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**ANTIBACTERIAL EFFICACY OF SILVER-CROSSLINKED HYDROGEL  
NANOCOMPOSITE VERSUS SODIUM HYPOCHLORITE AND CHLORHEXIDINE  
ON *ENTEROCOCCUS FAECALIS* FOR USE IN ROOT CANAL INFECTION**

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**ABSTRACT**

The aim of this study was to synthesize and evaluate the antibacterial properties of thermosensitive cross-linked poly(N-isopropylacrylamide-methacrylic acid-vinyl pyrrolidone) hydrogel (Poly (NIPAM-MAA-VP)) containing silver nanoparticles (silver cross-linked hydrogel nanocomposites (SCHNC)) at a concentration of 30 ppm in dental root canals infected with *Enterococcus faecalis* and to compare its efficacy with two commonly used irrigants; 2.5% sodium hypochlorite and 2% chlorhexidine gluconate.

Cross-linked poly (NIPAM-MAA-VP) hydrogel was synthesized by free radical copolymerization of monomers in 1,4-dioxane in the presence of N,N-Methylenebisacrylamide as crosslinking agent. Highly stable and uniformly distributed silver cross-linked hydrogel nanocomposite (SCHNC) was obtained via in situ reduction of silver nitrate (AgNO<sub>3</sub>) using sodium borohydride (NaBH<sub>4</sub>) as reducing agent. Minimum Inhibitory Concentration (MIC) of newly synthesized SCHNC was

determined against *E. faecalis* by broth macro dilution method. Also *in vitro* experiments were carried out on 100 human single rooted maxillary incisors infected with *E. faecalis*.

Mean MIC values for SCHNC and placebo cross-linked hydrogel (polymer without silver nanoparticles) were 15ppm and 200ppm respectively. In the dental root canals in the first inoculation SCHNC lowered the *E. faecalis* count below 10 CFU, the hypochlorite and chlorhexidine solutions killed all the bacteria. Furthermore, the synthesized SCHNC in double inoculation with the bacteria maintained its activity in reduction of bacteria more efficiently compared to hypochlorite and chlorhexidine solutions. Placebo cross-linked hydrogel failed to show significant change from the initial bacterial count in dental samples.

Findings of this study confirm the antibacterial effects of newly synthesized SCHNC against *E. faecalis*, and demonstrate that SCHNC can exhibit sustained antibacterial property and low toxicity. After re-inoculation of the dental root canal with the excess *E. faecalis* inoculum SCHNC successfully maintained its antibacterial activity compared to the hypochlorite and chlorhexidine solutions which were remarkably lost their disinfectant effects.

**Keywords:** *Enterococcus faecalis*, silver-cross-linked hydrogel nanocomposites, sodium hypochlorite, chlorhexidine, antibacterial activity

## INTRODUCTION

Studies have shown that the presence of microorganisms is the main factor in the development of pulpal and periradicular diseases. When tooth pulp undergoes pathological changes due to trauma or aggressive caries, the root canal system becomes susceptible to contamination by a variety of microbes. The microorganisms not only contribute in the anatomical irregularities of the root canal system but also invade the dentinal tubules and can re-infect a poorly treated root canal system [1]. The main objectives of endodontic therapy are to remove the infected tissue, eliminate the

microorganisms present in the root canal systems and dentinal tubules and prevent recontamination of the canal after the treatment [2]. Cleaning and shaping of the root canal through the use of appropriate intracanal irrigants reduces the bacterial load, thereby increases the likelihood of successful outcomes after root canal therapy [3].

Sodium hypochlorite (NaOCl) is a commonly used root canal irrigant. In addition to its bactericidal and proteolytic properties, NaOCl solution offers some advantages including its ability to dissolve organic substances, increase the permeability of the dentin, and

dissolve necrotic tissue [4-7]. However, the use of this irrigant is limited due to its undesirable taste and smell. Moreover, it has been proven to have cytotoxic effect on periradicular tissues in the case of extrusion [8, 9]. Recently with the major advances in nanotechnology, novel agents such as silver nanoparticles with advanced antimicrobial activity have been developed [10]. These nanoparticles possess improved characteristics include the high surface area to volume ratio, greater solubility and chemical reactivity and accordingly bactericidal activities [11]. The antimicrobial property of silver nanoparticles depends on its concentration and release rate. The antimicrobial activity of silver is attributed to the interaction of silver ions with specific thiol groups that contain sulphur and hydrogen and are found in different structural compounds of bacterial enzymes and proteins [12-15] thereby causing less bacterial resistance than other antibiotics. Silver-containing nanomaterials have been widely used in biomedical products due to the broad-spectrum antimicrobial properties. Already, silver nanoparticles have been shown to be effective against bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermis*, *Leuconostocme senteroides*,

*Bacillus subtilis*, *Klebsiella mobilis*, and *Klebsiella pneumonia* [16-17].

Different forms of silver nanomaterials have been already reported including metallic silver nanoparticles, silver chloride particles, silver-impregnated zeolite powders and activated carbon materials, dendrimer-silver complexes and composites, polymer-silver nanoparticle composites, and silver nanoparticles coated polymers [16-17].

Several factors have been reported to influence the antimicrobial activity of silver nanoparticle like particle size, shape, surface chemistry, coating agents, as well as, environmental factors such as pH, ionic strength, and the presence of macromolecules.

Stability of silver nanoparticles has a critical role in their properties since fast oxidation and also formation of silver aggregates tends to decrease their antimicrobial activity.

For biomedical applications, it is necessary to stabilize silver nanoparticles. Various methods for stabilizing and capping of nanoparticles have been reported [18-21]. Coating of silver nanoparticle, embedding silver nanoparticles in different polymeric composites and surface modification with functional polymers, have been used to increase nanoparticles stability.

We have previously reported the antibacterial activity of silver nanoparticles embedded in

smart poly (N-isopropylacrylamide)-based hydrogel networks [22]. A series of thermosensitive cross-linked poly(N-isopropylacrylamide-methacrylic acid-hydroxyethyl methacrylate) [P(NIPAAm-MAA-HEM)] have been obtained by cross-linking- free radical polymerization method. Highly stable and uniformly distributed silver nanoparticles have been obtained within the hydrogel networks via in situ reduction of silver nitrate using sodium borohydride ( $\text{NaBH}_4$ ) as reducing agent. The antibacterial activity of these hydrogel-silver nanocomposites has been studied.

In the present paper we have reported the synthesis of a novel silver nanocomposite prepared from silver nanoparticles embedded in a cross-linked thermosensitive N-isopropylacrylamide-based polymeric hydrogel. Cross-linked poly(N-isopropylacrylamide-methacrylic acid-vinyl pyrrolidone) [P(NIPAAm-MAA-VP)] copolymer was synthesized and used as hydrogel network. The antibacterial activities of silver-free hydrogel and hybrid silver-polymer nanocomposite were studied in dental root canals infected with *Enterococcus faecalis* and compare with two commonly used irrigants; 2.5% sodium hypochlorite and 2% chlorhexidine gluconate.

## MATERIALS AND METHODS

### Materials

N-isopropyl acrylamide (NIPAAm) (Fluka, Deisenhofen, Germany) was purified by recrystallization in hexane and dried under a vacuum at 25°C. Vinyl pyrrolidone (VP) (Merck, Hohenbrunn, Germany) was freed from the stabilizer by twice vacuum distillation with continuous bubbling argon. Methacrylic acid (MAA) (Fluka) was used as supplied. Benzoyl peroxide (BPO), silver nitrate ( $\text{AgNO}_3$ ), and sodiumborohydride ( $\text{NaBH}_4$ ) were from Merck Chemical Co. N,N-Methylenebisacrylamide (NNMBAAm) (Sigma-Aldrich Co.) was used directly without further purification. Muller-Hinton Broth medium and Agar-Agar from Merck (Germany), Todd Hewitt Broth, Bile Esculin Agar and Soybean Casein Digest Medium (tryptone Soya Broth) from Hi Media Laboratories (India) were used. *Enterococcus faecalis* (PTCC 1237) was purchased from Persian Type Culture Collection (Iran).

### Preparation and Characterization of Cross-Linked P (NIPAM-MAA-VP) Hydrogel

Poly (N-isopropylacrylamide-methacrylic acid-vinylpyrrolidone) (Poly (NIPAM-MAA-VP) copolymer was synthesized by free radical copolymerization of monomers in 1,4-dioxane under  $\text{N}_2$  atmosphere according to the method described previously [24]. The ratio

of NIPAM: MAA:VP was 85:5:10. Monomers were dissolved in 1,4-dioxane to form a 5 wt% solution containing PBO (0.1 wt% weight ratio of total monomers) and NNMBAAm (1 wt%) as a cross-linking agent. The polymerization was carried out at 70 °C for 10 h under N<sub>2</sub> atmosphere. The resulting copolymer was precipitated in excess cold n-hexane. The crude polymer was purified by dissolving in tetrahydrofuran (THF) and reprecipitation in diethyl ether to remove the reactant residues. The copolymer was finally dried by pumping under reduced pressure. Chemical structure of copolymers was determined by FT-IR (Shimadzu 8400, Kyoto, Japan) and <sup>1</sup>H-NMR (Bruker AC 80, Rheinsteten, Germany) spectroscopies.

#### **Preparation of Silver cross-Linked Hydrogel Nanocomposites (SCHNC)**

Precisely measured dry hydrogel was steeped in water for two days and the swollen hydrogels were transferred to a beaker containing aqueous solution of 50 ml of AgNO<sub>3</sub> (30 ppm) and stirred at 25 °C for 72 hours for equilibration. These silver salt absorbed hydrogels were then added to 50 ml of aqueous solution NaBH<sub>4</sub> (60 ppm) and stirred lightly for 4 hours to reduce the silver ions into silver nanoparticles. The resulted silver cross-linked hydrogel nanocomposite (SCHNC) was dried under vacuum oven at

25°C for 1 day. The dried specimens were examined by a LEO 906 transmission electron microscopy (TEM) operated at 80kV.

#### **Preparation of Tooth Samples**

The study was conducted on 100 human single-rooted upper anterior teeth with fully developed apices and straight roots devoid of any abnormalities, which were extracted due to periodontal reasons. The teeth were stored in a phosphate-buffered saline solution until used. Root surfaces were cleaned using an ultrasonic device and teeth appearing as cracked or calcified on radiographs were excluded from the study. Canal lengths of the selected teeth were measured by introducing a K-flexofile#20 (DentsplyMaillefer, Switzerland) into the canal until the tip was visible at the apical foramen.

Tooth crowns were cut-off at CEJ using diamond disks (D&Z, Diamond, Germany) such that the length of the roots were 12mm and then, using a Maillefer, Dentsply, Switzerland k-flexo file #20 the canal working length was set at 1 mm short of the apical foramen. Next, root canals were prepared using #3 and #4 Gates-Glidden drills and rotary files size 40 (10%) and size 35 (8%) of the RaCe system (FKG Dentaire, La-Chaux-de-Fonds, Switzerland) and according to the crown-down technique. Physiological serum was used for canal irrigation and the smear

layer was removed using 5.25% sodium hypochlorite (Taj Corp, Tehran, IRI) (3 minutes) and 17% EDTA (Pulpdent Corp, MA, USA). The teeth were divided into 10 groups, each group including 10 samples that were classified into A and B subgroups. All teeth were sterilized by autoclaving for 20 minutes at 121°C and 15 psi. To check the sterility of the samples, they were incubated for 24 hours at 37°C in brain-heart infusion broth (Merck, Darmstadt, Germany).

### Inoculum Preparation

*Enterococcus faecalis* (PTCC 1237) was obtained in lyophilized form Persian Type Culture Collection (Iran), which was activated by incubation in Todd Hewitt Broth for 24 hours at 37°C. 50 µl from the grown bacteria was transferred and spread on the surface of bile esculin sodium azide agar medium and incubated overnight at 37°C. The black colonies confirmed the identity of *enterococcus* species. A single colony from the plate was transferred into 4 ml fluid tryptone Soya Broth and incubated over night at 37°C and 200 rpm in shaking incubator. The cells were harvested by centrifugation at 3000 rpm for 15 min. Subsequently, they were washed twice and re-suspended in Ringer solution to provide bacterial concentrations between  $10^7$ – $10^8$  CFU/ml [25].

### Determination of the Antibacterial Activity of SCHNC Against *Enterococcus faecalis*

In order to evaluate the *in vitro* antibacterial activity of newly synthesized SCHNC, firstly the MIC value was determined by broth macrodilution technique according to CLSI [25]. In brief, serially diluted concentrations of SCHNC were prepared in sterile water ranging from 60 to 0.93 ppm. Muller-Hinton Broth medium containing the inoculum in the final concentration of  $10^6$  CFU/ml was added to the serially diluted concentrations of SCHNC. After 24 hours of incubation at 37°C, the tubes were checked for any evidence of bacterial growth. The MIC value was defined as the lowest concentration of SCHNC with no sign of bacterial growth. The tubes with placebo cross-linked hydrogel (polymer without silver nanoparticles) were also included in the test as negative control group.

In the next step, the teeth canals were inoculated, under aseptic conditions, with the bacterial inoculum and left in the room temperature for 4 hours. Then the samples were rinsed three times with 5 mL of 0.9% normal saline to eliminate un-attached bacteria. Accordingly, the samples in groups a and b (1 to 5) were filled with 250 µl of SCHNC (30 ppm), 2.5% sodium hypochlorite,

2% chlorhexidine gluconate, 30 ppm placebo cross-linked hydrogel and 0.9% normal saline as control respectively. After that, the samples of group A were rinsed three times with 5 mL of 0.9% normal saline and placed in a 15-mL tubes containing 1 mL of normal saline. The tubes were transferred to an ultrasonic bath cleaner (ModStar Sonic 1835, Italy) operating at 34 kHz and 180 W, and sonicated for 6 minutes to detach bacteria adhering to the surfaces of the specimens and to release them into the suspension. Finally the specimens were removed from the tubes and 100  $\mu$  L of the suspension containing detached bacteria was transferred onto a sterile plate for bacterial count via a standard pour plate technique.

The samples of group B were left in the room temperature for 4 hours and then re-inoculated with the bacterial inoculum and placed in the room temperature for another 4 hours. Finally, the samples were rinsed; their bacteria were detached and counted in the same conditions as for group a. All the experiments were performed in triplicates.

### Statistical Analysis

The Kolmogorov-Smirnov test indicated that the data obtained were not normally distributed, and so the Kruskal-Wallis test was used to compare the bacterial colony counts and the Bonferroni test was used for

pair-wise comparisons. The level of statistical significance was set at  $p < 0.05$ .

## Results

### Characterization of Chemical Structure of Cross-Linked P (NIPAM-MAA-VP) Copolymer

Chemical structure of cross-linked P (NIPAM-MAA-VP) copolymer were characterized by FT-IR and  $^1\text{H}$  NMR spectroscopies. FT-IR spectrum of cross-linked P(NIPAAm-VP-MAA) shows strong peaks in the range of 800–1000  $\text{cm}^{-1}$ . Absorbance of amide carbonyl groups in PNIPAAm occurs at 1650  $\text{cm}^{-1}$ , bending frequency of amide N–H appears at 1550  $\text{cm}^{-1}$ . An Intense and broad peak in 3300–3400  $\text{cm}^{-1}$  illustrates formation of hydrogel polymer due to the water of hydration attached to the polymer gives rise to broad spectrum. The C–H stretching vibration of the polymer backbone is manifested through strong peak at 2928  $\text{cm}^{-1}$ .

$^1\text{H}$ -NMR spectrum for synthesized novel copolymer, NIPAAm signal appeared in  $\delta = 3.9$  ppm, which represents  $N-\text{CH}-(\text{CH}_3)_2$ ,  $\delta = 1.1$  ppm represents  $\text{CH}-(\text{CH}_3)_2$ , and  $\delta = 1.6$  ppm relates to  $(\text{CH}_2-\text{H})$ . The peaks of vinyl pyrrolidone units appeared at  $\delta = 3.2$  ppm, which relates to  $N-\text{CH}-(\text{CH}_3)_2$ . No peak was observed between 5–7 ppm due to polymerization. If the polymeric chain did not

form or there was incomplete polymer formation, there should be some signals which relate to unbroken double bonds of NIPAAm, VP, or MAA.

### **Characterization of silver cross-linked hydrogel nanocomposites (SCHNC)**

#### **UV–Vis Absorption Spectra of Silvercross-Linked Hydrogel Nanocomposites (SCHNC)**

The existence of silver nanoparticles in the hydrogel networks was confirmed by UV–vis spectral analysis. The generation of silver nanoparticles was confirmed from the appearance of yellow color and an absorption maximum between 400 and 408nm. The color of the silver nanocomposite is depended on the concentrations of  $\text{AgNO}_3$ . By increasing the concentration of  $\text{AgNO}_3$  the color of solution was changed from golden yellow to red. **Figure 1** shows the UV–vis spectra of silvercross-linked hydrogel nanocomposites (SCHNC). As shown in **Figure 1**, aqueous solution of silver nanoparticles embedded in cross-linked P(NIPAAm-MAA-VP) hydrogel showed an absorption band at 408 nm, which is a typical absorption band of spherical Ag nanoparticles.

#### **Transmission Electron Microscopy (TEM) Analysis of Silvercross-Linked Hydrogel Nanocomposites (SCHNC)**

**Figure 2** shows the TEM image of silvercross-linked hydrogel nanocomposites (SCHNC). The produced nanoparticles were found to be spherical in shape and polydisperse. The average particle sizes were 20-30 nm.

#### **Antibacterial Properties of Silvercross-Linked Hydrogel Nanocomposites (SCHNC)**

Antibacterial activity of placebo cross-linked hydrogel (polymer without silver nanoparticles) and its silver cross-linked hydrogel nanocomposites (SCHNC) were studied.

**Figure 3** shows the mean MIC for the placebo cross-linked hydrogel and silvercross-linked hydrogel nanocomposites (SCHNC) against *E. faecalis*. The antibacterial activity of SCHNC was significantly greater than that of placebo cross-linked hydrogel in aqueous suspension with 5% DMSO added. As shown in the bar graph, mean MIC for placebo cross-linked hydrogel is 200 ppm, while that of SCHNC is 15 ppm.

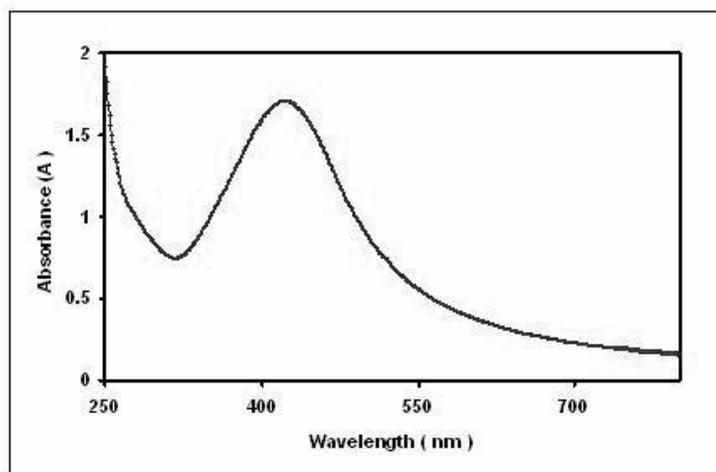
#### **Efficacy of Silvercross-Linked Hydrogel Nanocomposites (SCHNC) in Disinfecting Dental Root Canals**

The results pertaining to the efficacy of SCHNC for disinfecting dental roots infected with *E. faecalis* in comparison with

commonly used irrigants are presented in **Table 1**.

As can be seen from the bacterial counts in **Table 1**, the hypochlorite and chlorhexidine solutions in the used concentrations, killed all the bacteria inoculated in group a samples and no bacterial growth was observed. Our synthesized SCHNC reduced the bacterial count below 10 CFU. Since the initial amount of CFU ranged between  $24 \times 10^4 \pm 17$ , SCHNC achieved more than 5 log reduction of the bacterial count. Although placebo cross-linked hydrogel showed 2 log reductions in the bacterial count, it was failed to consider as a sufficient antibacterial agent.

On the other hand, after re-inoculation in group B, the obtained results were interesting. SCHNC maintained its antibacterial activity against *E. faecalis* with a mean count around 10 CFU, in spite of the hypochlorite and chlorhexidine solutions which were remarkably lost their disinfectant effects. The initial count of  $56 \pm 19 \times 10^4$  reduced to  $10 \pm 8$  in the case of SCHNC, compared to hypochlorite and chlorhexidine solutions that were lowered to  $67 \pm 25 \times 10^2$  and  $27 \pm 13 \times 10^3$  respectively. Furthermore, there was no significant difference between the initial count and placebo cross-linked hydrogel after re-inoculation.



**Figure 1: UV-Vis Spectra of Silver cross-Linked Hydrogel Nanocomposites (SCHNC)**



Figure 2: TEM Image of Silver cross-Linked Hydrogel Nanocomposites (SCHNC)

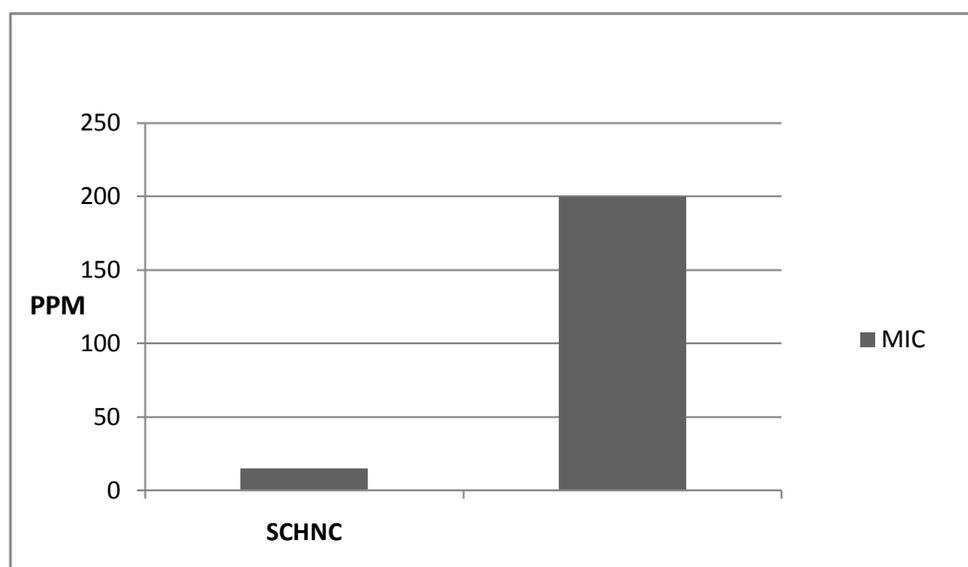


Figure 3: MICs (ppm) for Placebo Cross-Linked Hydrogel and its Silver cross-Linked Hydrogel Nanocomposites (SCHNC) Against *E. faecalis*

Table 1: Disinfectant Efficacy of the Tested Materials Against *E. faecalis*

Test groups	Mean bacterial Count (CFU±SD) after different treatment methods (n=10)				
	Untreated sample	30 ppm SCHNC	30 ppm placebo cross-linked hydrogel	2.5% Hypochlorite solution	2% Chlorhexidine solution
A	$24 \pm 17 \times 10^4$	$6 \pm 5$	$24 \pm 12 \times 10^2$	0	0
B	$56 \pm 19 \times 10^4$	$10 \pm 8$	$34 \pm 25 \times 10^4$	$67 \pm 25 \times 10^2$	$27 \pm 13 \times 10^3$

CFU: Colony Forming Units; SD: Standard Deviation; a: one time inoculation; b: two times inoculations

## DISCUSSION

In this study, the antibacterial activity of silvercross-linked hydrogel nanocomposites (SCHNC) at a concentration of 30 ppm in dental roots infected with *E. faecalis* was compared with that of two commonly used irrigants, 2.5% sodium hypochlorite and 2% chlorhexidine. Some natural or synthetic polymers have been examined for their antimicrobial activity by themselves or in combination formulations [26]. Cross-linked poly (NIPAAm-MAA-VP) copolymer is an amphiphilic copolymer. **Figure 3** shows that this copolymer has a slight antibacterial activity. The antibacterial activity of cross-linked hydrogel enhances by incorporation of silver nanoparticles. As shown in **Figure 3** the antibacterial activity of the silvercross-linked hydrogel nanocomposites (SCHNC) was significantly greater than that of silverfree cross-linked hydrogel in an aqueous suspension. The significant difference between the obtained MICs may be attributed to the increasing effect of antibacterial efficacy of silver. On the whole from the antibacterial point of view, the synthesized SCHNC was act as an antiseptic in the dental root canal model against *E. faecalis* (more than 5 log reduction in bacterial count), but not as effective as 2.5% sodium hypochlorite and 2% chlorhexidine (as fast acting

disinfectants) which completely eradicated the initial inoculum. In contrast, after re-inoculation of the dental root canal with the excess *E. faecalis* inoculum SCHNC successfully maintained its antibacterial activity compared to the hypochlorite and chlorhexidine solutions which were remarkably lost their disinfectant effects. This finding may be related to some reasons; firstly the sustained release behavior of the polymer that was embedded the silver, made it possible to preserve the antibacterial activity of silver in prolonged times. Secondly the lower concentration exponent of silver (0.9-1) prevents the significant changes of its efficacy after dilution [27].

In general, it is believed that except for the limitations arise from prolonged use, silver is nontoxic to mammalian cells. Silver poisoning occurs only in people who have a long history of close contact with silver [28]. One approach for decreasing the toxicity of silver nanoparticles would be the design of polymer-embedded silver nanoparticles. Our approach involves the combinations of antimicrobial activity of silver nanoparticles and biocompatibility of synthetic polymers by preparation of nanocomposites, in order to produce formulations with suitable performance and low toxicity *in vivo*.

The majority of studies have reported little tissue reaction to silver particles, especially to small amounts of it (23 and 46 ppm) [28-29].

Previous studies, such as those of Siren et al, have reported the prevalence of *E. faecalis* in filled root canals and the association of this species with periradicular diseases [30-32].

As a result, several substances have been used to eliminate these bacteria. Sodium hypochlorite is an effective antibacterial agent, but its effect depends on the concentration and duration of exposure [32].

This substance is very effective against endodontic micro-organisms, such as *E. faecalis*. Studies by Gomes and also Portenier et al., have confirmed the elimination of *E. faecalis* by sodium hypochlorite [33, 35]. Studies by Siqueira et al., on root canals infected with *E. faecalis* have demonstrated that sodium hypochlorite has greater anti-bacterial effects than physiological saline solution [35]. Chlorhexidine is another substance which has been used effectively against bacteria and fungi often found in endodontic infections [36]. The antibacterial effects of this substance against *A. israeli*, *E. faecalis* and *S. aureus* have been documented [37].

The incorporation of silver nanoparticles in repairing bones (as bone cement) has shown very strong antibacterial properties. The

advantage of these particles is their high surface area to volume ratio. These particles have greater solubility, chemical reactivity and antibacterial activities [38-41]. To obtain these characteristics, it is important to control the size and the shape of silver nanoparticles. Research has shown that the smaller the size of silver particles, the greater the contact with bacteria and the greater the likelihood of a chemical reaction [42]. The shape of the silver particles affects their antibacterial activities [43].

The advent of nanoscience has made it possible to use pharmaceutical substances in different fields of science like medicine. Of all the materials used as nanomaterials, silver nanoparticles have been found to possess the greatest anti-microbial effects [12]. Silver has been clearly demonstrated to be an effective anti-microbial agent against bacteria, viruses and fungi. According to studies by Moyer, silver nitrate has antibacterial effects against *S. aureus*, *E. coli* and *P. aeruginosa* [38]. The present study confirmed the disinfectant activity of the synthesized SCHNC against the *E. faecalis* *in vitro* experiments. The results obtained here, propose that development of a complex nanocomposite composed of NIPAAm-based hydrogel and silver nanoparticles can show enhanced antibacterial effect, sustained release behavior

and reduced toxicity in comparison to silver nanoparticles and antibacterial agents.

## CONCLUSION

A nanostructured, chemically crosslinked poly (NIPAAm-MAA-VP) hydrogel nanocomposites containing silver nanoparticles was prepared for promising antibacterial applications. This thermo and pH- sensitive hydrogel is a suitable candidate for the successful in situ synthesis of silver nanoparticles within the hydrogel networks, which also serve as an immobilizing matrix. Morphology studies using TEM, indicated that the average size of silvercross-linked hydrogel nanocomposites (SCHNC) was 20-30 nm. No aggregates of nanoparticles were observed. The existence of silver nanoparticles in the hydrogel networks was confirmed by UV-vis spectral analysis. UV-vis spectra showed a sharp peak at 408 nm which confirmed the presence of small-sized silver nanoparticles with a narrow size distribution. The antibacterial effects of the silver-nanocomposite hydrogel against *E.faecalis* was evaluated and compared with chlorhexidine and sodium hypochlorite. Findings of this *in vitro* study confirm the antibacterial effects of silvercross-linked hydrogel nanocomposites (SCHNC) against *E.faecalis*, and demonstrate that although, chlorhexidine and sodium

hypochlorite have greater antibacterial effects than SCHNC in an aqueous suspension but SCHNC can exhibit sustained release property and low toxicity.

## Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this article.

## REFERENCES

- [1] Torabinejad M, Khademi A A, Babagoli J, Cho Y, Johnson W B, Bozhilov K, *et al.*, A new solution for the removal of the smear layer, J. Endod., 29, 2003, 170-5.
- [2] Shabahang S, Pouresmail M and Torabinejad M, In vitro antimicrobial efficacy of MTAD and Sodium Hypochlorite, J. Endod., 29, 2003, 450-2.
- [3] Zhang W, Torabinejad M, Li Y, Evaluation of Cytotoxicity of MTAD using the MTT-Tetrazolium method, J. Endod., 29, 2003, 654-657.
- [4] Torabinejad M, Handysides R, Khademi A and Bakland LK, Clinical implications of the smear layer in endodontics: a review, Oral Surg Oral Med Oral Path Oral Radial Endod., 94, 2002, 658-66.
- [5] Marending M, Luder HU, Brunner TJ, Knecht S, Stark WJ and Zehnder M,

- Effect of sodium hypochlorite on human root dentine—mechanical, chemical and structural evaluation, *Int. Endod. J.*, 2007, 40:786-93.
- [6] Hand RE, Smith ML, Harrison JW, Analysis of the effect of dilution on the necrotic tissue dissolution property of sodium hypochlorite, *J. Endod.*, 4, 1978, 60–4.
- [7] Harrison JW, Irrigation of the root canal system, *Dent. Clin. N. Am.*, 1984, 797–808.
- [8] Chang YC, Huang FM, Tai KW, Chou MY, The effect of sodium hypochlorite and chlorhexidine on cultured human periodonal ligament cells, *Oral Surg Oral Med Oral Pathol Oral Radial Endod.*, 92, 2001, 446-450.
- [9] Mello I, Coil J, Antoniazzi J H, Paulo S, Does a final rinse to remove smear layer interfere on dentine permability of root canals, *Oral Surg Oral Med Oral Pathol Oral RadiolEndod.*, 107, 2009, e47-e51.
- [10] Alt V, Bechert T, Steinrucke P, Wagener M, Seidel P, Dingeldein E, *et al.*, An in vitro assessment of the antibacterial properties and cytotoxicity of nanoparticulate silver bone cement, *Biomaterials*, 25, 2004, 4383-91.
- [11] Martinez-Castanon GA, Nino-Martinez N, Martinez-Gutierrez F, Martinez-Mendoza JR, FR, Synthesis and antibacterial activity of silver nanoparticles with different sizes. *J Nanopart Res.*, 10, 2008, 1343-8.
- [12] Gong P, Li H, He X, Wang K, Hu J, Tan W, *et al.*, Preparation and antibacterial activity of Fe<sub>3</sub>O<sub>4</sub> and Ag nanoparticles, *Nanotechnol.*, 18, 2007,604–11.
- [13] Sharma VK, Yngard RA, Lin Y, Silver nanoparticles: green synthesis and their antimicrobial activities, *Adv Colloid Interface Sci.*, 145 (1-2), 2009, 83-96.
- [14] Morones JR, Elechiguerra JL, Camacho A and Ramirez JT, The bactericidal effect of silver nanoparticles, *Nanotechnol.*, 16, 2005, 2346–53.
- [15] Ovington LG, The truth about silver, *Ostomy Wound Manage*, 2004, 50-9A, Suppl: 1S-10S.
- [16] Catalina, Marambio-Jones ,Hoek, Eric M.V, A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the

- environment, *J. Nanoparticle Res.*, 12, 2010, 1531–1551.
- [17] Zhang Y, Peng H, Huang W, Zhou Y and Yan D, Facile preparation and characterization of highly antimicrobial colloid Ag or Au nanoparticles, *J. Colloid Interface Sci.*, 325, 2008, 371–376.
- [18] E. Filipo, A. Serra and D. Manno, Poly (Vinyl Alcohol) Capped Silver Nanoparticles as Localized Surface Plasmon Resonance-Based Hydrogen Peroxide Sensor, *Sensors and Actuators B: Chemical.*, 2 (138), 2009, 625-630.
- [19] Hemant K. Chitte, Narendra V. Bhat, Narayan S. Karmakar, Dushyant C. Kothari, Ganesh N. Shinde, Synthesis and Characterization of Polymeric Composites Embeded with Silver Nanoparticles, *World J. Nano Sci. and Engg.*, 2, 2012, 19-24
- [20] Falentin-Daudre C, Faure E, Svaldo-Lanero T, Farina F, Jerome C, Van De Weerd C, Martial J, Duwez AS and Detrembleur C, Antibacterial polyelectrolyte micelles for coating stainless steel, *Langmuir*, 2012, 28 (18), 7233–7241.
- [21] Khalil Abdelrazek Khalil, H. Fouad, T. Elsarnagawy, Fahad N. Almajhdi, Preparation and Characterization of Electrospun PLGA/silver Composite Nanofibers for Biomedical Applications, *Int.J. Electrochemical Sci.*, 8 (3), 2013, 3483
- [22] Valipour F, Davaran S, Esmhosseini M, Nejati M, Kianfar H, Pasdaran A, Synthesis and antibacterial activity of silver nanoparticles embedded in smart poly(*N*-isopropylacrylamide)-based hydrogel networks, *J. Nanotechnol. Eng. Med.*, 2(4), 2011, 041001-7.
- [23] Kandarp Mavani, Mihir Shah, Synthesis of Silver Nanoparticles by using Sodium Borohydride as a Reducing Agent, *Int. J. Engg. Res. & Technol.*, 2013, 2:3.
- [24] Rafie F, Javadzadeh Y, Javadzadeh A.R, Alizadeh Ghavidel L, Jafari B, Moogooee M, and Davaran S, *In Vivo* Evaluation of Novel Nanoparticles Containing Dexamethasone for Ocular Drug Delivery on Rabbit Eye, *Current Eye Res.*, 35 (12), 2010, 1081–1089.
- [25] The Clinical and Laboratory Standards Institute (CLSI), Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved

- Standard, Wayne, Pennsylvania, USA, 7<sup>th</sup> Ed., CLSI document M7-A7, 2006.
- [26] Carmona-Ribeiro AM, and Dias de Melo Carrasco L, Cationic Antimicrobial Polymers and Their Assemblies, *Int. J. Mol. Sci.*, 14, 2013, 9906-9946.
- [27] Russell AD, Hugo WB and Ayliffe GAJ, Principles and practice of disinfection, preservation and sterilization, Blackwell Scientific Publication, London, 1982.
- [28] Van de Voorde K, Nijsten T, Schelfhout K, Moorkens G and Lambert J, Long-term use of silver containing nose-drops resulting in systemic argyria, *Acta Clin. Belg.*, 2005, 60, 33-35.
- [29] Gomes-Filho JE, Silva FO, Watanabe S, Cintra LT, Tendoro KV, Dalto LG, Pacanaro SV, Lodi CS and de Melo FF, Tissue reaction to silver nanoparticles dispersion as an alternative irrigating solution, *J. Endod.*, 36, 2010., 1698-1702.
- [30] Siren EK, Haapasalo MP, Ranta K, Salmi P and Kerosuo EN, Microbiological findings and clinical treatment procedures in endodontic cases selected for microbiological investigation, *Int. Endod. J.*, 30, 1997, 91-95
- [31] Peciuliene V, Balciuniene I, Eriksen HM and Haapasalo M, Isolation of *Enterococcus faecalis* in previously root-filled canals in a Lithuanian population, *J. Endod.*, 26., 2000, 593-595
- [32] Siqueira JF, Jr., Rocas IN, Souto R, de Uzeda M and Colombo AP, *Actinomyces* species, streptococci, and *Enterococcus faecalis* in primary root canal infections, *J. Endod.*, 28, 2002, 168-172
- [33] Gomes BFP, Vianna MF and Matsumoto CU, Disinfection of gutta-percha cones with chlorhexidine and sodium hypochlorite, *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.*, (100), 2005, 512-7.
- [34] Portenier I, Waltimo T, Orstavik D and Haapasalo M, The susceptibility of starved, stationary phase, and growing cells of *enterococcus faecalis* to endodontic medicament, *J. Endod.*, (31), 2005, 380-6.
- [35] Sigueria JF Jr, Rocas IN and Santos SR, Efficacy of instrumentation techniques and irrigation regimens in reducing the bacterial population

- within root canals, *J. Endod.*, (28), 2002, 181-4.
- [36] Cervone F, Tronstad L and Hammond B, Antimicrobial effect of chlorhexidine in controlled release delivery system, *Endod Dental Traumatol.*, (16), 1990, 33-6.
- [37] Mohammadi Z, Abbott PV The properties and applications of chlorhexidine in endodontics, *IntEndod, J.*, 42, 2009, 288-302
- [38] Moyer CA, Brentano L, Gravens DL, Margraf HW and Monafo WW, Treatment of large human burns with 0.5% silver nitrate solution, *Arch. Surg.*, 90, 1965, 812-67.
- [39] Sharma VK, Yngard RA and Lin Y, Silver nanoparticles: green synthesis and their antimicrobial activities, *Adv Colloid Interface Sci.*, 145-1-2, 2009, 83-96.
- [40] Alt V, Bechert T, Steinrücke P, *et al.*, Nanoparticulate silver, A new antimicrobial substance for bone cement, *Orthopade*, 33 (8), 2004, 885-892.
- [41] Bellinger CG, Conway H, Effects of silver nitrate and sulfamylon on epithelial regeneration, *PlastReconstr Surg.*, 45, 1970, 582-5.
- [42] Lok, C.N, Ho CM, Chen R, He QY, Yu WY, Sun H, Tam PK, Chiu JF, Che CM. Proteomic analysis of the mode of antibacterial action of silver nanoparticles, *J. Proteome Res.*, 5, 2006, 916-924.
- [43] Morones JR, Elechiguerra JL, Camacho A, Ramirez JT, The bactericidal effect of silver nanoparticles, *Nanotechnol.*, 16, 2005, 2346-53.