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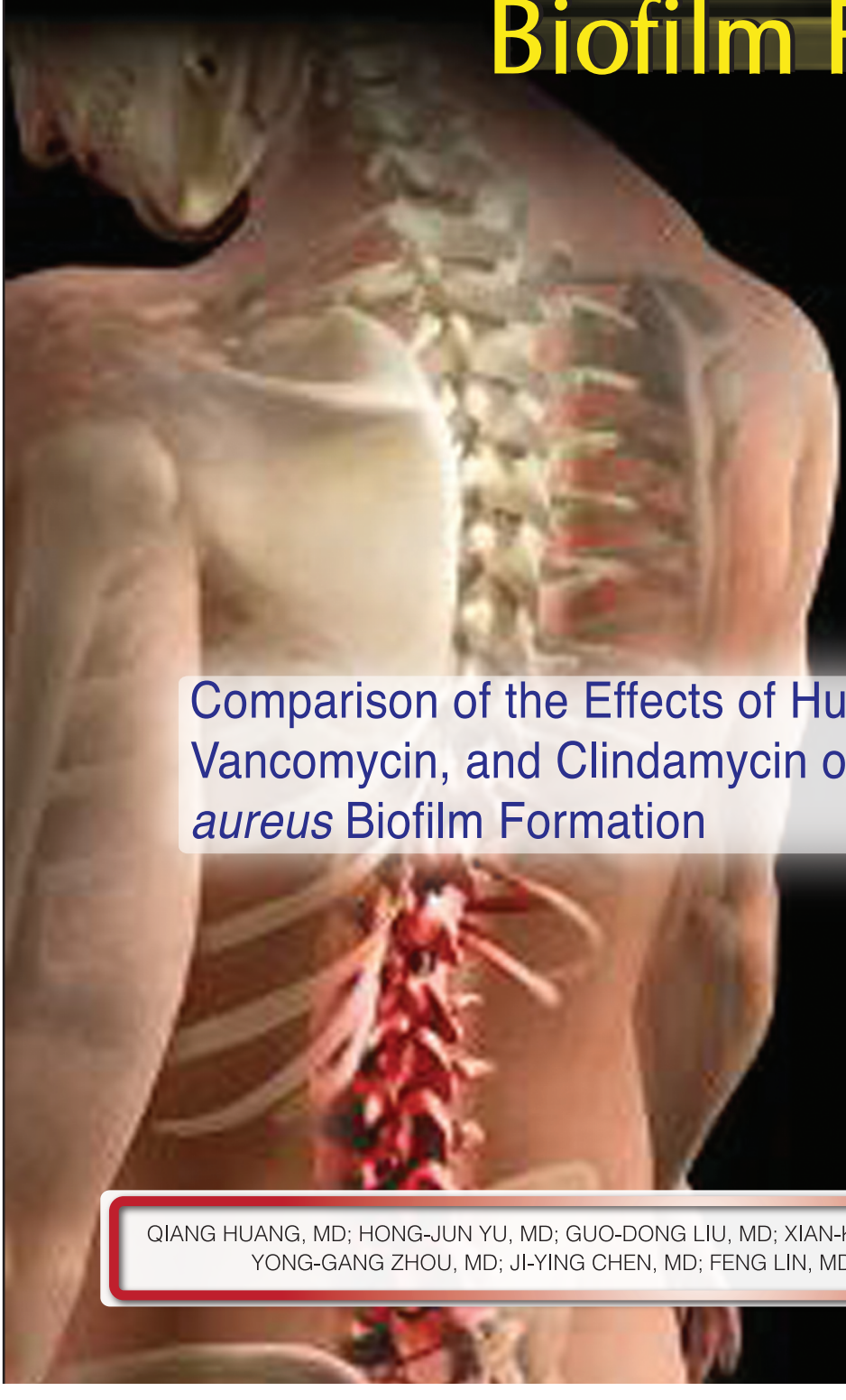
Feature Article on

Staphylococcus aureus Biofilm Formation



Comparison of the Effects of Human ß-defensin 3, Vancomycin, and Clindamycin on *Staphylococcus aureus* Biofilm Formation

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Abstract

Despite improvements in surgical techniques and implant design in orthopedic surgery, implantation-associated infections are still a challenging problem for surgeons. In 2006, trace quantities of human ß-defensin 3 (hBD-3) were found in human bone tissue and bone cells. Human ß-defensin 3 is a 45-amino-acid peptide that is considered the most promising class of defensin antimicrobial peptides and may help in the prevention and treatment of implantation-associated infections. Studies of the effectiveness of hBD-3 against *Staphylococcus aureus* showed that hBD-3 was more potent at low concen-trations than other antibiotics. The effect of hBD-3 on S aureus biofilms has not been reported. We studied the effect of hBD-3, vancomycin, and clindamycin on S aureus biofilms and on the survival of the bacteria in the biofilms.

Staphylococcus aureus biofilms were examined with confocal scanning laser microscopy. Staining with LIVE/DEAD BacLight viability stain (Molecular Probes Europe BV, Leiden, The Netherlands) differentiated between live and dead bacteria within the bio-films, and extracellular polymeric substances (slime) from the biofilms was evaluated after staining with calcofluor white (Sigma Chemical Company, Rocky Hill, New Jersey). Human ß-defensin 3 and clindamycin reduced the S aureus biofilm area. Human -defensin 3 was significantly more effective against bacteria from the S aureus biofilms than was clindamycin. Vancomycin did not reduce the *S aureus* biofilm area.

The use of orthopedic implants, cardiac devices, percutaneous intravascular catheters, and other implants is increasing. Infection of medically implanted material is associated with considerable morbidity.[1-3] Dependent on the site of implantation, infection rates range from 0.2% to 5% in orthopedic and trauma surgery and up to 40% in artificial hearts.[3] Given the high incidence of fracture stabilization devices (2 million per year), the number of implant infections amounts to 100,000 per year.[3] The major pathogens of implant-related infections are *staphylococci*, including *Staphy-lococcus aureus*.[1]

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Drs Huang (Qiang) and Huang (Xian-Kai) contributed equally to this study.

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Most strains of *S aureus* are capable of assuming the biofilm mode of growth. When bacteria grow in a biofilm state, they are resistant to antibiotic levels 10- to 1000-fold higher than genetically identical planktonic bacteria. Consequently, staphylococcal infections involving bio-film formation can be extremely difficult to treat with antibiotics, are often chronic or relapsing, and frequently necessitate invasive procedures, such as removal of the infected tissue or device.[4-5] The search for a new anti-biofilm method has become a focus of study and an urgent clinical need.

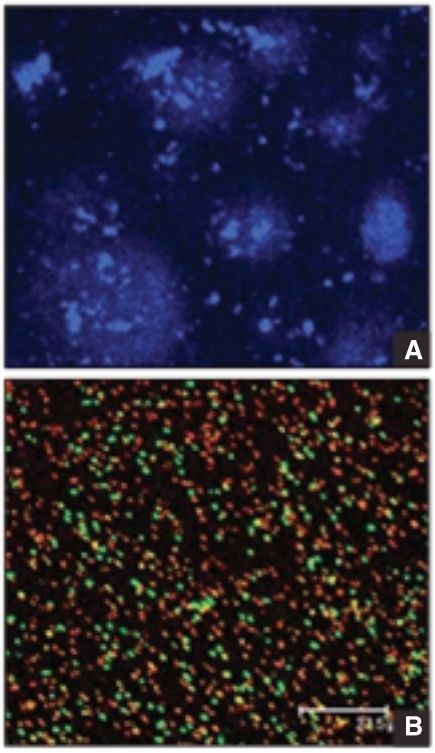

Figure: *Staphylococcus aureus* biofilm formation after 24-hour culture. The green stain is viable *S aureus*, the red fluorescent stain is dead *S aureus*, and the blue fluorescent stain is *S aureus* secretion of bacterial biofilm polysaccharide protein complexes. Biofilm formation of *S aureus* (A). Rate of viable colonies (B).

Numerous novel strategies have been proposed to more effectively prevent and control biofilms. Bacteriophages have been reported for prevention of biofilms in implants,[6-9] and maggot excretions/secretions have been used to break down *S aureus* and *Pseudomonas aeruginosa* biofilms.[10,11] However, they share a common defect in that they cause human allergic reactions because they are not natural substances in the human body.

Since the 1980s, antimicrobial peptides, as a biological composition of the innate immunity system, have been studied. KKVVFKVKFK-NH2 (KSL), an active decapeptide, and other bactericidal peptides inhibit biofilm formation. They are reduced by the bactericidal mechanism of biofilm formation of bacteria and lead to reduced formation of mature biofilms had no effect.[12] In 2006, trace quantities of human-defensin 3 (hBD-3) were found in human bone tissue and bone cells. Humandefensin 3 is a 45-amino-acid peptide that is considered the most promising class of defensin antimicrobial peptides and may help in the prevention and treatment of implantation-associated infections.[13] It has a strong lethal effect on *S aureus* compared with vancomycin and other antibiotics at low concentrations and can have a strong bactericidal effect.[14] Many antibiotics cannot break down bacterial biofilms but can effectively kill planktonic bacteria.[11] If a material is found to break down bacterial biofilms at an early stage after open fracture or orthopedic surgery, the infection rate of open fracture and incidence rate of implantation-associated infections can be decreased.

The effect of hBD-3 on *S aureus* biofilms has not been reported. We studied the effect of hBD-3, vancomycin, and clindamycin on S aureus biofilms and on the survival of the bacteria in these biofilms. Our study was also designed to evaluate the potential for surface treatment of medically implanted material with antibacterial peptides or agents to inhibit biofilm development. Biofilm formation and viability were assessed by confocal scanning laser microscopy after staining of the biofilm with calcofluor white.

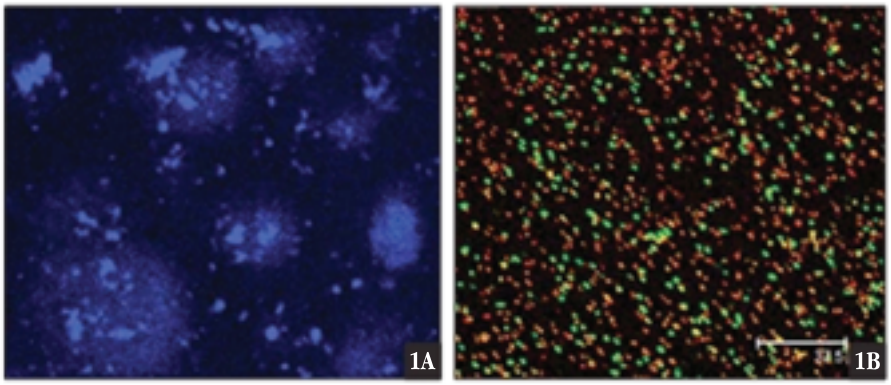

Figure 1: *Staphylococcus aureus* biofilm formation after 24-hour culture. The green stain is viable *S aureus*, the red fluorescent stain is dead *S aureus*, and the blue fluorescent stain is *S aureus* secretion of bacterial biofilm polysaccharide protein complexes. Biofilm formation of *S aureus* (A). Rate of viable colonies (B).

MATERIALS AND METHODS

Human ß-defensin 3 (Sigma Chemical Company, Rocky Hill, New Jersey) were reconstituted in 10 mM acetic acid to a concentration of 1.0 mg/mL. Vancomycin (Eli Lilly Japan K.K., Seishin Laboratories, Kobe, Japan) and clindamycin (Shuangcheng Corporation Ltd, Hainan, China) were dissolved in distilled water to a concentration of 10 mg/mL.

Staphylococcus aureus Cultures

The applied pathogen *S aureus* was purchased from the Chinese Ministry of Health (ATCC 25923 standard strain). This strain is sensitive to vancomycin. It was incubated for 24 hours in 10 mL Tryptone Soya Broth (Qingdao Hope Bio-Technology Co, Ltd, Qingdao, China) at 37°C in a water bath. The minimum inhibitory concentration (MIC) values for this strain are 1.6 to 8 mg/L for hBD-3,[15-18] 0.5 to 2 mg/L for vancomycin, and 0.06 to 0.25 mg/L for clindamycin.

Biofilm Formation

Biofilm formation of *S aureus* in 96-well polyvinyl chloride (PVC) plates was conducted as described.[10] In short, bacteria from overnight cultures were diluted 1:1000, and 5 μL of these bacterial suspensions were added to each well containing 100 μL of biofilm medium consisting of 0.5 mL Tryptone Soya Broth supplemented with 0.2% (w/v) glucose. In the bacterial culture 0, 6, and 24 hours later, with or without hBD-3 (4, 8, 16, and 32 mg/L), vancomycin (0.25, 0.5, and 2.8 mg/L) and clindamycin (0.125, 0.25, 0.5, and 1.0 mg/L) were added to the wells.Because drug concentrations too high or too low have no clinical significance, we chose the minimum one-half MIC concentration gradient to the highest 4 MIC. In every sample, we ensured that the drug action time was 6 hours. Because hBD-3 degrades gradually,[19] we added hBD-3 again after 3 hours.

Biofilm Evaluation With CSLM

Biofilm formation was visualized by confocal scanning laser microscopy (TCS-SP2; Leica Microsystems Heidelberg GmbH, Heidelberg, Germany) with beam path settings for fluorescein isothiocyanate- and tetraethyl rhodamine isothiocyanate-like labels after staining the disks for 30 minutes in the dark at room temperature with LIVE/DEAD BacLight viability stain (Molecular Probes Europe BV, Leiden, The Netherlands) containing 3.34 mM SYTO 9 dye (Molecular Probes Europe BV) and 20 mM propidium iodide. Stacks of approximately 20 images were collected using a 10× objective lens.

The stain differentiates live from dead bacteria by staining living bacteria fluorescent green and dead ones fluorescent red, where-as the background remains virtually non-fluorescent. Subsequently, the ratio of green bacteria to the total (red and green) bacteria was calculated and expressed as a percentage of viable cells. In each sample, 2 representative sites were chosen for visualization. To compare the images, laser power and pinhole settings were kept constant. The counting of stained scrapings from the wells was carried out in triplicate, for quantitative analysis of bacterial viability.

Evaluation of Slime With Confocal Scanning Laser Microscopy

Calcofluor white, a polysaccharide-binding dye, has been used to stain the extracellular matrix of biofilms formed by bacteria.[20] Thus, to determine whether the adhered structures of *S aureus* were encased in a polysaccharide matrix (slime), the biofilm was stained with 50 mM calcofluor white. Staining was done in duplicate for 15 minutes in the dark at room temperature, and slime production was then observed by confocal scanning laser microscopy.

Image and Statistical Analysis

Biofilm images were performed using Image-Pro Plus Version 6.0 (Media Cybernetics, Bethesda, Maryland). We measured the slime-stained area, the integrated optical density, and the number of live and dead colonies.

Data were expressed as mean±standard deviation. Chi-square test and *t* test were performed with SPSS 17.0 software (SPSS Inc, Chicago, Illinois). The level of significance was set at $P<.05$.

RESULTS

Staphylococcus aureus Biofilm Formation

To determine the role of experimental drugs on biofilms, we observed S aureus biofilm formation. Biofilm formation of *S aureus* in PVC plates was adhesion in 4 to 6 hours, secretion of the polysaccharide biofilm matrix in 6 to 8 hours, initial formation in 18 to 24 hours, and full maturity at 72 hours. When *S aureus* was cultured in 24 hours, the surface PVC plates formed biofilms, and the rate of viable cells was 0.746±0.013 (Figure 1).

Effect of hBD-3, Vancomycin, and Clindamycin on Biofilm Adhesion Phase

To assess the impact of hBD-3, vancomycin, and clindamycin on *S*

Table 1

Impact of hBD-3, Vancomycin, and Clindamycin on *S aureus* Biofilm Area, IOD Value, and Rate of Viable Colonies at the Adhesion Stage

	hBD-3			Vancomycin			Clindamycin			Control		
MIC	Area, μm	IOD Value	Rate of Viable Colonies	Area, μm	IOD Value	Rate of Viable Colonies	Area, μm	IOD Value	Rate of Viable Colonies	Area, μm	IOD Value	Rate of Viable Colonies
One-half	3213.00 ±672.5[a]	813.57 ±142.9[b]	0.515 ±0.04	3093.33 ±614.9[a]	729.96 ±57.6[a]	0.585 ±0.13	3643.53 ±745.9[a]	600.49 ±260.86[b]	0.528 ±0.14	414.33 ±66.1	145.87 ±69.3	0.535 ±0.05
1	3737.00 ±688.9[a]	1390.54 ±105.3[a]	0.406 ±0.02[b]	4890.00 ±2274.1[a]	1137.79 ±518.0[a]	0.413 ±0.09[b]	3280.06 ±1438.9[a]	1167.60 ±17.86[a]	0.522 ±0.10	414.33 ±66.1	145.87 ±69.3	0.535 ±0.05
2	2066.00 ±178.0[b]	370.10 ±35.5	0.396 ±0.02[b]	2512.00 ±353.2[b]	460.56 ±169.9	0.332 ±0.07[b]	2006.67 ±521.7[b]	437.04 ±61.42	0.407 ±0.01[b]	414.33 ±66.1	145.87 ±69.3	0.535 ±0.05
4	1627.33 ±1597.0	360.53 ±581.4	0.382 ±0.02[b]	1683.00 ±610.8	34.44 ±15.6	0.311 ±0.04[b]	1068.67 ±114.7	120.00 ±5.26	0.330 ±0.06b	414.33 ±66.1	145.87 ±69.3	0.535 ±0.05b

Abbreviations: hBD-3, human β-defensin 3; IOD, integrated optical density; MIC, minimum inhibitory concentration; S aureus, Staphylococcus aureus.
[a]*P<.01.*
[b]*P<.05.*

Table 2

Impact of hBD-3, Vancomycin, and Clindamycin on *S aureus* Biofilm Area, IOD Value, and Rate of Viable Colonies at the Process Stage

	hBD-3			Vancomycin			Clindamycin			Control		
MIC	Area, μm	IOD Value	Rate of Viable Colonies	Area, μm	IOD Value	Rate of Viable Colonies	Area, μm	IOD Value	Rate of Viable Colonies	Area, μm	IOD Value	Rate of Viable Colonies
One-half	6706.00 ±856.0	1708.07 ±183.8	0.779 ±0.09	8815.33 ±2800.2	1310.97 ±761.8	0.750 ±0.03	9590.33 ±4275.1	1471.93 ±213.0	0.795 ±0. 01	10072.33 ±1067.5	1736.83 ±161.7	0.767 ±0.04
1	2625.00 ±2689.8[a]	245.63 ±122.2[a]	0.682 ±0.08	8295.33 ±1255.4	2398.17 ±552.2	0.552 ±0.10[b]	5949.00 ±2932.9[b]	947.17 ±643.4[b]	0.681 ±0.07	10072.33 ±1067.5	1736.83 ±161.7	0.767 ±0.04
2	2034.00 ±1149.6[a]	171.17 ±144.5[a]	0.663 ±0.04	7801.33 ±3199.3	2815.57 ±655.0[b]	0.592 ±0.09[b]	5544.67 ±315.2[b]	796.93 ±151.0[b]	0.661 ±0.05	10072.33 ±1067.5	1736.83 ±161.7	0.767 ±0.04
4	2296.67 ±1275.8[a]	80.27 ±48.0[a]	0.576 ±0.05[b]	5394.67 ±1963.6[b]	3576.76 ±537.7[b]	0.541 ±0.11b	6230.33 ±659.0[b]	451.70 ±230.0[b]	0.626 ±0.13[b]	10072.33 ±1067.5	1736.83 ±161.7	0.767 ±0.04

Abbreviations: hBD-3, human β-defensin 3; IOD, integrated optical density; MIC, minimum inhibitory concentration; S aureus, Staphylococcus aureus.
[a]*P<.01.*
[b]*P<.05.*

aureus biofilm adhesion, we observed biofilm formation after 6 hours. In the one-half MIC and 1 MIC groups, hBD-3, vancomycin, and clindamycin significantly stimulated slime secretion (P<.05), and the 2 MIC group reached significance (P<.05). Itcan be seen from the integrated optical density value that 3 drugs stimulated *S aureus* slime secretion in the one-half MIC group. With the increase of drug concentration, the role of slime stimulation significantly increased. The results showed that with the increase of drug concentration, the rate of viable colonies decreased in hBD-3, vancomycin, and clindamycin from 1 MIC to 4 MIC (Table 1).

Effect of hBD-3, Vancomycin, and Clindamycin on Biofilm Formation Phase

After *S aureus* was cultured for 6 hours, hBD-3, vancomycin, and clindamycin were added, and biofilm formation was observed after 6 hours to assess the impact of the 3 drugs on *S aureus* biofilm formation.

When the hBD-3 concentration was >1 MIC, it significantly inhibited the formation of S aureus slime area (P<.01), as did the clindamycin (P<.05). An increased concentration of vancomycin inhibited slime area in the 2 MIC and 4 MIC groups. The variation trend in the integrated optical density value was the same as the area of slime variation. In the process stage of S aureus bacteria biofilm formation, vancomycin concentration >1 MIC significantly reduced the rate of viable colonies, whereas hBD-3 and clindamycin in the 4 MIC group significantly reduced the rate of viable colonies (Table 2).

Effect of hBD-3, Vancomycin, and Clindamycin on Biofilm Initial Mature Phase

After the *S aureus* biofilm was formed after 24 hours of culture, we added hBD-3, vancomycin, and clindamycin to observe their impact on the *S aureus* biofilm.

It was observed that the formation of biofilm was less than that in the control group by slime stain. From the slime area, the 2 MIC and 4 MIC groups of hBD-3 significantly reduced the area of biofilm formation (P<.01), and the 1 MIC group also reduced biofilm area (P<.05) (Figure 2). Vancomycin did not affect the S aureus biofilm in the experimental group (Figure 3). Clindamycin in the 2 MIC and 4 MIC groups reduced the area of biofilm formation (Figure 4). The variation trend in integrated optical density value was the same as the area of

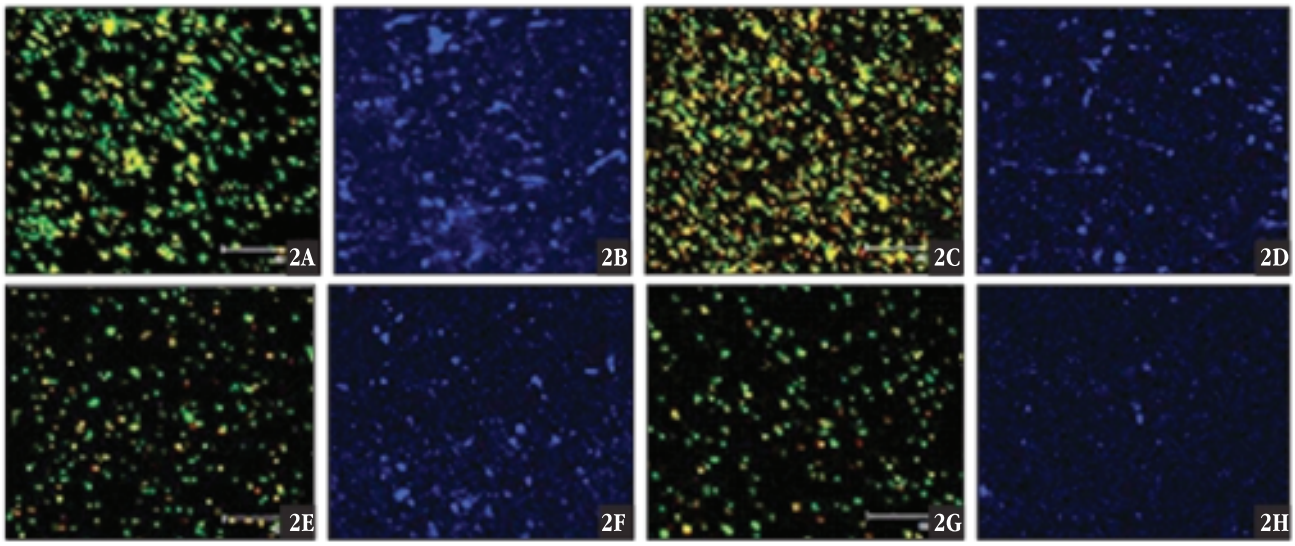

Figure 2: The effect of different concentrations of hBD-3 on the 24-hour area and rate of viable colonies of *Staphylococcus aureus* biofilm. Biofilm formation of S aureus in one-half MIC (A). Rate of viable colonies in one-half MIC (B). Biofilm formation of *S aureus* in 1 MIC (C). Rate of viable colonies in 1 MIC (D). Biofilm formation of *S aureus* in 2 MIC (E). Rate of viable colonies in 2 MIC (F). Biofilm formation of *S aureus* in 4 MIC (G). Rate of viable colonies in 4 MIC (H).

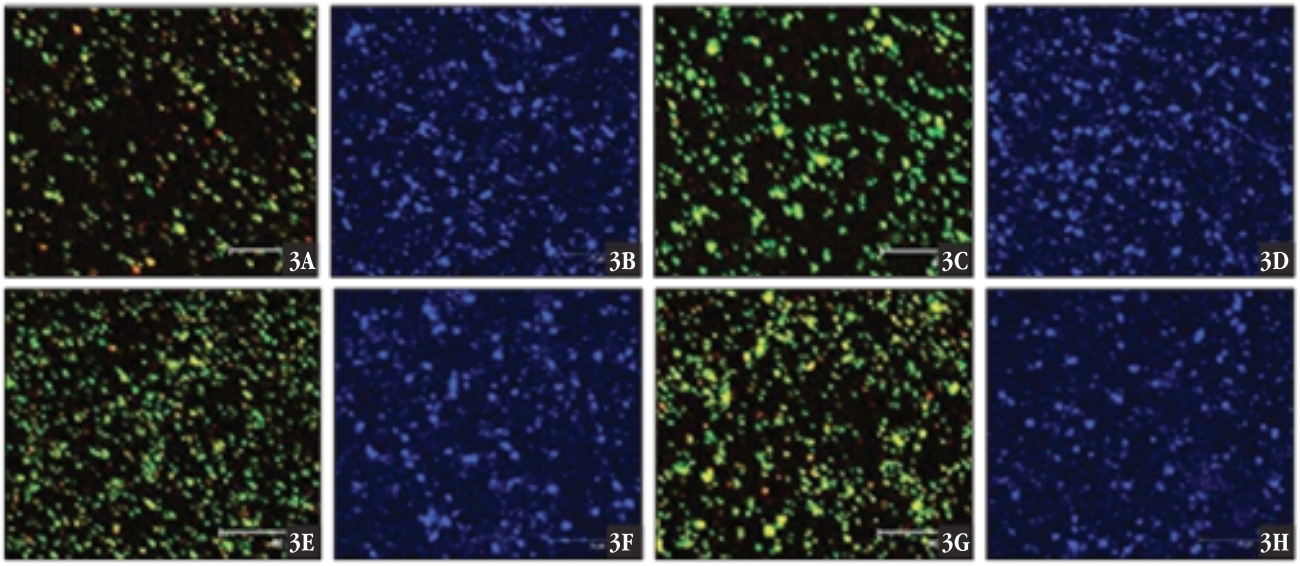

Figure 3: The effect of different concentrations of vancomycin on the 24-hour area and rate of viable colonies of *Staphylococcus aureus* biofilm. Biofilm formation of *S aureus* in one-half MIC (A). Rate of viable colonies in one-half MIC (B). Biofilm formation of *S aureus* in 1 MIC (C). Rate of viable colonies in 1 MIC (D). Biofilm formation of *S aureus* in 2 MIC (E). Rate of viable colonies in 2 MIC (F). Biofilm formation of *S aureus* in 4 MIC (G). Rate of viable colonies in 4 MIC (H).

slime variation.

The results showed that vancomycin and clindamycin concentrations in various experiments do not have an effect on biofilm bacteria, whereas hBD-3 in the 1, 2, and 4 MIC groups decreased the rate of viable colonies (Table 3).

DISCUSSION

In 2006, trace quantities of hBD-3 were found in human bone cells. Human ß-defensin 3 can exert a strong bactericidal effect on vancomycin-resistant S aureus and all aerobic bacteria at a low concentration of approximately 26.5 g/mL.[21] It shows efficient sterilization performance in the physiological concentration of sodium ions in muscle tissue and the physiological environment of low albumin.[21] However, because hBD-3 concentration in the bone tissue is low and insufficient to resist local infection, the local increase of hBD-3 concentration is necessary for replacement of the antibiotics to avoid the dysbacteriosis, toxicity, and resistance to a range of issues caused by antibiotics.

Previous studies have reported the bactericidal effect of hBD-3, but its role in inhibiting biofilm had not been reported. We first studied the effect of hBD-3 on the stages of *S aureus* biofilm formation so that we could distinguish the bactericidal or inhibiting effect of hBD-3. Our results showed that from one-half MIC to 4 MIC of hBD-3, vancomycin, and clindamycin significantly stimulated slime secretion at the adhesion stage. The results indicate that biofilm formation occurred at

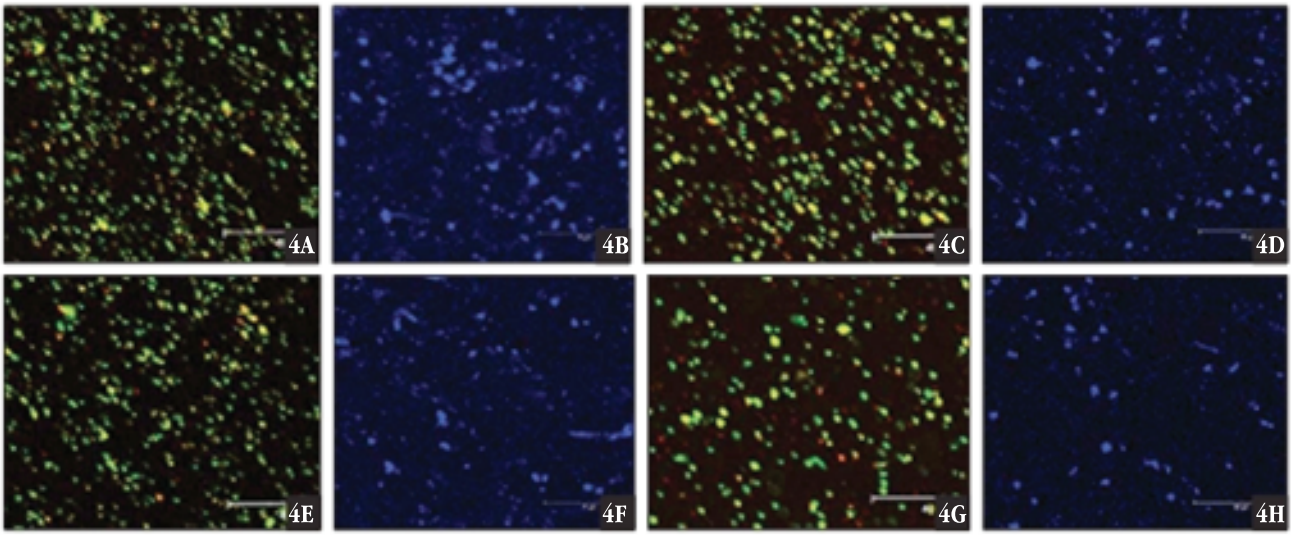

Figure 4: The effect of different concentrations of clindamycin on the 24-hour area and rate of viable colonies of *Staphylococcus aureus* biofilm. Biofilm formation of *S aureus* in one-half MIC (A). The rate of viable colonies in one-half MIC (B). Biofilm formation of *S aureus* in 1 MIC (C). The rate of viable colonies in 1 MIC (D). Biofilm formation of *S aureus* in 2 MIC (E). The rate of viable colonies in 2 MIC (F). Biofilm formation of *S aureus* in 4 MIC (G). The rate of viable colonies in 4 MIC (H).

Table 3

Impact of hBD-3, Vancomycin, and Clindamycin on *S aureus* Biofilm Area, IOD Value, and Rate of Viable Colonies at the Process Stage

	hBD-3			Vancomycin			Clindamycin			Control		
MIC	Area, μm	IOD Value	Rate of Viable Colonies	Area, μm	IOD Value	Rate of Viable Colonies	Area, μm	IOD Value	Rate of Viable Colonies	Area, μm	IOD Value	Rate of Viable Colonies
One-half	14681 ±2831	23566 ±4544	0.743 ±0.05	13388 ±3470	21492 ±5573	0.772 ±0.09	14766 ±2626	22465 ±4214	0.774 ±0.07	14350 ±2092	23040 ±3360	0.746 ±0.01
1	10967 ±805[a]	17615 ±1299[a]	0.581 ±0.03[a]	10422 ±219.7	19950 ±338	0.760 ±0.09	13432 ±1090	20391 ±1797	0.677 ±0.02	14350 ±2092	23040 ±3360	0.746 ±0.01
2	7926 ±186[b]	12787 ±357[b]	0.563 ±0.12[a]	13095 ±805.5	21020 ±1293	0.743 ±0.07	10657 ±1096[a]	17888 ±1759[a]	0.688 ±0.09	14350 ±2092	23040 ±3360	0.746 ±0.01
4	3908 ±2003[b]	6356 ±3331[b]	0.534 ±0.06[a]	11768 ±2016.2	18923 ±3295	0.732 ±0.03	6657 ±1001[b]	13896 ±1606[b]	0.671 ±0.14	14350 ±2092	23040 ±3360	0.746 ±0.01

Abbreviations: hBD-3, human ß-defensin 3; IOD, integrated optical density; MIC, minimum inhibitory concentration; S aureus, Staphylococcus aureus.
[a]*P<05.*
[b]*P<.01.*

the adhesion phase of *S aureus*. At this time, 3 drugs play the role of bactericide. Due to the defense response of bacteria, slime secretion increased significantly. Thus, the result reflects the slime area integrated optical density value was significantly higher than the control group, whereas the rate of viable colonies gradually decreased.

At the second stage of the biofilm formation process, when hBD-3 and clindamycin concentrations were >1 MIC, they could significantly inhibit the formation of *S aureus* slime, but hBD-3 had more effect than clindamycin. Although vancomycin also inhibited the slime area in the 2 MIC and 4 MIC groups, it significantly reduced the rate of viable colonies, even in the 1 MIC group. In the process stage of *S aureus* biofilm formation, vancomycin played the role of bactericide to inhibit biofilm formation. However, hBD-3 and clindamycin only significantly reduced the rate of viable colonies in the 4 MIC group. We speculate that hBD-3 not only inhibited biofilm formation by bactericidal mechanism, but that other mechanisms were involved.

When the *S aureus* biofilms were initially mature, hBD-3 in the 1 MIC group reduced biofilm formation and the ratio of viable cells in the biofilms. Clindamycin is also able to inhibit S aureus biofilms, but it must be >2 MIC to be effective, and its rate of viable colonies within the biofilm had no significant effect. Vancomycin, as an effective antibiotic for *S aureus*, did not demonstrate a result in biofilm formation and the bacteria within the biofilm. Studies have reported that large doses of vancomycin are required to have an effect on the biofilm.[11,20,22] In view of vancomycin nephrotoxicity, it has no clinical practical meaning.

Cell membrane permeability is an important bactericidal mechanism of hBD-3.[14,17] In the primary structure of hBD-3, N-terminal -helix is similar to KSL and 13-residue dermaseptin derivative, and the bactericidal mechanism is also similar.[23-25] C-terminal fragments, ß-sheet, and disulfide of hBD-3 have complex physiological functions. So far the mechanism is unclear, indicating that the hBD-3 mechanism of action may also have DNA, RNA, and protein effects. Considering the role of

hBD-3 in the various stages of biofilm formation, hBD-3 not only acted as a bactericidal agent, but it also effectively suppressed the role of *S aureus* biofilm formation. We found an inhibition of slime secretion in every stage of *S aureus* biofilm formation, and further study is needed.

In this study, clindamycin was effective against S aureus biofilm. In consideration of in vitro degradation of the hBD-3, further hBD-3 was added to maintain the concentration of hBD-3 in the experiments. As for biofilm bacteria, the bactericidal effect of hBD-3 was significantly more than clindamycin, enabling a significantly reduced the rate of viable colonies in the biofilm.

Taking into account that a 96-well plate is not flat, we divided every hole into 9 regions. This may lead to an increase in the standard deviation, thus affecting the results. However, that hBD-3 inhibited the ability of *S aureus* biofilm formation is evident. Similarly, to reduce systematic errors due to the different image acquisition area, our experiments assessed the rate of viable colonies instead of the number of viable colonies. In the next experiment, we will further reduce systematic errors.

Bacteriophages and maggot excretions/secretions can also prevent and control biofilms in vitro. However, potential obstacles to their use must be carefully considered, including the narrow host range, resistance of host bacteria, potential for inactivation by the patient's immune system, and safety of preparations in humans.[7] Compared with the bacteriophages and maggot excretions/secretions, hBD-3 as the body's own secretion has fewer side effects in suppressing bacterial biofilms. Therefore, hBD-3 has more clinical value.

With the recent widespread use of biological materials, implantation-associated infections have increased significantly. Mature biofilm prevents entrance of antibiotics,[26] and bacterial metabolism is slow.[27] The sol-gel method and biomimetic coating technology have been used to develop the vancomycin-loaded poly-DL-lactic acid or calcium-phosphate coating titanium alloy plate in previous studies,[28,29] with the aim of reducing the incidence of implantation associated infections. In further studies, we tried to find a substance that could directly inhibit bacterial biofilm formation for surface modification of the biomaterials. The early release of the drug from inside-out when implantation of the internal fixators was enabled directly inhibited the bacterial biofilm formation and further reduced the incidence of implantrelated infection rate. Our exploratory research confirmed that hBD-3 could inhibit *S aureus* biofilm and reduce the rate of viable colonies.

CONCLUSION

Human ß-defensin 3 inhibits *S aureus* biofilm and reduces the membrane of viable colonies. This finding may provide a feasible and effective method of antibiofilm infection treatment and has important clinical significance. Human ß-defensin 3 acts on biofilm mechanisms. An effective method for maintaining a stable concentration is an area for further study.

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ClindaX®

Clindamycin

Inhibits both gram-positive & anaerobes

- The exceptional antibiotic with wide antimicrobial coverage
- Possess adequate level of bone concentration (5.01 µgm/ml)
- 98% clinical success rate against MRSA
- No hypersensitivity and anaphylaxis reactions like penicillin derivatives
- Possess excellent efficacy in SSTIs, Dental infections and RTIs
- Largest number of possible dosage forms in Bangladesh

Clindax®
150 & 300 mg
Capsule

Clindax®
300 & 600 mg
Injection

FIRST TIME CLINDAMYCIN **ORAL** SOLUTION IN BANGLADESH

Clindax®
20 mg
Cream

Clindax®
25 ml Lotion

Clindax Plus®
10 mg
Gel

ClindaX®

Clindamycin

Injection 300 mg & 600 mg
Capsule 150 mg & 300 mg
Oral solution 100 ml
Lotion 25 ml &
Vaginal cream 20 mg

Inhibits both gram-positive & anaerobes

Dosage & Administration

IM or IV Administration

Adults

For serious infections: 600 mg - 1200 mg daily (in 2 - 4 divided doses).*
More severe infections: 1200 - 2700 mg/day in 2 - 4 equal doses is recommended.**

*Single intramuscular injection of greater than 600 mg are not recommended.
**Doses of as much as 4800 mg daily have been given intravenously to adults.

Child

Neonates (less than 1 month): 15 to 20 mg/kg/day in 3 to 4 equal doses. The lower dosage may be adequate for small prematures.

1 month to 16 years: 20-40 mg/kg/day in 3 or 4 equal doses. The higher doses would be used for more severe infections.

Dilution & Infusion Rates

ClindaX® Injection can be given as infusion with any of the following IV solutions:

1. Sodium Chloride Infusion
2. Dextrose 5% in water or saline
3. Dextrose 5% in ringer solution
4. Hartmann's solution

The usual infusion rates are as follows:

Dose	Diluent	Time
300 mg	50 ml	10 min
600 mg	50 ml	20 min
900 mg	50-100 ml	30 min
1200 mg	100 ml	40 min

Capsule/Oral Solution

Adults 150-300 mg every 6 hours & up to 450 mg every 6 hours in severe infections. For prophylactic use 600 mg orally or intravenously 30-60 minutes before procedure.

Child 3-6 mg/Kg every 6 hours. For prophylactic use 20 mg/kg orally or intravenously 30-60 minutes before procedure.

Topical

ClindaX® **Lotion:** Apply twice daily with the fingertip at the affected area for 6-8 weeks.

ClindaX® **Plus Gel:** Apply once daily with the fingertip (preferably at bed time) for 6-12 weeks.

ClindaX® **Vaginal Cream:** 1 applicatorful (containing 5 gm) at bet time for 3-7 consecutive days.

Skin Care Products

Brand Name	Generic Name
Lorix® 30 gm Cream	Permethrin 5%
Lorix® Plus 60 ml Lotion	Permethrin 5% + Crotamiton 10%
Dermex® 10 gm Cream & Ointment	Clobetasol Propionate 0.05%
Dermoban® 10 gm Ointment	Mupirocin 2%
Neocin® 10 gm Ointment & 5 gm Powder	Neomycin Sulphate 0.5% + Bacitracin 500 Units
Neocin® Plus 10 gm Ointment	Neomycin Sulphate 0.35% + Bacitracin zinc 400 IU + Polymyxin B 5000 IU
Betson® N 5 gm Cream	Betamethasone 0.1% + Neomycin Sulphate 0.5%
Dermex® NN 10 gm Cream	Clobetasol Propionate 0.05% + Neomycin Sulphate 0.5% + Nystatin 100000 units
Fusicort® 10 gm Cream	Fusidic Acid 2% + Hydrocortisone 1%
Clindax® 25 ml Lotion	Clindamycin USP 1%
Clindax® Plus 10 gm Gel	Clindamycin + Tretinoin BP
Azicin® 250 mg, 500 mg Tab, 250 mg, 500 mg Cap, 20 ml, 35 ml, 50 ml GFS & 1 gm Sachet	Azithromycin
Clavusef® 125 mg, 250 mg, 500 mg Tab.	Cefuroxime+ Clavulanic acid
Levox® 500 mg, 750 mg Tab. & 500 mg IV inj	Levofloxacin

Brand Name	Generic Name
Flucon® 50 mg,150 mg & 200 mg Capsule	Fluconazole
Ketocon® 60 ml Shampoo	Ketoconazole 2%
Betson® CL 10 gm Cream & Ointment	Betamethasone 0.05% + Clotrimazole 1%
Ecozol® Plus 10 mg Cream	Econazole Nitrate 1% + Triamcinolone Acetonide 0.1%
H-Trimazole® 10 gm Cream	Clotrimazole 1% + Hydrocortisone 1%
Unigal® HC 10 gm Cream	Miconazole Nitrate 2% + Hydrocortisone 1%
Esotid® 20 mg, 40 mg Tab, 20 mg, 40 mg Cap. & 40 mg inj	Esomeprazole
Ometid® 20 mg, 40 mg Cap., 40 mg inj	Omeprazole
Finix® 20 mg Tab	Rabeprazole Sodium
Ebanex® 10 mg Tab	Ebastine
Xyril® 10 mg, 25 mg Tab	Hydroxyzine Hydrochloride
Fixal® 30 mg, 60 mg, 120 mg 180 mg Tab. 50 ml Susp	Fexofenadine Hydrochloride
Citin® 10 mg Tab. 60 ml Syp	Cetrizine Hydrochloride
Trilock® 4 mg, 10 mg Tab., 4 mg Oral Granules Schet	Montelukast

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