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Bacteria Responsive Antibacterial Surfaces for Indwelling Device Infections

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Abstract

Indwelling device infections now represents life-threatening circumstances as a result of the biofilms' tolerance to antibiotic treatments. Current antibiotic impregnation approaches through sustained antibiotic release have some unsolved problems which include short life-span, narrowed antibacterial spectrum, ineffectiveness towards resistant mutants, and the potential to hasten the antibiotic resistance process. In this study, bacteria responsive anti-biofilm surfaces were developed using bioactive peptides with proved activity to antibiotic resistant bacteria and biofilms. Resulting surfaces were stable under physiological conditions and in the presence of high concentrations of salts (0.5 M NaCl) and biomacromolecules (1.0% DNA and 2.0% alginate), and thus showed good biocompatibility to various tissue cells. However, lytic peptide immobilized surfaces could sense bacteria adhesion and kill attached bacteria effectively and specifically, so biofilms were unable to develop on the lytic peptide immobilized surfaces. Bacteria responsive catheters remained biofilm free for up to a week. Therefore, the bacteria responsive antibacterial surfaces developed in this study represent new opportunities for indwelling device infections.

Keywords

Biofilms; Infections; Biomedical Devices; Responsible; Peptides; Controlled Release

1. Introduction

Bacteria prefer to attach and grow on any solid surfaces to develop into biofilms, an assemblage of microbial cells that is associated with a surface. It is estimated that over 95 % of bacteria existing in nature are found in biofilms. Bacterial cells of the same microbial species can exhibit extremely different physiological states in biofilms [1]. One of the most important features of biofilms is their tolerance towards antibiotic treatments. Bacteria living in biofilms can exhibit antibiotic tolerance up to several hundred times greater than

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planktonic bacteria [1–3]. As the wide use of biomedical devices and implants such as vascular grafts and catheters, biofilms infections pose potential life-threatening circumstances for patients [4–7]. Currently, the most effective strategy is antibiotic impregnation, which is accomplished by introducing antimicrobial agents onto biomedical device surfaces. Because a high local concentration of antibiotics is provided through sustained release, bacteria are killed before they grow in biofilms [8–10]. However, some unsolved problems are associated with current antibiotic impregnations: 1) since antibiotics for resistant bacteria such as MRSA are very limited at the present time [11, 12], antibiotic impregnations do not work in resistant mutant involved infections; 2) antibiotic impregnations usually have narrowed antibacterial spectrum and short life-spans because only limited amounts and types of antibiotics can be loaded; 3) the sustained antibiotic release feature of antibiotic impregnations has a fatal drawback with respect to the genetic feedback and can hasten the antibiotic resistance process.

Lytic peptides are a group of recently discovered membrane-acting antibacterial peptides. Besides hardly developed resistance and a wide antibacterial spectrum, lytic peptides are active to antibiotic resistant bacteria (including MRSA) [13] and demonstrate good activity to preformed biofilms [14]. In this study, bacteria responsive antibacterial surfaces designed for biofilms associated bio-fouling and biomedical device infections were constructed using lytic peptides. Resulting surfaces were stable under physiological conditions but could sense bacteria adhesion and biofilm formation to release bactericidal peptides at the contamination sites to kill attached bacteria specifically and effectively. Modified catheters with such responsive antibacterial surfaces maintained bio-fouling free after being continuously exposed to bacteria for a week.

2. Materials and Methods

2.1. Materials and bacterial strains

MTT (methylthiazolyldiphenyl-tetrazolium bromide), glucose, tryptic soy broth acrylic acid, acetic acid, and Toluidine Blue O were supplied by Sigma-Aldrich (St. Louis, MO). A LIVE/DEAD staining kit was purchased from Invitrogen Life Technologies (Carlsbad, CA). Peptides including fluorescent labeled peptides were synthesized by GenScript (Piscataway, NJ). The purity (>90%) of the synthesized peptide was confirmed by mass spectrometry and HPLC. *Staphylococcus aureus* (ATCC 25923, penicillin and methicillin sensitive, MSSA; ATCC 29213, penicillin resistant but methicillin sensitive, MSSA; ATCC 44300, penicillin and methicillin resistant, MRSA) and *Staphylococcus epidermidis* (ATCC 146), the two most common bacteria found in indwelling device infections, were purchased from the American Type Culture Collection (Manassas, VA). Human fetal osteoblasts (HFOB 1.19), human lung cells (A549), and mouse osteoblast precursor cells (MC-3T3) were also purchased from the American Type Culture Collection (Manassas, VA).

2.2. Lytic peptide characterization

The secondary structures of peptides were analyzed using CDPPO software based on the circular dichroism (CD) spectra of peptides recorded on a Jasco J-710 spectropolarimeter [15]. Peptide self-assembly in solution to form peptide aggregates was estimated by using 1-

anilinonaphthalene-8-sulfonic acid (1, 8-ANS) as a fluorescence probe [15]. The ANS (20 μ M) fluorescence emission spectrum changes associated with peptide aggregation were recorded on the fluorescence microplate reader (Biotek Inc.) by setting excitation wavelength at 369 nm.

2.3. Preparation of lytic peptides immobilized surfaces

The preparation process involved two steps: 1) plasma mediated surface modification (Figure 1A); and 2) lytic peptide immobilization (Figure 1B). Material samples (polyethylene terephthalate, PET, films and silicon wafers) were first activated using argon plasma produced in a Plasma Prep III device (SPI Supplies) [15] and then exposed to the air to generate hydroperoxide reactive centers (Figure 1A). The amount of hydroperoxides was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) [16]. Surface polymerization was carried out at 70°C under a nitrogen atmosphere in the presence of acrylic acid. Total poly(acrylic acid) grafted the surfaces was estimated through Toluidine Blue O staining [17]. Lytic peptide immobilization was done by immersing polymer grafted samples in lytic peptide solutions at room temperature for 1.0 hour (Figure 1B). After washing with distilled water to remove unbound peptides, samples were characterized and tested for peptide immobilization efficiency, stability, antibacterial activity, and biocompatibility.

2.4. Surface characterization

Graft polymerization and lytic peptide immobilization were analyzed using attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) (JASCO FT/IR-460Plus) and atomic force microscopy (AFM). ATR-FTIR was conducted in the percent transmittance mode in the range of 400–4000 cm^{-1} with a KRS-5 prism and an incident angle of 45°. AFM measurements were operated in air on a NSCRIPTOR dip pen nanolithography system (Pacific Nanotechnology, Inc.) using P-MAN-SICC-0 AFM cantilevers with a nominal force constant of 40 N/m [15].

2.5. Peptide release tests using fluorescent labeled peptides

Impregnations prepared from fluorescent labeled peptides were immersed in PBS solutions containing NaCl (0–5.0 M), DNA (1 %), alginate (2 %), or with varied pHs (pH=4.5–7.0) and incubated at room temperature for 60 minutes. Peptides released into the solutions were monitored by measuring solution fluorescence intensity changes at 535 nm ($\lambda_{\text{ex}} = 485 \text{ nm}$). Since pHs affected the fluorescence intensity of fluorescein, peptide release measured at different pHs were calibrated using fluorescein standard curves prepared at various pHs.

2.6. Biological stability in human plasma

Peptide immobilized samples were incubated with 1.0 mL pooled human sera and incubated at 37 °C for 4 hours. At the end of incubation, trifluoroacetic acid was added (0.05%, final concentration) to precipitate plasma protein and release peptides from sample surfaces. Peptides in supernatants were then purified through ZipTip_{C-18} column (Millipore). The amount of intact peptide was determined using MALDI-TOF mass spectrometry in the matrix containing α -cyano-4-hydrocinnamic acid (10 mg/mL in 50% acetonitrile with 0.05% trifluoroacetic acid). Measurements were made with Bruker UltraFLEXtreme mass

spectrometer and the data analyzed with Flex analysis version 3.3 software (Bruker Daltonik GmbH).

2.7. Biocompatibility tests

Peptide immobilized samples (2.5 × 2.5 cm) were placed in 6-well plates. Human tissue cells, MC-3T3, in alpha MEM medium supplemented with 10 % fetal bovine serum were seeded and incubated at 37 °C in a humidified atmosphere of 5 % CO₂ for 24 hours. At the end of incubation, samples were washed with saline and then stained with Live/Dead kit. Cell images were recorded using a Zeiss LSM510 Confocal Microscope by setting the excitation wavelength at 488 nm and the emission wavelengths at 505–530 nm (for the live cells) and 560 nm (for the dead cells). The percentage of green pixels out of total green and red pixels in captured cell images was calculated to estimate live/dead cell ratios [18].

2.8. Antibacterial and anti-biofilm activity assays

Peptide immobilized samples (2.5 cm × 2.5 cm) were placed in the wells of 6-well plates with mid-log phase *S. aureus* suspension in PBS and incubated at 37 °C for 2 hours. After this initial attachment phase, samples were gently washed and then transferred into the new wells containing diluted tryptic soy broth medium supplemented with 0.2% glucose (TSBG), the medium for *Staphylococcus aureus*. At the end of overnight cultivation at 37 °C, bacteria in the culture media were quantified by measuring the absorbance of culture supernatants at 570 nm. Live bacteria on samples (adhered or in the form of biofilms) were stained using LIVE/DEAD kit and quantified using a MTT-based viability assay established in previous publications [14, 18].

2.9. Bio-fouling tests

Bio-fouling assays were performed on a microfluidic cultivation system, which mimics the actual environment of indwelling devices *in vivo* (Figure 2). Silicone tubes (1.0 mm in diameter) with peptide immobilized inner surfaces were filled up with *S. aureus* suspension (2×10^4 cfu/mL PBS) through a multi-channel pump and incubated at 37 °C for 4 hours to simulate bacteria adhesion. After the initial attachment phase, tubes were fed with diluted TSBG continuously at a constant flow rate of 0.12 ml/min. Biofilm growth inside silicone tubes was monitored under microscopy for a week. In addition, silicone tubes were collected at different time points. Adhered live bacteria and formed biofilms inside silicone tubes were quantified using MTT-based viability assay [14] by gradually pumping MTT (0.5 mg/mL water) solution into the silicone tubes. This gradual process allowed for the diffusion of MTT through the treated tube and ensured that no bacteria/biofilm was released during the staining experiment. After 20 minute incubation, insoluble formazan inside silicone tubes were dissolved and diluted using SDS-HCl solutions. Total live bacteria in silicone tubes were estimated as previously described [14, 18].

3. Results and Discussions

3.1. Surface activation and graft polymerization

Plasma is considered a fourth state of matter and is distinguishable from the liquid, solid, and gaseous states. One of the attractive features of gas discharge plasma is the ability to

achieve enhanced gas phase chemistry at room temperature [18, 19]. Plasma treatment in the air can create short-live free radicals on material surfaces to form relatively stable hydroperoxides [20] as illustrated in Figure 1A. Contributions of discharge power, plasma treatment duration, and air exposure time to surface hydroperoxide generation were studied on PET films and silicon wafers. Although plasma discharge powers positively affected surface activation, significant amount of hydroperoxides could be generated at as low as 60 watts. Increasing discharge powers from 60 to 100 watts had limited effects on hydroperoxide generation (supplementary content). When discharge power was fixed, the optimal plasma treatment time was three minutes. Extended or shortened plasma exposure time led to low hydroperoxides. Interestingly, there was a big variation in optimal air exposure time for materials with distinct chemical properties. When plasma treatment time and discharge power were fixed (3 minutes and 80 watts, respectively), the exposure time to achieve maximal hydroperoxide production on silicon wafers and PET films was 75 minutes and 30 minutes, respectively.

Graft polymerization on activated material surfaces was conducted at 70 °C in the presence of the monomer acrylic acid (Figure 1A). Monomer concentrations and reaction time positively affected surface polymerization (supplementary content), but the effect from extended reaction time was limited and there was no benefit to keeping reaction time beyond 6 hours. Because high monomer concentrations increased both the grafting density and polymer chain length while extended reaction time mainly affected polymer chain length, surface modifications could be easily manipulated by controlling surface activation (grafting density), monomer concentration (grafting density and polymer chain length), and reaction time (polymer chain length) to generate polymer grafted surfaces with desired properties for both peptide immobilization and controlled release.

3.2. Assembly of lytic peptide immobilized surfaces

Immobilization of peptides happened automatically (Figure 1B) through the strong electrostatic interaction between negatively charged polymer chains and the positively charged lytic peptides. Four well studied lytic peptides with varied sequence, positive charges, and secondary structures but proved activity to antibiotic resistant mutants (Table 1) and biofilms [13, 14] were selected. Adsorption of peptides on to PAA grafted surfaces was rapid process and completed after 30 minutes of incubation (Figure 3A). Although slight difference was observed for two peptides with different sequences and charges, peptide adsorption onto PAA grafted surfaces was concentration dependent and followed the same S-shape curve with induction and saturated phases at low and high peptide concentration, respectively (Figure 3B).

Further studies revealed that peptide immobilization capacity was determined by surfaces grafted PAA, and there was good correlation between surface immobilized peptide and PAA content (Figure 4A). However, when the surfaces of PET sample was fixed, variations in peptide immobilization were observed at high (>120 μ M) peptide concentrations. Studies using fluorescent labeled peptides revealed that high concentration peptide led to unevenly coated surfaces (Figure 4B). Only big sizes of peptide aggregates were found when peptide concentrations reached 150 μ M and beyond. In previous studies, we reported that lytic

peptides tended to self-assemble in aqueous solutions to form peptide aggregates with defined super-molecular structures [21, 22]. Because peptide aggregates could hardly dissolve and have dramatically decreased activity [15], peptide solutions of moderate concentrations ($< 100 \mu\text{M}$) were Immobilized Peptide (used in immobilizations).

Each step of the graft polymerization and peptide immobilization was studied using ATR-FTIR and AFM. A FTIR peak of C-H bonds at 2850 cm^{-1} was found on PAA grafted silicon wafers (Figure 5A). The intensity of this 2850 cm^{-1} peak increased dramatically as antibacterial surfaces were assembled and peptides were successfully immobilized on silicon wafers. Meanwhile, a new broad band at 3700 cm^{-1} due to the N-H stretching of peptides was found. In addition, each modification was also associated with dramatic surface morphology changes. Surfaces grafted with PAA had greatly increased thickness and roughness (Figure 5B). Although surface thickness was further increased when peptides were loaded, peptide immobilization resulted in material samples with smooth surfaces.

3.3. Antibacterial and anti-biofilm activity of peptide immobilized surfaces

The effectiveness of peptide immobilized surfaces in preventing biofilm formation were tested *in vitro* by loading bacteria onto sample surfaces and incubating samples at 37°C in TSBG medium for overnight. Bacterial adhesion and biofilm formation on sample surfaces were stained using LIVE/DEAD kit and examined under confocal microscopy [13, 18]. As shown in Figure 6A, at the end of overnight incubation, *S. aureus* grew into mature biofilms with well developed architectures ($\sim 15 \mu\text{m}$ in thickness) on control (unmodified) PET films. However, no biofilms were formed on PTP-7 or PTP-7s immobilized PET films. Only single bacteria were found but all of them were dead (stained red by LIVE/DEAD kit). The activity of antibacterial surfaces was positively correlated with the amounts of surface immobilized peptides (Figure 6B). Interestingly, despite the excellent antibacterial ability of peptide PTP-7a and PTP-7b (Table 1), surfaces from these two peptides were less potent than those from PTP-7 and PTP-7s. Because close amounts of peptides were loaded ($5.5 \mu\text{g}/\text{cm}^2$ for PTP-7b and $6.5 \mu\text{g}/\text{cm}^2$ for PTP-7 as shown in Figure 3B), reasons other than peptide loading efficiency might be responsible for the low anti-biofilm activity of PTP-7a or PTP-7b immobilized surfaces.

3.4. Peptide release

Antibiotic impregnations work due to a high local concentration of antibiotics/antibacterial agents that are provided and bacteria are killed before they grow into biofilms. Therefore, sustained antibiotic release is set for all antibiotic impregnations. However, the lytic peptide immobilized surface proved to be stable under physiological conditions and in various culture media such as MEM and TSBG with distinct chemical compositions. Peptide leakage was detected in high concentration ($>500 \text{ mM}$) of salt solutions, but less than 10% of surface adsorbed PTP-7 peptides were released in the solution containing 1.85 M NaCl (Figure 7A). Bio-macromolecules secreted by bacteria such as DNA and polysaccharides had no effects on the stability of lytic peptide immobilized surfaces at reasonably high concentrations (1.0 % for DNA and 2.0% alginate). This was an expected result due to the extremely strong electrostatic interactions between positively charged lytic peptides and the negatively charged PAA chains. However, the high stability did not match the excellent anti-

biofilm activity of peptide immobilized surfaces, indicating that other mechanisms rather than sustained peptide release were responsible for the anti-biofilm activity of lytic peptides immobilized surfaces.

We know that bacteria metabolism also produces acidic substances, leading to a local pH drop at bacteria attached surfaces [23]. An obvious example is the formation of dental cavities as a result of the local acidification caused by bacteria adhered to teeth [24]. Therefore, the local pH drop affiliated with bacteria adhesion and colonization might have a dramatic effect on peptide interactions with polymer grafted surfaces. We tested peptide release from PTP-7 immobilized PET films in PBS with various pHs. As shown in Figure 7B, although PTP-7 immobilized surfaces were stable in solutions with a wide range of pHs (pH=6–10), peptide leakage occurred at pH=5.5 and all surface loaded PTP-7 were released when PET films were kept in pH=4.5 PBS for 60 minutes. It was interesting to notice that surfaces from PTP-7b were less sensitive to pHs than these from PTP-7. Under the same experimental condition at pH=4.5, only 20% of surface loaded PTP-7b was released. The pH-triggered peptide release profiles matched well with the anti-biofilm activities of PTP-7 and PTP-7b immobilized surfaces (Figure 6B), suggesting the role of bacteria adhesion caused local pH drop in the activation of lytic peptide impregnations. The bacteria responsive mechanism of lytic peptides immobilized surfaces was proposed and illustrated in Figure 8. We know that the development of bacterial resistance to antibiotics is caused by rapid evolution of the bacterial genome under selective antibiotic pressure. Unlike the sustained antibiotic release and high antibiotic loading in antibiotic impregnations, the “on-demand” release feature together with dramatically reduced peptide loading determine decreased selective antibiotic pressure and may lead to reduced resistance development to peptide immobilized surfaces.

3.5. Biological Stability and Biocompatibility

Biological instability is the problem faced by many therapeutic peptides. The abundance of lysine and arginine residues in the sequence of these peptides causes these lytic peptides to be sensitive to proteases in human plasma [15, 25, 26], resulting in short life-spans in circulation (Figure 9A). However, surface immobilization limited the access of proteases to lytic peptides. Although more than 80% of free PTP-7 in human plasma was degraded after 4 hours of incubation, most of the immobilized PTP-7 peptide remained intact under the same experimental conditions. Indeed, we had compared the anti-biofilm activity of lytic peptide immobilized surfaces from all L- and D-form amino acids. Despite the excellent biological stability of PTP-7s, surfaces from peptide PTP-7 were as potent as these from its D-form counterpart, PTP-7s (Figure 6B). The excellent physical (Figure 7A) and biological (Figure 9A) stability explained the same activity of anti-biofilm surfaces from L- and D-form lytic peptides.

Stability of the peptide surface in the presence of high salt and biomacromolecules is very important. Proteins, polysaccharides, and DNA did not lead to release of peptide (Figure 7A). Biomacromolecules in the environment may foul the peptide coated surface and affect peptide release or block the “sensing” of bacteria by buffering the local acidification. However, that was not the case. As a result of the grafted polyacrylic acid polymer brushes,

lytic peptide immobilized surfaces showed very low affinity to various biomacromolecules and maintained excellent antibacterial activity in the presence of or after exposure to the high concentrations of protein and DNA (Figure 9B).

Good stability (Figure 7A) together with bacteria responsive nature (Figure 8) also determined the biocompatibility of lytic peptides immobilized surfaces. Since there was no lytic peptide release in the absence of bacteria, tissue cells (Human fetal osteoblast HFOB1.19, human lung cell A549, and mouse osteoblast precursor cell MC-3T3) grew normally and healthy on lytic peptides immobilized surfaces (Figure 10). the surfaces (%)

3.6. Prevention of bio-fouling in catheters

A major advantage of antibacterial peptides over small molecular antibiotics is their low chance to develop resistance [28]. However, lytic peptides are unique because of their excellent activity to antibiotic resistant bacteria (Table 1) and biofilms [13, 14]. The bacteria responsive nature makes lytic peptide impregnation a perfect fit for various bio-fouling and indwelling device infections because it may solve all problems faced by current antibiotic impregnations, such as short life-span, fastening antibiotic resistance development, and inactivity to antibiotic resistant mutants and biofilms. In order to evaluate the effectiveness and efficiency of lytic peptide impregnations in preventing indwelling device infections, we prepared anti-biofilm catheters by immobilizing lytic peptides on the inner surfaces of silicone tubes. Bio-fouling studies were conducted on a microfluidic cultivation system (Figure 2), which mimicked the actual environment of catheters *in vivo*. An antibiotic resistant *S. aureus* (ATCC 29523) was used in order to test the capability of lytic peptides impregnations in killing antibiotic resistant bacteria.

As shown in Figure 11A, substantial amounts of bacteria and biofilms were detected on the inner surfaces of unmodified (control) catheters after 24 hours. From then, bacteria inside catheters grew rapidly and biofilms filled the entire inner space of unmodified catheters in less than 3 days of continuous cultivation. On the contrary, anti-biofilm catheters from lytic peptides demonstrated excellent anti-fouling ability. No biofilms were found in anti-biofilm catheters after 7 days of continuous cultivation. Bacteria responsive nature (Figure 8) determined the long life-span of lytic peptide impregnations. The excellent ability of anti-biofilm catheters was proved by live bacteria analysis (Figure 11B). No live bacteria were detected on the inner surfaces of anti-biofilm catheters during the entire period of cultivation. Total live bacteria in control (unmodified) catheters reached plateau after 3 day cultivation as the catheters were fully blocked by biofilms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

A) Chemistry of plasma-mediated surface graft polymerization; B) A schematic illustration of lytic peptide immobilization on material surfaces.

Figure 2.

A) Micro-fluidic biofilm cultivation system; B) Silicone tubes, 1.0 mm in diameter, mounted on multi-channel base; C) Images of *S. aureus* biofilms formed inside of silicone tubes after 24 hr cultivation. Biofilms (white color clusters) of various sizes are indicated by arrows.

Figure 3.

A) Binding kinetics of fluorescent labeled peptide PTP-7 and PTP-7b (70 μM in PBS solution) to PAA grafted PET films (2.5 $\times$ 2.5cm). Concentration changes of FITC-labeled peptides in solutions were monitored using a fluorescence spectrometer by setting excitation and emission wavelengths at 485 nm and 535 nm, respectively; B) Peptide concentrations affected peptide adsorption on PAA grafted PET films. Peptides were incubated with PAA grafted PET films for 30 minutes.

Figure 4.

A) A correlation between surfaces grafted PAA and immobilized peptide PTP-7; B) Confocal microscopy images of peptide concentrations affected PTP-7 (FITC-labeled) immobilization on silicon wafers. Concentrations of peptide solutions used in the immobilization were indicated in images.

Figure 5.

A) ATR-FTIR spectra of control, PAA grafted, and peptide PTP-7 immobilized silicon wafers; B) AFM images of control, PAA grafted, and peptide PTP-7 immobilized silicon wafers. Scan distance=5.00 μm ;

Figure 6.

A) Confocal microscopy images of live (green) and dead (red) bacteria on control (left) and PTP-7 immobilized (right) PET films. Penicillin resistant *S. aureus* (ATCC 29213) were seeded on PET films and incubated overnight before they were stained using Live/Dead staining kit; B) Peptide concentration affected anti-biofilm activity of lytic peptides immobilized surfaces. Live bacteria on PET films at the end of overnight cultivation were quantified using MTT assay.

Figure 7.

Release of immobilized PTP-7 from PET films. Samples were incubated at room temperature for 60 minutes. Studies were performed: A) in pH=7.4 PBS supplied with additionally added DNA (1 %), alginate (2 %), and NaCl (0~5.0 M); B) in PBS with varied pHs.

Figure 8.

Illustration of the proposed acting mechanism of bacteria responsive antibacterial surfaces: 1) peptides immobilized surfaces are stable under physiological conditions and thus there is no lytic peptide release; 2) bacteria adhesion caused local pH drop triggers peptide leakage at the contamination sites. Because bacteria have direct contact material surfaces, all released peptides are captured by attached bacterial cell membranes; 3) Adhered bacteria are selectively killed when the uncontaminated/unpopulated surfaces of peptide impregnations are left unaffected.

Figure 9.

A) Biological stability comparison between free and immobilized PTP-7 peptide surfaces. Free PTP-7 peptide (final concentration = 10 μ M) and PTP-7 immobilized samples were incubated with human plasma at 37 °C for 4 hours. After treatment with TFA, intact peptides in supernatants were quantified using mass spectrometry to estimate peptide degradation; B) Antibacterial activity of PTP-7 immobilized surfaces after exposure to high (1% w/v) concentration DNA (left) and BSA (middle). Only few and live bacteria were found in comparison with the Control surface (right).

Figure 10.

The biocompatibility of PTP-7 immobilized surfaces. MC-3T3 cells were seeded on unmodified (left) and PTP-7 immobilized PET films (right) and incubated at 37 °C for 48 hrs. Live and dead cells were then stained green and red under confocal microscopy using Live/Dead kit. The percentage of green pixels out of total green and red pixels in captured cell images was calculated to estimate live/dead cell ratios.

Figure 11. Catheter bio-fouling studies conducted using a penicillin resistant *S. aureus* (ATCC 29523). A) Images of biofilm growth inside catheters; B) Total live bacteria found on the inner surfaces of catheters as measured using modified MTT assay.

Table 1

Properties and antibacterial activities of lytic peptides

Peptide	Sequence	Net charge at pH=7.4	Secondary structure	P1	<u>S. epidermidis</u> (146)	MIC (μM) (25923)	<u>S. aureus</u> (29213)	(44300)
PTP-7	FLGALFKALSKLL	2	Random coil	10.6	4	4	4	4
PTP-7a	FLGALFHALSKLL	1	α-helix	10	8	4	4	4
PTP-7b	FLGALFKALSHLL	1	α-helix	10	8	4	8	4
PTP-7s	d{FL}Gq{ALFKALSKLL}	2	Random coil	10.6	4	4	4	4

25923, penicillin and methicillin sensitive (MSSA); 29213, penicillin resistant but methicillin sensitive (MSSA); 44300, penicillin and methicillin resistant (MRSA)