SILVER-NANOPARTICLES MODIFIED SOFT LINER MATERIAL FOR OBTURATOR WEARERS WITH ACQUIRED PALATAL DEFECT. MICROBIOLOGICAL CROSSOVER STUDY

Radwa M.K. Emera*, Ahmed K. Khalifa** and Noha Nabil Sheta***

ABSTRACT

Aim: This research aimed to clinically investigate the microbecidal power of silver nano-particles modified autopolymerized soft lining material for acquired palatal defect obturator wearers.

Materials and methods: A total of sixteen patients with acquired palatal defect were selected from the Mansoura Oncology center and referred to the Removable Prosthodontics Department, Faculty of Dentistry, Mansoura University. For each patient, immediate surgical obturator was constructed and then replaced by temporary obturator two weeks after the surgery. At definitive obturator construction, random assigning of patients into two equal groups (eight patients each) was done. Within patient cross over study design was followed with respect to silver nanoparticles (AgNps) percentages added to the self-cure soft liner material where: Patient’s obturators in group I were lined with untreated soft liner followed by modified soft liner with 0.05 % loading by weight of AgNps added to 5gm of the polymer powder followed by modified soft liner with 0.2 % loading by weight of AgNps for one month of each soft liner application. In group II the sequence was reversed. Microbiological investigation was performed to identify bacterial colonization count related to different soft liner samples.

Results: The highest mean of bacterial count was recorded with the soft liner samples without AgNps additives [mean = (762.5 ± 85.08) x10³]; while the lowest mean recorded with soft liner with 0.2% AgNps by weight [mean = (0.634 ± 0.73) x10³]. (ANOVA) test indicated a significant reduction in bacterial count within increased AgNps concentration. Wilks' lambda = 0.14, f = 40.75 and p = 0.001.

Conclusions: Within parameters of this study, it could be concluded that: Modification of autopolymerized acrylic-based soft liner with AgNps is a simple reliable method to enhance its bactericidal activity especially for obturator wearers. The antimicrobial efficacy of AgNps modified soft liner seemed to be concentration dependent.

KEYWORDS: Silver nano-particles, soft liner, obturator

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INTRODUCTION

Greater incidence of oral fungal infection was confirmed in cases received therapy for cancer. Chemotherapy and radiotherapy to head and neck region were accompanied by a seriously higher risk of oral fungal infections. This increased risk in patients receiving radiation therapy may be caused by diminished salivary secretion.\(^1\)

Additionally, clinical observations as well as preliminary investigations demonstrated post-surgical symptoms of defect inflammation caused by Candida strains usually occurring in cases of resected maxillary tumor who wear obturators to eliminate the passage between oral and nasal cavities. In these cases, the initiation of this pathological condition is accompanied by the adherence of pathogenic microorganisms to prosthesis contacting tissues. The contact affects proliferation of fungi both on the mucosa and on the prosthetic devices.\(^2,3\)

Soft lining materials are used to engage inaccessible undercuts, improve obturator retention and provide a cushion for soft tissues. There are numerous reports on soft lining materials being colonized by yeasts.\(^4,5\) Accordingly, maintenance of soft liners and the prevention of colony formation by microorganisms are important for oral tissue health.\(^6\)

Soft lining materials that possess antibacterial properties are preferred in use for obturators.\(^7\) Nano-particles that possess antimicrobial activity are of significant respect in the development of bactericidal materials.\(^8\) Modification of soft linings materials by silver nanoparticles (Ag NPs) can be an improvement in that respect. Fungicidal and bactericidal properties of silver have been known for centuries and they have been scientifically proved.\(^9,13\)

Silver nanoparticles showed antimicrobial effects as additives in irreversible hydrocolloid impression materials,\(^14\) denture base acrylic resins,\(^15\) tissue conditioners\(^16\) and Silicon soft lining materials.\(^17\)

Consequently, addition of AgNps into acrylic-based soft liners to modify their bactericidal effect was introduced. Recent in-vitro studies have proved the antifungal effect of nanosilver modified heat-cure acrylic-based soft denture liner\(^18\) and self-cure soft liner that showed a respectable antimicrobial effect.\(^19\) Therefore, this research aimed to clinically investigate the microbicidal power of silver nanoparticles modified self-cure soft lining material for acquired palatal defect obturator wearers.

The null hypothesis is no change in the bacterial count with different concentration of AgNps additives will be observed within the same patient.

MATERIALS AND METHODS:

A total of sixteen patients were selected from the Mansoura Oncology center and referred to the Removable Prosthodontics Department, Faculty of Dentistry, Mansoura University. They belong to both sexes (10 males, 6 females) and their ages ranged from 55 to 70 years (mean = 63 ± 7.3). Patients had either partially or completely edentulous arches with acquired palatal defect caused by resection of neoplastic lesion while the mandibular arches were intact partially or completely edentulous ones (Fig. 1). This research work was approved by the Faculty Ethical Committee under code number (1603-12).

After informing the patients about the treatment plane and their follow-up program, immediate surgical obturator was constructed and then replaced by temporary obturator two weeks after the surgery.

The patients were checked every 2 weeks for inspection of the defect tissues during wound healing and for the replacement of tissue conditioner lining of the obturators. After the complete healing approved by the oncologist, definitive obturator was constructed for each patient as follows

- Patient was seated in the upright position.
- Maxillary and mandibular hydrocolloid (CA 37.
Superior pink, cover, Holand bv) Impressions was poured in dental stone (Microstone®, USA)

- Maxillary and mandibular autopolymerizing acrylic resin (Acrostone, Egypt) custom trays were constructed.

- Design of the obturator metallic/acrylic frame work for patients with maxillary partially edentulous arches was planned followed by making the needed mouth preparations.

- For making the maxillary final impression, the custom tray was used to record final silicon impression after blocking of undesirable soft tissue undercuts in the resected side with a piece of gauze that was tied with a thread and lubricated with Vaseline. Impressions poured in dental stone.

- Wax pattern of the frame works of maxillary partially edentulous arches was constructed on the duplicated modified master casts, cast into cobalt chromium alloy, finished, polished and tried intraoral.

- Bite blocks were constructed followed by recording of maxillo-mandibular relation. Mounting on semi-adjustable articulator (Dentatus international, Arts, Hagertsen, Sweden) was done and non-anatomic acrylic resin artificial teeth (Acrostone™, Egypt) were arranged for harmonized occlusion.

- Obturators were processed into heat-cure acrylic resin following hollow pulp obturator technique, finished and polished (Fig.2).

- Verification of obturators was done intraoral, pressure areas of polished surfaces and borders were evaluated using pressure indicating paste. Bulb portion was checked by custom made pressure indicating paste.

Patients grouping:

Random assigning of patients to two equal groups (eight patients each) was done through computerized randomization to ensure comparability of the two groups.

Within patient crossover study design was followed with respect to silver nanoparticles (AgNps) (SigmaAldrich CO., USA) percentages added to the acrylic based autopolymerized soft liner material (Acrostone, Egypt), where:

Patient’s obturators in group I were lined with conventional soft liner. After one month, the liner was replaced by a soft liner modified by adding 0.05% by weight of AgNps added to 5gm of the polymer powder. After a month later the soft liner
was removed and a new layer of liner modified by adding 0.2 % by weight of AgNps added to 5gm of polymer powder was applied. In group II the sequence was reversed beginning with 0.2 % AgNps additive to the soft liner followed by 0.05 % AgNps additive to the soft liner and finally the conventional soft liner (Fig. 3, 4).

This study design was conducted to ensure that each patient would serve as a control for him/herself and eliminate any suspicious cumulative effect of AgNps added to the soft liner material. - Soft liner was mixed according to manufacture instructions, applied on the obturator pulp surface and allowed to set under guidance of the obturator adaptation to non-resected side. All patients were instructed for proper oral hygiene measures and regular periodic recalls for maintenance and evaluation.

**Microbiological examination:**

Soft liner samples were collected for each patient after one month of each studied soft liner application.

**Microbiological specimen collection:**
- Circular standardized diameter samples of soft liner were prepared using trephine bur.
- Samples were kept in sterile nutrient broth tubes and transferred immediately to the microbiology laboratory, Faulty of Medicine, Mansoura University.

**Bacterial culture:**
- Vials containing the soft liner samples were vortexed for 2 minutes to release bacteria then centrifuged for 5 minutes to help bacterial concentration.
- One microliter of the centrifuged solution was obtained by a pipette and plated on blood agar media in semiquantitative manner, then incubated at 37°C, for 48 hours.
- After bacterial culturing, identification of isolated bacteria was carried out according to standard protocols (20).
- The bacterial growth is represented in the form of CFU/mL

**RESULTS**

Due to the large reading numbers, all resulted readings were subdivided by 1000.

From the resulted date, the highest mean of bacterial count was recorded with the soft liner

![Fig. (3) samples of the soft liner with AgNps additives with 0% (A), with 0.05 (B) and 0.2% (C)]

![Fig. (4) Applied soft liner on the intaglio of obturator after intraoral application]
without AgNps additives (mean = 762.5 ± 85.08); while the lowest mean was recorded with modified soft liner with 0.2% AgNps by weight (mean = 0.634 ± 0.73) (Table 1). (Fig. 5)

A one way repeated measured analysis of variance (ANOVA), was conducted to evaluate the null hypothesis that there is no change in the bacterial count within different concentration of AgNps additives within the same patient (N=16). The results of the ANOVA indicated a significant reduction in bacterial count within increased AgNps concentration. Wilks’ lambda = 0.14, f = 40.75 and p = 0.001. Thus, there is a significant evidence for null hypothesis rejection.

The comparison proved that each pairwise difference was significant (p = 0.001). There was a significant decrease in bacterial count within increased AgNps concentration in soft liner. (Table 2)

**TABLE (1) Descriptive statistic for the bacterial count within different AgNP concentrations**

<table>
<thead>
<tr>
<th>AgNp Conc.</th>
<th>Mean</th>
<th>Std. Error</th>
<th>95% Confidence Interval</th>
<th>Lower Bound</th>
<th>Upper Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>762.500</td>
<td>85.086</td>
<td>581.144</td>
<td>943.856</td>
<td></td>
</tr>
<tr>
<td>0.05%</td>
<td>3.188</td>
<td>.390</td>
<td>2.357</td>
<td>4.018</td>
<td></td>
</tr>
<tr>
<td>0.2%</td>
<td>.634</td>
<td>.073</td>
<td>.479</td>
<td>.790</td>
<td></td>
</tr>
</tbody>
</table>

*All readings are subdivided by 1000*

**TABLE (2) Pairwise Comparisons within subject for the bacterial count with different AgNP concentrations**

<table>
<thead>
<tr>
<th>(I) conc</th>
<th>(J) conc</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig. b</th>
<th>95% Confidence Interval for Difference b</th>
<th>Lower Bound</th>
<th>Upper Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>0.05%</td>
<td>759.313*</td>
<td>84.895</td>
<td>.000</td>
<td>530.628</td>
<td>987.997</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.2%</td>
<td>761.866*</td>
<td>85.069</td>
<td>.000</td>
<td>532.713</td>
<td>991.019</td>
<td></td>
</tr>
<tr>
<td>0.05%</td>
<td>0%</td>
<td>-759.313*</td>
<td>84.895</td>
<td>.000</td>
<td>-987.997</td>
<td>-530.628</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.2%</td>
<td>2.553*</td>
<td>.410</td>
<td>.000</td>
<td>1.449</td>
<td>3.657</td>
<td></td>
</tr>
<tr>
<td>0.2%</td>
<td>0%</td>
<td>-761.866*</td>
<td>85.069</td>
<td>.000</td>
<td>-991.019</td>
<td>-532.713</td>
<td></td>
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<td>.000</td>
<td>-3.657</td>
<td>-1.449</td>
<td></td>
</tr>
</tbody>
</table>

*Based on estimated marginal means

*The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Bonferroni.

*All readings are subdivided by 1000*

Fig. (5) bar chart for bacterial count with different AgNps concentrations
DISCUSSION

Several studies confirmed the ability of Candida species especially Candida albicans to adhere to acrylic prostheses (Polymethyl methacrylate); this capability subjects a number of patients to medical complications requiring a long treatment course for candidiasis \(^{(21,22)}\), and can lead to serious infections with a high rate of mortality \(^{(23)}\).

Obturator wearers with oro-nasal communication often present prosthesis-induced stomatitis. The higher rate of Candida albicans colonization on the acrylic nasal surfaces must be considered as a serious matter because it is directly related to the nasal floor and adds to the nasal cavity contamination \(^{(24)}\). Adhesion of microbes on biomaterials surface relays on composition of biomaterials, the surface structure, as well as on the physic-chemical properties of the bacterial cell membrane; so that, AgNPs, have been proposed as antimicrobial agents in polymeric materials \(^{(25-28)}\).

Consequently, in this research AgNPs were introduced into soft liner of obturator as an attempt to enhance its bactericidal activity against Candida albicans and other microbial species which are the main predisposing factors of mucosal inflammation. The percentages of AgNPs used in this study (0.05\% and 0.2\% by Wight) were proven to decrease water sorption, and have no influence on shear-bond strength of the acrylic-based soft liner material \(^{(18)}\).

Results of this work indicated a statistically high significant decrease in Candida albicans colony formation (units/ml) after modification of the soft liner with AgNPs. The bactericidal activity appeared to be concentration dependent.

This result could be attributed to the fact that incorporating AgNPs into soft liner powder particles decrease their surface free energy, the interaction between cohesion forces that determine if wetting will occur or not, resulting in decreased possibility of microbial adhesion. This explanation is consistent with Dwairi et al.\(^{(29)}\) who reported that; the more the free surface energy, the more will be the adhesion of micro-organisms and alternatively, the more hydrophobic is the surface, the less adherence of cells is predicted.

After one month, it was found that all microbial species disappeared in case of soft liner modified with AgNPs with the higher concentration (0.2\%). This finding can be attributed to the role of freeing silver (Ag+) ions from nano-silver and its toxicity towards micro-organisms.\(^{(30)}\) The potential effect of nano-silver depends on its role as a source of Ag+ ions.\(^{(31)}\) Miao et al.\(^{(32)}\) refereed the effect of nano-silver to the dissolved Ag+ ions toxicity. Thus the higher concentration of AgNPs added to the soft liner material within the accepted biologic parameters, the more release of Ag+ ions and in turn the higher bactericidal effect.

However, some researches concluded that the antimicrobial effect was obtained from direct contact killing action; not due to Ag+ ions leached out of the copolymer.\(^{(33-35)}\) The metallic nano-particle size offers a significant large contact surface area of particle to the micro-organisms commensals \(^{(22)}\).

Previous researches were conducted to examine release of silver from a variety of polymeric materials, and a number of them concluded that the bactericidal effect of AgNPs was obtained through its direct contact to microorganisms.\(^{(36,18)}\) However, this was disagreed by some studies where different silver release concentrations were detected.\(^{(37-39)}\) This controversy may be explained by differences in polymeric materials types and their methods of polymerization, in addition to the differences in techniques of AgNPs incorporation.

CONCLUSIONS

Within parameters of this study, it could be concluded that:

1- Modification of acrylic-based soft liner with AgNPs is a simple method to enhance its bactericidal activity for obturator wearers.

2- The antimicrobial efficacy of AgNPs modified soft liner seemed to be concentration dependent.
RECOMMENDATIONS

1- Longer term clinical studies are still recommended to evaluate the cumulative effect of Ag-Nps on oral mucosa.

2- It is recommended to study the effect of nanoparticles of other metals to modify soft liners antimicrobial activity.

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REFERENCES


