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Effect of Hen Egg White on Microbial Adhesion and Biofilm Growth of Biomaterial Associated Infection Causing Pathogens

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Abstract

Microbial biofilms on biomaterial implant surfaces or devices are troublesome, since biofilm organisms are protected by matrix of exopolymeric substances, impenetrable for most antibiotics and immune cells. It is established that bacterial adhesion to surface is required for colonization and subsequent biofilm formation. The adherent state is important for bacterial survival and development of infection. Therefore, prevention of bacterial adhesion and biofilm growth on implant surfaces should ultimately prevent the occurrence of infection. Egg white was considered to play an important role in resistance against bacteria for developing embryo mainly during early incubation. Utilization of egg white as a coating to prevent bacterial adhesion and biofilm growth on biomaterials implants is novel. Here, we studied the effect of Hen egg white as a coating to surface, on bacterial (Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli) adhesion and biofilm growth. We report that a significant reduction in bacterial adhesion was observed for S.aureus on egg white coated surface compared to control (no egg white coating). Whereas P. aeruginosa and E. coli, do not show any significant difference compared to control. When bacteria were allowed to grow in the presence of egg white, a significant reduction in growth was observed on all bacteria compared to control. Thus Hen egg white potentially used as biological coating on implants for prevention of bacterial adhesion and biofilm formation.

Keywords: *Bacterial adhesion, Biofilms, Escherichia coli, Hen egg white, Implants, Staphylococcus aureus, Pseudomonas aeruginosa*

1. Introduction

The use of biomaterial implants in human functional restoration after damage has become inevitable. A serious complication associated with the use of biomaterial implants is the occurrence of microbial infections. According to the studies conducted by European Centre for Disease Prevention and Control, the average prevalence of healthcare associated infections in Europe was 7.1% i.e., approximately 4,131,000 patients were affected [1]. In India, an estimation of 10% to 30% patients admitted to hospitals acquire nosocomial infection and up to 70% of organisms causing infections are resistant to at least one antibiotic [2, 3]. *Staphylococcus aureus* and *Staphylococcus epidermidis* are the most frequently isolated pathogen from infections related to biomaterials implant surfaces. Additionally,

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isolated organisms include *Escherichia coli* and *Pseudomonas aeruginosa* [4]. *S. aureus* is detected in approximately 23% of the infections associated with prosthetic joints [5]. *P. aeruginosa* is responsible for around 12% of hospital acquired urinary tract infections, 10% of bloodstream infections and 7% of hip joint infections [5]. The microorganisms involved in implant infections are resistant to both the immune system and antibiotics due to their biofilm mode of growth. Biofilm formation is a step-by-step process, starting from bacterial adhesion to a substratum rapidly followed by aggregation, co-adhesion and growth leading to micro colony formation while continuous production of extracellular polysaccharides ultimately leads to the construction of biofilms [1].

Egg white was considered to play important role in bacterial resistance during the development of embryo in early stage of incubation. Hen egg white, a clear liquid, also called the albumen, surrounds the egg yolk and is formed from the secretions in the hen's oviduct [6]. Egg white protects the yolk and provides nutrition for the growth of the embryo. Egg white is composed of 90% water with 10% of dissolved proteins including albumins and globulins. Egg white constitutes over 50% of the total egg protein and two-thirds of the total egg weight. Egg white contains water in majority, together with proteins, minerals, glucose and vitamins [6, 7]. Egg white stores the nutrients for the yolk, protects the egg from possible microbial attacks and transports nutrients into the yolk. Lysozyme, a glycoside hydrolase present in egg white damages the cell walls of the bacteria by catalyzing a hydrolysis reaction. Egg white can thus be effectively used as an anti-bacterial agent. Alexander Fleming coined the term Lysozyme when he observed its anti-bacterial activity on the bacterial cultures, obtained from the nasal mucus of a patient [8]. Lysozyme brings out degradation of the cell wall of the bacteria by catalyzing the hydrolysis of the beta linkage between the N-acetylglucosamine and N-acetylmuramic acid [9]. Studies from Food science suggest that Hen egg white component, Lysozyme as an anti-microbial agent in preventing food spoilage and food-borne diseases [10–12]. Therefore, the aim of this study was to evaluate the effect of hen egg white on bacterial adhesion, and biofilm growth of implant infection causing pathogens such as *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*.

2. Materials and Methods

Fresh hen eggs used in the extraction of egg white were obtained from a supermarket in Chennai (India). Egg white extracted were stored in vials and kept at 4°C. *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* were used for this study. Bacteria were first grown aerobically overnight at 37°C on blood agar from a frozen stock. The plate was kept at 4°C. For each experiment, one colony was inoculated in 10 ml of tryptone soy broth (TSB; Hi media, Mumbai) and cultured for 16 h. Bacteria were harvested by centrifugation at 3000 rpm for 5 min. The bacteria are suspended in TSB to a concentration of 10^7 bacteria /ml.

Tissue culture polystyrene wells were filled with 500 µl of hen egg white and allowed to adsorb to the surface at 37°C for 10 min. Subsequently, wells were washed with sterile water. Then, 1 ml of bacterial suspension was added to wells and allowed to adhere aerobically at 37°C for 40 min. Bacterial adhesion in the absence of hen egg white was considered as control. Subsequently, wells were washed with sterile water to remove unbound bacteria and images (six images) were taken using phase contrast microscopy and number of adherent bacteria per cm^2 was determined using ImageJ software. Experiments were performed in triplicate with separately cultured bacteria.

In order to evaluate the effects of hen egg white on a developing biofilm, tissue culture polystyrene wells were filled with 1 ml of bacterial suspension and allowed to adhere aerobically at 37°C for 30 min. Subsequently, wells were washed with sterile water to remove unbound bacteria. Then, 1 ml of hen egg white was added to wells and allowed to grow aerobically at 37°C for 24 h. Biofilm growth in fresh medium was considered as control. Subsequently, wells were washed with sterile water to remove unbound bacteria and biofilm development was assessed by measuring the optical density using spectrophotometer. To this end, 500 µl of 1% crystal violet staining was added to each well. Plates were incubated for 5 min. Then, crystal violet was removed. The wells were washed with sterile water and 33% acetic acid was added to each well. The optical density (absorbance at 570 nm) was measured using spectrophotometer. Experiments were performed in triplicate. Data are represented as a mean with standard deviation. For statistical analysis ANOVA was performed followed by a Tukey's HSD post-hoc test and a *P*-value <0.05 was considered to be significant.

3. Results and Discussion

Bacterial adhesion and biofilm growth in the presence of hen egg white is studied. Phase contrast images of bacterial adhesion after 40 min on Hen egg white coated and uncoated surfaces were shown in Figure 1. Initial bacterial adhesion after 40 min showed a significant reduction in number of *S.aureus* adhesion on Hen egg white coated surface compared to uncoated surface.

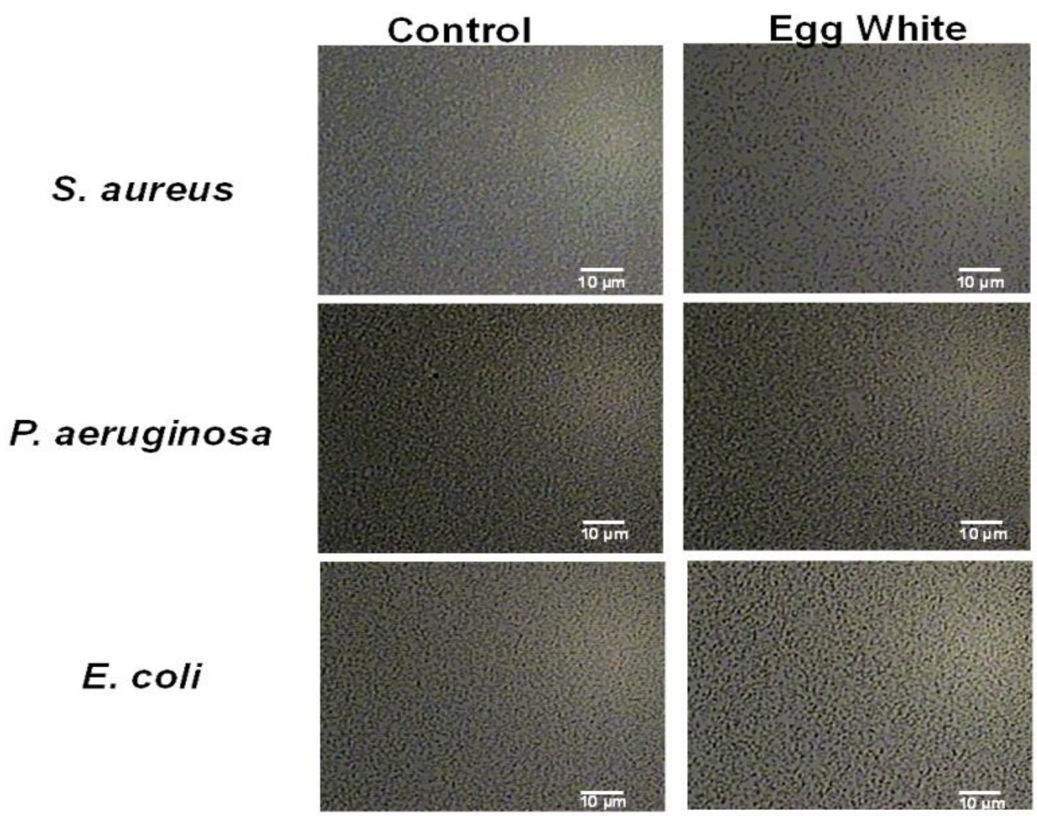

Figure 1. Initial bacterial adhesion of *S. aureus*, *P. aeruginosa* and *E. coli* after 40 min on egg white coated and uncoated (control) surfaces. Bar represents 10 µm

However, no significant reduction was observed on initial bacterial adhesion of *P. aeruginosa and E.coli* on egg white coated surface compared uncoated surface as observed from Figure 2. In order to evaluate the effect of egg white on biofilm formation, bacteria were allowed to grow in the presence of hen egg white for 24 h. Experimental results as seen in Figure 3 demonstrated significant effect in bacterial biofilm growth in the presence of egg white. A significant reduction in biofilm growth was observed in the presence of egg white compared to control (absence of egg white) in all three bacterial strains (*S.aureus*, *P. aeruginosa and E.coli*) as shown in Figure 4.

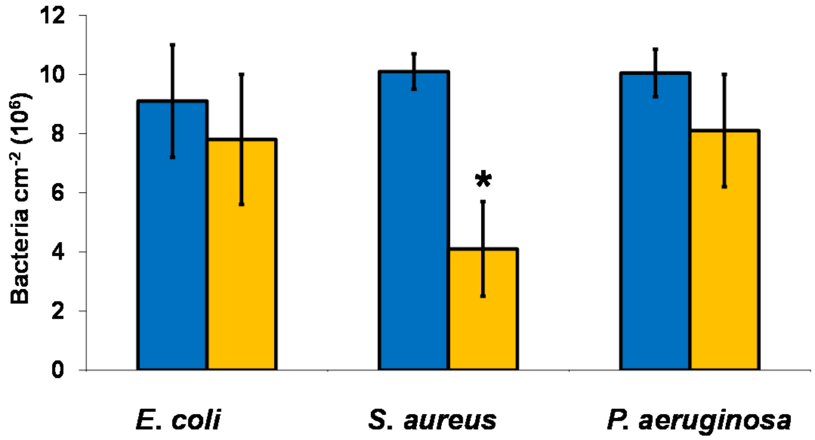

Figure 2. Number of adherent bacteria after 40 min on egg white coated surface (yellow column) compared to control (blue column). * denotes significant difference at $p<0.05$ compared to control

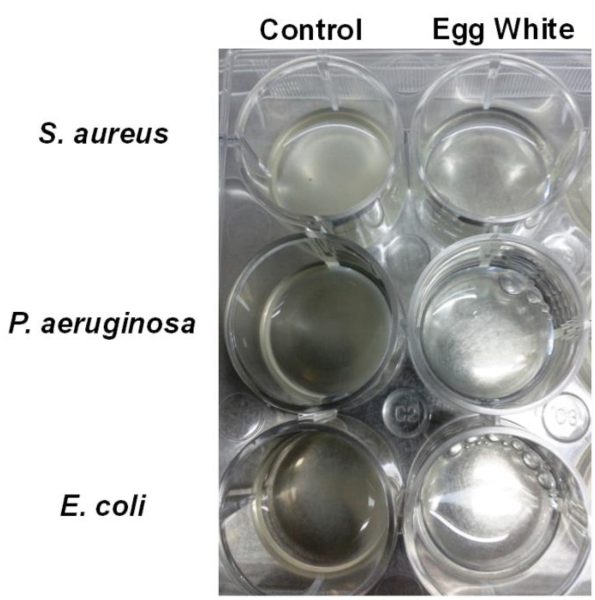

Figure 3. Biofilm formation of *S. aureus, P. aeruginosa* and *E. coli* after 24 h of growth in the presence of egg white compared to control (TSB)

The antibacterial activity could be due to different proteins present in egg white. Studies suggest that egg-white contains 3.4% Lysozyme, a glycoside hydrolase that damages the cell walls of the bacteria by catalyzing a hydrolysis reaction. Literature revealed the use of lysozyme as an anti-microbial agent in preventing food spoilage and food-borne diseases [6].

Lysozyme was found to have significant lysing properties that inhibited the growth of food spoiling bacteria. Strains of *C. botulinum*, *L. monocytogenes*, *C. thermosaccharolyticum* and *B. stearothermophilus* were found to be highly susceptible to lysozyme. The study also revealed that lysozyme can be used as an effective anti-food spoilage agent to inhibit the growth of pathogens. Lysozyme incorporated into Zein films showed antimicrobial effect against *Bacillus subtilis*, *Lactobacillus plantarum* and *Escherichia coli* [13].

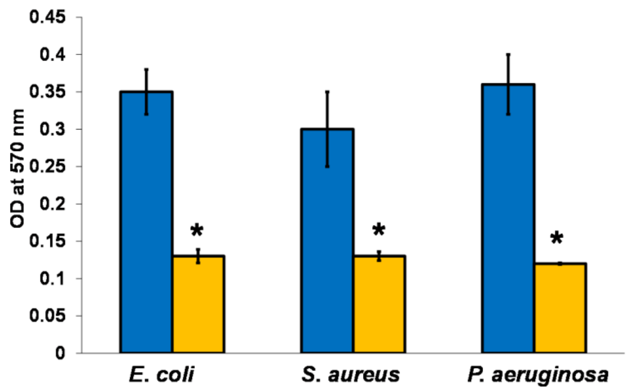

Figure 4. Optical density measurements after 24 h growth of bacteria in the presence of egg white (yellow column) compared to control (blue column). * denotes significant difference at p<0.01 compared to control

The study of literature revealed the presence of Conalbumin, an egg white protein, in the Hen's egg white that inhibited the growth of microbes [14]. The mechanism of action of the protein on *P. aeruginosa*, *M. phygenes* and several other organisms were studied and the results revealed that the protein had various rate of inhibitions on the growth of these organisms [14].

4. Conclusion

This study shows that hen egg white as a coating can reduce bacterial adhesion and significantly reduces biofilm growth. Thus Hen egg white can potentially be used as biological coating on implants for prevention of bacterial adhesion and biofilm formation.

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Conflict of Interest

No conflict of interest exits.

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