UNIT 2 RATIONALE OF ENDODONTICS AND MICROBIOLOGY OF ROOT CANALS

Structure

2.0 OBJECTIVES

After reading this unit, you should be able to:

- describe the historical misbelieves and the current concepts of endodontic infections;
- discuss the rationale of endodontic treatment;
- enumerate various methods available for detection of endodontic microbes; and
- list the microbes involved in failed endodontic treatment and various microbial virulence factors.

2.1 INTRODUCTION

Of the major dental diseases, infection of the root canal is unique for the oral cavity since infection establishes where micro-organisms have not previously been present. The other microbial diseases of the oral cavity, caries and periodontal disease, develop at sites where a microbial bio film (you must understand that microbes in an infected root canal do not occur in vivo as separate colonies but grow within an extra cellular matrix in interconnected communities as microbial biofilm) is already established and disease occurs with a change in the environmental conditions, the type and mix of microbial flora.

Endodontic treatment, whether surgical or non-surgical, essentially is debridement for removal of the microbial eco-systems associated with the disease process. You must understand that adequate knowledge of the micro-organisms associated with endodontic disease is necessary to develop a basic understanding of the disease process and a sound rationale for effective management of patients with endodontic infections.

In this unit we will come to know about the delicate balance between the host (the tooth) and micro-organisms in the pathogenesis of endodontic disease. Some recommendations for treatment of endodontic infections has also been addressed to.

2.2 HISTORICAL PERSPECTIVE

Endodontics is relatively a new branch in dentistry and my teacher used to call it the “youngest daughter of dentistry.” As such it will be interesting to view it from the historical angle.

In 1890, W.D. Miller associated the presence of bacteria with pulpal and periapical disease. E. C. Rosenow, in 1909 described the “Theory of Focal Infection” as a localized or generalized infection caused by bacteria traveling through the bloodstream from a distant focus of infection.

In 1910, a British physician, William Hunter, condemned the practice of dentistry in the United States, which emphasized restorations instead of tooth extraction. He believed that this was the cause of Americans’ many illnesses, including pale complexion, chronic dyspepsias, intestinal disorders, anemias, and nervous complaints. Soon pulpless teeth (teeth with necrotic pulps) and endodontically treated teeth were also implicated. The practice of root canal treatment suffered a severe set back for almost 40 years as a result of hypothesized association between oral focal sepsis and systemic illness.

In 1939, Fish recognised four zones of reaction formed in response to viable bacteria implanted in the jaws of guinea pigs. He described the bacteria as being confined by polymorphonuclear neutrophil leukocytes to a zone of infection. Outside the zone of infection is the zone of contamination containing inflammatory cells but no bacteria. Next, the zone of irritation contained histocytes and osteoclasts. On the outside was a zone of stimulation with mostly fibroblasts, capillary buds, and osteoblasts. Fish theorized that removal of the nidus of infection would lead to resolution of the infection. This theory became the basis for successful root canal treatment.

Today the medical and dental professions agree that there is no relationship between endodontically treated teeth and the degenerative diseases implicated in the theory of focal infection. This body of research of focal infection theory based on the poorly designed and outdated studies has been evaluated and disproved. Unfortunately, uninformed patients may receive this outdated information and believe it to be credible new findings, so it's the duty of the clinicians to make the patients understand and impart them with the logical information.

Check Your Progress 1

1) What do you mean by the 'theory of focal infection' and is it still valid?

....

....

....

....

2) What are the 'four zones of reaction' as described by Fish and its clinical significance?

....

....

....

....

2.3 PATHWAYS OF PULPAL INFECTION

You will be surprised to know that in the oral cavity, there are an estimated 1010 bacteria consisting of more than 500 different kinds of micro-organisms and all seek a niche and nutrition. As long as the enamel and cementum layers are intact, the pulp and root canal are protected from invasion, but loss of these structures by caries, cracks or trauma opens an avenue for penetration of bacteria through the dentinal tubules, Do you know that the dentinal tubules range from 1 to 4 micron meter in diameter, whereas most bacteria are less than 1 micron meter in diameter? The list of the causes of pulpal injury is very exhaustive which you must have read in previous units.

Bacterial movement is restricted by the outward flow of dentinal fluid (pulpal pressure is 24-30 mmHg), odontoblast processes, mineralized crystals, and macroglobulins including immunoglobulins in the tubules. The dentine-pulp complex of the tooth may react in a number of ways to the presence of micro-organisms, but irreversible inflammatory changes may ultimately occur with the development of an inflammatory front in the periradicular tissues causing a chronic periradicular periodontitis (Fig. 1). A potentially compromised pulp may present itself in a variety of ways, as mentioned above due to caries, surface tooth loss or injury. In each case you need to estimate the following points:

- What was the pre-existing state of the pulp? Sensitivity to hot and cold, sensitivity in tooth to touch,etc.
- What is the extent of the injury? Check whether the surfaces are involved with caries, what is the extension of caries etc?
- What is the degree of microbial contamination?

The root canal therapy is based on the rationale, whereby the root canal system is cleaned; chemomechanically shaped, and then obturated, which allows healing to take place. The objective is to reduce the microflora to a minimum, as it is not possible to get absolutely sterile root canal system and prevent recontamination, which usually occurs coronally. Basically you need to create an environment at the apex so that the healing of tissue occurs.

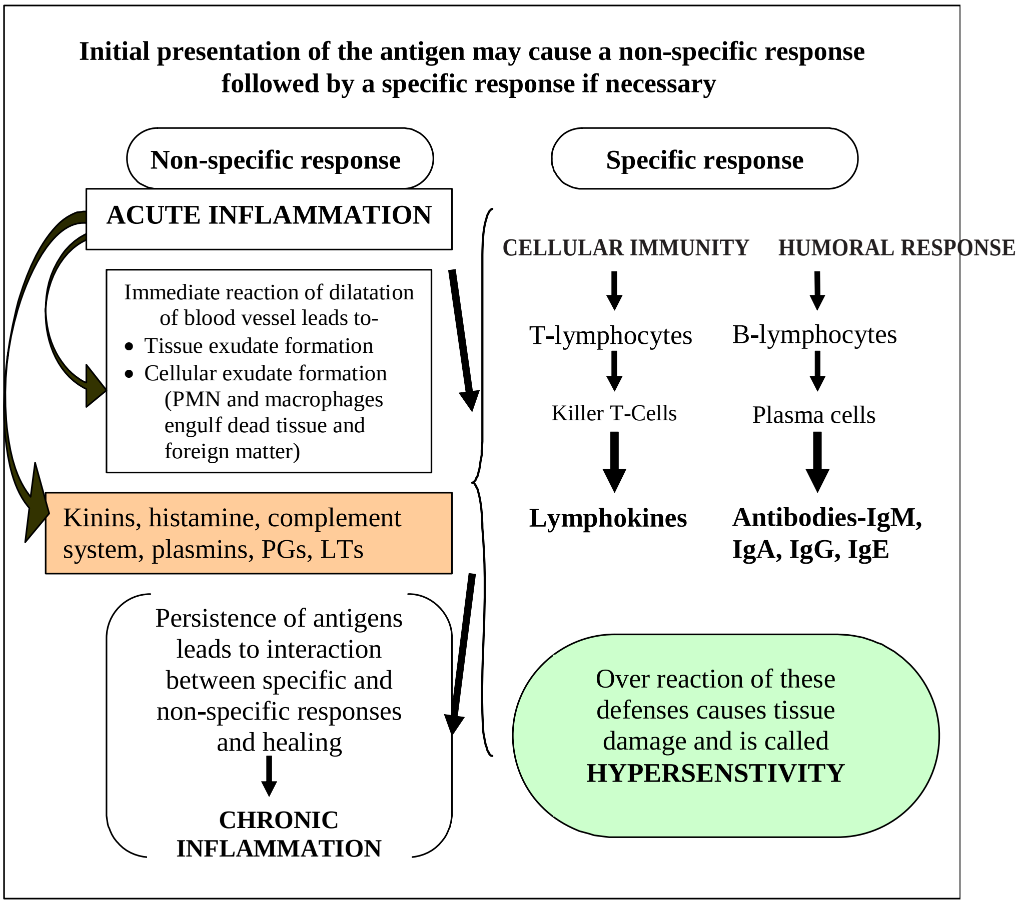

Fig. 2.1: Chart showing defense and immune reactions

2.3.1 Importance of Asepsis

Asepsis …. I know you know a lot about it…. Still, please do not skip this paragraph. Out of sight is out of mind.

The primary goal of doing the endodontic treatment is to eliminate the bacteria from the canal. It is therefore obvious to us all that we will perform the treatment procedure in a manner that we will not further contaminate the area. As in treatment procedures we say DO NO HARM, similarly here, DO NO CONTAMINATION.

You are lucky, you can do this. It is only in this treatment procedure and restorative work that it is possible to isolate the area from the rest of the oral cavity by use of rubber dam.

There are many other effficient methods available for disinfection of the operative field and the tooth. The importance of using sterile instruments and an aseptic technique in a disinfected field is always highly emphasized but rarely followed., Every effort should be made to exclude and eliminate micro-organisms from the operative field and the root canal itself. **Application of the rubber dam is mandatory for endodontic treatment**. The rubber dam must properly isolate the tooth from the oral cavity to ensure an aseptic field.

2.3.2 Antimicrobial Effect of Debridement

The control of bacteria within the root canal might appear to be straightforward since such a large proportion of the bacterial flora is sensitive to oxygen. However, the penetration of oxygen into the canal during treatment does not seem to have any significant effect on the bacteria. The reason for this is that many of the bacteria are protected in the irregularities and branches of the root canal system and in dentinal tubules. Only a few cells need to survive treatment so that when the canal is closed, the anaerobic milieu will be restored and the bacteria can re-multiply. The microbial flora within the root canal must be actively eliminated by a combination of physical debridement and antimicrobial chemical treatment.

Although the most important aspect of root canal instrumentation is undoubtedly the elimination of bacteria and the removal of remnants of pulp tissue and debris, the shaping of the root canal to accommodate the root filling material is also of importance.

Preparation of the root canal consists of two main phases: debridement by manual and mechanical instrumentation and chemical disinfection by irrigation and subsequent antibacterial dressing.

Check Your Progress 2

1) What is the most common cause of pulpal injury? State the rationale of vital pulp therapy?

....

....

....

....

2) What are the mechanisms restricting the dentinal ingress of bacteria?

....

....

....

....

2.4 MICROBES IN ENDODONTIC INFECTIONS

In 1967, Antony van Leewenhoek, became the first person to describe bacteria from root canals using one of single-lens microscopes.

The true significance of bacteria in endodontic disease was shown in the classic study by Kakehashi et al in 1965. They found that no pathologic changes occurred in the exposed pulps or periradicular tissues in germ-free rats. In conventional animals, however, pulp exposures led to pulpal necrosis and periradicular lesion formation. In contrast, the germ-free rats healed with dentinal bridging regardless of the severity of the pulpal exposure. Thus, the presence or absence of microbial flora was the major determinant for the destruction or healing of exposed rodent pulps.

2.4.1 Role of Enviornment

All bacteria within the oral cavity share the same opportunities for invading the root canal space, however only a restricted group of species have been identified in infected root canals.

The reason for the disproportionate ratio between potential and actual number of species is that the root canal is a unique environment where biological selection drives the type and course of infection. An anaerobic milieu, interactions between microbial factors and the availability of nutrition are principal factors that define the composition of the microbial flora.

In the initial phase of a root canal infection, the number of species is usually low. If the way of invasion is via caries, the bacteria in front of the carious process are the first to reach the pulp. In cases where there is no apparent communication with the oral cavity and the bacteria penetrate through dentinal tubules, as in trauma cases without pulp exposure, there is no clear pattern of primary bacterial invaders.

The number of bacterial species in an infected root canal may vary from one to more than 12, and the number of bacterial cells varies from $<10^2$ to $>10^8$ per sample. A correlation seems to exist between the size of the periapical lesion and the number of bacterial species and cells in the root canal. Teeth with long-standing infections and large lesions usually harbor more bacterial species and have a higher density of bacteria in their root canals than teeth with small lesions.

Of the varied number of microorganisms only a limited number have been consistently isolated from endodontic infections. These include species of the genera Streptococcus, Fusobacterium, Prevotella, Porphyromonas, Eubacterium, Peptostreptococcus, Bacteroides, and Lactobacillus.

The endodontic milieu is a selective habitat that supports the development of specific proportions of the anaerobic microflora. Oxygen and oxygen products play an important role as ecological determinants in the development of specific proportions of the root canal microflora. The consumption of oxygen and production of carbon dioxide and hydrogen along with the development of a low reduction-oxidation potential by the pioneer species favour the growth of anaerobic bacteria

Nutrition as an Ecological Driver

The type and availability of nutrients is important in establishing microbial growth. Nutrients may be derived from the oral cavity, degenerating connective tissue, dentinal tubule contents, or a serum-like fluid from periapical tissue. Exogenous nutrients, such as fermentable carbohydrates, affect the microbial ecology of the coronal part of an exposed root canal by promoting growth of species that primarily obtain energy by carbohydrate fermentation. Endogenous proteins and glycoproteins are the principal nutrients in the main body of the root canal system and this substrate encourages the growth of anaerobic bacteria capable of fermenting amino acids and peptides.

2.4.2 Microbial Ecology and Biofilms

The microflora ecosystem involved in root canal is appropriately described as **'polymicrobial'** in nature.

In carious exposed root canals most commonly found facultative anaerobic species are alpha-hemolytic streptococci, staphylococci, lactobacilli.

Large portion of microorganisms isolated from intact non-vital teeth are anaerobes e.g. Bacteriodes, peptococcus, peptostreptococcus, fusiform.

Strict anaerobes — These bacteria grow only in the absence of oxygen but vary in their sensitivity to oxygen. They function at low oxidation-reduction potentials and generally lack the enzymes superoxide dismutase and catalase. ***Prevotella nigrecens*** is the dark pigmented bacteria most often cultivated from endodontic infections. *Microaerophilic bacteria* can grow in an environment with oxygen but predominantly derive their energy from anaerobic energy pathways.

Facultative anaerobes grow in the presence or absence of oxygen and usually have the enzymes superoxide dismutase and catalase.

Obligate aerobes require oxygen for growth and possess both superoxide dismutase and catalase.

The interdependence of different bacterial species with their environment is the key to the success of root canal treatment. The treatment procedures (mechanical and chemical) essentially interfere with the environment, killing some bacteria and indirectly killing other species by altering the nutritional and toxic balance.

The surviving bacteria are usually those hardy enough to resist the treatment and capable of living independently of other species in unique nutrition-depleted conditions. This means a poor first attempt at root canal treatment may result in a more resistant and hardy infection to eradicate at the next step. **It is therefore best to launch the most comprehensive effort at eradicating the infection at the first attempt**.

Bacteria in a root canal infection do not occur in vivo as separate colonies, but grow within an extracellular matrix in interconnected communities as a **'bacterial biofilm'**. The ultrastructural appearance of these biofilms in the infected root canal is described as coaggregating communities with a palisade structure.

The clinical significance of a biofilm growth pattern is that bacteria are relatively protected within the coaggregated community compared with planktonic forms and are known to be more resistant to antimicrobial treatment measures. This is because of the several mechanisms, including the following:

- the exopolysaccharide in which the bacteria are embedded may restrict diffusion of the antibacterial agents to the cells;
- the different layers of cells similarly act as barriers to diffusion;
- some bacterial cells may be slower-growing or dormant and therefore may be more resistant to killing;
- cells may exhibit specific resistant mechanisms;
- biofilm phenotypes may be inherently resistant.

Currently, limited information is available on the development, physiology and antimicrobial management of biofilms in the root canal.

2.4.3 Flora in Untreated Root Canals

Pucnobial flora in intact nonvital canals and infected non vital canals is different.

There are 11 different phyla, representatives from only six of those have been reported in endodontic infections to date, namely, Bacteroids,

Spirochaetes,

Firmicutes,

Actinobacteria,

Fusobacteria, and

Proteobacteria.

Diversity of Endodontic Microflora

The microflora associated with endodontic infections is far more diverse than has been shown previously by cultural studies alone, mainly due to the advanced molecular detection methods available now.

Although bacteria are by far the most common micro-organisms involved in endodontic infection, recently studies even revealed possibility for ***fungi*** and even more recently ***viruses***. The various viruses which have been implicated are Herpes virus, Cytomegalovirus (CMV), Epstein - Barr virus (EBV).

Check Your Progress 3

1) What kind of microbes are most commonly found in root canal?

....

....

....

....

2) Which is the most common pathogen found in infected root canals?

....

....

....

....

3) What are the sources of nutrition for the endodontic pathogens?

....

....

....

....

4) Define biofilm. What are the defense mechanisms attained by the microbes in a biofilm?

....

....

....

....

2.4.4 Flora in Obturated Root Canals

It is generally acknowledged that persistence of disease is most commonly due to difficulties that occur during initial endodontic treatment. Inadequate aseptic control, poor access cavity design, missed canals, inadequate instrumentation, and breakdown of temporary or permanent restorations are examples of procedural pitfalls that may result in persistence of endodontic disease.

The reasons for disease persistence in *well-treated root-filled teeth* have been poorly characterized. Using block biopsy material from non-healed periapical tissues by correlative light and electron microscopy showed that there were four factors that may have contributed to persistence of periapical radiolucency after treatment. The factors were:

i) intraradicular infection;

ii) extraradicular infection by bacteria of the species Actinomyces israelii and Propionibacterium propionicum;

iii) foreign body reaction; and

iv) cysts, especially those containing cholesterol crystals.

Of all these factors, it is generally acknowledged that the major cause of post-treatment disease is the persistence of micro-organisms in the apical part of root-filled teeth.

Persistent endodontic disease, or apical periodontitis associated with a root-filled tooth, can continue for many years and may become apparent only when a tooth requires a new restoration or is detected on a routine radiograph.

The bacterial genera most frequently recovered from filled root canals were Enterococcus faecalis (most common) and streptococci. Other species found in higher proportions in individual studies are lactobacilli, Actinomyces species, peptostreptococci, Pseudoramibacter alactolyticus, Propionibacterium propionicum, Dialister pneumosintes, Filifactor alocis and Candida albicans.

2.4.5 Ecological Differences between Untreated and Root Canal Treated Teeth

The untreated infected root canal is an environment that provides micro-organisms with nutritional diversity in a shifting pattern over time. The available nutrients are mainly peptides and amino acids, which favour anaerobic proteolytic species. These microbes endure a static environment and starvation, but with some luck may encounter a serum like fluid transudate from the periapical tissue. The species that persist are those that either have survived the antimicrobial treatment, or have entered during treatment and found it possible to establish where others cannot do so. Where the coronal seal is defective or missing, there is the possibility for new infection of the root canal space.

Species commonly associated with persistent intraradicular infection such as Candida and Enterococci can be viewed as opportunistic pathogens.

In general, micro-organisms involved in persistent infections implement one of three strategies to evade the immune response — sequestration, cellular or humoral evasion.

- Sequestration involves a physical barrier between the microbe and the host (E.faecalis and Candida).
- Cellular evasion means that micro-organisms avoid leukocyte dependent antibacterial mechanisms.
- Humoral evasion means that those extracellular bacteria avoid the host's antibodies and complement.

Check Your Progress 4

1) Which pathogen is most commonly recovered from failed root canal? Enumerate the various reasons which favour its growth.

   ....

   ....

   ....

   ....

2) What are the reasons for persistence radiolucency, in well treated root filled teeth?

   ....

   ....

   ....

   ....

2.4.6 Bacterial Virulence Factors

Bacteria have a number of virulence factors that may be associated with disease. They include pili (fimbriae), capsules, extracellular vesicles, lipopolysaccharides, enzymes, short-chain fatty acids, polyamines, and low-molecular— weight products such as ammonia and hydrogen sulfide.

- Pili may be important for attachment to surfaces and interaction with other bacteria in a polymicrobial infection.
- Bacteria including gram-negative black-pigmented bacteria (BPB) may have capsules that enable them to avoid or survive phagocytosis.
- Lipopolysaccharides are found on the surface of gram-negative bacteria and have numerous biologic effects when released from the cell in the form of endotoxins. Endotoxins have been associated with periapical inflammation and activation of complement.
- Enzymes are produced by bacteria that may be spreading factors for infections or proteases that neutralize immunoglobulins and complement components.
- Gram-negative bacteria produce extracellular vesicles. These vesicles may contain enzymes or other toxic chemicals. It is believed that these vesicles are involved in hemagglutination, hemolysis, bacterial adhesion, and proteolytic activities.
- Anaerobic bacteria commonly produce short-chain fatty acids including propionic, butyric, and isobutyric acids. As virulence factors, these acids may

affect neutrophil chemotaxis, degranulation, chemiluminescence, and phagocytosis.

- Polyamines are biologically active chemicals found in infected canals.

Check Your Progress 5

Enumerate the various bacterial virulence factors involved in the pulpal disease?

....

....

....

....

2.5 DETECTION AND IDENTIFICATION OF PATHOGENS

The current knowledge in endodontic microbiology is based on the application of several methods, such as conventional histology, correlative light and electron microscopy, scanning confocal laser microscopy, microbial culturing, and biochemical and molecular techniques. All these methods have different degrees of limitations with respect to sensitivity, specificity, and etiologic relevance.

2.5.1 Cultivation Methods

Cultivation relies on the growth of micro-organisms in the laboratory under artificial growth conditions. This technique has some advantages:

1) It allows identification of a great diversity of microbial species in a sample, including those not being sought.

2) Also allows determination of the antimicrobial susceptibilities of the isolates.

Nevertheless, cultivation methods have a number of drawbacks:

1) They are scarcely able or unable to grow many micro-organisms, thus may lead to false-negative and underestimation of the pathogenic micro-organisms.

2) They are slow to provide diagnostic result.

3) They have low specificity and therefore have limitations in distinguishing among microbial species and strains.

4) They have low sensitivity i.e., failure to detect small numbers of micro-organisms.

5) They strictly depend on the mode of sample transport, which must allow the survival of anaerobic bacteria but not favour overgrowth of facultative bacteria.

6) They are time-consuming, laborious, and expensive.

In the past two decades a number of methods based on molecular biology have been introduced for microbial identification. However, researchers have also shown that 40-50 percent of bacterial clones in the oral cavity represent unknown and as yet uncultivable species. The question no longer is whether uncultivable micro-organisms exist but why huge proportion of the species living in diverse environments cannot be grown under laboratory conditions. Some micro-organisms may be uncultivable for a number of reasons:

1) The artificial culture medium may lack the required nutrients or growth factors.

2) Other micro-organisms in the sample may produce substances that may inhibit the target species e.g., Bacteriocins.

3) A species may depend on another species for growth.

4) Bacterial intercommunication may be disrupted, such as present in natural biofilms.

2.5.2 Molecular Diagnostic Methods

The advent of molecular genetic methods has revolutionized life sciences as a whole. It has its share of impact on microbiology in general and on oral and endodontic microbiology in particular. Molecular diagnostic methods for micro-organisms have several advantages over cultivation methods:

1) They can detect both cultivable and uncultivable microbial species and strains.

2) They have greater specificity.

3) They can detect microbial species directly in clinical samples, without the need for microbial.

4) They are more sensitive than other identification methods.

5) They are usually less time-consuming.

6) They do not require carefully controlled anaerobic conditions during sampling and transportations.

7) They can be used during antimicrobial treatment.

The molecular detection methods available are:

- DNA-DNA hybridization methodology — these methods use DNA probes.
- Polymerase Chain Reaction Method — It involves invitro replication of DNA and has often been referred to as the “Genetic Xeroxing”. A lot of variations of PCR are now available, such as Single PCR, Reverse Transcriptase (RT) PCR, Nested PCR, Real-Time PCR.

Molecular techniques have been used to detect bacteria in endodontic infections using oligonucleotide probes and checkerboard DNA-DNA hybridization analysis.

The checkerboard DNA-DNA hybridization has the following advantages:

- It permits the simultaneous determination of the presence of a multitude of bacterial species in single or multiple clinical samples.
- The method does not require bacterial viability and is particularly applicable in epidemiologic research.
- DNA-DNA hybridization technology has the additional advantage that DNA is not amplified. Thus, microbial contaminants are not cultured, nor are their DNA amplified.

However, the use of specific DNA probes limits the boundaries of the detection technique:

- As it assumes that these probes target the species of importance.
- There are inherent problems with checkerboard analysis, which stem from the lack of specificity of the whole genomic probes used. The technique cannot be used to determine the true diversity of potential pathogens from infected root canals.

Whilst molecular methods greatly facilitate identification of culture-difficult species and enhance the precision of taxonomic grouping, it is important to recognize the ***limitations of PCR-based*** methodology.

1) The high sensitivity of this method implies that it is essential that contamination controls be strictly applied, as contaminants may be easily picked up in the sample and amplified by PCR.

2) The PCR technique is based on recognition of gene sequences - not recovery of cultivable cells capable of growth — so the main drawback of PCR-based methods is that it ***may detect both living and dead bacteria***. Because DNA that persists after cell death may be detected by PCR, the findings from root canal samples may reveal more than just active contributors but could also reflect a historical record of the micro-organisms that have entered and not survived in the root canal.

Molecular diagnostic method have enabled the detection of some cultivable species in infected root canal or periradicular abscess in higher prevalence values than ever reported by cultural studies. These include *Porphyromonas endodontalis, Porphyromonas gingivalis, Fusobacterium nucleatum, Proprionibacterium propionicum, Actinomyces sp.*

Molecular diagnostic methods have also expanded the list of putative endodontic pathogens by including some fastidious bacterial species or even uncultivable bacteria. *T. forsythia*, which has never been detected from infected root canal by cultivation method.

Check Your Progress 6

1) Enumerate the various drawbacks of culture methods.

....

....

....

....

2) What are the various molecular methods available for identification of endodontic pathogens?

....

....

....

....

3) Enumerate the advantages of the recent molecular detection methods.

....

....

....

....

2.6 LET US SUM UP

Thus by now it can be truly quoted “a big role is being played by the very small - endodontic microbial flora” is very important for laying down a successful foundation for endodontic treatment. Infection of the root canal is not a random event. The type and mix of the microbial flora develop in response to the surrounding environment. Factors that influence whether species die or survive are:

a) the particular ecological niche, b) nutrition, c) anaerobiosis, d) pH e) Competition or cooperation with other microorganisms? and f) Whether it is A necrotic pulp or root-filled space?

The environment selects for micro-organisms that possess traits suited to establishing and sustaining the disease process. Reduction and elimination of micro-organisms from the infected root canal provides the optimal chance of treatment success. The goal of achieving a clean, microbe-free canal can best be realized by working in a sterile way using instrumentation with antibacterial irrigation, which is reinforced by an intracanal dressing with calcium hydroxide. All these form the biological and clinical rationale for a successful endodontic treatment.

2.7 ANSWERS TO CHECK YOUR PROGRESS

Check Your Progress 1

1) The “Theory of Focal Infection” was described by E. C. Rosenow, in 1909 as a localized or generalized infection caused by bacteria traveling through the bloodstream from a distant focus of infection. In the view of this theory practice of restorative dentistry including pulpless teeth and root canal treatment, all were considered as the main cause of the various systemic illnesses, due to the systemic spread of infection from the tooth, which was suspected as main focus of infection. However, it was later refuted as the earlier studies were flawed by inadequate controls, the use of massive doses of bacteria, and bacterial contamination of endodontically treated teeth during tooth extraction. This theory is no longer valid.

2) In 1939, Fish recognized four zones of reaction formed in response to viable bacteria implanted in the jaws of guinea pigs. The zones, from inside to outside are as follows:

   - Zone of infection: It is characterized by polymorphonuclear neutrophil leukocytes.
   - Zone of contamination: Containing inflammatory cells but no bacteria.
   - Zone of irritation: Contained histocytes and osteoclasts. In this area the histologic picture is one of much activity preparatory to repair.

- Zone of stimulation: It contains mostly fibroblasts, capillary buds, and osteoblasts, signifying that at the periphery the toxin was mild enough to be a stimulant.

Fish theorized that the significance of removal of the nidus of infection would lead to resolution of the infection. This theory became the basis for successful root canal treatment.

Check Your Progress 2

1) The most common cause of pulp injury is carious ingress. The treatment of pulp capping is based on the rationale to remove all infected hard and soft tissue and to restore the tooth with a bacteria-tight restoration in order to preserve the health of the residual pulp tissue.

2) The ingress of bacteria in the dentin is restricted because of the following reasons:

   - outward flow of dentinal fluid (pulpal pressure is 24-30 mmHg),
   - presence of odontoblast processes,
   - presence of mineralized crystals,
   - Macroglobulins including immunoglobulins in the tubules.

Check Your Progress 3

1) The endodontic infections are polymicrobial in nature. The bacteria micro-organisms most commonly found in root canal are obligate anaerobes. Of the varied number of micro-organisms only a limited number have been consistently isolated from endodontic infections. These include species of the genera Streptococcus, Fusobacterium, Prevotella, Porphyromonas, Eubacterium, Peptostreptococcus, Bacteroides, and Lactobacillus.

2) The bacteria found most frequently from infected root canal is a black pigmented bacteria Prevotella nigrecens.

3) Nutrient supply for the microbes may be derived from:

   - oral cavity;
   - degenerating connective tissue;
   - dentinal tubule contents;
   - a serum-like fluid from periapical tissue.

4) The aggregations of micro-organisms into communities at the surface interfaces, which exhibit well-defined structures and succession, are called biofilms. The various defense mechanisms involved by micro-organisms in the biofilms are:

   - the exopolysaccharide in which the bacteria are embedded may restrict diffusion of the antibacterial agents to the cells;
   - the different layers of cells similarly act as barriers to diffusion;
   - some bacterial cells may be slower-growing or dormant and therefore

may be more resistant to killing;

- cells may exhibit specific resistant mechanisms;
- biofilm phenotypes may be inherently resistant.

Check Your Progress 4

1) Enterococcus faecalis is the most frequent bacteria implicated from failed root canal treatment, it may be contributed due to :
   - Although most root canal bacteria are sensitive to the high pH of calcium hydroxide, it is known to have a capacity to resist a high pH.
   - It may undergo a period of starvation that may be crucial for survival.
   - An ability to utilize collagen within dentine may also be useful and there are indications that E. faecalis may have this property.
2) The major four factors that contribute to persistence of periapical radiolucency after treatment are as follows :
   - intraradicular infection;
   - extraradicular infection by bacteria of the species Actinomyces israelii and Propionibacterium propionicum;
   - foreign body reaction;
   - cysts, especially those containing cholesterol crystals.

Check Your Progress 5

1) A number of virulence factors that may be associated with disease. They include pili (fimbriae), capsules, extracellular vesicles, lipopolysaccharides, enzymes, short-chain fatty acids, polyamines, and low-molecular- weight products such as ammonia and hydrogen sulfide.

Check Your Progress 6

1) The various drawbacks seen with the cultivation methods are:
   - They are scarcely able or unable to grow many micro-organisms, thus may lead to false-negative and underestimation of the pathogenic micro-organisms.
   - They are slow to provide diagnostic result.
   - They have low specificity and therefore have limitations in distinguishing among microbial species and strains.
   - They have low sensitivity i.e., failure to detect small numbers of micro-organisms.
   - They strictly depend on the mode of sample transport.
2) The various molecular methods for detection of endodontic pathogens are:
   a) DNA-DNA hybridization methodology- these methods use DNA probes.

b) Polymerase Chain Reaction Method- It involves invitro replication of DNA and has often been referred to as the "Genetic Xeroxing". A lot of variations of PCR are now available, such as Single PCR, Reverse Transcriptase (RT) PCR, Nested PCR, Real-Time PCR.

3) The advantages of molecular detection methods are as follows:

- They can detect both cultivable and uncultivable microbial species and strains.
- They have greater specificity.
- They can detect microbial species directly in clinical samples, without the need for microbial.
- They are more sensitive than other identification methods.
- They are usually less time-consuming.
- They do not require carefully controlled anaerobic conditions during sampling and transportations.
- They can be used during antimicrobial treatment.

2.8 FURTHER READINGS

Endodontics (5th Edition) by Ingle.

Pathways of Pulp (8th Edition) by Stephen Cohen.

Endodontic Practice (11th Edition) by Gross man.

Endodontics (5th Edition) by Weine.