Effect of silver nanoparticles with polyelectrolyte multilayer against Aggregatibacter actinomycetemcomitans

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Abstract. Silver nanoparticles capped with alginate (SNP-DL) were prepared by chemical reduction method. Three concentrations of SNP-DL were produced by varied alginate into 0.1, 1, and 5 mM. UV-Vis spectroscopy was used to characterize their specific wavelength at 400 nm. Subsequently, 10 bilayers thin film were established between PDADMAC and SNP-DL via polyelectrolyte multilayer technique. These were characterized by SEM and AFM. Their toxicity to anaerobe bacteria Aggregatibacter actinomycetemcomitans (Aa) a common cause of periodontal disease, was tested. Findings showed that these silver nano-chips could release SNP and reduce the Aa colony by 90%.

1. Introduction
Silver nanoparticles (SNP) are increasingly being applied in the fields of medical and dentistry treatment because the unique properties of these particles make them especially effective in fighting bacterial infections [1],[2],[3]. In addition, they can be useful in treating patients who receive antibiotics regularly, and may be at risk of developing drug resistance [2],[3],[4]. They are also useful for treating patients who have been found to be allergic to some antibiotics such as Penicillin, etc [5],[6]. Thus, SNP offers a new alternative for inhibiting or destroying bacteria, especially for anaerobic bacteria, which are a significant cause of diseases such as periodontal disease. Aggregatibacter actinomycetemcomitans (Aa) is one of several bacteria causing periodontal disease [7],[8]. The toxins released from this bacteria can destroy the gum, causing dissolution of bone around the tooth, gum recession, tooth rocking, and eventual tooth loss[8],[9],[10]. In general treatment, after having cleaned the tooth, the dentist will wash the gum with an antibiotic drug or put the periochip, which is a film containing an antibiotic drug, into the gum to inhibit bacterial growth [11],[12],[13]. Therefore, the researcher is interested in developing this film by using SNP with PDADMAC to coat in several layers with polyelectrolyte multilayer technique as it is the technique that can coat several materials on several kinds of surface easily and not costly [14],[15]. Moreover, the features of multilayer coating and the porosity between layers can also control the release of SNP from the film [16].

Thus, this research aims to develop several layers of film between PDADMAC and SNP, in three concentrations, for use in testing their efficacy in inhibiting the growth of Aa over a 24 hour period.
2. Materials and methods

2.1 Chemicals
Poly(diallyldimethylammonium chloride) (PDADMAC, medium molecular weight, 20% by wt in water, typical Mw = 200,000 – 350,000), Alginic acid (Al, Typical Mw = 10,000 – 600,000), poly(sodium 4-styrenesulphonate) (PSS, typical Mw = 70,000) and sodium borohydride were purchased from Sigma-Aldrich, (Thailand) Co., Ltd. Silver nitrate was purchased from VWR, Thailand. Sodium chloride was purchased from Labscan Asia Co., Ltd., Thailand. All chemicals and solvents were used as received without any further purification. Doubly distilled water was used in all experiments.

2.2 Silver nanoparticles preparation
Chemical reduction was used to prepare SNP capped with alginate. 1 mM of silver nitrate was mixed with AL varying concentration from 0.1, 1 and 5 mM. then added 10 mM of sodium borohydried in the previous solution and stirred 15 min. the solution was change into yellow color. They were left overnight in room temperature. After that they were analyzed via UV-Vis spectroscopy.

2.3 Multilayer thin films preparation
Ten millimolar PDADMAC were prepared and then added to 0.1M NaCl in PDADMAC solution. A quartz slide was cleaned with piranha solution (H_2SO_4:H_2O_2 (70:30) and then coated with four layers of primer (PDADMAC/PSS). The multilayer thin film between PDADMAC and SNP-AL was prepared by dipping the quartz slide in PDADMAC for 2 min, followed by three rinses in distilled water. The slide was then dipped in SNP-AL 1:0.1 for 2 min, followed by three rinses with distilled water. This step achieved the formation of one bilayer. Dipping was continued in PDADMAC and SNP-AL 1:0.1 until ten bilayers of PDADMAC/SNP-AL 1:0.1 had formed. The preparation of PDADMAC and SNP-AL 1:1 and PDADMAC and SNP-AL 1:5 thin film followed the same method [17]. These SNP-AL films were analyzed their surface by scanning electron microscopy and measured their thickness by atomic force microscopy.

2.4 Antibacterial property against Aa
The antibacterial activity against Aa was tested using the standard method. The SNP films were exposed to 20 µl of Aa in brain heart infusion broth (2 ml). After 24 h incubation at 37°C, 5% CO_2 condition, the bacteria/broth mixture was diluted five times. Then 50 µl of diluted bacteria was placed onto brain heart infusion agar using the spread plate method. After 24 h incubation the bacteria were counted. The result was corrected by the dilution factor to give the number of colony forming units (CFU) per millilitre. The percentage of bacterial reduction was then calculated and compared to blank condition.

3. Results and discussion

3.1 UV-Vis spectroscopy
The SNP-AL with the concentration of alginate 0.1, 1, and 5 mM will be prepared through chemical reduction. The three substances have the concentration in yellow color as shown in Figure 1. When measuring the light absorbance with UV-VIS spectroscopy, the three substances have the peak value of light absorbance at 400 nm which is the specific absorbance value of SNP [18]. This can confirm the competence of SNP coated with alginate.

3.2 multilayers of PDADMAC/SNP-AL
When 20 layers of PDADMAC-SNP are coated on quartz slide, multicolored film will be gained as shown in Figure 2. For PDADMAC/SNP-AL 1:0.1, the color will be dark gray which is the feature indicating the film with much thickness as SNP-AL 1:0.1 has low negative charging value surrounding particles resulting in when being coated on PADAMAC, the particles will arrange in the short distance as there is low repulsive force between the charge. This causes the film in each layer contains the
particles of SNP-AL densely. At 20 levels, it will be in metallic gray color corresponding with the features of the film PDADMMAC/SNP-AL 1:0.1 [18],[19]. When being analyzed with SEM, it is found that the features of particles are large and spread densely on the film as shown in Figure 3. On the contrary, for PDADMAC/SNP-AL 1:1 and 1:5 mM with the increasing concentration of alginate causes the charge density surrounding SNP to increase. Consequently, the repulsive force between the particles us high. The SNP particles will spread resulting in when being coated as multilayer film, SNP will spread over PDADMAC layer non-densely. Multilayer film to be gained will be thin and in yellow color [19]. When analyzing it with SEM, the particles will be seen as smaller as shown in Figure 3D. The thickness of the film was analyzed by atomic force microscopy and the results are compiled in Table 1. An average thickness is can be measured when considering the height of the film with respect to the substrate.

![Figure 1. The UV–vis absorbance spectra of SNP-AL solution in a various concentration of alginate.](image1)

![Figure 2. Ten bilayer of PDADMAC/SNP-AL in a various conditions were coated on glass slide.](image2)

| samples                  | Thickness(µm) | Mean average ± SD |
|--------------------------|---------------|-------------------|
| Blank                    | 0             |                   |
| PDADMAC/SNP-AL 1:0.1     | 1.28± 0.16    |                   |
| PDADMAC/SNP-AL 1:1       | 0.52± 0.09    |                   |
| PDADMAC/SNP-AL 1:5       | 0.75±0.06     |                   |

3.3 Antibacterial effect (ATCC29523) 
For testing the three kinds of film on Aa, it is found that when the film is exposed to it for 24 hours, the increase in amount of colony of Aa can reduce compared to the condition without the film. As shown in Table 2, it can be seen that the Aa without the SNP film will have the amount of $2.02 \times 10^8$
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However, when the film is inserted for 24 hours, the amount of colony of Aa will reduce. PDADMAC/SNP-AL 1:0.1 causes colony to remain 1.45 x 10^7 CFU, PDADMAC/SNP-AL 1:1 remains 1.5 x 10^7 CFU and PDADMAC/SNP-AL 1:5 remains 1.75 x 10^7 CFU. When calculating in % of reduction, the amount is around 92.80, 92.57, and 91.34 %, respectively. It can be seen that three kinds of multilayer film cannot totally destroy Aa, only reduce the amount of colony. According to such result, it can be seen that SNP can spread from the film to Aa.

Figure 3. SEM image showing the surface of blank condition (A), PDADMAC/SNP-AL 1:0.1 film (B), PDADMAC/SNP-AL 1:1 film (C) and PDADMAC/SNP-AL 1:5 film (D).

According to the theory, the film PDADMAC/SNP-AL 1:0.1 can release SNP more easily than other kinds of film as the charge density surrounding the particles is less causing the adhesive force between molecules is less [18],[19]. When considering with this experimental result, it is found to follow the aforementioned principle. However, the difference of result on Aa of each kind of film is very little. The mechanism of SNP influencing the destruction of bacteria is unclear. However, there are three mechanisms which can be explained. The first mechanism is that the very tiny size of SNP in nanoparticle level causes it to penetrate the cell wall of bacteria to destroy the systems such as respiratory system [20],[21],[22]. The second mechanism is that SNP can penetrate into the bacteria and destroy the compound of sulfur and phosphorus such as DNA [20],[21],[22]. The last mechanism is that it can release silver ion which has the properties to kill the bacteria. Its positive charge will seize the cell wall of bacteria causing the cell wall to change in the structure by building electron-dense granules to destroy the cell. Moreover, silver ion can also reacts with thiol group which is the component of enzyme causing the enzyme not to react and the cell will die eventually [20],[21],[22]. From these mechanisms, it can be explained that SNP-AL can destroy Aa.
Table 2. Antibacterial activity of multilayer thin film

| samples                  | Colony of Aa (CFU) 24hr | % of reduction |
|--------------------------|-------------------------|----------------|
| Blank                    | 2.02 x 10^8             | 0              |
| PDADMAC/SNP-AL 1:0.1     | 1.45 x 10^7             | 92.80          |
| PDADMAC/SNP-AL 1:1       | 1.5 x 10^7              | 92.57          |
| PDADMAC/SNP-AL 1:5       | 1.75 x 10^7             | 91.34          |

4. Conclusion
Silver nano-chips are built through the method of polyelectrolyte multilayer technique in order to control the release of SNP. When testing the competence of film in anting Aa, it is found that the number of Aa can be reduced for 90% Therefore, it is the chance in developing silver nano-chips to be more effective. This can be done by increasing the numbers of layers of the film, increase the amount of SNP, and change the kind of coating substance on the nanoparticles, etc. For future study, silver nano-chips can be used to test with other antibacterial agents which are the causes of periodontal disease and then should be tested in humans to treat periodontal disease instead of local antibiotics.

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