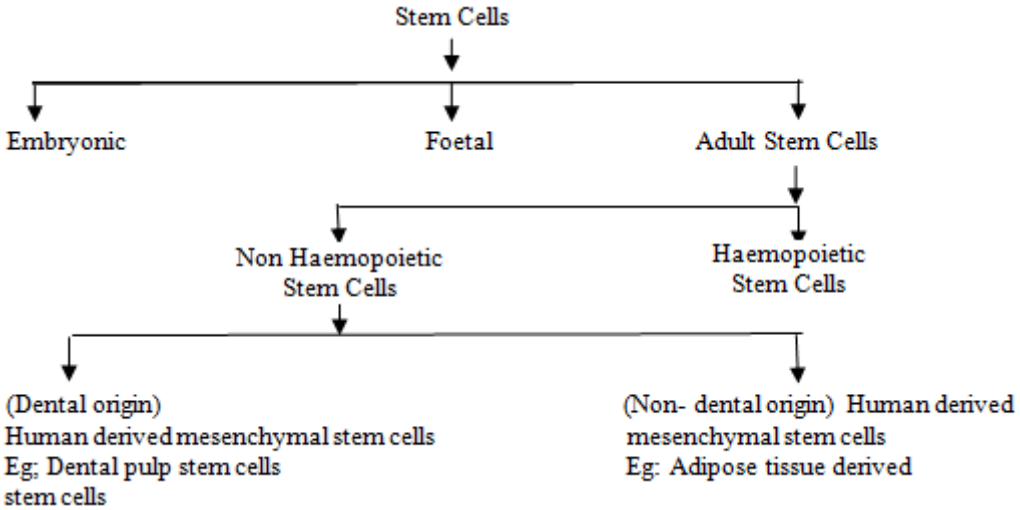
1. Classification based on their potency:

Stem cells can be classified by the degree to which they can differentiate into different cell types. These five main classifications are totipotent, pluripotent, multipotent, oligopotent and unipotent stem cells.[39]

a) **Totipotent (omnipotent) stem cells**: The ability to differentiate into all cell types like embryonic and extraembryonic stem cells. E.g.: The zygote formed at egg fertilization and the first few cells that result from the division of the zygote like 4 cell stage.
b) **Pluripotent stem cells**: The ability to differentiate into almost all cell types. E.g.: Embryonic stem cells and cells that are derived from the mesoderm, endoderm and ectoderm germ layers that are formed in the beginning stages of embryonic stem cell differentiation.
c) **Multipotent stem cells**: The ability to differentiate into a closely related family of cells. E.g.; Hematopoietic (adult) stem cells that can become red and white blood cells or platelets (bone marrow).
d) **Oligopotent stem cells**: It can differentiate into only a few type of cells, such as lymphoid or myeloid stem cells.
e) **Unipotent cells**: The ability to produce only cells of their own type, but have the property of self renewal. E.g.; (adult) muscle stem cells.

2. Classification based on their tissue of origin (flow chart 3)

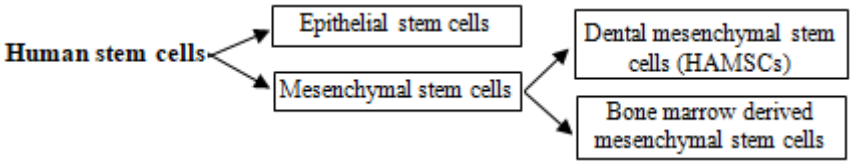
Based on their tissue of origin stem cells can be broadly classified into embryonic, foetal and adult stem cells. Adult stem cells are again classified into haemopoitic and and nonhaemopoietic stem cells. From the non haemopoetic stem cells human derived mesenchymal stem cells of dental origin and non-dental origin are deriving (flow chart 2).[39]



Flow chart 2: Classification of stem cells based on their tissue of origin

Dental stem cells

The existence of stem cells in the tooth will help for the odontogenesis. During the foetal development, teeth arise from the neural crest through a series of interactions between nueral, mesenchymal and epithelial tissues. Once embryonic oral epithelium and mesenchyme start interaction, the epithelial stem cells differentiate into ameloblasts; the mesenchymal stem cells differentiate into odontoblasts, fibroblasts and other cells; finally form functional teeth. Therefore, in order to regenerate a human tooth, two types of human stem cells are required (flow chart 3).



Flow chart 3: Types of human stem cells for tooth regeneration

Dental mesenchymal stem cells:

Dental mesenchymal stem cells have been identified in association with the mucosal tissues and both deciduous and permanent teeth in humans. These cells possesses the characteristics include the expression of specific markers, self-renewal, and the capacity to differentiate into multiple cell types. The relative accessibility of these cells means that they may represent a source of stem cells with great potential for use in tooth tissue regeneration.[40] This includes

1) Stem cells from the apical papilla (SCAPs)
2) Dental pulp stem cells (DPSCs)
3) Stem cells from exfoliated primary teeth (SHEDs)
4) Periodontal ligament stem cells (PDLSCs)
5) Dental follicle precursor cells (DFPCs)

Tissues engineering using stem cells in tooth tissue regeneration

The basis for this approach lies in the presence of a population of progenitor cells that can be induced, under the influence of these growth factors, to differentiate into the specific cells required for tissue regeneration. So tissue engineering using the dental stem cells can regenerate all the dental tissues like enamel, dentine, cementum, pulp and even whole tooth.

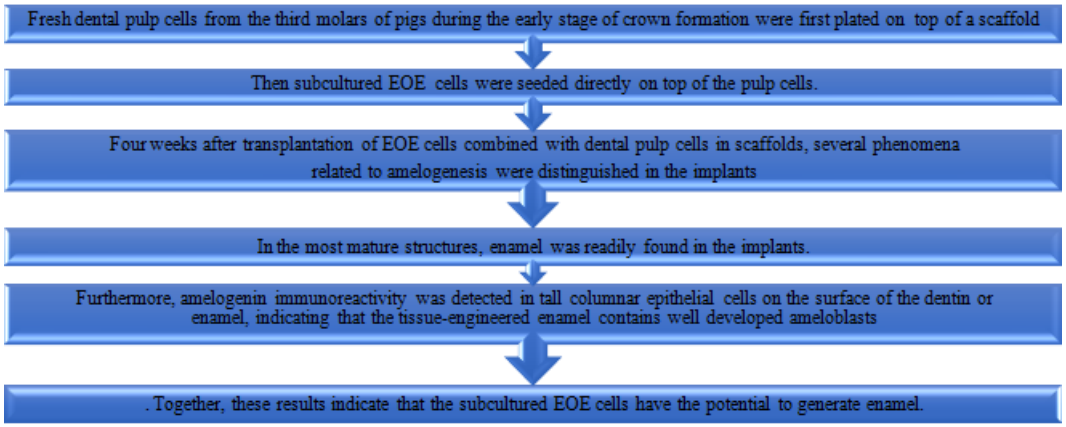
A. Enamel regeneration:

In human embryo, dental epithelial stem cells e.g.; enamel organ epithelial cells are the only cell source for enamel generation. However, because human dental epithelial stem cells are limited in the embryo, a key question is where to find the replacement from adult. One potential candidate of the replacement is oral epithelial stem cells because of that they derived from embryonic epithelium as same as the dental epithelial stem cells.[41]

In vivo study on mice

Developing a technique to manipulate enamel organ epithelial (EOE) cells is a significant advance towards enamel replacement and therefore attempted to develop a strategy to generate enamel based on subcultured enamel organ epithelial cells using tissue-engineering technology.[41]

Honda et al in 2010 examined the enamel-forming capability of subcultured EOE cells, by transplanting cells onto a biodegradable scaffold in vivo on mice (picture 18).[42]



Flow chart 4: Enamel regeneration based on subcultured EOE cells using tissue-engineering technology

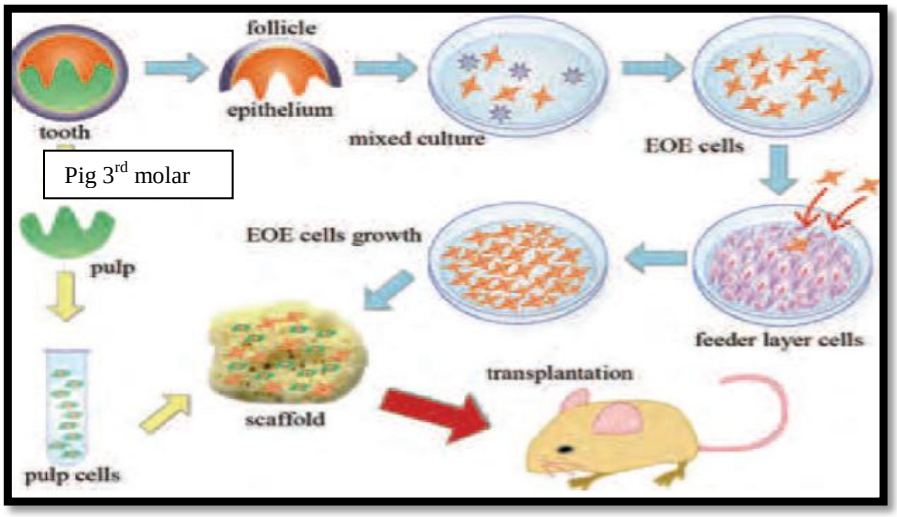


Figure 18: Enamel regeneration based on subcultured EOE cells using tissue-engineering technology

B. Dentine regeneration:

A number of recent studies have demonstrated that stem cells, of both dental and non-dental origin, are capable of inducing odontogenesis and regenerating dentin.[43] Human adult dental pulp contains a population of cells (dentin pulp stem cells; DPSCs) with stem cell-like properties such as self-renewal and the ability to differentiate into dentine. Deciduous teeth contain a population of more immature multipotent stem cells (stem cells from human exfoliated deciduous teeth; SHED), that in contrast to DPSCs, are capable of forming dentin-like structures but not a complete dentin-pulp complex.[44] The regeneration of dentin is feasible because dentin is in intimate contact with an underlying highly vascular and innervated pulpal tissue, forming a tightly regulated "dentin-pulp complex". During primary tooth formation, dentin is produced by odontoblastic cells located within the pulp. Following tooth eruption, the secretory activity of these cells is down-regulated, although they continue to produce secondary dentine at a low level.

C. Pulp regeneration:

Regenerative endodontics is the formation and delivery of tissues to replace diseased, missing and traumatized pulp. The main goal of dental pulp tissue engineering is to replace the inflamed or necrotic pulp by a healthy and functional tissue, capable of form in newdentin. The most futuristic approach for pulp regeneration is gene based therapies. These techniques will possibly involve some combination of disinfection or debridement of infected root canal system with apical enlargement to permit revascularization and use of adult stem cells, bioactive scaffolds and growth factors (diagram 3).[40]

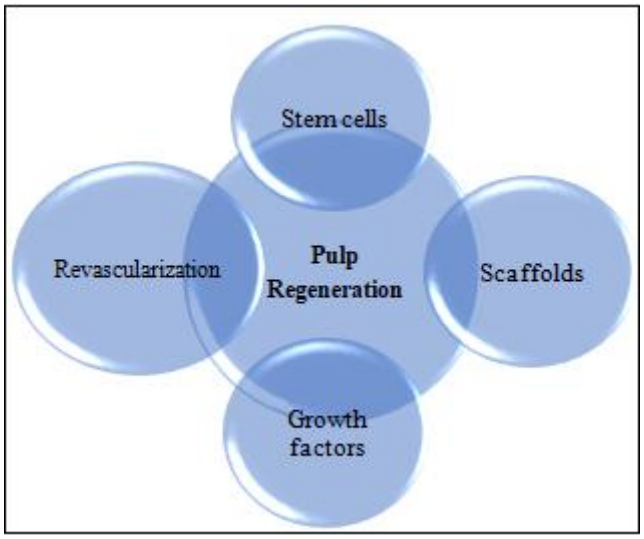


Diagram 3: Treatment options for pulp regeneration

Now will discuss about the potential role of stem cells, scaffolds & growth factors in the pulp regeneration using tissue engineering.

i) Potential role of stem cells

For the regeneration of the necrotic pulp, stem cells are required to achieve the goal of replacing diseased pulp into a healthy pulp that would continue normal dentinogenesis. So current clinical protocols for regenerating dental pulp are probably utilizing indigent stem cells to form progenitor cells. These indigent stem cells sources are; [40]

a) Stem cells of the apical papilla (SCAP)
These are a population of mesenchymal stem cells residing in the apical papilla of incompletely developed teeth. SCAP is the main source of primary odontoblast that are responsible for the formation of root dentin . because of its apical location, the apical papilla has collateral circulation, this will help it to survive during the process of pulp necrosis.

b) Dental pulp stem cells (DPSCs)
Human DPSCs cells having the capability of self renewal and multilineage differentiation potential including odontogenesis. These are the likely source of replacement odontoblast.

c) Periodontal ligament stem cells (PDLSCs)
PDLSCs has the potential to differentiate into cementoblast and osteoblast but they are not seem to be dentinogenic.

There are two main approaches for the delivery of these stem cells into the root canals; [40]

1) Cell transplantation-
This involve direct delivery of autologues and analogues stem cells into the root canal. There is still a need for good in-situ models for regenerative procedures.
2) Cell homing-
This involves chemotactic factors like stromal cell derived factors that can induce migration of stem cells from the periapical area into the root canal

ii) Potential role of growth factors

One use of gene delivery in endodontics would be to deliver mineralizing genes into pulp tissue to promote tissue mineralization.

In-vivo:

Half life of BMP's as recombinant proteins is limiting. Therefore gene therapy is a potential alternative to conquer disadvantages of protein therapy. Recombinant adenovirus containing BMP 7 gene induced only a small amount of poorly organized dentin after direct transduction in experimentally inflamed pulp. In vivo gene therapy does not have much effect on reparative dentin formation in case of severe inflammation.

Ex-vivo:

The transplantation of cultured dermal fibroblasts transduced with BMP-7 using a recombinant adenovirus, induced reparative dentin formation in the exposed pulp with reversible pulpitis. The great potency of BMP genes to provoke differentiation of pulp stem cells, even if' in reversible pulpitis, demonstrates the utility of ex vivo gene

therapy in reparative / regenerative dentin formation for clinical endodontic treatment.

iii) Potential role of bioactive scaffold

An ideal scaffold should have the following characteristics for successful pulp regeneration; capability of seeding of stem cells, growth factor supplement, controlled biodegradability to be eventually replaced with natural tissue etc. There are 2 types of scaffold delivery;

I) Scaffold implantation;

To create a more practical endodontic tissue engineering therapy, pulp stem cells must be organized into a three-dimensional structure that can support cell organization and vascularization. This can be accomplished using a porous polymer scaffold seeded pulp stem cells. A scaffold should contain growth factors to aid in stem cell proliferation and differentiation, leading to improved and faster tissue development. The scaffold may also contain nutrients promoting cell survival and growths, and possibly antibiotics to prevent any bacterial in-growth in the canal systems. In addition, the scaffold may exert essential mechanical and biological functions needed by replacement tissue.

To achieve the goal of pulp tissue reconstruction, scaffolds must meet some specific requirements. Biodegradability is essential, since scaffolds need to be absorbed by the surrounding tissues without the necessity of surgical removal. A high porosity & an adequate pore size are necessary to facilitate cell seeding and diffusion throughout whole structure of both cells and nutrients. The rat eats which degradation occurs has to coincide as much as possible with the rate of tissue formation; this means that while cells arc fabricating their own natural matrix structure around themselves, the scaffold is able to provide structural integrity within the body, and it will eventually break down, leaving newly formed tissue that will take over the mechanical load.

The principle drawbacks are related to the difficulties of obtaining high porosity and regular pore size. This has led researchers to concentrate efforts to engineer scaffolds at nanostructural level to modify cellular interactions with the scaffold. Some proteic materials have not been well studied. However, early results are promising in terms of supporting cell survival and function, although some immune reactions to these types of materials may threaten their future use as part of regenerative medicine.

II) Injectable scaffold delivery

Rigid tissue engineered scaffold structures provide excellent support for cells used in bone and other body areas where the engineered tissue is required to provide physical support. However, in root canal systems a tissue engineered pulp is not required to provide structure support of the tooth. This will allow tissue engineered pulp tissue to be administered in soft three-dimensional scaffold matrix, such as a polymer hydrogel. Hydrogels are injectable scaffolds that can be delivered by syringe. Hydrogels have the potential to be noninvasive and easy to deliver into the root canal systems. In theory, the hydrogel may promote pulp regeneration by providing a substrate for cell proliferation and differentiation into an organized tissues structure. Past problems with hydrogels included limited control over tissue formation and development, but advances in formulation have dramatically improved their ability support cell survival. Despite these advances, hydrogels are at an early stage of research, and this type of delivery system, although promising, has yet to be proven to be functional in vivo. To make hydrogels more practical, research is focusing on making them photopolymerizable to form rigid structures once they are implanted into the tissue site.

D. Whole tooth regeneration:

Tooth like tissues have been generated by the seeding of different cell types on biodegradable scaffolds. A common methodology is to harvest cells, expand and differentiate cells in vitro, seed cells onto scaffolds and implant them in vivo, in some cases the scaffolds are re-implanted into an extracted tooth socket or the jaw.[45]

The induction of odontogenic potential lies in the dentalepithelium. Dental epithelium from pre-bud stages can induce tooth formation when combined with non odontogenic mesenchyme as long as the mesenchymal cells have stem cell-like properties in common with neural crest cells. After epithelial induction of the mesenchyme, this becomes the inductive tissue and reciprocates inductive signals back to the now non inductive epithelium. Tooth regeneration can thus be approached in one of two ways; identification of either epithelial or mesenchymal cells than can induce tooth formation in the other cell type.[46]

Steps in entire process of tooth regeneration via stem cells

- Step 1: stem cell isolation and identification
- Step 2: culturing stem cells along with scaffold materials either in vitro or ex vivo
- Step 3: delivery of growth factors and transplantation into anatomical site.

Currently, the major challenges in whole tooth regeneration are to identify non-embryonic sources of cells with the same properties as tooth germ cells and to develop culture systems that can expand cells that retain tooth forming potential. This is even more challenging when considering the fact that tooth development requires two cell types, epithelial and mesenchymal.[45]

Future dental applications of bioengineering with stem cells

The future of bioengineering, in terms of pulp regeneration in immature teeth with affected tissue, focuses on the use of stem cells for root regeneration and the regulation of stem-cell growth by using appropriate growth factors. An essential part of this process is the development of scaffolds and support structures for hosting the cells and growth factors.[43]

Scaffolds are used in regenerative procedures to provide a framework for growing the cells and vasculature. Scaffolds can be infused with various factors that promote cell growth and cell differentiation. They can be constructed from synthetic materials, such as polyglycol, or from biological materials, such as a cellular, unmineralised tissue collagen. The matrix should not only promote the proliferation and differentiation of stem cells, but should also guarantee a good neurovascular supply to the new pulp tissue, which in turn must be organised

Orthodontic Tooth Movement

On an increased basis, malocclusion is considered an expression of normal biologic variation, and treatment need is often based as much on psychosocial concerns as on proven oral health risks attributable to malocclusion. It can be either due to dental discrepancies or skeletal discrepancies. Malocclusion will cause esthetic impairment, potential adverse effect on dental health and associated masticatory components, deviation from normal occlusion etc.

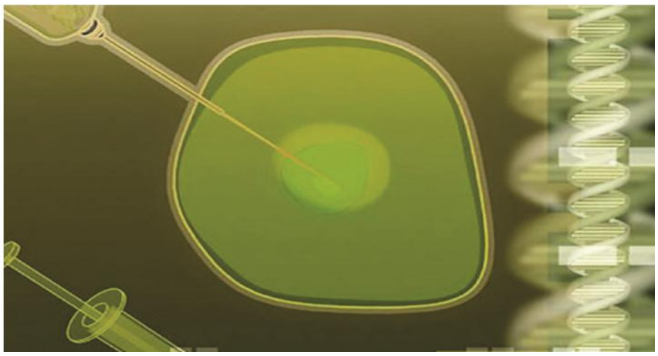
There are various treatment modalities to correct malocclusion, includes fixed orthodontic therapy, myofunctional therapy, using orthopeadic applinces, surgical orthodontic correction etc, based on the severity of malocclusion. Among this fixed orthodontic therapy is used for the correction of dental malocclusion.

The basic fundamental of fixed orthodontic therapy is orthodontic tooth movement. Orthodontic tooth movement (OTM) occurs during the bone remodeling procces that is induced by therapeutic mechanical stress. There occurs site-specific bone remodeling; bone resorption occurs on the compressed side, whereas bone formation occurs on the tensile side. Osteoclasts will form on the compressed side of an orthodontically moving tooth and will resorb the alveolar bone.

One of the major pitfall in fixed orthodontic therapy is lengthy time period for the treatment. Alveolar corticotomy surgery is an adjunctive therapy for reducing the orthodontic treatment period by almost a half. However, the short accelerated movement period and the morbidity rates from this kind of surgery make the dentist thought about alternative treatment modalities.

Numerous studies have reported the pharmacological acceleration of tooth movement through the activation of osteoclasts. Collins and Sinclair observed rapid tooth movement by vitamin D3 activated osteoclasts[47]. Orthodontic tooth movement can also induced by the local administration of prostaglandins, osteocalcin, or parathormone hormone. However, the daily systemic administration or daily local injection of these drugs are needed because these are rapidly flushed by blood circulation.[48]To overcome these disadvantages of pharmacological acceleration of tooth movement, osteoclastogenesis assisted by gene therapy were introduced in rapid orthodontic tooth movement.

The gene therapy is programmed by the help of either viral or non viral vectors. So the easiest way to implement local gene therapy is by injecting the vector into a specific tissue. The vector may be delivered systemically to all cells in the body or locally to the target tissue, only. For the orthodontic tooth movement we need to deliver these vectors in to the respective periodontal tissues locally (picture 19).[48]



Picture 19: Local gene delivery

Local gene transfer has two advantages;

1) It maintains local effective concentration and prolonged protein expression, regardless of blood circulation.
2) Protein expression occurs at a local site, thereby avoiding systemic effects.

Mechanism of bone resorption in orthodontic tooth movement in genetic level

Bone resorption is the result of osteoclast cells. Osteoclastogenesis is mainly regulated by two cytokines, receptor activator of nuclear factor kappa B ligand (RANKL) and macrophage colony-stimulating factor (M-CSF).Receptor activator for nuclear factor κB ligand (RANKL) is a trans membrane protein and is a member of the tumor necrosis factor super family that is expressed on preosteoblasts, osteoblasts and osteocytes. RANK is the receptor for RANKL and the binding between both of them stimulates the differentiation of preosteoclasts into mature osteoclasts. Osteoprotegerin (OPG) is a soluble extracellular tumor necrosis receptor protein that is secreted by preosteoblasts and osteoblasts. OPG is a decoy receptor for RANKL in regulating bone metabolism and inhibiting osteoclastogenesis and bone resorption. RANKL/OPG ratio is an important determinant of bone mass and skeletal integrity and also an indicator for the osteoclast function. This strong evidence is supported by several studies in various pathological conditions such as osteoporosis, periodontal disease and osteosarcoma. Oshiro et al. reported that RANKL induction was observed in the periodontal tissue of orthodontically moving tooth, and the RANK–RANKL regulation system was confirmed even in the site-specific bone remodeling that occurred during the orthodontic TM.[49]

Studies on genetic manipulation of tooth movement

The gene therapy experiments in orthodontic treatment are still limited to cell cultures or animal experiments. The

first attempt for gene therapy in orthodontic treatment done by Kanzaki H et al (2004)[50] aimed to transfer OPG gene into periodontal tissue to reduce osteoclast activity and inhibit tooth movement. The gene transfer approach using a hem agglutinating virus of Japan (HVJ) envelope vector carrying mouse OPG messenger RNA (mRNA) was performed in rats for 21 days of tooth movement. The vector solution was administered into rat's palatal gingiva by infiltration injection. The result showed that local OPG gene transfer reduced the number of osteoclasts and decreased tooth movement by 50% in rats in the experimental group compared to the ones in the control group. The effect of OPG gene transfer was local and did not affect bone mineral density of tibia of the animals.

The same group of investigators in 2006 performed another experiment using the same system to transfer mouse RANKL mRNA to periodontal tissue to activate osteoclastogenesis and accelerate tooth movement in rats. The results showed that local RANKL gene transfer induced increased numbers of osteoclasts and accelerated tooth movement by approximately 150% in the rats in the experimental group compared to the control group. The effect of RANKL gene transfer was local and did not elicit any systemic effects. Interestingly, the number of osteoclasts was reduced time dependently after gene transfer.

Iglesias-Linares et al in 2011[51]compared corticotomy with gene therapy using a hemagglutinating virus of Japan envelope vector containing mouse RANKL mRNA in rats for 32 days. The results showed increased level of RANKL protein 3 folds in the gene therapy group and 2 folds in the corticotomy group after 10 days; however, the level of RANKL protein was maintained in the gene therapy group but not in the corticotomy group. The number of osteoclasts in the RANKL gene therapy group was significantly higher at day 10 with or without tooth movement compared to the tooth movement only group. The tooth movement distance was 2 times more in the RANKL gene therapy group and 1.5 times in the corticotomy group; however, the rate of tooth movement slowed down in the corticotomy and controls groups but was constant in the RANKL gene therapy group. It was concluded that gene therapy was an alternative treatment for corticotomy to accelerate tooth movement and the efficacy of treatment was higher than corticotomy to accelerate tooth movement.

The OPG gene transfer experiment was performed by Zhao N et al in 2012[52]using the same viral envelope packaging and delivery system to investigate the inhibition of orthodontic relapse in rats. The first molars in the rats were moved mesially for 3 weeks then the springs were removed to generate orthodontic relapse in the rats. The rats received OPG gene therapy then were observed for 2 weeks. The results showed that relapse was significantly inhibited 2 times compared to the mock and control groups. The bone mineral density and bone volume fraction of alveolar bone were significantly increased in the gene therapy group compared to the mock and control groups. No difference of bone mineral density and bone volume fraction of tibia was found among groups. The investigators stated that local OPG gene therapy to periodontal tissues could inhibit relapse after orthodontic tooth movement via osteoclastogenesis inhibition.

The same group of investigators (2012) further investigated the effect of local OPG gene therapy on orthodontic root resorption with the same design of experiment. They utilized a microcomputed tomogram and histological analyses. The result showed no difference between root resorption at the beginning and the end of tooth movement in the OPG gene therapy group. However, the repair of root resorption in the gene therapy group was higher than other control groups.

Amuk NG et al in 2017[53] investigated the effect of local OPG gene therapy using mesenchymal stem cells as carriers for plasmid containing OPG mRNA. This cell mediated OPG gene transfer was generated by insertion of plasmid containing OPG mRNA into the mesenchymal stem cells and the cells were injected into the animals. The result showed that the cells containing OPG package grew in the animals' PDL and the number of osteoclasts, level of RANKL and bone resorption were reduced significantly after single injection. The level of OPG was highest in the gene therapy group.

Table 6: Summary of studies on genetic manipulation of tooth movement

Sl.No	*Year*	*Author*	*Experiment*
1	2004	Kanzaki H et al	Local OPG gene transfer to periodontal tissue inhibits orthodontic tooth movement
2	2006	Kanzaki H et al	Local RANKL gene transfer to the periodontal tissue accelerates orthodontic tooth movement
3	2011	Iglesias-Linares et al	The use of gene therapy vs. corticotomy surgery in accelerating orthodontic tooth movement.
4	2012	Zhao N et al	Effects of local osteoprotegerin gene transfection on orthodontic root resorption during retention: An in vivo micro-CT analysis.
5	2012	Zhao N et al	Local osteoprotegerin gene transfer inhibits relapse of orthodontic tooth movement
6	2017	Amuk NG et al	Effects of cell mediated osteoprotegerin gene transfer and mesenchymal stem cell applications on orthodontically induced root resorption of rat teeth.

Advantages

- Transfer of RANKL gene to periodontal tissue activated osteoclastogenesis and accelerated OTM without producing any systemic effects.
- RANKL gene transfer demonstrated higher efficacy and might substantially reduce orthodontic treatment time than standard surgical methods.
- Local OPG gene transfer has also been used to inhibit orthodontic tooth movement, which might be, in the

near future, an important tool to enforce the anchorage unit or increase stability of orthodontic results.

Local OPG gene transfer significantly inhibited RANKL-mediated osteoclastogenesis in the periodontium caused by experimental tooth movement. Moreover, local OPG gene transfer might be a biologic method employed to prevent or inhibit relapse after orthodontic treatment.

This approach is still in the developing process as an alternative approach to treat bone deformity or disease that conventional method could not achieved. Although many clinical trials have shown the efficacy of the treatment, the technique remains risky and is still under processes of investigation to make sure that it will be safe and do not elicit any systemic or hereditary effects for the patients.

In orthodontic field, the gene therapy approach will need several fundamental cell culture and animal experiments to demonstrate the safety and efficacy of the treatment concept.

2. Conclusion

Genetic engineering is the direct manipulation of an organism's genes using biotechnology; it is used to change the genetic makeup of cells, including the transfer of genes within and across species boundaries to produce improved or novel organisms. **Isaac Asimov** quoted that "The advance of genetic engineering makes it quite conceivable that we will begin to design our own evolutionary progress"**.** Based on this quote an attempt to describe the role of genetic engineering in the field of pediatric dentistry has been done through this library dissertation.

Virtually all cells in the human body contain genes, making them potential targets for gene therapy. However, these cells are divided into two major categories: somatic cells (most cells of the body) or germline cells (eggs or sperm). Based on this gene therapy is broadly classified into somatic gene therapy, is not passed on to future generations and germ line gene therapy, which results in permanent changes that are passed down to subsequent generations. Somatic gene therapy can be broadly split into two categories: ex vivo, where cells are modified outside the body and in vivo, where genes are changed in cells still in the body.

The concept genetic engineering were started years back and the he term "genetic engineering" was first coined by Jack Williamson in his science fiction novel Dragon's Island, published in 1951 – one year before DNA's role in heredity was confirmed by Alfred Hershey and Martha Chase. From there till the date so many clinical trials are going on in this field.

Vehicles of gene transfer called vectors, which facilitate the transfer of genetic information. It can be parted into viral and nonviral delivery systems. The most commonly used viral vectors are derived from retrovirus, adenovirus, and adeno associated virus (AAV). Nonviral vectors can be either plasmid deoxyribonucleic acid (DNA), which is a circle of double-stranded DNA that replicates in bacteria or chemicaly synthesized compounds that are or resemble oligodeoxynucleotides.

Applications of gene therapy to dental and oral problems illustrate the potential impact of this technology on dentistry. In the past six years, remarkable progress has been made in the field of gene therapy, including several areas relevant to dental practice: caries prevention and management, bone repair, salivary glands, autoimmune disease, pain, keratinocytes, cancer etc. There is a potential role of genetic engineering in pediatric dentistry includes caries vaccine, genetically modified food, replacement therapy, stem cells in tooth regeneration and rapid orthodontic tooth movement.

Under the prevention of dental caries, caries vaccine, genetically modified food and replacement therapy were included. Dental caries vaccines and genetically modified foods directed to key components of S. mutans colonization and enhanced by safe and effective adjuvants and optimal delivery vehicles, are likely to be forthcoming. However replacement therapy for the prevention of dental caries used none or less virulent strains of tooth-cloning bacteria pre-empting the tooth surface and replacing the more cariogenic counterparts like streptococcus mutans. If ultimately successful, the use of genetic engineering to tailor an effector strain for replacement therapy for dental caries will encourage similar efforts to prevent other infectious diseases as well.

The regenerative medicine aims to repair or re-grow parts or tissues which are lost due to disease or injury. One of the building blocks of this therapy is stem cells. So the management of dental caries is possible by tooth tissue regeneration through coupling of stem cell therapy and genetic engineering, and it found to be a very promising approach for the dental caries management.

Bone resorption is the result of osteoclast cells. Osteoclastogenesis is mainly regulated by two cytokines, receptor activator of nuclear factor kappa B ligand (RANKL) and macrophage colony-stimulating factor (M-CSF).The rapid orthodontic tooth movement by the local injection of genetically modified RANKL-mediated osteoclastogenesis and local OPG gene transfer to prevent or inhibit relapse after orthodontic treatment were also discussed in detail.

Thus it can be concluded that genetic engineering can create drastic changes in the prevention and treatment modalities of different pediatric as well as other dental problems. But the limitation because of the myths exists in our world the practical implementation is delaying since years. So we can hope for a promising future in the field of paediatric dentistry using genetic engineering by giving faith in james lovelock's words "I suspect any worries about genetic engineering may be unnecessary. Genetic mutation have always happened naturally anyway"

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