ISSN: 2717-8161 RESEARCH ARTICLE

New Trend Med Sci 2024; 5(3):181-186. https://dergipark.org.tr/tr/pub/ntms

From Lab to Clinic: The Potential of Nanobubble Ozone Stored in Liposome with Pantothenic Acid (NOSLIP) in Treating Vaginal Infections with Long-lasting Effectiveness

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Article History
Received 03 June 2024
Accepted 24 Aug 2024
Published Online 30 Sep 2024

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Doi:10.56766/ntms.1492707

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Abstract: In our study, pantothenic acid nanoparticle liposomal ozone solution (NOSLIP) with patent application number PCT/TR2022/050177 was used and to show that the solution maintains its effectiveness for a long time, it is developed as an antibacterial and antifungal agent and can be used for vaginal antisepsis, and it is a suitable drug that can be used for the treatment of vaginitis in the future. The antibacterial tests of NOSLIP, which was developed with a new technique, with the CLSI M07 A9 standard test method, and its antifungal activity with CLSI M27-A3 were studied. The stability test of the NOSLIP solution was kept at 55 oC for 74 days, corresponding to 2-year stability, according to the ASTM F 1980 standard. The product's particle was determined as 363nm. No growth was observed after 24-hour hemodynamic incubation with Streptecoccus agalactia (ATCC13813), E. coli (ATCC25922) bacterial suspensions adjusted to 0.5 MacFarland value and Broth medium. Again for Candida albicans (ATCC 10231), in the time-dependent efficacy test performed with a concentration of 1600 ppm, a 90% reduction in 24-hour plaque and no growth was observed at the 48th hour. In terms of effectiveness, the solution was still found to be effective after 2 years according to the ASTM F 1980 standard. It is thought that NOSLIP can be used for vaginal antisepsis with solutions to be prepared in appropriate doses due to its natural and slow release, prevent bacteria and fungi from settling on the mucosal membranes. ©2024 NTMS.

Keywords: Nanobubble Ozone; Nanoliposome; Vajinitis; Candida Albicans; Antibacterial Agent.

1. Introduction

Every year, 5-10 million women apply to various centers for sexually transmitted diseases due to infectious vaginitis [1]. The three most notable causes of infectious vaginitis are bacterial vaginosis (BV), trichomoniasis, and vulvovaginal candidiasis (VVC). BV and VVC, which are endogenous genital infections, are the agents most responsible for the etiology of vaginal discharge [2]. The most common symptoms of infectious vaginitis are vaginal discharge, itching, and a burning sensation. However, some cases are asymptomatic and are untreated [3].

Group B streptococci (Streptococcus agalactiae; GBS) are gram-positive encapsulated bacteria that can colonize the intestinal and vaginal flora in 10-30% of healthy adults [4]. *Streptococcus agalactiae* causes serious infections such as meningitis, sepsis, skin and soft tissue infections, pneumonia, urinary tract infections, and postpartum endometritis in newborns,

Cite this article as: Erkan Alkan P and Karabiyik T. From Lab to Clinic: The Potential of Nanobubble Ozone Stored in Liposome with Pantothenic Acid (NOSLIP) in Treating Vaginal Infections with Long-lasting Effectiveness. *Trend Med Sci.* 2024; 5(3):181-186.Doi:10.56766/ntms.1492707.

pregnant women and adults with underlying diseases [4-6].

Ozone is a reactive oxygen species consisting of three oxygen atoms produced by ultraviolet light and high-pressure diatomic oxygen, and is recognized as a strong oxidative antimicrobial agent. Ozone therapy has received increasing attention in recent years and is widely known for its positive effects on infection, reperfusion injury, cancer, and dental caries [7-8]. Currently, ozone therapy is a new concept in the clinical treatment of vaginitis. The medical integrated ozone therapeutic apparatus uses an ozone generator to prepare a certain concentration of ozone and mixes it with filtered tap water to form ozonated water. Ozone and active molecules are in a liquid state and play a role in the sterilization of the vagina [9].

2. Material and Methods

2.1 *Solutions Preparations*

The nanobubble ozone stored in a liposome solution (NOSLIP), which was prepared with a method that is different from standard ozonation mechanisms, is protected by patent PCT/TR2022/050177. While preparing the solution, pantothenic acid (vitamin B5) was attached to the carrier nanomolecules to support the vaginal mucosa. The antibacterial, antiviral, biocompatibility and cytotoxicity tests of the NOSLIP solution before it was decorated with panthotenic acid were studied and published [12].

2.2 *Characterization of NOSLIP*

Size polydispersity (PDI), zeta potential, hydrodynamic diameter (Z-average size), dynamic Light Scattering (DLS) measurements were taken at 20 °C from three independent samples with a Zetasizer Nano ZS instrument (Malvern Instruments Ltd., UK) containing a solid-state HeNe laser (λ=633nm) at a scattering angle of 173°.

2.3. *In vitro Anti-Fungal Activity of the NOSLIP Solution*

According to CLSI M27-A3 [11] recommendations, antifungal drugs were diluted in an RPMI 1640 medium containing 0.2% glucose and were distributed at the appropriate concentration onto U-bottom microdilution plates. The inoculum suspension was adjusted to a final concentration of 0.5 x 10^3-2.5 x 10^3 cells/ml and it was dispensed into microdilution wells with different antifungal concentrations. Plates were incubated at 35 °C. While determining the MIC value for Candida species according to the CLSI standard, the concentration at which a 50% decrease was observed at the end of the 24th hour from the prepared dilutions was considered to be the MIC (minimal inhibitory concentration) value. In this study, it was determined that the MIC value was 1600 ppm by performing the standard study with 3200 ppm, 2400 ppm, 1600 ppm, and 800 ppm concentrations (Table 1).

Table 1: MIC values against Candida albicans (ATCC 10231) according to the CLSI M27-A3 method.

Sample	Tube	Dilution	ppm	*Candida albicans*
NOSLIP Solution	1	1	3200	-
	2	1/3	2400	-
	3	1/2	1600	-
	4	1/4	800	+

3. Results

3.1. The NOSLIP Solution Characterization

The NOSLIP solution dimensions ranged between 48 nanometers and 2 microns. Most of the particles were found to be concentrated at 4844 and 106,1 nanometers (Figure 1).

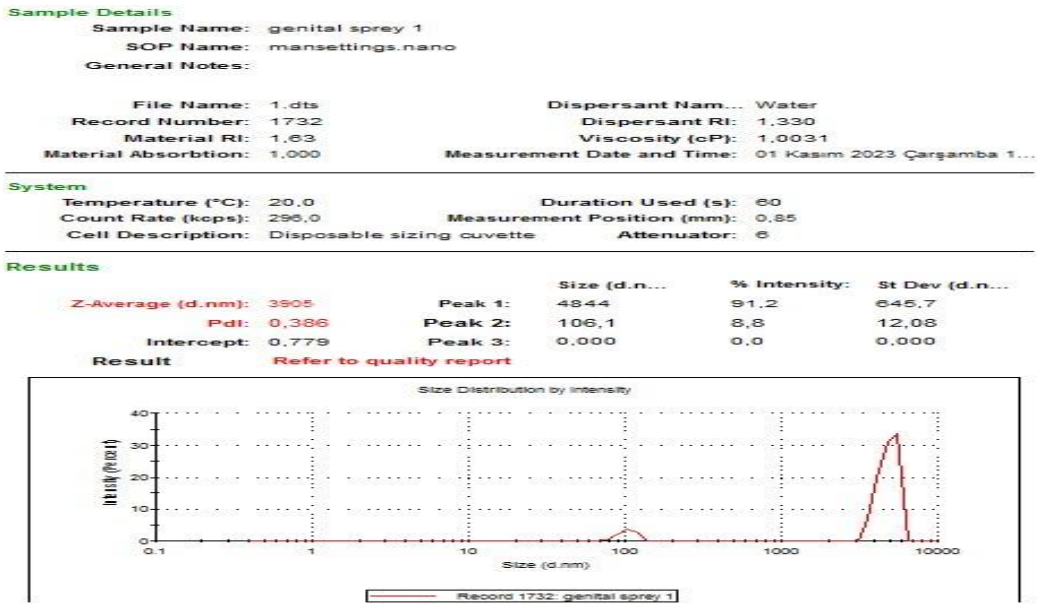

Figure 1: The Zeta size and poly dispersity index of the NOSLIP solution.

The NOSLIP solution was imaged for the first time by Scanning Electron Microscopy (SEM) and it was determined that the product was a nanomolecule (Figure 2).

3.2. Analysis of time-dependent Antibacterial Effects of the NOSLIP Solution

The MIC of the nanobubble liposomal ozone solution was determined using the CLSI M07 A9 (Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard) standard test method for Methicillin-resistant *Staphylococcus aureus* (ATCC 12493), *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25922). The ATCC25923 strains were calculated as 1562 ppm. To evaluate the time-dependent effects of the solution, the MIC value was above 1600 ppm. No growth was observed as a result of the 24-hour 37 ˚C hemodynamic incubation with *Streptococcus agalactia* (ATCC13813) and *Escherichia coli* (ATCC25922) bacterial suspensions, and Broth, a medium which was adjusted to a 0.5 MacFarland value (Table 2).

Table 2: Tests of Streptococcus agalactia (ATCC13813) and Escherichia coli (ATCC 25922) bacteria at different ppm levels nanoparticle liposomes at different times.

Time	*Streptococcus agalactia (ATCC13813)*	*Escherichia coli (ATCC25922)*
2 min.	+	+
10 min.	+	+
30 min.	+	+
1 h.	Reduction	Reduction
2 h.	-	-
3 h.	-	-
4 h.	-	-
5 h.	-	-
6 h.	-	-
24 h.	-	-

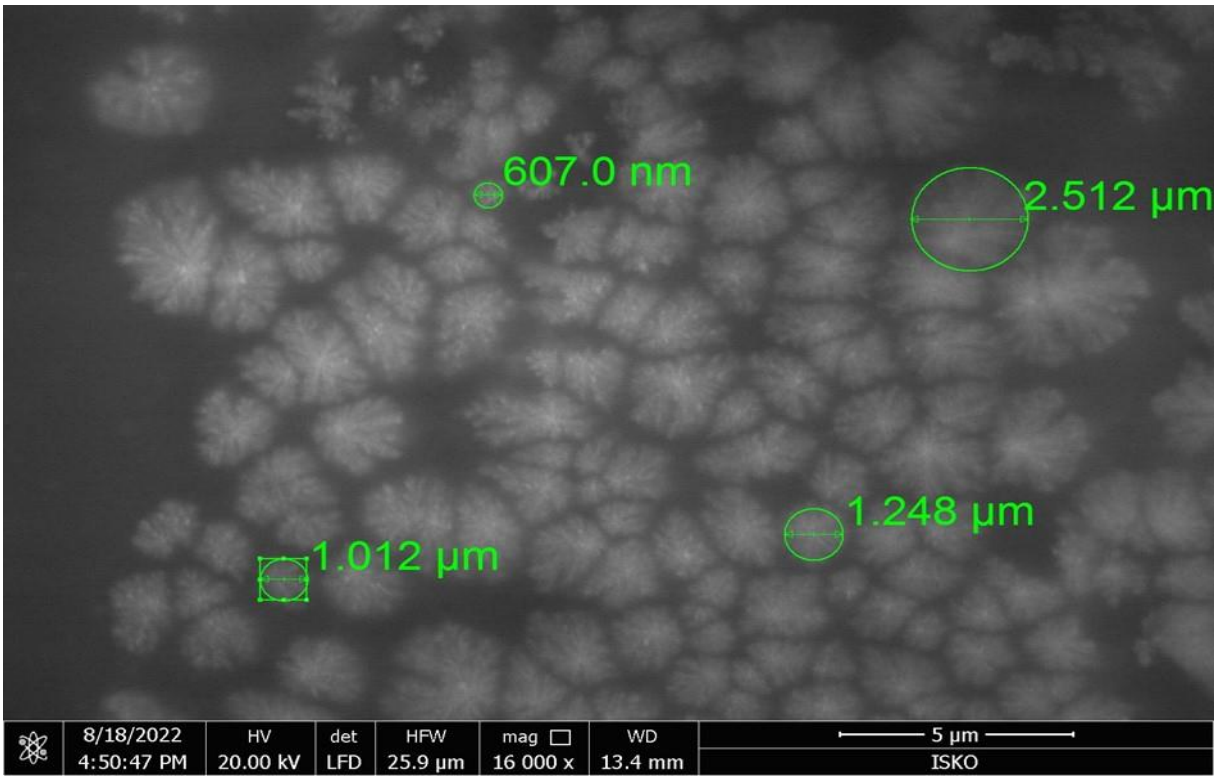

Figure 2: SEM image of NOSLIP Solution at 16 000 magnification.

3.3. Analysis of time-dependent Antifungal Effects of the NOSLIP Solution

For Candida albicans (ATCC 10231), in the time-dependent efficacy test performed with a concentration of 1600 ppm, a 90% reduction in 24-hour plaque and no growth at 48 hours were observed (Figure 3a-b).

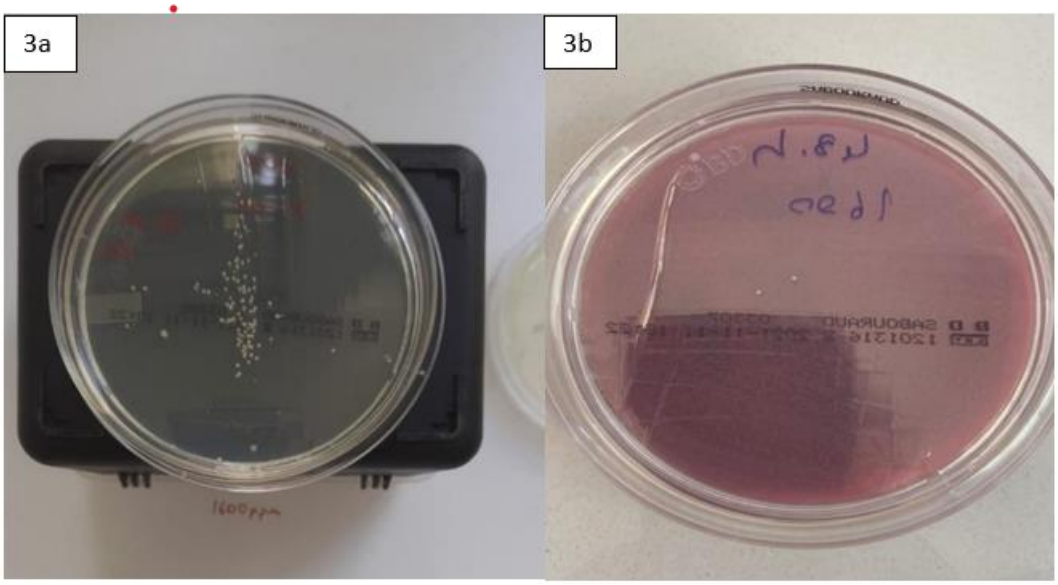

Figure 3: 3a,3b- The time-dependent efficacy test.

3.5 *A Stability Test of the NOSLIP Solution*

The ASTM F1980 (standard guide for accelerated aging of sterile barrier systems for medical devices) was used as a reference to prepare the ozone solutions in their active concentrations, and these solutions were stored at 55 °C for 74 days to determine their stability after two years. After the years, *Staphylococcus aureus* (ATCC 25922), Methicillin-resistant *Staphylococcus aureus* (ATCC 12493) and *Escherichia coli* (ATCC 25922) suspensions regulated to 0.5 McFarland turbidity were readded to the solutions. As before, the samples were obtained from the solutions at 2 min, 10 min, 30 min, 1 h, 2 h, 3 h, 4 h, 5 h, and finally, 6 h (Table 3). The blood agar medium (Germany-Becton Dickinson) was used for cultivation of the samples and they were incubated for 24 hours at 37 °C. The presence of bacterial growth was assessed on the plates after the incubation period. The stability was defined as the preserved effectiveness of the solution during the contact period, at the concentration where the antibacterial activity was previously recorded.

Table 3: ASTM F 1980 Stored at 55°C for 74 Days NOSLIP Solution.

Time	*Streptococcus agalactia (ATCC13813)*	*Escherichia coli (ATCC25922)*
2 min.	+	+
10 min.	+	+
30 min.	+	+
1 h.	Reduction	Reduction
2 h.	-	-
3 h.	-	-
4 h.	-	-
5 h.	-	-
6 h.	-	-
24 h.	-	-

The products and results used in our study are available to Data Availability.

4. Discussion

The exploration of NOSLIP as a potential treatment for vaginitis presents a promising avenue for addressing both the pathogenic and ecological aspects of vaginal health. Traditional treatments often focus solely on eradicating pathogens, which can inadvertently disrupt the delicate balance of the vaginal microbiome. This disruption can lead to further complications, including recurrent infections, as evidenced by the high rates of recurrence associated with bacterial vaginosis (BV) treatments that do not restore the normal flora [13] NOSLIP, with its dual action of pathogen elimination and preservation of beneficial bacteria, could represent a significant advancement in the management of vaginitis.

The vaginal microbiome is predominantly composed of Lactobacillus species, which play a crucial role in maintaining a healthy vaginal environment by producing lactic acid and other metabolites that inhibit pathogenic growth [14-15]. The introduction of NOSLIP, which utilizes ozone and pantothenic acid in a slow-release formulation, could enhance the proliferation of these beneficial bacteria while simultaneously reducing the concentration of harmful pathogens16. This aligns with findings that suggest treatments promoting Lactobacillus growth can significantly improve vaginal health and reduce the incidence of infections [17-18].

Moreover, the stability of NOSLIP solutions for at least two years, in contrast to the short half-life of ozone in water, suggests a sustained therapeutic effect that could be beneficial in clinical settings [20]. This prolonged efficacy is critical, as many existing treatments require frequent application, which can be burdensome for patients and may lead to inconsistent outcomes. The slow-release mechanism of NOSLIP not only ensures a continuous antimicrobial effect but also supports the

recovery of the vaginal microecology, which is essential for long-term health [20-21].

Clinical evidence supporting the efficacy of NOSLIP in treating vaginitis is still limited, necessitating further studies to establish its role within the broader context of vaginal health management. Previous studies have indicated that restoring the vaginal microbiome can significantly alleviate symptoms associated with vaginitis and reduce inflammatory responses [22]. For instance, the use of prebiotics and probiotics has shown promise in promoting the growth of Lactobacillus, thereby enhancing the natural defenses of the vagina against infections [23-24].

Conclusion

The potential of NOSLIP as a treatment for vaginitis lies in its ability to address both the immediate symptoms of infection and the underlying microbial imbalances. By fostering a healthy vaginal environment, NOSLIP could not only alleviate discomfort but also reduce the risk of recurrent infections, thereby improving the overall quality of life for affected individuals. Future clinical trials will be essential to validate these findings and explore the full therapeutic potential of NOSLIP in the context of vaginal health.

Limitations of the Study

In the study, evaluations were made based on in vitro experiments.

Acknowledgement

None.

Conflict of Interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Financial Support

The authors received no financial support for the research and/or authorship of this article.

Author Contributions

All authors contributed equally to the article.

Ethical Approval

None.

Data sharing statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Consent to participate

None.

Informed Statement

None.

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