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Research Article

Scanning Electron Microscopic study on biofilm forming *Proteus mirabilis*

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Abstract

The scanning electron microscope (SEM) is a type of electron microscope that uses a focused beam of high-energy electrons in producing a variety of signals at the surface of a solid specimen. SEM study on the biofilm formation will help to understand the thickness of biofilm matrix and to develop strategy to present biofilm formation. Hence a SEM study on the major bacteria isolated from biofilm is needed. SEM study was also used to find out bacterial colonization. In the present study biofilm forming bacteria *P. mirabilis* was chosen for SEM study. The microbe was allowed to form a biofilm matrix in a sample piece of catheter tube with a coating of silver nanoparticles. The nature of biofilm formed on the catheter with and without silver nanoparticles coating was tested. Scanning electron microscopic study was made on normal catheter with bacterial biofilm and silver nanoparticles coated catheter. The evaluation of silver nanoparticles treated and untreated was done to find out whether silver nanoparticles inhibit the growth of microbial biofilm over catheter.

Keywords: Intrauterine devices (IUDs), Copper-T and cervical swab, Cystine lactose electrolyte deficient agar medium (CLED), microtitre plate (MTP), SEM, *Proteus mirabilis.*

Introduction

The scanning electron microscope (SEM) is a type of electron microscope that uses a focused beam of high-energy electrons in producing a variety of signals at the surface of a solid specimen. The signals produced by the interacting electrons contain useful information such as the shape, atomic structure and conductivity. When an electron hits the surface, it may be reflected (*backscattered*), absorbed, or conducted away. Electrons that are absorbed can cause the atom that they hit to become unstable, forcing it to give off another electron (a secondary electron), or to give off light in order to stabilize. Different detectors, for the different types of reactions, may be fitted to an electron microscope, depending on what is being looked for (Merli *et al.,* 1995).

The magnification that can be achieved in a scanning electron microscope depends on how narrow the beam of electrons that strikes the surface can be, and can reach 1 nanometer, about the size of 3 to 5 atoms. The control of the beam is achieved using magnetic fields, with other magnetic fields being used to shape the beam, and to move it across the sample. The range of magnification may range from 30 x to as high as 500,000x (Merli *et al.,* 1995).

SEM study on the biofilm formation will help to understand the thickness of biofilm matrix and to develop strategy to present biofilm formation. Hence a SEM study on the major bacteria isolated from biofilm is needed. SEM study was also used to find out bacterial colonization (Muller *et al.,* 1997).

Materials and Methods

In the present study biofilm forming bacteria *P. mirabilis* was chosen for SEM study. The microbe was allowed to form a biofilm matrix in a sample piece of

catheter tube with a coating of silver nanoparticles. The nature of biofilm formed on the catheter with and without silver nanoparticles coating was tested. Catheter biofilm (*Proteus mirabilis)* were fixed with 2.5% (v/v) glutaraldehyde in 0.15M PBS for 1 h at room temperature. 1% (w/v) osmium tetra oxide for 1h, washed thrice with distilled water. They were then treated with 1 % (w/v) urinal acetate for 1h and washed again with distilled water. The samples were dehydrated in ethanol. As *Proteus mirabilis* contained proteins and a high proportion of water in their cells, it was fixed first in order to preserve their structure while they are being further prepared for SEM. To accomplish such steps in reasonable time, the specimens was relatively thin (<2 mm) and small (only a few mm). All samples were dried to critical point, gold coated and viewed under SEM (Fujiyoshi, 1998).

Results and Discussion

Scanning electron microscopic study was made on normal catheter with bacterial biofilm and silver nanoparticles coated catheter. The evaluation of silver nanoparticles treated and untreated was done to find out whether silver nanoparticles inhibit the growth of microbial biofilm over catheter.

A scanning electron microscopic and atomic force microscopic study of the surfaces of catheter biofilm had already been reported by Marrie and Costerton (1983). Their scanning electron microscopy study showed highly organized and often densely packed micro-colonies of bacteria, a reflection of the possibility that the majority of these bacteria had been present on these surfaces for a long time. In our case, a catheter was examined for biofilm formation in parallel with culture. Biofilm formation, involving both coccal and bacillary forms, was detected on the surface of the catheter by scanning electron microscopy. Quantitative culture of aerobic and anaerobic bacteria showed a dominance of anaerobic bacteria in this biofilm. Bacteria living in such a biofilm are usually resistant to attack by antimicrobial agents and host phagocytes.

SEM analysis was carried out to detect the inhibition activity of silver nanoparticle against the biofilm formed by *Proteus mirabilis*. The SEM analysis showed the porosity in the range of 73-142 µm in the positive control (Fig 1). The biofilm produced by *Proteus mirabilis* in the presence of PBS buffer (which is used for diluting nanoparticles) showed the porosity value in the range of 111-173µm (Fig 2). The biofilm produced by *Proteus mirabilis* in the presence of PBS buffer with silver nanoparticles was in the range of 218-422µm (Fig 3). From the result it is evident that a silver nanoparticle has inhibitory activity against biofilm produced by *Proteus mirabilis.* As the pore size in the biofilm matrix was high, it indicates poor colonization of biofilm forming bacteria.

Figure: 1 Positive control for biofilm and the PBS buffer produced by *P. mirabilis* (the formed porosity in the range of 73-142 µm)

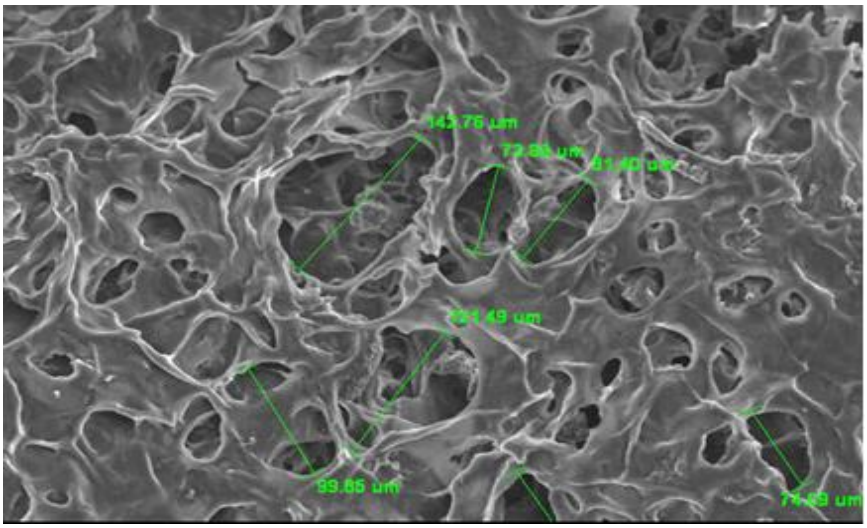


Figure: 2 Positive control for biofilm produced by *P. mirabilis* in the presence of PBS buffer which is used for diluting nanoparticles (the formed porosity in the range of 111 -173µm)

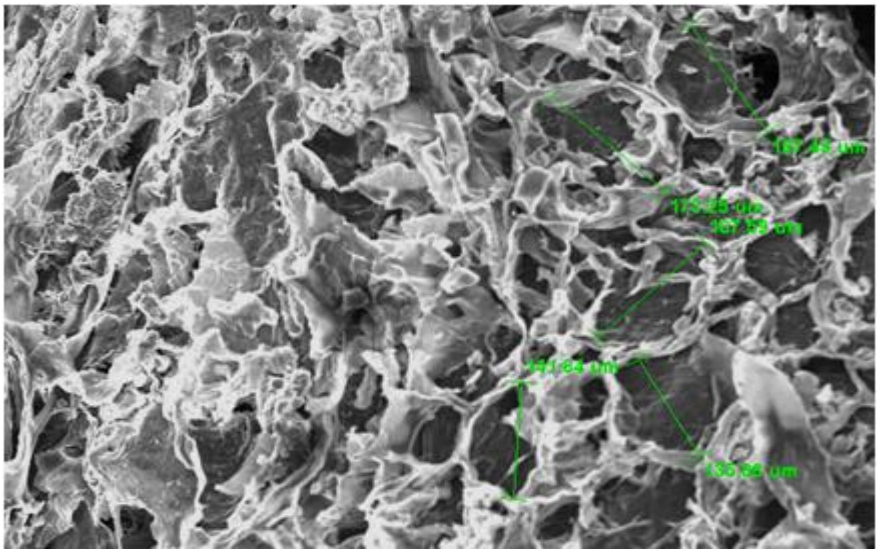
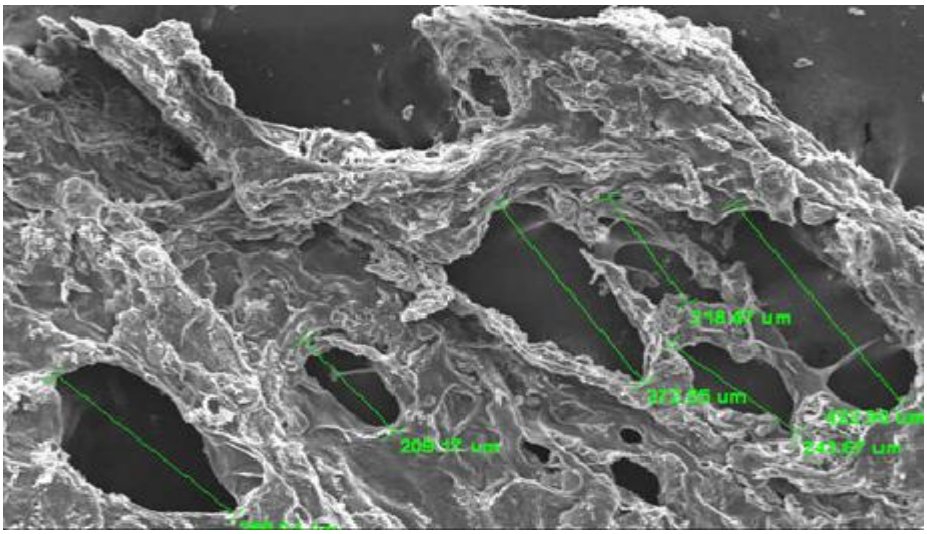


Figure: 3 The biofilm produced by *P. mirabilis* in the presence of PBS buffer with silver nanoparticles (the formed porosity in the range of 218 -422µm).



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