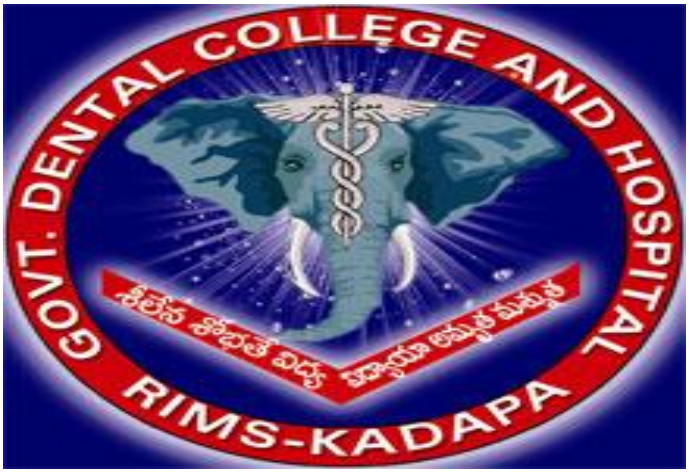
GOVERNMENT DENTAL COLLEGE & HOSPITAL, KADAPA.

DEPARTMENT OF PERIODONTICS



SEMINAR PRESENTATION ON "GENETICS IN PERIODONTICS"

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INTRODUCTION

- Periodontitis is a chronic inflammatory disease of supporting tissues of the teeth.
- In an advanced destructive periodontitis there is an imbalance between host immune system and microflora.
- Oral cavity – most complex ecosystem of the human body with a myriad of micro-organisms.
- The normal flora protect the host from pathogenic organisms and the host immune system controls the growth of the micro-organisms.
- The complex interplay between environmental factors like pathogens in oral cavity, the immune system & consequences of lifestyle factors is largely regulated by genes.
- Genes encode immune receptors as well as molecules, which influence receptor specificity & sensitivity to bacterial species.
- They regulate & influence the intensity of the inflammatory response by encoding & adapting the signal transduction pathways up & down – stream of the inflammatory signals & allow a flexible response of the organism to external & internal stimuli.

- Genetic research improves the understanding of the factors that mediate the immune response & explain why this response often greatly differs between individuals who have the same environmental context & comparable lifestyle habits.
- An objective of genetic research is to identify the genes underlying disease & to estimate the genetic effects of potential risk variants within these loci.
- Genetic variation most often affects the regulatory regions of the genes, which lead to susceptible changes in their expression in the quantity of the transcribed gene products, but also the tissue & development specific gene expression.
- The genetic basis of periodontitis was demonstrated by formal genetic studies & many genetic variants were analysed for their involvement in the disease physiology.

TERMINOLOGIES

- **GENOME**: The entire hereditary information of an organism. This term refers to all of the genes and other non-gene portions of DNA carried by an individual cell.
- **GENE**: The segment of the genome that carries the instructions for a specific protein synthesis.
- **GENOTYPE**: The genetic makeup of an organism or cell.
- **ALLELE**: Alternative forms of a gene differing in DNA sequence.
- **LOCUS**: The physical location of a gene occupying within a chromosome or portion of genomic DNA.

GENETICS – AN INSIGHT

- **GENETICS** – study of inheritance or heredity of living organisms. It is a wide ranging science that explores the transmission of biological properties from parents to the offspring.
- Pioneer – ***Gregor Johann Mendel***.
- Human genome consists of 23 pairs of chromosomes- 22 pairs are autosomes and 1 pair is allosomes or sex chromosomes
- Human genome – 30,000-40,000 genes.
- Humans share 99.9% of their genetic information, remaining 0.1% differs from one person to the others.
- Genes are composed of nucleotides & are organised in the chromosomes within the cell nucleus.
- The sequence of nucleotides determines the expression of the gene.

GENETIC DISEASE MODELS

The search for genes that are important in gene susceptibility are identified and broadly divided into the followed:

Major Disease Gene, which is responsible for disease expression, according to mendel's law when a given aberrant allelic form is present.

- To date, only one major disease gene has been revealed in relation to periodontitis; gene on chromosome 11 responsible for a severe form of prepubertal periodontitis.

Modifying Disease Gene - is identified to be involved in complex multifactorial diseases.

- Most forms of periodontitis are likely to be associated with multiple modifying genes.
- It is estimated that for periodontitis, between 10 to 20 genes may be involved.

Gene Diseases

- Geneticists have traditionally divided genetic diseases into two broad groups:
    - Simple mendelian diseases.
    - Complex diseases.
- The distinction of this groups are based on the pattern of transmission of the disease, which reflects the manner in which genes contribute to each disease.

SIMPLE MENDELIAN DISEASES

- Diseases that follow predictable & generally simple patterns of transmission – Mendelian diseases.
- These diseases occur in simple patterns in families & in most cases genetic alterations at a single gene locus are the major determinants of the clinical disease phenotype.
- The disease phenotype usually manifests over a broad range of environmental factors & other genes can modify the clinical presentation, in most cases the mutation will manifest in a remarkably similar phenotype.
- Prevalence – rare (0.1%).
- Examples – Amelogenesis imperfecta,

    Crouzon syndrome,

    Cliedocranio dysplasia,

    Papillon-Lefevre syndrome.

- When the gene responsible for a mendelian disease has been identified, it is often possible to develop a diagnostic test to identify individuals who carry a disease causing mutation in the responsible gene.
- Depending on the mode of transmission, it is also possible to make specific determinations of the probability of the mutant gene being passed to a child & often it is possible to predict the course of clinical disease.

COMPLEX GENETIC DISEASES

- The complex traits are the result of interaction of alleles at multiple different gene loci.
- Environmental factors are usually etiologically important, & often necessary in the development of complex diseases.
- On a population level – complex diseases are much more common than simple mendelian disease (prevalence – 1%).
- The individual genetic variants that are important in complex diseases are much less disruptive, & usually function within normal range.
- The presence of one disease associated allele is not sufficient to cause disease.
- There is no one-to-one correlation of presence of a specific genetic allele & occurrence of disease.
- The alleles reported to be associated with a disease are also found in unaffected individuals & some individuals with disease who do not have the allele.
- Thus, presence of disease associated allele is not diagnostic for a disease.

METHODS OF GENETIC ANALYSIS:

- Clinical & scientific data from a variety of sources suggest that genetic variants are major determinants of syndromic & non-syndromic periodontitis.
- To evaluate the quality of supporting studies requires an understanding of the formal genetic analytical methods that have been used.
- Geneticists use a variety of techniques to demonstrate the genetic basis of diseases, some are general methods where as others permit precise identification of genetic variants that cause or contribute to disease.
- Familial aggregation, twin studies, segregation analysis, linkage analysis & association studies are some important methods in evaluation of genetic diseases including periodontitis.

FAMILIAL AGGREGATION

- Familial aggregation of a trait or disease can suggest genetic etiology.
- However, families also share many aspects of a common environment, including diet & nutrition, exposes to pollutants & behaviours such as smoking.
- Certain infectious agents may cluster in families. Thus, familial aggregation may result from shared genes, shared environmental exposures and similar socioeconomic influences.
- To determine the evidence for genetic factors in familial aggregation of a trait, more formal genetic studies are required.
- There have been many clinical reports suggesting a familial aggregation of periodontitis, but until recently the research tools to pursue these reports were lacking.

TWIN STUDIES

- Through the phenomenon of twins, in particular monozygous twins, nature has provided a wonderful tool for the examination of genetic influences in disease and for partitioning the relative contribution of genes and environment to a trait.
- Monozygous twins are genetically identical. Dizygous twins are only as genetically similar as brothers and sisters would be, on average they share 50% of their genes in common.
- Discordance or differences in the presence of disease between monozygous twins must be due to environmental factors.
- Disease discordance between dizygous twins could arise from both environmental and genetic differences.
- The difference in concordance between monozygous and dizygous twins for a particular phenotype can be used to estimate the relative contribution of genes (heredity) and environmental factors to a disease and studying disease presentation in twins is often a valuable first step in this process.

SEGREGATION ANALYSIS

- Genes are passed from parents to children in a predictable manner, and usually segregate in families as predicted by Mendel's laws.
- Geneticists can study the pattern of disease transmission in families using a method called segregation analysis.
- Segregation analysis evaluates the relative support for different transmission models to determine which model can account for the observed segregation of a trait through families.

- By sequentially comparing models to each other, segregation analysis identifies the model that best accounts for the observed transmission of a trait in a given population.
- Geneticists generally apply segregation analyses to determine whether a trait transmission appears to fit a Mendelian or another mode of genetic transmission.
- When comparing genetic models of transmission, genetic characteristics including mode of transmission (e.g. autosomal, X-linked, dominant, recessive, complex, multilocus, or random environmental), penetrance, phenocopy rates and frequencies for disease and non-disease alleles are some of the characteristics included in the different models evaluated.
- But segregation analysis does not necessarily provide the true model.
- Since it is a comparison of two models, segregation analyses are only as good as the models tested.
- If important assumptions of the model tested are incorrect, this will limit the results.
- This limitation of segregation analysis must be realized, as it has resulted in inaccurate conclusions for the transmission of at least one form of early onset periodontitis.
- It is most appropriately applied to data sets of many families to determine the best fitting model.
- Segregation analysis does not find or aim to find a specific gene responsible for a trait.

LINKAGE ANALYSIS

- Linkage analysis is a technique used to localize the gene for a trait to a specific chromosomal location.
- Genetic linkage studies are based on the fact that alleles at syntenic gene loci in close proximity on the same chromosome tend to be passed together from generation to generation, as a unit.
- Such genes are said to be ‘linked’ and violate Mendel’s law of independent assortment.
- Geneticists can apply quantitative analyses to detect this lack of independent assortment of genetic loci, and can use it to map (localize) genes to specific chromosome locations.
- Over the past 15 years, genetic maps have been developed that show the position of millions of polymorphic genetic loci spanning the human genome.
- Scientists can follow a specific trait as it segregates through families of interest and determine whether the trait appears to segregate with a known genetic polymorphism that has been localized to a specific chromosomal location.
- Linkage can therefore prove the genetic basis of disease.

- Linkage is often used as a first step to determine the approximate location of a gene of interest, permitting subsequent studies to identify the mutation responsible for a disease trait.
- Linkage studies have been particularly effective in identifying the genetic basis of simple Mendelian traits (OMIM 2004), where mutation of a single gene can cause a disease.
- Linkage studies of complex genetic traits have not been as successful for a variety of reasons.
- A limiting factor in the traditional application of linkage to complex diseases that occur due to the combined effect of 'multiple genes of minor effect'.
- When multiple genes each contribute a small amount to the disease phenotype, traditional parametric linkage studies are much less powerful.
- The newer adaptations of the linkage approach and the availability of Association testing approaches offer a practical alternative.

ASSOCIATION STUDIES

- Genes contributing to common, complex diseases such as periodontitis have proven more difficult to isolate.
- When multiple, perhaps many, genes act with environmental factors to contribute to disease liability, it is difficult to formulate disease models.
- In the absence of specific genetic models, the etiology of complex diseases is often conceptualized as due to multiple factors, i.e. several genetic loci interacting with each other to produce an underlying susceptibility, which in turn interacts with additional environmental factors to produce an actual disease state.
- For complex traits, such as bipolar disorder, diabetes, obesity and oral–facial clefting, traditional parametric linkage analysis has produced either negative results or a plethora of weak, positive results not easily replicated.
- Theoretical research suggests several reasons for the ambiguity of the linkage results in these cases.
- First, if a disease gene is neither necessary nor sufficient to cause a disease, but rather is a 'modifier gene' that elevates a nonzero baseline risk, conventional parametric linkage analysis may not detect the gene.
- Second, if the relative contribution of a gene to a disease phenotype is small, linkage analysis using affected sibling pairs will not be powerful enough to detect the gene, given realistic sample sizes.

- Consequently, attention has shifted away from model-dependent parametric linkage analysis to model-free, nonparametric 'association' analysis as an alternative means of locating disease susceptibility genes, especially since association studies can sometimes detect weaker effects than can linkage analysis.
- Two types of association analysis are commonly employed in genetic studies:
    - population-based and
    - family-based.
- The population-based approach utilizes a standard case-control design, in which marker allele frequencies are compared between cases (affected individuals) and controls (either unaffected individuals or individuals randomly chosen from the population).
- When a positive association is found, several interpretations are possible:
    - the associated allele itself is the disease-predisposing allele;
    - the associated allele is in linkage disequilibrium with the actual disease-predisposing locus;
    - the association is due to population stratification;
    - the association is a sampling, or statistical, artifact.
- The first two interpretations represent the alternative hypotheses of interest in a gene mapping context. In the first case, the marker itself is the disease-susceptibility locus.
- This outcome is the rationale behind candidate gene studies, in which alleles of the genes being tested have some *a priori* expectation of being directly involved in the disease process.
- Evidence of a positive association can be followed up by investigations to establish a functional role.
- In the second case, the associated allelic polymorphism itself does not play a functional role in causing disease, but rather the polymorphism is in close physical proximity to the gene that does contribute to susceptibility.
- A classic example is the human leukocyte antigen (HLA) system, in which various HLA haplotypes are associated with a number of diseases, including insulin dependent diabetes mellitus, rheumatoid arthritis, and ankylosing spondylitis.

GENETICS IN ETIOPATHOGENESIS OF PERIODONTAL DISEASES:

- Research on the role of genes & pattern of inheritance in periodontal disease says that, there are chromosome regions that potentially harbour susceptibility genes for periodontal diseases.

- Among chronic & aggressive periodontitis, aggressive periodontitis showed genetic predisposition in the affected person.
- Various investigators conducted a familial study based on the hypothesis that it is inherited.
- Saxen concluded that the aggressive periodontitis is inherited in an autosomal recessive mode.
- Shapira et al, showed family pedigree is consistent with an autosomal dominant mode of transmission in aggressive periodontitis.
- Michalowicz et al analyzed periodontal finding which included probing depth, clinical attachment level & plaque score in 110 adult twins who were both reared together & reared apart.

EVIDENCE FOR THE ROLE OF GENETIC VARIANTS IN PERIODONTITIS

- The risk for many diseases, including periodontal diseases, is not borne equally by all individuals in a population.
- A variety of microbial, environmental, behavioral, and systemic disease factors are reported to influence the risk for periodontitis.
- An individual's genetic makeup is a crucial factor influencing their systemic or host response-related risk.
- There are reports in the literature on familial aggregation of periodontal diseases, but it is difficult to compare them.
- Most familial reports of aggressive periodontitis are noted.
- Reports of the familial nature of chronic forms of periodontitis are less frequent but the aggregation within families is consistent with a genetic predisposition.
- We should remember, however, that familial aggregation of periodontal disease may also reflect exposure to common environmental factors.
- Shared environmental factors include education, socioeconomic grouping, oral hygiene, shared transmission of bacteria, diseases such as diabetes and environmental features such as passive smoking, sanitation, etc.
- Complex interactions between genes and environment are difficult to quantify, but are likely to be important when considering the familial risk for the periodontal diseases.
- The effects of the environment, for example plaque accumulation and smoking, also have major long-term influences on disease experience and these confound the diagnosis of aggressive periodontitis.

- While there is evidence for a gene of major effect in aggressive periodontitis, it appears to be etiologically complex and heterogeneous.
- Although bacterial transmission between subjects has been suggested to explain aggressive periodontitis clustering within families, this observation alone is insufficient to account for familial clustering.
- While the heterogeneity paradigm discussed by Potter is borne out in subsequent familial studies of aggressive periodontitis, the striking familial aggregation of the trait is consistent with a significant genetic etiology.
- Characterization of the specific genetic components in the etiology of this disease requires more formal genetic analyses.

TWIN STUDIES

- Twin studies have been invaluable in studying the genetic basis of simple and complex traits.
- Large, worldwide registers of data on twins and their relatives have been established.
- Such twin studies registers offer unique opportunities for selected sampling of quantitative trait loci linkage and association studies.
- Twin studies of periodontitis, however, have generally been limited in scope and in subject numbers.
- Most twin studies have studied the more prevalent form of periodontitis, which is chronic periodontitis, as well as chronic gingivitis.
- Corey et al. studied self-reported periodontal health in 4908 twin pairs and found that 9% of subjects, consisting of 116 identical and 233 nonidentical twin pairs, reported a history of periodontitis.
- The concordance rate, or level of similarity in disease experience, ranged from 0.23 to 0.38 for monozygous twins, and was much lower (0.08–0.16) for dizygous twins.
- These findings suggest that heritable factors are important in the reported periodontitis experience.
- Environmental factors such as smoking status were not factored into this analysis and could introduce a bias towards finding a correlation between twins.
- Michalowicz et al. studied dizygous twins reared apart (dizygous-A) and reared together (dizygous-T) and monozygous twins reared apart (monozygous-A) and reared together (monozygous-T).

- The mean probing depth and clinical attachment level scores were found to vary less for monozygousT than for dizygous-T twin pairs, further supporting the role of genetics in this disease.
- Michalowicz et al. investigated alveolar bone height and showed significant variations related to genotype.
- The twin groups had similar smoking histories and oral hygiene practices.
- It was concluded that genetics plays a role in susceptibility to periodontal disease.
- In a subsequent study of 117 adult twin pairs, Michalowicz and coworkers estimated genetic and environmental variances and heritability for gingivitis and chronic periodontitis using models with maximum likelihood estimation techniques.
- Monozygous twins were found to be more similar than dizygous twins for all clinical measures.
- Chronic periodontitis was estimated to have approximately 50% heritability, and was unaltered after adjusting for behavioral variables, including smoking.
- Monozygous twins were also more similar than dizygous twins for gingivitis scores but there was no evidence of heritability for gingivitis after behavioral covariates such as utilization of dental care and smoking were incorporated.
- These results confirm previous studies and indicate that approximately half of the variance for chronic periodontitis is attributable to genetic variance.
- The basis for the heritability of periodontitis appears to be biological and not behavioral.

SEGREGATION ANALYSIS

- Genetic segregation analysis needs accurate clinical identification of affected individuals and familial relationships as well as genetic assumptions of the analysis.
- If inaccurate assumptions or data are used, the outcomes will reflect this.
- Early studies of aggressive forms of periodontitis were hampered by diagnostic classification issues and by an overrepresentation of affected females, falsely supporting X-linked transmission.
- A segregation analysis of North American families was performed by Marazita and coworkers, who studied more than 100 families segregating aggressive forms of periodontitis; their results supported an autosomal dominant transmission.
- They concluded that autosomal dominant inheritance with approximately 70% penetrance occurred for both Blacks and nonBlacks.

- The currently held theory on the genetics of aggressive periodontitis is probably due to a major gene locus transmitted in an autosomal manner with reduced penetrance; there is evidence for both autosomal recessive and autosomal dominant forms.
- It is likely that these aggressive forms of periodontitis are genetically heterogeneous, meaning that while the mutated gene responsible for the condition is likely to be the same in any given family, there are probable several different genetic loci that, if mutated, can cause aggressive periodontitis.
- The expression 'reduced penetrance' means that some subjects with the genotype may not actually express the phenotype, i.e. the clinical manifestations of aggressive periodontitis, whereas others may express it fully.
- Environmental factors (such as smoking and plaque control) as well as epigenetic interactions of multiple susceptibility genes may play a large role in whether the phenotype is expressed clinically.

LINKAGE STUDIES IN AGGRESSIVE PERIODONTITIS

- Three linkage studies have been performed to date on families with localized aggressive periodontitis.
- Boughman et al. identified an autosomal dominant form of localized aggressive periodontitis in an extended family from Southern Maryland.
- In this family, type III dentinogenesis imperfecta and a localized form of aggressive periodontitis were segregating as dominant traits.
- Since the gene for dentinogenesis imperfecta-III had been previously localized to chromosome 4, they performed a linkage analysis on this chromosome and demonstrated a relatively close linkage with the suspected locus for aggressive periodontitis.
- This was an important study because it supported autosomal dominant inheritance of a single major gene locus, clearly indicating a major genetic component to the aggressive periodontitis disease etiology.
- Hart et al. evaluated support for linkage to this region of chromosome 4 in a different population of families (14 African American and 4 Caucasian).
- Their findings supported genetic locus heterogeneity of aggressive periodontitis, as they excluded a chromosome 4 major gene locus for aggressive periodontitis in the families they studied.
- Thus, this Brandywine population appears to have a different form of periodontal disease with a different gene being responsible compared with the African-American and Caucasian families studied by Hart and coworkers.

- These findings support genetic heterogeneity, with at least one gene locus responsible for aggressive periodontitis located on chromosome 4.
- Li and coworkers reported evidence of a gene responsible for localized aggressive periodontitis located on chromosome 1q25.
- A gene of major effect for aggressive periodontitis has not been identified.

GENOME WIDE ASSOCIATION STUDY

- GWAS approach is that because the entire human genome is searched, we no longer have to depend on prior hypotheses about the disease's molecular pathology.
- In most GWAS studies, about half of the statistically definitive findings point to genes that experts in the field had no suspicion whatsoever were involved in the disease's etiology.
- This allows researchers to open up entirely new pathways for investigation that may lead to insights about the disease's biologic mechanism and suggest novel molecular strategies for pharmaceutical or other therapeutic interventions.
- In more than a few cases, robust GWAS findings implicate regions of the human genome in which no genes appear to be present, thereby highlighting the limitations of our current knowledge of basic genome functions.
- Although great progress has been made toward understanding the etiology of many complex human diseases by using GWAS methods, the approach has nevertheless usually failed to account for most of the heritability known to exist for these conditions.

SYNDROMIC FORMS OF PERIODONTITIS

- Severe periodontitis presents as a part of the clinical monogenetic syndromes as well as gene mutation & biochemical defect is known for many of these conditions.
- A commonality of these conditions is that they are inherited as simple Mendelian traits due to genetic alterations of a single gene locus.

Table 1. Examples of syndromic forms of periodontitis in which inheritance is Mendelian and due to a genetic alteration at a single gene locus

Condition	Biochemical/tissue defect	Inheritance	OMIM
Papillon–Lefèvre syndrome	Cathepsin C	Autosomal recessive	245000
Haim–Munk syndrome	Cathepsin C	Autosomal recessive	245100
Ehlers–Danlos syndrome type IV	Collagen	Autosomal dominant	130050
Ehlers–Danlos syndrome 8	Collagen	Autosomal dominant	130080
Cyclic neutropenia	Neutrophil elastase	Autosomal dominant	162800
Chronic familial neutropenia	Defect unknown	Autosomal dominant	162700
Chediak–Higashi syndrome	Lysosomal trafficking regulator gene	Autosomal recessive	214500
Congenital disorder of glycosylation type IIc (Leukocyte adhesion deficiency type 2)	Glucose diphosphate-fucose transporter-1	Autosomal recessive	266265
Leukocyte adhesion deficiency type I	Leukocyte chain adhesion molecule CD18	Autosomal recessive	116920

- The significance of these conditions is that they clearly demonstrate that a genetic mutation at a single locus can impart susceptibility to periodontitis.
- Additionally, these conditions illustrate that this genetic susceptibility may segregate by different transmission patterns.

NEUTROPHIL FUNCTIONAL DISORDERS:

- The defect in the number of these receptors may lead to increased susceptibility to infectious disease.
- Page et al. proposed that the generalized form of prepubertal periodontitis is a localized oral manifestation of the leukocyte adhesion deficiency syndromes.
- Leukocyte adhesion deficiency occurs in two forms, leukocyte adhesion deficiency syndrome type 1 and type2, both of which are autosomal recessive traits.
- Circulating leukocytes have reduced or defective surface receptors and do not adhere to vascular endothelial cells; thus they do not accumulate in sites of inflammation where they are needed.

- Other disorders of neutrophil function are associated with severe forms of periodontal destruction.
- The Chediak–Higashi Syndrome is a rare disease transmitted as an autosomal recessive trait.
- Those affected are very susceptible to bacterial infections due to alterations in the functional capacity of the polymorphonuclear leukocyte.
- Humans and other animals with Chediak–Higashi syndrome exhibit generalized, severe gingivitis and extensive loss of alveolar bone and premature loss of teeth.
- The polymorphonuclear leukocyte chemotactic and bactericidal functions are thought to be abnormal in these patients.

DEFICIENCY IN NEUTROPHIL NUMBERS (NEUTROPENIAS)

- Neutrophil deficiency is found in infantile genetic agranulocytosis, a rare autosomal recessive disease where polymorphonuclear leukocyte numbers are very low and which has been associated with aggressive periodontitis.
- Cohen's syndrome is another autosomal recessive syndrome and is characterized by mental retardation, obesity, dysmorphia, and neutropenia.
- Individuals with Cohen's syndrome show more frequent and extensive alveolar bone loss than do age-, sex-, and mental ability-matched controls.
- Not all neutropenias result in periodontal disease. Familial benign chronic neutropenia has variable expressivity and although several individuals within a family may be neutropenic, not all are affected by recurrent infections or periodontal disease.
- These findings might be explained by the variable genetic expression of the disorder or by the variable effects of the environment (such as plaque or smoking) on these patients.

GENETIC DEFECTS OF STRUCTURAL COMPONENTS

- Papillon–Lefe`vre Syndrome is a condition in which the cardinal clinical features are severe periodontitis and great variation in the severity and extent of palmar plantar hyperkeratosis.
- Genetic linkage studies narrowed the Papillon–Lefe`vre gene locus to chromosome 11 and subsequent mutational analyses permitted identification of mutations in the cathepsin C gene in patients with Papillon–Lefe`vre syndrome.
- Subsequent studies have identified more than 40 different cathepsin C mutations in individuals from many different ethnic groups.
- This is an excellent example of the success of genetic studies in contributing to the identification of a gene defect of periodontal importance.

- Genetic linkage studies permitted localization of the gene defect to a specific chromosome, permitting focused mutational analyses on genes within an area of the chromosome.
- Mutations of this gene are associated with the loss of protease activity of the cathepsin C protein.
- Additional work has demonstrated that Papillon–Lefe`vre syndrome and Haim– Munk syndrome (a slightly different clinical variant within the Papillon–Lefe`vre syndrome group of disorders) are allelic variants of cathepsin C gene mutations, as predicted by Gorlin et al.
- Ehlers–Danlos types IV and VIII are related to an increased susceptibility to periodontitis and are inherited in an autosomal dominant manner.
- Clinical characteristics of type VIII Ehlers–Danlos syndrome include fragility of the oral mucosa and blood vessels, and a severe form of aggressive periodontitis.

GENE POLYMORPHISM

- The coexistence of multiple alleles at a locus is called "**Genetic polymorphism"** when they occur at more than one percent in a population.
- There may be multi (poly) forms (morphism) of a gene & the altered forms of a gene's structure are referred to as "genetic polymorphism".
- Researchers have estimated that every person has 6 billion nucleotides & 0.1% is polymorphic.

Types of Polymorphism

- **SNP**: Single Nucleotide polymorphisms
- **STR**: Simple Tandem repeats. (1-10) - Microsatellite
- **VNTR**: Variable number of Tandem Repeats. (>10)- Minisatellite

Gene Polymorphism In Periodontal Diseases

- 1. Cytokine gene polymorphisms
    - a. IL-1 gene polymorphism
    - b. TNF-α gene polymorphism
    - c. IL-10 gene polymorphism
- 2. Receptor and other gene polymorphisms
    - a. FCγR gene polymorphisms
        - • FcγRIIa-131 H/R polymorphism

- • FcγRIIIa-158 F/V polymorphism
- • FcγRIIIb polymorphism
- b. Cytokine and chemokine receptor gene polymorphisms
- c. Immune receptor gene polymorphism
- • FMLP receptor gene polymorphism

- 3. Metabolism – related gene polymorphism
  - a. Vitamin D receptor gene polymorphism
  - b. Calcitonin receptor gene polymorphism
- 4. Antigen – recognition related gene polymorphism
  - HLA gene polymorphism
- 5. Polymorphisms in the innate immunity receptors
  - a. TLR2 and TLR4 gene polymorphisms
  - b. CD 14 gene polymorphism
  - c. CARD 15 gene polymorphism
- 6. Miscellaneous gene polymorphisms

CYTOKINE GENE POLYMORPHISMS

IL-1 GENE POLYMORPHISM

- Polymorphisms in the IL-1 cluster have been the main focus of attention in recent studies because of the fundamental role of IL-1β in the pathogenesis of periodontal disease.
- Kornman et al., suggested that periodontitis associated genotype (PAG) had an approximately 7 times greater chance of having severe periodontitis than those who were PAG negative.
- PAG is a composite IL-1 genotype found by the combination of two rare alleles at separate single nucleotide polymorphisms (SNP) in this cluster at position -889 in the IL-1A promoter and at + 3954 (now referred to as +3953) of the IL-1B gene.
- Frequency of allele 2 of the IL-1B +3953 SNP was significantly increased in patients with advanced periodontitis.

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- Recently, a study tested polymorphisms derived from genes of the IL-1 cluster for association with generalized aggressive periodontitis (GAP) through both allelic association and by constructing a linkage disequilibrium map of the 2q13-14 disease candidate region.
- This study suggested that there is some evidence for an association between GAP and the IL-1β (+3953) polymorphisms.
- Diehl et al.1999 showed the association of IL-1 gene cluster and aggressive periodontitis, but in direction different from the previous ones. He confirmed that IL-1 gene cluster acts as putative susceptibility factor for periodontitis.
- Anne Havemose – Poulsen et al. demonstrate that in localized aggressive periodontitis patients, allele 2 of IL – 1 RN VNTR was associated with significantly higher levels of IL – 1 α, 6, 10 and TNF – α, whereas allele 2 of IL - 1β +3954 was associated with significantly lower levels of the same cytokine.
- Moreira PR Costa et al. evaluated the association of IL - 1A (-889) gene polymorphism in Brazilian individuals with different clinical forms and severity of periodontitis and demonstrated a significant association between the two.
- In 2008, Stefan Reichert et al. studied the expression of IL – 12 R β2 molecule in a crucial regulatory factor in the T – helper type differentiation of T cells. They found that single nucleotide polymorphism of the 5'flanking region of IL – 12RB2 leads to a very weak cellular immune response. They reported that the frequencies of variant alleles of IL – 12 RB2 were significantly higher in aggressive periodontitis patients as compared with healthy controls or chronic periodontitis patients.

IL-10 GENE POLYMORPHISMS

- IL-10 gene is located on chromosome 1, in a cluster with closely related IL-genes IL-19, 20, 24. IL-10 has an inhibitory effect on IL-1α, IL-1β, TNF-α, IL-6, IL-8 and IL-12. Functional disturbances in IL-10 due to genetic polymorphisms could be detrimental to host tissue and linked to periodontal disease susceptibility.

RECEPTOR AND OTHER GENE POLYMORPHISMS

FCΓR GENE POLYMORPHISMS

- The Fc-gamma receptor is the receptor present on phagocytes, which binds IgG.

- Polymorphisms that influence the binding affinity between the FcɣR and IgG of different subclasses are considered important in susceptibility to periodontal disease.
- There are three main classes of FcR: FcɣRI, FcɣR II, FcɣR III.
- Sugita et al., reported that FcɣR IIIb has a NA1-NA2 polymorphism. NA1 is a more efficient opsonophagocytic agent than NA2. NA1 is found to a greater extent in Japanese patients who are resistant chronic periodontitis.
- Meisel et al., analysed the association between FcɣR IIIa (high affinity receptor) and FcɣR IIIb (low affinity receptor) and chronic periodontitis in German population. They found that FcɣR IIIa was associated with chronic periodontitis but this is in linkage disequilibrium with FcɣR IIIb, which is also associated chronic periodontitis.

IMMUNE RECEPTOR GENE POLYMORPHISM

- FMLP Receptor polymorphism depressed chemotactic response to n-formyl-1-methionyl-1-leucyl-1-phenylalanine peptides has been confirmed in studies done by VanDyke et al. and Serhan CN.

METABOLISM RELATED

VITAMIN D RECEPTOR GENE POLYMORPHISM

- The 3' portion of the VDR gene includes a cluster of linked polymorphisms: Bsml, Apal and Taql sites.
- The first two sites are in the region of the gene from intron 8 to the 3' untranslated region.
- A silent mutation within codon 352 of the ninth exon alters a Taql site.
- The presence of the restriction endonuclease site has been denoted by b, a, t; the absence of the restriction endonuclease site has been denoted by B, A, T.
- VDR gene polymorphisms are normally determined by polymerase chain reaction (PCR) and restriction enzyme digestion.
- The VDR gene polymorphisms are commonly present. If these polymorphisms influence the level or function of the VDR, they may be pathogenic.
- Li et al. found in his study that F O K I polymorphism of vitamin D receptor gene might be associated with generalized aggressive periodontitis in Chinese patients.
- The carriage of F allele increases the risk of developing generalized aggressive peridontitis.

- Nibali et al. found that Vitamin D receptor Taq – 1 TT polymorphism was moderately associated with both the presence and the progression of periodontitis in smokers, while no association was detected in non-smoking individuals.

CALCITONIN RECEPTOR POLYMORPHISM

- Nosaka et al. have found that patients with this polymorphism were 20 times more likely to suffer buccal marginal bone loss than patients who were calcitonin receptor genotype negative.

ANTIGEN – RECOGNITION RELATED GENE POLYMORPHISM

HLA GENE POLYMORPHISM

- Human leukocyte antigen (HLA) is involved in genetically predetermined humoral response via recognition of foreign antigens.
- The MHC genes are the most polymorphic genes present in the genome of every species analyzed.
- The various alleles associated with disease in Periodontics are: HLA-DRB1.1501-DQB1.0602 genotype, HLA-DR4 and its subtypes.

POLYMORPHISMS IN THE INNATE IMMUNITY RECEPTORS

a. TLR2 AND TLR4 GENE POLYMORPHISMS

- The TLR2 gene polymorphism has been reported to decrease the ability of TLR2 to mediate a response to bacterial cell wall components.
- The TLR4 gene polymorphism has been reported to attenuate the efficacy of lipopolysaccharide (LPS) signaling and decrease the capacity to elicit inflammation.
- These polymorphisms have been correlated with hyporesponsiveness to inhaled LPS, sepsis and infection caused by gram negative bacteria.
- However, despite the perceived importance of these functional TLR polymorphisms, no relation with periodontitis has been observed.
- James JA et al. studied whether there is an association between the frequency of functional polymorphisms in the toll-like receptor 4 and cluster of differentiation 14 (CD 14) genes and periodontitis. The results concluded that TLR4 gene polymorphism is associated with a decreased risk of aggressive periodontitis but not chronic periodontitis.

b. CD 14 GENE POLYMORPHISM

- Increased serum levels of sCD14 have been known to be associated with periodontitis. There are contradictory findings from the studies of Holla et al. and Yamazaki et al. which did not find any association between CD14 genome polymorphism and chronic periodontitis.

c. CARD 15 GENE POLYMORPHISM

- Polymorphism led to impaired activation of nuclear factor-κB, which in turn led to decreased expression of pro-inflammatory cytokines. However, no association was found with periodontitis.

MISCELLANEOUS GENE POLYMORPHISMS

a. CATHEPSIN C GENE POLYMORPHISM

- Cathepsin C is a proteinase and is expressed in the hyperkeratotic epithelial lesions such as palms, knees and oral keratinized gingiva.
- Hart et al. identified a gene on chromosome 11 containing the cathepsin C gene, responsible for prepubertal periodontitis as well as Papillon – Lefevre syndrome (PLS).
- All patients with pre-pubertal periodontitis were found to be homozygous for an A-G mutation at gene position +1040, resulting in a substitution of the amino acid tyrosine by a cysteine. This gene polymorphism was shown to be functional as there was a diminished activity of cathepsin C in PLS.
- Another study by Noack et al. reported two novel gene mutations at positions 947 and 1268, which were associated with these two diseases.

b. MMP GENE POLYMORPHISM

- MMP-1 is an important mediator of connective tissue destruction in periodontal disease. Cao Z et al., evaluated the association between the MMP-1 promoter gene polymorphism and chronic periodontitis susceptibility and/or severity in a Chinese population. This study suggests that a single nucleotide polymorphism in the MMP-1 promoter region of -1607 bp may be associated with severe chronic periodontitis in a Chinese population.

GENETICS TEST FOR DIAGNOSIS AND THERAPEUTIC TREATMENT

GENETIC COUNSELING

- Genetic counseling is defined as a communication process involved in human problems associated with the occurrence and recurrence of a genetic disorder in a family.
- This process involves the expertise of a trained counselor to guide individuals, family to the medical facts related to diagnosis, prognosis, and management of a disorder, the role of

heredity in genetic disorder, the probable impact on the other members of the family, and preventive measures for further recurrence of such disorders in the family.

Steps in genetic counseling:

- a. Family history
    - • To construct and analysis pedigree
- b. Clinical Examination
- c. Investigation*
    - • Chromosomal analysis
    - • Enzyme assays
    - • Metabolite measurements
    - • DNA analysis.

*These are specialized tests which may be essential to arrive at the final diagnosis

- d. Disease managements.

GENETIC TESTING FOR PERIODONTITIS

- At present, it is possible to perform genetic testing to identify individuals carrying gene mutations responsible for several syndromic forms of periodontitis including LAD types 1 and 2, Papillon-Lefèvre syndrome, Haim-Munk syndrome, Chédiak-Higashi syndrome, and some forms of Ehlers-Danlos syndrome.
- To date, there is no evidence that mutations in the genes responsible for these conditions are responsible for the more prevalent forms of aggressive or chronic periodontitis.
- Genetic testing for mutations of specific genes is not currently utilized for testing for aggressive periodontitis and chronic periodontitis is unknown.
- In the field of periodontics, most work in evaluating genetic polymorphisms and their relationship to periodontitis has been performed for several IL-1 genetic polymorphisms, and these tests show promise, especially among certain preselected populations, but for reasons stated previously, more genotypic information that identifies additional genomic risk markers would likely provide even better diagnostic and prognostic tools in the future.

Human Genome project

- It is an international scientific research project with the goal of determining identification of a large number of disease - causing genes. It opened the floodgate of DNA diagnostic tests which

have found a firm place in clinical management of various diseases by way of genetic counseling, carrier detection, and presymptomatic.

CANDIDATE GENE APPROACH

- Gene mapping is a test which is used to find whether one allele of a gene occurs more often in patients with the disease than in participant without a disease. Gene, i.e., candidate gene is chosen on the basis of their presumed or known function.

GENE THERAPY

- Gene therapy is defined as the genetic modification of cells for therapeutic purposes.
- The goal of gene therapy is to transfer the DNA of interest into the cell.
- Currently genetic principles are being applied along with tissue engineering for periodontal rehabilitation.

HISTORY

- The first gene therapy trials on humans began in 1990 on patients with Severe Combined Immunodeficiency (SCID).
- In 2000, the first gene therapy "success" resulted in SCID patients with a functional immune system. These trials were stopped when it was discovered that two of ten patients in one trial had developed leukemia resulting from the insertion of the gene-carrying retrovirus near an oncogene.

FUNDAMENTALS OF GENE THERAPY

There are a variety of methods to replace or repair the genes targeted in gene therapy.

• A normal gene may be inserted into a nonspecific location within the genome to replace a nonfunctional gene. This approach is most common

• An abnormal gene could be swapped for a normal gene through homologous recombination

• The abnormal gene could be repaired through selective reverse mutation, which returns the gene to its normal function

• The regulation (the degree to which a gene is turned on or off) of a particular gene could be altered

• Spindle transfer is used to replace entire mitochondria that carry defective mitochondrial DNA.

TYPES OF GENE THERAPY

- Gene therapy may be classified into the following types:

Germ line gene therapy

- In the case of germ line gene therapy, germ cells, i.e., sperm or eggs are modified by the introduction of functional genes, which are ordinarily integrated into their genomes. Therefore, the change due to therapy would be heritable and would be passed on to later generations.

Somatic gene therapy

- In the case of somatic gene therapy, the therapeutic genes are transferred into the somatic cells of a patient. Any modifications and effects will be restricted to the individual patient only, and will not be inherited by the patient's offspring.

GENE DELIVERY

- In general, gene therapy involves the transfer of genetic information to target cells, which enables them to synthesize a protein of interest to treat disease.
- The technology can be used to treat disorders that result from single point mutations.
- The preferred strategy for gene transfer depends on the required duration of protein release and the morphology of the target site production.
- There are various methods for gene delivery:

Viral

- A carrier molecule called a vector must be used to deliver the therapeutic gene to the patient's target cells. Currently, the most common vector is a virus that has been genetically altered to carry normal human DNA. Viruses have evolved a way of encapsulating and delivering their genes to human cells in a pathogenic manner. Scientists have tried to take advantage of this capability and manipulate the virus genome to remove disease-causing genes and insert therapeutic genes.

  Different vectors are:

- Retroviruses
- Adenoviruses
- Adeno-assosiated viruses
- Herpes simplex viruses

Non viral

- The simplest method is the direct introduction of therapeutic DNA into target cells. This approach is limited in its application because it can be used only with certain tissues and requires large amounts of DNA
- Another non viral approach involves the use of an artificial lipid sphere with an aqueous core. This liposome, which carries the therapeutic DNA, is capable of passing the DNA through the target cell's membrane.

MAJOR DEVELOPMENTS IN GENE THERAPY

In 1999

- Trial treatments of SCID have been gene therapy's only success; since 1999, gene therapy has restored the immune systems of at least 17 children with two forms (ADA-SCID and X-SCID) of the disorder.

In 2002

- In 2002 a question was raised when two of the ten children treated developed a leukemia-like condition.

In 2006

- Scientists have successfully treated metastatic melanoma in two patients using killer T cells genetically retargeted to attack the cancer cells . As well as in March again, scientists announced the successful use of gene therapy to treat two adult patients for a disease affecting myeloid cells.
- Italy reported a breakthrough for gene therapy in which they developed a way to prevent the immune system from rejecting a newly delivered gene. Similar to organ transplantation.

In 2007

- The world's first gene therapy trial was done for inherited retinal disease. The sub retinal delivery of recombinant adeno associated virus (AAV) carrying RPE65 gene, was found to be safe and yielded positive results.

In 2009

- The journal Nature reported that researchers at the University of Washington and University of Florida were able to give trichromatic vision to squirrel monkeys using gene therapy, a hopeful precursor to a treatment for color blindness in humans.

IMPLICATIONS OF GENE THERAPY IN PERIODONTICS

There are three approach of tissue engineering in periodontics:

- 1. Protein based approach: Growth and differentiation factors are used for regeneration of periodontal tissues likes TGF-β, BMP-2,6,7,12, bFGF, VEGF and PDGF.
- 2. Cell based approach: Several studies using mesengymal stem cell have demonstrated efficient reconstruction of bone defect that are too large to heal spontaneously.
- 3. Gene delivery approach: To overcome the short half-lives of growth factor peptides in vivo, gene therapy that uses a vector that encodes the growth factor is utilized to stimulate tissue regeneration. So far, two main strategies of gene vector delivery have been applied to periodontal tissue engineering.

APPLICATIONS

They are as following:

- 1. Gene Therapeutics-Periodontal Vaccination
- 2. Genetic Approach to Biofilm Antibiotic Resistance
- 3. An In vivo Gene Transfer by Electroporation for Alveolar Remodeling
- 4. Antimicrobial Gene Therapy to Control Disease Progression
- 5. Designer Drug Therapy in Treating Periodontal Disease

GENE THERAPEUTICS-PERIODONTAL VACCINATION

A salivary gland of a mouse when immunized using plasmid DNA encoding the Porphyromonas gingivalis (P. gingivalis) fimbrial gene produces fimbrial protein locally in the salivary gland tissue resulting in the subsequent production of specific salivary immunoglobulin's A, or IgA and immunoglobulin G, or IgG, antibodies and serum IgG antibodies. This secreted IgA could neutralize P. gingivalis and limit its ability to participate in plaque formation.

- Scientists have also demonstrated the efficacy of immunization with genetically engineered Strepto-cocci gordoni vectors expressing P. Gingivalis is fimbrial antigen as vaccine against P. gingivalis associated periodontitis in rats.
- The gene hemagglutinin which is an important virulence factor of P. gingivalis has been identified, cloned and expressed in Escherichia coli. The recombinant hemagglutinin B (rHag B) when injected subcutaneously in Fischer rats infected with P. gingivalis showed serum IgG antibody and interleukin-2 (IL-2), IL-10, and the IL-4 production which gave protection against P. gingivalis induced bone loss.

GENETIC APPROACH TO BIOFILM ANTIBIOTIC RESISTANCE

- Researchers have found bacteria growing in biofilms become up to 1,000 fold more resistant to antibiotics as compared to a planktonic counterpart making them hard to control.
- Recently Mah et al., identified gene ndvB encoding for glycosyltransferase required for the synthesis of periplasmic glucans in wild form of Pseudomonas aeuroginosa RA14 strain.
- This remarkably protected them from the effects of antibiotics biocides, and disinfectant .
- Using a genetic approach.
- Researchers have isolated ndvB mutant of Pseudomonas aeuroginosa still capable of forming biofilm but lacking the characteristic of periplasmic glucans there by render- ing microbial communities in biofilm more susceptible to conventional antibiotic therapy.

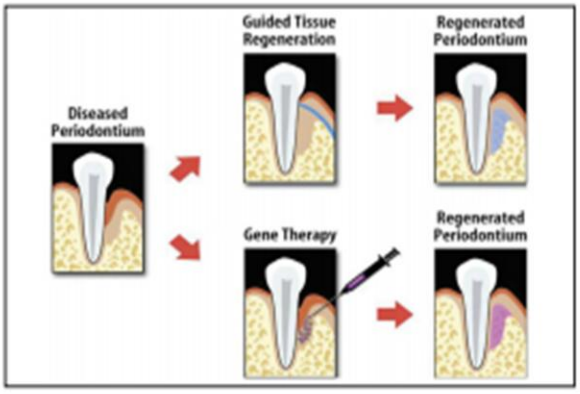


Figure 1: Approaches for regenerating tooth supporting structure

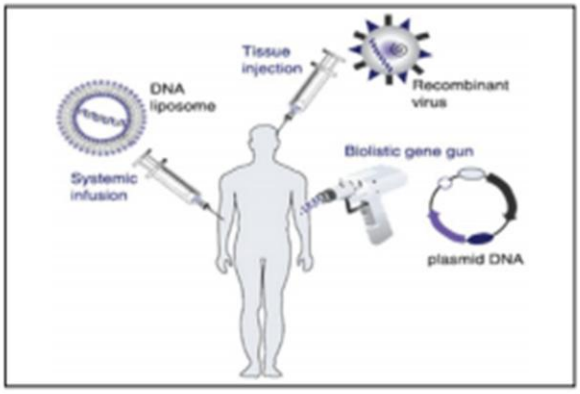


Figure 2: *In vivo*

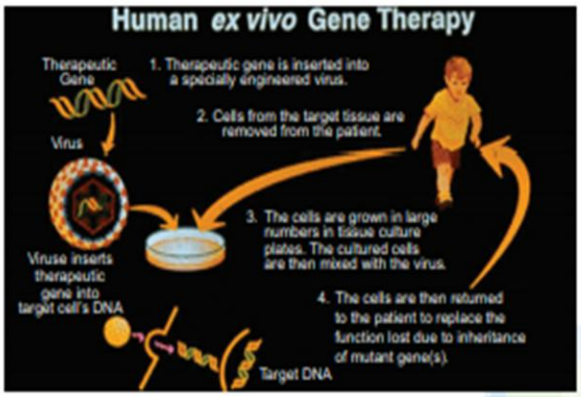


Figure 3: *Ex vivo*

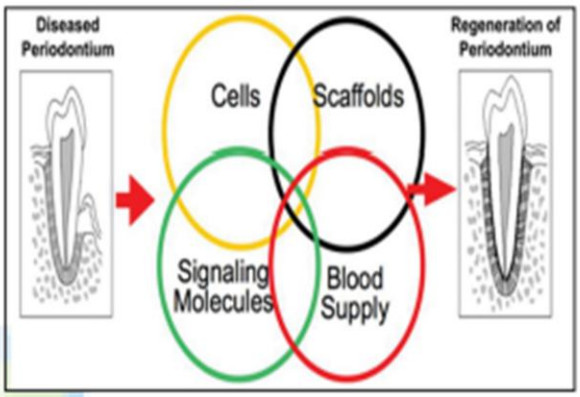
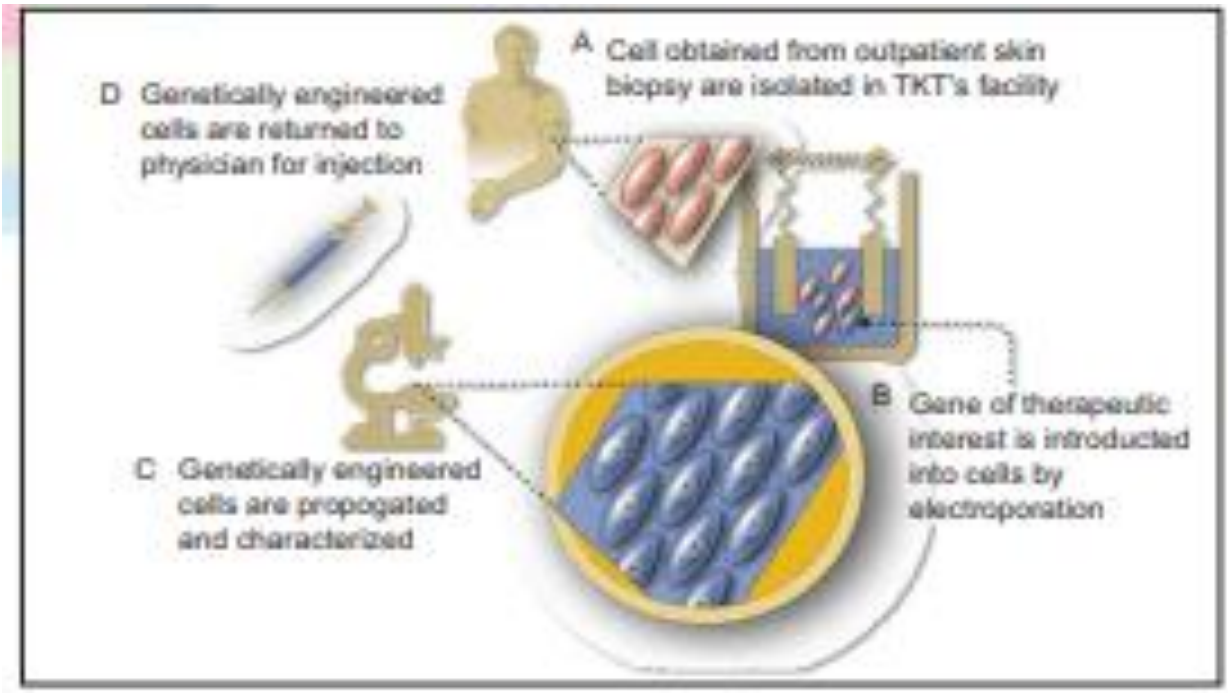
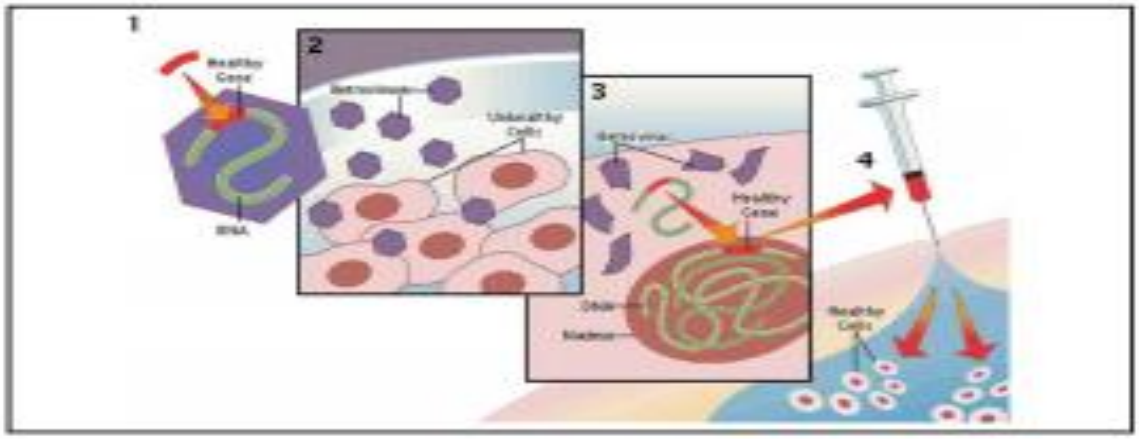


Figure 4: Tissue engineering

ELECTROPORATION OF ALVEOLAR BONE:





CONCLUSION:

- A multitude of host factors are involved in the response of microbial challenge & in the subsequent immune response.
- Genetic polymorphism probably exist in may inflammatory & immune mediators.
- Correlation of genetic polymorphism in immune responses with phenotypes for certain patient groups, such as FcγIIa receptor for early-onset periodontitis & IL-1 for adult periodontitis, currently appears to provide the most promising application of genetic determinants of periodontitis.

- Correlation of the genetic polymorphisms with stable phenotypic characteristics of periodontitis patient groups may provide the framework for identification of molecular biomarkers to be incorporated to set the foundation for developing new treatment strategies.

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