[Frontiers in Bioscience, Scholar, 8, 129-142, January 1, 2016]

Chemical immobilization of antimicrobial peptides on biomaterial surfaces

Rafael R. Silva[1], Karen Y.P.S. Avelino[2], Kalline L. Ribeiro[1], Octavio L. Franco[3], Maria D.L. Oliveira[2], Cesar A.S. Andrade[1,2]

[1]Programa de Pos-Graduacao em Inovacao Terapeutica, Universidade Federal de Pernambuco, 50670-901 Recife, PE, Brasil, [2]Departamento de Bioquímica, Universidade Federal de Pernambuco, 50670-901, Recife, PE, Brasil, [3]Centro de Analise Proteomicas e Bioquimicas de Brasilia, Universidade Catolica de Brasília, 71966-700, Brasilia, DF, Brasil

TABLE OF CONTENTS

1. ABSTRACT

Hospital infections associated with surgical procedures and implants still present a severe problem in modern societies. Therefore, new strategies to combat bacterial infections mainly caused by microorganisms resistant to conventional antibiotics are necessary. In this context, antimicrobial peptides have gained prominence due to their biocompatibility, low toxicity and effectiveness. The immobilization of antimicrobial peptides (AMPs) onto biomaterial surfaces is an excellent alternative for the development of new biodevices with microbicidal properties. Herein, we describe reports related to physical-chemical characterization, *in vitro*/*in vivo* studies and the clinical applicability of such active surfaces. In this review, we focused on the mechanisms of action, different peptide immobilization strategies on solid surfaces and the microbicidal effectiveness of AMPs.

2. INTRODUCTION

Bacterial resistance is still one of the major problems facing public health in modern societies (1). This can occur due to the indiscriminate use of antibiotics, which induces the formation of new species that are resistant to conventional antibiotics (2). A major problem facing modern societies is the health risks stemming from the transmission of new infections that are difficult to treat. In addition, the transmission routes (e.g. airborne or direct contact) can lead to epidemic episodes (3). Thus, many studies have aimed to minimize microbial contamination by developing new drugs. Although there are thousands of new compounds synthesized per year, the difficult process of validation and clinical phase studies decrease the possibility of new drug manufacturing. In this context, the discovery of antimicrobial peptides (AMPs) has shown promise as a way to eradicate some resistant bacterial strains (4).

AMPs are small amino acid sequences obtained by their extraction from many types of organisms (plants, insects and animals) and play an important role in inhibiting the growth of multiple microorganisms (5, 6). Currently, the database that contains detailed information on these peptides (antimicrobial peptides database, APD) contains 2495 types with different functions: antibacterial, antifungal, antiparasitic, anti-cancer, antioxidant, etc. (7). Chemically modified AMPs are obtained from the modifications of natural ones derived from magainin and histidine (8, 9) or through new synthetic forms (e.g. E14LKK, RK1, RK2), aiming to improve microbicidal effectiveness (10, 11).

Some hospital infections come from the adherence of microbes, especially bacterial species on the surfaces of medical devices and implants (e.g. dental or orthopedic) or during surgical procedures due to the lack of adequate hygienization (12, 13). The massive bacterial colonization on solid surfaces could contribute to biofilm formation (14). This process involves the transport of bacterial cells and further attachment commonly mediated by van der Waals and electrostatic charges. The adhesion of proteins and extracellular polymeric substances (EPS) can reinforce bacterial adhesion (15). Once implanted, these microorganisms can maturate and differentiate, forming microcolonies and propagating

infections with detached cells (16). As a result, infections associated with bacteria are difficult to treat, and the removal or replacement of infected medical implants or devices reflects considerable costs for the healthcare system, which leads to patient suffering, prolonged hospitalization and eventually death (17).

In this scenario, the development of new biomaterials to prevent adhesion and microbial infection in hospital equipment and implants are of increased interest. The use of AMPs in adsorption processes, functionalization and immobilization of organic coatings onto substrate surfaces (titanium or silicone) has demonstrated good activity with clinical potential, summarized in Table 1.

Therefore, this review addresses three important aspects for the use of AMPs in antimicrobial coatings: basic concepts and mechanisms of action of peptides against microorganisms (with the emphasis on bacteria), chemical immobilization strategies for the inclusion of peptides associated to solid substrates and the effectiveness of these models in antimicrobial testing.

2.1. Antimicrobial peptides

AMPs are important components of the innate immune systems of living organisms and contribute effectively against exogenous pathogens (18). After a microbial infection, most of these peptides act to neutralize a wide range of microorganisms. In addition, AMPs are efficient at low concentrations, are less likely to promote bacterial resistance and have antitumor properties. Therefore, AMPs are promising candidates for their use as novel therapeutic drugs (4). They comprise a chemically and structurally heterogeneous family yet share molecular masses lower than 5 kDa, aside from having cationic and amphipathic properties (19, 20).

Due to the enormous variety of amino acid sequences and structural features, the exact action mechanism of AMPs is still a controversial issue. However, there is a consensus about the mechanism of positively charged peptides. The cationic charges on the peptide surface favor the electrostatic interaction of negatively charged microbial membranes through bivalent cation exchange (20). In addition, AMPs assume an amphipathic structure after interacting with the bacterial membrane, resulting in a lethal permeabilization (20, 21).

The antimicrobial activity of AMPs is based on four model mechanisms named barrel, carpet, toroidal and detergent. In the barrel model, the hydrophobic part of AMPs interacts with the lipid hydrocarbon chains of membranes, and their hydrophilic part exposes the lumen as a result of transmembrane aqueous channel formation. The carpet model occurs due to the saturation of the bacterial membrane by AMP molecules, resulting in its permeabilization (21, 22). In the toroidal model, the peptides are interspersed with phospholipids, and the polar groups of both molecules interact with each other, resulting in pore formation in the lipid membrane, where peptides are assumed to adopt a transmembrane orientation (23). Finally, the detergent-like model can occur as a consequence of the carpet model. Once attached to membrane surface by electrostatic interactions, AMPs interleave the lipid bilayer until reaching a saturation point with subsequent micelle formation and bacterial membrane destruction (24).

The systemic use of AMPs is restricted, mainly due to their toxicity when used at high concentrations, as well as by its relatively short half-life and susceptibility to proteases (10). The interaction of peptides with bacteria is initially driven by weak attraction forces (van der Waals and electrostatic interactions), which are further enhanced by specific interactions involving peptides and biofilm formation (25). Therefore, the most feasible way to use peptides is through their immobilization onto solid surfaces with the aim of developing new antimicrobial structures. In general, the use of intermediary linkers between solid surfaces and AMPs is required to obtain better microbicidal activity (25).

Bacterial infections associated with implanted devices still present a significant threat to patients and are a serious challenge for physicians. High rates of infection are observed for orthopedic implants, dental devices, vascular grafts, urinary and venous catheters, which results in low performance of these devices in terms of safety and longevity (26, 27). Central venous catheters, commonly used in clinical cases (e.g. chemotherapy, prolonged parenteral nutrition and hemodialysis), contain silicone or polyurethane in their constitution. In addition, catheters are useful for peptide immobilization and, therefore, acquire the potential to be used against biofilms involved in hospital infections post-surgery (28). Furthermore, limitations of biomolecular immobilization could spur the development of new antimicrobial surfaces capable of preventing initial bacterial colonizations or at least reduce active bacterial titles. Thus, modified surfaces could directly affect patient health and reduce costs in public health, making the use of AMPs an excellent alternative to remedy these issues.

3. PHYSICAL METHODS FOR IMMOBILIZATION OF AMPS

Recently, several studies have demonstrated AMP coupling with different substrates and their effectiveness against biofilm formation (29-33). AMPs have unique bioactive properties capable of overcoming limitations of other antibacterial coatings, such as the risk of developing bacterial resistance, short-term antimicrobial protection, limited antimicrobial spectrum and high cytotoxicity (34-37). Therefore, AMP immobilization onto diverse medical devices, including implants, urinary

Table 1. AMP covalent immobilization on solid surfaces and their antimicrobial activity against bacteria, fungi and yeasts

Substratum	AMP	Chemistry Immobilization Strategy	Evaluated microorganisms	References
Polyamide resin (pepsynK)	Novel synthetic peptides and Magainin 2	Grafting of C-terminus to the polymer support during solid-phase peptide synthesis	*E. coli, K. pneumoniae, S. aureus, B. subtilis, C. albicans*	10
PEG[1]-Polystyrene (PEG-PS) resin beads	6K8L	Peptide synthesized by solid-phase peptide synthesis on a PEG-PS resin using Fmoc[2] chemistry	*B. subtilis, E. coli. Kluyveromyces marxianus, L.monocytogenes, P. fluorescens, S. typhimurium, Serratia liquefaciens, S. aureus*	53
PEGylated resin beads (TentaGel S NH2, HypoGel 400 NH2 and HypoGel 200 NH2)	KLAL and Magainin-derived peptide (MK5E)	C-terminal immobilization by standard solid-phase peptide synthesis and Fmoc chemistry; N-terminal and side-chain immobilization by thioalkylation and oxime formation	*E. coli, B. subtilis*	8
PEGylated resin beads (TentaGel S NH2 resin beads)	Melittin, Buforin 2 and Tritrpticin	Thetered modified beads by oxime-forming ligation strategy	*E. coli, B. subtilis*	90
PEG-Polystyrene (PEG-PS) resin beads	amphipathic β-sheet peptides	Covalent binding by Fmoc chemistry on PEG-PS beads	*S. aureus, Micrococcus luteus, P. aeruginosa, E. coli.*	51
Glass coverslips	Melimine	Grafting via ABA[3] and FNA[4] linkers	*P. aeruginosa, S. aureus*	72
Indium-tin-oxide glass	Polymixin B	Silane containing epoxy rings to couple peptides by catalyst	*E. coli. NCTC 8007*	94
Polydimethylsiloxane (PDMS)	CW11	Cross-Linking of peptides to allylglycidil ether modified PDMS surface (PDMS-AGE[5]-PEG) via Sulfhydryl Chemistry	*E. coli, S. aureus, P. aeruginosa*	100
Silicone Urinary Catheter and Polydimethylsiloxane (PDMS)	RK1 and RK2	Cross-linking of peptides to allylglycidil ether modified PDMS surface (PDMS-AGE-PEG) via Sulfhydryl Chemistry	*E. coli, S. aureus, C. albicans*	11
Pretreated Ti with amino silane and epoxy silane	LL-37	Site-specific conjugation through amine reactive NHS-group and the Thiol-reactive maleimide-moiety	*E. coli*	105
Pretreated Tisilanization with CPTES or APTES	hLF[6]-11	Peptide physical adsorption and covalent binding with CPTES[7] or APTES[8]	*S. sanguinis, L. salivarius*	31
-	Nisin, Trp-11, 4K-C16	Covalent immobilization via reaction between amine groups on the peptides and surface epoxy groups on the plasma polymer interlayer	*E. coli, B. subtilis*	85
-	Nisin, Magainin I.	Covalent immobilization through grafting of chitosan, cross-linking agents and peptides	*L. ivanovii*	111

[1]PEG: Polyethylene glycol, [2]Fmoc: 9-fluorenylmethyloxycarbonyl, [3]ABA: 4-azidobenzoic acid, [4]FNA: 4-fluoro-3-nitrophenyl azide, [5]AGE: allylglycidyl ether, [6]hLf: human lactoferrin, [7]CPTES: chloropropyltriethoxysilane, [8]APTES: 3-aminopropyltriethoxysilane

and intravenous catheters, has an undeniable potential for clinical use (38-41). However, new immobilization strategies are required to ensure the applicability of AMPs on solid surfaces, improving the effectiveness and functionality of modified biodevices (35, 42).

AMPs can be immobilized onto solid surfaces through physical methods such as adsorption, self-assembled monolayers (SAM) or through chemical methods via selective or non-selective covalent bonding (35, 42, 43). Physical methods are based on hydrogen bonds, permanent or induced dipole interactions (van der Waals' force or London dispersion force), and hydrophobic or ionic interactions between AMPs and surfaces (44-47). Some substrates have been used for AMP anchoring such as gold (32), titanium (31, 34, 40, 48), titanium dioxide (49), silicon (50), silicone (11, 41), polymeric brushes and resins (8, 51-54). Of note, immobilization onto substrate surfaces can be performed without any shape restriction, such as

planar, spherical or curved geometries (55). However, specific surface properties (nature, composition, charge, hydrophilic or hydrophobic character, topography and roughness) and AMP characteristics (type, charge, molecular size and conformational stability) interfere in the immobilization process (46). In addition, experimental conditions such as time, peptide concentration, pH and temperature should be also considered (46, 53).

The self-assembly technique is one of the main strategies used for physical AMP immobilization (56, 57). This approach is based on the alternating deposition of anionic and cationic layers on a solid substrate (58-60), enabling the obtainment of functionalized films through the insertion of AMPs between polyelectrolytes layers (55) and the control of film thickness and adsorbed biomolecule amounts (56). Moreover, self-assembly is an effective procedure since it does not cause chemical changes in the functional peptide and maintains its conformational stability by retaining water molecules between the matrices (57, 61). One of the main features that make SAM films promising for biotechnological applications is the possibility of temporal control over incorporated AMP release in hydrolytically degradable polymers through surface erosion (57, 62-64).

Several studies have demonstrated good prospects for AMP application in polymeric films. However, some disadvantages could be associated with the self-assembly technique, limiting its application for obtaining implants coated by biomaterials and medical devices (55). An important disadvantage comprises AMP incorporation into the lower layers of the film, which restricts their direct contact with the surrounding bulk (31). AMP bioactivity is dependent on the diffusion process at the interface, being influenced by the tortuosity of the diffusion (64) via film thickness (65) and intermolecular interactions between the polymer and peptide (66). A relatively fast peptide release from the polymeric films results in a decreased amount of anchored AMPs, interfering in the minimum AMP concentration required to inhibit the growth of microorganisms and increase the number of biomolecules in the bulk (55). In addition, a fast release can provide conditions for the development of bacterial resistance, local toxicity and hemolytic activity (45,55). Therefore, the limitations of the self-assembly technique should be considered prior to its use as a strategy for obtaining more effective antimicrobial coatings.

The achievement of antimicrobial coatings with adequate properties results from the improvement of immobilization techniques (16, 39). The development of appropriate methodologies for coating surfaces ensures biological peptide properties including mechanism of action, wide spectrum bioactivity, stability in adverse conditions and low propensity for the development of bacterial resistance (3, 40-42). However, this requires some factors that influence the performance of these biomolecules, such as peptide orientation, surface concentration of bounded AMPs, and spacer length and flexibility, which determine the lateral mobility of AMPs (5, 7). Under these conditions, the use of chemical methods for the immobilization of AMPs onto solid surfaces has increased (27).

4. CHEMICAL APPROACHES FOR COVALENT IMMOBILIZATION OF AMPS ONTO SURFACES

The chemical immobilization of AMPs is an alternative strategy for coating surfaces and improving peptide stability. Consequently, the duration of the antimicrobial efficacy is increased and is further associated with a reduction in the toxicological risks for patients by reducing the leaching of peptides (8, 37, 65, 66). Besides these advantages, adequate AMP orientation on the substrate can provide greater bioactivity (20, 67). For these reasons, covalent bonds have been extensively studied as a tool to overcome the limitations of the physical anchoring methods (20, 54, 68). However, it is important to emphasize that chemical immobilization can alter the conformational structure of the molecule, restrict its mobility and interfere in the mechanism of action (46). Therefore, molecular coupling mechanisms should be thoroughly evaluated for the maintenance of bioactive AMP properties (52).

Chemical immobilization involves the formation of at least one covalent bond between the surface and the responsible biomolecule to provide stability to antimicrobial film (20). Stability is obtained through the strength of covalent bonds, which prevents spontaneous peptide uncoupling (8,10,69). Covalent bonds are classified as selective or non-selective (70). A selective bond between AMPs and substrates can be obtained through the insertion of a specific functional group in the molecular structure of the peptide via chemical synthesis (35). In addition, is possible control the route of reaction and orientation of the biomolecule (8, 48, 71). On the other hand, non-selective immobilization occurs naturally without requiring additional chemical modifications in the peptides. In this case, covalent binding uses the intrinsic functional groups (e.g. carboxylic acid, amino, sulfhydryl and hydroxyl groups) of the peptide sequence to react chemically with activated surfaces. Non-selective immobilization can result in more than one type of covalent bonding with different orientations of the biomolecule (42, 72). However, for non-reactive surfaces, the functional groups should be inserted through spacers to obtain a covalent bond between AMPs and surfaces (73). Substrate modification can be performed using SAM composed by chemically-reactive organic molecules as a simple and effective strategy (35, 74-76). The spacer length can be varied from one to several carbon atoms with a direct influence on AMP bioactivity (35).

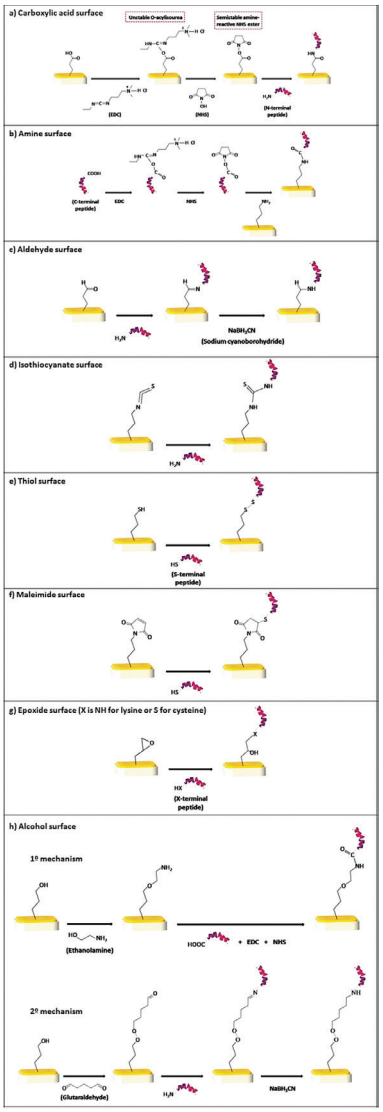

Figure 1. Examples of chemical strategies for controlled covalent attachment of AMPs on surfaces functionalized with different reactive groups. a) Surfaces functionalized with carboxylic acid groups can be used to covalently bind AMPs via coupling agents 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) that activate the chemical groups on the surface. b) AMPs can be attached to amino-functionalized surfaces through activation of their carboxylic groups with EDC and NHS before incubating on the surface. c) Aldehyde groups present on functionalized surfaces can chemically react with amine groups of AMPs and establish stable covalent bonds through reducing agents, such as sodium cyanoborohydride ($NaBH_3CN$). AMPs can be immobilized on solid supports functionalized with d) isothiocyanate, e) thiol, f) maleimide and g) epoxide groups. Except for anchoring of AMPs on isothiocyanate modified-surfaces, the use of thiol-bearing peptides is verified for covalent immobilization of AMPs on surfaces functionalized with thiol, maleimide and epoxide groups. In all cases, the chemical coupling occurs in a single step and without the need for additional reagents. h) Surfaces functionalized with alcohol can anchor AMPs via two reaction mechanisms. In the first mechanism, hydroxyl groups of spacers are derivatized with an amino alcohol, such as ethanolamine, and the carboxylic acid groups of AMPs are activated with EDC and NHS for the attachment of the peptides on the surface. In the second mechanism, hydroxyl groups of spacers are derivatized with an aldehyde (e.g. glutaraldehyde), subsequently, the immobilization of AMPs proceeds similarly to the anchoring of peptides on aldehyde-modified surfaces.

Another strategy to covalently immobilize AMPs involves the use of functionalized polymer resins such as polyethylene glycol (PEG) or other 'brushes' that bear reactive groups suitable for coupling peptides (8, 10, 51, 53). PEG is one of the main spacers used in the activation of surfaces by presenting the anti-adhesive property that prevents or minimizes bacterial colonization (77-79). In addition, PEG is an amphiphilic and flexible polymer that allows for a greater lateral mobility of the AMPs and retention of water molecules in its interior. PEG is capable of maintaining the bactericidal activity of peptides after immobilization onto solid supports (25). However, a disadvantage of the use of polymers as spacers consists in the possibility of polymer chain degradation and premature AMP release (53).

A wide variety of chemical coupling methods for the immobilization of AMPs onto surfaces are shown in Figure 1. Carboxylic acid-functionalized surfaces can react with primary amines, leading to the formation of peptide bonds (amide bonds) (73). However, the reactive chemical groups on the surface should be initially activated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) (Figure 1a) (35, 73). On the other hand, the AMP immobilization onto amino-functionalized surfaces is similarly obtained, as explained previously, for carboxylic acid-functionalized surfaces. In this case, the accessible carboxylic groups of the peptide should be activated with the EDC:NHS coupling method before being added to the functionalized surface (Figure 1b) (35). The carboxyl-amine conjugation reactions based on the use of EDC and NHS occur in two sequential steps. EDC first reacts with a carboxyl group, forming an amine-reactive O-acylisourea intermediate. This unstable intermediate is susceptible to hydrolysis and stabilized through the addition of NHS by converting it to a semistable amine-reactive NHS ester (Figure 1a). After the addition of NHS, it is possible to obtain a stable amide bond with a 10-20 fold increase in coupling efficiency. Carbodiimide cross linker chemistry is widely used for the immobilization of peptides on modified surfaces with carboxyl (Figure 1a) or amino groups (Figure 1b) (80-82). Another strategy for biomolecule immobilization is based on aldehyde groups displayed on functionalized surfaces. This approach can be used to covalently bind AMPs from primary amines, resulting in the formation of imine bonds. However, since the imine bonds are unstable, they should be converted to amine bonds through reducing agents, as sodium cyanoborohydride ($NaBH_3CN$), for the stabilization of anchored peptides (Figure 1c) (71, 83).

The surface coupling strategy also uses isothiocyanate for the attachment of peptides via primary amine groups (Figure 1d) (35). Disulfide bonds can also be used to immobilize many peptides and proteins. Surfaces modified with thiol groups can covalently immobilize AMPs through disulfide bonds established

between the surface and cysteine residues of the peptide (Figure 1e) (70,84). AMPs can be attached to maleimide-functionalized surfaces through covalent bonds established between the thiol group of the peptide and the α,β-unsaturated carboxyl of the maleimide (Figure 1f) (25,52). AMPs containing thiol or primary amino group derivatives (e.g. amino acids cysteine and lysine, respectively) can be easily immobilized onto epoxide-functionalized surfaces through a nucleophilic ring opening in a spontaneous reaction (Figure 1g) (83,85).

There are currently two reaction mechanisms for AMP immobilization onto alcohol-functionalized surfaces (Figure 1h). In the first mechanism, the spacers containing hydroxyl groups are derivatized with an amino alcohol such as ethanolamine. Subsequently, the peptides whose carboxylic acid groups were previously activated with the EDC: NHS solution are added to the modified surface, resulting in a covalent bond (35). In the second mechanism, the spacers presenting the alcohol function are derivatized with an aldehyde (e.g. glutaraldehyde). Therefore, the immobilization of AMPs on aldehyde-modified surfaces occurs similarly, as has been shown previously (35). As explained before, diverse methodologies for AMP immobilization onto solid surfaces are available. However, the advantages and disadvantages of each technique should be evaluated for obtaining effective antibacterial films(46). Therefore, the molecular coupling process should be rigorously controlled for the maintenance of the bioactive properties of the peptides and the functionality of the final product (42,43).

5. EFFECTIVENESS OF AMPS IMMOBILIZED ONTO A BIOMATERIAL SURFACE

Antimicrobial peptides have a well-defined mechanism of action described by interaction models that evaluate the association between the sequence of peptides and the bacterial cell wall (86-88). The search for improving the efficiency of AMP-bacteria interaction also leads to the development of artificial peptides and the discovery of novel, natural peptides extracted from various living organisms. In recent years, there has been increased interest in developing new antimicrobial biomaterials in pre/post-surgery processes to avoid nosocomial infections (25). The applications of immobilized AMPs are an excellent alternative for use against nosocomial infections caused by common pathogens and antibiotic-resistant microorganisms (8, 42). AMPs have been tested on diverse solid surfaces such as resins, glass, silicone, titanium and stainless steel for clinical use (43,89).

Resins are solid polymers obtained from plants or chemical synthesis. Some resins are chemically modified and useful as a substrate for AMP immobilization due to their high molecular weight and amide/amine moieties in its molecular chain (10,90) (Table 1). However, physicochemical characteristics of resins do not contribute to clinical applications. Of note, these materials are important to obtain a better understanding of the mechanisms of action of surface-active peptides (8,53).

Haynie *et al.* studied the bactericidal effects of magainin 2 and various synthetic peptides mainly containing lysine, leucine and glycine in their compositions, utilizing an ethylenediamine-modified polyamide resin (Pepsin K). Altogether, 70% of the tested peptides, which included magainin2, showed bactericidal activity against fungi and Gram-positive and Gram—negative strains. Among them, E14LKK showed the highest antibacterial activity, except for *P. aeruginosa* and *A. niger*. Other studies also used E14LKK immobilized onto polyethylene film containing PEG as the intermediate binder. The peptide remained active and reduced *E. coli* strains up to 3 log (54).

The covalent immobilization of AMPs at different binding sites and with different length spacers also influences biocidal and hemolytic activities. Two α-helical cationic AMPs, MK5E and KLAL, were immobilized on polystyrene resin beads (Table 1). MK5E is an AMP derived from magainin2 having only microbicidal activity while KLAL has both biocidal and hemolytic activities. Bagheri *et al.* used resin beads with different sizes (TentaGel S NH2, HypoGel 200, and 400 NH_2) with covalently-linked peptides via N and C terminal chains. They demonstrated that the antibacterial activity of cationic AMPs was influenced by spacer length. AMP biocidal activity is directly dependent on the spacer length, providing more flexibility and capacity to interact with microorganism surfaces (8).

Bioactive glasses also provide an advantage in forming an interface between implant and organism without inducing immune responses. This type of glass has potential applications in dentistry (91), ophthalmology (92) and orthopedics (93). However, there are still few studies on the immobilization of AMPs onto glass (72,94). These materials have been used for the immobilization of melamine, a cationic peptide modified by covalent bonding. In addition, ligands such as 4-azidobenzoic acid or 4-fluoro-3-nitrophenyl azide are used to modify glass substrate surfaces (72) (Table 1). Glass coated by indium-tin-oxide was also functionalized with silane coatings containing epoxy rings (3-glycidyloxypropyl-trimethoxysilane (94).

Silicone is a synthetic polymer obtained from fluid resin or elastomer forms. Silicone is one of the most cited materials for peptide immobilization and is extensively used in the medical and pharmaceutical fields due to its biocompatibility and wide range of physical forms. In addition, this polymer is applied

in manufacturing prostheses and medical devices (e.g., catheters and stents) (95,96). New approaches have been developed to prevent biofilm formation on pre/post-surgical procedures aiming to decrease mortality rates and costs to public health (97-99).

Some peptides have been immobilized on venous catheters composed of silicone for the development of bactericidal surfaces against bacterial strains (38). The understanding of the interactions between AMPs and bacteria has led to the development of novel synthetic peptides (e.g. CW11, RK1 and RK2) (11,100). CW11, a synthetic peptide, was chemically immobilized on a polydimethylsiloxane (PDMS) surface maintaining an *in vitro* bactericidal effect (Table 1). CW11 is mainly composed of tryptophan and arginine, which contribute to a better adhesion process on bacterial surfaces even under saline conditions. CW11 has low cytotoxicity and excellent antibiotic activity against *E. coli*, *P. aeruginosa* and *S. aureus* as compared to conventional antibiotics. Thus, the CW11 peptide is an alternative for modifying medical devices based on silicone as a substrate (100).

In addition, other peptides such as RK1 and RK2 (both derived from the human beta-defensin-28 variant) showed a broad antimicrobial spectrum, tolerance to saline conditions and use for the modification of silicone surfaces (101) (Table 1). These peptides are rich in arginine, lysine and tryptophan residues, showing antimicrobial activity against *E. coli, S. aureus* and *C. albicans* in phosphate buffered solutions and urine samples. Cultures of smooth muscle cells showed no signs of toxicity, demonstrating biocompatibility for use in urinary catheters. Therefore, the development of new peptides is an alternative for covering catheters and preventing urinary tract infections (11). In addition, new, natural and/or synthetic peptides have been used to modify metal surfaces such as titanium, and have been applied in clinical and preclinical research studies (9, 31, 40, 102).

Titanium (Ti) is another material used for implants in dental and orthopedic applications (12, 13). Likewise, the properties of biocompatibility, corrosion resistance and ability to bind to bone has induced new research studies (103). The use of AMPs and Ti is associated with the lack of toxicity and low immune response. The bactericidal effectiveness of LL-37 on Ti surfaces was evaluated (104, 105) (Table 1). LL-37 is a peptide derived from cathelicidin and extracted from epithelial cells and neutrophil granules. Zanetti *et al.* (104) performed the immobilization of LL37 by covalent bonding strategies using PEG as a spacer between solid surfaces and the peptide. In addition, LL-37 with cysteine (Cys-LL37) was used to assess binding to the spacer. The presence of cysteine ensured stable bonding with PEG and better mobility of LL-37, facilitating the interaction with the evaluated bacterial cell membrane. AMPs conjugated to spacers (copolymer "brushes") with a low density provide a high number of peptide/polymer chains, which results in higher antimicrobial activity (48).

Besides LL-37, another example of AMPs extracted from humans are human lactoferrins (hLF). In addition, hLFs are involved in the activation and differentiation processes and immune responses against microorganisms (106-108). The peptide hLF1-11 is effective against strains of *Streptococcus sanguinis* and *Lactobacillus salivarius*, preventing the formation of biofilms at early stages. Recently, the antimicrobial effect of the hLF1-11 attached to Ti surfaces was demonstrated to be effective for dental applications (31).

On the other hand, stainless steel (SS) is a metal present in several areas where hygiene is essential, including the home, the food industry and the medical field. The presence of SS in hospital environments highlights its importance for developing antimicrobial surfaces in order to avoid pre/postoperative infections (109, 110). In this context, the functionalization of organic polymers deposited by plasma (glow discharge plasma) is essential to immobilize covalent peptides. The process occurred by binding the peptide amine groups and epoxy groups from polymerized interlayers deposited via plasma on SS surfaces. These systems reduced the growth of *E. coli* and *B. subtilis* ranging from 3 to 6 $\log^{10}$ in accordance with the tested peptide (85). In addition, chitosan polymer layers (1,4 linked N-acetyl glucosamine and glucosamine) were used on SS surfaces to immobilize the AMPs nisin and Magainin I. This strategy was possible due to the insertion of terephthalaldehyde cross linker (111). New studies, even at early stages, are essential to contribute to the development and improvement of biomaterials for clinical use and biodevice implantation.

6. CONCLUSION

Since bacterial resistance is still one of the major problems facing modern societies, new strategic therapies to combat microorganisms resistant to conventional antibiotics are required. In this context, antimicrobial peptides are promising due to their biocompatibility, low toxicity and high effectiveness. Synthetic forms of peptides also have increased their microbicidal activity. Currently, chemical methods for immobilization of AMPs on surfaces are considered valuable tools for the construction of modified biodevices. Chemical immobilization provides improvements in peptide stability and ensures an adequate orientation of the biomolecule. Covalent bonding overcomes limitations of the physical anchoring methods, creating films with greater antimicrobial efficacy. Chemical approaches to covalent immobilization of AMPs on solid surfaces, such as orthopedic implants and surgical instruments, represent an advance in the prevention of nosocomial infections. Although many studies are still

in the initial phase, they represent the first step towards the development and improvement of new antimicrobial biomaterials with the capability of reducing infections in hospital environment and improving human health.

7. ACKNOWLEDGEMENTS

The authors are grateful for the support provided by the Brazilian National Council for Scientific and Technological Development (CNPq), Pernambuco State Foundation for Research Support (FACEPE), Nanobiotechnology/CAPES. Silva and Ribeiro would like to thank FACEPE for scholarships.

8. REFERENCES

1. SB Levy, B Marshall: Antibacterial resistance worldwide: causes, challenges and responses. *Nat Med* 10, 122-129 (2004) DOI: 10.1038/nm1145
2. MF Mohamed, MN Seleem: Efficacy of short novel antimicrobial and anti-inflammatory peptides in a mouse model of methicillin-resistant Staphylococcus aureus (MRSA) skin infection. *Drug Des Dev Ther* 8, 1979-1983 (2014) DOI: 10.2147/DDDT.S72129
3. K Molbak: Spread of resistant bacteria and resistance genes from animals to humans--the public health consequences. *J Vet Med B Infect Dis Vet Public Health* 51, 364-369 (2004) DOI: 10.1111/j.1439-0450.2004.00788.x
4. KV Reddy, RD Yedery, C Aranha: Antimicrobial peptides: premises and promises. *Int J Antimicrob Agents* 24, 536-547 (2004) DOI: 10.1016/j.ijantimicag.2004.09.005
5. M Zasloff: Antimicrobial peptides of multicellular organisms. *Nature* 415, 389-395 (2002) DOI: 10.1038/415389a
6. T Ganz: Defensins: antimicrobial peptides of innate immunity. *Nat Rev Immunol* 3, 710-720 (2003) DOI: 10.1038/nri1180
7. Z Wang, G Wang: APD: the Antimicrobial Peptide Database. *Nucleic Acids Res* 32, 590-592 (2004) DOI: 10.1093/nar/gkh025
8. M Bagheri, M Beyermann, M Dathe: Immobilization reduces the activity of surface-bound cationic antimicrobial peptides with no influence upon the activity spectrum. *Antimicrob Agents Chemother* 53, 1132-1141 (2009) DOI: 10.1128/AAC.01254-08
9. S Makihira, H Nikawa, T Shuto, M Nishimura, Y Mine, K Tsuji, K Okamoto, Y Sakai, M Sakai, N Imari, S Iwata, M Takeda, F Suehiro: Evaluation of trabecular bone formation in a canine model surrounding a dental implant fixture immobilized with an antimicrobial peptide derived from histatin. *J Mater Sci Mater Med* 22, 2765-2772 (2011) DOI: 10.1007/s10856-011-4440-2
10. SL Haynie, GA Crum, BA Doele: Antimicrobial activities of amphiphilic peptides covalently bonded to a water-insoluble resin. *Antimicrob Agents Chemother* 39, 301-307 (1995) DOI: 10.1128/AAC.39.2.301
11. X Li, P Li, R Saravanan, A Basu, B Mishra, SH Lim, X Su, PA Tambyah, SS Leong: Antimicrobial functionalization of silicone surfaces with engineered short peptides having broad spectrum antimicrobial and salt-resistant properties. *Acta Biomater* 10, 258-266 (2014) DOI: 10.1016/j.actbio.2013.09.009
12. S Bencharit, WC Byrd, S Altarawneh, B Hosseini, A Leong, G Reside, T Morelli, S Offenbacher: Development and applications of porous tantalum trabecular metal-enhanced titanium dental implants. *Clin Implant Dent Relat Res* 16, 817-826 (2014) DOI: 10.1111/cid.12059
13. E Vanderleyden, S Van Bael, YC Chai, JP Kruth, J Schrooten, P Dubruel: Gelatin functionalised porous titanium alloy implants for orthopaedic applications. *Mater Sci Eng C Mater Biol Appl* 42, 396-404 (2014) DOI: 10.1016/j.msec.2014.05.048
14. JW Costerton, PS Stewart, EP Greenberg: Bacterial biofilms: a common cause of persistent infections. *Science* 284, 1318-1322 (1999) DOI: 10.1126/science.284.5418.1318
15. CC de Carvalho: Biofilms: recent developments on an old battle. *Recent Pat Biotechnol* 1, 49-57 (2007) DOI: 10.2174/187220807779813965
16. WMD Jr: Bacterial adhesion: seen any good biofilms lately? *Clin Microbiol Rev* 15, 155-166 (2002) DOI: 10.1128/CMR.15.2.155-166.2002

17. L Zhao, PK Chu, Y Zhang, Z Wu: Antibacterial coatings on titanium implants. *J Biomed Mater Res B Appl Biomater* 91, 470-480 (2009) DOI: 10.1002/jbm.b.31463

18. A Schmidtchen, M Pasupuleti, M Malmsten: Effect of hydrophobic modifications in antimicrobial peptides. *Adv Colloid Interface Sci* 205, 265-274 (2014) DOI: 10.1016/j.cis.2013.06.009

19. D Andreu, L Rivas: Animal antimicrobial peptides: an overview. *Biopolymers* 47, 415-433 (1998) DOI: 10.1002/(SICI)1097-0282(1998)47:6 <415:AID-BIP2>3.0.CO;2-D

20. YJ Gordon, EG Romanowski, AM McDermott: A review of antimicrobial peptides and their therapeutic potential as anti-infective drugs. *Curr Eye Res* 30, 505-515 (2005) DOI: 10.1080/02713680590968637

21. W van 't Hof, EC Veerman, EJ Helmerhorst, AV Amerongen: Antimicrobial peptides: properties and applicability. *Biol Chem* 382, 597-619 (2001)

22. LAC Carvalho, MT Machini: Hemocidinas derivadas da hemoglobina: estruturas, propriedades e perspectivas. *Quim Nova* 36, 1021-1029 (2013) DOI: 10.1590/S0100-40422013000700017

23. KA Brogden: Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? *Nat Rev Microbiol* 3, 238-250 (2005) DOI: 10.1038/nrmicro1098

24. RR Silva, KY Avelino, KL Ribeiro, OL Franco, MD Oliveira, CAS Andrade: Optical and dielectric sensors based on antimicrobial peptides for microorganism diagnosis. *Front Microbiol* 5, 443 (2014) DOI: 10.3389/fmicb.2014.00443

25. F Costa, IF Carvalho, RC Montelaro, P Gomes, MC Martins: Covalent immobilization of antimicrobial peptides (AMPs) onto biomaterial surfaces. *Acta Biomater* 7, 1431-1440 (2011) DOI: 10.1016/j.actbio.2010.11.005

26. G Gao, D Lange, K Hilpert, J Kindrachuk, Y Zou, JT Cheng, M Kazemzadeh-Narbat, K Yu, R Wang, SK Straus, DE Brooks, BH Chew, RE Hancock, JN Kizhakkedathu: The biocompatibility and biofilm resistance of implant coatings based on hydrophilic polymer brushes conjugated with antimicrobial peptides. *Biomaterials* 32, 3899-3909 (2011) DOI: 10.1016/j.biomaterials.2011.02.013

27. RO Darouiche: Treatment of infections associated with surgical implants. *N Engl J Med* 350, 1422-1429 (2004) DOI: 10.1056/NEJMra035415

28. J Vila, A Soriano, J Mensa: (Molecular basis of microbial adherence to prosthetic materials. Role of biofilms in prosthesis-associated infection). *Enferm Infecc Microbiol Clin* 26, 48-54 (2008) DOI: 10.1157/13114395

29. RT Cleophas, J Sjollema, HJ Busscher, JA Kruijtzer, RM Liskamp: Characterization and activity of an immobilized antimicrobial peptide containing bactericidal PEG-hydrogel. *Biomacromolecules* 15, 3390-3395 (2014) DOI: 10.1021/bm500899r

30. LH Eckhard, A Sol, E Abtew, Y Shai, AJ Domb, G Bachrach, N Beyth: Biohybrid polymer-antimicrobial peptide medium against Enterococcus faecalis. *PLoS One* 9, e109413 (2014) DOI: 10.1371/journal.pone.0109413

31. M Godoy-Gallardo, C Mas-Moruno, MC Fernandez-Calderon, C Perez-Giraldo, JM Manero, F Albericio, FJ Gil, D Rodriguez: Covalent immobilization of hLf1-11 peptide on a titanium surface reduces bacterial adhesion and biofilm formation. *Acta Biomater* 10, 3522-3534 (2014) DOI: 10.1016/j.actbio.2014.03.026

32. A Lombana, Z Raja, S Casale, C M Pradier, T Foulon, A Ladram, V Humblot: Temporin-SHa peptides grafted on gold surfaces display antibacterial activity. *J Pept Sci* 20, 563-569 (2014) DOI: 10.1002/psc.2654

33. M Dawgul, M Maciejewska, M Jaskiewicz, A Karafova, W Kamysz: Antimicrobial peptides as potential tool to fight bacterial biofilm. *Acta Pol Pharm* 71, 39-47 (2014)

34. M Kazemzadeh-Narbat, J Kindrachuk, K Duan, H Jenssen, RE Hancock, R Wang: Antimicrobial peptides on calcium phosphate-coated titanium for the prevention of implant-associated infections. *Biomaterials* 31, 9519-9526 (2010) DOI: 10.1016/j.biomaterials.2010.08.035

35. SA Onaizi, SS Leong: Tethering antimicrobial peptides: current status and potential challenges. *Biotechnol Adv* 29, 67-74 (2011) DOI: 10.1016/j.biotechadv.2010.08.012

36. G Wang, ML Hanke, B Mishra, T Lushnikova, CE Heim, V Chittezham Thomas, KW Bayles, T Kielian: Transformation of human cathelicidin LL-37 into selective, stable, and potent antimicrobial compounds. *ACS Chem Biol* 9, 1997-2002 (2014) DOI: 10.1021/cb500475y

37. L Ferreira, A Zumbuehl: Non-leaching surfaces capable of killing microorganisms on contact. *J Mater Chem* 19, 7796-7806 (2009) DOI: 10.1039/b905668h

38. MB Fontana, MC Bastos, A Brandelli: Peptide-like substances as antimicrobial barriers to Corynebacterium sp. adhesion to silicone catheters. *Lett Appl Microbiol* 45, 324-329 (2007) DOI: 10.1111/j.1472-765X.2007.02193.x

39. PH Kwakman, AA te Velde, CM Vandenbroucke-Grauls, SJ van Deventer, SA Zaat: Treatment and prevention of Staphylococcus epidermidis experimental biomaterial-associated infection by bactericidal peptide 2. *Antimicrob Agents Chemother* 50, 3977-3983 (2006) DOI: 10.1128/AAC.00575-06

40. XW Tan, TW Goh, P Saraswathi, CL Nyein, M Setiawan, A Riau, R Lakshminarayanan, S Liu, D Tan, RW Beuerman, JS Mehta: Effectiveness of antimicrobial peptide immobilization for preventing perioperative cornea implant-associated bacterial infection. *Antimicrob Agents Chemother* 58, 5229-5238 (2014) DOI: 10.1128/AAC.02859-14

41. B Mishra, A Basu, RRY Chua, R Saravanan, PA Tambyah, B Ho, MW Chang, SS J Leong: Site specific immobilization of a potent antimicrobial peptide onto silicone catheters: evaluation against urinary tract infection pathogens. *J Mater Chem B* 2, 1706-1716 (2014) DOI: 10.1039/c3tb21300e

42. K Rapsch, FF Bier, M Tadros, M von Nickisch-Rosenegk: Identification of antimicrobial peptides and immobilization strategy suitable for a covalent surface coating with biocompatible properties. *Bioconjug Chem* 25, 308-319 (2014) DOI: 10.1021/bc4004469

43. D Alves, MO Pereira: Mini-review: Antimicrobial peptides and enzymes as promising candidates to functionalize biomaterial surfaces. *Biofouling* 30, 483-499 (2014) DOI: 10.1080/08927014.2014.889120

44. D Leckband, J Israelachvili: Intermolecular forces in biology. *Q Rev Biophys* 34, 105-267 (2001) DOI: 10.1017/S0033583501003687

45. AD Buckingham: Permanent and induced molecular moments and long-range intermolecular forces. In: Advances in Chemical Physics. Ed: JO Hirschfelder, New Jersey (2007)

46. L Karam, C Jama, P Dhulster, N Chihib: Study of surface interactions between peptides, materials and bacteria for setting up antimicrobial surfaces and active food packaging. *J Mater Environ Sci* 4, 798-821 (2013)

47. JC Stendahl, MS Rao, MO Guler, SI Stupp: Intermolecular forces in the self-assembly of peptide amphiphile nanofibers. *Adv Funct Mater* 16, 499-508 (2006) DOI: 10.1002/adfm.200500161

48. G Gao, K Yu, J Kindrachuk, DE Brooks, RE Hancock, JN Kizhakkedathu: Antibacterial surfaces based on polymer brushes: investigation on the influence of brush properties on antimicrobial peptide immobilization and antimicrobial activity. *Biomacromolecules* 12, 3715-3727 (2011) DOI: 10.1021/bm2009697

49. AR Statz, JP Park, NP Chongsiriwatana, AE Barron, PB Messersmith: Surface-immobilised antimicrobial peptoids. *Biofouling* 24, 439-448 (2008) DOI: 10.1080/08927010802331829

50. A Basu, B Mishra, SSJ Leong: Immobilization of polybia-MPI by allyl glycidyl ether based brush chemistry to generate a novel antimicrobial surface. *J Mater Chem B* 1, 4746-4755 (2013) DOI: 10.1039/c3tb20805b

51. WM Cho, BP Joshi, H Cho, KH Lee: Design and synthesis of novel antibacterial peptide-resin conjugates. *Bioorg Med Chem Lett* 17, 5772-5776 (2007) DOI: 10.1016/j.bmcl.2007.08.056

52. K Glinel, AM Jonas, T Jouenne, J Leprince, L

Galas, WT Huck: Antibacterial and antifouling polymer brushes incorporating antimicrobial peptide. *Bioconjug Chem* 20, 71-77 (2009) DOI: 10.1021/bc800280u

53. P Appendini, JH Hotchkiss: Surface modification of poly(styrene) by the attachment of an antimicrobial peptide. *J Appl Polym Sci* 81, 609-616 (2001) DOI: 10.1002/app.1476
54. MD Steven, JH Hotchkiss: Covalent immobilization of an antimicrobial peptide on poly(ethylene) film. *J Appl Polym Sci* 110, 2665-2670 (2008) DOI: 10.1002/app.27638
55. O Etienne, C Picart, C Taddei, Y Haikel, JL Dimarcq, P Schaaf, JC Voegel, JA Ogier, C Egles: Multilayer polyelectrolyte films functionalized by insertion of defensin: a new approach to protection of implants from bacterial colonization. *Antimicrob Agents Chemother* 48, 3662-3669 (2004) DOI: 10.1128/AAC.48.10.3662-3669.2004
56. D Alves, MO Pereira: Mini-review: Antimicrobial peptides and enzymes as promising candidates to functionalize biomaterial surfaces. *Biofouling* 30, 483-499 (2014) DOI: 10.1080/08927014.2014.889120
57. A Shukla, KE Fleming, HF Chuang, TM Chau, CR Loose, GN Stephanopoulos, PT Hammond: Controlling the release of peptide antimicrobial agents from surfaces. *Biomaterials* 31, 2348-2357 (2010) DOI: 10.1016/j.biomaterials.2009.11.082
58. K Ariga, JP Hill, Q Ji: Layer-by-layer assembly as a versatile bottom-up nanofabrication technique for exploratory research and realistic application. *Phys Chem Chem Phys* 9, 2319-2340 (2007) DOI: 10.1039/b700410a
59. G Decher, JD Hong, J Schmitt: Buildup of ultrathin multilayer films by a self-assembly process: III. Consecutively alternating adsorption of anionic and cationic polyelectrolytes on charged surfaces. *Thin Solid Films* 210-211, Part 2, 831-835 (1992) DOI: 10.1016/0040-6090(92)90417-A
60. G Decher, M Eckle, J Schmitt, B Struth: Layer-by-layer assembled multicomposite films. *Curr Opin Colloid Interface Sci* 3, 32-39 (1998) DOI: 10.1016/S1359-0294(98)80039-3
61. W Soliman, S Bhattacharjee, K Kaur: Adsorption of an antimicrobial peptide on self-assembled monolayers by molecular dynamics simulation. *J Phys Chem B* 114, 11292-11302 (2010) DOI: 10.1021/jp104024d
62. DM Lynn: Peeling back the layers: controlled erosion and triggered disassembly of multilayered polyelectrolyte thin films. *Adv Mater* 19, 4118-4130 (2007) DOI: 10.1002/adma.200701748
63. E Vázquez, DM Dewitt, PT Hammond, DM Lynn: Construction of hydrolytically-degradable thin films via layer-by-layer deposition of degradable polyelectrolytes. *J Am Chem Soc* 124, 13992-13993 (2002) DOI: 10.1021/ja026405w
64. KC Wood, HF Chuang, RD Batten, DM Lynn, PT Hammond: Controlling interlayer diffusion to achieve sustained, multiagent delivery from layer-by-layer thin films. *Proc Natl Acad Sci USA* 103, 10207-10212 (2006) DOI: 10.1073/pnas.0602884103
65. JM Goddard, JH Hotchkiss: Polymer surface modification for the attachment of bioactive compounds. *Prog Polym Sci* 32, 698-725 (2007) DOI: 10.1016/j.progpolymsci.2007.04.002
66. K Vasilev, J Cook, HJ Griesser: Antibacterial surfaces for biomedical devices. *Expert Rev Med Devices* 6, 553-567 (2009) DOI: 10.1586/erd.09.36
67. N Aumsuwan, RC Danyus, S Heinhorst, MW Urban: Attachment of ampicillin to expanded poly(tetrafluoroethylene): surface reactions leading to inhibition of microbial growth. *Biomacromolecules* 9, 1712-1718 (2008) DOI: 10.1021/bm800176t
68. X Qi, G Poernomo, K Wang, Y Chen, MB Chan-Park, R Xu, MW Chang: Covalent immobilization of nisin on multi-walled carbon nanotubes: superior antimicrobial and anti-biofilm properties. *Nanoscale* 3, 1874-1880 (2011) DOI: 10.1039/c1nr10024f
69. P Appendini, JH Hotchkiss: Review of antimicrobial food packagingInnov. *Food Sci Emerg Technol* 3, 113-126 (2002) DOI: 10.1016/S1466-8564(02)00012-7
70. F Rusmini, Z Zhong, J Feijen: Protein

immobilization strategies for protein biochips. *Biomacromolecules* 8, 1775-1789 (2007) DOI: 10.1021/bm061197b

71. P Jonkheijm, D Weinrich, H Schröder, CM Niemeyer, H Waldmann: Chemical strategies for generating protein biochips. A*ngew Chem Int* 47, 9618-9647 (2008) DOI: 10.1002/anie.200801711
72. R Chen, N Cole, MD Willcox, J Park, R Rasul, E Carter, N Kumar: Synthesis, characterization and *in vitro* activity of a surface-attached antimicrobial cationic peptide. *Biofouling* 25, 517-524 (2009) DOI: 10.1080/08927010902954207
73. V Humblot, JF Yala, P Thebault, K Boukerma, A Héquet, JM Berjeaud, CM Pradier: The antibacterial activity of Magainin I immobilized onto mixed thiols self-assembled monolayers. *Biomaterials* 30, 3503-3512 (2009) DOI: 10.1016/j.biomaterials.2009.03.025
74. JJ Gooding, F Mearns, W Yang, J Liu: Self-assembled monolayers into the 21st century: recent advances and applications. *Electroanalysis* 15, 81-96 (2003) DOI: 10.1002/elan.200390017
75. TP Sullivan, WTS Huck: Reactions on monolayers: organic synthesis in two dimensions. *Eur J Org Chem* 2003, 17-29 (2003) DOI: 10.1002/1099-0690(200301)2003:1<17 :AID-EJOC17>3.0.CO;2-H
76. A Ulman: Formation and structure of self-assembled monolayers. *Chem Rev* 96, 1533-1554 (1996) DOI: 10.1021/cr9502357
77. F Boulmedais, B Frisch, O Etienne, P Lavalle, C Picart, J Ogier, JC Voegel, P Schaaf, C Egles: Polyelectrolyte multilayer films with pegylated polypeptides as a new type of anti-microbial protection for biomaterials. *Biomaterials* 25, 2003-2011 (2004) DOI: 10.1016/j.biomaterials.2003.08.039
78. LG Harris, S Tosatti, M Wieland, M Textor, RG Richards: Staphylococcus aureus adhesion to titanium oxide surfaces coated with non-functionalized and peptide-functionalized poly(l-lysine)-grafted-poly(ethylene glycol) copolymers. *Biomaterials* 25, 4135-4148 (2004) DOI: 10.1016/j.biomaterials.2003.11.033
79. P Kingshott, J Wei, D Bagge-Ravn, N Gadegaard, L Gram: Covalent attachment of poly(ethylene glycol) to surfaces, critical for reducing bacterial adhesion. *Langmuir* 19, 6912-6921 (2003) DOI: 10.1021/la034032m
80. Y Gao, I Kyratzis: Covalent immobilization of proteins on carbon nanotubes using the cross-linker 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide-a critical assessment. *Bioconjug Chem* 19, 1945-1950 (2008) DOI: 10.1021/bc800051c
81. D Sehgal, IK Vijay: A method for the high efficiency of water-soluble carbodiimide-mediated amidation. *Anal Biochem* 218, 87-91 (1994) DOI: 10.1006/abio.1994.1144
82. JV Staros, RW Wright, DM Swingle: Enhancement by N-hydroxysulfosuccinimide of water-soluble carbodiimide-mediated coupling reactions. *Anal Biochem* 156, 220-222 (1986) DOI: 10.1016/0003-2697(86)90176-4
83. LS Wong, F Khan, J Micklefield: Selective covalent protein immobilization: strategies and applications. *Chem Rev* 109, 4025-4053 (2009) DOI: 10.1021/cr8004668
84. SJ Oh, BJ Hong, KY Choi, JW Park: Surface modification for DNA and protein microarrays. *OMICS* 10, 327-343 (2006) DOI: 10.1089/omi.2006.10.327
85. C Vreuls, G Zocchi, B Thierry, G Garitte, SS Griesser, C Archambeau, C Van de Weerdt, J Martial, H Griesser: Prevention of bacterial biofilms by covalent immobilization of peptides onto plasma polymer functionalized substrates. *J Mater Chem* 20, 8092-8098 (2010) DOI: 10.1039/c0jm01419b
86. M Rahnamaeian: Antimicrobial peptides: modes of mechanism, modulation of defense responses. *Plant Signal Behav* 6, 1325-1332 (2011) DOI: 10.4161/psb.6.9.16319
87. JJ Lopez Cascales, A Garro, RD Porasso, RD Enriz: The dynamic action mechanism of small cationic antimicrobial peptides. *Phys Chem Chem Phys* 16, 21694-21705 (2014) DOI: 10.1039/C4CP02537G
88. KL Lam, H Wang, TA Siaw, MR Chapman, AJ Waring, JT Kindt, KY Lee: Mechanism

of structural transformations induced by antimicrobial peptides in lipid membranes. *Biochim Biophys Acta* 1818, 194-204 (2012) DOI: 10.1016/j.bbamem.2011.11.002

89. RE Hancock, HG Sahl: Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. *Nat Biotechnol* 24, 1551-1557 (2006) DOI: 10.1038/nbt1267
90. M Bagheri, M Beyermann, M Dathe: Mode of action of cationic antimicrobial peptides defines the tethering position and the efficacy of biocidal surfaces. *Bioconjug Chem* 23, 66-74 (2012) DOI: 10.1021/bc200367f
91. A Kirsten, A Hausmann, M Weber, J Fischer, H Fischer: Bioactive and thermally compatible glass coating on zirconia dental implants. *J Dent Res* 94, 297-303 (2015) DOI: 10.1177/0022034514559250
92. J Ye, J He, C Wang, K Yao, Z Gou: Copper-containing mesoporous bioactive glass coatings on orbital implants for improving drug delivery capacity and antibacterial activity. *Biotechnol Lett* 36, 961-968 (2014) DOI: 10.1007/s10529-014-1465-x
93. N Drnovsek, S Novak, U Dragin, M Ceh, M Gorensek, M Gradisar: Bioactive glass enhances bone ingrowth into the porous titanium coating on orthopaedic implants. *Int Orthop* 36, 1739-1745 (2012) DOI: 10.1007/s00264-012-1520-y
94. M Mohorcic, I Jerman, M Zorko, L Butinar, B Orel, R Jerala, J Friedrich: Surface with antimicrobial activity obtained through silane coating with covalently bound polymyxin B. *J Mater Sci Mater Med* 21, 2775-2782 (2010) DOI: 10.1007/s10856-010-4136-z
95. B Ashar, RSW Jr, LR Turcotte: Development of a silica-free silicone system for medical applications. *J Biomed Mater Res* 15, 663-672 (1981) DOI: 10.1002/jbm.820150505
96. E Briganti, P Losi, A Raffi, M Scoccianti, A Munao, G Soldani: Silicone based polyurethane materials: a promising biocompatible elastomeric formulation for cardiovascular applications. *J Mater Sci Mater Med* 17, 259-266 (2006) DOI: 10.1007/s10856-006-7312-4
97. MC Musolf: Altering the physical properties of silicone elastomers for medical device applications. *Med Device Technol* 1, 26-29 (1990)
98. L Azevedo Calderon, AA Silva, P Ciancaglini, RG Stabeli: Antimicrobial peptides from Phyllomedusa frogs: from biomolecular diversity to potential nanotechnologic medical applications. *Amino Acids* 40, 29-49 (2011) DOI: 10.1007/s00726-010-0622-3
99. A Giacometti, O Cirioni, MS Del Prete, F Barchiesi, AM Paggi, E Petrelli, G Scalise: Comparative activities of polycationic peptides and clinically used antimicrobial agents against multidrug-resistant nosocomial isolates of Acinetobacter baumannii. *J Antimicrob Chemother* 46, 807-810 (2000) DOI: 10.1093/jac/46.5.807
100. K Lim, RR Chua, R Saravanan, A Basu, B Mishra, PA Tambyah, B Ho, SS Leong: Immobilization studies of an engineered arginine-tryptophan-rich peptide on a silicone surface with antimicrobial and antibiofilm activity. *ACS Appl Mater Interfaces* 5, 6412-6422 (2013) DOI: 10.1021/am401629p
101. R Saravanan, X Li, K Lim, H Mohanram, L Peng, B Mishra, A Basu, JM Lee, S Bhattacharjya, SS Leong: Design of short membrane selective antimicrobial peptides containing tryptophan and arginine residues for improved activity, salt-resistance, and biocompatibility. *Biotechnol Bioeng* 111, 37-49 (2014) DOI: 10.1002/bit.25003
102. M Yoshinari, T Kato, K Matsuzaka, T Hayakawa, K Shiba: Prevention of biofilm formation on titanium surfaces modified with conjugated molecules comprised of antimicrobial and titanium-binding peptides. *Biofouling* 26, 103-110 (2010) DOI: 10.1080/08927010903216572
103. E Vasilescu, P Drob, D Raducanu, VD Cojocaru, I Cinca, D Iordachescu, R Ion, M Popa, C Vasilescu: *In vitro* biocompatibility and corrosion resistance of a new implant titanium base alloy. *J Mater Sci Mater Med* 21, 1959-1968 (2010) DOI: 10.1007/s10856-010-4057-x
104. M Zanetti, R Gennaro, D Romeo: Cathelicidins: a novel protein family with a common proregion

and a variable C-terminal antimicrobial domain. *FEBS Lett* 374, 1-5 (1995) DOI: 10.1016/0014-5793(95)01050-O

105. M Gabriel, K Nazmi, EC Veerman, AVN Amerongen, A Zentner: Preparation of LL-37-grafted titanium surfaces with bactericidal activity. *Bioconjug Chem* 17, 548-550 (2006) DOI: 10.1021/bc050091v
106. WJ Velden, TM van Iersel, NM Blijlevens, JP Donnelly: Safety and tolerability of the antimicrobial peptide human lactoferrin 1-11 (hLF1-11). *BMC Med* 7, 1-8 (2009) DOI: 10.1186/1741-7015-7-44
107. AM van der Does, PJ Hensbergen, SJ Bogaards, M Cansoy, AM Deelder, HC van Leeuwen, JW Drijfhout, JT van Dissel, PH Nibbering: The human lactoferrin-derived peptide hLF1-11 exerts immunomodulatory effects by specific inhibition of myeloperoxidase activity. *J Immunol* 188, 5012-5019 (2012) DOI: 10.4049/jimmunol.1102777
108. AM van der Does, SJ Bogaards, L Jonk, M Wulferink, MP Velders, PH Nibbering: The human lactoferrin-derived peptide hLF1-11 primes monocytes for an enhanced TLR-mediated immune response. *Biometals* 23, 493-505 (2010) DOI: 10.1007/s10534-010-9322-4
109. H Ceylan, AB Tekinay, MO Guler: Selective adhesion and growth of vascular endothelial cells on bioactive peptide nanofiber functionalized stainless steel surface. *Biomaterials* 32, 8797-8805 (2011) DOI: 10.1016/j.biomaterials.2011.08.018
110. EM Davis, DY Li, RT Irvin: A peptide-stainless steel reaction that yields a new bioorganic-metal state of matter. *Biomaterials* 32, 5311-5319 (2011) DOI: 10.1016/j.biomaterials.2011.04.027
111. A Hequet, V Humblot, JM Berjeaud, CM Pradier: Optimized grafting of antimicrobial peptides on stainless steel surface and biofilm resistance tests. *Colloids Surf B Biointerfaces* 84, 301-319 (2011) DOI: 10.1016/j.colsurfb.2011.01.012

Abreviations: AMP: antimicrobial peptide, PEG: Polyethylene glycol, CPTES: chloropropyltrie thoxysilane, APTES: 3-aminopropyltriethoxysilane, hLF: human lactoferrin, Fmoc: 9-fluorenylmethyloxycarbonyl, AGE: allylglycidyl ether, ABA: 4-azidobenzoic acid, FNA: 4-fluoro-3-nitrophenyl azide, SAM: self-assembly monolayers

Key Words: Antimicrobial peptides, Immobilization, Surface, Biomaterial, Review

Send correspondence to: Cesar Andrade, Departamento de Bioquimica, Universidade Federal de Pernambuco, 50670-901 Recife, PE, Brasil, Tel: 55-81-2126-8-450, Fax: 55-81-2126-8-576, E-mail: csrandrade@gmail.com