Porous silicon nanoparticles prepared via an improved method: a developing strategy for a successful antimicrobial agent against *Escherichia coli* and *Staphylococcus aureus*

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Abstract. One of the commonest nanoparticles with unique properties is porous silicon nanoparticles (PSNPs). This study aims to prepare PSNPs via an improved method, followed by the investigation of the antimicrobial activity of the synthesized PSNPs against *Escherichia coli* and *Staphylococcus aureus*. The synthesis of the PSNPs was executed via a modified electrochemical etching process. After the synthesis, the obtained liquid PSNPs were subjected to laser treatment under an Nd-YAG laser condition of laser energy 350 mJ, and wavelength 1064 nm. The synthesized PSNPs were further characterized for functional groups and surface morphology using Fourier transfer infrared (FTIR) and Scanning Electron Microscopy (SEM), respectively. With *E. coli* and *S. aureus* as the commonest implicated organisms in both adult and children infections, there is a need to explore novel antimicrobial agents rather than antibiotics for curtailing these bacterial species. The synthesized PSNPs showed potential antibacterial activity against the studied organisms, although the observed antibacterial activities after the combination of PSNPs with Amoxicillin and Cephalexin were higher compared to the activity of PSNPs alone. These complexes were observed to act on the bacterial cytoplasmic membrane and nucleic acid which resulted in an improved cellular permeability due to the loss of membrane integrity and nucleic acid damage. However, there is a need to further investigate the antibacterial activity of PSNPs and its complexes with other antibacterial agents against other disease-causing by other bacterial species.

Keyword: Porous silicon nanoparticles; Antimicrobial activity; improved electrochemical etching; FTIR; SEM.

1. Introduction

Recently, much interest has been devoted to PSNPs as a channel for controlled drug delivery [1] due to its possession of an open interior pore volume which can be exploited for drug loading and protection of therapeutic agents [2]. It also has a large surface area which can allow the conjugation of targeting moieties like antibodies and peptides [3-5] to enhance a selective homing to targeted tissues. The porous nature and surface chemistry of PSNPs can be easily modified to facilitate the solubility of adsorbed or loaded agents as has been proven for the delayed release of both antibacterial and chemotherapeutic agents from porous silicon (PS) carriers (6-7). The intrinsic near-infrared photoluminescence of PSNPs can also be exploited in theranostic applications for the monitoring of post-delivery targeting and degradation processes. Luminescent silicon nanoparticles [8] are considered as a harmless alternative to cadmium or indium-based quantum dots [9] due to the non-
respectively. It was observed that the PSNPs at the concentrations of 250, 500 and 750 µg/mL had particle sizes 35.5, 65 and 75 nm, respectively. It was also observed that the antimicrobial activity of the prepared PSNPs was investigated against two bacterial species S. aureus and E. coli.

2. Material and Method

2.1 Preparation of PSNPs

The PS samples were produced via an electrochemical etching technique from silicon wafers (100) oriented boron doped of resistivity 1.5-4 Ω cm, diameter 7.6 cm, central circular 5.5 cm², and thickness 550±50 nm. An electrolyte solution composed of 24% HCl and absolute ethanol (1:1 v/v) was used during the etching process. The preparation of the PS films was executed by the anodization of the Si wafers for about forty minutes etching period at 80 mA cm⁻² current density. The HF container was Teflon-based due to the high resistivity of Teflon to HF. The porous layer on the Si substrate was removed by a 30-sec etching process using a current density of 250 mA cm⁻². The sample was further rinsed with changes of pentane and ethanol before being dried and milled to the nano-size using a ball mill. The obtained NPs were dissolved in double deionized water (DDW) prior to a 4-h ultrasonication process. Next, the NPs were passed through a filter of 0.22 µm size for size uniformity. Finally, the obtained NPs were subjected to laser treatment at an Nd:YAG laser condition of laser energy 350 mJ and wavelength 1064 nm to obtain the smaller sized PSNPs. The obtained PSNPs were surface characterized via SEM (INSPECTS50-USA), while the functional groups were identified via FTIR.

2.2 Agar well diffusion assay for anti-bacterial activities

A well diffusion method was employed to investigate the antibacterial activity of the prepared PSNPs. Nutrient agar plates were prepared in sterile Petri dishes; then, the bacterial species (S. aureus and E. coli) were cultured on the prepared plates. After culturing the microbes on the plates, agar wells of approximately 6 mm in diameter were bored into the agar. Into the wells, the solutions of the prepared PSNPs and its complexes with amoxicillin and cephalexin were added to maximum capacity, while distilled water was used to fill the negative control wells. The inoculated plates were further incubated overnight at 37°C [15]. After the incubation period, the plates were observed for zones of inhibition (areas surrounding the wells where the growth of the respective organisms was inhibited). The experiments were performed in triplicate. For the synergistic antibacterial activity of the PSNPs when combined with amoxicillin and cephalexin, a thin smear of the organisms was made on glass slides and observed for morphological changes in the bacterial cells via SEM. The membrane integrity-based discrimination of dead cells from the viable cells after exposure to PSNPs and its complexes with amoxicillin and cephalexin was performed using a fluorescent microscopic imaging technique [16,17].

2.3 Statistical analysis

An unpaired t-test was employed for analyzing the level of significance in the observed values among the treatment groups at a p < 0.05 level of significance [18].

3. Result and Discussion

3.1 SEM-based morphological features of PSNPs

An SEM-based morphological characterization of the prepared PSNPs was carried out. The prepared PSNPs at the concentrations of 250, 500 and 750 µg/mL had particle sizes 35.5, 65 and 75 nm, respectively. It was observed that the sizes of the PSNPs prepared via high-power-induced
electrochemical etching process were smaller compared to those prepared by only electrochemical etching at different concentrations (Figure 1). The results indicates that the increased concentration is accompanied by an increase in the nanosize. The size of PSNPs is depends on the concentration. Collectively, these findings suggest the significance of laser treatment in the reduction of the nanoparticle sizes owing to the electromagnetic wave effects on the solution [19].

![Image Fig.1](image)

**Figure 1.** The SEM images of PSNPs prepared by electrochemical etching methods at different concentration.

3.2 FTIR-based chemical characteristics of PSNPs

The FTIR is an important laboratory investigation for the determination of the chemical groups contained in compounds. These chemical groups are identified by the absorbance of their inherent chemical bonds at a wavelength range of 3500-1000 cm\(^{-1}\). The chemical groups in the prepared PSNPs were scanned, identified, and compared to previous reports. The outcome of the analysis is presented in Table 2. At 750 µg/mL concentration, the absorption spectra of PSNPs indicated the presence of vibratory patterns at the wavelength ranges of, 1392.73, 2112.56, 1636.55, 3331.05, 1050 cm\(^{-1}\) which respectively corresponds to the presence of C-O, CH\(_3\), OH, CH\(_3\), and SiO. At 500 µg/mL concentration, the observed IR spectra indicated the presence of OH, C-O, CH\(_2\) and SiO at the respective wavelengths of 3332.03, 1501.81, 2112.05 and 1048.72. Lastly, the PSNPs at the concentration of 250 µg/mL showed the presence of CO Bending, CH\(_2\) and OH at the respective wavelengths of 1637.66, 2118.41 and 3331.92 cm\(^{-1}\).

**Table 1.** Active groups of infrared spectra PSNPs prepared by electrochemical method at different concentration.

| Functional groups                  | Concentration750µg / ml. | Ref. |
|------------------------------------|--------------------------|------|
| SiO Stretching in (O-SiO)          | 1050                     | [20] |
| CH\(_3\) symmetric Stretching     | 1392.73                  | [21] |
| CO Bending                         | 1636.55                  | [22] |
| CH\(_3\) symmetric Stretching     | 2112.56                  | [23] |
| OH Stretching (SiOH)               | 3331.05                  | [20] |
| **Concentration 500 µg/ml**        |                          |      |
| SiO                                | 1048.72 - 1097.89        | [20] |
| C-O                                | 1501.81 - 1637.93        | [21] |
| CH\(_3\) symmetric Stretching     | 2112.05                  | [23] |
| OH Stretching (SiOH)               | 3332.03                  | [20] |
| **Concentration 250 µg / ml.**     |                          |      |
| CO Bending                         | 1637.66                  | [22] |
| CH\(_2\) symmetric Stretching     | 2118.41                  | [23] |
| OH Stretching (SiOH)               | 3331.92                  | [20] |
3.3 In vitro-based antibacterial activity of PSNPs

The in vitro-based antibacterial activity of PSNPs at different concentrations against *E. coli* and *S. aureus* showed the growth inhibitory effects of the nanoparticles against *S. aureus* and *E. coli* at the concentrations 250 µg/mL and 2500 µg/mL, respectively (Figure 2A and 2B). The PSNPs showed more growth inhibitory activity against *S. aureus* compared to *E. coli*. Based on this observation, it is evident that the gram-positive organism is more susceptible to the nanoparticles compared to the gram-negative organism. Even though the mechanism of this antimicrobial activity of PSNPs is yet to be understood, they are known to denature microbial proteins and interfere with the replication of bacterial DNA [24].

![Figure 2. Antibacterial activity of PSNPs against (a) *S. aureus*, and (b) *E. coli*. 1 = negative control, 2 = 250 µg/mL, 3 = 500 µg/mL. The bars represent the standard deviation of triplicate experiments. ** and *** represent significant differences at p<0.01 and p<0.001 respectively compared to the negative control.](image)

The synergistic activity of PSNPs when combined with amoxicillin and cephalexin was studied via well diffusion method at the concentration of 250 µg/mL. The observed synergistic effect of PSNPs-cephalexin against *S. aureus* is shown in figure 3A with an observed inhibition zone of 43 mm. Cephalexin alone recorded an inhibition zone of 33 mm against *S. aureus*, while PSNPs alone recorded an inhibition zone of 26 mm. Regarding the synergistic effect of a PSNPs-amoxicillin complex against *E. coli*, an inhibition zone of 37 mm was recorded by the complex, while amoxicillin and PSNPs individually recorded inhibition zones of 25 mm and 22 mm (figure 3B). The observed synergistic activity against the microbes could be attributed to the reaction between the nanoparticles and the antibiotics [25]. Previously, silver nanoparticles have been observed to interact with amoxicillin to achieve the best synergic activity on the inhibition of *E. coli* growth [26]. This demonstrates the ability of PSNPs to facilitate the uptake of cephalexin across the cell membrane. Additionally, the large surface area of the NPs provided more rooms for the interactions that enhanced the bactericidal activity of the complex compared to the larger sized particles. Hence, the impact enhanced the cytotoxicity of the complex to the microorganisms. The gram-positive organisms showed more zones of inhibition compared to the gram-negative organism in each treatment group. This improved sensitivity of the gram-positive organisms can be due to the differences in the cell wall of both organisms. The gram-negative organisms have an exo-polysaccharide membrane which holds and maintains the integrity of the cell membrane, as well as impacts resistance to the permeation...
of lipophilic solutes. However, the cell wall of gram-positive organisms is composed of an outer peptidoglycan layer [27]. Several hypotheses have explained the mechanism of synergistic activities of nanoparticles with antibiotics as earlier discussed. An antibiotic-NPs complex can exert bactericidal effects via several mechanisms. Bacterial species with proven resistance could be susceptible to other components of a complex in one way or the other. This plays particularly important in situations where a bacteria species acquire resistance against an agent. But in situations where there is no antimicrobial resistance, the bonding reaction between the antibiotic and the NPs can result in a synergistic effect [24]. Most antibiotics contain several functional groups such as amido and hydroxy groups which can easily react with NPs via chelation. The antibiotic can also bind with each other via other weak bonds interaction such as van der Waals. Ultimately, the antimicrobial groups could be composed of a nanoparticles core and an antibiotic surrounding. Antimicrobial groups act on bacterial cell surfaces to bring about a considerable alteration in morphology and integrity, thus, strong antimicrobial group scan be formed by increasing the concentration of antimicrobial agents.

**Figure 3.** The synergistic activity of PSNPs and antibiotics (1) negative control, (2) PSNPs only, (3) cephalexin, (4) PSNPs-antibiotics complex against (A) S. aureus, and (B) E. coli. The bars represent the standard deviation of triplicate measurements. * and ** indicates significant differences (at p<0.05 and p<0.01 respectively) compared to PSNPs alone and the antibiotics.

### 3.4 The mechanism of the bactericidal action of PSNP

The morphology of the bacterial cells after treatment with the agents was observed using SEM. First, the microbes were cultured for 24h in a broth medium supplemented with PSNPs. The SEM images showed distinct morphological alterations in the exposed bacterial cell wall. The images of the treated and untreated cells are shown in figure 4 and 5. The image of S. aureus treated with and without PSNPs is shown in figure 4, while the image of E. coli treated with and without PSNPs is shown in figure 5. The untreated cells showed no morphological changes compared to the treated cells. The PSNPs caused disruptions on the surface of the treated cells, leading to the loss of bacterial binding power and weakened cell membrane integrity.
Figure 4. SEM images of *S. aureus* treated with and without PSNPs.

Figure 5. SEM images of *E. coli* treated with and without PSNPs, showing membrane damage, blebbing, and membrane clumping in the treated bacterial cells.

To study the mechanism of PSNPs binding to the free radicals inside the microbes, and to observe the nuclear breakdown in the treated and untreated bacterial cells, the cells were stained with acridine orange-ethidium bromide and examined under a fluorescent microscope. Under the fluorescent microscope, the dead cells were colored red due to the nuclear breakdown, while the viable cells were stained greenish (figures 6 and 7).
Figure 6. Fluorescent images of the viable (green) and dead (red) *S. aureus* after treatment with PSNPs.

Figure 7. Fluorescence images of the viable (green) and dead (red) *E. coli* after treatment with PSNPs.

4. Conclusions
In this study, PSNPs were synthesized via a high-power laser-based electrochemical etching process. The synthesized particles were characterized morphologically and chemically using SEM and FTIR, respectively. From the characterization studies, it was observed that the post-laser treatment of the PSNPs using a high-powered Nd-YAG laser at a wavelength 1064 nm facilitated the reduction of the size of the PSNPs via fragmentation into smaller sizes. This observation was proven via FTIR tests where increases in the concentration of silicon resulted in increases in the size of the nanoparticles. The PSNPs were also found to have antibacterial activity against gram-positive and gram-negative bacteria which was mediated by their ability to penetrate the bacterial wall and bind with the generated ROS within the cells, thereby, breaking the nuclear DNA molecules of the cells.

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