ANTI-INFECTIVE MEDICAL DEVICES: THE EMPLOYMENT OF HOST DEFENCE PEPTIDES IDENTIFIED IN HUMAN APOLIPOPROTEIN B

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ABSTRACT

Introduction of medical devices in modern medical practise has significantly improved patients' well-being. However, the employment of these "foreign" devices into human body can also be associated with serious complications, among which biofilm-associated infections remain a major public health concern. The emergence and fast diffusion of antibiotic-resistant bacterial strains makes the management of such infections increasingly difficult. AntiMicrobial Peptides (AMPs), also named Host Defence Peptides (HDPs) because of their wide range of bioactivities, are small molecules present in all the living organisms. They are generally endowed not only with direct antimicrobial activity, but also with anti-biofilm, antifungal, antiviral, anticancer, wound healing, and immunomodulatory properties. Because of this wide range of properties and because they generally don't lead to the development of multidrug resistance phenotype, they attracted considerable attention as an effective weapon to generate antimicrobial surfaces.

The main aim of the present PhD Thesis has been the evaluation of the applicability of novel HDPs identified in human Apolipoprotein B to design infection-resistant medical devices. The attention has been focused on the functionalization of two kinds of medical devices, such as urinary catheters and contact lenses, with a selected ApoB-derived peptide. Polydimethylsiloxane (PDMS) polymer, generally employed to fabricate urinary catheters, and hydroxyethylmethacrylate (HEMA) hydrogel, the main component of commercial soft contact lenses, have been selected as prototypes materials for functionalization studies. In both cases, a physicochemical characterization of obtained functionalized material has been performed together with analyses of peptide loading and release from surfaces under test. Importantly, anti-infective properties and cytocompatibility of obtained functionalized surfaces have been also evaluated by using bacterial strains commonly associated to medical devices infections and proper eukaryotic cell lines simulating those generally in contact with medical devices.

Altogether, obtained results open interesting perspectives to the applicability of ApoB-derived peptides to functionalize surfaces of biotechnological interest.

RIASSUNTO

I dispositivi biomedicali sono diventati parte integrante del moderno sistema sanitario. Negli ultimi trenta anni l'utilizzo di cateteri, protesi, *pacemaker*, valvole cardiache e lenti a contatto è stato implementato al fine di migliorare la qualità della vita di milioni di persone in tutto il mondo. Bisogna, però, sottolineare che il loro utilizzo presenta anche alcuni svantaggi, quali l'elevato tasso di insorgenza di infezioni nosocomiali o ospedaliere che rappresentano una delle principali cause di morbidità e mortalità, con un conseguente forte impatto economico a causa del prolungamento dell'ospedalizzazione e la necessità di ulteriori trattamenti farmacologici. Il fallimento delle prestazioni dei dispositivi medici viene comunemente attribuito alla colonizzazione della superficie di tali dispositivi da parte del *biofilm* batterico, un'organizzazione di batteri racchiusi in una matrice extracellulare autoprodotta costituita principalmente da acqua, polisaccaridi, proteine e DNA extracellulare. L'adesione del *biofilm* batterico alle superfici è facilitata dal fatto che, subito dopo l'inserzione del dispositivo medico, composti presenti nei fluidi corporei si depositano sulla sua superficie formando uno strato particolarmente adatto all'adesione dei microrganismi. Inoltre, nel sito di inserzione, la risposta immunitaria risulta alterata e questo contribuisce a favorire la contaminazione batterica. Una volta sviluppate, le infezioni sono molto difficili da trattare in quanto i batteri all'interno della matrice del *biofilm* risultano più resistenti sia all'azione dei trattamenti farmacologici convenzionali sia all'azione del sistema immunitario dell'ospite. Sebbene, subito dopo la loro scoperta, gli antibiotici abbiano rappresentato un'arma preziosa e rivoluzionaria per combattere le infezioni batteriche e siano tuttora farmaci preziosi per salvare vite umane, il loro utilizzo eccessivo ed improprio ha favorito la rapida diffusione di ceppi patogeni resistenti alla loro azione. Secondo dati dell'Organizzazione Mondiale della Sanità (OMS), da qui al 2050 i batteri resistenti agli antibiotici convenzionali saranno causa di almeno 10 milioni di decessi all'anno, situazione che ha portato a classificare il fenomeno della resistenza agli antibiotici come una delle principali minacce per la salute umana. Nonostante ciò, la ricerca, la produzione e la commercializzazione da parte delle industrie farmaceutiche di nuovi antibiotici è notevolmente rallentata negli ultimi 20 anni poiché risulta poco vantaggiosa dal punto di vista economico.

In tale contesto si inquadra la necessità di progettare dispositivi biomedicali dotati di superfici in grado di resistere e/o prevenire la

colonizzazione batterica e la formazione del *biofilm*. Le possibili strategie per ottenere ciò sono due e sono classificate come passive e attive. Quelle passive si basano sulla produzione delle cosiddette "superfici anti-adesive" con caratteristiche di carica e di idrofilicità tali da interferire con l'adesione dei microrganismi; le strategie "attive" prevedono, invece, la produzione sia di superfici in cui i composti antimicrobici sono covalentemente legati ad esse, e quindi in grado di determinare la morte dei microrganismi per contatto diretto, sia di superfici in grado di rilasciare nell'ambiente circostante tali sostanze ad azione antimicrobica. Diverse tipologie di agenti antimicrobici, quali antibiotici, composti quaternari dell'ammonio e composti metallici, sono state impiegati allo scopo di conferire alle superfici proprietà antimicrobiche. Purtroppo, tali strategie hanno mostrato limitazioni legate all'insorgenza di effetti tossici e alla selezione di ceppi batterici resistenti. Pertanto, nuove tipologie di agenti antimicrobici sono fortemente richieste. In questo scenario, l'utilizzo dei peptidi antimicrobici, denominati anche peptidi di difesa dell'ospite, sembra essere una valida alternativa per la generazione di superfici con proprietà antimicrobiche. I peptidi antimicrobici sono un gruppo di molecole del sistema immunitario innato conservate durante l'evoluzione e presenti in tutti gli organismi viventi complessi. Tali peptidi sono risultati dotati di numerose proprietà biologiche, quali attività antimicrobica, anti-biofilm, anti-infiammatoria, anticancro e di rimarginazione delle ferite. Dal momento che, in molti casi, non sono né tossici né emolitici nei confronti di cellule di mammifero in coltura, essi risultano essere buoni candidati per applicazioni in ambito biomedico. Le caratteristiche chimico-fisiche alla base della loro attività antimicrobica e selettività risiedono nella loro carica netta positiva e nella presenza di un'elevata percentuale di residui idrofobici. Infatti, la membrana batterica dotata di carica netta negativa rappresenta il principale bersaglio dell'azione dei peptidi di difesa dell'ospite. Degno di nota è il fatto che il loro impiego generalmente non determina l'insorgenza di ceppi batterici resistenti, dal momento che il batterio dovrebbe completamente riorganizzare la sua struttura esterna.

Nel 2006 è stato introdotto per la prima volta il termine "criptoma" per descrivere l'insieme delle proteine che agiscono come precursori di peptidi bioattivi, noti come "criptidi". Questi ultimi, a loro volta, possono presentare proprietà correlate o migliorate rispetto al precursore proteico oppure possono esplicare funzioni biologiche completamente diverse. I precursori proteici in questione, le cui funzioni non sono necessariamente legate alla difesa dell'ospite, rilasciano i peptidi bioattivi in seguito a scissioni proteolitiche da parte di proteasi

batteriche e/o dell'ospite. Dato il forte interesse nello studio delle proprietà di tali peptidi, diversi metodi e strategie volti alla loro identificazione sono stati sviluppati. Negli ultimi anni, il gruppo di ricerca presso cui è stato svolto il presente progetto di Dottorato, ha identificato una sequenza dalla putativa azione antimicrobica nell'apolipoproteina B umana. Di tale sequenza sono state prodotte tre diverse varianti, due più lunghe, qui denominate r(P)ApoB$_L^{Pro}$ e r(P)ApoB$_L^{Ala}$, che differiscono per la sostituzione di un residuo di prolina con un'alanina in posizione sette, e una versione più breve di tale sequenza, qui denominata r(P)ApoB$_S^{Pro}$. Tali peptidi sono stati prodotti efficientemente per via ricombinante in cellule di *Escherichia coli* sotto forma di proteina chimerica. I peptidi derivanti dall'apolipoproteina B umana sono stati poi oggetto di caratterizzazione strutturale e funzionale. Essi sono risultati dotati di attività antimicrobica ad ampio spettro, in quanto in grado di inibire la crescita di batteri Gram-positivi e Gram-negativi. Si sono, inoltre, rivelati degli efficienti agenti anti-biofilm e in grado di modulare la risposta immunitaria. Inoltre, i peptidi non hanno mostrato né effetti tossici su linee cellulari eucariotiche né effetti emolitici quando analizzati su globuli rossi, il che ha aperto interessanti prospettive per la loro applicabilità. Alla luce dei promettenti risultati pregressi, l'obiettivo principale del presente progetto di Dottorato è stato la valutazione dell'utilizzo di uno dei tre peptidi antimicrobici prodotti, r(P)ApoB$_L^{Pro}$, per la funzionalizzazione di superfici di dispositivi biomedicali. Nello specifico, l'attenzione è stata focalizzata sue due tipologie di dispositivi biomedicali, ossia i cateteri urinari e le lenti a contatto. I cateteri sono tra i dispositivi biomedicali più frequentemente adoperati in ambito ospedaliero. Secondo l'Organizzazione Mondiale della Sanità (OMS), circa il 70-80% delle infezioni urinarie sono riscontrante in pazienti con cateteri. I principali microrganismi responsabili di tali infezioni sono *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Escherichia coli*, *Proteus mirabilis* e *Staphylococcus aureus*. Le infezioni urinarie associate ai cateteri, se non trattate tempestivamente ed in modo adeguato, possono diffondersi ai reni (pielonefrite) o al sangue (setticemia) e, in alcuni casi, possono anche provocare la morte del paziente. Nonostante le rigorose procedure igieniche adottate, è difficile evitare completamente tali infezioni.

In tale lavoro di Tesi, una delle superfici selezionate per la funzionalizzazione con il peptide antimicrobico di interesse è stata il polidimetilsilossano (PDMS), un polimero comunemente utilizzato per la produzione di cateteri urinari grazie alle sue proprietà, quali stabilità chimica, biocompatibilità ed economicità. Al fine di funzionalizzare il

PDMS con il peptide derivato da ApoB, la soluzione polimerica è stata miscelata con la soluzione peptidica di interesse. Una volta ottenuto il polimero funzionalizzato, è stata effettuata una caratterizzazione chimico-fisica del materiale ottenuto mediante misurazione dell'angolo di contatto (WCA), analisi di spettroscopia a infrarossi (FTIR) e di microscopia elettronica che hanno indicato la presenza del peptide antimicrobico di interesse non solo nella matrice del polimero, ma anche sulla sua superficie. Sono state, inoltre, effettuate analisi mediante la tecnologia delle microbilance a cristalli di quarzo (QCM) che hanno indicato un quasi totale rilascio del peptide dalla matrice di PDMS nell'arco delle 24 ore di incubazione. Alla luce di tali risultati, si è proceduto poi alla valutazione dell'attività antimicrobica del PDMS funzionalizzato con il peptide di interesse. A tal proposito, sono stati utilizzati due ceppi batterici, un Gram-positivo e un Gram-negativo, che sono risultati entrambi altamente sensibili all'attività antimicrobica del peptide immobilizzato. Sono state, inoltre, effettuate analisi mediante l'impiego del colorante *crystal violet* e della microscopia elettronica a scansione, le quali hanno dimostrato l'abilità della superficie funzionalizzata di ostacolare l'adesione delle cellule batteriche. Il PDMS funzionalizzato è risultato, inoltre, biocompatibile, dal momento che non è risultato alterare la vitalità di fibroblasti murini e umani in saggi *in vitro*.

Ulteriori esperimenti sono stati condotti con l'obiettivo di funzionalizzare con lo stesso peptide un altro tipo di dispositivo biomedicale. A tal proposito, sono state selezionate lenti a contatto composte da un opportuno materiale. Tali esperimenti sono stati effettuati durante il periodo all'estero (6 mesi) presso il laboratorio di ricerca diretto dalla Prof.ssa Dr. Carmen Alvarez Lorenzo presso il Dipartimento di Farmacia e Tecnologie Farmaceutiche dell'Università di Santiago Di Compostela, Spagna. Le lenti a contatto sono ampiamente utilizzate per correggere difetti di rifrazione o ametropia. Quelle maggiormente diffuse sono definite lenti a contatto "morbide" e sono a base di polimeri di *hydrogel* di idrossietilmetacrilato (pHEMA). Negli ultimi decenni si è sviluppato un crescente interesse nell'utilizzo delle lenti a contatto come sistemi di *drug delivery* di sostanze utili al trattamento di diverse condizioni che affliggono la salute dell'occhio, quali, ad esempio, il glaucoma, le infezioni, le congiuntiviti e la cataratta. Nel presente lavoro di Tesi, diverse composizioni polimeriche di *hydrogel* sono state preparate tramite co-polimerizzazione del monomero con i cosiddetti monomeri funzionali (ad esempio, acido metacrilico, MAA). Al termine della polimerizzazione, sono stati ottenuti dei dischi usati come prototipi di lenti a contatto. L'immersione di tali dischi in una soluzione

contenente il peptide a concentrazioni prestabilite ha consentito, nel caso dei dischi di HEMA/MAA, di caricare tutto il peptide presente in soluzione. La valutazione del rilascio, effettuata tramite cromatografia liquida ad alta pressione (HPLC), ha mostrato che solo una piccola quantità di peptide caricato viene poi eluito nel tempo. Saggi di attività antimicrobica in *vitro* hanno messo in luce che purtroppo tale quantità non è sufficiente ad uccidere i batteri presenti in soluzione. Ulteriori esperimenti saranno condotti in futuro, al fine di effettuare la funzionalizzazione con maggiori quantità di peptide e di testare differenti tipi di lenti a contatto la cui composizione potrebbe garantire un'interazione più blanda tra la superficie della lente e le molecole di peptide.

Nel complesso, i risultati ottenuti nel presente progetto di Tesi aprono interessanti prospettive all'applicabilità dei peptidi di interesse nella funzionalizzazione di superfici di interesse biotecnologico.

CHAPTER 1

General Introduction

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General introduction

1.1 Medical device-associated infections

A medical device is an instrument, apparatus, implant, machine, tool, *in vitro* reagent, or any similar article used to diagnose, prevent, mitigate, treat, or cure disease or other conditions, and, unlike a pharmaceutical or biologic tool, achieves its purpose by physical, structural, or mechanical properties (Zhang and Wagner, 2017). Permanent medical devices are also called implants, and include orthopedic devices, prosthetic joints and internal fixation devices (nails, plates, and screws), artificial heart valves, intravascular stents (Figure 1). Temporary medical devices are, instead, represented by catheters, sutures, and contact lenses (Andrea, Molchanova and Jenssen, 2018) (Figure1). The material selected to fabricate biomedical devices depends on the specific requirements that they should fulfil; the most common materials are ceramics, polymers and metal alloy (Olmo *et al.*, 2020). One of the earliest biomedical devices was an artificial toe discovered in Egypt; it was obtained by using leather and appears to have been sewn in combination with another material to facilitate walking by the wearer (Cooper, 2015). To date, biomedical devices and implants have become an integral part of the modern health care system; over the last decades, their use is markedly increased to save lives and to improve the quality of life of millions of people worldwide (Crnich and Drinka, 2012). However, the huge benefits deriving from their use are affected by increasing risks of infections (Zimmerli and Sendi, 2011), with a consequent important economic impact (Umscheid *et al.*, 2011). Indeed, medical devices might be contaminated by bacteria from droplets or air before implantation (Phillips, Patwardhan and Jayan, 2015). Furthermore, due to peri-implant tissue damage occurring during the implantation procedure, the local immune responses are dysregulated, thus making the tissues around the foreign body more vulnerable to infections (Zimmerli and Sendi, 2011). Indeed, it was demonstrated that polymorphonuclear leukocytes (PMN) around the implant were unable to kill bacteria, thus confirming the increased susceptibility to infections that allows to a minimal number of bacteria to colonize the implant (Zimmerli, Lew and Waldvogel, 1984). Although the impact of bacterial contaminations has been reduced by the use of aseptic surgical techniques and severe hygiene procedures, microorganisms are still isolated from the surfaces of removed implants (Schneeberger *et al.* 2002; Nablo *et al.* 2005). In the early 1980s, it was demonstrated the presence of microbial biofilms on medical devices, such as pacemaker leads and prosthetic joints recovered from infected

patients (Reviews, 1998; Nobile and Mitchell, 2007). Bacterial biofilms are communities of microorganisms enclosed within an extracellular polymeric matrix (EPM) composed by exopolysaccharides, proteins, extracellular DNA and water, with the ability to attach to abiotic and biotic surfaces (Bryers, 2008). The development of biofilm infections on medical device surfaces occurs through different steps (Darouiche, 2001; Arciola, Campoccia and Montanaro, 2018) (Figure 2). Firstly, a non-specific and reversible adhesion occurs through weak and non-covalent forces favoured by the deposition on the surface of the medical device of organic compounds and macromolecules present in physiological fluids that form the so-called “conditioning film” (Arciola, Campoccia and Montanaro, 2018). At this stage, bacteria are still susceptible to antibiotics and can be easily dispersed by physical or even chemical methods (Mihai *e*t al., 2015). A specific irreversible adhesion is due to covalent bonds between bacterial surface proteins and human host proteins present on the surface of the device. At this point, bacterial cells division is accompanied by biofilm development through quorum sensing, up-regulation of virulence factors and extracellular matrix constitution (Michael, 2002). Finally, detachment of encapsulated within the matrix can happen, leading to subsequent dispersion and dissemination of bacterial cells that, through bloodstream, can reach and colonize different sites of the body and cause systemic infections (Veerachamy *et al.*, 2014). It is also noteworthy that bacteria within biofilms are less responsive to conventional antimicrobial therapy and are able to escape from the host’s physiological immune response (Li and Webster, 2018).

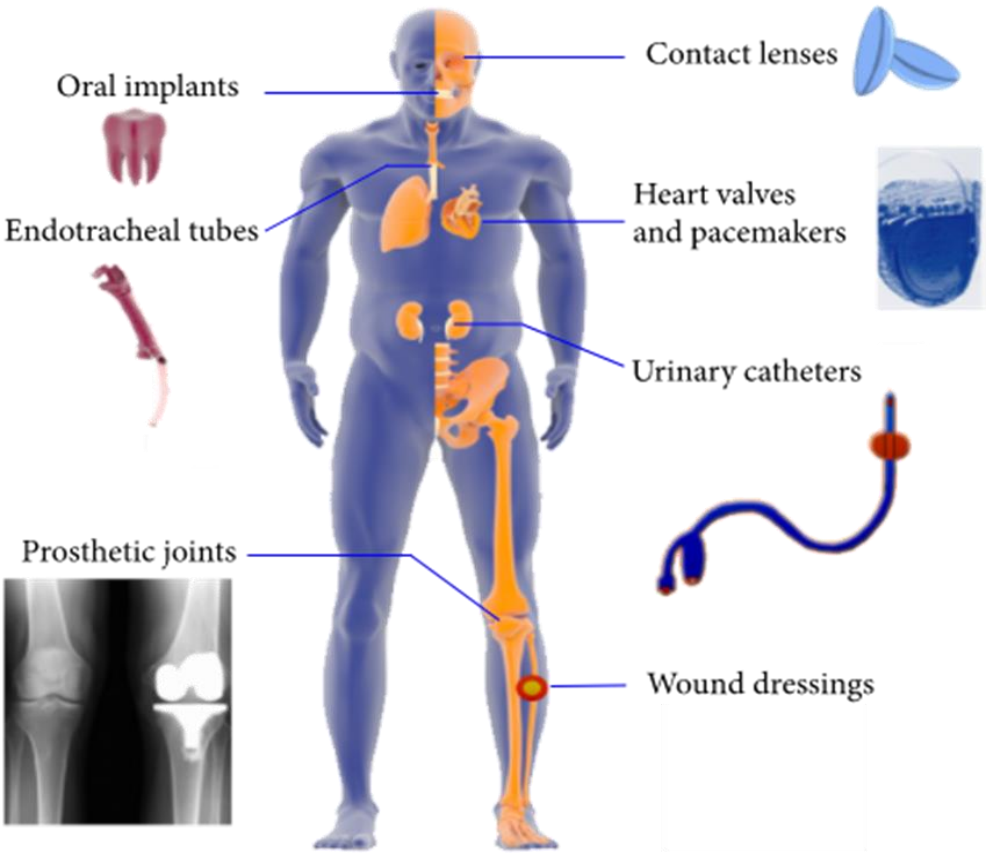

Figure 1. Schematic representation of the common types of medical devices (Ramasamy and Lee, 2016).

1.2. Antibiotic resistance

According to recent statistics, in 2015 antibiotic-resistant infections will be responsible for 33,000 annual deaths in Europe (Cassini *et al.*, 2019), thus killing approximately 700,000 persons worldwide each year (Organization, 2018) with a prevision of up to 10 million deaths by 2050 (de Kraker, Stewardson and Harbarth, 2016). Indeed, several important health organizations, such as the Centre for Disease Control and Prevention (CDC), Infectious Diseases Society of America, World Economic Forum, and the World Health Organization (WHO), have declared that antibiotic resistance is a “global public health concern” (Wang *et al.*, 2018), thus highlighting the necessity of global action plans (Wernli *et al.*, 2017). The “golden era” of antibiotics began with penicillin discovery in 1928 (Davies, 1996; Aminov, 2010). Novel

antibiotics were then immediately introduced, such as streptomycin, chloramphenicol, and tetracycline. Since then, antibiotics' employment completely revolutionized medical approaches for the treatment of infectious diseases (Debabov, 2013). However, in 1945, Sir Alexander Fleming was the first one to warn about the inappropriate use of penicillin that could lead to the selection of resistant ''mutant forms'' of *Staphylococcus aureus* strains (Alanis, 2005). Indeed, the fast spread of antibiotic resistance phenotype can be attributed to several causes including (i) overconsumption of antibiotics ascribable to over-prescription, self-medication and over-the-counter accessible antibiotics, (ii) absence of standardized guidelines for antibiotic usage, and (iii) lack of appropriate sanitation/hygiene practices (Debabov, 2013). The ability of bacteria to acquire resistance to antibiotics can be classified as "intrinsic" when it depends on innate structural features of bacteria (Blair *et al.*, 2015) or "acquired" when it is the result of endogenous genes' mutations or obtained through the transfer of resistance determinants from one microorganism to another (Jorge *et al.*, 2019). Resistance to antibiotics is acquired through several mechanisms: (i) antibiotic inactivation *via* hydrolysis (*e.g.*, β-lactamase activation) or modification; (ii) modification of intracellular targets that become unrecognizable (*e.g.,* DNA gyrase mutation in the case of resistance to fluoroquinolone); (iii) activation of mechanisms that inhibit drug access into the cell; (iv) activation of membrane-bound efflux transporters that pump drugs out from the cells (Motta, Cluzel and Aldana, 2015). Based on this, the emergence of "MultiDrug-Resistant Organisms (MDROs)" contributed to endanger antibiotics (Kim, Kim and Rhee, 2019). Indeed, bacteria belonging to the so-called ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter* species) are of particular concern as they are resistant to many available antibiotics (Mulani *et al.*, 2019; De Oliveira *et al.*, 2020). Furthermore, it has to be highlighted that microorganisms within biofilms result even more recalcitrant to antibiotics with respect to free-floating planktonic cells (Roy *et al.*, 2018). This is mainly due to the presence of the extracellular matrix that acts as a physical barrier, to modifications of metabolic processes and to the presence of the so called "persister" cells, dormant cells that form spontaneously within a biofilm and that are highly recalcitrant to antimicrobial challenge (Hall and Mah, 2017). Indeed, to kill bacteria within biofilm, higher amounts of antibiotics (*i.e.,* from 10 to 1,000 times more) are required with respect to the antimicrobial doses commonly used to kill planktonic bacterial cells, with the main disadvantage that the efficacy is not

guaranteed (Haney et al., 2018). Despite this dramatic situation, economic investments in the discovery of novel antibiotics decreased over the last decades, since financial returns are likely to be limited. Because of this scenario, novel effective systemic antibacterial agents able to cure infections associate to multidrug resistant strains and to biofilm formation are urgently needed.

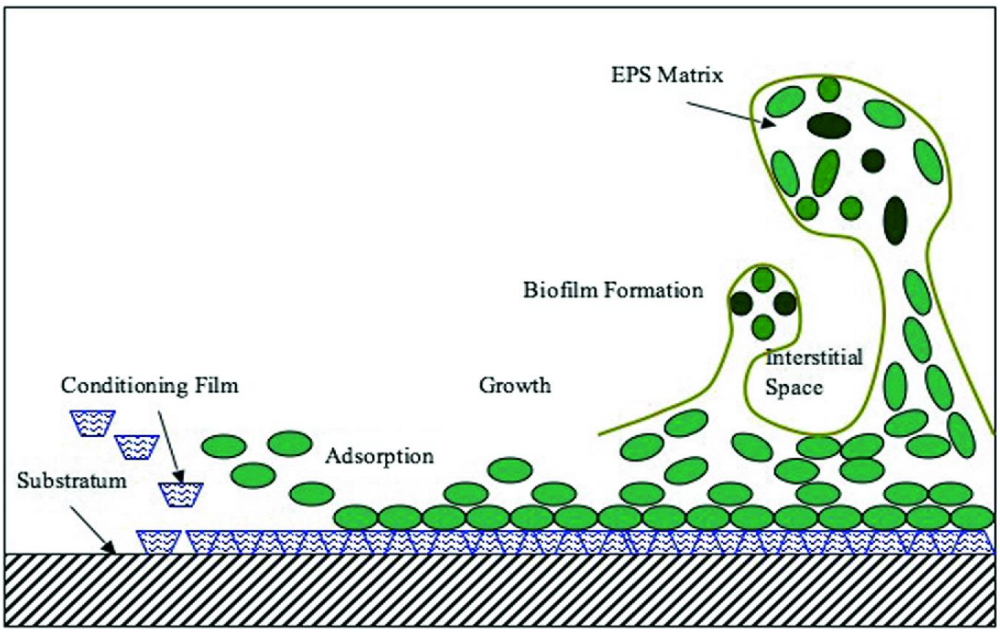

Figure 2. Main stages of biofilm formation on medical devices (Carvalho *et al.*, 2021)**.**

1.3 Infections of medical devices resistant to conventional antibiotics

Failure of medical devices due to microbial contamination and subsequent biofilm formation is difficult to deal with because of biofilm resilience to conventional antimicrobial treatments and for the fast spread of antibiotic resistance (Sun *et al.*, 2015). The development of anti-infective biomaterials, able to resist or to prevent bacterial colonization, has progressively become an interesting strategy to effectively threat infections affecting medical devices and to mitigate the related complications (Campoccia, Montanaro and Arciola, 2013; Olmo *et al.*, 2020). Indeed, Food and Drug Administration (FDA) approved an increasing number of anti-infective biomaterials in the last decades (Francolini *et al.*, 2017; Liang, Wang and Libera, 2019). To

date, different approaches have been explored to design biomaterials endowed with “self-defensive” properties (Bruellhoff *et al.*, 2010; Benčina *et al.*, 2018). Such strategies can be classified into two large main groups: active and passive (Lichter, Van Vlietpa and Rubner, 2009) (Figure 3). The passive strategies are based on the use of “antifouling surfaces” that are able to repel bacteria adhesion without killing bacterial cells deposited on the surface (Keum *et al.*, 2017). This strategy is based on the optimization of the physico-chemical properties of the materials, with the aim to reduce attractive forces between bacteria and surface components (Alves and Olívia Pereira, 2014a). Bacteria repelling is realized through three mechanisms: (i) steric, (ii) electrostatic and (iii) superhydrophobic, that can be observed in hydrophilic, charged and superhydrophobic surfaces, respectively (Yeo *et al.*, 2012; Olmo *et al.*, 2020). Conversely, the “active” strategies are represented by contact-killing or biocide-leaching surfaces and allow the direct killing of bacteria upon contact or in the peri-implant area, respectively (Râpă *et al.*, 2013). In first case, the antimicrobial agent can be either covalently immobilized or physically adsorbed on the surface of the device, whereas, in the latter case, the antimicrobial agent is ionically loaded/entrapped within the bulk of the material and subsequently released from the device into the surrounding tissue (Pihl *et al.*, 2021). Antifouling surfaces can be obtained *via* chemical modification. This is the case of hydrophilic polymers, such as poly(ethylene glycol) (PEG) (Francolini et al., 2017) chains that, being highly hydrated, create a steric hindrance to bacteria attachment. Indeed, when bacteria approach to the surface, a compression of the hydrated layer occurs, with a consequent decrease of conformational entropy of polymer chains and the development of repulsive elastic forces (Yang and Deng, 2008; Lopez-Mila *et al.*, 2018). The efficiency of this kind of coatings can be enhanced by increasing the density of the graft polymer and the polymer chain length (Gunkel-Grabole *et al.*, 2016). Passive strategies also comprise “superhydrophobic surfaces” characterized by a water contact angle value (θ) higher than 150° and by the ability to form a barrier between bacteria and biomaterials, thus preventing a direct contact between them (Zhang, Wang and Levänen, 2013). An example is represented by superhydrophobic coatings for dental applications that have been prepared by applying fluoroalkylated acrylic acid oligomers into resin composite substrates and that have been found to be endowed with interesting anti-infective properties (Francolini *et al.*, 2017). As an example, Yang and Deng produced a superhydrophobic paper by multi-layer deposition of polydiallyldimethylammonium chloride and silica particles on cellulose

fibres, followed by a fluorination surface treatment; the obtained biomaterial was found to be effective in preventing bacteria adhesion (Yang and Deng, 2008). A further example is represented by a biomaterial obtained by the deposition of TiO_2 nanotubes on wettable Ti surfaces followed by functionalization with fluoroalkyl silane. Also in this case, electron microscopy analyses revealed the ability of the obtained biomaterial to prevent bacteria adhesion (Lin et al., 2011). Layer by layer (LbL) is a methodology that was found to be useful to prepare superhydrophobic coatings (Olmo *et al.*, 2020). Zwitterionic polymers, such as poly(carboxybetaine) (PCB), poly(phosphataine) (PPB) and poly(sulfobetaine) (PSB), have been recently investigated for their ability to create a hydrated layer characterized by "superhydrophilicity", thus effectively preventing the adhesion of proteins and bacteria. For example, glass surfaces coated with zwitterionic poly(carboxybetaine methacrylate) were found to be able to interfere with *P. aeruginosa* biofilm accumulation on the surface for 240 h (Cheng et al., 2009). Among the antibacterial agents most commonly used to produce active bactericidal surfaces, they are included quaternary ammonium compounds (QACs) (Rauner *et al.*, 2018), antimicrobial enzymes (AMEs), antibiotics, metal ions and antimicrobial peptides (AMPs) (He *et al.*, 2017; Drexelius and Neundorf, 2021; Li *et al.*, 2021). As an example, polyhexamethylene guanidine hydrochloride (PHGH), a cationic antimicrobial oligomer, has been chemical grafted onto a starch carrier and incorporated into Polylactic acid (PLA) polymers to produce an active antimicrobial material (Ojogbo, Ward and Mekonnen, 2020). In several studies, the coating with polydopamine (pDA) has been adopted. Indeed, under oxidative and alkaline conditions, dopamine is able to self-polymerize, thus forming a polymer film (polydopamine) on different types of material surfaces and acting as a platform for the immobilization of active compounds (Alves and Olívia Pereira, 2014b; Andrea, Molchanova and Jenssen, 2018; Balaure and Grumezescu, 2020). Bone implants have been coated with cephalothin-releasing hydroxyapatite (HAP), in order to both prevent infections and to promote bone ingrowth (Forsgren *et al.*, 2012). To prevent implant-associated osteomyelitis caused by methicillin-resistant *S. aureus* (MRSA), a polymer-lipid encapsulation matrix (PLEX) has been used as a nanoplatform to allow a controlled release and local delivery of doxycycline (DOX) (Metsemakers *et al.*, 2015). Catheters impregnated with combinations of conventional antibiotics were found to display a long-term antimicrobial activity against *P. mirabilis*, *S. aureus*, and *E. coli* bacterial strains (Fisher *et al.*, 2015). An antimicrobial and biodegradable coating for surgical sutures has

been prepared by loading in poly lactic-co-glycolic acid (PLGA) polymers antibacterial totarol, a naturally occurring diterpenoid isolated from *Podocarpus totara.* The obtained biomaterial was found to be able to inhibit the growth of *Staphylococcus aureus* over a period of 15 days (Reinbold *et al.*, 2017). The antimicrobial properties of silver ions and silver nanoparticles are well-known and, for this reason, this material has been widely used to produce antibacterial surfaces (Fu, Reinhold and Woodbury, 2011; Green, Fulghum and Nordhaus, 2011; Cooper, Pollini and Paladini, 2016; Burdușel *et al.*, 2018). However, it has to be highlighted that, although some of these strategies have resulted appropriate for specific applications, there is an urgent need to develop and identify broad spectrum antimicrobials able to prevent bacterial colonization of biomaterials, with low cytotoxicity and negligible propensity to develop bacterial resistance (Costa *et al.* 2011). In the search for compounds that satisfy the aforementioned criteria, AntiMicrobial Peptides (AMPs) gained great interest.

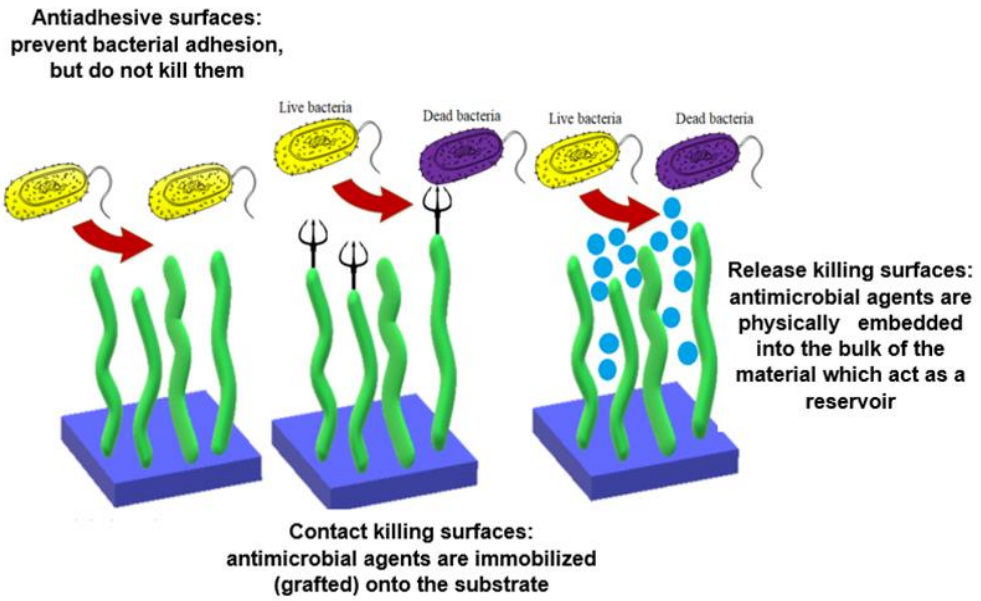

Figure 3. Schematic representation of the strategies employed to confer anti-infective properties to surfaces (Balaure and Grumezescu, 2020).

1.4 AntiMicrobial Peptides (AMPs)

Nowadays, researchers are intensively focused on the identification of novel and non-conventional anti-infective therapies, including adjunctive or preventive approaches, such as antibodies targeting a virulence factor, probiotics, vaccines, and phage therapy (Sillankorva, Pereira and Henriques, 2019). Interestingly, AntiMicrobial Peptides (AMPs) have rapidly captured the attention as novel drug candidates (Czaplewski *et al.*, 2016) in a scenario characterized by the fast spread of antibiotic resistance and worsened by the slow-down of the development of newer antibiotics (Moazzezy *et al.*, 2020; Qian *et al.,* 2020; Sarkar, Chetia and Chatterjee, 2021). AMPs are evolutionary conserved molecules virtually produced by all forms of life from prokaryotes to humans (Andrès and Dimarcq, 2001). In complex organisms, AMPs act as a first line of defence against pathogens. In bacteria, produced AMPs play a key role in the killing of other bacteria, thus allowing to different strains to compete for the same ecological niche (Hassan *et al.*, 2012). Besides being endowed with direct antimicrobial activity against both Gram-positive and Gram-negative bacterial strains, fungi (Kang *et al.*, 2017) and viruses (Jenssen, 2009), AMPs have been also found to be endowed with antibiofilm properties (Pletzer, Coleman and Hancock, 2016; Hancock, Alford and Haney, 2021). Moreover, the huge interest in AMPs is also due to their ability to display immunomodulatory activities (Hancock, Haney and Gill, 2016). Because of this extension of functionalities, AMPs have been also referred to as 'Host Defence Peptides' (HDPs). Indeed, HDPs have been found to be able to modulate the activation of pro-inflammatory responses and to improve the clearance of bacterial biofilms by host immune system by mitigating the derangement of immune responses after the implantation of a foreign body (Riool *et al.*, 2017). Naturally occurring HDPs are small bioactive peptides with a length ranging from 12 to 50 (or more) amino acids. They are predominantly cationic because of their high content in lysine and arginine residues, and they have been found to contain about 50% hydrophobic amino acids (Hassan *et al*., 2012). HDPs can be classified into four major groups according to their structures: β-sheet (*e.g.,* human α and β defensins), α-helical (*e.g*., LL-37, magainins and mellitin), loop peptides with one disulfide bridge (*e.g*., bactenecin), and peptides enriched in specific amino acid, such as proline, tryptophan histidine or glycine without a well-defined structure (*e.g*., indolicidin) (Mookherjee, Chow and Hancock, 2012). Cationic AMPs selectively

interact with negatively charged prokaryotic membranes that represent the key target of their direct antimicrobial activity (Mookherjee *et al*., 2020) Moreover, it has been reported that some AMPs can be translocated into bacterial cells where they may interact with multiple cytosolic targets, thus interfering with vital cellular processes, such as cell wall biogenesis, protein synthesis, protein folding, enzymatic activity (Scocchi *et al*., 2016). Given to AMPs peculiar mechanism of action, the development of resistance phenotype is highly improbable, since it would require a deep reorganization of bacterial membrane, a "costly" process for most microbial species (Nguyen, Haney and Vogel, 2011,) (Brogden, 2005). The mechanism at the basis of AMPs interaction with membranes has been extensively studied and several models have been proposed, such as 'carpet' model, 'barrel-stave' model, and the 'toroidal-pore' model (E., A. Aguilar and D., 2011). In the case of carpet model, peptides assemble and cover as a carpet the surface of target membranes, thus causing the collapse of the cell membrane in a "detergent-like" manner with formation of micelles (Huan *et al.*, 2020). In the barrel-stave model, peptides interact with each other on the surface of the plasma membrane and aggregate, thus forming pores into the membrane (Raheem and Straus, 2019). According to toroidal-pore model, instead, peptides orient parallelly to the plane of the plasma membrane and locate closely to the region of phospholipid polar heads in a functionally inactive state. When AMPs concentration reaches a critical value, peptides aggregation occurs, thus determining membrane curvature and toroidal pores formation (Figure 4).

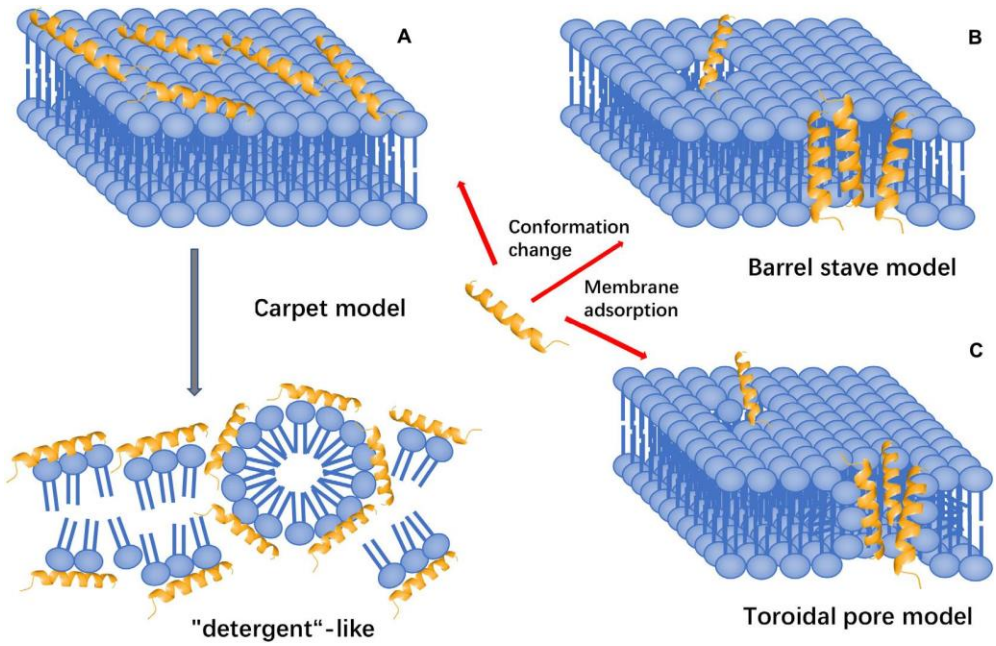

Figure 4. Representation of AMPs mechanisms of interaction with bacterial membranes (Huan *et al.*, 2020)**.**

1.5 Cryptides

In 2006, the term "cryptome" has been introduced to describe a unique subset of proteins acting as precursors of bioactive peptides named "cryptides" (Autelitano *et al.*, 2006). Indeed, different kinds of proteins involved in a wide range of cellular processes, such as haemoglobin, thrombin, lactoferrin, lysozyme, histone-like proteins, vertebrate secretory ribonucleases, apolipoproteins, cytochromes, laminins, and collagen, act as a source of functional peptides (Kobori *et al.*, 2007). Identified cryptides have been classified into three classes. Cryptides with related or increased properties with respect to precursor proteins belong to class 2, whereas those possessing completely unrelated biological functions belong to class 1. The third class of cryptides is represented by peptide molecules generated *in vitro* and endowed with novel bioactivities with respect to the precursor protein (Gaglione, Pizzo, *et al.*, 2020). Due to the increasing interest towards cryptides as novel putative antimicrobial agents, several approaches have been investigated to discover novel cryptides, such as high-throughput diagnostic screening, mass spectrometry-based proteomic analyses, and computational biology techniques (Messana *et al*., 2013).

Furthermore, many antimicrobial cryptides have been obtained from a mixture of peptides upon digestion of the “precursor” protein with one or more proteases (Papareddy *et al.*, 2010). Other antimicrobial cryptides have been discovered on the basis of their homology with already known antimicrobial cryptides. This is the case of the cryptides from the C-terminus of S1 proteinases (Kasetty *et al.*, 2011). Notably, several research groups have developed *in silico* methods to identify AMPs sequences in protein precursors (Wang *et al.*, 2011; Falcao *et al.*, 2016; Santos *et al.*, 2021)(Santos *et al.*, 2021). Recently, a novel bioinformatic tool has been set up (Pane *et al.* 2017), and it allows to both localize cryptic AMPs within a protein sequence and to estimate their antimicrobial potency. The method is based on the finding that the antimicrobial efficacy of an AMP is directly proportional to a score – named “absolute score” (AS) – that can be calculated on the basis of net charge (C), hydrophobicity (H) and length (L) of the peptide and on the basis of two bacterial strain-dependent variables defining the relative contribution of charge and hydrophobicity to peptide antimicrobial activity. By using this algorithm, the entire human proteome has been scanned to identify putative antimicrobial peptides ranging from 8 to 50 residues in length (Torres *et al.*, 2021). This method has allowed the discovery of hidden antimicrobial peptides in the sequences of human apolipoproteins, such as Apolipoprotein B (Gaglione et al., 2017) and Apolipoprotein E (Zanfardino et al., 2018). Upon the identification of a putative antimicrobial region in human Apolipoprotein B, our research group recombinantly produced three versions of the identified HDP, named r(P)ApoB$_L^{Pro}$, r(P)ApoB$_S^{Pro}$ and r(P)ApoB$_L^{Ala}$. The term “(P)” is related to the presence a Pro residue at the N-terminus of each recombinant peptide due to the release, during the purification process, of the peptide from the carrier protein upon acidic hydrolysis of an Asp-Pro bond. Apices “Pro” and “Ala” refer, instead, to the amino acid present in position seven of the peptide, being Ala the most common amino acid present in human ApoB,and being Pro present in an isoform of ApoB (Gaglione *et al.*, 2021). A cost-effective production strategy was set-up (Gaglione *et al.*, 2019), and the three recombinant ApoB-derived peptides, once purified, were extensively characterized. They have been found to be endowed with antibacterial and anti-biofilm properties against both Gram-positive and Gram-negative bacterial strains and towards resistant bacterial strains clinically isolated from cystic fibrosis (CF) patients (Gaglione, Cesaro, *et al.*, 2020). Furthermore, peptides were found to be neither toxic, when tested on murine and human cells, nor hemolytic when tested on murine erythrocytes (Gaglione, R. *et al.*, 2017). All the three ApoB-

derived HDPs were also found to be able to act in synergism with either commonly used antibiotics or EDTA, to exert immunomodulatory and wound-healing properties (Gaglione, R. *et al.*, 2017), and to be endowed with antifungal properties and with the ability to prevent chicken meat samples contaminations when immobilized on chitosan films (Dell'Olmo *et al.*, 2021).

1.6 Aims of the Thesis

The main goal of the present Thesis has been the investigation of the applicability of ApoB-derived peptides to functionalize medical devices surfaces, thus preventing and counteracting their infection. Aims and obtained results are described through the chapters of the Thesis as indicated below:

Chapter 1 provides a general introduction on the state of the art and describes the main findings obtained for the host defence peptides selected for the experimental work;

Chapter 2 describes the functionalization of polydimethylsiloxane (PDMS), the material commonly used to manufacture urinary catheters, with the selected antimicrobial peptide; furthermore, it is described the characterization of the obtained biomaterial that has been analysed for its anti-infective and biocompatibility properties;

Chapter 3 describes the functionalization of polyhydroxyethylmethacrylate (pHEMA)-hydrogel contact lenses with selected ApoB-derived peptide and the characterization of the properties of the obtained material;

Chapter 4 comprises a general discussion on the main obtained findings at the light of the most recent data published in the literature and general conclusions derived from the present research are also clearly described.

References

Alanis, A. J. (2005) 'Resistance to antibiotics: Are we in the post-antibiotic era?', *Archives of Medical Research*, 36(6), pp. 697–705. doi: 10.1016/j.arcmed.2005.06.009.

Alves, D. and Olívia Pereira, M. (2014a) 'Mini-review: Antimicrobial

peptides and enzymes as promising candidates to functionalize biomaterial surfaces', *Biofouling*, 30(4), pp. 483–499. doi: 10.1080/08927014.2014.889120.

Alves, D. and Olívia Pereira, M. (2014b) 'Mini-review: Antimicrobial peptides and enzymes as promising candidates to functionalize biomaterial surfaces', *Biofouling*, 30(4), pp. 483–499. doi: 10.1080/08927014.2014.889120.

Aminov, R. I. (2010) 'A brief history of the antibiotic era: Lessons learned and challenges for the future', *Frontiers in Microbiology*, 1(DEC). doi: 10.3389/fmicb.2010.00134.

Andrea, A., Molchanova, N. and Jenssen, H. (2018) 'Antibiofilm peptides and peptidomimetics with focus on surface immobilization', *Biomolecules*, 8(2). doi: 10.3390/biom8020027.

Andrès, E. and Dimarcq, J. L. (2001) 'Cationic antimicrobial peptides: Towards clinical applications', *Antibiotiques*, 3(4), pp. 201–205.

Arciola, C. R., Campoccia, D. and Montanaro, L. (2018) 'Implant infections: Adhesion, biofilm formation and immune evasion', *Nature Reviews Microbiology*, 16(7), pp. 397–409. doi: 10.1038/s41579-018-0019-y.

Autelitano, D. J. *et al.* (2006) 'The cryptome: a subset of the proteome, comprising cryptic peptides with distinct bioactivities', *Drug Discovery Today*, 11(7–8), pp. 306–314. doi: 10.1016/j.drudis.2006.02.003.

Balaure, P. C. and Grumezescu, A. M. (2020) *Recent advances in surface nanoengineering for biofilm prevention and control. Part i: Molecular basis of biofilm recalcitrance. passive anti-biofouling nanocoatings*, *Nanomaterials*. doi: 10.3390/nano10061230.

Benčina, M. *et al.* (2018) 'The Importance of Antibacterial Surfaces in Biomedical Applications', *Advances in Biomembranes and Lipid Self-Assembly*, 28, pp. 115–165. doi: 10.1016/bs.abl.2018.05.001.

Blair, J. M. A. *et al.* (2015) 'Molecular mechanisms of antibiotic resistance', *Nature Reviews Microbiology*, 13(1), pp. 42–51. doi: 10.1038/nrmicro3380.

Brogden, K. A. (2005) 'Antimicrobial peptides: Pore formers or metabolic inhibitors in bacteria?', *Nature Reviews Microbiology*, 3(3), pp. 238–250. doi: 10.1038/nrmicro1098.

Bruellhoff, K. *et al.* (2010) 'Surface coating strategies to prevent biofilm

formation on implant surfaces', *International Journal of Artificial Organs*, 33(9), pp. 646–653. doi: 10.1177/039139881003300910.

Bryers, J. D. (2008) 'Medical biofilms', *Biotechnology and Bioengineering*, 100(1), pp. 1–18. doi: 10.1002/bit.21838.

Burdușel, A. C. *et al.* (2018) 'Biomedical applications of silver nanoparticles: An up-to-date overview', *Nanomaterials*, 8(9), pp. 1–25. doi: 10.3390/nano8090681.

Campoccia, D., Montanaro, L. and Arciola, C. R. (2013) 'A review of the biomaterials technologies for infection-resistant surfaces', *Biomaterials*, 34(34), pp. 8533–8554. doi: 10.1016/j.biomaterials.2013.07.089.

Carvalho, F. M. *et al.* (2021) 'The use of probiotics to fight biofilms in medical devices: A systematic review and meta-analysis', *Microorganisms*, 9(1), pp. 1–26. doi: 10.3390/microorganisms9010027.

Cassini, A. *et al.* (2019) 'Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis', *The Lancet Infectious Diseases*, 19(1), pp. 56–66. doi: 10.1016/S1473-3099(18)30605-4.

Cheng, G. *et al.* (2009) 'Zwitterionic carboxybetaine polymer surfaces and their resistance to long-term biofilm formation', *Biomaterials*, 30(28), pp. 5234–5240. doi: 10.1016/j.biomaterials.2009.05.058.

Cooper, I. R. (2015) 'Introduction to biomaterials and medical device-associated infections', *Biomaterials and Medical Device - Associated Infections*, pp. 3–17. doi: 10.1533/9780857097224.1.3.

Cooper, I. R., Pollini, M. and Paladini, F. (2016) 'The potential of photo-deposited silver coatings on Foley catheters to prevent urinary tract infections', *Materials Science and Engineering C*, 69, pp. 414–420. doi: 10.1016/j.msec.2016.07.004.

Crnich, C. J. and Drinka, P. (2012) 'Medical Device-Associated Infections in the Long-Term Care Setting', *Infectious Disease Clinics of North America*, 26(1), pp. 143–164. doi: 10.1016/j.idc.2011.09.007.

Czaplewski, L. *et al.* (2016) 'Alternatives to antibiotics-a pipeline portfolio review', *The Lancet Infectious Diseases*, 16(2), pp. 239–251. doi: 10.1016/S1473-3099(15)00466-1.

Darouiche, R. O. (2001) 'Device-associated infections: A macroproblem that starts with microadherence', *Clinical Infectious*

Diseases, 33(9), pp. 1567–1572. doi: 10.1086/323130.

Davies, J. (1996) 'Origins and evolution of antibiotic resistance.', *Microbiología (Madrid, Spain)*, 12(1), pp. 9–16. doi: 10.1128/mmbr.00016-10.

Debabov, D. (2013) 'Antibiotic resistance: Origins, mechanisms, approaches to counter', *Applied Biochemistry and Microbiology*, 49(8), pp. 665–671. doi: 10.1134/S0003683813080024.

Drexelius, M. G. and Neundorf, I. (2021) 'Application of Antimicrobial Peptides on Biomedical Implants : Three Ways to Pursue Peptide Coatings'.

E., J., A. Aguilar, A. O.-Z. J. and D., P. (2011) *Antimicrobial Peptides: Diversity and Perspectives for Their Biomedical Application*, *Biomedical Engineering, Trends, Research and Technologies*. doi: 10.5772/13058.

Falcao, L. L. *et al.* (2016) 'Antimicrobial properties of two novel peptides derived from Theobroma cacao osmotin', *Peptides*, 79, pp. 75–82. doi: 10.1016/j.peptides.2016.03.006.

Fisher, L. E. *et al.* (2015) 'Biomaterial modification of urinary catheters with antimicrobials to give long-term broadspectrum antibiofilm activity', *Journal of Controlled Release*, 202, pp. 57–64. doi: 10.1016/j.jconrel.2015.01.037.

Forsgren, J. *et al.* (2012) 'Co-loading of bisphosphonates and antibiotics to a biomimetic hydroxyapatite coating To cite this version : HAL Id : hal-00665450 Co-loading of bisphosphonates and antibiotics to a biomimetic hydroxyapatite coating'.

Francolini, I. *et al.* (2017) 'Antifouling and antimicrobial biomaterials: an overview', *Apmis*, 125(4), pp. 392–417. doi: 10.1111/apm.12675.

Fu, J., Reinhold, J. and Woodbury, N. W. (2011) 'Peptide-modified surfaces for enzyme immobilization', *PLoS ONE*, 6(4), pp. 2–7. doi: 10.1371/journal.pone.0018692.

Gaglione, R. *et al.* (2017) 'Novel human bioactive peptides identified in Apolipoprotein B: Evaluation of their therapeutic potential', *Biochemical Pharmacology*, 130, pp. 34–50. doi: 10.1016/j.bcp.2017.01.009.

Gaglione, R. *et al.* (2019) 'Cost-effective production of recombinant peptides in Escherichia coli', *New Biotechnology*, 51(December 2017), pp. 39–48. doi: 10.1016/j.nbt.2019.02.004.

Gaglione, R., Cesaro, A., *et al.* (2020) 'Cryptides identified in human apolipoprotein b as new weapons to fight antibiotic resistance in cystic fibrosis disease', *International Journal of Molecular Sciences*, 21(6). doi: 10.3390/ijms21062049.

Gaglione, R., Pizzo, E., *et al.* (2020) 'Host Defence Cryptides from Human Apolipoproteins: Applications in Medicinal Chemistry', *Current Topics in Medicinal Chemistry*, 20(14), pp. 1324–1337. doi: 10.2174/1568026620666200427091454.

Gaglione, R. *et al.* (2021) 'Impact of a single point mutation on the antimicrobial and fibrillogenic properties of cryptides from human apolipoprotein B', *Pharmaceuticals*, 14(7). doi: 10.3390/ph14070631.

Green, J.-B. D., Fulghum, T. and Nordhaus, M. a (2011) 'Immobilized antimicrobial agents: a critical perspective', *Science against microbial pathogens: communicating current research and technological advances*, (3), pp. 84–98.

Gunkel-Grabole, G. *et al.* (2016) 'PEG Brushes on Porous, PDMS-Coated Surfaces and Their Interaction with Carbon Dioxide', *Macromolecular Chemistry and Physics*, 217(8), pp. 966–973. doi: 10.1002/macp.201500450.

Hall, C. W. and Mah, T. F. (2017) 'Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria', *FEMS Microbiology Reviews*, 41(3), pp. 276–301. doi: 10.1093/femsre/fux010.

Hancock, R. E. W., Alford, M. A. and Haney, E. F. (2021) 'Antibiofilm activity of host defence peptides: complexity provides opportunities', *Nature Reviews Microbiology*, 0123456789. doi: 10.1038/s41579-021-00585-w.

Hancock, R. E. W., Haney, E. F. and Gill, E. E. (2016) 'The immunology of host defence peptides: Beyond antimicrobial activity', *Nature Reviews Immunology*, 16(5), pp. 321–334. doi: 10.1038/nri.2016.29.

Haney, E. F. *et al.* (2018) 'Critical assessment of methods to quantify biofilm growth and evaluate antibiofilm activity of host defence peptides', *Biomolecules*, 8(2), pp. 1–22. doi: 10.3390/biom8020029.

Hassan, M. *et al.* (2012) 'Natural antimicrobial peptides from bacteria: Characteristics and potential applications to fight against antibiotic resistance', *Journal of Applied Microbiology*, 113(4), pp. 723–736. doi: 10.1111/j.1365-2672.2012.05338.x.

He, J. *et al.* (2017) 'Immobilization of an antimicrobial peptide on silicon surface with stable activity by click chemistry', *Journal of Materials Chemistry B*, 6(1), pp. 68–74. doi: 10.1039/c7tb02557b.

Huan, Y. *et al.* (2020) 'Antimicrobial Peptides: Classification, Design, Application and Research Progress in Multiple Fields', *Frontiers in Microbiology*, 11(October), pp. 1–21. doi: 10.3389/fmicb.2020.582779.

Jenssen, H. (2009) 'Therapeutic approaches using host defence peptides to tackle herpes virus infections', *Viruses*, 1(3), pp. 939–964. doi: 10.3390/v1030939.

Jorge, P. *et al.* (2019) 'Antimicrobial resistance three ways: Healthcare crisis, major concepts and the relevance of biofilms', *FEMS Microbiology Ecology*, 95(8), pp. 1–17. doi: 10.1093/femsec/fiz115.

Kang, H. K. *et al.* (2017) 'The therapeutic applications of antimicrobial peptides (AMPs): a patent review', *Journal of Microbiology*, 55(1), pp. 1–12. doi: 10.1007/s12275-017-6452-1.

Kasetty, G. *et al.* (2011) 'The C-terminal sequence of several human serine proteases encodes host defense functions', *Journal of Innate Immunity*, 3(5), pp. 471–482. doi: 10.1159/000327016.

Keum, H. *et al.* (2017) 'Prevention of bacterial colonization on catheters by a one-step coating process involving an antibiofouling polymer in water', *ACS Applied Materials and Interfaces*, 9(23). doi: 10.1021/acsami.7b06899.

Kim, S. A., Kim, H. W. and Rhee, M. S. (2019) 'Cranberry extract with enhanced bactericidal activities against uropathogenic Escherichia coli within one minute of treatment', *Lwt*, 113(June), p. 108318. doi: 10.1016/j.lwt.2019.108318.

Kobori, T. *et al.* (2007) 'Biopolymers Volume 85 / Number 4 295', *Biopolymers*, 85(4), pp. 392–406. doi: 10.1002/bip.

de Kraker, M. E. A., Stewardson, A. J. and Harbarth, S. (2016) 'Will 10 Million People Die a Year due to Antimicrobial Resistance by 2050?', *PLoS Medicine*, 13(11), pp. 1–6. doi: 10.1371/journal.pmed.1002184.

Li, B. and Webster, T. J. (2018) 'Bacteria antibiotic resistance: New challenges and opportunities for implant-associated orthopedic infections', *Journal of Orthopaedic Research*, 36(1), pp. 22–32. doi: 10.1002/jor.23656.

Li, W. *et al.* (2021) 'Surface Design for Antibacterial Materials: From

Fundamentals to Advanced Strategies', *Advanced Science*, 8(19), pp. 1–23. doi: 10.1002/advs.202100368.

Liang, J., Wang, H. and Libera, M. (2019) 'Biomaterial surfaces self-defensive against bacteria by contact transfer of antimicrobials', *Biomaterials*, 204(December 2018), pp. 25–35. doi: 10.1016/j.biomaterials.2019.03.006.

Lichter, J. A., Van Vlietpa, K. J. and Rubner, M. F. (2009) 'Design of antibacterial surfaces and interfaces: Polyelectrolyte multilayers as a multifunctional platform', *Macromolecules*, 42(22), pp. 8573–8586. doi: 10.1021/ma901356s.

Lin, C. *et al.* (2011) 'Effect of superhydrophobic surface of titanium on staphylococcus aureus adhesion', *Journal of Nanomaterials*, 2011. doi: 10.1155/2011/178921.

Lopez-Mila, B. *et al.* (2018) 'Effect of shear stress on the reduction of bacterial adhesion to antifouling polymers', *Bioinspiration and Biomimetics*, 13(6). doi: 10.1088/1748-3190/aadcc2.

Messana, I. *et al.* (2013) 'Unraveling the different proteomic platforms', *Journal of Separation Science*, 36(1), pp. 128–139. doi: 10.1002/jssc.201200830.

Metsemakers, W. J. *et al.* (2015) 'A doxycycline-loaded polymer-lipid encapsulation matrix coating for the prevention of implant-related osteomyelitis due to doxycycline-resistant methicillin-resistant Staphylococcus aureus', *Journal of Controlled Release*, 209, pp. 47–56. doi: 10.1016/j.jconrel.2015.04.022.

Michael, W. (2002) 'Bacterial Adhesion : Seen Any Good Biofilms Lately ? Bacterial Adhesion : Seen Any Good Biofilms Lately ?', *Clinical Microbiology Reviews*, 15(2), pp. 155–166. doi: 10.1128/CMR.15.2.155.

Mihai, M. M. *et al.* (2015) 'Send Orders for Reprints to reprints@benthamscience.ae Microbial Biofilms: Impact on the Pathogenesis of Periodontitis, Cystic Fibrosis, Chronic Wounds and Medical Device-Related Infections', *Current Topics in Medicinal Chemistry*, 15, pp. 1552–1576.

Moazzezy, N. *et al.* (2020) 'Inhibition and eradication activity of truncated α-defensin analogs against multidrug resistant uropathogenic Escherichia coli biofilm', *PLoS ONE*, 15(7), pp. 1–13. doi: 10.1371/journal.pone.0235892.

Mookherjee, N. *et al.* (2020) 'Antimicrobial host defence peptides: functions and clinical potential', *Nature Reviews Drug Discovery*, 19(5), pp. 311–332. doi: 10.1038/s41573-019-0058-8.

Mookherjee, N., Chow, L. N. Y. and Hancock, R. E. W. (2012) 'Immunomodulatory cationic peptide therapeutics: A new paradigm in infection and immunity', *ACS Symposium Series*, 1095, pp. 1–19. doi: 10.1021/bk-2012-1095.ch001.

Motta, S. S., Cluzel, P. and Aldana, M. (2015) 'Adaptive resistance in bacteria requires epigenetic inheritance, genetic noise, and cost of efflux pumps', *PLoS ONE*, 10(3). doi: 10.1371/journal.pone.0118464.

Mulani, M. S. *et al.* (2019) 'Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: A review', *Frontiers in Microbiology*, 10(APR). doi: 10.3389/fmicb.2019.00539.

Nguyen, L. T., Haney, E. F. and Vogel, H. J. (2011) 'The expanding scope of antimicrobial peptide structures and their modes of action', *Trends in Biotechnology*, 29(9), pp. 464–472. doi: 10.1016/j.tibtech.2011.05.001.

Nobile, C. J. and Mitchell, A. P. (2007) 'Microbial biofilms: e pluribus unum', *Current Biology*, 17(10), pp. 349–353. doi: 10.1016/j.cub.2007.02.035.

Ojogbo, E., Ward, V. and Mekonnen, T. H. (2020) 'Functionalized starch microparticles for contact-active antimicrobial polymer surfaces', *Carbohydrate Polymers*, 229(October 2019), p. 115422. doi: 10.1016/j.carbpol.2019.115422.

De Oliveira, D. M. P. *et al.* (2020) 'Antimicrobial resistance in ESKAPE pathogens', *Clinical Microbiology Reviews*, 33(3). doi: 10.1128/CMR.00181-19.

Olmo, J. A. Del *et al.* (2020) 'Antibacterial coatings for improving the performance of biomaterials', *Coatings*, 10(2). doi: 10.3390/coatings10020139.

Organization, W. H. (2018) *WHO Report on Surveillance of Antibiotic Consumption*, *Who*. Available at: https://apps.who.int/iris/bitstream/handle/10665/277359/9789241514880-eng.pdf.

Pane, K. *et al.* (2017) 'Antimicrobial potency of cationic antimicrobial peptides can be predicted from their amino acid composition:

Application to the detection of “cryptic” antimicrobial peptides’, *Journal of Theoretical Biology*, 419(June 2016), pp. 254–265. doi: 10.1016/j.jtbi.2017.02.012.

Papareddy, P. *et al.* (2010) ‘Proteolysis of human thrombin generates novel host defense peptides.’, *PLoS pathogens*, 6(4). doi: 10.1371/journal.ppat.1000857.

Phillips, K. S., Patwardhan, D. and Jayan, G. (2015) ‘Biofilms, medical devices, and antibiofilm technology: Key messages from a recent public workshop’, *American Journal of Infection Control*, 43(1), pp. 2–3. doi: 10.1016/j.ajic.2014.09.019.

Pihl, M. *et al.* (2021) ‘Osseointegration and antibacterial effect of an antimicrobial peptide releasing mesoporous titania implant’, *Journal of Biomedical Materials Research - Part B Applied Biomaterials*, 109(11), pp. 1787–1795. doi: 10.1002/jbm.b.34838.

Pletzer, D., Coleman, S. R. and Hancock, R. E. W. (2016) ‘Anti-biofilm peptides as a new weapon in antimicrobial warfare’, *Current Opinion in Microbiology*, 33, pp. 35–40. doi: 10.1016/j.mib.2016.05.016.

Qian, Y. *et al.* (2020) ‘Using in Vivo Assessment on Host Defense Peptide Mimicking Polymer-Modified Surfaces for Combating Implant Infections’, *ACS Applied Bio Materials*. doi: 10.1021/acsabm.0c01066.

Raheem, N. and Straus, S. K. (2019) ‘Mechanisms of Action for Antimicrobial Peptides With Antibacterial and Antibiofilm Functions’, *Frontiers in Microbiology*, 10(December), pp. 1–14. doi: 10.3389/fmicb.2019.02866.

Ramasamy, M. and Lee, J. (2016) ‘Recent nanotechnology approaches for prevention and treatment of biofilm-associated infections on medical devices’, *BioMed Research International*, 2016. doi: 10.1155/2016/1851242.

Râpă, M. *et al.* (2013) ‘Preparation of medical devices with antimicrobial properties’, *Journal of Optoelectronics and Advanced Materials*, 15(7–8), pp. 807–816.

Rauner, N. *et al.* (2018) ‘A Coating that Combines Lotus-Effect and Contact-Active Antimicrobial Properties on Silicone’, *Advanced Functional Materials*, 28(29), pp. 1–9. doi: 10.1002/adfm.201801248.

Reinbold, J. *et al.* (2017) ‘Preventing surgical site infections using a natural, biodegradable, antibacterial coating on surgical sutures’,

Molecules, 22(9), pp. 1–15. doi: 10.3390/molecules22091570.

Reviews, B. O. O. K. (1998) '1232 B O O K REVIEWS November', 84(2), pp. 1232–1234.

Riool, M. *et al.* (2017) 'Antimicrobial peptides in biomedical device manufacturing', *Frontiers in Chemistry*, AUG(2017), pp. 1–13. doi: 10.3389/fchem.2017.00063.

Roy, R. *et al.* (2018) 'Strategies for combating bacterial biofilms: A focus on anti-biofilm agents and their mechanisms of action', *Virulence*, 9(1), pp. 522–554. doi: 10.1080/21505594.2017.1313372.

Santos, B. P. O. *et al.* (2021) 'Schistocins: Novel antimicrobial peptides encrypted in the Schistosoma mansoni Kunitz Inhibitor SmKI-1', *Biochimica et Biophysica Acta - General Subjects*, 1865(11). doi: 10.1016/j.bbagen.2021.129989.

Sarkar, T., Chetia, M. and Chatterjee, S. (2021) 'Antimicrobial Peptides and Proteins: From Nature's Reservoir to the Laboratory and Beyond', *Frontiers in Chemistry*, 9(June). doi: 10.3389/fchem.2021.691532.

Scocchi, M. *et al.* (2016) 'Send Orders for Reprints to reprints@benthamscience.ae Non-Membrane Permeabilizing Modes of Action of Antimicrobial Peptides on Bacteria', *Current Topics in Medicinal Chemistry*, 16, pp. 76–88.

Sillankorva, S., Pereira, M. O. and Henriques, M. (2019) *Editorial: Antibiotic alternatives and combinational therapies for bacterial infections*, *Frontiers in Microbiology*. doi: 10.3389/fmicb.2018.03359.

Sun, D. *et al.* (2015) 'Antimicrobial materials with medical applications', *Materials Technology*, 30(B2), pp. B90–B95. doi: 10.1179/1753555714Y.0000000239.

Torres, M. D. T. *et al.* (2021) 'Mining for encrypted peptide antibiotics in the human proteome', *Nature Biomedical Engineering*. doi: 10.1038/s41551-021-00801-1.

Umscheid, C. A. *et al.* (2011) 'Estimating the Proportion of Healthcare-Associated Infections That Are Reasonably Preventable and the Related Mortality and Costs', *Infection Control & Hospital Epidemiology*, 32(2), pp. 101–114. doi: 10.1086/657912.

Veerachamy, S. *et al.* (2014) 'Bacterial adherence and biofilm formation on medical implants: A review', *Proceedings of the Institution of Mechanical Engineers, Part H: Journal of Engineering in Medicine*,

228(10), pp. 1083–1099. doi: 10.1177/0954411914556137.

Wang, P. *et al.* (2011) 'Prediction of antimicrobial peptides based on sequence alignment and feature selection methods', *PLoS ONE*, 6(4). doi: 10.1371/journal.pone.0018476.

Wang, W. *et al.* (2018) 'Antibiotic resistance : a rundown of a global crisis', *Infection and Drug Resistance*, pp. 1645–1658.

Wernli, D. *et al.* (2017) 'Antimicrobial resistance: The complex challenge of measurement to inform policy and the public', *PLoS Medicine*, 14(8), pp. 1–9. doi: 10.1371/journal.pmed.1002378.

Yang, H. and Deng, Y. (2008) 'Preparation and physical properties of superhydrophobic papers', *Journal of Colloid and Interface Science*, 325(2), pp. 588–593. doi: 10.1016/j.jcis.2008.06.034.

Yeo, I. S. *et al.* (2012) 'Implant surface factors and bacterial adhesion: A review of the literature', *International Journal of Artificial Organs*, 35(10), pp. 762–772. doi: 10.5301/ijao.5000154.

Zanfardino, A. *et al.* (2018) 'Human apolipoprotein E as a reservoir of cryptic bioactive peptides: The case of ApoE 133-167', *Journal of Peptide Science*, 24(7). doi: 10.1002/psc.3095.

Zhang, X., Wang, L. and Levänen, E. (2013) 'hesionSuperhydrophobic surfaces for the reduction of bacterial ad', *RSC Advances*, 3(30), pp. 12003–12020. doi: 10.1039/c3ra40497h.

Zhang, Z. and Wagner, V. E. (2017) 'Antimicrobial coatings and modifications on medical devices', *Antimicrobial Coatings and Modifications on Medical Devices*, (March 2018), pp. 1–273. doi: 10.1007/978-3-319-57494-3.

Zimmerli, W., Lew, P. D. and Waldvogel, F. A. (1984) 'Pathogenesis of foreign body infection. Evidence for a local granulocyte defect', *Journal of Clinical Investigation*, 73(4), pp. 1191–1200. doi: 10.1172/JCI111305.

Zimmerli, W. and Sendi, P. (2011) 'Pathogenesis of implant-associated infection: The role of the host', *Seminars in Immunopathology*, 33(3), pp. 295–306. doi: 10.1007/s00281-011-0275-7.

CHAPTER 3

HEMA hydrogel contact lenses functionalized with an ApoB-derived antimicrobial peptide

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1.Introduction

Contact lenses (CLs) are medical devices generally used to correct vision problems, such as astigmatism, presbyopia, farsightedness, and myopia. It has been estimated that worldwide over 200 million people wear contact lens (Efron *et al.*, 2013). However, their beneficial effects are accompanied by the development of cornea infections associated to their use. It has to be noticed that this is a common drawback associated to the employment of almost all medical devises (Arciola, Campoccia and Montanaro, 2018). Indeed, biomaterials surface favour the adhesion of microorganisms that, in the case of contact lenses, may then reach cornea surface (Zimmerman, Nixon and Rueff, 2016). Under physiological conditions, cornea is able to protect itself by producing chemokines, cytokines, antimicrobial peptides and surfactants (Fleiszig *et al.*, 2020). However, when CLs are inserted into the eyes, the innate mechanisms of defence are hindered. In these conditions, proteins, glycoproteins and lipids present in the tear film rapidly accumulate on CLs surface, thus creating a suitable environment for microorganisms' attachment. The risk to develop CLs-associated infections increases when these devices are extensively worn (Xiao *et al.*, 2018).

It has to be highlighted that bacteria adhesion on medical devices is generally followed by biofilm development (Garrett, Bhakoo and Zhang, 2008). Biofilm infections are difficult to menage, since bacteria embedded in self-produced extracellular matrix (Bryers, 2008) are characterized by a lower susceptibility to the effects of conventional antibiotics and of the immune system cells (De la Fuente-Núñez *et al.*, 2013). Bacterial contaminations of CLs are generally accompanied by adverse events, such as Contact Lens-Induced Acute Red Eyes (CLARE), Contact Lens Peripheral Ulcers (CLPU), and Infiltrative Keratitis (IK) (Fleiszig *et al.*, 2020), generally due to inflammatory complications. It has also to be noticed that microbial keratitis is a rare serious infection of the cornea which, if untreated, may cause corneal injury and vision loss (Zimmerman, Nixon and Rueff, 2016). Different types of microorganisms may be responsible for ocular complications. Among them, the most common are *Pseudomonas aeruginosa* and *Staphylococcus aureus*; furthermore, cases of fungal and *Acanthamoeba* contamination have been also reported (Khan and Lee, 2020). Ocular diseases, such as cataract, dry eye, conjunctivitis, glaucoma, and infections, are generally treated by topical administration of proper drugs as eye drops, even if the method has several limitations (Bertens *et al.*, 2018). Indeed, it has been reported

that drug bioavailability is very low (in the range of 5%–10%) (Hughes *et al.*, 2005). Moreover, it has been described that several factors, such as tear flow, lachrymal drainage and blinking, are responsible for a very short (1-3 min) permanence of eye drops on eye surface (Maulvi, Soni and Shah, 2016). Hence, in order to reach the required therapeutic doses or to treat chronic diseases, frequent applications of concentrated drug drops are required. This has adverse effects, since the use of concentrated solutions can determine fluctuations of drug amount in the tear film with the concomitant presence, in different eye areas, of toxic and subtherapeutic levels of the drug (Alvarez-Lorenzo *et al.*, 2019). In the case of the application of antibiotic eye drops, this might have very severe effects, since the misuse and exposition to sublethal doses promote the raise of antibiotic-resistance microorganisms (Farkouh, Frigo and Czejka, 2016). In the last decades, the use of contact lenses as a drug delivery system is increasing thanks to the possibility to overcome some of the limitations associated to other topical ophthalmic therapies (Weissman *et al.*, 2017; Haider, Haider and Kang, 2018). Indeed, the use of contact lenses allows to extend the residence time of drugs on eye surface to more than 30 min by entrapping the drug in the lachrymal fluid between the lens and the cornea, thus increasing drug bioavailability on the cornea up to 50%. Treatment of glaucoma through drug delivery *via* contact lenses showed an efficiency 10 times higher than that obtained through eye drops instillation (Silva *et al*., 2020). The first use of contact lenses as a potential platform for drug delivery dates back to 1965 (González-Chomón, Concheiro and Alvarez-Lorenzo, 2013). In particular, soft contact lenses (SLCs) based on HEMA hydrogel have become very popular due to their peculiar properties, such as easy preparation, transparency, high water content, drug loading possibilities and high biocompatibility (Efron *et al.*, 2013). CLs might act as pre and/or post-surgical corneal bandages to improve healing processes (Jacobs *et al.*, 2021). It has been reported that wearing therapeutic bandage CLs after cataract surgery significantly improves the comfort feeling and the tear film, thus promoting corneal healing (Xiao *et al.*, 2018). In addition, the use of CLs as drug delivery system could provide a pharmaceutical treatment simultaneously with a refractive error correction. Based on reported evidence, CLs loaded with antimicrobial agents might be used to avoid infections. Indeed, some in *vitro* and in *vivo* studies have already been performed on antibiotic-loaded contact lenses, that were found to be able to prevent bacterial keratitis provoked by infections associated to *S. aureus* and *S. epidermidis* (Hui, Boone and Jones, 2008; Maulvi *et al.*, 2018).

Noteworthy, due to the rapid increase of infections caused by microorganisms resistant to conventional antibiotics, a great interest has been devoted to novel antimicrobial agents (Wernli *et al.*, 2017). Among them, Antimicrobial Peptides (AMPs) are gaining great attention because of their broad spectrum antimicrobial activity, being active against Gram-positive and Gram-negative bacteria, protozoa, fungi, and viruses (Michael Zasloff, 2002). Several mechanisms have been proposed to explain their mode of action (not yet completely understood). In particular, cationic antimicrobial peptides are able to selectively interact with negatively charged bacterial membranes determining destabilization, pore formation and cell lysis. AMPs have been also reported to be able to penetrate bacterial cells, and to bind to various organelles, thus interfering with different cell functions (Zhang and Gallo, 2016). Furthermore, despite from conventional antibiotics, their use is generally not associated to the development of resistance phenotype, since this would require a deep reorganization of bacterial membrane.

Here, the possibility to create AMPs-loaded CLs has been investigated, with the main purpose to prevent bacterial contamination of CLs surfaces and to treat ocular infections (Sultana, Luo and Ramakrishna, 2021). To this purpose, the antimicrobial peptide named r(P)ApoB$_L^{Pro}$, recently identified in the sequence of human Apolipoprotein B (ApoB) by using a bioinformatic tool (Gaglione *et al.*, 2017a) has been recombinantly produced to be immobilized on CLs. To do this, disks of pHEMA (poly-2-Hydroxyethyl methacrylate) hydrogel alone and copolymerized with hydrophilic monomers, *i.e*., methacrylic acid (MAA) and 2-Acrylamido-2-methylpropane sulfonic acid (AMPSA), have been prepared. The use of MAA and AMPSA has the main goal to introduce monomers (named functional monomers) able to establish non-covalent interactions with the drug, thus increasing the affinity of the hydrogel network for the drug. In this case, anionic monomers have been selected because of the cationic nature of the antimicrobial peptide under test. The peptide was loaded into CLs by the soaking method that represents a simple and cost-effective strategy based on the immersion of hydrogel disks in a peptide solution with a proper concentration (Xu *et al.*, 2018). Once obtained functionalized CLs, their optical properties and their capability of drug loading/release were investigated. Afterwards, antimicrobial properties of functionalized CLs were also evaluated towards two of the most common bacterial strains responsible for ocular infections (Guo *et al.*, 2020).

2. Methods and materials

2.1 Materials

2-Hydroxyethyl methacrylate (HEMA) was supplied by Merck (Darmstadt, Germany). 2-Acrylamido-2-methylpropane sulfonic acid (AMPSA), ethylene glycol dimethacrylate (EGDMA), dichlorodimethylsilane, 2,20-azobis(2-methylpropionitrile) (AIBN), and methacrylic acid (MAA) were from Sigma-Merck (Steinheim, Germany). Sodium chloride (NaCl) was from Scharlau (Barcelona, Spain), and absolute ethanol 99.9% was from VWR Chemicals (Leuven, Belgium). Ultrapure water (resistivity >18.2MΩ cm) was obtained by reverse osmosis (MilliQ®, Millipore Ibérica, Madrid, Spain). r(P)ApoB$_L^{Pro}$ antimicrobial peptide was recombinantly produced in bacteria cells as previously described (Gaglione *et al.*, 2017a).

2.2 Hydrogel Preparation

In order to prepare CLs, monomers solutions were obtained at room temperature under magnetic stirring (400 rpm) by using EGDMA as cross-linker and AIBN as initiator. To obtain H2 (average weight approximatively 14 mg) and H3 (average weight approximatively 10 mg), functional monomer was added (MAA and AMPSA, respectively). The monomer solutions were injected (25 G needle) into molds composed by glass plates (12 X 14 cm) previously treated with dichlorodimethylsilane and fixed with 0.20 mm Teflon frame. To obtain H3 solution, glass plates were also separated by using plastic sheets. The polymerization was carried out at 50 ºC for 12 hrs and at 70 ºC for other 24 hrs. After polymerization, hydrogel sheets were demolded by injecting a small amount of water into the molds with a syringe, washed in 1 L of boiling distilled water for 15 min, in order to remove unreacted monomers, and cut with punches into 10 mm size disks.

Hydrogel pieces were washed alternatively in MilliQ® water and 0.9 % NaCl at room temperature and by replacing the medium two or three times *per* day under magnetic stirring (200 rpm). The cleaning process was carried out until the complete removal of unreacted monomers was reached. This was monitored by measuring the absorbance of aliquots of the washing medium (UV–Vis spectrophotometer Agilent 8453, Waldbronn, Germany). Finally, hydrogel samples were dried at 70 ºC for 24 hrs.

Table 1. Hydrogel composition*

Code	HEMA (mL)	MAA (µL)	AMPSA (g)	EGDMA (µL)	DMSO (mL)	AIBN (g)
H1	4	0	0	6.04	0.5	0.0082
H2	4	68.9	0	6.04	0.5	0.0082
H3	4	0	0.166	6.04	0.5	0.0082

* HEMA: 2-hydroxyethyl methacrylate; MAA: methacrylic acid; AMPSA: 2-Acrylamido-2-methylpropane sulfonic acid; EGDMA: ethyleneglycol dimethacrylate; DMSO: Dimethyl sulfoxide; AIBN:2,20-azobis(2-methylpropionitrile

2.3 Silanization of glassware

Peptide solutions were always placed in silanized glass, in order to avoid peptide adsorption to glass surface. All glass items were immersed in 2% v/v dimethyldichlorosilane in chloroform for 1 min. After that, the glassware was removed from the solution, placed on a paper towel in a hood, and dried overnight. Then, items were transferred to an oven at 70 °C for 2 hrs. Finally, the glassware was washed by using soap and deionized water and dried to air.

2.4 Hydrogel Characterization

2.4.1 Water uptake

Dried hydrogel disks were weighted (W_0) and placed in 2 mL of water or 0.5 mL of peptide solution (160 µM, in water) at room temperature. The experiment was performed in triplicates.

At predefined time intervals, hydrogels were removed from the solution, carefully wiped with absorbent paper, and weighted (W_t). The water uptake (W_U %) was calculated as follows:

$$Water\ uptake(\%) = \frac{Wt - Wo}{Wo} \times 100$$

2.4.2 Analysis of transmittance

Experiments to determine the transmittance of disks hydrated in water and in peptide solution (160 µM, in water) were performed in triplicate, in a range between 200–800 nm (UV–Vis spectrophotometer, Agilent 8453, Boeblingen, Germany).

2.4.3 Analysis of Young's modulus

Disks (10 mm) of HEMA hydrogel swollen in water or in peptide solution were fixed at room temperature to the upper and lower clamps (gap 4 mm) of a TA. XT Plus Texture Analyzer (Stable Micro Systems, Ltd., Surrey, UK) was fitted with a 5 Kg load cell. Stress–strain plots were recorded at a crosshead speed of 0.1 mm · s^{-1} at least in triplicate. Young's modulus was calculated from the slope of the linear portion of the stress *versus* strain curves.

2.5 Peptide production

Expression and isolation of the recombinant r(P)ApoB$_L^{Pro}$ peptide was carried out as previously described (Gaglione *et al.*, 2017b) with the only exception of a final gel-filtration step, that was added in order to remove salts used along the purification process and that tend to attach to the peptides.

2.6 Peptide loading and release experiments

Peptide loading and release analyses were carried out by using HEMA-hydrogel-dried disks, here indicated as H1 (average weight approximatively15mg), H2 (average weight approximatively 14 mg), and H3 (average weight approximatively 10 mg). To determine peptide loading, disks were placed in Eppendorf tubes and soaked into water solutions (0.5 mL) containing ApoB-derived peptide at two different concentrations (80 µM or 160 µM). Mixtures were then kept at 4 °C for 24 hrs. Following incubation, the amount of loaded peptide was determined by keeping 50 µL aliquots from each Eppendorf tube and determining the amount of not loaded peptide. This allowed to estimate the percentage of peptide loading. The test was carried out in quadruplicate. Release experiments were then carried out by placing the peptide-loaded disks (previously rinsed with 0.9% NaCl, in order to remove the excess of peptide) in vials containing 2 mL of 0.9% NaCl saline solution. The vials were then kept at 37 °C under stirring (180 rpm). At defined time intervals, aliquots of 100 µL were taken from each vial, in order to quantify peptide amount, and replaced with the same volume of fresh medium (to maintain sink conditions and avoid false plateaus). Analyses were conducted in triplicate. Samples were collected every hour for the first 8 hrs, and then once a day. Peptide amount (loaded and released) in collected samples was quantified by reversed-phase high-performance liquid chromatography (RP-HPLC). HPLC experiments were carried out by using a JASCO (Tokyo, Japan) HPLC (AS-4140 Autosampler, PU-4180 Pump, LC-NetII/ADC Interface

Box, CO-4060 Column Oven, MD-4010 Photodiode Array Detector). A Europa Protein 300 C18 column (5 µm, 25×1) from Teknokroma (Barcelona, Spain) was used. The mobile phase consisted of 50 % (v/v) 0.05% trifluoroacetic acid (TFA) in water (solvent A) and 50% (v/v) 0.05% TFA in acetonitrile (solvent B). Analyses were conducted at 37 °C with a flow rate of 2 mL/min. An injection volume of 20 µL was used and the eluent was detected at 214 nm. Peptide retention time was found to be 5.3 min. r(P)ApoB$_L^{Pro}$ amount was calculated by using a calibration curve prepared with solutions of pure peptide dissolved in NaCl 0.9% characterized by increasing concentration values (2.5-80 µM in NaCl 0.9%). The dilution effect due to medium replacement was considered when analysing the cumulative release curves.

2.7 Bacterial strains and growth conditions

Two bacterial strains were used in the present study, *i.e*., *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* (MRSA WKZ-2). Both bacterial strains were grown in Muller Hinton Broth (MHB, Becton Dickinson Difco, Franklin Lakes, NJ) and on Luria Bertani Agar (LBA; Oxoid Ltd., Hampshire, UK). In all the experiments, bacteria were inoculated and grown over-night in Muller Hinton Broth (MHB) at 37 °C. The next day, bacteria were transferred to a fresh MHB tube and grown to mid-logarithmic phase.

2.8 Evaluation of antimicrobial activity

H2 disks, composed by HEMA hydrogel functionalized with methacrylic acid, were sterilized by UV treatment for 15 min and then soaked in peptide solutions characterized by two different concentrations of r(P)ApoB$_L^{Pro}$ (80 -160 µM) for 24 hrs. Following incubation, disks were washed, in order to remove the excess of peptide, and incubated with bacterial solutions that were prepared starting from a single colony of *P. aeruginosa* ATCC 27853 or *S. aureus methicillin-resistant* (MRSA WKZ-2) that was transferred into 5 mL of MHB and grown at 37 °C to mid-log phase. Afterwards, 1 mL of bacterial suspension was diluted in 0.5X NB at a density of 1 x 10^3 CFU mL^{-1} and then added upon each disk at 37 °C for 16 hrs. Following incubation, bacterial supernatant was transferred from each well to a sterile Eppendorf tube, diluted and plated for the evaluation of planktonic cells growth. Each disk was rinsed with 1 mL of sterile 0.9% NaCl, in order to remove non-adherent bacteria that were transferred into a sterile Eppendorf tube containing 1 mL of HBSS. Adherent bacteria were, instead, detached by vortexing for 1 min, sonicating by ultrasounds for 3 min in a water bath, vortexing again for 1 min and centrifuging at 10,000 rpm for 5 min. Upon dilution,

100 µL of each sample were deposited on LBA plates as duplicates. Plates were incubated at 37 °C for 16 hrs and colonies were counted.

3. Results

3.1 HEMA-based CLs preparation

In Table 1, the composition of all the prepared and designed hydrogel disks, named H1, H2 and H3, is reported. They have all been obtained by a free-radical polymerization reaction. Specifically, H1 was produced by polymerization of HEMA monomer, while H2 and H3 have been obtained by co-polymerization of HEMA monomer with functional monomers, *i.e*., methacrylic acid (MAA) and 2-Acrylamido-2-methylpropane sulfonic acid (AMPSA), respectively. Upon polymerization, hydrogel sheets were cut, in order to obtain disks mimicking contact lenses shape.

3.2. Analysis of HEMA-based CLs swelling properties

Swelling of hydrogel disks in water and in peptide solution was monitored at room temperature by recording weight increase of the disks at defined time intervals. In all the cases, dried disks rapidly swelled in water by reaching equilibrium status in 30 min. Swelling degree was estimated to be about 50% in the case of H1 and around 80% in the case of H2 and H3. It is well-known that the presence of functional monomers is responsible for an increase in water content of HEMA hydrogel, with a consequent higher permeability to oxygen with respect to conventional hydrogels (Musgrave and Fang, 2019). Notably, the presence of ApoB-derived peptide r(P)ApoB$_L^{Pro}$ within hydrogel matrix is responsible for a very slight decrease of water uptake (Figure 1).

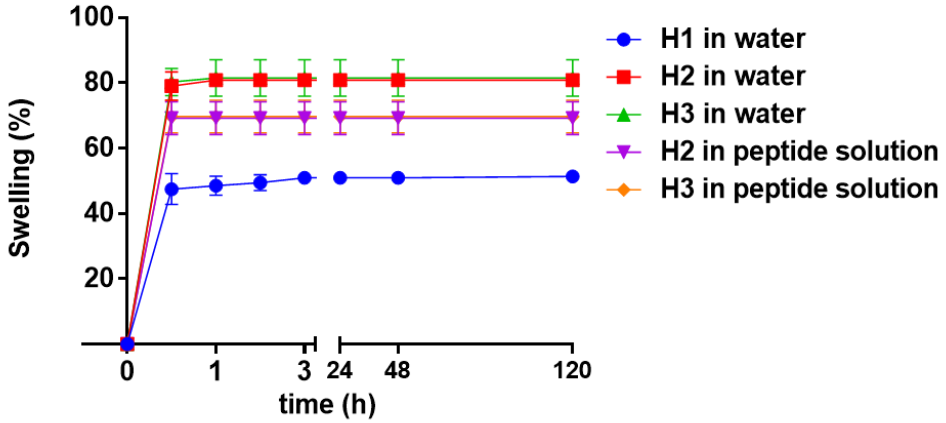

Figure1. Swelling degree of HEMA-based hydrogel disks in water and in peptide solution.

3.3 Analysis of HEMA-based CLs light transmittance

All hydrated hydrogels were transparent in the visible range (around 600 nm), with transmittance values above 90% (Mun *et al.*, 2019). No significant differences in light transmittance were observed for the different kinds of analysed hydrogels. Interestingly, the presence of the drug did not alter light transmittance in the visible region (Table 2).

3.4 Analysis of HEMA-based CLs Young's modulus

The Young's modulus of commercially available hydrogel contact lenses is typically in the range of 0.3-0.6 MPa. A Young's modulus exceeding this values indicates a risk of corneal inflammatory disease (Chang *et al.*, 2020). In Table 2, Young's modulus registered for all the kinds of HEMA-based hydrogel disks are reported. Values were found to be in the range accepted for commercial soft CLs (Kim, Saha and Ehrmann, 2018). Furthermore, the presence of the peptide in H2 and H3 hydrogels didn't determine an alteration of this parameter (Table 2).

Table 2.
Properties of not loaded hydrogels and of hydrogels loaded with the selected ApoB-derived peptide.

Hydrogel code	Transmittance (%)	Young's Modulus (MPa)
H1	96.56 ± 2.38	0.44 ± 0.04
H2	95.96 ± 1.31	0.43 ± 0.03
H2 - r(P)ApoB$_L^{Pro}$	94.70 ± 1.77	0.45 ± 0.05
H3	95.09 ± 0.92	0.59 ± 0.012
H3 - r(P)ApoB$_L^{Pro}$	92.05 ± 0.57	0.55 ± 0.02

3.5 Analysis of peptide loading and release from HEMA-based CLs

To load HEMA-based disks with r(P)ApoB$_L^{Pro}$ peptide, disks were dried and immersed in the peptide solution at two different concentrations (80 and 160 µM) for 24 hrs at 4 °C. Temperature value was selected to preserve peptide stability. No uptake of peptide was evaluated in the case of HEMA-based hydrogels not containing functional monomers (H1). Conversely, in the case of HEMA-based hydrogels H2 and H3, a complete absorption of the peptide was observed under the experimental conditions tested for both peptide concentrations (80 and 160 µM). A similar result was obtained for both H2 and H3 hydrogels. Loading was monitored by performing HPLC analyses of peptide solution in contact with HEMA-based hydrogels at the end of 24 hrs incubation; precisely, after this period of time no presence of peptide was detected in the loading solution. Once obtained loaded HEMA-based CLs, peptide release was evaluated. To this purpose, loaded disks were rinsed with 0.9% NaCl and immersed in 2 mL of 0.9% NaCl at 37 °C in agitation at 180 rpm. At defined time intervals, aliquots of peptide solutions in contact with the disks were taken, in order to quantify peptide amount. By this way, a slow and progressive release of the peptide from the disks was observed over the first 8 hrs, with the maximum amount of released peptide detected after 24 hrs. In both cases (H2 and H3), the total amount of released peptide was found to be proportional to initially loaded peptide amount. Indeed, in both cases, the percentage of peptide release was found to be about 10% of initial peptide amount (**Figure 2A and B**). No burst release was observed in both cases. Altogether, these results indicate that strong electrostatic and ionic interactions occur between negatively charged hydrogel network and positively charged peptide molecules. It is also noteworthy

that peptide appears degraded after 72 hrs. Since peptide degradation was found to be higher in the case of H3 disks, only H2 disks were selected to perform further analyses.

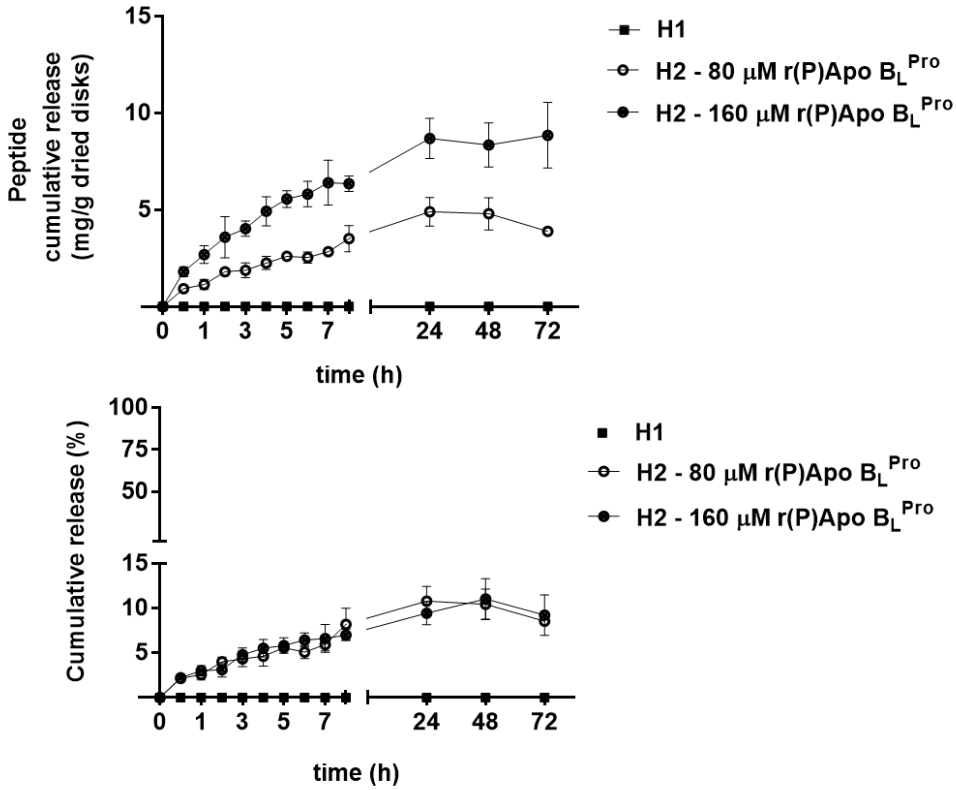

Figure 2. Cumulative release of r(P)ApoB$_L^{Pro}$ from hydrogel disks reported as mg of peptide related to the weight (in mg) of the disk (**A**) and as percentage values related to the total initially loaded peptide amount (**B**).

3.6 Analyses of the antimicrobial activity of HEMA-based CLs

HEMA-based hydrogels functionalized with methacrylic acid (H2) and loaded with ApoB-derived antimicrobial peptide were analysed for their

antimicrobial properties. To this purpose, loaded and non-loaded H2 disks were incubated with two bacterial strains, such as *P. aeruginosa* ATCC 27853 and *S. aureus* methicillin-resistant (MRSA WKZ-2) selected as prototypes of Gram-negative and Gram-positive bacterial strains responsible for ocular infections (Bourcier *et al.*, 2003; Agi *et al.*, 2021). After 16hrs of incubation, viability of microbial cells was evaluated by analysing both bacterial cells in the medium surrounding the disks and those attached to the surface of the disks. In all the cases, no significant differences were detected between control (non-loaded disks) and loaded disks (80 and 160 μM) (**Figure 3A and B**). For this reason, further experiments will be performed by loading H2 disks with higher amount of Apo-derived antimicrobial peptide.

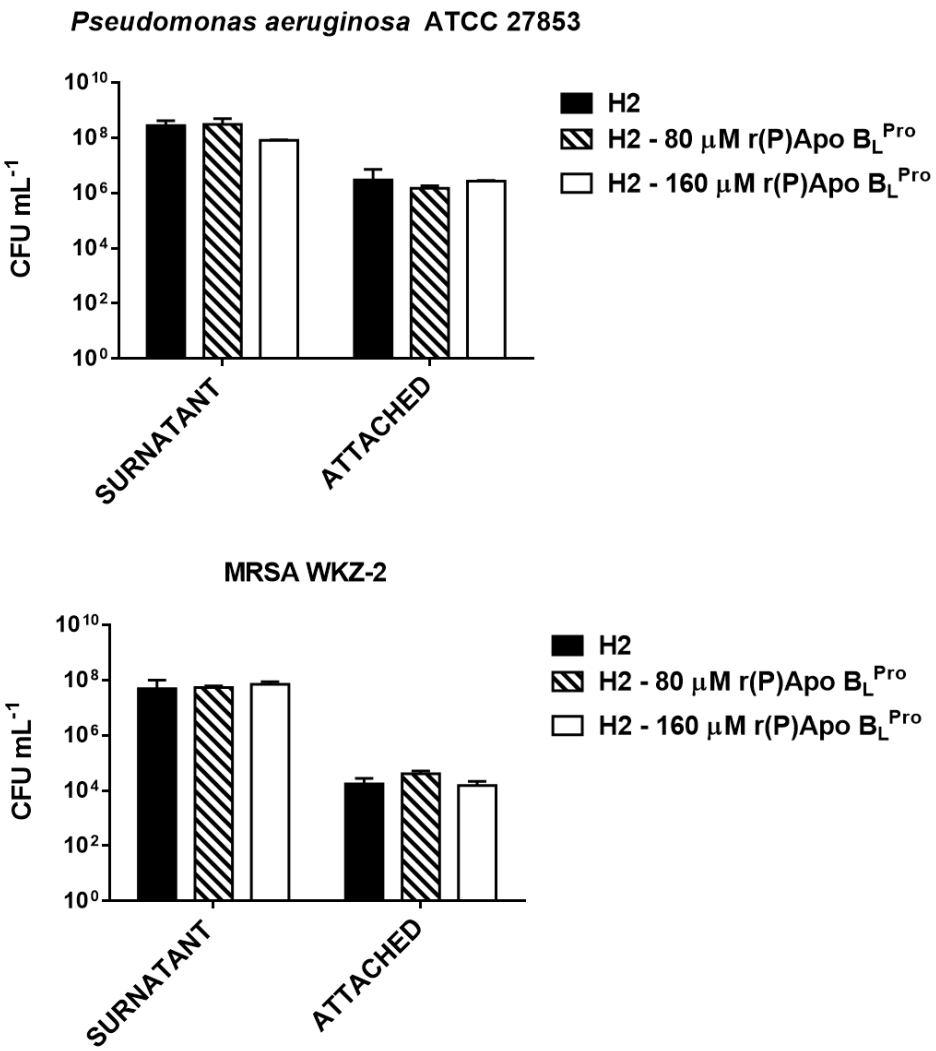

Figure 3. Evaluation of the antimicrobial activity of peptide-loaded HEMA/MAA (H2) disks by colonies counting of bacterial cells present in the supernatant surrounding the disks and of those attached to disks surface after 16hrs of incubation with *P. aeruginosa* ATCC 27853 (**A**) or with *S. aureus* methicillin-resistant (MRSA WKZ-2) (**B**) bacterial strains.

4. Discussion

Ocular infections generally cause pain and sensitivity to light and, if left untreated, might lead to damage of eye physiological structures, with consequent visual impairments till blindness depending on the severity of the infection. Common treatments involve frequent applications of antibiotic eye drops (Zidan *et al.*, 2018). These drug formulations show several disadvantages, such as limited bioavailability due to eye barriers, improper absorption and nasolacrimal drainage, with consequent significant (≈ 95%) drug losses (Souza *et al.*, 2014). For this reason, more efficient drug delivery approaches are required to obtain proper drug administration. Soft contact lenses (SCLs) are attracting considerable attention not only as a precious tool to correct mild ametropia but also as a suitable system to efficiently deliver drug molecules to the anterior segment of the eye (Rykowska, Nowak and Nowak, 2021). Hydrogel-based SCLs are widely used to correct eye refractive problems, since they are well tolerated even after a prolonged contact with wearer eye (Filipe *et al.*, 2016). Because of these properties, SCLs appear also a suitable system to deliver antimicrobial peptides (AMPs) able to counteract ocular infections. Despite their great potentialities and their ability to overcome antibiotic resistance phenomenon, AMPs applicability is limited by several factors (Sarkar, Chetia and Chatterjee, 2021) that could be addressed by using a proper system to deliver them to the site of infection (Nordström and Malmsten, 2017). In the present Thesis, HEMA-based hydrogel disks have been prepared with the main aim to obtain a system for the controlled delivery of the ApoB-derived AMP under test to the anterior chamber of the eye, in order to treat ocular infections (Yang *et al.*, 2018). To do this, HEMA-based hydrogel disks have been prepared by functionalization with suitable monomers, selected to allow an improvement of the properties of HEMA, the most common material employed to manufacture contact lenses (Nesic *et al.*, 2016; Weissman *et al.*, 2017). Methacrylic acid (MAA), the second main component of commercially available CLs, has been selected to be used as co-monomer with HEMA (Efron *et al.*, 2020). Furthermore, 2-Acrylamido-2-methylpropane sulfonic acid (AMPS) has been also selected for its properties, such as stability, non-toxicity and cheapness. It contains a hydrophilic sulfonic acid (SO_2) functional group and a non-ionic amide group in its structure, and has been largely employed in medicine, bioengineering, and catalysis (Matsumura *et al.*, 2008; Anirudhan and Rejeena, 2014). In this work, pHEMA/MAA hydrogel polymers were loaded with r(P)ApoB$_L^{Pro}$ antimicrobial peptide that was found to be completely adsorbed by the disks, in agreement with previously reported studies (Alvarez-Lorenzo

et al., 2002; Alvarez-Lorenzo, Hiratani and Concheiro, 2006; Forbes *et al.*, 2013). This could be explained with the formation of electrostatic and ionic interactions between positively charged peptide molecules and negatively charged co-monomers. In agreement with this, it was also demonstrated that hydrogel matrix strongly retains the peptide. Indeed, only a very small percentage (less than 10%) of initially loaded total amount of the peptide was found to be release by the matrix after 24 hrs incubation independently from peptide concentration. Once obtained HEMA-based hydrogel disks loaded with ApoB-derived antimicrobial peptide, their main properties, such as water uptake, transmittance and Young´s modulus, have been analyzed. Collected results clearly indicate that obtained loaded CLs are endowed with properties that satisfy the standards required for CLs commercialization. Indeed, all the collected values are comprised in the range recommended to avoid discomfort and visual quality affection during CLs use (Silva *et al.*, 2021), thus indicating that the presence of the peptide doesn't affect CLs properties. Despite these promising results, when peptide-loaded disks were analysed for their antimicrobial ability *in vitro*, no significant antimicrobial properties and no differences between loaded and non-loaded disks were detected. For this reason, further experiments will be performed in the future by preparing CLs loaded with significantly higher amounts of ApoB-derived antimicrobial peptide. It has to be highlighted that ApoB-derived peptides were previously characterized and found to be endowed with direct antimicrobial activity against a wide spectrum of Gram-positive and Gram-negative bacterial strains at very low concentrations, being MIC_{100} values comprised in the range of 5-20 µM (Gaglione *et al.*, 2017b, 2020). Although the exact mechanism of action of antimicrobial peptides has not been fully elucidate yet, it is known that a key event is represented by the selective electrostatic interaction between positively charged AMP molecules and negatively charged bacterial membranes, an event leading to membrane destabilization responsible for bacterial cells death (Costa *et al.*, 2011). Since ApoB-derived peptides have been found to exert their bactericidal activity after a few hours of incubation with target cells (Gaglione *et al.*, 2019, 2021), it is plausible that the low peptide amount released by CLs is not enough to exert a strong antimicrobial activity. Indeed, bacterial cells escaping peptide activity would continue to grow exponentially (Fujikawa, Kai and Morozumi, 2004), thus making ineffective the killing of a small portion of the cells due the low amount of released peptide. In order to overcome this problem, different kinds of material could be employed, such as silicone-based hydrogel characterized by a more hydrophobic

nature that could allow a greater peptide release. Hence, further studies will be performed in the future to select a hydrogel composition able to allow a burst release of the selected peptide, with the consequent delivery to the site of infection of a proper amount of the peptide, able to completely kill bacterial cells, thus efficiently counteracting infections.

References

Agi, J. *et al.* (2021) 'Three Decades of Contact Lens-associated Microbial Keratitis in a Referral Hospital in São Paulo, Brazil', *Arquivos Brasileiros de Oftalmologia*, 84(5), pp. 474–480. doi: 10.5935/0004-2749.20210079.

Alvarez-Lorenzo, C. *et al.* (2002) 'Soft contact lenses capable of sustained delivery of timolol', *Journal of Pharmaceutical Sciences*, 91(10), pp. 2182–2192. doi: 10.1002/jps.10209.

Alvarez-Lorenzo, C. *et al.* (2019) 'Bioinspired hydrogels for drug-eluting contact lenses', *Acta Biomaterialia*, 84, pp. 49–62. doi: 10.1016/j.actbio.2018.11.020.

Alvarez-Lorenzo, C., Hiratani, H. and Concheiro, A. (2006) 'Contact lenses for drug delivery: Achieving sustained release with novel systems', *American Journal of Drug Delivery*, 4(3), pp. 131–151. doi: 10.2165/00137696-200604030-00002.

Anirudhan, T. S. and Rejeena, S. R. (2014) 'Poly(acrylic acid-co-acrylamide-co-2-acrylamido-2-methyl-1-propanesulfonic acid)-grafted nanocellulose/poly(vinyl alcohol) composite for the in vitro gastrointestinal release of amoxicillin', *Journal of Applied Polymer Science*, 131(17), pp. 8657–8668. doi: 10.1002/app.40699.

Arciola, C. R., Campoccia, D. and Montanaro, L. (2018) 'Implant infections: Adhesion, biofilm formation and immune evasion', *Nature Reviews Microbiology*, 16(7), pp. 397–409. doi: 10.1038/s41579-018-0019-y.

Bertens, C. J. F. *et al.* (2018) 'Topical drug delivery devices: A review', *Experimental Eye Research*, 168(January), pp. 149–160. doi: 10.1016/j.exer.2018.01.010.

Bourcier, T. *et al.* (2003) 'Bacterial keratitis: Predisposing factors, clinical and microbiological review of 300 cases', *British Journal of Ophthalmology*, 87(7), pp. 834–838. doi: 10.1136/bjo.87.7.834.

Bryers, J. D. (2008) 'Medical biofilms', *Biotechnology and Bioengineering*, 100(1), pp. 1–18. doi: 10.1002/bit.21838.

Chang, W. H. *et al.* (2020) 'Reduction of Physical Strength and Enhancement of Anti-Protein and Anti-Lipid Adsorption Abilities of Contact Lenses by Adding 2-Methacryloyloxyethyl Phosphorylcholine', *Macromolecular Research*, 28(12), pp. 1064–1073. doi: 10.1007/s13233-020-8149-2.

Costa, F. *et al.* (2011) 'Covalent immobilization of antimicrobial peptides (AMPs) onto biomaterial surfaces', *Acta Biomaterialia*, 7(4), pp. 1431–1440. doi: 10.1016/j.actbio.2010.11.005.

Efron, N. *et al.* (2013) 'An international survey of daily disposable contact lens prescribing', *Clinical and Experimental Optometry*, 96(1), pp. 58–64. doi: 10.1111/j.1444-0938.2012.00773.x.

Efron, N. *et al.* (2020) 'Thirty years of "quiet eye" with etafilcon A contact lenses', *Contact Lens and Anterior Eye*, 43(3), pp. 285–297. doi: 10.1016/j.clae.2020.03.015.

Farkouh, A., Frigo, P. and Czejka, M. (2016) 'Systemic side effects of eye drops: A pharmacokinetic perspective', *Clinical Ophthalmology*, 10, pp. 2433–2441. doi: 10.2147/OPTH.S118409.

Filipe, H. P. *et al.* (2016) 'Contact lenses as drug controlled release systems: A narrative review', *Revista Brasileira de Oftalmologia*, 75(3), pp. 241–247. doi: 10.5935/0034-7280.20160051.

Fleiszig, S. M. J. *et al.* (2020) 'Contact lens-related corneal infection: Intrinsic resistance and its compromise', *Progress in Retinal and Eye Research*, 76, pp. 1–90. doi: 10.1016/j.preteyeres.2019.100804.

Forbes, S. *et al.* (2013) 'Comparative surface antimicrobial properties of synthetic biocides and novel human apolipoprotein E derived antimicrobial peptides', *Biomaterials*, 34(22), pp. 5453–5464. doi: 10.1016/j.biomaterials.2013.03.087.

Fujikawa, H., Kai, A. and Morozumi, S. (2004) 'A new logistic model for Escherichia coli growth at constant and dynamic temperatures', *Food Microbiology*, 21(5), pp. 501–509. doi: 10.1016/j.fm.2004.01.007.

Gaglione, R. *et al.* (2017a) 'Novel human bioactive peptides identified in Apolipoprotein B: Evaluation of their therapeutic potential', *Biochemical Pharmacology*, 130, pp. 34–50. doi: 10.1016/j.bcp.2017.01.009.

Gaglione, R. *et al.* (2017b) 'Novel human bioactive peptides identified in Apolipoprotein B: Evaluation of their therapeutic potential', *Biochemical Pharmacology*, 130, pp. 34–50. doi: 10.1016/j.bcp.2017.01.009.

Gaglione, R. *et al.* (2019) 'Effects of human antimicrobial cryptides identified in apolipoprotein B depend on specific features of bacterial strains', *Scientific Reports*, 9(1), pp. 1–13. doi: 10.1038/s41598-019-43063-3.

Gaglione, R. *et al.* (2020) 'Cryptides identified in human apolipoprotein b as new weapons to fight antibiotic resistance in cystic fibrosis disease', *International Journal of Molecular Sciences*, 21(6). doi: 10.3390/ijms21062049.

Gaglione, R. *et al.* (2021) 'Impact of a single point mutation on the antimicrobial and fibrillogenic properties of cryptides from human apolipoprotein B', *Pharmaceuticals*, 14(7). doi: 10.3390/ph14070631.

Garrett, T. R., Bhakoo, M. and Zhang, Z. (2008) 'Bacterial adhesion and biofilms on surfaces', *Progress in Natural Science*, 18(9), pp. 1049–1056. doi: 10.1016/j.pnsc.2008.04.001.

González-Chomón, C., Concheiro, A. and Alvarez-Lorenzo, C. (2013) 'Soft contact lenses for controlled ocular delivery: 50 years in the making', *Therapeutic Delivery*, 4(9), pp. 1141–1161. doi: 10.4155/tde.13.81.

Guo, Y. *et al.* (2020) 'Reversible antibiotic loading and pH-responsive release from polymer brushes on contact lenses for therapy and prevention of corneal infections', *Journal of Materials Chemistry B*, 8(44), pp. 10087–10092. doi: 10.1039/d0tb01508c.

Haider, A., Haider, S. and Kang, I. K. (2018) 'A comprehensive review summarizing the effect of electrospinning parameters and potential applications of nanofibers in biomedical and biotechnology', *Arabian Journal of Chemistry*, 11(8), pp. 1165–1188. doi: 10.1016/j.arabjc.2015.11.015.

Hughes, P. M. *et al.* (2005) 'Topical and systemic drug delivery to the posterior segments', *Advanced Drug Delivery Reviews*, 57(14 SPEC. ISS.), pp. 2010–2032. doi: 10.1016/j.addr.2005.09.004.

Hui, A., Boone, A. and Jones, L. (2008) 'Uptake and release of ciprofloxacin-HCl from conventional and silicone hydrogel contact lens materials.', *Eye & contact lens*, 34(5), pp. 266–271. doi:

10.1097/icl.0b013e3181812ba2.

Jacobs, D. S. *et al.* (2021) 'CLEAR – Medical use of contact lenses', *Contact Lens and Anterior Eye*, 44(2), pp. 289–329. doi: 10.1016/j.clae.2021.02.002.

Khan, S. A. and Lee, C. S. (2020) 'Recent progress and strategies to develop antimicrobial contact lenses and lens cases for different types of microbial keratitis', *Acta Biomaterialia*, 113, pp. 101–118. doi: 10.1016/j.actbio.2020.06.039.

Kim, E., Saha, M. and Ehrmann, K. (2018) 'Mechanical Properties of Contact Lens Materials', *Eye & contact lens*, 44(July 2018), pp. S148–S156. doi: 10.1097/ICL.0000000000000442.

De la Fuente-Núñez, C. *et al.* (2013) 'Bacterial biofilm development as a multicellular adaptation: Antibiotic resistance and new therapeutic strategies', *Current Opinion in Microbiology*, 16(5), pp. 580–589. doi: 10.1016/j.mib.2013.06.013.

Matsumura, S. *et al.* (2008) 'Stability and Utility of Pyridyl Disulfide Functionality in RAFT and Conventional Radical Polymerizations', *Journal of Polymer Science: Part A: Polymer Chemistry*, 46(April), pp. 7207–7224. doi: 10.1002/pola.

Maulvi, F. A. *et al.* (2018) 'Contact lenses with dual drug delivery for the treatment of bacterial conjunctivitis', *International Journal of Pharmaceutics*, 548(1), pp. 139–150. doi: 10.1016/j.ijpharm.2018.06.059.

Maulvi, F. A., Soni, T. G. and Shah, D. O. (2016) 'A review on therapeutic contact lenses for ocular drug delivery', *Drug Delivery*, 23(8), pp. 3017–3026. doi: 10.3109/10717544.2016.1138342.

Michael Zasloff (2002) 'Antimicrobial peptides of multicellularorganisms', *Nature*, 415(January), pp. 389–395.

Mun, J. *et al.* (2019) 'Drug-eluting contact lens containing cyclosporine-loaded cholesterol-hyaluronate micelles for dry eye syndrome', *RSC Advances*, 9(29), pp. 16578–16585. doi: 10.1039/c9ra02858g.

Musgrave, C. S. A. and Fang, F. (2019) 'Contact lens materials: A materials science perspective', *Materials*, 12(2), pp. 1–36. doi: 10.3390/ma12020261.

Nesic, A. *et al.* (2016) 'Physical-chemical behavior of novel copolymers composed of methacrylic acid and 2-acrylamido-2-methylpropane

sulfonic acid', *Materials Chemistry and Physics*, 174, pp. 156–163. doi: 10.1016/j.matchemphys.2016.02.063.

Nordström, R. and Malmsten, M. (2017) *Delivery systems for antimicrobial peptides*, *Advances in Colloid and Interface Science*. Elsevier B.V. doi: 10.1016/j.cis.2017.01.005.

Rykowska, I., Nowak, I. and Nowak, R. (2021) 'Soft contact lenses as drug delivery systems: A review', *Molecules*, 26(18), pp. 1–31. doi: 10.3390/molecules26185577.

Sarkar, T., Chetia, M. and Chatterjee, S. (2021) 'Antimicrobial Peptides and Proteins: From Nature's Reservoir to the Laboratory and Beyond', *Frontiers in Chemistry*, 9(June). doi: 10.3389/fchem.2021.691532.

Silva, D. *et al.* (2020) 'Contact lenses as drug-delivery systems: a promising therapeutic tool', *Applied Sciences (Switzerland)*, 44(1), pp. 16578–16585. doi: 10.1080/1040841X.2017.1313813.

Silva, D. *et al.* (2021) 'Moxifloxacin-imprinted silicone-based hydrogels as contact lens materials for extended drug release', *European Journal of Pharmaceutical Sciences*, 156(September 2020), p. 105591. doi: 10.1016/j.ejps.2020.105591.

Souza, J. G. *et al.* (2014) 'Topical delivery of ocular therapeutics: Carrier systems and physical methods', *Journal of Pharmacy and Pharmacology*, 66(4), pp. 507–530. doi: 10.1111/jphp.12132.

Sultana, A., Luo, H. and Ramakrishna, S. (2021) 'Antimicrobial peptides and their applications in biomedical sector', *Antibiotics*, 10(9), pp. 1–24. doi: 10.3390/antibiotics10091094.

Weissman, B. A. *et al.* (2017) 'Optometric Clinical Practice Guideline Care of the Contact Lens Patient', *American Optometric Association*, 31(6), pp. 1–46. Available at: http://www.nei.nih.gov/nehep/kap/.%0Ahttps://linkinghub.elsevier.com/retrieve/pii/S2452232517300227%0Ahttps://synapse.koreamed.org/DOIx.php?id=10.3341/kjo.2016.0107%0Ahttp://dx.doi.org/10.2147/AHMT.S55834%0Ahttps://www.clspectrum.com/content/alcon/18/CLS_.

Wernli, D. *et al.* (2017) 'Antimicrobial resistance: The complex challenge of measurement to inform policy and the public', *PLoS Medicine*, 14(8), pp. 1–9. doi: 10.1371/journal.pmed.1002378.

Xiao, A. *et al.* (2018) 'Strategies to design antimicrobial contact lenses and contact lens cases', *Journal of Materials Chemistry B*, 6(15), pp.

2171–2186. doi: 10.1039/c7tb03136j.

Xu, J. *et al.* (2018) ‘A comprehensive review on contact lens for ophthalmic drug delivery’, *Journal of Controlled Release*, 281(March), pp. 97–118. doi: 10.1016/j.jconrel.2018.05.020.

Yang, K. *et al.* (2018) ‘Antimicrobial hydrogels: Promising materials for medical application’, *International Journal of Nanomedicine*, 13, pp. 2217–2263. doi: 10.2147/IJN.S154748.

Zhang, L. J. and Gallo, R. L. (2016) *Antimicrobial peptides*, *Current Biology*. doi: 10.1016/j.cub.2015.11.017.

Zidan, G. *et al.* (2018) ‘Medicated ocular bandages and corneal health: potential excipients and active pharmaceutical ingredients’, *Pharmaceutical Development and Technology*, 23(3), pp. 255–260. doi: 10.1080/10837450.2017.1377232.

Zimmerman, A. B., Nixon, A. D. and Rueff, E. M. (2016) ‘Contact lens associated microbial keratitis: Practical considerations for the optometrist’, *Clinical Optometry*, 8, pp. 1–12. doi: 10.2147/OPTO.S66424.

CHAPTER 4

General discussion and concluding remarks

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Microbial infections represent an everlasting concern for people across the world, affecting different fields, such as medicine and food chain (Marshall and Levy, 2011). The predominant bacterial lifestyle is represented by communities of sessile microorganisms referred to as biofilm. Biofilm infections, responsible for to up to 80% of human infections, start with bacteria adhesion to both biotic and abiotic surfaces. In the first case, bacteria adherence to host tissue can lead to chronic infections like osteomyelitis, endocarditis, cystic fibrosis-associated lung infections (Römling and Balsalobre, 2012), and to inflammatory diseases as Crohn's disease as well as dental caries and periodontitis (Claret *et al.*, 2007; Do, Devine and Marsh, 2013). In the second case, bacteria can colonize surfaces of medical devices with subsequent biofilm establishment leading to development of infections both in the site of the insertion and in the peri-implant area (Cooper, 2015). Indeed, at the implant-tissue interphase, local immune responses are dysregulated, thus rendering the peri-implant area more vulnerable to infections. Several cases of peri-implantitis are frequently related with dental implants (Berglundh, Persson and Klinge, 2002). Biofilm formation on contact lenses may result in various severe ocular diseases, such as microbial keratitis (Zimmerman, Nixon and Rueff, 2016). Urinary Catheter associated infections are one the most common cause of secondary bloodstream infections (Neoh *et al.*, 2017). The Centre for Disease Control reported that around 50-70% of the nearly 2 million healthcare-associated infections was provoked by medical device-associated infections (Yu *et al.*, 2021). Despite the use of medical devices has become part of our life and the strong efforts to design smart biomaterials (Altomare *et al.*, 2018), contamination of these tools by microorganisms, with consequent failure and malfunctioning, is still a huge concern (Drexelius and Neundorf, 2021). Furthermore, biofilm-based infections are tough to treat since the unique organization of microorganisms within biofilm matrix makes them recalcitrant to common antibiotic treatments and to host immune action (Hall and Mah, 2017). Moreover, conventional therapeutic approaches, based on systemic or locally administration of antibiotics, failed to fulfil the expectations, since their unconstraint utilization has caused the development of microorganisms non-responsive to their activity (Debabov, 2013). Indeed, none of the antibiotics currently available in the clinics have been specifically designed to eradicate microbial biofilms (Bi *et al.*, 2021). Nowadays, antibiotic resistance and biofilm infections have a negative impact on people quality of life across the world, constituting also a strong economic burden. In the search for new antimicrobial agents, antimicrobial peptides are considered very

promising drug candidates to combat both bacterial biofilms and antibiotic resistant strains (Czaplewski *et al.*, 2016). They have attracted considerable attention because of their antimicrobial, anti-biofilm and immunomodulatory properties, but above all because they generally do not lead to selection of resistant strains (Magana *et al.*, 2020)**.** Moreover, AMPs display wide applicability in medicine, since they can regulate pro-inflammatory reactions, promote wound healing by modulating cell migration, promote angiogenesis, chemotaxis and cytokine release (Mookherjee, Chow and Hancock, 2012). To date, more than 4,000 AMPs have been identified or synthetized and their sequences are available on the web, collected in the antimicrobial peptide database (Fan *et al.*, 2016). Several AMPs are under clinical trials to treat health care infections (Mirski *et al.*, 2018). However, during those trials, different candidates demonstrated high susceptibility to enzymatic degradation and fast renal clearance leading to a short peptide in *vivo* half-life or unexpected toxicity. Hence, localized delivery of antimicrobial peptides has been selected as an effective strategy to prevent such disadvantages (Teixeira *et al.*, 2020). For this reason, a good compromise to treat medical devices infections and to overcome limitations to peptides applicability could be the functionalization of medical devises' surfaces with AMPs endowed with anti-infective properties. During the last years, different types of strategies have been developed and tested; several biomaterials have been functionalized with AMPs, including titanium (Kazemzadeh-Narbat *et al.*, 2010; Chen *et al.*, 2016), catheter-like surfaces (Lim *et al.*, 2015; Alves *et al.*, 2016) and contact lenses (Willcox *et al.*, 2008; Dutta *et al.*, 2013). Among the proposed strategies, those based on active release procedure, where the antimicrobial compound is not covalently bound to the surface, allow a continuous release of the antimicrobial agent in the surrounding area, thus taking care of peri-implant infections (Riool *et al.*, 2017). In recent years, our research group identified, by means of a bioinformatic approach, a putative AMP hidden in the sequence of human Apolipoprotein B. Three versions of the identified peptide have been recombinantly produced in bacterial cells and their biological properties have been characterized. Indeed, ApoB-derived peptides have been found to be endowed with antimicrobial, anti-biofilm, wound healing and immunomodulatory properties (Gaglione *et al.*, 2017a). Furthermore, ApoB-derived peptides have been found to synergistically act in combination with conventional antibiotics and EDTA (Gaglione *et al.*, 2017b), to be endowed with antifungal properties (Dell'Olmo, Gaglione, Pane, *et al.*, 2021), and to be able to prevent chicken meat contaminations when immobilized on chitosan edible films (Dell'Olmo,

Gaglione, Sabbah, *et al.*, 2021). In this context, the present PhD Thesis aimed to provide a first attempt to use ApoB-derived peptides to tackle the issue of medical devices infections. Specifically, one of the three available ApoB-derived antimicrobial peptides, namely r(P)ApoB$_L^{Pro}$, has been selected to develop anti-infective urinary catheters (chapter 2) and AMP-loaded contact lenses (chapter 3), since they represent two of the most common medical devices associated with high infection rates (Li *et al.*, 2021). Indeed, in order to handle CAUTI problem, the selected peptide has been used to functionalize polydimethylsiloxane **(**PDMS) polymer, which is one of the most commonly used material to fabricate commercial urinary catheters. Physicochemical analyses of PDMS functionalized with the peptide under test have been performed by FTIR, scanning electron microscopy and water contact angle studies. By this way, the presence of the peptide was revealed both inside and on PDMS surface. Moreover, QCM analyses indicated a total release of the peptide from PDMS surface over time, thus suggesting that the eluted peptide might exert effective antimicrobial properties. Indeed, antimicrobial activity tests on a Gram-negative and a Gram-positive bacterial strain demonstrated the ability of functionalized PDMS to exert strong antimicrobial effects. Furthermore, functionalized PDMS was found to be able to inhibit the adhesion of bacteria, whereas no toxicity was detected when functionalized material was tested on eukaryotic cell lines. Altogether, obtained results pave the way to the applicability of the obtained functionalized material in *in vivo* animal models. Furthermore, future investigations could be also aimed at evaluating the applicability of the obtained material in flow model systems in artificial urine media (Azevedo *et al.*, 2017).

In the second part of the Thesis project, the surface of contact lenses has been functionalized with r(P)ApoB$_L^{Pro}$ peptide, in order to develop a proper material to solve the problem of infections related to CLs wear. To this purpose, 2-hydroxyethyl methacrylate (HEMA) hydrogel has been selected being the most common material employed to manufacture soft contact lenses. It has also to be highlithed that, in the last decades, a great interest has been devoted to contact lenses as drug delivery systems (Filipe *et al.*, 2016). Drug delivery has the main advantage to increase the residence time of a drug at the site of infection, thus reducing dosing frequency and toxic effects and improving patient compliance (Taleb, Hegazy and Mahmoud, 2014). To obtain antimicrobial contact lenses, HEMA hydrogel disks characterized by different compositions have been prepared by co-polymerization of HEMA monomers with “functional monomers” able to

confer to HEMA-based hydrogels suitable properties. Indeed, it has been demonstrated that the presence of such monomers (*i.e*, MAA and AMPSA), by conferring a net negative charge to hydrogel surface, allowed the complete absorption of the peptide present in the soaking solution in direct contact with hydrogel disks. Mechanical and physicochemical properties of the obtained functionalized material have been analyzed, and, interestingly, the presence of the peptide was found not to alter the optical properties of hydrogel disks. However, no release of the peptide from hydrogel disks was evaluated over time. In agreement with this, obtained functionalized material was found not to be endowed with significant antimicrobial properties in *in vitro* assays. For this reason, further studies will be performed in the future to test different hydrogel compositions able to establish less strong interactions with peptide molecules and to allow their gradual release over time.

In conclusion, the results collected in the present PhD Thesis demonstrated the suitability of ApoB-derived peptides as model molecules to functionalize the surface of medical devices, thus preventing their colonization by microorganisms. Noteworthy, the release of the peptide from the surface of interest is a key feature to consider, since an insufficient release of the peptide could be related to a very low antimicrobial activity, not sufficient to counteract the infection. In recent years, a great interest in the use of nanosystems to deliver AMPs is emerging (Martin-Serrano *et al.*, 2019; Gera, Kankuri and Kogermann, 2021), thus allowing a more efficient employment of these precious molecules for biotechnological applications. The present study demonstrated the applicability of AMP under test to functionalize the surfaces of medical devices making them antimicrobial. Further studies will be performed in the future to optimize the procedure and to perform further steps towards the applicability of the proposed system.

References

Altomare, L. *et al.* (2018) 'Biopolymer-based strategies in the design of smart medical devices and artificial organs', *International Journal of Artificial Organs*. SAGE Publications Ltd, pp. 337–359. doi: 10.1177/0391398818765323.

Alves, D. *et al.* (2016) 'Co-immobilization of Palm and DNase I for the development of an effective anti-infective coating for catheter surfaces', *Acta Biomaterialia*, 44, pp. 313–322. doi: 10.1016/j.actbio.2016.08.010.

Azevedo, A. S. *et al.* (2017) 'An in vitro model of catheter-associated urinary tract infections to investigate the role of uncommon bacteria on the Escherichia coli microbial consortium', *Biochemical Engineering Journal*, 118, pp. 64–69. doi: 10.1016/j.bej.2016.11.013.

Berglundh, T., Persson, L. and Klinge, B. (2002) 'A systematic review of the incidence of biological and technical complications in implant dentistry reported in prospective longitudinal studies of at least 5 years', *Journal of Clinical Periodontology*, 29(SUPPL. 3), pp. 197–212. doi: 10.1034/j.1600-051X.29.s3.12.x.

Bi, Y. *et al.* (2021) 'Therapeutic strategies against bacterial biofilms', *Fundamental Research*, 1(2), pp. 193–212. doi: 10.1016/j.fmre.2021.02.003.

Chen, R. *et al.* (2016) 'Antimicrobial peptide melimine coating for titanium and its in vivo antibacterial activity in rodent subcutaneous infection models', *Biomaterials*, 85, pp. 142–151. doi: 10.1016/j.biomaterials.2016.01.063.

Claret, L. *et al.* (2007) 'The flagellar sigma factor FliA regulates adhesion and invasion of Crohn disease-associated Escherichia coli via a cyclic dimeric GMP-dependent pathway', *Journal of Biological Chemistry*, 282(46), pp. 33275–33283. doi: 10.1074/jbc.M702800200.

Cooper, I. R. (2015) 'Introduction to biomaterials and medical device-associated infections', *Biomaterials and Medical Device - Associated Infections*, pp. 3–17. doi: 10.1533/9780857097224.1.3.

Czaplewski, L. *et al.* (2016) 'Alternatives to antibiotics-a pipeline portfolio review', *The Lancet Infectious Diseases*, 16(2), pp. 239–251. doi: 10.1016/S1473-3099(15)00466-1.

Debabov, D. (2013) 'Antibiotic resistance: Origins, mechanisms, approaches to counter', *Applied Biochemistry and Microbiology*, 49(8), pp. 665–671. doi: 10.1134/S0003683813080024.

Dell'Olmo, E., Gaglione, R., Pane, K., *et al.* (2021) 'Host defence peptides identified in human apolipoprotein B as promising antifungal agents', *Natural Product Research*, 35(10), pp. 1686–1689. doi: 10.1080/14786419.2019.1624961.

Dell'Olmo, E., Gaglione, R., Sabbah, M., *et al.* (2021) 'Host defense peptides identified in human apolipoprotein B as novel food biopreservatives and active coating components', *Food Microbiology*, 99(October 2020), p. 103804. doi: 10.1016/j.fm.2021.103804.

Do, T., Devine, D. and Marsh, P. D. (2013) 'Oral biofilms: Molecular analysis, challenges, and future prospects in dental diagnostics', *Clinical, Cosmetic and Investigational Dentistry*, 5, pp. 11–19. doi: 10.2147/CCIDE.S31005.

Drexelius, M. G. and Neundorf, I. (2021) 'Application of Antimicrobial Peptides on Biomedical Implants : Three Ways to Pursue Peptide Coatings'.

Dutta, D. *et al.* (2013) 'Broad spectrum antimicrobial activity of melimine covalently bound to contact lenses', *Investigative Ophthalmology and Visual Science*, 54(1), pp. 175–182. doi: 10.1167/iovs.12-10989.

Fan, L. *et al.* (2016) 'DRAMP: A comprehensive data repository of antimicrobial peptides', *Scientific Reports*, 6, pp. 1–7. doi: 10.1038/srep24482.

Filipe, H. P. *et al.* (2016) 'Contact lenses as drug controlled release systems: A narrative review', *Revista Brasileira de Oftalmologia*, 75(3), pp. 241–247. doi: 10.5935/0034-7280.20160051.

Gaglione, R. *et al.* (2017a) 'Novel human bioactive peptides identified in Apolipoprotein B: Evaluation of their therapeutic potential', *Biochemical Pharmacology*, 130, pp. 34–50. doi: 10.1016/j.bcp.2017.01.009.

Gaglione, R. *et al.* (2017b) 'Novel human bioactive peptides identified in Apolipoprotein B: Evaluation of their therapeutic potential', *Biochemical Pharmacology*, 130, pp. 34–50. doi: 10.1016/j.bcp.2017.01.009.

Gera, S., Kankuri, E. and Kogermann, K. (2021) 'Antimicrobial peptides – Unleashing their therapeutic potential using nanotechnology', *Pharmacology and Therapeutics*, (xxxx), p. 107990. doi: 10.1016/j.pharmthera.2021.107990.

Hall, C. W. and Mah, T. F. (2017) 'Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria', *FEMS Microbiology Reviews*, 41(3), pp. 276–301. doi: 10.1093/femsre/fux010.

Kazemzadeh-Narbat, M. *et al.* (2010) 'Antimicrobial peptides on calcium phosphate-coated titanium for the prevention of implant-associated infections', *Biomaterials*, 31(36), pp. 9519–9526. doi: 10.1016/j.biomaterials.2010.08.035.

Li, X. *et al.* (2021) 'Novel approaches to combat medical device-associated biofilms', *Coatings*, 11(3), pp. 1–31. doi: 10.3390/coatings11030294.

Lim, K. *et al.* (2015) 'Development of a catheter functionalized by a polydopamine peptide coating with antimicrobial and antibiofilm properties', *Acta Biomaterialia*, 15, pp. 127–138. doi: 10.1016/j.actbio.2014.12.015.

Magana, M. *et al.* (2020) 'The value of antimicrobial peptides in the age of resistance', *The Lancet Infectious Diseases*, 20(9), pp. e216–e230. doi: 10.1016/S1473-3099(20)30327-3.

Marshall, B. M. and Levy, S. B. (2011) 'Food animals and antimicrobials: Impacts on human health', *Clinical Microbiology Reviews*, 24(4), pp. 718–733. doi: 10.1128/CMR.00002-11.

Martin-Serrano, Á. *et al.* (2019) 'Nanosystems as vehicles for the delivery of antimicrobial peptides (Amps)', *Pharmaceutics*, 11(9), pp. 1–24. doi: 10.3390/pharmaceutics11090448.

Mirski, T. *et al.* (2018) 'Utilisation of peptides against microbial infections – a review', *Annals of Agricultural and Environmental Medicine*, 25(2), pp. 205–210. doi: 10.26444/aaem/74471.

Mookherjee, N., Chow, L. N. Y. and Hancock, R. E. W. (2012) 'Immunomodulatory cationic peptide therapeutics: A new paradigm in infection and immunity', *ACS Symposium Series*, 1095, pp. 1–19. doi: 10.1021/bk-2012-1095.ch001.

Neoh, K. G. *et al.* (2017) 'Surface modification strategies for combating catheter-related complications: recent advances and challenges', *Journal of Materials Chemistry B*. doi: 10.1039/c6tb03280j.

Riool, M. *et al.* (2017) 'Antimicrobial peptides in biomedical device manufacturing', *Frontiers in Chemistry*, AUG(2017), pp. 1–13. doi: 10.3389/fchem.2017.00063.

Römling, U. and Balsalobre, C. (2012) 'Biofilm infections, their resilience to therapy and innovative treatment strategies', *Journal of Internal Medicine*, 272(6), pp. 541–561. doi: 10.1111/joim.12004.

Taleb, M. A., Hegazy, D. E. and Mahmoud, G. A. (2014) 'Characterization and in vitro drug release behavior of (2-hydroxyethyl methacrylate)-co-(2-acrylamido-2-methyl-1-propanesulfonic acid) crosslinked hydrogels prepared by ionizing radiation', *International*

Journal of Polymeric Materials and Polymeric Biomaterials, 63(16), pp. 840–845. doi: 10.1080/00914037.2014.886221.

Teixeira, M. C. *et al.* (2020) 'Nanomedicines for the delivery of antimicrobial peptides (Amps)', *Nanomaterials*, 10(3), pp. 1–23. doi: 10.3390/nano10030560.

Willcox, M. D. P. *et al.* (2008) 'A novel cationic-peptide coating for the prevention of microbial colonization on contact lenses', *Journal of Applied Microbiology*, 105(6), pp. 1817–1825. doi: 10.1111/j.1365-2672.2008.03942.x.

Yu, K. *et al.* (2021) 'Rapid Assembly of Infection-Resistant Coatings: Screening and Identification of Antimicrobial Peptides Works in Cooperation with an Antifouling Background', *ACS Applied Materials and Interfaces*, 13(31), pp. 36784–36799. doi: 10.1021/acsami.1c07515.

Zimmerman, A. B., Nixon, A. D. and Rueff, E. M. (2016) 'Contact lens associated microbial keratitis: Practical considerations for the optometrist', *Clinical Optometry*, 8, pp. 1–12. doi: 10.2147/OPTO.S66424.