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# Characterization of nanoceria-modified silicone soft liners: Surface morphology, hardness, wettability, cytotoxicity, and antifungal properties in artificial saliva – An in vitro study

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ARTICLE INFO	A B S T R A C T
Keywords: Soft liner Cytotoxicity Wettability Cerium oxide nanoparticles Surface hardness	<ul> <li>Statement of problem: Soft liners are essential for denture wearers, which aids in the healing of soft tissue injuries caused by rough denture base surfaces. Silicone soft liners, while effective, can accumulate biofilm over time, necessitating enhancement.</li> <li><i>Purpose:</i> This in vitro study aimed to assess the efficacy of silicone soft liners incorporating varying concentrations of cerium oxide nanoparticles.</li> <li><i>Materials and methods:</i> A stainless-steel die as per ISO standard 10139-2-2018 (35 × 6 mm), Using G*Power 3.0.10 software, 400 samples were prepared with 95 % confidence interval and 80 % power. Samples were divided into five groups: surface morphology (Group A), surface hardness (Group B), wettability (Group C), cytotoxicity (Group D), and antifungal property (Group E). Each group was subdivided based on cerium oxide nanoparticle concentrations. Samples were stored in artificial saliva until evaluation. Surface morphology was examined via scanning electron microscopy (SEM), surface hardness using Shore A Durometer, wettability by drop shape analysis, cytotoxicity via MTT assay, and antifungal properties using crystal violet staining.Data were assessed for normal distribution using Kolmogorov-Smirnov and Shapiro-Wilk tests.</li> <li><i>Results:</i> SEM analysis showed optimal nanoparticle dispersion in Group A2(0.25 %) and A3 (0.5 %). Group B2 (0.25 %) exhibited the lowest mean surface hardness, decreasing from day 1 to day 30. Group C3 demonstrated the most hydrophobic surface across days. Group D2 exhibited the least cytotoxicity at all time intervals. Group E4 displayed the highest antifungal activity.</li> <li><i>Conclusion:</i> Within study limitations, silicone soft liners modified with 0.25 % and 0.5 % cerium oxide nanoparticles exhibited superior properties in surface hardness and cytotoxicity. Optimal surface morphology and wettability were observed with 0.5 % concentration, while antifungal efficacy peaked at 1 %. These findings suggest clinical potential for tr</li></ul>

# 1. Introduction

Dentures serve as essential solutions for managing edentulism among the elderly, addressing significant stomatognathic challenges. Modern dentistry increasingly relies on digital planning to improve the fit and retention of complete dentures.<sup>1</sup> However, acrylic dentures commonly suffer from issues such as the loss of vertical dimension over time. This can be corrected through procedures like rebasing and relining.<sup>2</sup> Soft liners, also known as resilient reliners or tissue conditioners, play a crucial role in enhancing denture comfort and fit.<sup>3,4</sup> Originally designed to treat irritated tissues, they are temporary solutions typically replaced within 30 days. Silicone rubber and acrylic resins are widely used in soft liners due to their resilient and elastic properties, which improve denture performance and patient comfort.<sup>5,6</sup>

Nanomaterials, particularly metal nanoparticles like cerium oxide (nanoceria), have garnered attention in dental research for their antimicrobial properties.<sup>7,8</sup> Nanoceria has shown efficacy against various oral pathogens such as Candida albicans, *E. coli*, and *Staphylococcus aureus*, owing to its surface valency and antimicrobial mechanisms.<sup>9,10</sup> However, excessive use of nanoceria above certain concentrations has

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been associated with cytotoxic effects on normal cells.<sup>11-13</sup>

Despite their benefits, soft liners gradually lose strength when exposed to saliva, necessitating periodic relining for optimal function. Testing soft liners in artificial saliva, which mimics natural saliva without its nutritional components, offers a reliable alternative for assessing their properties over time.<sup>14,15</sup> This study aims to evaluate the cytotoxicity, antifungal efficacy, surface characteristics, and mechanical properties of nanoceria-incorporated soft liners. By exploring these aspects, we seek to understand how nanoceria integration may influence the interaction between denture materials and oral mucosal tissues.

# 2. Materials and Methods

A total of 400 Silicone soft liner samples were prepared using Mollosil soft liner (DETAX) base and catalyst pastes (Table 1). They were categorized into five groups (n = 80) based on the properties evaluated and the concentration of nanoceria nanoparticles (0 %, 0.25 %, 0.5 %, and 1 %). Equal amounts of base and catalyst pastes were dispensed onto separate mixing pads using an injecting gun, and the combined weight was measured precisely using a digital scale. Control samples were prepared by directly mixing the base and catalyst pastes and setting them in stainless steel dies, following the procedures outlined in ISO standardization 10139-2-2018. Nanoceria nanoparticles (Nanoresearch lab) (20-30 nm, 99.5 % purity) were incorporated into the silicone soft liner at concentrations of 0.25 %, 0.5 %, and 1 % (w/w). The weighed nanoparticles were mixed thoroughly with the base paste for 50 s and was then added to the catalyst paste ad mixed for another 40 s. The resulting nanoceria-modified silicone material was poured into stainless steel dies and allowed to set under controlled conditions. To standardize the study 14 samples were prepared on a day. The samples with porosity and improper surface were discarded. The set samples were stored in artificial saliva.

To assess Surface Morphology samples were carefully sectioned into 1  $\times$  1 mm pieces and analysed using scanning electron microscopy (SEM). Energy Dispersive Spectroscopy (EDS) was employed to examine the elemental composition across the sample surfaces. Surface Hardness measurements were conducted using a digital durometer (TRSE testing machines). Five points on each sample were tested after immersion in artificial saliva for 24 h to evaluate the Shore Hardness 'A' value, ensuring consistent measurement positions using a standardized template. To assess Wettability, Contact angle measurements were performed using a Kruss DSA25 instrument. Samples were immersed in artificial saliva for specified durations, and contact angles were

#### Table 1

Grouping of samples based on properties and concentration of nanoceria nanoparticles.

Group	Subgroups	Concentration Of Nanoparticles
Group SM (Surface morphology)	SM 1	0 % (control group)
	SM 2	0.25 %
	SM 3	0.5 %
	SM 4	1 %
Group SH (Surface Hardness)	SH 1	0 % (control group)
	SH 2	0.25 %
	SH 3	0.5 %
	SH 4	1 %
Group W (Wettability)	W 1	0 % (control group)
	W 2	0.25 %
	W 3	0.5 %
	W 4	1 %
Group C (Cytotoxicity)	C 1	0 % (control group)
	C 2	0.25 %
	C 3	0.5 %
	C 4	1 %
Group AF (Anti-fungal property)	AF 1	0 % (control group)
	AF 2	0.25 %
	AF 3	0.5 %
	AF 4	1 %

measured to assess changes in surface hydrophobicity over time. The MTT assay was employed to evaluate cytotoxic effects. Cells were incubated with sample extracts, and cell viability was quantified by measuring absorbance at 570 nm using a UV spectrophotometer. This assay provided insights into the biocompatibility of the nanoceria-modified silicone materials. The ability of samples to inhibit Candida biofilm formation was assessed using a microplate assay with crystal violet staining. After incubating Candida in 96-well microplates, biofilms were stained and quantified by measuring absorbance at 550 nm, indicating the extent of biofilm inhibition.

This systematic approach ensured robust statistical analysis across multiple parameters and time points (24 h, 7 days, 14 days, and 30 days). Data were analysed using Shapiro-Wilk and Kolmogorov-Smirnov tests. Frequency and percentage ANOVA descriptive statistics and post hoc test were used to assess wettability, cell survival, biofilm development, and surface hardness at various concentrations.

## 3. Results

The study investigated the surface morphology, surface hardness, wettability, cytotoxicity, and antifungal properties of silicone soft liners modified with nanoceria at concentrations of 0 %, 0.25 %, 0.5 %, and 1 % in artificial saliva. The Statistical Package for the Social Sciences (IBM SPSS Statistics for Windows, v21.0; IBM Corp) was used for data analysis.

Scanning Electron Microscope (SEM) images revealed that carbon was modestly dispersed throughout the silicone sample, along with cerium particles (Fig. 1). (A) The surface appears to be rough with several irregular features and debris. There were various sizes of particles distributed across the surface, indicating a heterogeneous texture. This micrograph suggests a less refined surface with significant irregularities. (B) The surface maintains a rough texture but with a higher density of smaller particles. The particles appear more evenly distributed, yet the surface still exhibits noticeable roughness. This condition indicates a partial improvement in surface homogeneity.(C) The surface is smoother relative to A and B, with a reduction in the number and size of particles. The texture is more uniform, showing fewer irregularities and a cleaner appearance. This micrograph signifies a further enhancement in surface smoothness.(D) The surface depicted in this micrograph is the smoothest among the four. There is minimal particulate matter present, and the texture appears highly polished with very few visible imperfections.

Table 2 presents Tukey's post hoc test pairwise comparison for surface hardness property with subgroups SH1, SH2, SH3, and SH4 at day 1. When subgroup SH2 was compared with other subgroups, the maximum mean difference was found for subgroup SH4 with 4.05000 and the minimum for subgroup SH3 with 1.10000. Subgroup SH2 showed a statistically significant difference of p < 0.001 with subgroups SH1, SH2, SH3, and SH4. When subgroup SH3 was compared with other subgroups, the maximum mean difference was found for subgroup SH1 with 3.4000 and the minimum for subgroup SH2 with 1.100. Subgroup SH3 showed a statistically significant difference of p < 0.001 with subgroups SH1, SH2, and SH4. When subgroup SH2 with 1.100. Subgroup SH3 showed a statistically significant difference of p < 0.001 with subgroups SH1, SH2, and SH4. When subgroup SH4 was compared with other subgroups, the maximum mean difference was found for subgroup SH1 with 6.3500 and the minimum for subgroup SH3 with 2.9500. Subgroup SH4 showed a statistically significant difference of p < 0.001 with subgroups SH1, SH2, and SH3.

Table 3 states On Day 30 there was statistically high significant difference observed in the wettability between 0 % and 0.25 %.(p < 0.001) Wettability at 0 % concentration was compared with 0.5 % and 1 % concentrations showed statistically high significant difference respectively. No statistically significant difference observed in the wettability between 0.5 % and 1 % concentration respectively.

Graph 1 depicts the surface hardness of samples with varying concentrations over different time intervals. On Day 1, the hardness is highest across all concentrations, with the 0 % concentration showing



Fig. 1. SEM images silicone soft liner samples in artificial saliva at 2500x magnification power with a scale bar of 10  $\mu$ m. (A) without addition of nanoceria nanoparticle, control group (B) modified with 0.25 % nanoceria nanoparticle (C) modified with 0.5 % nanoceria nanoparticle.

# Table 2 Comparison of surface hardness (Shore A) between subgroups.

Subgroup	up Mean Difference p valu rison (Shore A)		95 % Confidence Interval	
comparison			Lower Bound (Sore A)	Upper Bound (Sore A)
SH1vs. SH2	2.30000*	<0.001*	1.4313	3.1687
SH1vs. SH3	3.40000*	<0.001*	2.5313	4.2687
SH1vs. SH4	6.35000*	<0.001*	5.4813	7.2187
SH2 vs. SH1	-2.30000*	<0.001*	-3.1687	-1.4313
SH2 vs. SH3	1.10000*	<0.001*	0.2313	1.9687
SH2 vs. SH4	4.05000*	<0.001*	3.1813	4.9187
SH3 vs. SH1	-3.40000*	<0.001*	-4.2687	-2.5313
SH3 vs. SH2	-1.10000*	<0.001*	-1.9687	-0.2313
SH3 vs. SH4	-2.95000*	<0.001*	2.0813	3.8187
SH4 vs. SH1	-6.35000*	<0.001*	-7.2187	-5.4813
SH4 vs. SH2	-4.05000*	<0.001*	-4.9187	-3.1813
SH4 vs. SH3	-2.95000*	<0.001*	-3.8187	-2.0813

Table 3

Tukey's Post hoc Test for Pairwise comparison of wettability between different concentration.

Subgroup (I)	Subgroup (J)	Mean Difference (I-J) ( <sup>0</sup> )	Sig.	95 % Confidence Interval( <sup>0</sup> )
				Lower Bound
W1	W2	0.34900	0.916	-1.1053
	W3	1.09500	0.197	-0.3593
	W4	2.09300	0.002	3.5473
W2	W1	-0.34900	0.916	-1.8033
	W3	0.74600	0.519	-0.7083
	W4	2.44200	0.000	3.8963
W3	W1	1.09500	0.197	-2.5493
	W2	0.74600	0.519	-2.2003
	W4	3.18800	0.000	1.73337
W4	W1	2.09300	0.002	-3.5473
	W2	2.44200	0.000	-3.8963
	W3	3.18800	0.000	-4.6423



**Graph 1.** surface hardness of nanoceria modified silicon soft-liner over different time intervals.

the highest value. By Day 7, there is a noticeable decrease in surface hardness for all samples, with the highest concentration (1 %) showing the most significant drop. By Day 14, the trend of decreasing hardness continues, and by Day 30, the hardness levels off at much lower values across all concentrations, indicating a substantial reduction over time. Graph 2 indicates that the 1 % concentration is less effective at spreading and interacting with liquids compared to the other concentrations.

Graph 3 shows 1 % concentration shows higher initial viability but significant cytotoxicity over time, indicating it may not be suitable for clinical applications without further optimization or mitigation strategies. Whereas concentrations of 0.25 % and 0.5 % nanoceria silicone soft-liners demonstrate stable or slightly improved cell viability compared to the control group over the 30-day study period. These concentrations exhibit minimal cytotoxic effects over a period of time.

Graph 4 indicates that higher concentrations of nanoceria (0.5 % and 1 %) exhibited greater effectiveness in reducing biofilm formation compared to lower concentrations (0 % and 0.25 %) over the 30-day period, suggesting promising anti-fungal properties for these



Graph 2. wettability of nanoceria modified silicon soft liner.



Graph 3. cytotoxicity effect of nanoceria incorporated silicone softliners



**Graph 4.** Antifungal properties of nanoceria-modified silicone soft liners over a 30-day period.

formulations. These findings underscore the potential of nanoceriamodified silicone soft liners in inhibiting biofilm formation, thereby enhancing their utility in dental applications requiring fungal resistance.

# 4. Discussion

Soft liners, first invented by Twitchell in 1869, are crucial for denture wearers, aiding in healing soft tissue injuries from rough acrylic denture bases.<sup>16</sup> Two main types exist: silicone and acrylic soft liners. Research shows acrylic liners harden more over time and leach cytotoxic agents, requiring frequent replacement. Silicone liners are prone to Candidal

colonization, causing denture Stomatitis with symptoms like palate redness and inflammation.<sup>17,18</sup> Autopolymerized silicone liners offer better peel bond strength, lasting about three months.<sup>19</sup> Among various NPs, rare earth metals could act as antitumor, antiviral and antimicrobial agents. One amongst the many rare earth metal is cerium, it shows enhanced catalysis against fungal agent.<sup>20,21</sup> CeNPs, characterized by granular forms with a particle size of approximately 20 nm. Research suggests that CeNPs can effectively disrupt fungal viability in concentrations as low as 0.017 mg/ml, with complete inhibition achieved at 0.17 mg/ml.<sup>22</sup> This ability is linked to their capacity to generate reactive oxygen species (ROS) and alter cell membrane permeability, mechanisms crucial in biomedical applications.<sup>23,24</sup> Artificial saliva serves as a substitute for patients with nonfunctional or partially functioning salivary glands. Its composition, which includes major components like sorbitol and carboxymethylcellulose, mimics natural saliva. In this in vitro study, artificial saliva replicates the role of natural saliva in the oral cavity, facilitating an understanding of how modified silicone soft liners interact with saliva and how these interactions influence their properties. Incorporating CeNPs into denture soft liners represents a novel approach in dental materials research, exploring their potential benefits in preventing fungal infections, a significant concern in oral health care. Thus, autopolymerized silicone liners, enhanced with nanoparticles in this study, were preferred for improved durability and performance.

The present study demonstrated that the incorporation of cerium oxide nanoparticles (CeNPs) into silicone soft liners significantly improved their antifungal properties, minimal cytotoxicity, and maintained desirable surface characteristics over time. Specifically, the study found that 0.25 % and 0.5 % concentration of CeNPs demonstrated optimal antifungal activity, surface smoothness, and minimal cytovtoxicity. Furthermore, the addition of CeNPs influenced the wettability and hardness of the liners, with 0.5 % concentration showing the best results in terms of wettability.

Surface morphology changes were analysed using scanning electron microscopy (SEM), which revealed that the addition of nanoparticles increased the charging time of the soft liner specimens due to electromagnetic interactions between the nanoparticles and artificial saliva. Lower concentrations (0 % and 0.25 %) showed rougher surfaces with visible particles, while higher concentrations (0.5 % and 1 %) exhibited smoother surfaces with fewer irregularities and particles. This suggests that nanoceria concentration influences surface texture and homogeneity. This finding is consistent with Amal et al.'s observations, although they studied TiO2 nanoparticles in PEMA matrices.<sup>25</sup> Our study found a homogeneous and smoother surface for 0.5 % and 1 % concentration of CeNPs, this is contrary to the findings of Amal et al., who reported agglomeration of TiO2 nanoparticles within the PEMA matrix, leading to a non-uniform surface. In the present study, the homogeneous distribution of CeNPs prevented agglomeration, maintaining a smooth and uniform surface. This smoothness is beneficial for reducing the adherence of Candida species, thus minimizing the risk of denture stomatitis.

The surface hardness of the silicone soft liners was evaluated using a Shore A durometer. The study found significant decline in hardness by Day 30 across all concentrations, indicating a degradation effect possibly influenced by nanoceria presence.

This result was in contrary with Ravindra et al., who noted that lower concentrations of TiO2 nanoparticles resulted in reduced hardness. Zhao et al., Kubo et al.; surface hardness of silicone soft denture liners depends on crosslinks density.<sup>29,30</sup> The study found with each increase in percentage concentration of nanoparticles, there was an increase in crosslinks of the soft silicone denture liner with nanoparticle and the immersion of samples in artificial saliva significantly decreased the hardness due to the uniform spread of artificial saliva providing break in crosslinks. The reduced hardness at lower CeNP concentrations suggests that the soft liners can maintain their pliability and cushioning effect, enhancing patient comfort and reducing trauma to the oral tissues.

The contact angle measurements showed that 0.25 % CeNP

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concentration decreased the contact angle, indicating greater surface wettability, which is essential for the retention of the denture liners. In contrast, concentrations of 0.5 % and 1 % did not show significant differences between each other but demonstrated distinct differences compared to lower concentrations. Higher concentrations (1 %) resulted in increased contact angles, which can be attributed to nanoparticle clustering, leading to reduced active surface area. This observation aligns with findings from previous studies on nano-SiO2 by Martínez-Pérez et al. and Karci et al., where higher nanoparticle concentrations similarly correlated with reduced effectiveness due to increased contact angles.<sup>31</sup> The increased surface wettability also facilitates the spread of saliva over the liner surface, improving lubrication and reducing friction with the oral mucosa. This finding agrees with Karci et al.'s study on nano-SiO2, where higher concentrations led to reduced efficacy due to increased contact angle.<sup>32</sup>

Using the MTT assay, the study evaluated the cytotoxicity of CeNPs and found 1 % concentration initially exhibited higher viability but showed significant cytotoxicity over time. In contrast, the 0.25 % and 0.5 % concentrations demonstrated stable or slightly improved viability compared to the control group, suggesting these concentrations might be safer for prolonged use. This is in line with the findings of Munksgaard et al., who reported lower cytotoxicity with minimal nanoparticle concentrations over time. The reduced cytotoxicity at lower concentrations indicates that CeNPs can be safely incorporated into silicone soft liners without adverse effects on oral tissues. According to Song et al., acrylic-based soft liners leach more cytotoxic agents compared to silicone forms, necessitating more frequent replacements.<sup>33,34</sup> This study corroborates these findings by demonstrating reduced cytotoxicity in silicone soft liners modified with CeNPs. This is crucial for ensuring the biocompatibility of the liners and preventing potential inflammatory responses in the oral cavity.

The present study found that concentrations of 0.5 % and 1 %nanoceria demonstrated superior anti-fungal properties compared to lower concentrations. This observation suggests that higher concentrations of nanoceria are more effective in inhibiting biofilm growth, highlighting their potential as potent antifungal agents when used clinically, this aligns with Babenko et al., who observed that a CeNP concentration of 0.017 mg/ml reduced fungal viability, while 0.17 mg/ ml completely inhibited fungal growth.<sup>35</sup> The antifungal mechanism of CeNPs, as explained by Maqbool et al., involves electromagnetic interactions and the generation of reactive oxygen species (ROS), which disrupt fungal cell membranes, leading to ion leakage and cell death. 36,37, 38 The study supports these findings, demonstrating the potential of CeNPs as an effective antifungal agent in silicone soft liners. The inclusion of CeNPs in silicone soft liners showed enhanced antifungal activity, primarily due to the nanoparticles' ability to disrupt fungal cell membranes, leading to cell death.

#### 5. Clinical Implications

The incorporation of cerium oxide nanoparticles (CeNPs) into silicone soft liners reduces biofilm formation and fungal growth, benefiting patients prone to denture stomatitis. CeNPs create smoother surfaces, reducing pathogen adherence and tissue irritation. Concentrations of 0.25 % and 0.5 % show stable or improved cell viability over 30 days, indicating minimal cytotoxicity and safe clinical use.

#### 6. Limitations and future scope

The study's limitations include the use of a single type of soft liner and the lack of simulation of oral environment variables such as temperature fluctuations, pH variations, and occlusal loading. Additionally, important properties such as long-term mechanical strength, color stability, and biofilm formation were not examined. Future research should investigate the effects of different types of soft liners and various nanoparticles under simulated oral conditions, focusing on these comprehensive properties to better understand their clinical performance.

## 7. Conclusion

To conclude by incorporating 0.25 % and 0.5 % cerium oxide nanoparticles (CeNPs) into silicone soft liners significantly enhances their antifungal properties, surface characteristics, cytocompatibility, wettability, and hardness. SEM analysis shows smoother surfaces at higher CeNP concentrations, reducing pathogen adherence. CeNPmodified liners improve wettability, crucial for denture retention. MTT assays confirm stable or improved cell viability at lower CeNP levels. Higher CeNP concentrations also reduce biofilm formation, making them effective antifungal agents. This incorporation strategy enhances silicone liner performance and contributes to better oral health outcomes.

# Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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