

## **INTRODUCTION**

Root canal therapy is a sequence of treatment for the infected pulp of a tooth which seeks an elimination of infection and the protection of the decontaminated tooth from future microbial invasion.

Elimination of microorganisms from infected root canals is a complicated task. The chances of a favorable outcome with root canal treatment are particularly high if infection is eradicated effectively before the root canal system is obturated. However, if microorganisms persist at the time of obturation, or if they penetrate into the canal after obturation, there is a high risk of treatment failure.

Using of various instrumentation techniques, irrigation regimens, Using an intracanal medicaments, and chemical agents during instrumentation to completely clean all aspects of the root canal system is central to successful endodontic treatment. The root canal system is complex and a structure of dentine features, such as dentinal tubules, and intracanal communication, are entrapped by microorganisms. Self-aggregates of mono bacterial morpho types and co aggregates of different bacterial morpho types are also found a dhering to teeth.

Bacteria within a biofilm have increased resistance to a variety of external hostile influences, such as the host defense responses, antibiotics, antiseptics, and shear forces, compared with isolated bacterial cells.

Charcoal (Carbon) is one of the most exist elements in nature and is the second most abundant element in the human body by mass (about 18.5%) after oxygen. Due to its high microporosity, just one gram of activated carbon has a surface area in excess of 3,000 m<sup>2</sup>, as determined by gas adsorption. Its useful application may be attained from high surface area; however, further chemical treatment often enhances adsorption properties.

The objective of this study is to investigate in vitro the feasibility of using an activated charcoal (carbon) treatment in infected root canals with complex microbes biofilm, which is a major challenge in endodontic treatment.

## **REVIEW OF LITERATURE**

**This part of the study is reviewing the literature regarding:-**

A. Anti-bacterial effects of activated (charcoal) carbon.

B. Antibacterial effect of calcium hydroxide.

### **A. Anti-bacterial effects of activated (charcoal) carbon:**

**Shi et al.(2007)<sup>1</sup>** functionalized Activated carbon (AC) with two types of quaternary ammonium groups to achieve antibacterial properties. The first route utilized covalently coupled 3-(trimethoxysilyl) propyldimethyloctadecyl ammonium chloride (QAS) on the AC surface, while the second route employed a polycation, poly-(vinyl-N-hexylpyridinium bromide). They found that both types of functionalized ACs show highly effective antibacterial activities against *Escherichia coli* and *Staphylococcus aureus*. Furthermore. Also, they investigated the adsorption capacity of the different ACs. They found that the different degrees of decrease in the adsorptive capacity of the two types of functionalized ACs can be related to the changes in the surface area and pore size distribution arising from the different functionalization routes.

**Arias and Yang (2009)**<sup>2</sup> studied the antimicrobial activities of carbon nano-tubes with different walls, single-walled nano-tubes (SWNTs) and multi walled carbon nano-tubes (MWNTs) with different surface groups to different bacteria. They found that, SWNTs' antimicrobial activity increased with time and concentration. SWNTs with surface functional groups like hydroxyl ions and carboxylate displayed very strong antimicrobial activity to both Gram-positive and Gram-negative bacterial cells in distilled water and normal solution. They mentioned, antimicrobial activities of SWNTs-OH and SWNTs-COOH showed their antimicrobial activity at approximately 50 micro g/mL. SWNTs-NH<sub>2</sub> only displayed antimicrobial activity at higher concentrations. MWNTs with surface groups of -OH, -COOH, and -NH<sub>2</sub> did not present any significant antimicrobial activity to all tested bacterial cells in any of the tested buffers at high concentrations. SWNTs – NH<sub>2</sub> only exhibited antimicrobial activity at higher concentrations.

**Liu et al. (2009)**<sup>3</sup> studied antibacterial activity of single dispersed purified single-walled carbon nano-tube and the results demonstrated that single purified SWCNTs were more toxic than SWCNT aggregates toward bacteria (gram-negative *Escherichia coli*, *Pseudomonas aeruginosa*, and gram-positive *Staphylococcus aureus*, *Bacillus subtilis*). They suggested that inhibiting cell growth of

cells and oxidative stress were not the major causes responsible for the death of cells. They proved that the death rates of bacteria were strongly correlated with their mechanical properties; soft cells were more vulnerable to SWCNT piercing. They concluded that the antimicrobial effects of SWCNTs can be improved by enhancing the SWCNT physical damage on bacteria in the these ways: (1) scattered individual SWCNTs (2) increasing SWCNT concentration and (3) increasing the shaking speed of incubation to speed up the nano darts.

Venkata et al. (2009)<sup>4</sup> provided a comprehensive review on application of Carbon nanotube CNTs as adsorbent media to concentrate and remove pathogens, natural organic matter NOM, and cyanobacterial (microcystin derivatives) toxins from water systems. They also surveyed on consideration of CNT based adsorption filters for removal of these contaminants from cost, operational and safety standpoint. Based on the studied literature, they found that support point of use (POU) \ based CNT technology looks promising, that can possibly facilitate treating biological contaminants in conventional water treatment plants.

**Vecsiti et al. (2010)<sup>5</sup>** observed that Single-walled carbon nano-tubes (SWNTs) is strong antimicrobial material, and coated SWNT can significantly reduce

biofilm formation. However, antimicrobial mechanism of SWNT is not completely understood. They suggested that membrane stress (i.e., direct contact with bacteria resulting in membrane irregular movement and the release of intracellular contents) was the primary cause of cell death. They noticed an increasing fraction of metallic SWNTs lead to increasing in a loss of *Escherichia coli* viability. They concluded that in all cases antimicrobial action of SWNT occurs shortly after (<15min) bacteria SWNT contact. The SWNT toxicity mechanism was involving (i) firstly SWNT bacteria contact (ii) irregular movement of the cell membrane, and (iii) oxidative stress.

**Yang et al. (2010)**<sup>6</sup> investigated the effect of SWCNTs length on their antimicrobial activity to bacterial cells in suspensions. They tested three different lengths of SWCNTs (<1  $\mu\text{m}$ , 1-5  $\mu\text{m}$ , and  $\sim 5 \mu\text{m}$ ). They found that at the same weight concentration, longer SWCNTs exhibited stronger antimicrobial activity. They demonstrated that the longer SWCNTs aggregated with bacterial cells more effectively, whereas short length SWCNTs tended to aggregate themselves without involving many bacterial cells. They concluded that longer SWCNTs exhibited more pronounced concentration and treatment time-dependence.

**Dong et al. (2011)**<sup>7</sup> investigated the antibacterial activity of single-walled carbon nano-tubes (SWCNTs)

dispersed in surfactant solutions of sodium cholate, sodium dodecylbenzene sulfonate, and sodium dodecyl sulfate. They demonstrated the weakest antibacterial activity against *Salmonella enteric* was sodium cholate. They found that SWCNTs displayed antibacterial characteristics for both *S. enterica* and *E. coli*. They concluded that carbon nano-tubes could become an effective alternative to antibiotics in dealing with drug-resistant and multidrug-resistant bacterial strains. Due to the physical mode of bactericidal action that SWCNTs display antimicrobial activity.

**Zhao et al. (2012)**<sup>8</sup> investigated silver-containing activated carbon (Ag/AC) exhibiting controlled release of silver. They found that increasing concentration of CH<sub>3</sub>COOAg, increasing in silver content, changing the samples from exhibiting no antibacterial activity to inhibition of bacteria growth and then to antibacterial activity due to the smaller size of the silver particles. They noticed that Ag/AC composites showed a lower release rate of silver than that of a composite prepared by a traditional AgNO<sub>3</sub> impregnation method, which suggested a strong interaction between the silver particles and carbon. They concluded that antimicrobial effects and controlled release of silver showed that promise for purification of drinking water.

**Varghese et al. (2013)<sup>9</sup>** studied antimicrobial activity of carbon nano-particles isolated from natural sources against pathogenic Gram-negative and Gram-positive bacteria. They described the isolation of carbon nano-particles (CNPs) from kitchen soot. They tested antibacterial activity of the isolated carbon nano-particles against various pathogenic bacterial strains such as gram-negative proteus refrigere and pseudomonas aeruginosa and gram-positive staphylococcus aureus and Streptococcus haemolyticus. They found that the carbon nano-particles isolated from natural sources are active against these gram-negative and gram-positive bacterial strains. They concluded, the nano-particles might cross the cell membranes, penetrating into the interior of the cell and interacting with intracellular sites, by preventing bacteria from dividing and multiplying. It induces cell lysis and kills the bacteria.

**Karnib et al. (2013)<sup>10</sup>** investigated the ability of the activated carbon, silver impregnated activated carbon, and silica sand to remove and destroy water pathogens. They found that silver nanoparticles showed the highest antibacterial effect against *E. coli*. One hour of bacteria incubation with silver impregnated activated carbon was quite enough for bacteria reduction. While no bacterial growth was detected after 2, 3 and 24 hours using the activated carbon. They concluded, bacterial growth was

completely inhibited on using silver impregnated activated carbon at all the tested concentrations after one hour of incubation.

**Choi et al. (2014)<sup>11</sup>** investigated the effect of bamboo charcoal on reducing Streptococcus mutans, in operative dentistry. They found that 58% was the percentage colony count reduction of S. mutans. They demonstrated an antibacterial activity of bamboo charcoal is a concentration dependent. Finally, they concluded that the bamboo charcoal proved to be bactericidal effect on S. mutans.

**Sim et al. (2014)<sup>12</sup>** investigated antibacterial activity of activated carbon fiber (ACF) filters against various pathogens. They observed, ACF filters was a source of microbial contamination and their filter efficacy declined. In this study they used Sophora flavescens in antimicrobial nanoparticles coated onto ACF filters. They found that extract from S. flavescens is an effective against various pathogens. They concluded that the efficiency of Staphylococcus epidermidis in activation is with the of S. flavescens nanoparticles concentration dependent in the ACF filter coating.

**Yang et al. (2014)<sup>13</sup>** investigated antibacterial properties of a synthesized activated nanosized carbon

spheres (CS) (400–500 nm in diameter) for adsorption of a quaternary phosphonium salt. They demonstrated, the activated CS (ACS) exhibited a high adsorption capacity ( $2325 \text{ m}^2 \text{ g}^{-1}$ ) for the quaternary phosphonium salt (QPS) and have excellent antibacterial properties. They found that the QPS on ACS were more stable than the QPS itself, resulting in long-term antibacterial effects. They concluded that the excellent adsorption performance and reusability of the steam activated CS could represent a new type of cheap and efficient adsorbent nanomaterial for antibacterial materials. The content of the QPS in the ACS played a key role in the antimicrobial effects of the spheres and the effect increased with higher content.

**Karthik and Saraswathy (2015)**<sup>14</sup> studied the antimicrobial activity of *Tribulus terrestris* weed modified as activated carbon using conc.  $\text{H}_2\text{SO}_4$ . They used agar well diffusion method on the *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* & *Klebsiella pneumoniae*. They found that activated carbon prepared from *Tribulus terrestris* has a maximum antibacterial activity against the tested bacterial species.

**Dong et al (2017)**<sup>15</sup> investigated the antimicrobial effects of newly discovered photo-activated CDots in combination with other antimicrobial reagents, including  $\text{H}_2\text{O}_2$ ,  $\text{Na}_2\text{CO}_3$ , and  $\text{AcOH}$  (acetic acid).. They observed

antibacterial synergistic effects when CDots combined with  $\text{H}_2\text{O}_2$ . However, the combination of CDots with  $\text{Na}_2\text{CO}_3$  or AcOH did not show synergistic effects. They concluded that the combination of CDots with their synergistic antimicrobial reagents, such as  $\text{H}_2\text{O}_2$ , could inhibiting bacteria growth by using lower concentration of each individual chemical in the combination than using one chemical treatment alone.

**Ilomuanya et al. (2017)**<sup>16</sup> investigated the effect of pore size and morphology of activated charcoal prepared from midribs of *Elaeis guineensis* on adsorption of poisons using metronidazole and *Escherichia coli* O157:H7. They obtained activated charcoal from *Elaeis guineensis* mid ribs. They found that commercial activated charcoal / metronidazole combination in vitro- pharmacodynamic model reflected no re-growth after 4 hours, however for charcoal formulated from *Elaeis guineensis* via chemical activation with phosphoric acid and metronidazole no regrowth was seen at the second hour. They concluded that Activated charcoal obtained from *Elaeis guineensis* dried mid ribs consisting of macroporosity with mixed meso/micro porosity and antibacterial metronidazole is the best model for bacterial adsorption and will be useful in the treatment of diarrhea caused by *E. coli* O157:H7.

**Tang et al. (2017)**<sup>17</sup> investigated preparation, mechanism, and antibacterial activity of Silver nanoparticles-loaded activated carbon fibers using chitosan as binding agent. They used silver nanoparticles (AgNPs), Chitosan as a binding agent for the effective loading of AgNPs on activated carbon fibers (ACF) surface to fabricate the antibacterial material. They found The AgNPs-loaded ACF material showed antibacterial activity for *S. aureus* and *E. coli*. Finally, they concluded chitosan is proved to be a promising binding agent for the fabrication of AgNPs-loaded ACF material with excellent antibacterial activity.

## **B. Antibacterial effect of calcium hydroxide:**

**Leonardo et al. (2000)**<sup>18</sup> used two methods to test in vitro the antimicrobial activity of 4 root canal sealers (AH plus, Sealapex, Ketac Endo and Fill Canal), two calcium hydroxide pastes (Calen and Calasept) and a zinc oxide paste against seven bacterial strains by placing sealers and pastes directly into 4.5 x 4.0 mm wells on agar plates or by using absorbent paper points after immersion in pastes and sealers. They found that sealers and pastes exhibited an antimicrobial activity for all bacterial strains used in this study.

**Han et al. (2001)**<sup>19</sup> conducted a study on sixty-eight standardized human root specimens infected with *Enterococcus faecalis* after removal of the smear layer. After 3 weeks of infection, the smear layer was reformed and in half of the specimens the smear layer was again removed. Specimens were divided into four groups: a) non smeared aqueous CaOH group, b) non smeared silicon oil-based CaOH group, c) smeared aqueous CaOH group, (d) smeared silicon oil based CaOH group. They placed medications in the canals for 7 days then the quantity of bacteria was assessed. They found that all calcium hydroxide pastes were effective in the elimination of bacteria in the dentinal tubules, except in the smeared group with silicone-oil base CaOH.

**Estrela et al. (2001)**<sup>20</sup> investigated the influence of different vehicles on the antimicrobial efficiency of CaOH. They used intracanal dressings of: CaOH+1% chlorhexidine solution; CaOH +3% sodium lauryl sulphate; CaOH + otosporin. They found that an antimicrobial effect occurred after 48h with *S. mutans*, *E. faecalis*, *S. aureus*, *P.aeruginosa*, *B. subtilis*, *C. albicans*. It also occurred with a mixed suspension containing all the microorganisms irrespective of the vehicle associated with. CaOH pastes.

**Peters et al. (2002)**<sup>21</sup> evaluated the fate of microorganisms in root canals of teeth with infected pulps

and periapical bone lesions with and without the use of calcium hydroxide medication. They instrumented forty three tooth and filled half of the teeth with a thick slurry of calcium hydroxide in sterile saline while the other teeth obturated After 4 weeks with calcium hydroxide. They accessed the teeth again and after microbiological sampling they obturated them. They concluded that a calcium hydroxide and sterile saline slurry limits but does not totally prevent regrowth of endodontic bacteria.

**Filho et al. (2002)**<sup>22</sup> evaluated the effect of calcium hydroxide on bacterial endotoxin lipopolysaccharide (LPS; endotoxin) induced in dog's teeth. They showed that bacterial endotoxin (I,PS) caused radiographically visible periapical lesions. This endotoxin was detoxified when associated with calcium hydroxide. They also showed that even after bacterial death they release endotoxin. They concluded that from a clinical view point the use of medication that leads only to the death of bacteria for the treatment of teeth with pulp necrosis and chronic periapical lesion is not sufficient but medication must also inactivate bacterial endotoxin.

**Law and Messer (2004)**<sup>23</sup> reviewed the literature concerning the antibacterial effectiveness of intracanal medicaments used in the management of apical periodontitis. The total sample size in these studies was 164 teeth. Microbiologic sampling was performed before endodontic treatment ( $S_1$ ) after instrumentation and irrigation ( $S_2$ ) and after intracanal medication ( $S_3$ ). They

concluded that CaOH remains the best medicament available reduce residual microbial flora.

**Zehdenr et al. (2006)**<sup>24</sup> evaluated the effects of bioactive glass S53 p4 versus CaOH when used as dressing in premolars infected with *Enterococcus faecalis*. They dressed Teeth with bioactive glass S53 P4 (BAG) or CaOH suspension for 10 days. They found that CaOH had a strong antibacterial effect and was significantly more effective than BAG in preventing residual bacterial growth.

**Chu et al. (2006)**<sup>25</sup> compared the disinfection potential of antitiotic steroid medicaments or a calcium hydroxide paste in root canals with periapical radiolucencies. They took premedication samples by paper points. They instrumented, irrigated and divided root canals into 3 groups: 1<sup>st</sup> group: dressed with Ledermix, 2<sup>nd</sup> group: dressed with Septomixine forte and 3<sup>rd</sup> group: dressed with Calasept. After 1 week, they removed the medication and took samples from root canal. They found that 1 week interappointment period is acceptable and that the use of CaOH has been considered as the preferable option over antibiotic containing combinations for management of infected canals.

**Herrera et al. 2008**<sup>26</sup> assessed antibacterial synergism on *Enterococcus faecalis* and *Pseudomonas*