The Emergence of Peptides and Dendrimers in Dentistry

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Edited by

Shashikiran N. D.,
Sachin Gugawad,
Namrata Gaonkar,
Savita Hadakar,
Sonali Waghmode,
Pranjali Pokharkar
and Snehal Patil

Contributors: Ankita Maurya, Gouri Reddy,
Prathamesh Nikam and Vaishaki Pawar

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PART A:

PEPTIDES IN DENTISTRY

1. Introduction to Peptides and Peptide Bonds

Almost all aspects of life are engineered at the molecular level, and without understanding molecules we can only have a very sketchy understanding of life itself.

The structure of peptides has been known for more than a century, but it was not until du Vigneaud published the synthesis of oxytocin in 1953 that peptides began to be utilized in the pharmaceutical field. The ability to chemically produce peptides using solid-phase techniques has led to an explosion in research activity in a number of fields and has brought peptide chemistry to the forefront of many medical applications.

However, the use of synthetic peptides in the oral and dental research field is relatively new. These areas are poised to expand rapidly due, in part, to the application of genomic and proteomic techniques to the oral tissues and pathogens and the ability to reliably grow, in batch and continuous culture, a number of oral bacterial pathogens.

Peptides play a significant role in cell signaling and function and can be used as an important tool for research and therapeutic treatments.

Both peptides and proteins fall under a category called polypeptide chains. These chains contain two or more amino acids (forming amino acid polymers) that are coupled by a peptide bond. The bond is a special linkage between the nitrogen atom of one amino acid and the carboxyl carbon atom of another.

Peptides differ from proteins by the amount of amino acid residues the molecule contains. Polymer molecules with ten or fewer amino acid residues are called oligopeptides. Peptides often contain up to 50 amino acid residues, proteins are molecules with more than 50 amino acid residues.[1]

Peptide Bond

All 20 amino acids are characterized by a central alpha carbon covalently bonded to a carboxyl group, an amine group, a hydrogen atom, and a variable R' group.

Peptide Bond Formation Mechanism

A peptide bond forms when the **carboxylic acid** group (R-C[O]OH) of one amino acid reacts with the **amine group** ($R-NH_2$) of another. The resulting molecule is an amide with a C–N bond (R-C(O)-NH-R). This condensation reaction results in a dipeptide, and the release of a water molecule—with a hydroxyl (OH) leaving the carboxyl group, and the hydrogen atom from the amine—with the carboxyl group releasing a hydroxide, and the amine releasing a hydrogen atom. Normally, when we refer to this process in biology, we call it a «dehydration synthesis», since we are building a higher order structure at the expense of a loss of water.[2]

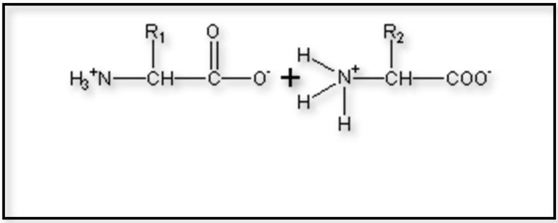

Fig 1-1: Peptide Bond Formation

Some examples of peptides

Synthetic peptides have been studied for over a century. The first synthetic peptide glycyl-glycine (see Fig 1-4) was discovered by Emil Fischer in collaboration with Ernest Fourneau in 1901.

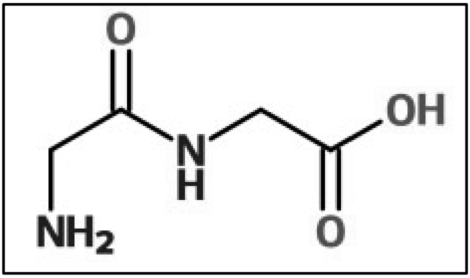

Fig 1-4: The first synthetic peptide glycyl-glycine

The first polypeptide (oxytocin—nine amino acid sequence) was synthesized by Vincent du Vigneaud in 1953.

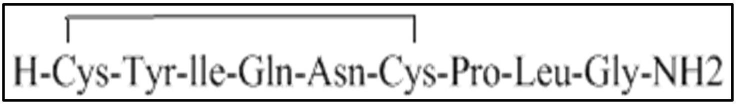

Fig 1-5: The first polypeptide (oxytocin—nine amino acid sequence)

The development of peptide therapeutics has made many advances over the years. Insulin was the first therapeutic protein to be introduced to treat insulin-dependent diabetes in the 1920s. It was initially isolated from bovine or porcine pancreases, but now human insulin is manufactured using genetically engineered E. coli. There are currently 60 FDA approved peptide drugs on the market, and pharmaceutical companies are increasingly interested in adding to that number. About 140 peptide drugs are in clinical trials and over 500 are in pre-clinical development.[3]

2. PEPTIDE HISTORY AND SYNTHESIS

A Brief History of Peptide Synthesis

A «peptide synthesis» includes a large range of techniques and procedures that enable the preparation of materials ranging from small peptides to large proteins. The pioneering work of Bruce Merrifield, which introduced solid phase peptide synthesis (SPPS), dramatically changed the strategy of peptide synthesis and simplified the tedious and demanding steps of purification associated with solution phase synthesis. Moreover, Merrifield's SPPS allows the development of automation and the extensive range of robotic instrumentation. After defining a synthesis strategy and programming the amino acid sequence, machines can automatically perform all the synthesis steps to prepare multiple peptide samples. SPPS has now become the method of choice to produce peptides, although solution phase synthesis can still be useful for large-scale production of a given peptide.[4]

The first peptide synthesis, as well as the term «peptide», was reported by Hermann Emil Fischer and Ernest Fourneau in 1901. Fischer and Fourneau published their preparation of the first dipeptide, glycylglycine by partial HCl hydrolysis of glycine diketopiperazine.[5]

Emil Fischer and his co-workers synthesized about 100 peptides within the first decade of the 20th century using the methods developed in his laboratory, with the peptides containing from 2 to 18 amino acid residues.[6]

Synthetic work with peptides was resumed by Bergmann in a systematic way (along the lines of the Fischer school) with Joseph S. Fruton, who joined Bergmann in 1934, soon after his arrival at the Rockefeller Institute. Fruton utilized the potential of the new carbobenzoxy method to synthesize inaccessible peptides for testing as substrates for enzymatic hydrolysis. He and Bergmann published a syntheses of simple substrates which were cleaved by papain, chymotrypsin, trypsin and pepsin at certain peptide bonds.[7]

The history of peptide chemistry stretches over a century, from compounds as simple as diglycine to enzymes with more than 100 amino acids. Due to the individual character of each amino acid and the combination of many amino acids forming large molecules, peptides show a great variety of specific functions. They can act as chemical messengers, hormone, intra- or inter-cellular mediators, highly specific stimulators and inhibitors and as biologically active peptides in the brain and nervous systems.[8]

3. PEPTIDE FAMILIES

The peptide families in this section are ribosomal peptides, usually with hormonal activity. All of these peptides are synthesized by the cells as longer "propeptides" or "proproteins" and are truncated prior to exiting the cell. They are released into the bloodstream, where they perform their signaling functions.[9]

Antimicrobial peptides

- Magainin family
- Cecropin family
- Cathelicidin family
- Defensin family

Tachykinin peptides

- Substance P
- Kassinin
- Neurokinin A
- Eledoisin
- Neurokinin B

Vasoactive intestinal peptides

- VIP (**V**asoactive **I**ntestinal **P**eptide; PHM27)
- PACAP (**P**ituitary **A**denylate **C**yclase **A**ctivating **P**eptide)
- Peptide PHI 27 (**P**eptide **H**istidine **I**soleucine 27)
- GHRH 1-24 (**G**rowth **H**ormone **R**eleasing **H**ormone 1-24)
- Glucagon
- Secretin

Pancreatic polypeptide-related peptides

- NPY **(N**euro**P**eptide **Y**)
- PYY (**P**eptide **YY**)

- APP (**A**vian **P**ancreatic **P**olypeptide**)**
- PPY **P**ancreatic **P**ol**Y**peptide

Opioid peptides

- Proopiomelanocortin (POMC) peptides
- Enkephalin pentapeptides
- Prodynorphin peptides

Calcitonin peptides

- Calcitonin
- Amylin
- AGG01

Self-Assembling peptides

- Aromatic short peptides
- Biomimetic peptides
- Peptide amphiphiles
- Peptide dendrimers

Other peptides

- B-type Natriuretic Peptide (BNP) - produced in the myocardium and useful in medical diagnosis
- Lactotripeptides - Lactotripeptides might reduce blood pressure, although the evidence is mixed.
- Peptidic components from traditional Chinese medicine Colla Corii Asini in hematopoiesis.

4. APPLICATIONS OF PEPTIDES

Peptide applications may soon be as varied as peptides themselves. Indeed, cell-penetrating peptides (CPP) have served to deliver various molecules and particles into cells. Biomedical research is vastly improving and gaining ground due to the use of CPPs and synthetic peptides.

In addition to the medical and pharmaceutical arena, synthetic peptides (throughout we will interchangeably refer to synthetic peptides and cell-penetrating peptides [CPP]) have found their place in biochemistry, molecular biology and immunology. Synthetic peptides are extremely useful in studies regarding polypeptides, as well as:

- Peptide hormones
- Hormone analogues
- Cross-reacting antibody preparation
- Design of novel enzymes

The design of synthetic peptides had begun mainly due to the availability of secondary structure prediction methods, and by the discovery of finding protein fragments that are >100 residues that can assume or maintain their native structures as well as their activities. Here it is important to note that, due to the biological activity of peptides, they must take on conformational aspects which mirror their conformational properties. These are all determined by the:

- Amino acid sequences
- Polarity of the medium
- Ligand interactions, such as: nucleic acids, receptors and metal ions

Taking all of this into account, peptide chemists have been able to perform experimental and computational techniques in order to focus on peptide conformational stability and the accompanying and resulting dynamics of such. Molecular biologists, on the other hand, perform protein engineering processes at the level of the DNA, but this work was initiated by peptide chemists. Peptide chemists were also the first to prepare synthetic peptides (a/k/a peptide libraries) in vast amounts.[10]

Synthetized Bioactive Peptides

Synthetic peptides and proteins also have their place in the market, for which they have yielded billions of USDs. New chemical entities (NCE) have remained steady over the last decade, other than peptide and protein NCEs, which have been increasing. Synthetic peptides can positively influence the functions and conditions of humans. In fact, several useful properties for human health, using bioactive peptides, have been realized, such as for these activities:

- Antimicrobial
- Antifungal
- Antiviral
- Antitumor
- Antioxidant
- Antithrombotic

All peptides exist in limited quantities within natural settings or nature. Therefore, researchers have re-produced them in their labs, and these are known as synthesized bioactive peptides. As we can see, their applications and properties have been applied in various areas. Synthesized peptides are categorized by class and the mechanisms that they serve.[11]

Classes of Biosynthetic Peptides and Peptidomimetics

Delving further into the particular classes of peptides is important. In order to better understand these classes, first we will explore peptidomimetics, which are known as compounds that contain crucial elements (pharmacophore) which mimic a protein in 3D space, or a natural peptide. They maintain a striking ability to interact with designated biological targets. They then create the same biological effect as their natural counterparts.

Peptidomimetics serve to avoid some of the issues that are associated with natural peptides, such as their duration of activity and unavailability. In addition, properties such as potency or receptor selectivity can be improved, giving mimicking proteins the ability to aid in drug discovery. Now, for the classes of biosynthetic peptides and peptidomimetics:

- Gonadorelin Super-agonists
- Somatostatin Analogues
- Angiotensin Converting Enzyme (ACE) Inhibitors
- HIV Protease Inhibitors

- Calcitonins
- Immunostimulating Peptides[10]

Peptide-based Vaccines

Specific tumor antigens have advanced the field of medicine, specifically oncology, to target cancer cells. Cancer vaccines have been developed as a result of the heightened knowledge regarding the molecular basis of antigen recognition. This has resulted in human class I or class II major histocompatibility (MHC) motifs which bind to these human classes. Here, synthetic peptides have been used for these cancer vaccines while presenting the following advantages of their uses:

- Ease of construction and production
- Chemical stability
- Lack of infectious or oncogenic potential
- Improved manipulation of immune responses via epitopes that stimulate T-cell subsets
- Improved effectiveness for generating immune responses to self-proteins

Clinical trials of single peptides have shown that cancer patients can be vaccinated against self-tumor antigens, with some studies showing positive early results. On the horizon are continuing efforts for multiple peptide vaccinations for the prevention and treatment of malignant human cancers. Other efforts include the focus on improving the immunogenicity of individual MHC-binding peptides.[12]

Peptide Nanotubes

Nanotechnology is known as molecular manufacturing. Nanotubes are grown in laboratories and they hold thermal and electrical properties that are used in chips, for instance. Nanotubes also have the ability to be used as semiconductors, with the possibility of replacing silicon. Peptide nanotubes (PNTs) are surfacing as one of the most compelling nanostructures within the field of nanotechnology. These smart self-assemblies have various applications such as:

- Sensors
- Catalysis
- Electronics
- Stimulus-response materials

• Nanoreactor designs.[13]

Antimicrobial Peptides for Food Safety

Antimicrobial peptides have also found their place as a beneficial application in the area of biomedical devices, food processing equipment, and food preservation. In the latter, peptides can be incorporated into materials that create an antimicrobial packaging (Appendini and Hotchkiss, 2002). This type of packaging serves to sustain food safety and quality by reducing bacterial growth on product surfaces (Soares, et al., 2009). It works to inhibit, reduce, or retard the growth of unhealthy microorganisms within the food. Specific food preservation is enacted via peptide synthesis and peptide bioproperties, making food safer for public consumption.[14]

Advantages and Disadvantages of Peptides as Drugs

For all their usefulness and varied applications, synthesized peptides and CPPs, when used as drugs, do have their disadvantages. For instance, they have to be injected, or they must consist of specific formulations due to their low bioavailability. Also, synthesis is very costly, but this could change with increased production of the necessary products such as protected amino acids, coupling reagents, and resins. However, their advantages far outweigh any disadvantages, which include the fact that, to be effective, only small doses of peptide are needed, and the total amount to be produced is small. They also have low systemic toxicity since they do not accumulate within the body tissues, organs and blood due to their short half-lives.

New non-injectable peptide (and protein) formulations are being developed such as non-degradable implants, liposomes, inhalation, oral administration and more. While dozens of companies are supporting new formulations, not all are focusing on peptides and proteins. It is considered that this will change as more and more research reveals the steady benefits of synthesized peptides for human therapeutics, diagnostics, and treatments, as well as nanotechnology, food safety and food quality.[15]

5. APPLICATIONS OF PEPTIDES IN DENTISTRY

Dental caries is a common disease that affects a large proportion of the world's population.[16] Extensive research indicates that dental caries is the result of bacterial infection and is also influenced by host and dietary factors.[17]

Human saliva possesses several functions involved in oral health and homeostasis, with an active protective role in maintaining oral health. It also contains appreciable quantities of free amino acids and low-molecular-weight peptides.[18,19] These molecules are exchangeable with plaque fluid, being not only potential metabolic substrates for the plaque microflora, but also products of microorganic metabolic pathways.[20]

Peptides have a wide range of applications in dentistry. Anti-microbial and self-assembling classes of peptides are commonly used.

The oral cavity is observed to be in continuous communication with the external environment. One of the major colonization sites for microorganisms in the oral cavity are the dental hard tissues and gingival pockets. These sites can serve as major reservoirs of oral disease and, along with the airway passage (upper respiratory tract), can act as gateways inside the body.[21] The first source of immunity in the mouth is provided by the epithelial cells of the oral mucosa; they function as a physical barrier between the intra and extra oral environments and play an active role in providing innate immunity by secreting factors such as interleukin (IL)-8, chemokines and cytokines which can signal and attract neutrophils.[22] Furthermore, they can also produce antimicrobial proteins and peptides constitutively or indelibly.

Antimicrobial Peptides (AMPs)

Antimicrobial peptides are natural antibiotics that provide a first line of defense against a wide spectrum of pathogens. These peptides may be particularly present in the oral cavity, where members of the microbial flora are present in high numbers at all times. Hence, the levels of these molecules in saliva may express a higher or lower risk of developing this disease. The ability of peptides to kill or inhibit the proliferation of pathogenic microorganisms has been previously described.[23]

The various applications of AMPs are:

1. Prevention of caries
2. Remineralization of the tooth structure
3. Adhesion of restorative materials
4. As implant coatings
5. Role in periodontal health
6. Prevention of biofilm
7. In mouthwash
8. In oral spray, etc.
9. Another class of peptide i.e., self-assembling peptide is also commonly used.

The various other peptides that are used are:

1. Leucine-rich amelogenin peptide as hydrogel
2. Peptide nisin in adhesives
3. Milk derived casein phosphopeptide
4. Lys-a1 peptide
5. 3DSS peptide
6. Cemp-1-p3 peptide, etc.

Dental researchers are applying current peptide chemistry techniques to diagnose, prevent and potentially cure oral diseases. The combination of peptide chemistry and oral/dental research is in its early stages but it has already produced exciting results which, if taken to their full potential, are likely to provide medical benefits for us all as well as advancing the research dialectic in both fields.

6. INTRODUCTION TO ANTIMICROBIAL PEPTIDES

The global public health threat of antimicrobial resistance has led the scientific community to highly engage in research on alternative strategies to the traditional small molecule therapeutics. Synthetic antimicrobial peptides are one of the most recent and popular alternatives amongst basic and applied research scientists. Synthetic antimicrobial peptides carry with them the ease of peptide chemical synthesis combined with emerging engineering principles and potent broad-spectrum activity, including against multidrug-resistant strains.[24]

Various antibiotics have been broadly applied to treat human infectious diseases since the first antibiotic, Penicillin, was discovered by Alexander Fleming in 1928.[25,26] Antibiotics are usually effective in the treatment of pathogenic bacteria.[27,28] However, antibiotics gradually lose their antibacterial ability and drug-resistant bacteria appear with the use of large amounts of antibiotics and even the abuse of various antibiotics.[29,30] Thus, the findings of new antibiotics or other new antibacterial resources has become an urgent need and are catching the attention of more and more scientists all over the world. Antimicrobial peptides (AMPs) or host defense peptides were firstly discovered in the 1980s.[31]

Antimicrobial peptides and proteins (AMPs) are a diverse class of naturally occurring molecules that are produced as a first line of defense by all multicellular organisms. These proteins can have broad activity to directly kill bacteria, yeasts, fungi, viruses and even cancer cells. Insects and plants primarily deploy AMPs as an antibiotic to protect against potential pathogenic microbes, but microbes also produce AMPs to defend their environmental niche.[32]

Although AMPs induce significant damage in bacteria, including inhibition of DNA, RNA, and protein synthesis, their main mode of action is disruption of the bacterial membranes.[34-36] Several mechanisms for this have been suggested, including detergency-like packing disruption and formation of a barrel-stave, carpet-like model or toroidal pores.[34-38]

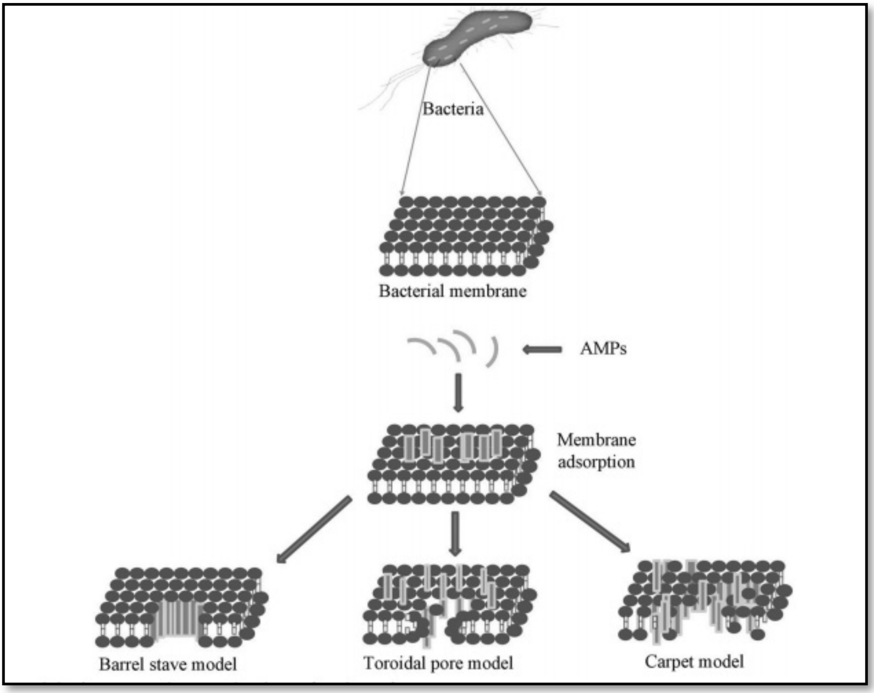

Fig 6-1: Formation of a barrel-stave, carpet-like model or toroidal pores

Potential chemical gradients may also cause transient defects due to peptide translocation through the membrane.[39] Furthermore, lateral membrane expansion due to peptide binding allows relaxation of the alkyl chains and causes membrane thinning, further facilitating membrane destabilization.[40] Also peptide-induced lipid segregation and/or phase transition may contribute to peptide induced membrane rupture.[41,42]

There are at least two good reasons for our current focus on host defense AMPs. First, AMPs have remained potent for millions of years. Therefore, AMPs constitute useful templates for developing a new generation of antimicrobials to meet the growing antibiotic resistance problem worldwide. Second, AMPs are key components of the innate immune system universally required for the survival of both invertebrates and vertebrates. Thus, research in this direction improves our understanding of innate immunity and its relationship with the adaptive immune system in vertebrates.

7. Current Progress and Applications of Antimicrobial Peptides

AMPs constitute useful templates for developing a new generation of antimicrobials to meet the growing antibiotic resistance problem worldwide. They are key components of the innate immune system universally required for the survival of both invertebrates and vertebrates. Thus, research in this direction improves our understanding of innate immunity and its relationships with the adaptive immune system in vertebrates.

Medicine

Antimicrobial peptides can regulate pro-inflammatory reactions, recruit cells, stimulate the proliferation of cells, promote wound healing, modify gene expression and kill cancer cells to participate in the immune regulation of human skin, respiratory infections and inflammatory diseases.[43] For example, α-defensins HNP-1, HNP-2, and HNP-3 showed effective antibacterial activity against the adenovirus, human papilloma virus, herpes virus, influenza virus and cytomegalovirus. Pulmonary diseases, such as idiopathic pulmonary fibrosis, alveolar proteinosis and acute respiratory distress syndrome, show elevated levels of AMPs.[44] Likewise, AMPs secreted by the Paneth cells in the mammalian gut are important in shaping the gut microbiota.[45]

The application of AMPs in medicine, such as dental, surgical infection, wound healing and ophthalmology is developing now. But there are only three AMPs that have been approved by the FDA including gramicidin, daptomycin and colistin.

Dental caries, endodontic infections, candidiasis, and periodontal disease are common diseases in the human oral cavity. Dental caries is a prevalent oral disease and some acidogenic bacteria like Streptococcus sp. are the main caries-associated pathogens.[46] Several AMPs have good application potential. For instance, peptide ZXR-2 (FKIGGFIKKLWRSLLA) has shown potent activity against the pathogenic bacteria of dental caries, Streptococcus mutans, Streptococcus sobrinus, and Porphyromonas gingivalis and peptide PAC-113 (Clinical trial identifier: NCT00659971) that has been sold over the counter in Taiwan for treating oral candidiasis.[47]

In surgical infection and wound healing: surgical infection occurs after surgery, burns, accidental injury, and skin disease, and chronic wound infections are a serious hazard to human life (Thapa et al., 2020). Several AMPs have shown their therapeutic potential for these diseases. For example, AMP PXL150 shows pronounced efficacy as an anti-infective agent in burn wounds in mice and AMP D2A21 has been in the third phase of clinical trials for treating burn wound infections.[48]

In ophthalmology: human eyes are prone to infection from several organisms including bacteria and fungi in which S. aureus, Streptococcus pneumoniae, P. aeruginosa, Aspergillus spp., and C. albicans are the most relevant pathogens.[49] Although AMPs such as Lactoferricin B, and Protegrin1 have exhibited antimicrobial activity against these pathogenic bacteria, their application in the field of ophthalmology is only at the theoretical stage. With the popularity of contact lenses and the increase in cases of related eye infections, antimicrobial peptides have shown good application potential in ophthalmology.[50]

Additional methods need to be performed for the application of AMPs as drugs in medicine. The main strategies include (1) constructing precursors to reduce cytotoxicity and improve protease stability, (2) using AMPs in combination with existing antibacterial agents, and (3) inducing the correct expression of AMPs with appropriate drugs and using engineering probiotics as vectors to express AMPs. For example, in the field of wound repair, different formulation strategies, such as loading AMPs in nanoparticles, hydrogels, creams, gels, ointments, or glutinous rice paper capsules, have been developed to effectively deliver AMPs to the wound.[51] In recent research, the sponges developed from modified starch and HS-PEG-SH and covalently immobilized with AMP showed effective antibacterial activity.[52]

More technical means, including pheromone-labeled AMPs and local environment-triggered AMPs (enzyme precursor drug release system, pH-activated AMPs, etc.), have been developed to improve the targeting mechanism of AMPs. Furthermore, nanotubes, quantum dots, graphene, and metal nanoparticles have been proposed as potential methods to enhance drug delivery of AMPs.[53] Hybrid peptides have also been used to build targeting peptides. For example, PA2, which is a P. aeruginosa-targeting peptide, was combined with GNU7 (a broad-spectrum AMP) to construct a hybrid peptide (PA2–GNU7) that targets OprF protein and has good bactericidal activity and specificity.[54] Furthermore, some antibiotics, for instance, daptomycin (a lipopeptide), lugdunin which is a 21-membered cyclic peptide consisting of 6 amino acid residues plus a thiazolidine moiety and telavancin (a glycopeptide) have been widely used for clinical purposes.[55] Although they are antibiotics, they have provided broader ideas for the design of AMPs.

Food

Food preservatives can cause potential harm to the human body. Therefore, natural preservatives are being advocated by more people. AMPs have a good inhibitory effect on common bacteria and fungi in food, and many AMPs are resistant to acids and alkalis, and high temperatures are easily hydrolyzed by proteases in the human body. Thus, AMPs are a promising alternative to preservatives. Nisin is a bacteriocin produced by the L. lactis subspecies. Lactic acid bacteria have been widely used as food preservatives.[48]

Animal Husbandry and Aquaculture

The European Union banned the use of animal growth promoters in animal feed in 2006. Thus, a new antibacterial strategy is needed. Many AMPs have the potential to be used in poultry, swine and ruminants' breeding and aquaculture because they can improve production performance (Liu et al., 2008; Bao et al., 2009), immunity and promote intestinal health and some of them have a stronger inhibitory effect on bacterial inflammation if used with antibiotics (Wang et al., 2019; Cote et al., 2020). For example, swine intestine antimicrobial peptides (SIAMPs) have a good effect on the treatment of Infectious Bronchitis Virus (IBV) in chickens (Sun et al., 2010). By adding swine gut intestinal antimicrobial peptides (SGAMP), broilers showed higher average daily gain and feed efficiency under chronic heat stress conditions (Hu et al., 2017). Frog caerin 1.1, European sea bass dicentracin and NK-lysine peptides (NKLPs) have a good inhibitory effect on Nodavirus, Septicaemia haemorrhagic virus, Infectious pancreatic necrosis virus and Spring viremia carp virus, which are devastating to fish farming (León et al., 2020). The AMP in soybean meal fermented by B. subtilis E20 effectively inhibits V. parahaemolyticus and Vibrio alginolyticus and enhances the resistance level of Litopenaeus vannamei against V. parahaemolyticus when added to feeds (Chen et al., 2017).[47]

Agriculture

For agriculture, the plant pathogenic infection of bacteria and fungi causes the loss of economy, for instance, the Aspergillus flavus infection of corn and peanuts, citrus green mold caused by Penicillium digitatum, gray mold disease caused by Botrytis cinerea on strawberries and the Geotrichum citri-aurantii infection of citrus fruit all cause great harm to the growth and post-harvest of agricultural products (Liu et al., 2007; Liu et al., 2019). Several AFPs have shown the potential to control these problems. However, the practical application of antimicrobial peptides in the transportation and preservation of agricultural products is still lacking, because the use of antimicrobial peptides will greatly increase the cost in the transportation of fruits and vegetables (application examples of AMPs in these four fields are shown in Huan et al., (2020).[48]

8. ANTI-MICROBIAL PEPTIDES IN THE ORAL ENVIRONMENT

The oral cavity is a unique environment. Oral mucosa is a critical protective interface between the external and internal environments and must serve as a barrier to the large number of microbial species present in this warm, moist environment. The oral cavity is the only area of the body in which hard tissues break through the epithelial surface. The periodontal epithelium surrounding the tooth is specialized in forming an attachment and seal around each tooth. This unique function imparts special challenges to the tissue and leads to certain vulnerabilities associated with periodontal disease, especially in view of the continual exposure to the bacterial biofilm (dental plaque) that forms on the tooth surface at the junction of the soft tissue. Thus, this anatomical region is one where there is a significant risk of bacterially induced infection and inflammation. Antimicrobial peptides are important contributors to maintaining the balance between health and disease in this complex environment.[56]

Antimicrobial peptides (AMPs) are a wide-ranging class of host-defense molecules that act early to contest against microbial invasion and challenges. These are small cationic peptides that play an important role in the development of innate immunity. In the oral cavity, the AMPs are produced by the salivary glands and the oral epithelium and serve defensive purposes.[57]

Oral epithelia are constantly exposed to a variety of microbial challenges. Bacterial infection in the mouth is resisted by the stratified squamous epithelium, which acts as a mechanical barrier, and saliva, which contains unique AMPs as well as providing a mechanical rinsing action. Saliva contains a large number of protective proteins, such as lactoferrin, lysozome, peroxidases, immunoglobulins, agglutinin and mucins and several types of antimicrobial peptides, including histatins, defensins and cathelicidin/LL-37, that may have an important role in innate host defense. Histatins, which are major AMPs in human saliva, are constitutively produced and directly secreted by the submandibular, sublingual and parotid glands. Since the histatin family was first described in the early 1980s, many papers have been published on their antibacterial and fungicidal properties.

Histatin 5 has the strongest antimicrobial activity, and most of the research on histatins has focused on this peptide. The salivary glands also secrete small amounts of defensins and cathelicidin/LL-37, and these peptides are also produced by neutrophils and oral epithelial cells. Certain types of defensins and cathelicidin/LL-37 are inducible by inflammatory cytokines, indicating that these peptides may be of crucial importance under inflammatory conditions.[58]

The β-defensins are expressed in the epithelium, the α-defensins are expressed in the neutrophils, and cathelicidin/LL-37, is expressed in both the epithelium and the neutrophils. These peptides are part of the host innate immune response in this environment. Epithelia, polymorphonuclear leukocytes (neutrophils), and saliva all contribute to maintaining the health of the oral cavity in overlapping but independent ways. Thus, AMPs are important contributors to maintaining the balance between health and disease in this complex environment.[56]

The timeline of each human salivary protein and peptide identification is listed below and each peptide and its role in the oral cavity will be discussed in detail.

Proteins and peptides	Year	Amino acids	Functions
Statherin	1977	43	Potent inhibitor of calcium phosphate precipitation
α-Defensins (HNP-1-4)	1985	< 50	Antibacterial, antifungal and antiviral activities because of six cysteine disulfide bonds
Histatin-1	1988	38	Responsible for maintenance of oral hemostasis, role in forming acquired tooth pellicle and help in bonding of some metal ions
Histatin-3	1988	32	Responsible for maintenance of oral hemostasis, role in forming acquired tooth pellicle and help in bonding of some metal ions
Histatin-5	1988	24	Responsible for maintenance of oral hemostasis, role in forming acquired tooth pellicle and help in bonding of some metal ions
Adrenomedullin	1993	52	Mitogenic, inducible, vasodilator and antibacterial peptide
β-Defensins (hBD-1)	1995	36–47	Poor antibacterial activity, antiviral and antifungal activity, Also help in tissue repair
Cathelicidins (LL-37)	1995	37	Chemo-attractant to immune cells and act as "alarmin" by inducing immune response
β-Defensins (hBD-2)	1997	41	Antibacterial against Gram-negative and Gram-positive, antiviral and antifungal activity against HIV
β-Defensins (hBD-3)	2001	45	Antibacterial against Gram-negative and Gram-positive, also drug resistant microbes, chemo-attractant antiviral and antifungal activity
C-C motif Chemokine 28	2008	128	Act as chemokine, salt sensitive and broad spectral antibacterial
Azurocidin		251	Strong antibacterial activity against Gram-negative bacteria
Neuropeptides	1997	–	They have five types; Substance P (SP), Neurokinin A (NKA), Calcitonin Gene Related Peptide (CGRP), Neuropeptide Y (NPY) and Vasoactive Intestinal Polypeptide (VIP), effective against *Candida albicans* and bacteria
Calprotectin	1999	93–114	Act as calcium sensors and potent zinc binder, also play role in tissue repair
Mucins	1990	3750	Aggregate oral flora bacteria and prevent dental caries

Table 8-1: Timeline of identification of the human salivary proteins

Human Oral Antimicrobial Peptides	Function
α- Defensins (HNP1-4)	Antibacterial, antifungal, and antiviral. Functional levels in GCF Expression defective in Morbus Kostmann syndrome (congenital neutropenia associated with periodontal disease).
β- Defensins (hBD 1-6)	Chemotactic to immune cells, Anti-inflammatory function, Promote wound healing, help in degranulation of mast cells and also help in releasing of chemokine from non-resident and resident cells of oral cavity.
Histatins	Antifungal. Histidine-rich group of peptides. Histatin 5 (24 amino acids) is most active. Anti-fungal action requires metabolic activity.
Cathelicidins (calgranulin)	Promotion of keratinocytes migration, Induction of angiogenesis, Inhibition of keratinocyte apoptosis, Promotion of wound healing, Cytokine/Chemokine release from keratinocytes or leukocytes.
Adrenomedullin	Role in endocrine effects, Multifunctional peptides (Antibacterial, mitogenic, vasodilator, inducible peptide), act as biomarker in periodontal diseases, wound healing process.
Calprotectin	Act as calcium sensor, Role in tissue repair and remodelling, Weak chemo-attractant to leucocytes, Help in epithelial cell proliferation and differentiation.
Statherin	Restrains the crystallization of calcium phosphate, provide protection from plaque formation and also work as infection biomarker in oral cavity.
C-C motif chemokine 28	Act against broad-spectrum antimicrobial agent and as a chemokine activating agent.
Azurocidin	Strong bactericidal activity against Gram-Ve bacteria.
Neuropeptides	Active against *Candida Albicans*.

Table 8-2: List of human oral antimicrobial peptides (AMPs) with their functions