# Antimicrobial Peptides in Skin Diseases

By

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A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy

School of Pharmacy
Brac University
March 2022

## Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

**Student's Full Name & Signature:**

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# Approval

The thesis/project titled “Antimicrobial Peptides in Skin Diseases” submitted by Monika Tabassum Pinkey (18146038) of Summer, 2021 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on March 10, 2022.

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## Ethics Statement

This study does not involve any kind of animal or human trial.

## Abstract

Skin diseases, including inflammatory and infections, result from disruptions in the skin's barrier. Antimicrobial peptides (AMPs) have recently gained attention as potential treatments for inflammatory, soft skin tissue and wound infections. This review discusses AMPs in skin diseases. It highlights the therapeutic properties of AMPs and their potential utility in treating skin disorders. Even though only a few AMPs, such as oritavancin and daptomycin, have been approved for use in clinical trials. Moreover, omiganan, pexiganan, and DPK-060 have also been studied extensively for treating skin problems. This paper also mentions some strategies to increase AMPs' clinical trial success rate.

**Keywords:** Skin diseases, inflammatory disease, soft skin tissue infections, antimicrobial peptides, therapeutic agents.

# Dedication

*Dedicated to my beloved parents and respected supervisor for their immense support and for having faith in me.*

## Acknowledgement

First, I express my heartfelt gratitude to Allah, the Almighty, for providing me with the knowledge, wisdom, strength, and patience that enabled me to reach this point. Without these blessings, I would not have made it this far. It aided me in overcoming all barriers and developing my ability to deal with situations as I worked towards achieving my objective and following my dream, respectively. I would never be able to complete this project without His mercy.

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## List of Acronyms

AMPs Antimicrobial Peptides

SSTIs Soft Skin Tissue Infections

LPS Lipopolysaccharides

IL Interleukin

TNF Tumor Necrosis Factor

# Chapter 1

# Introduction

## 1.1 Skin diseases

The skin is the body's outermost layer, comprising nearly 1.8 $m^2$, and serves as the first line of protection against a variety of external infections and environmental toxins. The skin's structure and cellular component act in conjunction to protect against infection and to respond with physical and chemical threats from outside (Chambers & Vukmanovic-Stejic, 2020). Deficiency in skin barrier and disruption of commensal-pathogen equilibrium can lead to skin illness (Byrd et al., 2018). The skin's epidermis not only provides a physical barrier against the majority of infectious organisms, but it also triggers an instant immune reaction, allowing humans to fight off infection. Non-specifically, innate immune defense systems detect and destroy bacteria within a short period of time, distinguishing between host cells and pathogens with remarkable accuracy (Marcinkiewicz & Majewski, 2016). Tears in the skin's integrity as the tissue loses its intrinsic elasticity and suppleness as it ages, are commonly infected by bacteria that infiltrate the dermis, resulting in skin infections (Lam et al., 2018). Also, skin damage from traumas such as scratches, burns, ulcers, skin abnormalities, wounds, skin thinning may undermine the skin's defense allowing microbial penetration of the epidermis and resulting in moderate to severe soft skin tissue infections (Marques-Neto et al., 2018). Infections involving the skin and the soft tissues beneath it, such as the muscle, fat, and fascia, are known as soft skin tissue infections (SSTIs) (Ki & Rotstein, 2008). Gram-positive bacteria, *S. aureus*, and *S. pyogenes* can cause bacterial soft skin tissue infections such as carbunculosis, impetigo, necrotizing fasciitis, follicular folliculitis, cellulitis (Kuhlmann et al., 2018). These infections are also caused by the ESKAPE pathogens: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and

*Enterobacter species* (Thapa et al., 2020). These bacteria are resistant to practically all antibiotics now in use. The most common bacteria found in chronic wounds are *S. aureus* and *P. aeruginosa*, and the evolution of resistance to topical antibiotics becomes a critical concern (Pfalzgraff et al., 2018a). Noninfectious skin disorders, including eczema, can induce infections that are pathogenic by causing skin damage, raising the risk of subsequent infection with *Staphylococcus aureus* (Bacalum et al., 2019). Pathogens such as *E. coli*, *P. aeruginosa*, and *S. aureus*, as well as inflammation, blood flow, and pain, all influence the healing rates of a chronic wound. As a result, reducing disease as well as inflammation may help you heal faster (Luong et al., 2020).

## 1.2 Conventional treatment options and challenges

Infections caused by atopic dermatitis are often treated with antibiotics topically. The most prevalent are fusidic acid and mupirocin. The combined formulations of gentamycin and corticosteroids are also famous in atopic dermatitis treatments. Another alternative is to use antiseptics in the form of emollients, such as triclosan or chlorhexidine, which can dramatically prevent colonization. Moreover, retinoids, corticosteroids, dithranol, vitamin D3, calcineurin inhibitors, emollients are common topical treatments for psoriasis, while immunomodulators such as cyclosporine and retinoids are widely used as systemic treatments. Due to the prevalence of streptococci bacteria in various types of psoriasis, antibiotics are used in psoriasis (Herman & Herman, 2019). Bacterial infections are mainly treated by antibiotics. Bacteria of both gram-positive and negative species commonly cause acute skin infections, and it is essential to determine whether or not the infection will develop resistance to antibiotics. Several novel antibiotics, including oritavancin, tedizolid, delafloxacin, dalbavancin, are used to treat SSTIs. SSTIs are cellulitis, erysipelas, abscesses, diabetic foot infection, wound. In contrast, doxycycline and trimethoprim-sulfamethoxazole are proposed standards for the optimal period of antibiotic therapy, with a duration of 7–10 days (Golan, 2019). The most

common microorganism linked with skin infections is *Staphylococcus aureus*, and it increases community-associated methicillin-resistant, making treatment challenging. Gram-positive bacteria, notably methicillin-resistant *Staphylococcus aureus*, are the primary target of antibiotics. However, no oral drug has been produced that can successfully cure MRSA and associated gram-negative infections. Intermittent skin hosts typically cause SSTIs with several virulence factors, such as *Staphylococcus aureus* and *Streptococcus pyogenes* group A streptococcus. As a result, if antibiotic therapy is necessary, it must target these often associated bacterial species. Group A streptococcus is consistently susceptible to penicillins, including amoxicillin. However, this antibiotic is rendered inactive by beta-lactamase, generated by 90 percent of SA strains. Clavulanate efficiently inhibits beta-lactamase. For this reason, the combination of amoxicillin and clavulanate, with its excellent pharmacokinetics and pharmacodynamics properties for *S. pyogenes* and *S. aureus*, is the preferable choice when broad antibiotic therapy is necessary. Moreover, Intravenous vancomycin, linezolid, daptomycin, tigecycline, telavancin, and ceftaroline are all options for treating severe MRSA infections.

## 1.3 Antimicrobial peptides

A class of tiny proteins known as antimicrobial peptides (AMPs) can be found in a range of plants and animals, and they serve as the first level of defense against pathogens (Sarkar et al., 2021). An antimicrobial peptide may have around five and more than one hundred amino acids. These AMPs give a counter to a broad spectrum of pathogens, notably viruses, gram-positive and negative bacteria, fungus, and other parasites. Almost all these peptides have a positive charge and are amphipathic, so they can be dissolved in water without losing their capacity to attach to bacterial cell walls. The peptides kill infections in various ways, including physical perforation and activation of the host immune response once attached to the target membrane (Gaspari et al., 2017).

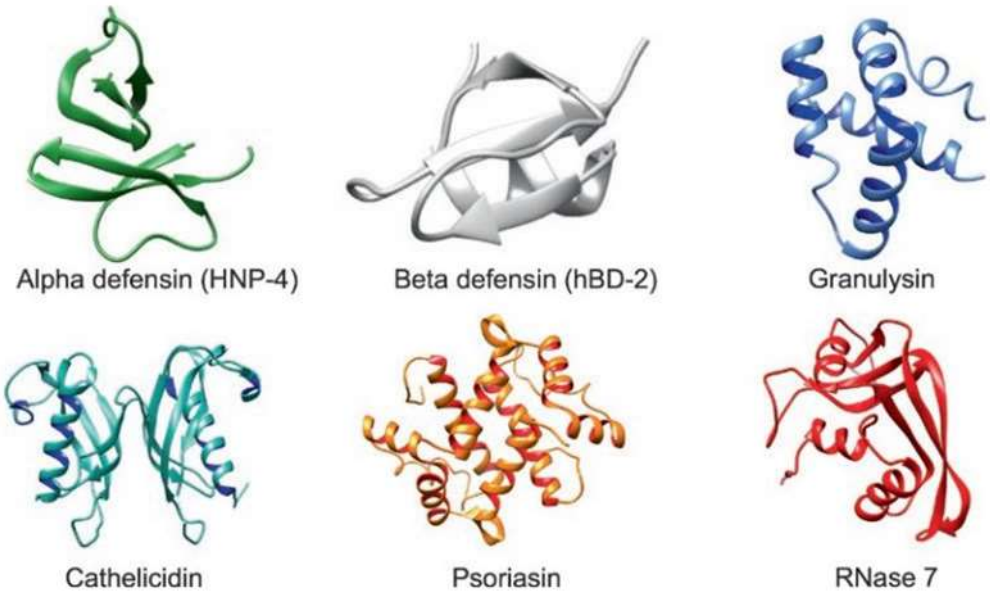


*Figure 1: Some common examples of antimicrobial peptides (Gaspari et al., 2017)*

There are some common characteristics among most AMPs, such as their amino acid composition ranging from 10 to 50, their charge ranging from +2 to +11, their high concentration of hydrophobic residues up to 50%, and their utilization of alpha-helical linear and a beta-sheet circular amphipathic second structure, contribute to their antibacterial activity via interactions with bacterial cell membranes (S. Wang et al., 2021).

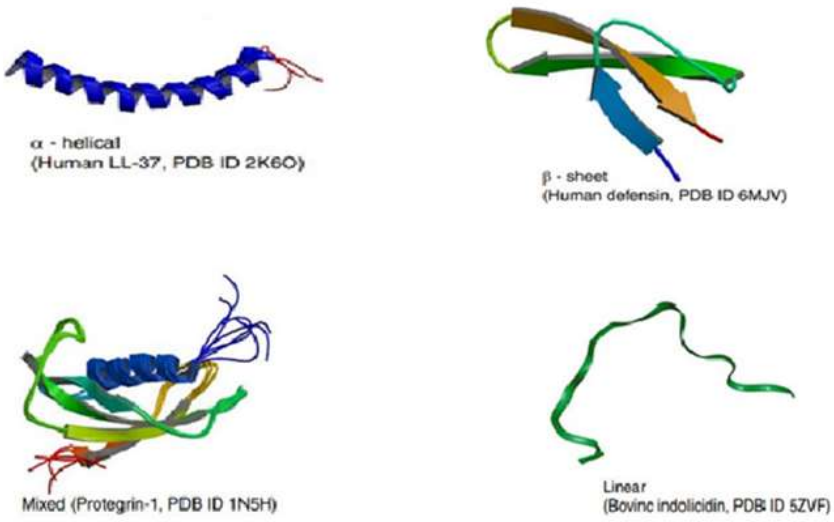


*Figure 2: Structure based examples of AMP ;α-helical, β-sheets ,Mixed(αβ), linear (non-αβ) (Liang et al., 2020)*

## 1.4 Application of AMPs in skin diseases

Antimicrobial peptides stimulate a robust immune response to skin illness. As a result, cytokines are produced, inflammation occurs, and a cell response occurs. Certain AMP qualities are essential to keep the skin barrier in equilibrium. Also, an unbalanced skin microbiota modulates. The expression of AMP in the skin is crucial for developing a range of skin illnesses, including acne vulgaris, atopic dermatitis, and psoriasis. Understanding the basic biology of AMP-related skin diseases will enable the development of AMP-based treatment options. AMPs may be attractive possibilities for innovative treatment strategies in humans as pathogen-specific antibiotics for skin infections, immunological modulators, and active wound healing agents (Herman & Herman, 2018).

## 1.5 Aim of the study

The goal of this review paper is to look at the therapeutic potential of AMPs on skin inflammatory and infectious diseases and to find ways to use AMPs to cure skin infections and soft skin tissue infections. As a result, use of AMPs for skin diseases will show their benefits and drawbacks, and will try to figure out if AMPs are great options for treating inflammatory and bacterial skin and soft skin tissue infections, or not.

## 1.6 Objective of the study

- To better understand the role of AMPs in inflammatory and infectious skin diseases.
- Classification & working mechanisms of AMPs skin diseases.
- Identify the potential application of AMPs on the treatment of skin diseases as a drug.
- Challenges and Prospects of AMP Therapeutics.

# Chapter 2

# Methodology

This review paper has been conducted on the basis of relevant recently published research papers and articles from high-impact factor journals. A comprehensive search has been performed through official reports, peer-reviewed journals, and articles. Basic and additional information has been collected from different books to enrich the review paper. The data for this paper was gathered by using the search engines listed below- Research Gate, PubMed, Science Direct, Cell Press, Elsevier, Google Scholar etc. in which the significant publications include- AACR (American Association for Cancer Research), Nature, ACS (American Chemistry Society), Science, Journal of Molecular Biology, Molecular Cell, Cancer Cell, Journal of Medicine, etc. To construct a high-quality review of the role of Antimicrobial Peptides in Skin Diseases, an extensive scan of the journals was followed by filtering down to the most current (within the previous five years) and relevant ones.

# Chapter 3

# Skin Diseases in Human

## 3.1 Inflammatory skin diseases

Skin inflammation is characterized by the stimulation of the innate and adaptive immune systems and the production of pro-inflammatory cytokines. Their development and maintenance result from abnormal reactions by epidermal immune cells. Epidermal keratinocytes control acute and chronic phases of skin inflammation by producing cytokines and chemokines and the expression of surface molecules on the skin's surface. Some types of inflammatory skin diseases are psoriasis, atopic dermatitis, acne.

### 3.1.1 Pathophysiology of psoriasis

Exogenous initiating variables for psoriasis include mechanical skin injury, streptococcal infections, and some medications. T17 and T22 cells are implicated in the pathogenesis of psoriasis, and their mediators, as well as upstream and downstream molecules, are plentiful in lesions. Along with T cells, type 3 innate lymphoid cells contribute to the generation of interleukin-17 (IL-17) and IL-22. IL-23 is a critical cytokine for the expression of IL-17 and IL-22 by immune cells. IL-23 interrupts T17 cells' synthesis of IL-10, resulting in T17 cells that are more susceptible to inflammation. There is a high concentration of TNF-α that is predominantly released by T17 cells and T22 cells also, IFN- α, which is primarily released by T1 cells, in psoriatic skin. Psoriatic lesions, on the other hand, do not produce any IL-4 (Chovatiya & Silverberg, 2019). Keratinocytes are the major target of IL-17A, IL-17F, and IL-22 in the skin, despite the fact that IL-17 effects have been demonstrated on immune cells and other tissue cells. All neutrophilic granulocytes and other cytokines in the skin are attracted to the skin by the IL-17 induced synthesis of specific chemokines in the keratinocytes. Probably, when IL-17 is used alone, it causes only mild cell responses. This is mostly because it works

well with TNF and IL-22. IL-17, in conjunction with IL-22, stimulates the generation of AMPs and hence plays a critical part in the psoriatic plaque's exceptional immunological response against external bacteria and fungi. Psoriasis patients are unique in that decreased skin barrier function is not linked to an increased risk of skin infections (Choi & Di Nardo, 2018).

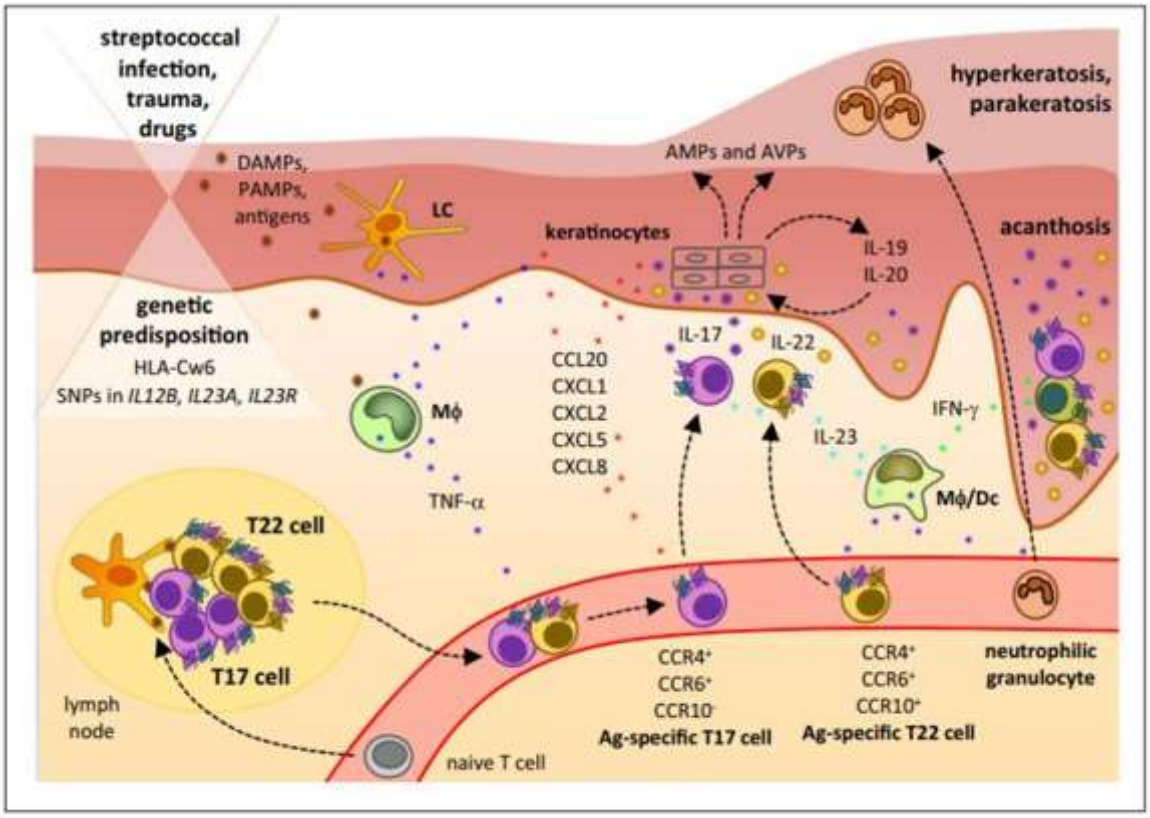


*Figure 3: Pathophysiology of psoriasis*

Increased release of hBD-2 and LL-37 is associated with the development of psoriasis, and LL-37 tends to develop complexes with self-RNA, activating TLR7 in plasmacytoid dendritic cells and TLR8 in myeloid dendritic cells, as well as permitting plasmacytoid dendritic cells to identify self-DNA via TLR9 and thus contributing to the pathophysiology of psoriasis. Similarly, hBD2, hBD3, and lysozyme have all been shown to activate plasmacytoid dendritic cells in the context of psoriasis.

### 3.1.2 Pathophysiology of atopic dermatitis

Atopic dermatitis is defined by a compromised skin barrier, which serves an essential role in the pathogenesis of the disease and is associated with its severity. The condition has also been connected to genetic abnormalities in the genes that code for the T2 pathway-associated cytokine receptors IL-4, IL-13, IL-4RA, and IL-31, as well as downstream components such as signal transducer and activator of transcription 6 and protein coded gene GATA3 (Sabat et al., 2019). Microbial antigens, allergens, superantigens, skin itching, emotional stress are all exogenous disease triggers. T2 cell mediators are required for atopic dermatitis etiology. Additionally, the T22 mediator IL-22 is important during the chronic disease stage. Inhibitors of keratinocyte terminal differentiation have been identified in the interleukin-4, interleukin-13, interleukin-31, and interleukin-22 pathways. Filaggrin and other molecules essential for the formation of the skin's barrier function are reduced even in patients who do not have a filaggrin mutation. Also, Irritation, a typical sign of atopic dermatitis, has been linked to IL-31. IL-5 has been shown to activate eosinophilic granulocytes detected histologically in individuals with skin lesions. IL-4 also helps to make IgE, which is found in the blood of most people with atopic dermatitis. In addition, chronic lesions don't have much of the T1 cell cytokine IFN-γ, and IL-17 isn't at all. The mentioned cytokine pattern also contributes to the epidermal synthesis of AMPs being weak. Indeed, elevated levels of the T2 cytokine IL-17 cause keratinocytes to produce less AMP (Nakatsuji et al., 2017). Atopic dermatitis is associated with low AMP levels. Patients with compromised skin are more likely to develop atypical cutaneous colonization with Staphylococcus aureus and access to microbiological pathogens and their immuno-stimulatory elements into the skin, and infection with this pathogen. Subclinical *S. aureus* colonization occurs in the non-lesional skin of patients and is associated with reduced skin barrier function and illness severity.

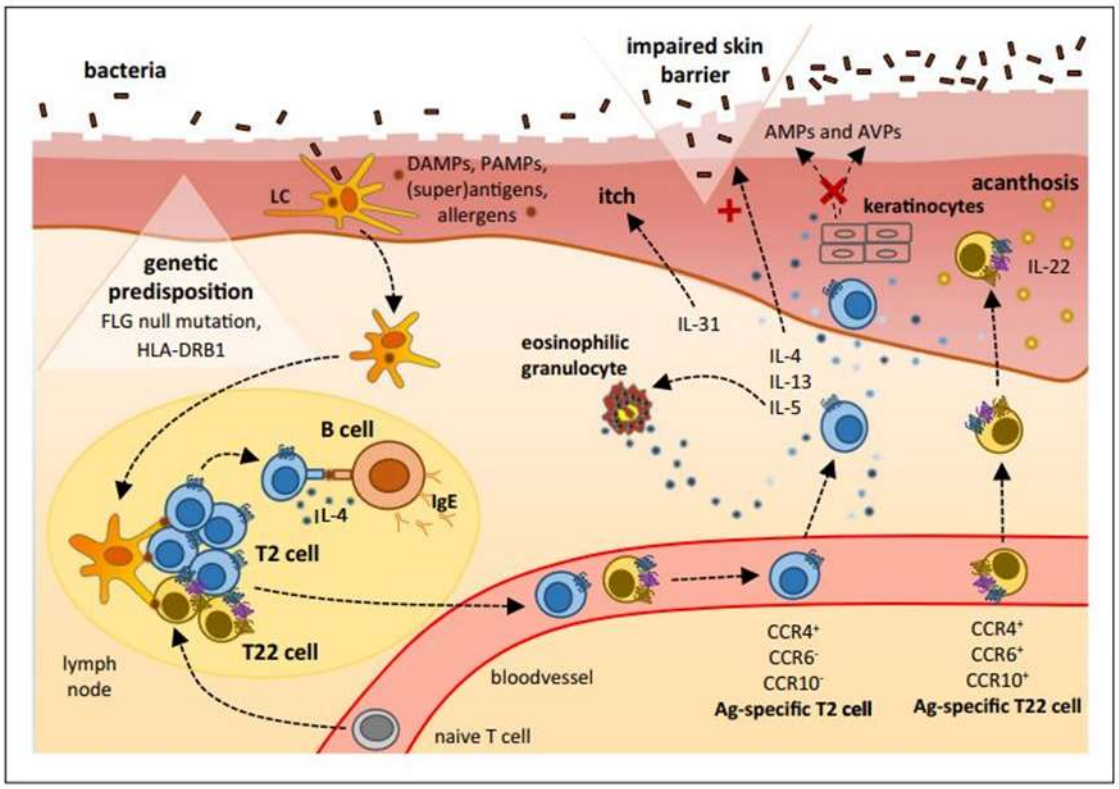


*Figure 4: Immunopathophysiology of atopic dermatitis (Sabat et al., 2019)*

### 3.1.3 Physiopathology of acne

Acne typically begins with an increase in sebum secretion by the sebaceous gland above the pore's capacity; this changes in the cycle of epithelial desquamation, accumulating dead keratin cells at the follicular canal's top, resulting in the generation of comedones or fat plugs that obstruct adequate fluid eviction. Whenever dead corneocytes, sebum, and microbial flora become trapped within a blocked pore, the stress of the retained mass causes a white papule, which can burst internal epithelial tissue, infiltrating the dermis and causing internal inflammation. If the cyst ruptures, it has the capability to interfere with the bacterial germs surrounding the pore, such as Propionibacterium acnes, causing irritation and advancement to the pustule or papule stage (Felgueiras, 2021). Propionibacterium acnes and other commonly found members of the skin's microbiota thrive in the pilosebaceous unit when this mass accumulates (Gaspari et al., 2017). Because the presence of P bacteria triggers an instant

response in the host's innate immune system, the outcome is redness or swelling. Toll type receptors on neutrophils, macrophages, and keratinocytes detect the appearance of an invading microorganism when acne in the sebocytes causes an infection, which activates the classic immune system pathways, resulting in the generation of pro-inflammatory cells like interleukin gamma interferon (IFN-γ) and tumor necrosis factor alpha (TNF-α), which cause tissue damage and necrosis in the skin. AMPs, such as beta-human defensin peptide, can also be produced by this mechanism, which is derived from skin inflammation and plays a significant role in interfacing electrostatic interactions with the foreign invader's microorganism's membrane, leading to an imbalance in osmotic pressure. Nodules or cysts may form as a result of these inflammatory processes (M. Gómez et al., 2019).

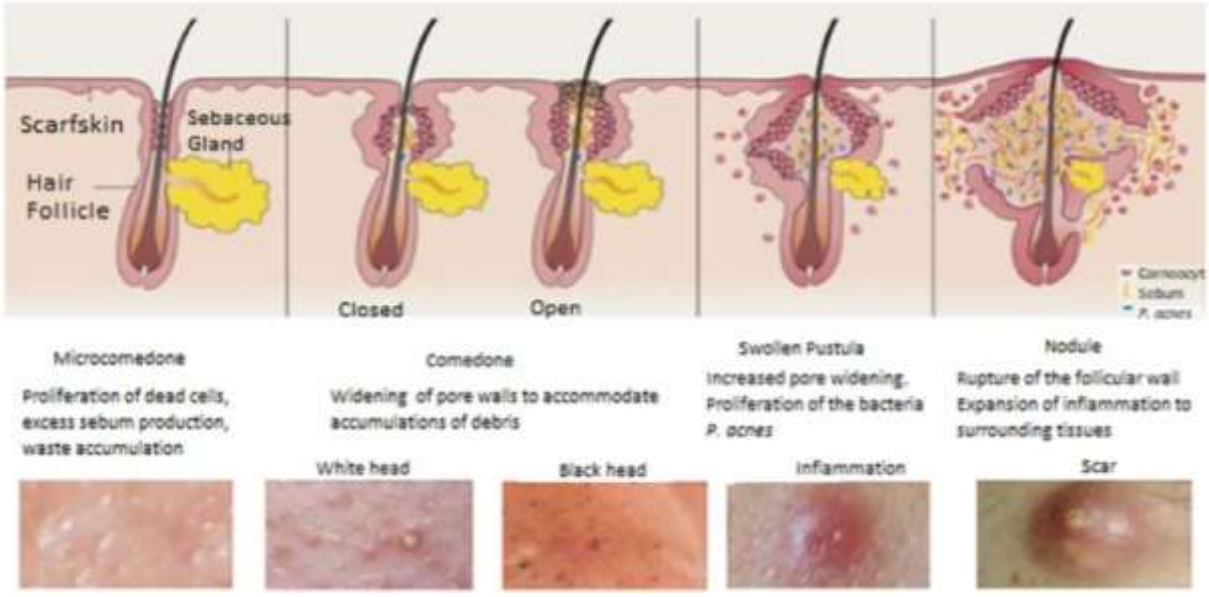


*Figure 5: Different stages of acne formation (M. Gómez et al., 2019)*

## 3.2 Skin and soft skin tissue infections

SSTIs are caused by a breakdown of the skin's defenses, as well as microbial invasion and interaction with the skin. Due to the fact that SSTIs are typically caused by bacteria, the majority of standards and guidelines exclude viral, fungal, or parasite etiologies. There are

numerous ways to compromise the skin barrier; among them, traumatic injury and surgical intervention are two of the most prevalent methods. SSTIs are caused by the invasion of otherwise healthy skin; secondary SSTIs are caused by infection of previously damaged skin, such as that caused by trauma or an underlying disease. While infections are frequently localized, they can also spread across the blood circulation or lymphatic system (Silverberg, 2021).

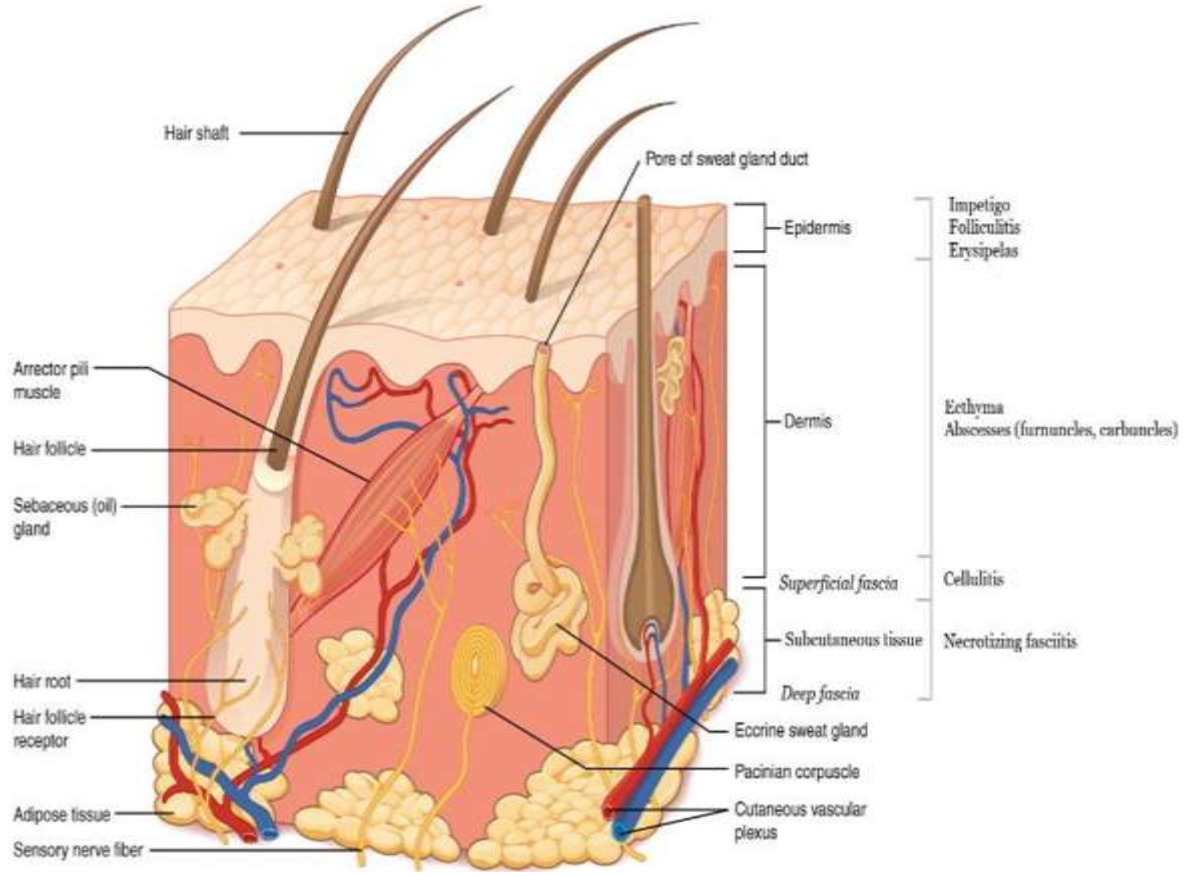


*Figure 6: Regions of Soft skin tissue Infections in the skin (Silverberg, 2021)*

Bacteria invade several levels of the skin structure, including the epidermis, dermis, subcutaneous and adipose tissues, and muscle fascia, initially in low numbers (Watkins & David, 2021). When the barrier of the skin is compromised, bacteria colonize and an SSTI develops. Pores in the epidermis may become involved, resulting in folliculitis, furuncles, or

carbuncles. Erysipelas refers to infection of the surface layers of skin, whereas cellulitis refers to infection of the dermis or subcutaneous tissues. There are also risks of fasciitis and myositis, which happen when deeper skin structures become inflamed. People who have a lot of fat, like people who are overweight or obese, get panniculitis when fat tissue gets infected. Most SSTIs show up in people's bodies after two steps. There is first an invasion. Then, a process happens that leads to clinical effects because of the bacteria's interaction with the body's defenses. There are a lot of ways bacteria can get into skin. The most common way to get through the skin barrier is to breaking the barrier. Lesions like chicken pox or ulcer, instrumentation like needles, preexisting skin problems, wounds such systems allow flora of normal skin and native flora to penetrate into the skin and cause infections. Water entering skin pores and, infrequently, hematogenous seeding are other routes of penetration (Ki & Rotstein, 2008).

The most often encountered pathogens in SSTIs are

- *S. aureus* and Methicillin-resistant *Staphylococcus aureus*
- *S. pyogenes*
- *P. aeruginosa*
- *E. coli*
- K. pneumoniae

Moreover, diabetic foot infections and superficial wounds also classified as SSTIs.

### 3.2.1 Diabetic foot infections

Most diabetic foot infections present as skin ulcers, commonly caused by an unnoticed injury to a neuropathic foot. Animal or human bites, prolonged pressure, mechanical trauma, chemical or thermal burns, surgery, venous or arterial insufficiency, fundamental dermatologic disorders can result in infected wounds in people with or without diabetes (A. Gomes et al., 2017). The lymphatic system or direct extension from the epidermis may be used to transport pathogenic

microorganisms deeper into the skin after they have invaded the epidermis. When an infection occurs underneath the dermal papillae, the existence of a dense capillary network is critical for the localization of the infection as well as the induction of an immediate inflammatory reaction (Lipsky et al., 2017).

### 3.2.2 Wounds

Some varieties of SSTI, such as diabetic foot infections, get their infectious agents from the normal skin around it, which can be permanent or temporary flora. Endogenous pathogens are found in the anterior nares, or the perineum is deposited directly in the wound by direct inoculation. Another kind of pathogens, known as exogenous, are brought into the body due to harm to the skin like accidental ,surgical or during the wound healing process (Shedoeva et al., 2019). The risk that an infection will occur in a colonized wound is directly proportional to the inoculum size of the contaminating organism and the intrinsic virulence. It is inversely proportional to the host's ability to defend itself. The susceptibility of some species, such as β -hemolytic streptococci and *Staphylococcus aureus*, is essentially high in wounds. Still, other organisms only become pathogens when their combinations are present in abundance, inoculate injured skin, or colonize a wound in an immunocompromised host. The existence of a foreign body, necrotic tissue, local ischemia enhances the likelihood of a wound becoming infected as well as the severity of the infection.

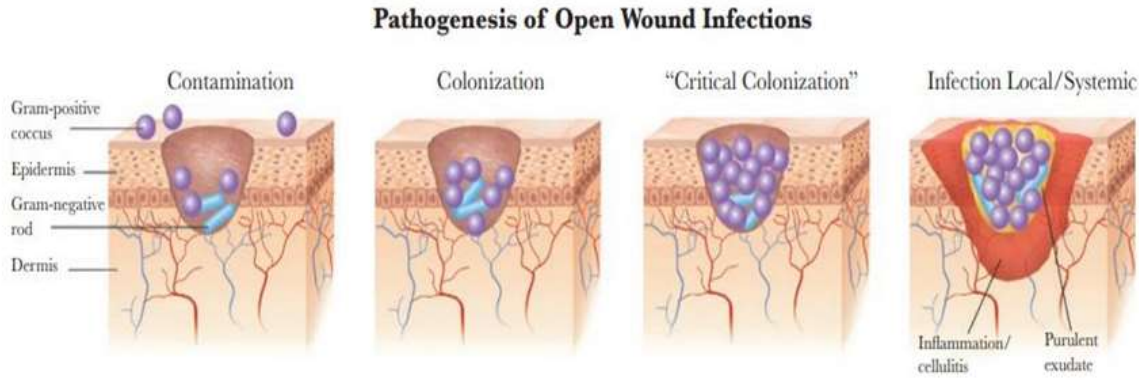


*Figure 7: Evolution of a superficial wound infection and growth of microorganisms (Lipsky et al., 2017)*

Especially *S. aureus*, aerobic gram-positive cocci, and less frequently, streptococci, are the most common pathogens, but specific exposures to water-related trauma or bite wounds are associated with less common pathogens such as Enterobacteriaceae, *Pseudomonas aeruginosa*, and obligate anaerobes (Lipsky et al., 2017).

# Chapter 4

# Antimicrobial Peptides

## 4.1 Antimicrobial peptides

Antimicrobial peptides (AMPs) are cationic peptides that are found in nature and have the capacity to destroy bacteria. They usually have a length of less than 50 amino acids. They may be found in both vertebrates and invertebrates and they work in both adaptive and non-adaptive immune systems (Eton & Lepore, 2008). The general features of antimicrobial peptides, such as net charge, hydrophobic character, secondary structure, and conformation, determine their activity on target cells. AMPs have antimicrobial efficacy against a broad range of viruses, fungi, bacteria, as well as immunomodulatory effects on skin surfaces (Pfalzgraff et al., 2018b).

## 4.2 Source

According to the source, AMPs are divided into natural AMPs and synthetic AMPs.

### 4.2.1 Natural AMPs

AMPs are made in nature in either of the following ways: ribosomal mRNA translation or nonribosomal synthesis. Nonribosomal synthesis of peptides is created mainly by bacteria, whereas all living forms produce ribosomally synthesis of AMPs. Antibiotics derived from nonribosomal peptides have been utilized for a long time, but in recent years, ribosomally generated peptides have received increasing attention due to their therapeutic potential (Bin Hafeez et al., 2021).

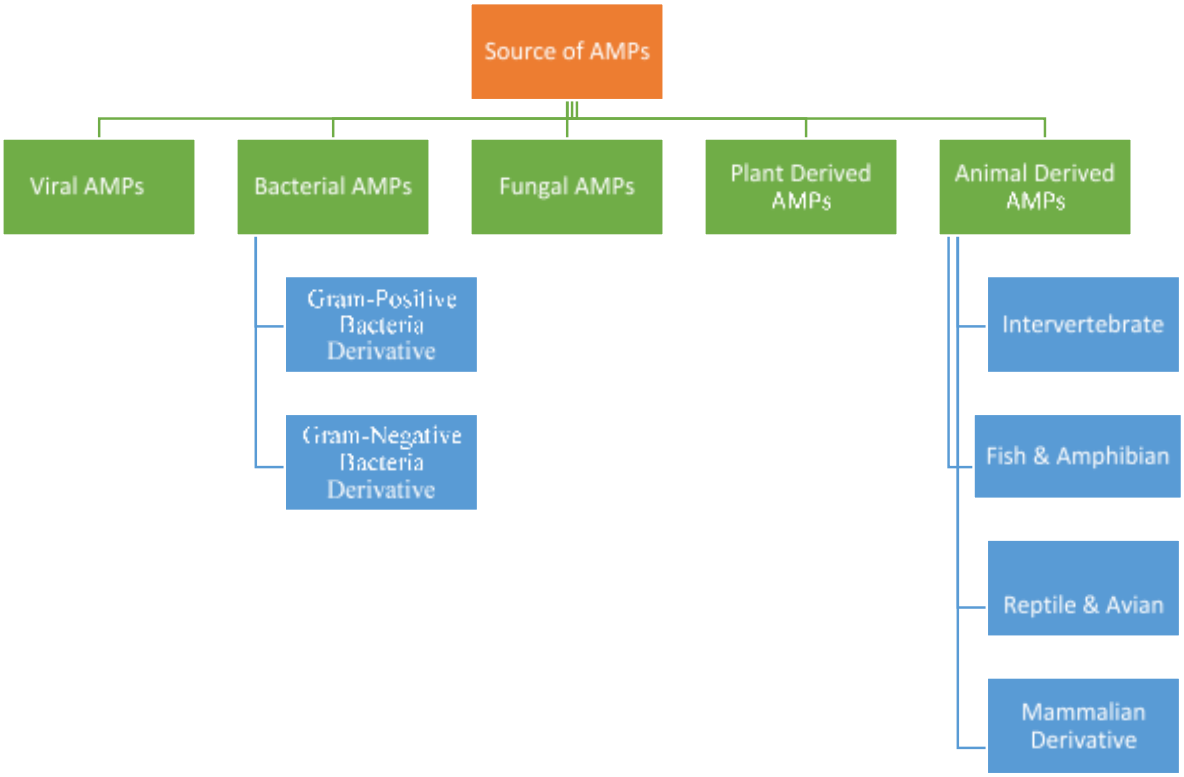


*Figure 8: Source of Natural AMPs modified from-(Bin Hafeez et al., 2021)*

### 4.2.2 Synthetic AMPs

Isolating AMPs from natural sources, can lead to the discovery of new synthetic AMPs generated from natural sources have certain negative features such instability due to poor selectivity, hemolytic activity, host protease degradation, host toxicity, salt sensitivity and low hydro solubility. Synthetic AMPs were developed to address these issues. The purpose of synthesizing AMP sequences is to remove the better piece, which has antibacterial effect, while excluding bad half, which has toxicity and low proteolysis resistance. The logical design of these peptides is based on the relationship between their structure and activity, as each amino acid is successively added to the AMP, allowing for precise alterations, analysis, and optimization. One of the most common approaches is to take the sequence of naturally existing

AMPs as a template and then construct a new synthetic peptide. Another possibility is to use AMP libraries and bioinformatics technologies that help identify and combine new AMP structures. Specific amino acids may be altered with the use of in silico technology, and designed AMPs can be tested for increased antibacterial action (Erdem Büyükkiraz & Kesmen, 2021). Addition of cationic as well as hydrophobic residues are some modifications which helps to show higher antimicrobial activity also acetylation, cyclization, d-amino acids, peptidomimetics are needed to enhance the stability. For example, the natural antimicrobial peptide AamAP1 derived from *Androctonus amoreuxi* demonstrates antimicrobial activity in the case of *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* at concentration levels ranging from 20 to 150 μM, whereas the synthetic version of this peptide, AamAP1-lysine, demonstrates antimicrobial activity towards the same pathogens at a concentration range of 5–7.5μM. Some other example of synthetically synthesized peptides is Rs-AFP2, which is 2.6 times more effective against *Fusarium culmorum* than the natural Rs-AFP2. In spite of the difference in efficiency between natural and synthetic peptides, it is worth mentioning that both synthetic and natural antimicrobial peptides have the same mode of action against infections, indicating that natural and synthetic antimicrobial peptides share common attributes (Lima et al., 2021).

## 4.3 AMP structures

According to structure-based categorization, AMPs are classified into four separate groups depending on the presence of secondary structures: (i) α-helical, (ii) β-sheets (at least two), (iii) αβ, and (iv) non-αβ. Also, another group of AMPs are available based on structure, and it is cyclic and unusual AMPs.

### 4.3.1 α-helix AMPs

α-helical AMPs are found in mammals, insects, amphibians, plants, fish, and. The α -the helical structure of these AMPs is strongly dependent on membrane contact, and for achieving

membrane targeting function, the peptide must adopt an amphipathic configuration. A typical barrel-stave model can clarify the basic structure of this kind of AMPs. This model makes a transmembrane pore that allows things to pass through. The hydrophobic part of the α- helix is connected to the membrane lipid core via bundles in the membrane. In contrast, the hydrophilic portion of the helix is connected to the inside of the membrane by a pore. The amino acids Leu, Ala, Gly, and Lys, are commonly found in abundance in the α -helices. Example: melittin, cecropin, moricin, magainin.

### 4.3.2 β-sheet AMPs

β-sheet AMPs form at least two strands and several linear structures arranged in a β-hairpin-like pattern. Peptides typically include between 2 to 8 cysteine residues, resulting in 1 to 4 pairs of intramolecular disulfide bonds. These peptides' structure and biological activity are both dependent on disulfide bonds. For instance, defensins' disulfide bonds offer structural stability and inhibit protease-mediated destruction. Hydrophobic side chains and cationic residues found on antiparallel β-sheets are usually attributed with antibacterial activity. This class of peptides is frequently high in Lys, Gly, Leu, Ala, and polyphemusin I, protegrin-1, tachyplesin, gomesin, and thanatin, are a few examples (Q. Y. Zhang et al., 2021).

### 4.3.3 αβ AMPs

AMPs with α-helices and β-sheets have a considerable affinity for membranes. Defensins, which interact with sphingolipids in fungal membranes or microsomal membranes, are the most well-known members of this family, exhibiting antifungal activity. For instance, in *Neurospora crassa*, the antifungal peptide pisum sativum defensin-1 possesses a fold that inhibits cyclin F, interfering with the cell cycle. Also, RsAFP2, a defensin from *Raphanus sativus* that interacts with yeast and fungal glucosylceramides, causes cell death in Candida albicans by activating a

signalling cascade implicated in reactive oxygen species. The beta-defensins (hBD3, hBD1, hBD2), which include an αβββ fold, are examples of αβ-AMPs in humans.

### 4.3.4 Non-αβ AMPs

Non-AMPs, or extended or loop peptides, are peptides that lack α -helix and β -sheet structures. These peptides include variants with a high tryptophan content, a high glycine content, and a high proline content. Numerous peptides high in tryptophan have an amphipathic structure. Indolicidin, for example, is an amphipathic molecule with a tryptophan-rich core domain necessary for peptide anchoring. The aromatic rings Trp9 and Trp6 packed against Pro7 and Pro10 interacted with indolicidin connected to dodecylphosphocholine (DPC) micelles. When lactoferrin B2 interacts with SDS micelles, the Trp-rich regions take on an irregular non αβ-conforming shape with a bent backbone. On the other hand, proline-rich peptides range in length from 15 to 39 residues and operate on intracellular targets.

### 4.3.5 Cyclic and unusual AMPs

These peptides form a new class of AMPs, called the fifth class. Crosslinks (for example, thioether or disulfide bonds) and cyclic topology (head-to-tail or head-to-side-chain) can also be used to categorize this category further. The N-to-C-terminal covalent bond and lack of further connections distinguish cyclic bacteriocins, a class of peptides produced by ribosomes. Examples are carnocyclin A from *Carnobacterium maltaromaticum* UAL307 and enterocin NKR-5-3B from *Enterococcus faecium*, which have four α -helices and are connected at the N-terminus and C-terminus. A linker connects N-terminal Leu at position 1 of carnocyclin A to C-terminal Leu at position 60.

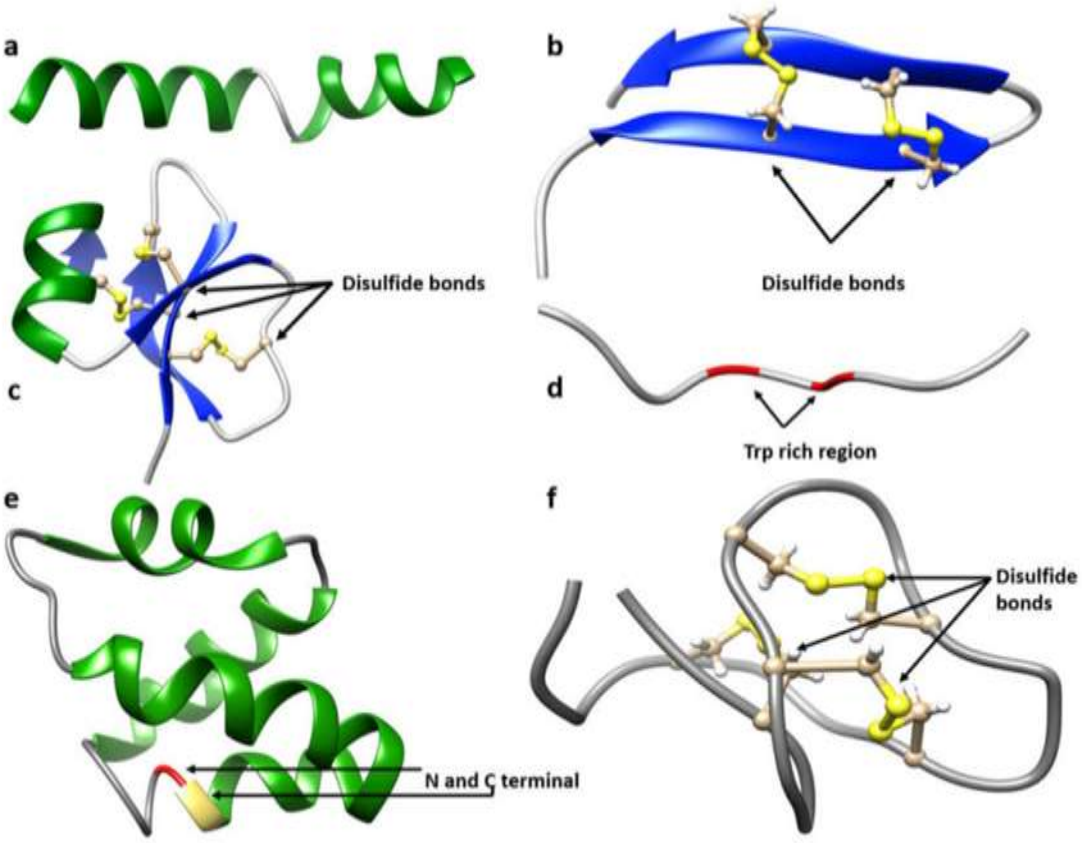


*Figure 9: Classification of AMPs based on structure*

*(a) Melittin (α-helix AMPs)*

*(b) Protegrin-1(β-sheet AMPs)*

*(c) hBD2 (αβ-AMPs)*

*(d) Indolicidin (non-αβ AMPs)*

*(e) Carnocyclin A (Cyclic AMPs) (Bin Hafeez et al., 2021)*

## 4.4 Mechanism of action of AMPs

In various microbes and bacteria, AMP molecules have a variety of methods of action for resistance. The form of their cationic charge is amphipathic. The principal mechanism of action is electrostatic interaction with negatively charged bacterial surfaces, followed by membrane breakage. AMPs can disrupt metabolic processes, reduce protein production or enzyme performance, and even act intracellularly.

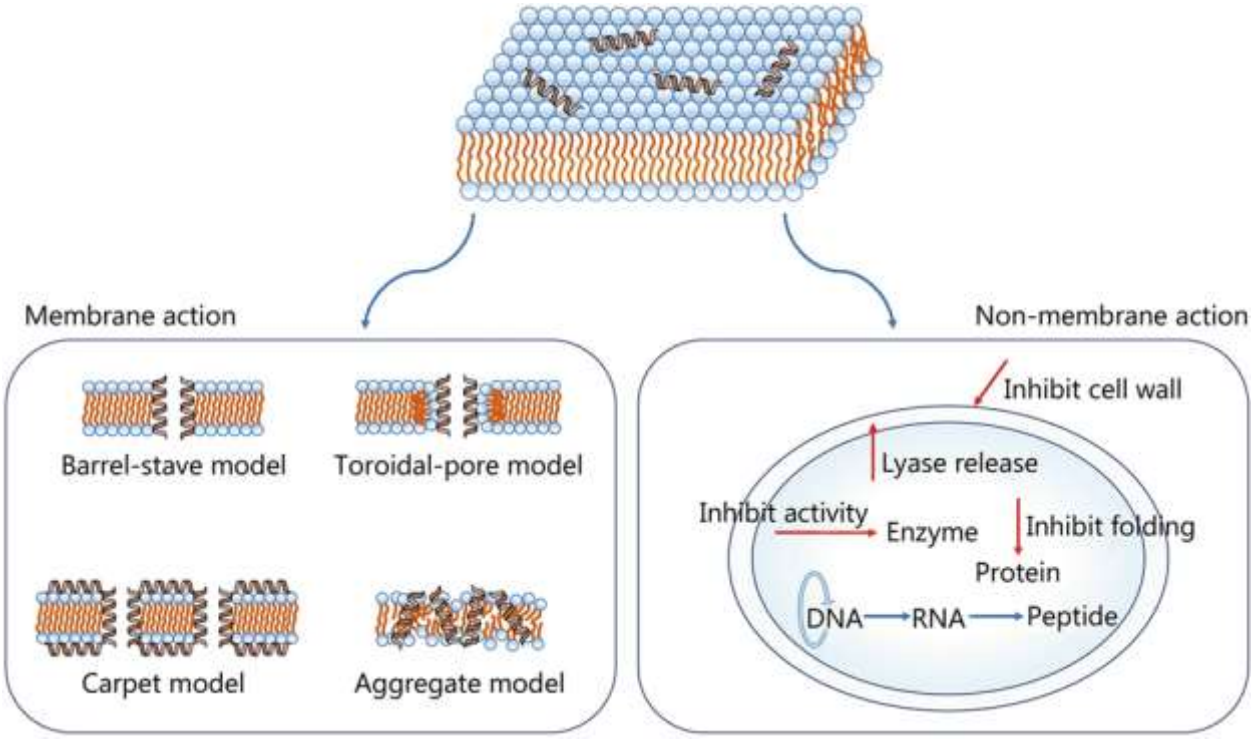


*Figure 10: Mechanism of Action of AMPs (Q. Y. Zhang et al., 2021)*

### 4.4.1 Membrane disruptive mechanism

In the Membrane Disruptive Model, cationic AMPs interact with bacterial membranes, which have a negative charge and promote the membrane's permeability, resulting in the lysis of cell membrane and discharge the cell content. These proteins bind to the microbial membrane and interact with the plasma membrane's anionic components as they approach the cytoplasmic membrane through electrostatic interaction with it. AMPs must first pass via the capsule containing polysaccharides as well as other cell wall components, such as gram-negative bacteria's lipopolysaccharides and gram-positive bacteria's lipoteichoic acid and peptidoglycan (Q. Y. Zhang et al., 2021). Membrane disruptive model is also divided as toroidal-pore, barrel-stave, aggregate, and carpet models for the formation of cavity. After attachment, each of these models creates a hole in the surface of the cell membrane, impairing the cell membrane's stability. Peptides are parallel to the lipid surface, and they align themselves perpendicular to

the cell membrane as its concentration rises. At greater peptide concentrations, antimicrobial action is induced via the breakdown of the cell membrane. In the 'Barrel-stave' model, the hydrophobic region is oriented toward the lipidic section, while the hydrophilic region comprises the interior of the pore. According to the Carpet model, peptides are electrostatically bonded to the negatively charged cell membrane and are distributed throughout the whole cell. The additional peptides induce the lipidic portions of the "Toroidal pore" model to bend, resulting in the formation of a pore structure (Mukhopadhyay et al., 2020).

### 4.4.2 Non-membrane disruptive mechanism

Direct Killing is another phrase used to describe modes of action that do not target membranes. These non-membrane targeting AMPs can be classified into two categories: those that target the bacterial cell wall and those that target intracellular targets. In addition to a wide spectrum of precursor molecules essential for cell wall synthesis, AMPs typically interact with a highly conserved lipid II. The negatively charged sugar moiety of the lipid II molecule, for example, is bound by AMPs such as defensins, which may induce the formation of holes and the disintegration of the membrane molecule. AMPs such as human α-defensin-1,β-defensin-3 are bactericidal because of their ability to selectively attach to lipid II (Kumar et al., 2018). Numerous AMPs target is intracellular due to their ability to kill bacteria at the lowest effective dosage without causing membrane permeabilization. For this, AMPs first interact with the cytoplasmic membrane before accumulating intracellularly, where they might disrupt vital cellular functions. Many novel strategies involving intracellular targets have been found, including nucleic acid or protein synthesis inhibition and disruption of the function of protein. For example, buforin II, a frog-derived histone AMP, does not permeabilize the bacterial membrane and bonds to *E.coli* RNA and DNA. Cytoplasm of *E.coli* contains human alpha defensin-5, which concentrates at the cell division plate and the opposite poles, indicating that cytoplasmic targets may account for some of the antibacterial action. The antibacterial activity

of some additional AMPs, such as indolicidin, human defensin-4, human defensin-one, has been demonstrated to target bacterial components present within the cell's membrane.

### 4.4.3 Immune modulation mechanism

There are several ways that antimicrobials may help the body's immune system fight against illness besides directly killing the microorganisms. AMPs have been shown to reduce inflammation by reducing the quantities of proinflammatory cytokines released in response to pathogen signature molecules and thus to modulate chemokine activity. It is advantageous to have these effects during microbial infections because they may prevent excessive and dangerous proinflammatory responses that can lead to organ failure and sepsis from occurring. It has also been shown that AMPs may improve wound healing by increasing the synthesis of restructuring metalloproteinases, increasing epithelial cell metabolism, and increasing the migrating of epithelial as well as keratinocyte cells. Chemotaxis, angiogenesis, and the formation of macrophages (leukocytes) and dendritic cells may all be influenced by AMPs. Cell surface receptors may be activated by AMP-induced receptor signaling; AMPs may affect the receptor's activation state and function by modifying the membrane portion that contains the receptor; AMPs may release a membrane-bound component that may then bind to its receptor (Lima et al., 2021).

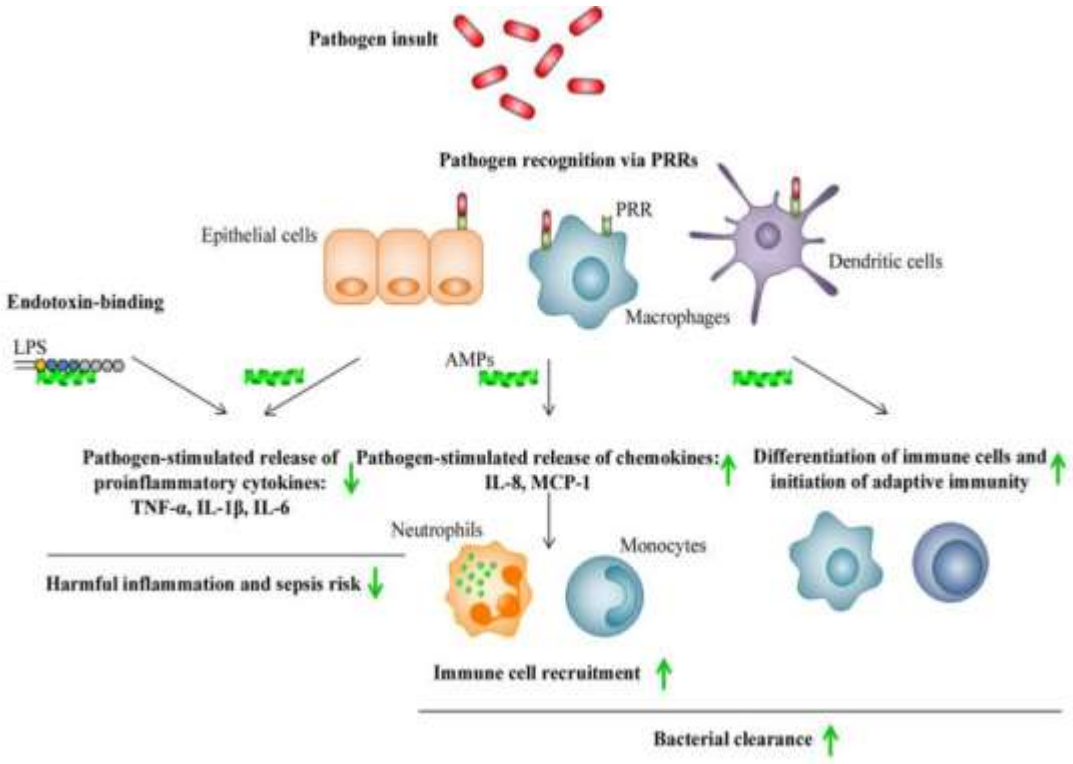


*Figure 11: Immunomodulatory activities of AMPs (Mahlapuu et al., 2016)*

AMPs have a wide range of immunomodulatory effects that help the body get rid of bacteria. These include encouraging chemotaxis, influencing immune cell differentiation, and initiating adaptive immunity, all of which help the body get rid of bacteria. Toll-like receptors, cytokine-mediated synthesis of proinflammatory cytokines, and an anti-endotoxin activity are also included in the immunomodulatory actions, which effectively prevent detrimental proinflammatory responses such as sepsis. For example, LL37 and bovine lactoferricin has shown to suppress the lipopolysaccharides induced release of TNF-α and IL-6 in THP-1 cells, respectively, while LL-37 reduces the lipoteichoic acid and lipopolysaccharides induced generation of TNF-α, IL-1β, IL-6, and IL-8 in primary monocytes (Mahlapuu et al., 2016).

# Chapter 5

# AMPs for The Treatment of Skin Diseases

## 5.1 Treatment of inflammatory skin diseases

The relationship between the severity of various skin disorders and AMP expression is particularly fascinating for applied dermatology. Positive or inverse correlations exist between the expression of AMPs and the disease. For example, comparison to normal skin, burns and chronic wounds have decreased levels of AMPs. AMP upregulation in human skin appears to be associated with enhanced protection against infection in psoriasis, rosacea, and other inflammatory skin conditions. These data suggest that AMPs may be used therapeutically (Korting, 2012).

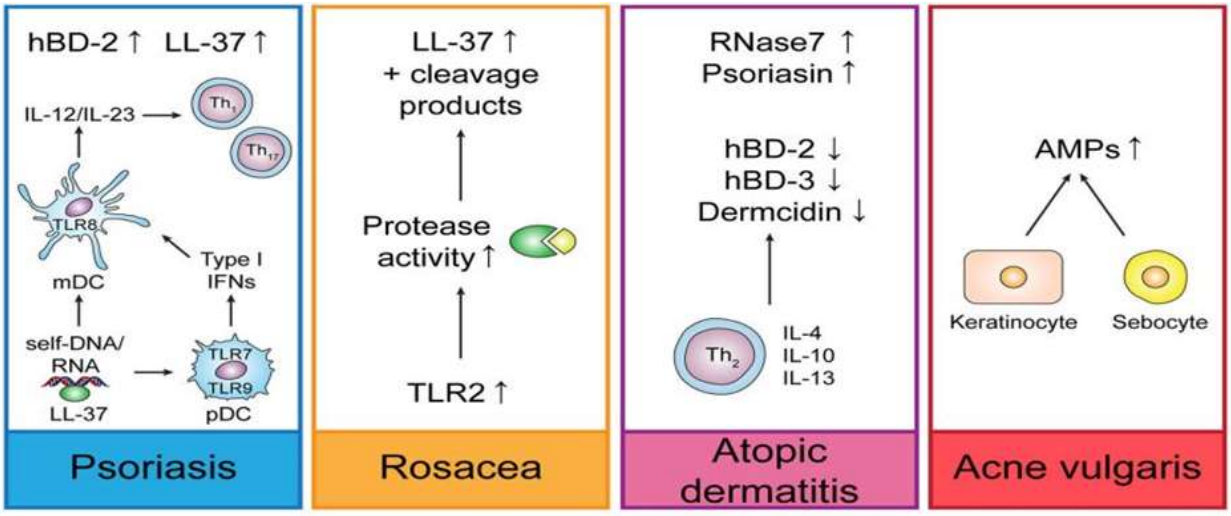


*Figure 12: AMPs regulation of different skin diseases (Pfalzgraff et al., 2018a)*

It is possible that patients with atopic dermatitis are more susceptible to *S. aureus* superinfections as a consequence of lower AMP expression. In addition, studies have indicated decreased expression of hBD-2, LL-37, and hBD-3 in atopic dermatitis skin due to the absence of important inducers like as IL-22, IL-1, IL-17 as well as an increase in the production of Th2

cytokines. Due to the inflammation in atopic dermatitis, individuals had considerably decreased expression of sweat gland-derived dermcidin. This may be in accordance with the finding that UVB phototherapy promotes eczema healing by increasing vitamin D production, the same vitamin that promotes LL-37 production in atopic dermatitis patient's skin through oral supplementation. Even in severe atopic dermatitis, there was no *E. coli* skin superinfection due to the expression of psoriasin that is an *E. coli* killing AMP (Marcinkiewicz & Majewski, 2016). Certain AMPs, such as hBD-2 and hBD-3, were initially detected in patients with psoriasis. Skin superinfections are rare in this chronic, inflammatory, and hyperproliferative skin disease. It has been postulated that lesional psoriatic skin releases antimicrobial peptides as a consequence, hBD-2, hBD-3, psoriasin, RNase7, LL-37, and calprotectin are all increased in psoriatic skin. LL-37, a dermal cathelicidin, adheres to self-DNA and activates dermal plasmacytoid dendritic cells, whereas LL-37, an epidermal cathelicidin, binds to keratinocyte cytosolic DNA and suppresses pro-inflammatory activity. This could explain why vitamin D3, which increases cathelicidin synthesis in keratinocytes and monocytes, is helpful in the treatment of psoriasis and reduces inflammation in psoriatic lesions (V. Wang et al., 2021). Psoriasis and other inflammatory skin disorders have been linked to colonization with Staphylococcus and Streptococcus bacteria. Toll-like receptors are thought to initiate this reaction by recognizing foreign microbial compounds and eliciting a strong immune response as a result. Inflammation is triggered when *S. aureus* interacts directly with psoriatic keratinocytes. *S. aureus* produces inflammation by inducing keratinocytes to express some proinflammatory cytokines, such as vascular endothelial growth factor, TNF-α, IL-6, and IL-8, as well as by increasing the generation and release of AMPs through TLR-dependent pathways, all of which contribute to the inflammation.

Synthetic AMPs have an antibacterial and anti-inflammatory effect due to their direct bactericidal activity and their ability to attach to bacterial components such as peptidoglycan,

lipoteichoic acid, protein A, α-toxins, and superantigens. Synthetic AMPs inhibit the release of pro-inflammatory cytokines such as tumor necrosis factor, interleukin-8, chemokines, endogenous AMP, and VEGF in keratinocytes is shown in (Figure 13). As a result, these characteristics make them appealing therapeutic options for the treatment of psoriasis, a chronic inflammatory disease with bacterial components (Ryu et al., 2019).

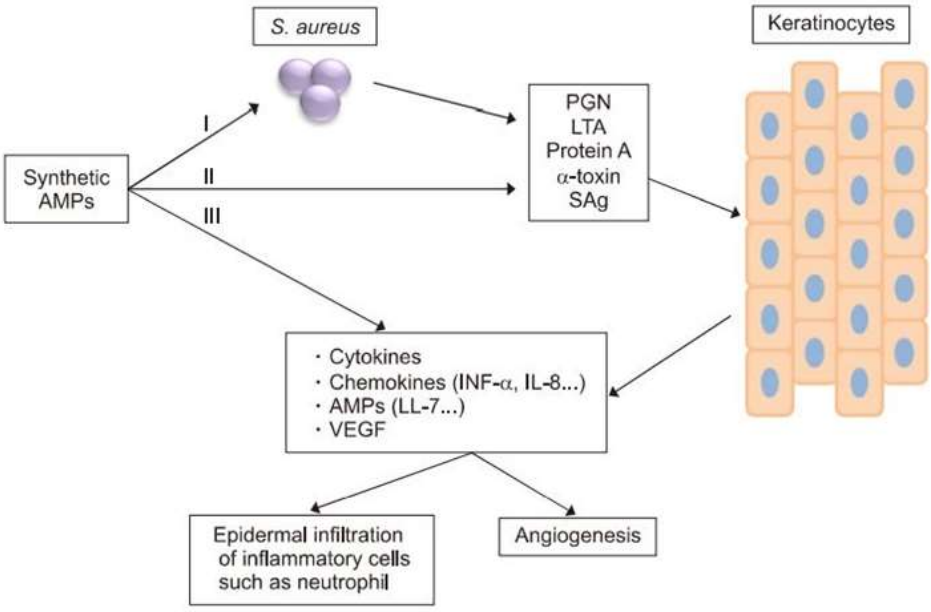


*Figure 13: Antimicrobial and anti-inflammatory actions by the synthetic AMPs in psoriatic keratinocytes (Ryu et al., 2019)*

Aside from direct AMP treatment, several strategies are being taken to boost endogenous AMP production. Vitamin D3, for example, has shown to increase the expression of several AMPs, including hCAP18 (a precursor to LL-37), neutrophil gelatinase-associated lipocalin, defensin b2. Vitamin D3's most researched target gene is the hCAP18 gene, significantly activated in vitro by 1,25-dihydroxyvitamin D3. Furthermore, topical 1,25(OH)2D3 treatment increased the number of hCAP18 in human skin in vivo (Mahlapuu et al., 2020).

Furthermore, Antimicrobial peptides like as endogenous bacteriocin, β-defensin, granulysin, and, as well as exogenously synthesized P5, pentobra, and granulysin-derived peptides, have been shown to have antimicrobial activity against *P. acnes* in experimental studies, and this

may result in a reduction in the expression of inflammatory cytokines (Aslan Kayiran et al., 2020). HP (2–20) and its derivatives, particularly HPN3 and HPA3NT3, are being studied for their potential therapeutic use in the treatment of acne produced by *Propionibacterium acnes*, according to one of the patents. HP (2–20) and HPA3NT3 suppressed the development of *P. acnes* in early microbiological tests with the same potency as the antibiotic clindamycin, with MIC values of 0.8 and 0.4 M (Wollenberg et al., 2018). Furthermore, HPA3NT3 demonstrated a minimal inhibitory concentration (MIC) that was 156 times lower than benzoyl peroxide, a commonly used antibiotic for *P. acnes* skin infections (Kosikowska & Lesner, 2016). Additionally, cathelicidin LL-37, beta-defensins, and psoriasin generated in keratinocytes as well as sebocytes are capable of killing *P. acnes*, indicating that these AMPs may be possible therapeutic targets for acne etiology in the near future (B. Gomes et al., 2018). Another AMP with potential for use in acne treatment is omiganan, a novel synthetic cationic analogue of the AMP indolicidin, which was first isolated from bovine neutrophil cytoplasmic granules. Omiganan has the potential to be used in the treatment of acne in the future. In vitro, it has been shown to be effective against a wide range of microorganisms, including gram-positive and gram-negative bacteria and fungus. Furthermore, omiganan is the most advanced medicine at the forefront of AMP uses. It is currently being investigated in clinical studies to treat acne, rosacea, and atopic dermatitis. Certain AMPs have also been promoted for the treatment of acne vulgaris. They are as follows:

Defensins, which are determined by keratinocyte cells, are one of the most studied compounds and play an important role in the innate immune system, particularly in the skin. As cationic sequences rich in cysteines, they make it easier to form disulfide bridges and are hence more robust, due to the beta-leaf structure they take on. Based on current research, it appears that these chemicals interact with microorganisms by creating pores in the lipid membrane of the host, which could lead to death through osmotic imbalance (Gaspari et al., 2017).

Psoriasis and acne lesions triggered by *Propionibacterium acnes* bacteria, human beta-defensin type 2 was discovered as a pro-inflammatory molecule. Cathelicidine is a 37-amino-acid cationic peptide with alpha-helicoidal propensity and amphipathic sections. Keratinocytes, mast cells, neutrophils, epithelial cells express this peptide. It interferes with the *P. acnes* membrane electrostatically at first. Then because of its tertiary structure, it shows that it can be incorporated into the lipidic bilayer, attempting to promote the formation of pores-channels that allow cellular material to enter and exit, ultimately killing the pathogen.

Granulisine is a peptide that has a high molecular weight. It is found in natural killer (NK) and cytotoxic T cells with a wide range of bacteria-killing abilities, including *P. acnes*. It has five alpha helicoidal sections within the tertiary structure composed of 74 amino acids, two of which have disulfide bonds. This polypeptide has been shown to have antibacterial and anti-inflammatory properties, mainly when produced with modified d-amino acids. Its bactericidal effect is owing to the molecule's natural capacity to be poisonous to the presence of foreign substances such as Propionibacterium acnes, which helps to inhibit the growth of this microorganism (M. Gómez et al., 2019).

The LZ1 peptide generated by Zhang and colleagues which is derived from the combination of known antimicrobial peptide families. This fusion assembles a molecule with antibacterial activity that has been optimized for length, load, hydrophobicity, and amphipaticity. It is composed of 15 monomer residues with helical alpha potential and amphipathic properties. With the native sequences of each original family, it was designed to inhibit *P. acnes* and *Staphylococcus epidermis* growth while also inhibiting cytokine release.

The antimicrobial peptide CEN1HC-Br is produced from the Centrocin1 peptide, a 30 amino acid heterodimeric peptide with an intramolecular disulfide bond. The halogenated atom in the CEN1HC-Br peptide confers antibacterial action against microorganisms. The Anti-inflammatory benefits of this peptide against *Propionibacterium acnes* need a lower

concentration per dosage supplied (in vivo) than conventional acne medicine Climdamicin® or Eritromicin®. However, it's still being explored because bromine could produce adverse effects in hypothetical human treatment (M. Gómez et al., 2019).

## 5.2 AMPs for the treatment of SSTIs and wounds

The development of antibiotic resistance challenges the therapy for SSTIs and wound care. A novel therapeutic alternative may so emerge from the AMP family. AMPs have a broader scope than conventional antibiotics. Due to its low bloodstream permeability, AMP preparations allow for high concentration at the target site for topical application. AMPs influence cell motility, chemotaxis, cytokine release, and angiogenesis, limiting pathogen proliferation and promoting wound healing. As a result of these favorable benefits, AMPs may be used to treat both infectious and non-infectious wounds. Indeed, the majority of peptide-related antibiotics licensed by the FDA for skin infections are topical, such as oritavancin, daptomycin, while others, such as LL37 for difficult-to-heal venous leg ulcers, are still in clinical studies (Luong et al., 2020). Frequently, many bacteria colonize non-healing wounds and the most commonly identified bacteria from such damages are *Staphylococcus aureus*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa*. The innate immune system in healthy people produces gene-encoded AMP, which suppresses pathogenic microbe proliferation on the skin and triggers a signaling cascade in response to injury. Diabetic patients, on the other hand, are immunocompromised as a result of their venous insufficiency and other diabetes-related problems that encourage the formation of bacterial biofilms that are highly resistant to antibiotics (A. Gomes et al., 2017).

Glycopeptides and lipopeptides are utilized widely to treat and prevent surgical site infections and severe skin and skin structure infections. Numerous naturally occurring AMPs are effective against methicillin-resistant *staphylococcus aureus,* enterococcal pathogens, and resistant gram-negative isolates in vitro, implying the possibility of preventing and treating these serious

organisms prevalent in SSTIs. In experimentally infected mouse soft tissue wounds, MRSA is significantly killed by lactoferrin-derived topical AMP (Fry, 2018).

Wound healing can be divided into three stages: inflammatory, proliferative, and remodeling, and endogenous AMPs play a vital role in each. The insufficient healing process resulting from infection or bacterial colonization can lead to difficult-to-treat chronic wounds that commonly persist in an inflammatory state (Golan, 2019). Chronic wounds are mostly polymicrobial, with *S. aureus* and *Pseudomonas aeruginosa* being the most prevalent pathogens, posing a treatment challenge given the rising resistance to conventional antibiotics, including topical medicines like mupirocin, fusidic acid. AMPs have a lot of healing properties, which makes them good options for treating wounds, SSTIs. The AMPs, on the other hand, should be able to withstand high salt concentrations, and proteases at the wound site also have some characteristics like low cytotoxicity and increased stability, which makes them capable of treating both noninfected and infected wounds on the surface. Aside from antibacterial activity, AMPs may have immunomodulatory features such as anti-inflammatory effects, frequently more clinically significant.

*Table 1: Antimicrobial peptides with wound-healing activities (Pfalzgraff et al., 2018a)*

| Peptides | Primary amino acid sequence |
|---|---|
| AH90 | ATAWDFGPHGLLPIRPIRIRPLCG |
| Catestatin | SSMKLSFRARAYGFRGPGPQL |
| CW49 | APFRMGICTTN |
| DRGN1 | PSKKTKPVKPKKVA |
| Epi-1 | GFIFHIIKGLFHAGKMIHGLV |
| Esculentin-1a(1-21)$NH_2$ | GIFSKLAGKKIKNLLISGLKG |
| LL-37 | LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES |
| Melittin | GIGAVLKVLTTGLPALISWIKRKRQQ |
| Pep19-2.5 | GCKKYRRFRWKFKGKFWFWG |
| Pep19-4LF | GKKYRRFRWKFKGKLFLFG |
| SHAP1 | APKAMKLLKKLLKLQKKGI |
| SR-0379 | MLKLIFLHRLKRMRKRLkRK |
| Tiger17 | WCKPKPKPRCH |
| Tylotoin | KCVRQNNKRVCK |
| WRL3 | WLRAFRRLVRRLARGLRR |

It has also been demonstrated that the antibacterial agents Pep19-2.5 and Pep19-4LF can inhibit the response mediated by TLR2 and TLR4 induced by cell wall-derived inflammatory toxins from gram-positive and gram-negative bacteria in skin cells such as dendritic cells, dermal fibroblasts, and keratinocytes. Furthermore, it has been demonstrated that both natural and synthetic AMPs can activate critical wound healing processes such as cell migration, proliferation, and angiogenesis. The transactivation of the epidermal growth factor receptor by specific peptides promotes cell migration, which is a critical mechanism during the re-epithelialization process. In keratinocytes, the epidermal growth factor receptor (EGFR), Pep19-2.5, and Pep19-4LF accelerated artificial wound closure by activating purinergic P2X7 receptors, resulting in calcium influx and mitochondrial reactive oxygen species release, followed by metalloprotease-dependent transactivation of the EGFR and downstream activation of either ERK1 or ERK2. Pep19-2.5 mediated keratinocyte migration was suppressed by ATPase hexokinase is shown in (Figure 14). However, Pep19-2.5 failed to

increase extracellular levels of ATP, indicating that Pep19-2.5 either indirectly activates the P2X7R or increases the P2X7 receptor's sensitivity to adenosine triphosphate. Comparatively to other AMPs that promote cell migration and proliferation by trans activating the epidermal growth factor receptor (EGFR), SALPs promote keratinocyte migration but not proliferation. Furthermore, synthetic anti-lipopolysaccharide peptides (SALPs) may also effectively cure non-infected wounds and polymicrobial wound infections, according to in vitro and in vivo tests. Combining SALPs with antibiotics may increase therapeutic efficacy through a synergistic effect between the anti-inflammatory and re epithelialization promoting action of SALPs and the antibiotic's direct antibacterial impact (Correa et al., 2019).

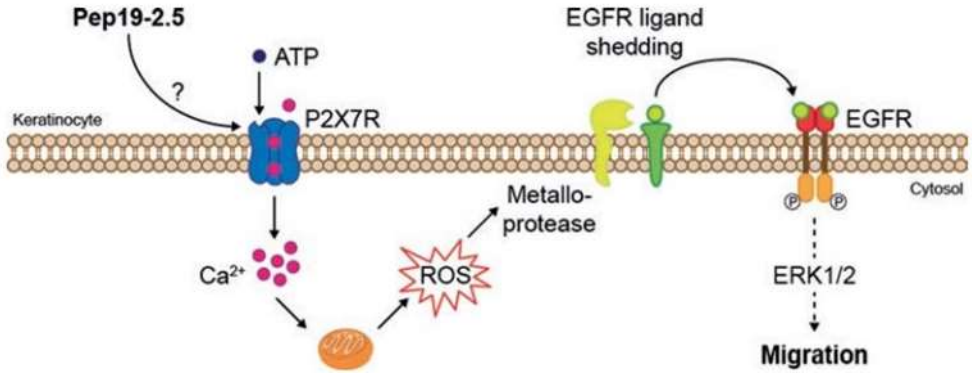


*Figure 14: Mode of action of Pep19-2.5-induced keratinocyte migration (Correa et al., 2019).*

Generally, pro-inflammatory cytokines are overproduced in chronic wounds, such as diabetic wounds, leading to wound healing that lasts longer in the inflammatory phase and lacks angiogenesis. If the AMPs are used in this case, they kill bacterial pathogens or regulate and balance the human immune response. It can be either by improving infection outcomes by boosting numbers of immune cells that clear infections or at the same time decreasing pathogenicity factor-induced production of cytokines that cause inflammation (Pfalzgraff et al., 2018b).

*Table 2: FDA Approved AMP Drugs list (Divyashree et al., 2019)*

| **Generic name** | **Source** | **Medical use** | **Mode of action** | **FDA approval** |
|---|---|---|---|---|
| Daptomycin | Cyclic lipopeptide | Bacterial infections of skin and underlying tissues | Membrane interruption, and inhibition of DNA, RNA and protein synthesis | 2003 |
| Telavancin | Semi-synthetic derivative of vancomycin | Skin and skin structure infections | Interference in cell wall and peptidoglycan synthesis | 2009 |
| Dalbavancin | Semisynthetic lipoglycopeptide | Bacterial skin and skin structure infection | Disruption of cell wall biosynthesis | 2014 |
| Oritavancin | Semisynthetic glycopeptide | Acute bacterial skin and skin-structure infections | Disruption of Gram-positive bacteria cell membrane and inhibition of trans glycosylation and transpeptidation. | 2014 |

## 5.3 Dosage forms of AMPs

Clinical trials are currently being undertaken on topical creams and gels, hydrogels, polyvinyl alcohol-based wound bed solutions, and hyaluronic acid-based surgical site hydrogels. Additionally, topical antibiotic therapy has a number of advantages since it results in a high antibacterial local load (B. Gomes et al., 2018). Furthermore, AMPs can aid in the fight against ESKAPE infections and regulate a range of activities, including the host's inflammatory and wound-healing processes, according to their pleiotropic mechanisms of action (Felgueiras, 2021). In contrast, AMPs used to treat chronic skin and soft tissue infections should not enter the bloodstream by absorption via the infection site and wound, nor should they provoke allergic sensitization. Since drug transport can occur through layers of the skin and hair follicles, topical administration of AMPs might induce systemic adverse effects. Furthermore, when peptides are created for therapeutic use, stability against enzyme degradation must be improved. Nevertheless, various peptidases like as, zinc-dependent endopeptidases, leukocyte elastase, interstitial collagenase cathepsins B and D are present at the epithelial cell membrane boundary. They should be evaluated since they have a broad specificity for degrading exogen peptides. Additionally, the use of nanocarriers for AMP administration has lately gained attention due to the particular advantages provided by nanoparticles in terms of AMP adsorption/encapsulation and suppression of peptide self-aggregation. For example, unique formulation techniques enable the development of DPK-060 for topical administration and the definition of a variety of dosage formulations, including Poloxamer Gel Formulation, Lipid Nanocapsule Formulation, and Cubosome Formulation (Håkansson et al., 2019). Peptide DPK-060 was created by adding three tryptophan residues to the C-terminus of the native 17-amino acid sequence of human protein kininogen. GKH17-WWW, the generic name for DPK-060, is a skin infection treatment medication. Furthermore, DPK060 is susceptible to a wide variety of microorganisms in vitro, including methicillin-resistant *S. aureus* Patients with atopic

dermatitis were tested for the safety and efficacy of a polyethylene glycol (PEG)-based ointment containing 1 percent DPK-060. After 14 days of twice-daily dosing, the microbiological density in eczematous lesions treated with DPK-060 1 percent ointment was dramatically reduced compared to untreated.

## 5.4 Routes of administration for AMP drugs

Intramuscular or subcutaneous administration, in comparison to other modes of administration, may not need an excessive quantity of peptide stability (Narayana et al., 2020). Similarly, these means of administration may overlook AMP's physicochemical and biological properties. At the same time, its size, poor penetration through the gastrointestinal wall, low stability at pH of gastric juice, and susceptibility to proteolytic enzymes all impede oral delivery. Thus, injection is the optimal mode of delivery for the majority of AMPs. Intravenous injection, on the other hand, places the peptides in contact with serum's esterase and peptidase activity. Due to its non-invasive and painless administration, the oral route is still a patient-friendly option (R. Zhang et al., 2018). However, the formulation of AMPs through oral pharmaceutical technology has not shown substantial progress in terms of bioavailability. The primary focus is on peptide stability, which is facilitated by pancreatic peptidases, such as pancreatic elastase a-chymotrypsin, trypsin which are produced by the pancreas into the gastrointestinal tract. Additionally, when a medicine is designed for lung administration, the high dosage and minimal systemic exposure allow for the minimization of systemic side effects. Inhaled peptide medicines have been shown to be superior in terms of quick onset. Peptide macrocycles with antibacterial action that operate as protein epitope mimics can also be synthesized for inhalation due to their chemical stability. Presently, topical treatments involving the incorporation of AMPs into nanoparticles, hydrogels, creams, gels, and ointments are the most widely used and developed AMPs applications, and additional research is required to exploit novel appropriate delivery methods. Simultaneously, AMPs have limited metabolic stability due to proteolytic

degradation, cytotoxicity, and low oral bioavailability. Digestive enzymes that hydrolyze the amide bonds in ingested proteins are likewise capable of hydrolyzing the amide bonds in AMPs. Additionally, the molecular weight, polarity of AMPs limits their intestinal permeability. Additionally, due to their quick proteolysis in the bloodstream and rapid clearance from circulation by the kidneys and liver, AMPs are not ideal for systemic administration. As a result, while certain AMPs are intended for intravenous and oral administration, it is acknowledged that local application remains the most prevalent mode of administration for AMPs (Mahlapuu et al., 2020)

*Table 3: List of AMPs that are selected in clinical phase of development (Mahlapuu et al., 2020)*

| **Antimicrobial peptides** | **Source** | **Indication** | **Route of administration** | **Clinical Phase** |
|---|---|---|---|---|
| Gramicidin | Polycyclic Peptide | Infected wound | Topical | III |
| Daptomycin | Lipopeptide | Skin infection | Intravenous | III |
| Pexiganan | Magainin analog | Diabetic foot ulcers | Topical | III |
| Omiganan (CLS001 or MBI-226) | Derived from bovine indolicidin | Atopic dermatitis, acne vulgaris, and rosacea | Topical gel | III |
| Brilacidin | Defence mimetic | Acute bacterial infection | Intravenous | II |
| LTX-109 (Lytixar) | Synthetic peptidomimetic | Gram-positive skin infections, impetigo | Topical hydrogel | II |

| | | | | |
|---|---|---|---|---|
| Lenopepden | Synthetic hydrazide | Bacterial skin infection | Oral | II |
| DPK-060 | Derived from human kininogen | Bacterial infections in atopic dermatitis | Ointment for local application | II |

## 5.5 Necessities to develop AMP drugs as an alternative

Antibiotic resistance is becoming a prominent topic due to antibiotic misuse and overuse, and a lack of effective antimicrobial agents against multidrug resistant bacteria causes a big challenge in treating the potentially fatal skin and soft skin tissue infection. Because of their unique membrane penetration method to kill germs, antimicrobial peptides have immense promise as one of the most promising antibiotic choices for fighting pathogenic illnesses (Z. Wang et al., 2021).

In most situations, antibiotics function by influencing bacterial metabolic activities such as the formation of cell walls, membranes, proteins, DNA, and RNA, which render bacteria more susceptible to antibiotic resistance. Bacteria use four main ways to become more resistant. They are:

- preventing cellular penetration,
- ejecting via efflux pumps,
- deterioration or alteration and
- alteration of the target.

However, antibiotics kill germs relatively slowly, which increases the likelihood of bacteria developing resistance through multiplication during the process. In comparison to conventional antibiotics, AMPs work directly on the bacterial membrane even in a faster way , which is more

energy-intensive to repair after disruption and thus less prone to produce development of resistance (Liang et al., 2020)

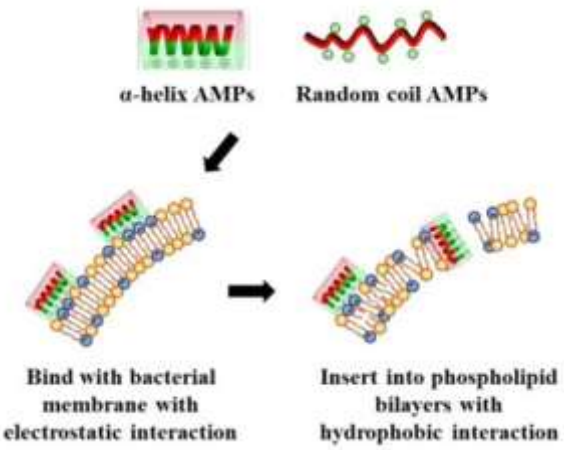


*Figure 15: General mechanism of action of Antimicrobial Peptide (Liang et al., 2020)*

## 5.6 AMPs over conventional antibiotics

Antimicrobial resistance has emerged as one of the most serious threats to global public health, owing to the widespread use of conventional antibiotics in healthcare and the general population. Even minor illnesses or accidents can be deadly if this disease is not adequately managed. Consequently, there is a necessity to develop new antibiotics. There are various advantages to using AMPs for this purpose, including that they are naturally produced by the human body to prevent infection by bacteria or microorganism growth (Luong et al., 2020). There is less possibility for antibiotic resistance, lower drug residues, a quicker time to action, and more selective toxicity with AMPs than with antibiotics. An AMP's several low-affinity targets make it harder for bacteria to use a certain resistance mechanism than with traditional antibiotics. It is also widely accepted that AMPs will not cause widespread bacterial resistance in the future. This means that AMPs might replace antibiotics as a viable option (S. Wang et al., 2021)

# Chapter 6

# Conclusion

The skin serves as an active organ of the immune system continually in contact with the external environment. Deficiencies in the skin's barrier or the balance between commensals and pathogens can lead to skin disease and even systemic illness. As well as providing a physical barrier, the skin's epidermis also elicits an immediate immunological response, allowing humans to fend off infection. The skin's epithelial cells not only act as physical barriers but also produce endogenous antibiotics known as AMPs. AMPs are small proteins that serve very first layer of protection against microorganisms. An antimicrobial peptide's amino acid count might range from five to over one hundred. Although AMPs come from various natural and synthetic sources, their different structural and physicochemical properties and mechanisms of action enable these peptides for therapeutic application. Additionally, many therapeutic development programs involving AMPs to treat human disease share common strengths and opportunities. These antimicrobial peptides (AMPs) protect against viruses, bacteria, fungi, and parasites. Host defense peptides, also known as AMPs, have recently attracted attention as possible therapeutics for infectious disease and novel immunomodulatory therapies. Psoriasis, atopic dermatitis, and acne vulgaris skin inflammatory conditions are associated with an abnormal expression of AMP in the skin. It is typical for germs to invade the dermis and cause skin infections due to the loss of elasticity and suppleness that occurs as we age. SSTIs can be mild to severe if the skin is damaged by trauma such as scratches, burns, ulcers, skin anomalies, wounds, or skin thinning. SSTIs are infections of the skin and underlying soft tissues, such as muscles, fat, and fascia, that are caused by a microbial invasion. Bacterial soft tissue diseases including carbunculosis, necrotizing fasciitis, and erysipellis are all caused by *S. aureus* and *S. pyogenes*, which are Gram-positive bacteria. AMPs offer

enormous promise as one of the most promising antibiotic alternatives for battling pathogenic infections due to their unique membrane penetration method of killing microorganisms. Antibiotic resistance is less likely, drug residues are smaller, the time to act is faster, and toxicity is more selective with AMPs than with antibiotics. As an AMP has a lot of low-affinity targets, bacteria have a hard time protecting themselves with a specific resistance mechanism than they do with traditional antibiotics, which have fewer targets. The spread of microbial resistance to traditional antibiotics has led to more efforts to make anti-infectives with new mechanisms of action that are less likely to be resistant to bacterial resistance. AMP medicines such as LTX-109, Omiganon, and DPK-060, for reference, are currently in the clinical development phase as topical formulations to treat Gram-positive infectious skin diseases, impetigo a soft skin tissue infection, bacterial infections in acne vulgaris, rosacea, atopic dermatitis. So far, only a few AMPs have reached the clinic to treat skin diseases, including daptomycin, telavancin, oritavancin, teicoplanin. The development of AMP-based pharmaceutical product candidates is projected to accelerate in the coming years as a greater understanding of how AMPs work, novel packaging strategies, and more sophisticated chemical synthesis methods become available. Costs of production, peptide absorption, cytotoxicity, and efficacy are all significant challenges to AMP medical application. Numerous strategies have been devised to overcome these barriers, including the synthesis of ultra-short AMPs, delivery systems and particularly targeted AMPs, chemical modifications, and careful selection of a counter-ion during the last phase of AMP production. While not all AMPs in the clinical pipeline will reach the market, these strategies may aid in the success of AMPs in clinical trials. Notably, even after local therapy, AMPs are prone to degradation by tissue proteolytic enzymes. Optimizing the pharmacokinetic properties of the next generation of peptide-based therapeutics is another challenge. Enhancing peptides' interaction with

membranes, increasing their protease resistance, and minimizing their clearance should be prioritized.

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