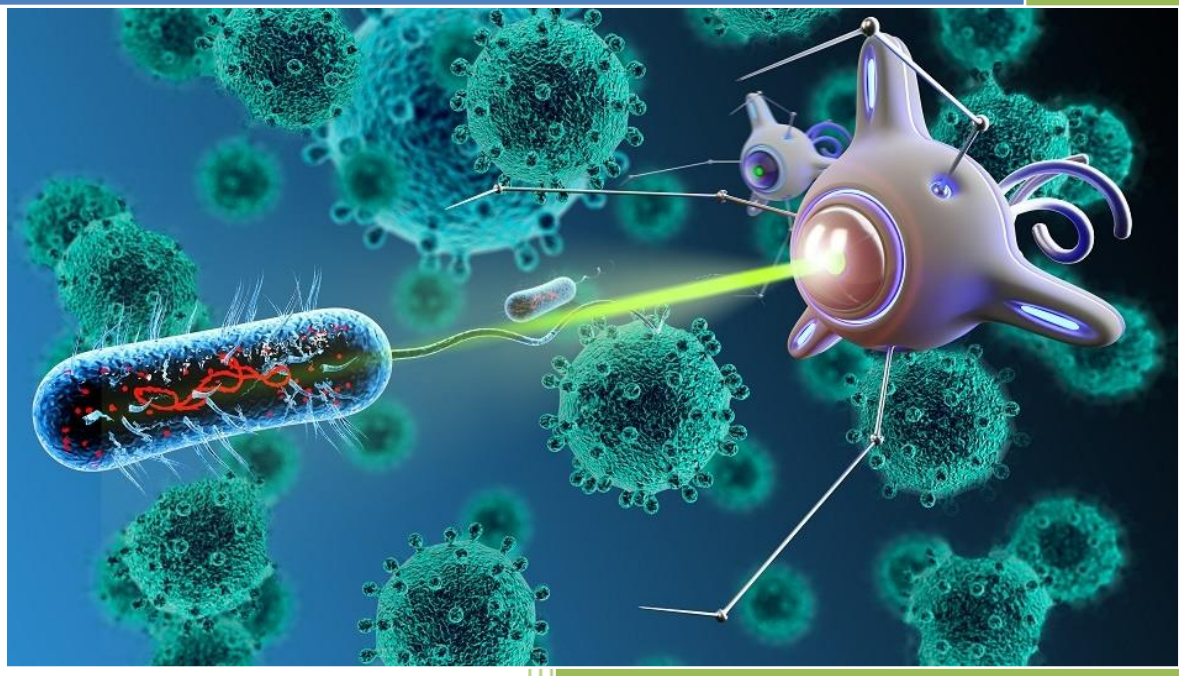
2019

NANOTECHNOLOGY IN ENDODONTICS



DR. RICHA MODI
DR. MANOJ CHANDAK
DR. RAKESH GOGIYA
DR. ABHILASHA DASS MISHRA

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NANOTECHNOLOGY IN ENDODONTICS
"THERE IS PLENTY OF ROOM IN THE BOTTOM"

DR.RICHA R.MODI
DR. MANOJ CHANDAK
DR. RAKESH GOGIYA
DR. ABHILASHA DASS MISHRA

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Preface

Today's world is full of modern research and rapid advances in dentistry which helps students with a wealth of ever increasing literature. No attempt has been made yet to make a comprehensive text on the subject of nanotechnology in the endodontics. Readers will be benefitted by having the knowledge of various nanoparticles used in endodontics mentioned in this monograph. We are thankful to those who have contributed to bring out this monograph with the hope that this venture proves useful to those for whom it is meant.

Sincerely,

Dr. Manoj Chandak (Professor)

Department of Conservative and Endodontics,

Sharad Pawar Dental College, Sawangi, Wardha, Maharashtra,

India.

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Authors

DR.RICHA MODI

Third Year Postgraduate, Department of Conservative Dentistry and Endodontics, Sharad Pawar Dental College, Sawangi (Meghe) Wardha, Maharashtra, India.

Email-richa.modi55@gmail.com

DR. MANOJ CHANDAK

Professor,Department of Conservative Dentistry and Endodontics, Sharad Pawar Dental College, Sawangi (Meghe) Wardha, Maharashtra, India.

Email-drmanojchandak@yahoo.com

DR. RAKESH GOGIYA

Third Year Postgraduate, Department of Conservative Dentistry and Endodontics, Sharad Pawar Dental College, Sawangi (Meghe) Wardha, Maharashtra,India.

Email- rakesh.gogiya@gmail.com

DR. ABHILASHA DASS MISHRA

Lecturer, Department of Conservative Dentistry and Endodontics, Sharad Pawar Dental College, Sawangi (Meghe) Wardha, Maharashtra,India.

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CHAPTER 1 – INTRODUCTION

Nanomaterials are the particles of size ranging from 1–100 nm. (1)

Nanodentistry consists of nanomaterials and nanorobots. In endodontics, these advancements are used to reduce the antimicrobial load from the root canal. (2)

Prof. Kerie E. Drexeler, in 1980's first introduced the word nanoparticles derived from Greek word which means 'dwarf. (3)

Physicist Dr Richard Feynman in 1959 introduced the concept of NANOTECHNOLOGY. The idea was entitled as "There's Plenty of Room at the Bottom" and presented at California Institute of Technology.

Japanese scientist Dr. Nori Taniguchi in 1974 defined nanotechnology as "the processing of separation, consolidation, and deformation of materials by one atom or one molecule".

Dr. Drexler further studied and published a book titled "Engines of Creation-The Coming Era of Nanotechnology" around late 1980s. ´ In 1991, the publication by Dr SumioLijima "Helical microtubules of graphitic carbon" introduced the concept of nanotubes and boosted nanomaterials research . ´

DRR.A FREITAS in 2000 coined the term "Nano Dentistry"

On the basis of application, there are 4 approaches of nanotechnology in dentistry.

1.Top down approach 2.Bottom up approach 3.Functional approach 4.Biomimetic approach

Nanoparticles are developed through two approaches, either top down or bottom up.

Top down approach: In this, the bulk material are sliced or cut down into pieces until it reaches the size of a nanoparticles.

Eg. Nanocomposites, nanotweezers, impression materials, nanosolutions (bonding agents)

These are synthesized by mechanical milling or by electro explosion. (4)

Bottom up approach: This approach refers to a method of building nanoparticles atom by atom, molecule by molecule, in order to achieve desired properties.

Eg. Micron size dental robot containing active local anesthetic, nanorobotic dentifrices for hypersensitivity cure. (5)

These are synthesized by atomic or molecular condensation or by laser pyrolysis.(6) (FIGURE 1)

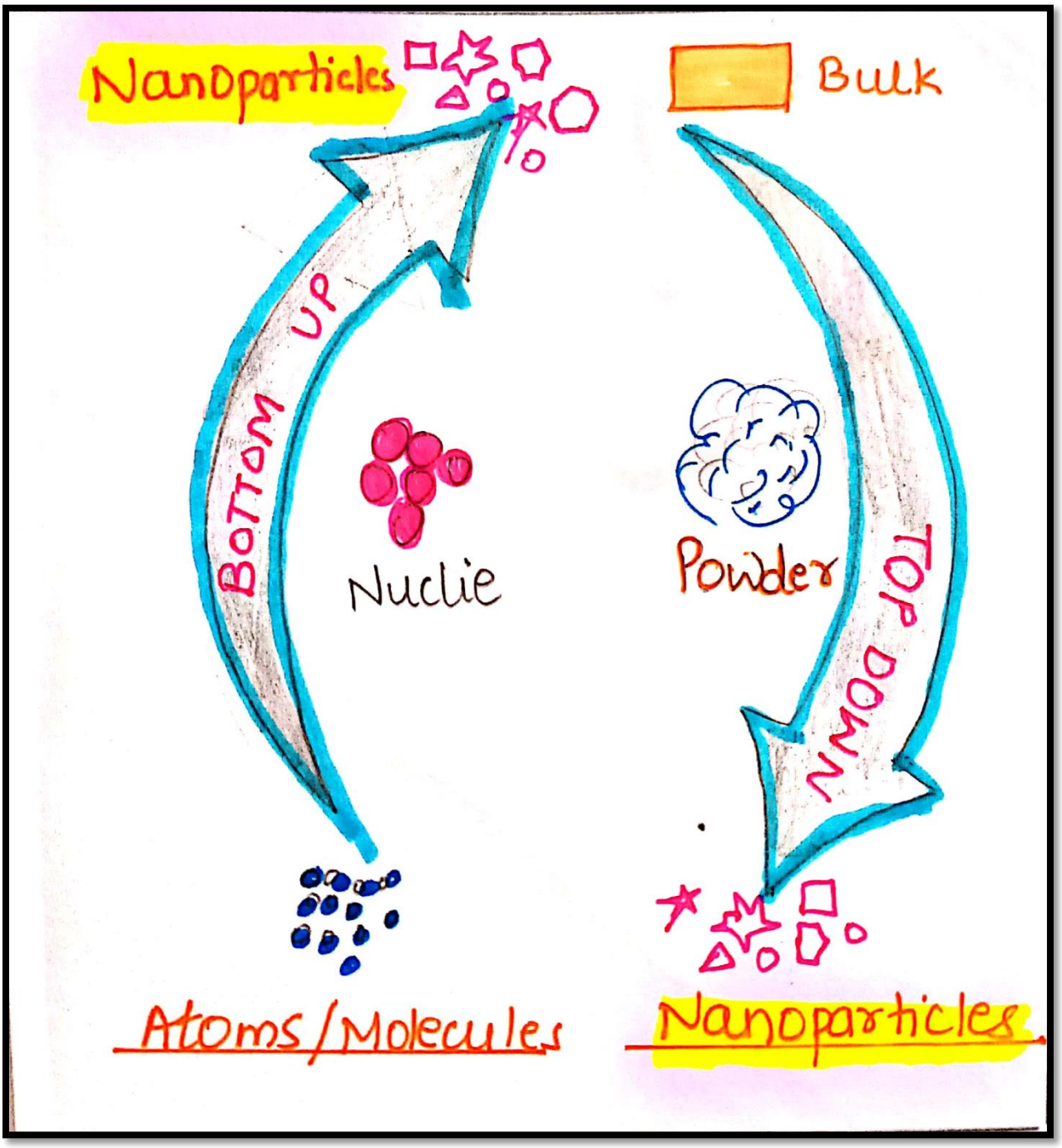


FIGURE 1: SHOWING TOP DOWN AND BOTTOM UP APPROACH

Nanoparticles are composed of three layers-

(a) **The Uppermost layer also called as surface layer**, is functionalized with a variety of small molecules, metal ions, surfactants and polymers.

When biomolecules are conjugated with the nanoparticles, the term biofunctionalization has been used. Functionalization alters the surface structure and composition of the material while keeping the properties of the core material intact.

(b) **The shell layer**, which is chemically different material from the core in all aspects, and

(c) **The core**, which is essentially the central portion of the NP and usually refers the NP itself (Shin et al., 2016) (7)

Classification of nanoparticles:

(1)Based on the composition

Naturally occurring

Synthetic

(2) Based on nature

Organic- alginate and chitosan

Inorganic- Zinc oxide, Iron oxide, Titanium dioxide, Aluminum oxide bioactive glass

(3)Based on the shape

Particles

Spheres

Tubes

Rods

Plates

Nanoparticles are unique in size, shape and structure. They are rigid and stable. They have high surface area and nanoscale size.

Nanotechnology provides a good platform for developing important metal properties in the form of nanoparticles. These are effective in various fields such as diagnostics, antimicrobial agents, drug delivery systems and for treatment of various diseases. Therefore researches have shifted towards the use of nanoparticles to combat multi drug resistance. (8)

Since antiquity silver has been a potent antimicrobial agent. It is active against pseudomonas, E coli, streptococci pyogens. Silver ions have the capability to denature the proteins and destruct the bacterial DNA. Various compounds of silver such as silver zeolite, silver sulfadiazine, and silver nitrate have been used in different zones. (9)

Silver nitrate has application in the treatment of burns venereal diseases and eye drops.

Silver sulfadiazine in a concentration of 1% as water soluble cream is broad spectrum bactericidal and mainly used for treating burn wounds. (10)

Silver zeolite is bactericidal compound used for food preservation and disinfection of medical products. (11)

Silver nanoparticles are the nano size materials that have different properties than the bulk size materials. The various metallic nanoparticles such as copper, gold, magnesium, titanium, have strong antibacterial properties owing to their large surface. (12)

CHAPTER 2 - BIOFILMS:

Biofilm consists of various microbial colonies that are adhered to each other and to the substrate by the extracellular matrix secreted by them i.e. self made..

Characteristics of biofilm ¨ Biofilms should possess

1. Autopoiesis- ability to self organize

2. Homeostasis- resists environmental perturbations

3. Synergy- effective in association than in isolation ¨

4. Community- responds to environmental changes as a unit rather than single individual (13)

Stages in development of biofilm:

Formation of conditioning layer i.e. adsorption of macromolecules

Adhesion and co adhesion – initial phase includes nonspecific microbial –substrate adhesion, later stages include specific microbial –substrate adhesion

Bacterial growth and microbial expansion i.e multiplication of microorganisms.

Detachment and dispersion phase. (14) (FIG

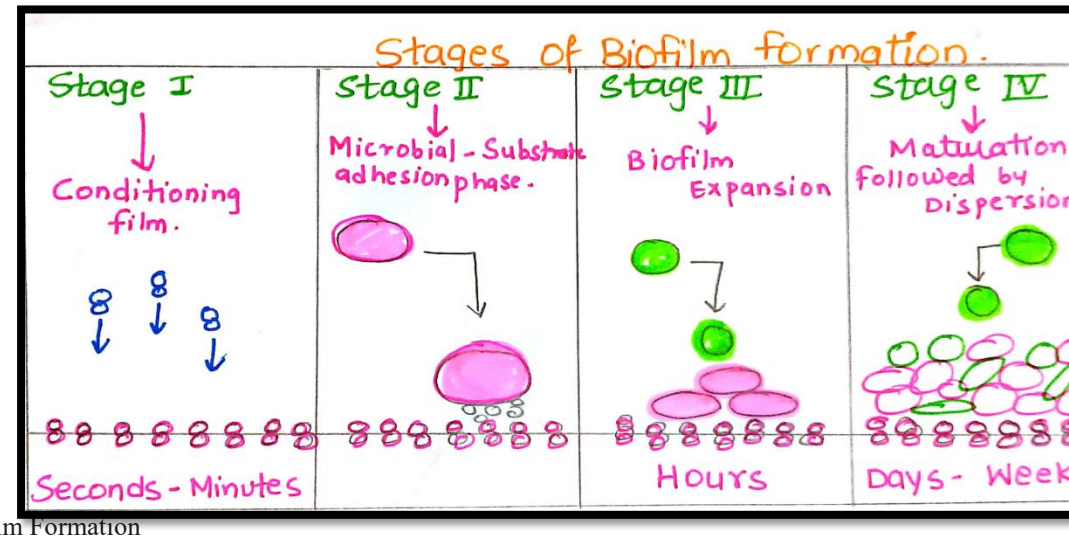


Figure 2: Stages In Biofilm Formation

Various types of biofilms include:

Intracanal biofilm

Extra radicular biofilm

Periapical biofilm

Biomaterial centered biofilm

Endodontic microbiology can be classified as-

Gram positive micro organisms— Streptococcus, Enterococcus, Candida , Actinomyces , Lactobacillus

Gram negative micro organisms – Fusobacterium, Neisseria, Campylobacter, Bacteroids ,Veilloniela.,etc. (15)

How to combat microbes in the endodontic therapy?

Through cleaning and shaping of the root canal system.

Oxygenating the canal simply by opening it is detrimental to anaerobes.

Antibiotics

Intracanal medicaments and sealers.

METHODS TO ERADICATE BIOFILMS:

Sodium hypochlorite

Chlorhexidine glucunate

QMix 2 in 1 (mixture of 17% EDTA ,2% CHLORHEXIDIE, TWEEN 80 detergent.)

EDTA

MTAD, Tetraclean

Calcium hydroxide

Ultrasonically activated irrigation, Endoactivator

Ozonated water (Viera et al in 1999 reported that 0.1 to 0.3 ppm concentration is able to completely kill bacteria in 15 to 30 minutes of contact time).

Lasers ,Plasma dental probe

Photoactivted disinfection

Antibacterial Nanoparticles. (16)

CHAPTER 3 - ANTIBIOTIC RESISTANCE:

Antibiotic resistance is a constant threat and a major hindrance in the success of root canal therapy. The major cause for this is its widespread use which causes untoward effects on commensal microbial flora. (17)

Antibiotic resistance often leads to superinfection. Therefore search for an alternative option is constantly rising and search of the newer antibiotics are declining. (18)

Various antibiotic resistant strains are – methcillin resistant staphylococcus aureus, vancomycin reisistant enterococci.

Mechanisms of antibiotic resistant:

Exchange of genetic materials: Changes in the composition or structure of the target in the bacterium (resulting from mutations in the bacterial DNA) can stop the antibiotic from interacting with the target.

Deficiency of specific porin channels.

Promotion of active drug efflux i.e. Pump the antibiotic out from the bacterial cell. (20)

Thickening of the peptidoglycan layer of the outer wall.

Destroy the antibiotic**:** There are bacterial enzymes that can inactivate antibiotics. One example is β-lactamase that destroys the active component (the β-lactam ring) of penicillins.

Modify the antibiotic**:** Bacteria can sometimes produce enzymes that are capable of adding different chemical groups to antibiotics. This in turn prohibits binding between the antibiotic and its target in the bacterial cell.

CHAPTER 4 –CHITOSAN NANOPARTICLES

Chitin is a principal component of crustacean exoskeletons. It is a non-toxic cationic biopolymer. It is synthesized from alkaline deacytelation of chitin. (21)

It basically involves contact mediated killing by electrostatic attraction. Chitosan forms an impermeable layer around the bacterial cells thus cutting off their nutrient supply.

The structure of chitin closely resembles cellulose. It can be formulated in various forms such as scaffolds, hydrogels, powder, films and capsules.

Its structure is similar to extracellular ground substance and therefore can be used to reinforce the collagen meshwork.

Properties of chitosan:

Biocompatibility (non toxic towards mammalian cells)

Color compatibility to tooth structure

Chelating capacity

Antimicrobial effects against a broad range of gram-positive and gram-negative bacteria as well as fungi

Remove the smear layer from a root-dentin surface and simultaneously inhibit bacterial recolonization

Insoluble in most solvents,(insolubility in water)

Soluble in dilute organic acids such as acetic acid, formic acid, succinic acid, lactic acid, and malic acid (22). The Degree of Deacytelation (DD) is known to influence the antibacterial activity. With higher DD, chitosan showed higher antibacterial efficacy.(23) Kishan et al. and Shertha et al. showed that chitosan nanoparticles can completely eliminate E. faecalis pathogens present in a planktonic state, and can cause a significant reduction of bacteria in the biofilm state.(24)

Chitosan nanoparticles were incorporated into a zinc oxide eugenol based sealer to be effective against E. faecalis biofilm on bovine root dentine. Chitosan nanoparticles engulf the bacteria by surrounding it. (FIGURE 3)

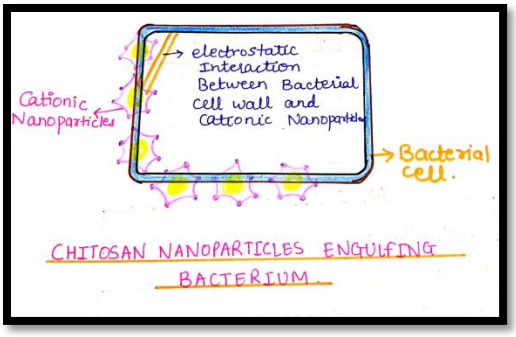


FIGURE 3: MECHANISM OF ACTION OF CHITOSAN NANOPARTICLES.

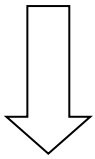
CHAPTER 5– SILVER NANOPARTICLES

"Javidi et al. evaluated the antimicrobial effect of $Ca(OH)_2$ with and without silver nanoparticles on E. faecalis from root canals. The study result found that the number of CFUs observed after the use of $Ca(OH)_2$ plus silver nanoparticles suspension was significantly less than the number observed with $Ca(OH)_2$alone."(25)

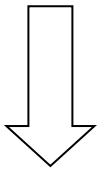
In early 1800, silver was available in different forms such as metallic silver, silver nitrate, and silver sulfadiazine, for treating burns and severe bacterial infected wounds and injuries. But due to the advent of antibiotics the use of these compounds declined. The use of silver reappeared in the form of nanoparticles.

MECHANISM OF ACTION:

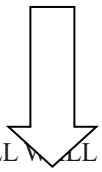
ACTS ON MULTIPLE TARGETS



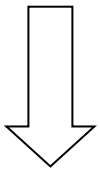
STARTING FROM SULHYDRYL GROUP OF PROTIENS AND DNA



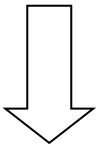
ALTER THE HYDROGEN BONDING/RESPIRATORY CHAIN



INTERFERE WITH CELL WALL SYNTHESIS



INCREASE PERMEABILITY OF CELL



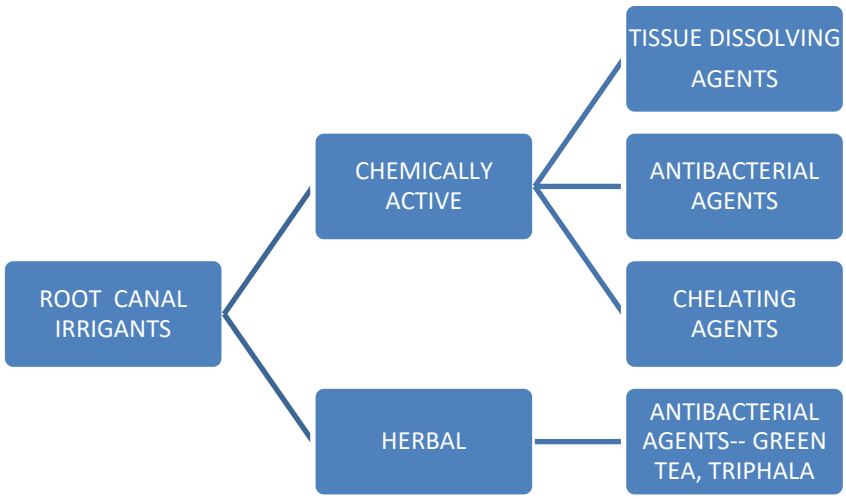
CAHPTER 6-- NANOPARTICLES AS ROOT CANAL IRRIGANT:

Root canal irrigants are the chemically active solutions that are used along with the mechanical instrumentation to disinfect the canals. A large portion of the root canal system remains untouched by mechanical preparation therefore use of irrigants are an important step in disinfecting the hard to reach areas of complex root canal anatomy .

Functions of root canal irrigants:

- Lubrication
- Emulsification
- Dissolve nectrotic tissues
- Removes debris
- Germicidal
- Bleaching action

Classification of root canal irrigants :



TISSUE DISSOLVING AGENTS – e.g. sodium hypochlorite

ANTIBACTERIAL AGENTS – chlorhexidine , MTAD

CHELATING AGENTS – EDTA,HEBP

RECENT ADVANCEMENTS –

"MTAD (Torabinejad *et al*. developed a irrigant with combined chelating and antibacterial properties.MTAD is a mixture of 3% doxycycline, 4.25% citric acid, and detergent (Tween-80).)"(26)

Tetraclean

SILVER NANOPARTICLES SOLUTION

ZINC OXIDE NANOPARTICLES SOLUTION

MAGNESSIUM OXIDE NANOPARTICLES SOLUTION

Electrochemically activated solutions

Ozonated water

Photon-activated disinfection

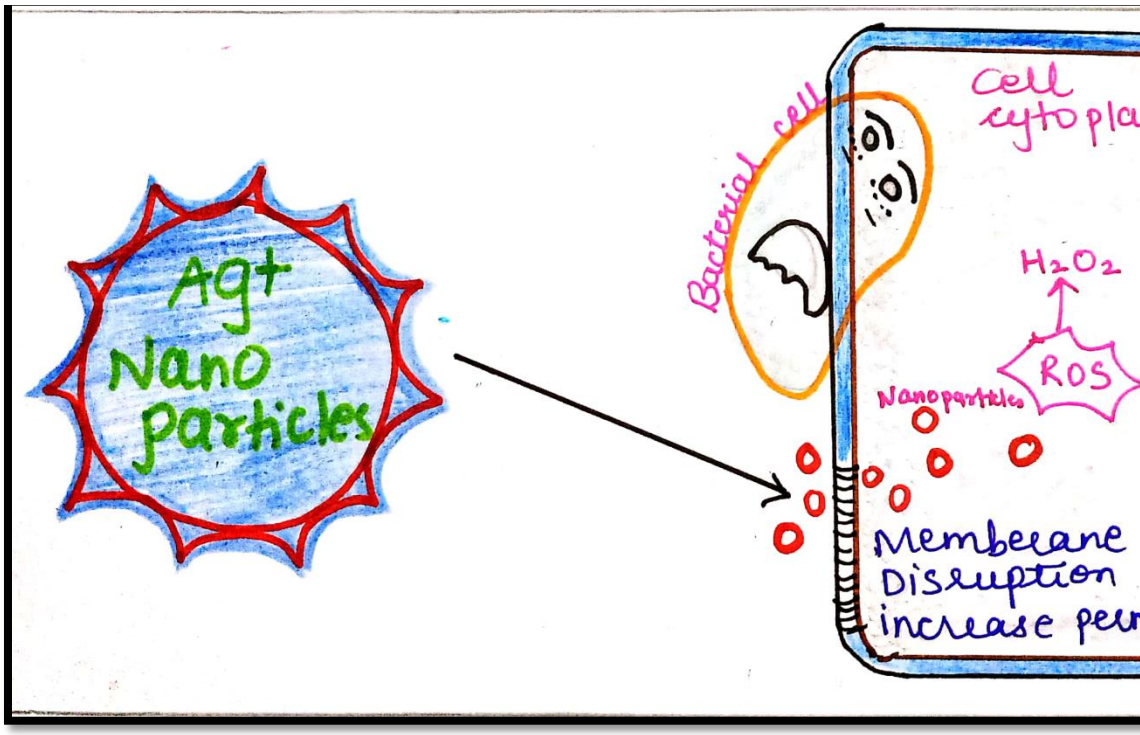
Herbal irrigants.(Green tea polyphenols, the traditional drink of Japan and China is prepared from the young shoots of the tea plant Camellia sinensis. (27)

SILVER NANOPARTICLES:

"A 3.8% w/v silver diamine fluoride (Ag $[NH_3]_2F$) solution has been developed for intracanal irrigation. This represents a 1:10 dilution of the original 38% Ag $(NH_3)_2$ F solutions used for root canal infection.
The study on the antibacterial effect of 3.8% $Ag(NH_3)_2F$ against a E faecalis biofilm model concluded that $Ag(NH_3)_2F$ has potential for use as an antimicrobial root canal irrigant or interappointment medicament to reduce bacterial loads."(28)

Around 1 hour is required by $Ag(NH^3)^2F$ to kill E.Feacalis (29)

The silver deposits blocks the tubular openings after removal of the smear layer.(30)



The antimicrobial properties of silver nanoparticles were first demonstrated by Jose Ruben *et al*.

Wu *et al*. evaluated the effect of silver nanoparticles in a concentration of 0.1% as an endodontic irrigant solution and as a gel in two different concentrations (0.02% and 0.1%) against *Enterococcus faecalis*biofilm.(31)

The solution did not cause any major change to the structure of *E. faecalis*biofilm.

However, the use of silver nanoparticles in a gel form with a concentration of 0.02% had the ability to disrupt the structural integrity of the *E. faecalis* biofilm.(32)

Zinc oxide nanoparticles (ZnO-NPs):

Creates high pH environment.

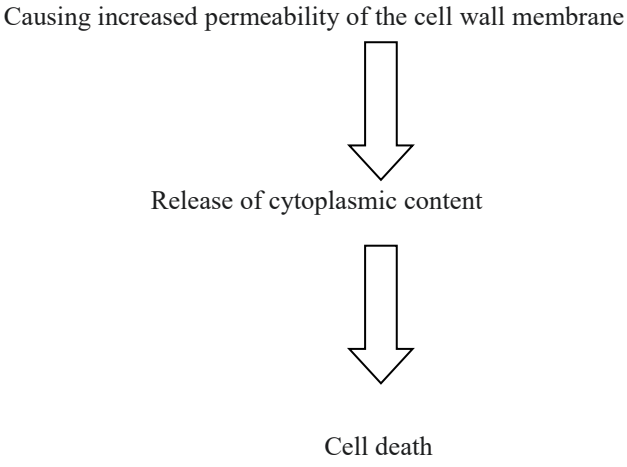
Higher antibacterial efficacy (33)

Production of reactive oxygen species such as hydrogen peroxide when in contact with an aqueous medium.

More effective on gram positive bacteria than on gram negative

Used in the concentration of 50 ppm

MECHANISM OF ACTION:



Magnesium-containing nanoparticles (Mg-NPs):

Magnesium-oxide nanoparticles were found to be bactericidal when present in an aqueous form as a result of the action of superoxide anions that formed on the bacterial cell surface.

The antibacterial efficacy of different concentrations of magnesium oxide nanoparticles (5 mg/L and 10 mg/L) and 5.25% sodium hypochlorite and 2% chlorhexidine against endodontic pathogens such as *E. faecalis*, *S. aureus* and *Candida albicans*was studied by Monzavi*et al*.

The results showed no significant differences in the antimicrobial efficacies of the irrigant solutions used against the tested endodontic pathogens. However, the inclusion of magnesium oxide nanoparticles in an irrigant solution produced extended antibacterial activity when compared with sodium hypochlorite.

CHAPTER 7: NANOPARTICLES AS INTRACANAL MEDICAMENT:

Intracanal medicaments are the inter appointment chemical adjunct for cleaning and disinfecting the canal system. The main objective of the intracanal medicament is to reduce inter appointment pain and reduce the overgrowth of microorganisms in root canal.

Various chemical agents have been tried as an intracanalmediacament.

According to Grossman (1990), intracanal medicaments can be classified as

1.Essential oils • Eugenol

2.Phenolic compounds • Phenol • Parachlorophenol • Camphorated para chlorophenol • Cresol • Formocresol • Creosote • Cresatin • Cresanol

3. Halogens • Sodium hypochlorite • Iodides • Chlorhexidine

4.Calcium hydroxide

5. Antibiotic medications

Ledermix paste (mixture of 1% triamcinilone and 3.2% demeclocycline)

Septomixine forte paste (Neomycin, Polumixin B and hydrocortisone)

Pulpomixine paste

Sulphonamides.

6. Combining medicaments

Ledermix and Calcium hydroxide

Calcium hydroxide and silver nanoparticles

Zinc oxide nanoparticles

IDEAL REQUIREMENTS OF ROOT CANAL MEDICAMENTS

"1. It should be an effective germicide and fungicide.

2. It should be non irritating to the periapical tissues

3. It should remain stable in solution.

4. It should have a prolonged antimicrobial effect.

5. It should be active in the presence of blood, serum and protein derivatives of the tissues.

6. It should have low surface tension.

7. It should not interfere with repair of periapical tissues.

8. It should be capable of penetrating the tissues deeply

9. It should not stain tooth structure.

10.1t should be easily introduced into the canal

11. It should not induce cell mediated immune response

12. It should be capable of being inactivated or neutralized in culture medium

13. Inexpensive and long shelf life." (34)

Calcium hydroxide is considered as the most efficient root canal medicament, but it is ineffective against well established E.faecalis biofilm. Various combinations have been tried with calcium hydroxide such as calcium hydroxide with chlorhexidine, calcium hydroxide with silver nanoparticles.
AgNPs should ideally be used as medicament rather than as an irrigant as a prolonged interaction time is required by Ag- NPs for effective bacterial killing.

Different studies had shown that using Silver or Copper alongside Calcium hydroxide improved Antibacterial properties.8-12 Metal oxides have been used for centuries as antibacterial agents. Metal oxides like Zinc Oxide and Magnesium Oxide have antibacterial properties in various forms.

BIOACTIVE NANOPARTICLES:

Bioactive glass has antibacterial properties. It consists of SiO2 , Na 2 O, CaO 2, and P 2 O 5 at different concentrations
“Nanometric bioactive glass ranges from 20 to 60 nm in size. The increase in pH is mainly responsible for the antimicrobial activity. Furthermore, the release of Ca 2+ , Na + , PO4 3− , and Si 4+ could lead to formation of bonds with the mineralized hard tissues”.(35)

Antibacterial Properties

The antibacterial mechanism of BAG has been attributed to several factors acting together :

- Alkaline pH
- Osmosis
- Induce mineralization on bacterial surface due to high calcium and phosphate content.

BAG was used for in vitro root canal disinfection studies.(36)

BAG nanometric slurry had a 12-fold higher specific surface area than the micrometric counterpart.

Nano BAG has ten folds more silica release capacity and thus has got highpH. (37)

CHAPTER 8: NANOPARTICLES MODIFICATION FOR PHOTODYNAMIC THEORY:

SEM studies have shown that bacteria can penetrate to a depth of 1000 um in the dentinal tubules. (38)

Presence of smear layer during and after instrumentation has reduced the penetration of intracanal medicaments and irrigants. High proportions of gram positive and facultative anaerobe reside in the complex root canal anatomy and lead to secondary infections or apical periodontitis. (39)

“PDT is based on the concept that non-toxic photosensitizers can be preferentially localized in certain tissues and subsequently activated by light of the appropriate wavelength to generate singlet oxygen and free radicals that are cytotoxic to cells of the target tissue.”(40)

"The concept of photodynamic inactivation requires microbial exposure to either exogenous or endogenous photosensitizer molecules, followed by visible light energy, typically wavelengths in the red/near-infrared region that cause the excitation of the photosensitizers resulting in the production of singlet oxygen and other reactive oxygen species that react with intracellular components and consequently produce cell inactivation and death."(41)

The mechanism of damage is based on both oxygen tension.

It was first introduced by Wainwright in **1998**.

In dentistry it was introduced by Bergmans *et al*. **2008**.

A photosensitizer (PS) is a dye with the capacity to absorb energy from a light source and transfer this energy to another molecule (Plaetzer *et al*. **2009**).

Dyes most commonly used are phenothiazine salts, namely toluidine blue O (TBO) and methylene blue (MB), with wavelengths of absorption of 600–660 nm. (42)

Antimicrobial efficacy is due to high-power diode laser. (43)

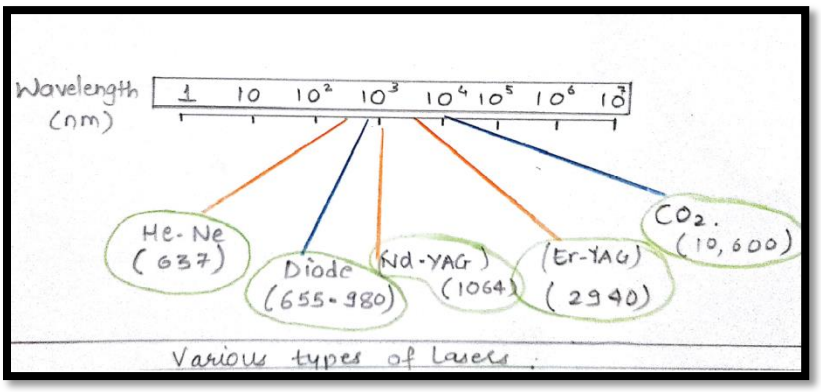


Figure 4: Showing different types of LASERS

Recently, the studies have focused on photosenstizers encapsulated in polymer based nanoparticles. "The advantage of this is --

1) A larger critical mass (concentrated package of photosensitizer) for the production of reactive oxygen species that destroy cells,

2) Limit the target cell's ability to pump the drug molecule back out thus reducing the possibility of multiple-drug-resistance,

3) Selectivity of treatment by localized delivery agents, which can be achieved by either passive targeting or by active targeting via the charged surface of the nanoparticle, and

4) The nanoparticle matrix is non-immunogenic. Engineered biodegradable polymeric nanoparticles, made R. Drug delivery anof FDA-approved poly(lactic-co-glycolic acid) (PLGA).

5) Control the release of the photosensitizer molecules."(44)

Once encapsulated within PLGA, the excited state of the photosensitizer is quenched, which results in loss of phototoxicity . When the nanoparticles were incubated with the targeted cells, they showed a time-dependent release of the photosensitizer, which then regained its phototoxicity and resulted in an activatable PDT nanoagen.(45)

The nanoparticle matrix PLGA is a polyester co-polymer of polylactic acid (PLA) and polyglycolic acid (PGA) that has received FDA approval due to its biocompatibility and its ability to degrade in the body through natural pathways. (46)

Recently, MB-containing silica-coated magnetic nanoparticles were proposed as potential carriers for PDT.(47)

Sensitization of MB with light leads to the production of singlet oxygen (1O2), which can migrate approximately 0.02 μm after its formation, targeting important intracellular components.(48)

CHAPTER 9: NANOPARTICLES CONTAINING ROOT CANAL SEALERS:

Root canal sealers are used in conjunction with biologically acceptable semisolid or solid obturating materials to establish an adequate seal of the root canal system.

Absence of root canal sealant application of root canal sealant prevents the bacterial growth and penetration

FUNCTIONS OF ROOT CANAL SEALERS

Serves as a filler for canal irregularities and minor discrepancies between the root canal wall and core filling material.

To obturate the lateral canals

Acts as lubricant

Enhances the possible attainment of an impervious seal

Can assist in microbial control.

For radiopacity

CLASSIFICATION OF CURRENTLY EMPLOYED ROOT CANAL SEALANTS: (GROSSMAN)

Zinc oxide eugenol based sealers

Calcium hydroxide based sealers

Glass ionomer based sealers

Resin based sealers

Calcium silicate based sealers.

RECENT ADVANCES- Nanoparticles containing root canal sealers- eg. Zinc oxide nanoparticles, silver nanoparticles, chitosan nanoparticles.

ZINC OXIDE NANOPARTICLES:

Since last 8 decades zinc oxide eugenol andguttapercha has been used as a root canal filling material. Root canal sealer plays an important role in filling the spaces between the filling material and root dentinal wall.

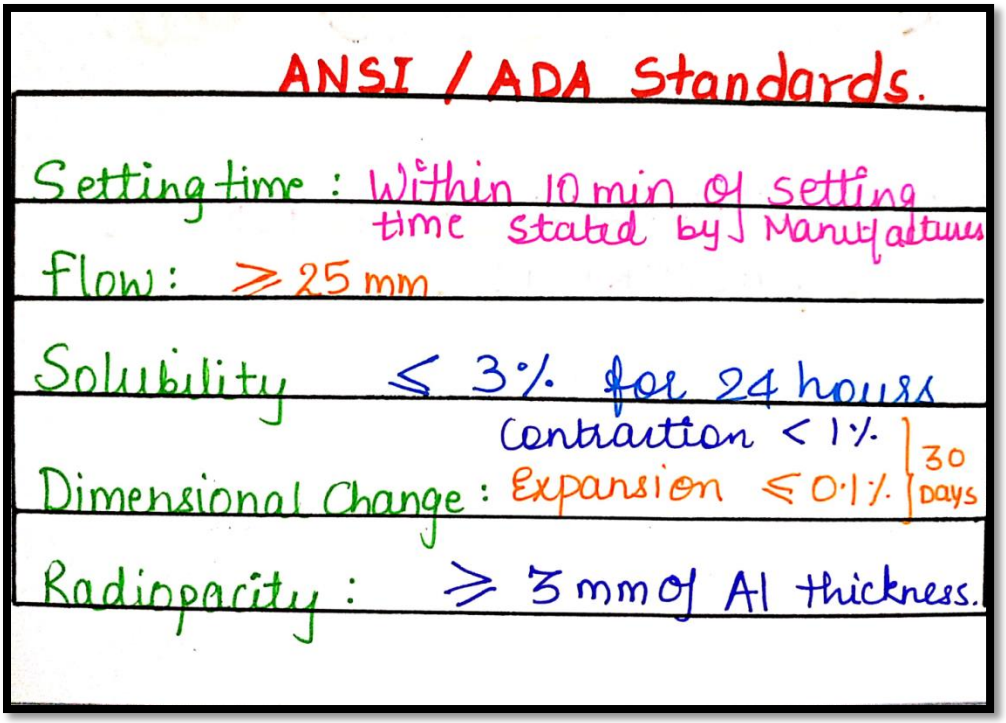
It also fills the isthmuses, fins, deltas and lateral canals.

Incorporating nanosized particles into zinc oxidebased sealer can be useful to improve its technical and practical features as well as desirable physicochemical properties for the optimal functioning of the root canal filling and sealing.

Precipitated silver used for radiopacity produced sulfides, which caused tooth discoloration., silver was eliminated from the composition, whereas zinc chloride was substituted with almond oil to avoid tooth discoloration and at the same time increase setting time. (49)

Modification of conventional zinc oxide sealers were made by adding zinc oxide nanoparticles.. This modification inhibited biofilm formation within the sealer dentin interface., reduced cytotoxicity , and improved sealing ability.(50)

"Marco Aurelio Versiani et al studied the physicochemical properties of zinc oxide based Grossman sealer s to evaluate the effect of incorporating different degrees of ZnO Np on the setting time, flow, solubility, dimensional changes, and radiopacity properties of Grossman sealer."(51)



The physicochemical properties of ZnO-Np–based sealers:

Setting time— 86 ± 2.55 min

Flow --

Solubility – 4.57%

Dimensional change—0.34%

Radiopacity – 9.08 mmAl

The replacement of 25% of conventional ZnO powder with ZnO-Np improved the setting time, flow, solubility, dimensional stability, and radiopacity of Grossman sealer, which were all in adherence to ANSI/ADA requirements. *(52) (53)*

"MTA Fillapex (Angelus dental solutions, Londrina, PR, Brazil) is another currently available calcium silicate based root canal sealer. This sealer consists of salicylate resin, diluting resin, natural resin, bismuth oxide, nanoparticulated silica, and MTA."(54)

CONCLUSION:

Nanoparticles have the antimicrobial potential. They have exclusive advantage of increased surface area which can be positively used in the field of dentistry/ endodontics.

Functionalization of nanoparticles i.e surface medication can add to its penetration capacity thus eradicating biofilms. It also has applications in drug delivery and targeted antibacterial efficacy.

The whole concept of nanotechnology in health care should be accepted with positive zeal and caution for future development.

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