

Subgingival Application of Amla Extract Mediated Copper and Graphene Oxide Nanocomposite Gel in the Treatment of Periodontal Pocket: A Randomized Clinical Study.

Running Title: Effect of copper and graphene oxide nanocomposite gel in periodontal pocket

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Abstract:

Background: Beneficial effects of the novel nanocomposite made with copper and graphene oxide nanoparticles using amla extract have already been proven in our previous studies. As only scaling and root planing fails to give expected outcomes in some cases several adjective measures are taken to achieve the optimal outcome. Contact killing properties of copper metal, antioxidant effects of amla are well known. Hence this study evaluated the adjunctive application of the novel gel as an adjunct to the conventional scaling and root planing.

Aim: To evaluate clinical efficacy of amla extract mediated copper (Cu) and graphene oxide (GO) nanocomposite gel as an adjunct to conventional scaling and root planing to treat periodontal pockets.

Materials and Methods: Novel nanocomposite gel was made with 20 μ L concentration. Selected patients were divided into 2 groups. Each group had 10 individuals with periodontal pockets of ≥ 5 mm. Group 1 patients were treated with conventional scaling and root planing (SRP). Group 2 individuals were treated with scaling root planing + subgingival application of the novel gel with help of wide bore blunt ended syringe. Subgingival application was done at initial SRP appointment followed by 7 and 14 days. Clinical parameters including probing depth (PD), plaque index (PI) and gingival index (GI) at baseline and 90 days.

Results: Significant reduction in GI, PI, and PD was observed when compared to baseline. Intergroup comparison yielded significant reduction in the clinical parameters. Higher reduction in the test group was observed.

Conclusion: Subgingival application of Cu and GO nanocomposite containing novel gel as an adjunct to SRP is an effective treatment for treating periodontal pockets.

Key Words: Copper, Graphene oxide, Nanocomposite, Subgingival application, Periodontitis

Introduction:

Nanotechnology is a relatively new technology that has ushered in a new era in every aspect of research. In recent years, nanoparticles have attracted a lot of attention in the scientific world. Optics, electronics, health, and materials sciences have all benefited from this technique^{1,2}.

Chronic periodontitis is a condition caused by bacterial accumulation and invasion of the periodontium, primarily gram-ve anaerobic or facultative bacteria present in dental plaque, which causes chronic inflammation and

attachment degradation of the periodontal ligament, as well as alveolar bone loss^{3,4}. It was previously assumed that chronic periodontitis is caused by specific infectious bacteria that can affect the inflammatory and immune systems, later it was found that the quality, not the amount, of pathogenic local factors determines the disease's course⁵.

Copper was the first recognized metal having antimicrobial properties by US-EPA for its superior owing to their nontoxicity, biocompatibility, use in drug and bactericidal activity^{6,7}. Copper's contact killing property has been extensively researched in recent years. Increased bacterial intracellular oxidative stress in the bacterial cell wall due to ion release from the copper surface results in bacterial cell lysis, according to studies. Synthesis of copper nanoparticles is highly technique sensitive due to its high incidence of oxide layer formation on the nanoparticle surface which will result in reduced antibacterial property⁸.

Graphene oxide was selected for its excellent mechanical strength, electrical conductivity and most importantly the barrier properties, also easy step down preparation of graphene oxide nanoparticles makes it one of the most efficient carriers of nanoparticles in any nanocomposite⁹.

Ideal treatment for chronic periodontitis cases differed according to the clinical parameter. Lindhe et al have postulated critical probing depth values that help the clinician to advocate the correct treatment protocol to the patient. Destruction of the bacterial biofilms is the basic goal of any periodontal treatment as biofilm provides increased resistance to the bacteria.

Scaling and root planing is still the gold standard for non surgical periodontal therapy but recently multiple adjunctive methods have been studied to achieve optimal treatment outcome and faster tissue healing post SRP. Adjunctive use of lasers, subgingival irrigators, local drug delivery systems etc are few of the main adjuncts being tried in various settings¹⁰. The present study was done to evaluate the clinical efficacy of amla extract mediated copper (Cu) and graphene oxide (GO) nanocomposite gel as an adjunct to conventional scaling and root planing (SRP) to treat periodontal pockets.

Material and Methods:

Preparation of amla extract

Freshly collected organic amla fruits were thoroughly washed multiple times in distilled water. Seed was taken out and the pulp was cut into small pieces using a sterile knife and was ground into small particles by means of a mortar and pestle. Amla extract was prepared by 1 grams of amla pulp with 100 ml distilled water to make 1 molar solution of amla extract.¹¹

Synthesis of CuGO nano-composite

Nanocomposite synthesis was done by mixing 50 ml of both 1M solutions of copper and graphene oxide nanoparticles as mentioned in the previous steps. The nanocomposite solution was stirred overnight on an orbital shaker followed by a magnetic heated stirrer till color change was observed. UV-vis spectrometric readings were taken hourly to check the synthesis of copper-graphene oxide nano composite. The resultant mixture was centrifuged and CuGO nanocomposite was obtained.¹¹

Preparation of CuGO nano-composite gel

In order to create a gel from the nanocomposite, Xanthan based gel formulations containing the final CuGO nanocomposite (20 μ L concentration) was prepared. This gel formulation was packaged under sterile conditions, labeled with appropriate details and stored at room temperature for further use.

Study design

The present study was carried out on 20 patients seeking periodontal treatment in the department of Periodontics Saveetha dental college and hospital, Chennai. These patients were diagnosed to have chronic periodontitis with at least two posterior quadrants with pocket depth ≥ 5 mm. No history of any preceding oral infections or periodontal treatment for at least three months before starting the study. Smokers and alcoholic patients have been excluded also with pregnant, post-menopausal and lactating women with age range of patients is between 35 to 55 years old. Patients with poor systemic health like uncontrolled diabetes, hypertension, osteoporosis, collagen disorders are excluded also with patients who were on or expected to take antibiotics or anti-inflammatory drugs within duration of the study. Patients were divided into 2 groups randomly with a lottery system. Group 1 patients were treated with SRP alone and group 2 individuals were treated with SRP+subgingival application of the novel Cu and GO nanocomposite gel. Clinical parameters including probing depth (PD), plaque index (PI) and gingival index (GI) at baseline and 90 days.

Results:

Statistical analysis was done using Version 23 of SPSS software. Paired t test was done to compare means of the PD, GI, PI within the group at baseline and 90 days. It was found to be statistically significant (p value<0.05). independent t test was done to compare means of the PD,GI,PI between the group at baseline and 3 months. It was found to be statistically significant (p value<0.05). (Table 1)

Table 1: Comparing the clinical parameters between test (CuGO) and (CHX) groups.

Parameters	Groups (Mean ± SD)		P value
	Group 1 (test)	Group 2 (control)	
PD			
Baseline	4.80 ±0.41	4.60±0.488	0.000
90 days	2.20±0.41	3.20±0.59	0.000
P value	0.000		
PI			
Baseline	2.47±0.65	2.47±0.64	1.000
90 days	0.80±0.56	1.93±0.44	0.000
P value	0.000		
GI			
Baseline	2.46±0.64	2.47±0.64	1.000
90 days	0.80±0.56	1.94±0.45	0.000
P value	0.000		

Discussion:

The results obtained in the present randomized multicenter trial showed that the adjunctive subgingival administration of novel Cu-GO nanocomposite infused gel significantly improved the positive therapeutic effects of extensive SRP on chronic periodontitis. These conclusions are supported by the clinical parameters and they were

particularly evident at 3 months. Irrespective of their initial PD, the sites treated with SRP + novel Cu-GO nanocomposite infused gel showed significantly lower PD and CAL scores compared to the SRP treatment group. Although the parameters were found to be significant in both the groups the percentage of reduction of the PD and gain in the attachment loss was seen greater in the test group.

Chlorhexidine is a gold standard adjunctive proven by many studies. Multiple studies have been to compare the adjunctive use of CHX gel with SRP alone¹². Application of CHX gel subgingivally has shown beneficial effects on the improvement in clinical parameters^{3,13}.

The present results are in contrast with findings from studies on the effects of subgingival administration of a CHX gel as an adjunct to SRP. In particular, Oosterwaal et al. investigated the effects of a 2% CHX gel used as an adjunct to SRP; similar clinical results were obtained with SRP treatment alone and when subgingival administration of 2% CHX or placebo gels were associated with SRP. Unsal et al.¹⁵ found less CAL gain in periodontal sites treated with SRP and subgingival administration of 1% CHX gel compared to those treated with SRP alone. This observation was explained by the mechanical interference of the CHX gel with the early healing process.

Subgingival application of subgingival 1 % gel was shown to give negligible beneficial effects when used as an adjunct for single stage full mouth disinfection^{14,15}. As proven by previous studies Cu and GO nanocomposite has extremely beneficial properties which can help overcome the current issue^{11,16}. Furthermore, the lack of adherence of CHX to root surfaces and its high affinity for blood and serum proteins were also hypothesized to be among the causes of its low subgingival substantivity.

Xanthan gum has been shown to have bioadhesive properties, although it is not well established which physical properties of the polymer vehicle are important in the retention at specific oral sites. It was reported that a greater retention of xanthan gum over polyethylene oxide within periodontal pockets. Furthermore, xanthan gum provided the most prolonged adhesion time on the oral mucosa with respect to other delivery vehicles.¹⁶

Emblica officinalis (Amla) has a lot of medicinal properties and has been used since ancient times¹⁶. A wide range of phytochemical components present in amla including alkaloids, tannins, and flavonoids are responsible for its variety of medicinal uses. It belongs to the family: Euphorbiaceae. It is cytoprotective, antitussive, gastroprotective and has antioxidant, immunomodulatory property. Keeping all these beneficial effects of amla fruit and easy availability we decided to choose amla for the green preparation of the nanocomposite in the present study.

When certain bacteria come in contact with the uncoated copper surface it results in formation of intracellular oxidative stress in the bacterial cell wall due to release of ions from the copper surface which results in bacterial cell lysis This phenomenon was well known since ancient times but, recently it has gotten renewed attention from the researchers. The term 'contact killing' was coined for the above phenomenon. United States Environmental Protection Agency (US EPA) recognized copper as the first antimicrobial metal in the year of 2008. One of the most important advantages of copper as an antimicrobial agent is its low levels of resistance in the microorganisms. The large surface area and high charge density of NPs enable them to interact with the negatively-charged surface of bacterial cells to a sizable extent resulting in enhanced antimicrobial activity.

As we can see in the result table the nano gel group showed significant improvement in the clinical parameters. Further studies to compare the nano gel with CHX gel, have to be done to compare both the gels.

Conclusion:

Within the limits of the study, it can be suggested that adjective use of CuGO nanocomposite has significantly beneficial properties when compared with SRP alone. Further studies to compare the novel nano gel with commercially available CHX gel should be done to reach a conclusion.

References:

1. Ketkar Gn, Malaiappan S. Green Preparation Of Nano Copper (Cu) With Nano Graphene Oxide (Go) Nano Composite Characterization And Antimicrobial Activity *Plant Cell Biotechnology And*, <https://www.ikppress.org/index.php/pcbmb/article/view/5550> (2020).
2. Yasmin A, Ramesh K, Rajeshkumar S. Optimization And Stabilization Of Gold Nanoparticles By Using Herbal Plant Extract With Microwave Heating. *Nano Conver*2014; 1: 12.
3. Cosyn J, Sabzevar Mm. A Systematic Review On The Effects Of Subgingival Chlorhexidine Gel Administration In The Treatment Of Chronic Periodontitis. *J Periodontol*2005; 76: 1805–1813.
4. Jia L, Jia J, Xie M, Et Al. Clinical Attachment Level Gain Of Lasers In Scaling And Root Planing Of Chronic

- Periodontitis: A Network Meta-Analysis Of Randomized Controlled Clinical Trials. *Lasers Med Sci*2020; 35: 473–485.
5. Cobb Cm. Clinical Significance Of Non-Surgical Periodontal Therapy: An Evidence-Based Perspective Of Scaling And Root Planing. *Journal Of Clinical Periodontology*2002; 29: 22–32.
 6. Konieczny J, Rdzawski Z. Antibacterial Properties Of Copper And Its Alloys. *Archives Of Materials Science And Engineering*2012; 56: 53–60.
 7. Samavati A, Ismail Af. Antibacterial Properties Of Copper-Substituted Cobalt Ferrite Nanoparticles Synthesized By Co-Precipitation Method. *Particuology*2017; 30: 158–163.
 8. Vincent M, Duval Re, Hartemann P, Et Al. Contact Killing And Antimicrobial Properties Of Copper. *J Appl Microbiol*2018; 124: 1032–1046.
 9. Zhu Y, Murali S, Cai W, Et Al. Graphene And Graphene Oxide: Synthesis, Properties, And Applications. *Adv Mater*2010; 22: 3906–3924.
 10. Brayer Wk, MellonigJt, Dunlap Rm, Et Al. Scaling And Root Planing Effectiveness: The Effect Of Root Surface Access And Operator Experience. *J Periodontol*1989; 60: 67–72.
 11. Ketkar Gn, Malaiappan S. Green Preparation Of Nano Copper (Cu) With Nano Graphene Oxide (Go) Nano Composite Characterization And Antimicrobial Activity Against Oral Aerobic Pathogens. *Plant Cell Biotechnology And Molecular Biology*2020; 31–39.
 12. Zhao H, Hu J, Zhao L. Adjunctive Subgingival Application Of Chlorhexidine Gel In Nonsurgical Periodontal Treatment For Chronic Periodontitis: A Systematic Review And Meta-Analysis. *Bmc Oral Health*2020; 20: 34.
 13. Figueiredo De Almeida Gomes Bp, Vianna Me, SenaNt, Et Al. In Vitro Evaluation Of The Antimicrobial Activity Of Calcium Hydroxide Combined With Chlorhexidine Gel Used As Intracanal Medicament. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, And Endodontology*2006; 102: 544–550.
 14. OosterwaalPjm, MikxFhm, Hof Ma, Et Al. Short-Term Bactericidal Activity Of Chlorhexidine Gel, Stannous Fluoride Gel And Amine Fluoride Gel Tested In Periodontal Pockets. *Journal Of Clinical Periodontology*1991; 18: 97–100.
 15. KalaitzakisCj, Tynelius-Bratthall G, Attström R. Clinical And Microbiological Effects Of Subgingival Application Of A Chlorhexidine Gel In Chronic Periodontitis. A Pilot Study. *Swed Dent J*1993; 17: 129–137.
 16. Ketkar Gn, Malaiappan S, Muralidharan. Comparative Evaluation Of Inherent Antimicrobial Properties And Bacterial Surface Adherence Between Copper And Stainless Steel Suction Tube. *J Pharm Res Int*2020; 149–156.