Assessment and comparison of the antimicrobial and clinical efficacy of copper nanoparticles gel with scaling and root planing (SRP) against chlorhexidine gel with scaling and root planing (SRP) as a local drug delivery agent in patients with periodontitis – A randomized interventional clinical trial.

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Research Article

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Abstract

BACKGROUND: Nanotechnology provides a novel approach for delivering and stopping alveolar infections. Nano-materials have improved and distinctive physicochemical properties like ultra-small sized ranging from 1–100 nm tiny solid molecules, substantial ratio of mass to surface area and increased chemical responsiveness makes it encouraging antibacterial therapy. Cu NPs exhibits excellent antiviral behavior which inactivates virus by inhibiting its RNA replication while its protein shell remains intact, which suggests that ions penetrated into the virus to act against the RNA. In addition to direct killing, NPs modulates immune responses, and enhances innate antimicrobial immune defenses. Phagocyte-derived reactive oxygen and nitrogen species remain vital for the host to fight against infective pathogens. Nitric oxide gets oxidized to Cu, present in protective proteins in microbes, to free Cu ions, which boosts toxicity to microbial cells. The aim of the study is to appraise physical properties, antimicrobial, cytotoxicity, time kill assay, anti-biofilm formation of Cu nanoparticles gel against periodontal pathogens (Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Fusobacterium nucleatum, Prevotella intermedia and Tannerella forsythia). In-vivo analysis to compare clinical efficacy of copper nanoparticle gel + SRP against chlorhexidine gel + SRP as a local drug delivery agent in patients with periodontitis.

METHODS: Copper nanoparticles were procured from Nano research Laboratory. Gel was prepared and assessed for Minimum Inhibitory Concentration and Minimum Bactericidal Concentration (MIC, MBC), MTT assay to find out its cytotoxicity against periodontal and mouse fibroblasts, Time Kill Assay at different time intervals against periodontal pathogens. Anti-biofilm formation was done by cell culture method.

DISCUSSION:
The pH of the prepared CU-NP gel was 5.76, viscosity was 322.5 cPs and spread diameter of 425% IC50 value of CUNP against mouse fibroblast was 49.12±1.67 whereas against periodontal fibroblast at 3.12 μg/ml, displaying 98% of cell viability. At 1hr interval, time kill assay had revealed no growth of periodontal pathogens when carried out for 48 hrs. MIC value was within the range of 0.4-3.12 μg/ml, whereas MBC value was 0.8-3.12 μg/ml. Anti-biofilm activity did not demonstrate promising result as compared to control.

Since the results of Copper nanoparticles gel were promising which encouraged us to evaluate in -vivo analysis of gel as a local drug delivery in the treatment of periodontal pocket in periodontitis patients.

Trial registration: CTRI/2022/07/044047 (Clinical Trial Registry of India www.ctri.nic.in)

Administrative information

Note: the numbers in curly brackets in this protocol refer to SPIRIT checklist item numbers. The order of the items has been modified to group similar items (see http://www.equator-network.org/reporting-guidelines/spirit-2013-statement-defining-standard-protocol-items-for-clinical-trials/).
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<td><strong>Author details (5a)</strong></td>
<td>1] Designation: Dr. Swapna Arunkumar Mahale, PhD scholar Department of Periodontology and Implantology, Sharad Pawar Dental College, Datta Meghe Institute of Higher Education and Research (Deemed to be University), Sawangi, Wardha</td>
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<tr>
<td><strong>Name and contact information for the trial sponsor (5b)</strong></td>
<td>In Vitro analysis Partially sponsored by I-STEM (Indian Science, Technology and Engineering Facilities Map, Ref: I-STEM/catalyticgrant/acad_17/2022-23)</td>
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Background and rationale

Background:

Nanotechnology furnishes an opportunity to resolve antibiotic resistance of microbes to drugs. Nanoparticles tackle "common antibiotic resistance mechanisms like regulation of permeability, multi-drug efflux pumps, antibiotic degradation and target site binding affinity mutations". The introduction of copper fittings, and work surfaces acts as an additional blockade because of its ability to speedily eradicate MRSA to prevent cross-contamination. Copper represents two ends of the viability spectrum for micro-organisms, being both an essential nutrient at one end but also highly toxic at the other end. The metals used for these NPs are almost exclusively heavy metals, such as Ag and Cu. The mechanism of action recognized by the nanoparticles is not fully understood thereby underscoring the need for more studies. A necessity for efficacious clinical trial is required to estimate the usefulness in conjunction with the safety. The current information on NPs inspires that further research should be conducted to invent the potential applications of NPs in the controlling of dental infections.

Objectives (7) - Phase I-In Vitro

- To assess antimicrobial activity of copper nanoparticles against periodontal pathogens (Porphyromonas gingivalis, P. intermedia, F. nucleatum, Tannerella forsythia, and Actinobacillus actinomycetemcomitans).

- To assess cell viability (MTT assay) for its efficacy.

- To assess Safety and dose determination of the prepared copper nanoparticle gel

- To assess Time kill assay for copper nanoparticle gel against periodontal pathogens.

- To assess Anti-biofilm formation for copper nanoparticle gel

Phase II-In Vivo

- To assess clinical efficacy of copper nanoparticle gel +SRP on the basis of Papillary Bleeding Index (PBI), Modified Plaque Index (PI), Probing Pocket Depth (PPD), Relative Attachment Level (RAL) in patients with periodontitis.
To assess clinical efficacy of chlorhexidine gel +SRP on the basis of Papillary Bleeding Index (PBI), Modified Plaque Index (PI), Probing Pocket Depth (PPD), Relative Attachment Level (RAL) in patients with periodontitis

To compare clinical efficacy of copper nanoparticle gel +SRP against chlorhexidine gel +SRP on the basis of Papillary Bleeding Index (PBI), Modified Plaque Index (PI), Probing Pocket Depth (PPD), Relative Attachment Level (RAL) in patients with periodontitis

Trial design {8} In-Vitro, In-Vivo

Methods

Study setting {9} The subjects to be studied will be selected from the outpatient section, Department of Periodontology and Implantology, Datta Meghe Institute of Higher Education and Research (Deemed to be University), Sawangi, Wardha. The study procedure was first approved by the Institutional Ethics Committee (DMIMS (DU)/IEC/2021/581) and will be in accordance with the Declaration of Helsinki for conduct of biomedical research on human subjects.

Participants-A total of 40 subjects with Generalized Periodontitis Stage 2 Grade B will be divided into two groups. It will be made clear to the potential subjects that participation will be voluntary and written informed consent will be gained from those who accept to contribute in the study.

Interventions Group A- scaling and root planing followed by copper nanoparticles gel application in residual pockets, Group B - Scaling and root planing followed by Chlorhexidine gel application in residual pockets.

Outcomes-To assess the clinical outcomes in terms of Papillary Bleeding Index (PBI), Modified Plaque Index (PI), Probing Pocket Depth (PPD), Relative Attachment Level (RAL) at baseline, 1 month and 3 month as local drug delivery agent in patients with periodontitis.

Eligibility criteria {10}

Inclusion Criteria:
Both males and female patients, Age range between 35 - 55 years, Subjects should have at least 20 natural teeth. Patients diagnosed with Generalized Periodontitis Stage 2 Grade B currently
unstable without any risk factor (2017 World Workshop on the classification of periodontal and peri-implant diseases and conditions). Patients ready to give informed consent and ready to collaborate with the study protocol

**Exclusion Criteria:** Pregnant/ lactating mothers, Smokers/tobacco in any form, Patients who have undergone periodontal therapy, antibiotic or antiseptic therapy 6 months former to the study, Patients with bleeding disorders or under immunosuppressive chemotherapy. Any other systemic disease which can alter the course of periodontal disease

**Who will take informed consent?** {26a}

Informed consent or assent from potential trial participants will be taken by the primary investigator.

**Additional consent provisions for collection and use of participant data and biological specimens** {26b}

Additional consent provisions for collection and use of participant data will be taken by the primary investigator.

**Interventions**

- **Group A** - scaling and root planing followed by copper nanoparticles gel application in residual pockets,
- **Group B** - Scaling and root planing followed by Chlorhexidine gel application in residual pockets.

**Explanation for the choice of comparators** {6b} - As Chlorhexidine is considered as a gold standard in the prevention of gingival inflammation, reduction of pocket depth and gain in clinical attachment level.

**Intervention description** {11a}
Criteria for discontinuing or modifying allocated interventions (11b)

Participant request, not willing to follow up

Strategies to improve adherence to interventions (11c)
Patient education, motivation, Recall visits

**Relevant concomitant care permitted or prohibited during the trial (11d)**

Other Oral Hygiene measures are not permitted

**Provisions for post-trial care (30)**

Follow up at regular intervals

**Outcomes (12)**

Primary outcome - Probing Pocket Depth (PPD), Relative Attachment Level (RAL)

Secondary outcome - Papillary Bleeding Index (PBI), Modified Plaque Index (PI),

**Participant timeline (13)**

Baseline, 1 month and 3 month interval

**SAMPLE SIZE: (14)**

**Recruitment (15)** A power analysis was established by G*Power, version 3.0.1 (Franz Faul University, Kiel, Germany). Total calculated minimum sample size of 38 rounded to 40 subjects (i.e. 20 participants in each group; total 2 study groups) would yield 80% power to detect significant differences, with effect size of 0.95 and significance level at 0.5.

**Assignment of interventions: allocation**

After reviewing the randomization sheet, the pharmacist will dispense the therapy for the patient. The randomization sheet will contain information about the study, protocol number, randomization number and the information concerning the assignment of treatments i.e, test product.

**Sequence generation (16a)**

CRO provides the Investigator with a randomization list with IP codes for patients enrolled at the site. A randomization list will be generated by the Biostatistician for this study by using the Statistical program in the SAS environment by the random number generation method when including a patient in the study, investigator will assign the patient next available randomization number. It is mandatory to adhere to the randomization numbers in an increasing order. (For example, after the patient with the number “001”, the next patient should be assign the number “002” etc.).

**Concealment mechanism (16b)**

Envelopes will be prepared with defined randomized treatment as per randomization schedule for all the subjects. All the envelopes will be kept in controlled access at the site and will only be broken by the Site
PI for the subject(s)

Implementation (16c)

Assignment of interventions: Blinding

Who will be blinded (17a)

Trial participants, outcome assessors, data analysts.

Procedure for unblinding if needed (17b)

NA.

Data collection and management- All the results will be tabulated and statistically analyzed using SPSS software (version 26© SPSS, Chicago, IL).

Plans for assessment and collection of outcomes (18a)- Clinical parameters will be assessed by using different indices (PBI, MPI, PPD and CAL), values will be calculated at different time intervals.

Plans to promote participant retention and complete follow-up (18b)- Patients will be educated and motivated for oral hygiene and recall visit.

Data management (19)- Data will be listed as mean and standard deviation. 95% confidence interval and 80% of power will be considered. Values will be considered significant when P < 0.05.

Confidentiality (27)- It will be maintained by primary investigator.

Plans for collection, laboratory evaluation and storage of biological specimens for genetic or molecular analysis in this trial/future use (33)- NA

Statistical methods- All the results will be tabulated and statistically analyzed using SPSS software (version 26© SPSS, Chicago, IL).

Statistical methods for primary and secondary outcomes (20a)-

Paired “t” test, Unpaired “t” test

Interim analyses (21b)

NA

Methods for additional analyses (e.g. subgroup analyses) (20b)
Discussion

The pH of the prepared CU-NP gel was 5.76, viscosity was 322.5 cPs and spread diameter of 425%, IC50 value of CUNP against mouse fibroblast was 49.12±1.67 whereas against periodontal fibroblast at 3.12μg/ml, displaying 98% of cell viability. At 1hr interval, time kill assay had revealed no growth of periodontal pathogens when carried out for 48 hrs. MIC value was within the range of 0.4-3.12μg/ml whereas MBC value was 0.8-3.12μg/ml. Anti-biofilm activity did not demonstrate promising result as compared to control.

Copper nanoparticles prepared by biological synthesis is cost-effective and eco-friendly. In -vivo analysis of copper nanoparticle gel as a local drug delivery in the treatment of periodontal pocket need to be
Declarations

**ACKNOWLEDGEMENTS:** Maratha Mandal's central research Laboratory, Belgaum for supporting and smooth conduction of in-vitro study, I-STEM (Indian Institute of Science, Technology and Engineering Facilities Map) for catalytic research grant (academic) to accomplish in-vitro test.

**AUTHORS’ CONTRIBUTIONS {31b}**

1) SM 2) PD

SM is the Primary Investigator, conceived the study, steered the proposal and protocol development. PD contributed to study design and development of the proposal.

All the authors read and approved the final manuscript.

**FUNDING {4}**
Partly by I-STEM (IN-Vitro) (Indian Science, Technology and Engineering Facilities Map, Ref: ISTEM/catalyticgrant/acad_17/2022-23)

AVAILABILITY OF DATA AND MATERIALS (29)

Available

ETHICS APPROVAL AND CONSENT TO PARTICIPATE (24)

Ethical approval for the study is obtained from the Institutional Ethics Committee of Datta Meghe Institute of Higher Education and Research (Deemed to be University), Wardha.

[Ref no: (DMIMS(DU)/IEC/2021/581)]. Written informed consent in patients own mother tongue will be taken from each participant.

CONSENT FOR PUBLICATION (32)

Yes.

COMPETING INTERESTS (28)

The authors declare that they have no competing interests.

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References


**Figures**

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**Figure 1**

The schedule of enrolment, interventions, and assessments.*