

In Silico Approach for the Identification of Potential Targets and Specific Antimicrobials for *Streptococcus mutans*

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Abstract

Tooth decay affects most of the population in developed countries. The multifactorial etiology of the disease includes multiple bacterial species, *S. mutans* is the main pathogen associated with the disease. This bacterium adheres to the tooth surface and allows the colonization of other microorganisms resulting in dental biofilm. Several therapeutic agents are available to treat or prevent tooth decay, but none, with the exception of fluoride, has significantly influenced the disease's global burden. Moreover, the probable development of resistance of microorganisms to existing antibacterial agents and the scarcity of good antimicrobial agents motivates this effort for innovation. The detailed knowledge obtained in recent years on the *S. mutans* allowed the identification of potential targets in this microorganism, enabling the development of specific drugs to combat tooth decay. Thus, the identification of potential targets in these pathogens is the first step in the discovery process of new therapeutic agents. Currently, the experimental assays used for this purpose are expensive and time consuming. In contrast, bioinformatics methods to predict drug targets are cheap, quick and workaday in the biotechnology. This article will review the potential drug targets in *S. mutans*, as well as the bioinformatics methods used to identify these targets and effective drugs for specific pharmacological treatment of dental caries.

Keywords

Bioinformatics; Antimicrobial Targets; *S. mutans*

1. Introduction

Dental caries still has high prevalence and incidence in the world population [1]. It is an irreversible microbial disease of hard dental tissues, characterized by demineralization of the inorganic portion and destruction of the tooth organic material, which often leads to cavitation. [2] Bacteria in the biofilm produce acids from the metabolism of carbohydrates from the host diet [3].

Streptococcus mutans is a Gram-positive, aciduric and acidogenic bacteria, being considered the most frequent microorganism associated with tooth decay [4]. This bacterium is able to organize itself in bacterial communities through cell-cell interaction and connection with other components present in the medium, such as polysaccharides, proteins and DNA, forming the biofilm. The bacteria included in this system are more resistant and sessile than those planktonic [5], forming an effective barrier against the penetration of antimicrobial agents capable of providing them with a high level of antibiotic resistance [6].

In addition to traditional measures that are routinely used in the control of caries, such as oral care products of mechanical action (toothbrushes and dental floss) and methods of population prevention (water fluoridation) [7] [8], the products of chemical action (toothpastes and mouthrinses) have been considered an interesting alternative to control biofilm and thus dental caries. However, these antimicrobial dental use should be able to significantly inhibit the cell growth of cariogenic pathogens without causing adverse effects to the host [9] [10] effects.

In this context, modern strategies have been used for the rational design of new compound-prototypes allowing to plan the chemical structure of a new molecule based on the previous definition of the therapeutic action mechanism, *i.e.* the biomacromolecule to which the new drug will bind to change a particular biochemical process. Knowing the three-dimensional molecular structure of the elected therapeutic target, *i.e.*, from the region responsible for chemical interaction, it is possible to identify a compound capable of binding that active site and changing its properties through computational molecular modeling techniques [11] [12]. For dentistry, the understanding of mechanisms involved in the formation, virulence and drug resistance of dental biofilms is vital and it has necessary support in computer science to obtain greater precision in the development of substances capable of destabilizing the structure of the biofilm formed specially by *S. mutans*. This paper will highlight the potential molecular targets in *S. mutans* as the main computational methods used to detect these targets and specific drugs for treatment of dental caries.

2. Dental Caries, Biofilms and *Streptococcus mutans*

Dental caries is a localized multifactorial disease, dependent on diet, oral microbiota and host response [1]. The reduced pH caused by acid production by cariogenic microorganisms embedded in the biofilm by means of carbohydrates metabolism causes dissolution of dental enamel ions into the medium, thereby causing cavitation in the tissue. [2] *Streptococcus mutans*, a Gram-positive bacterium, is considered the most frequent microorganism associated with dental caries [4]

The biofilm can be defined as a diverse community of microorganisms present on the tooth surface as a biofilm embedded in a polymer matrix of microbial origin and from the host. This microbial organization system has been extensively studied in recent years, particularly in studies on antimicrobials, since it presents particular characteristics that allow the microorganisms residing on it better resist to drugs than planktonic forms [13].

Imbalance in these dental microbial biofilms can allow the development of dental caries, since it is caused by microorganisms belonging to the resident oral flora. Before the establishment of the carious lesion, an environment with low pH caused by microbial fermentation of dietary carbohydrates selects a population of producer and resistant strains to acids such as streptococci from the mutans group and lactobacilli. These species increase the acid formation which can cause demineralization [14].

Biofilms have a more tolerant phenotype to antimicrobial agents, stress and host defenses than planktonic cultures, making them difficult to handle [15]. This means that the effectiveness of products used in the prevention of dental caries should be evaluated in biofilms and not in traditional liquid cultures [16]. Patterns of gene expression in biofilms are also distinguished from those of planktonic cells [17], justifying the need to perform laboratory tests under both conditions to better understand the effect of antimicrobials in these cells.

Streptococcus mutans can develop by forming biofilm on dental tissues [6]. The initial phase of colonization depends on the specific interaction between *S. mutans* and proteins/glycoproteins of salivary and microbial origin adsorbed on the acquired pellicle that coats the tooth enamel [18]. During the biofilm formation, bacteria aggregate to each other, surrounded by a network of extracellular polysaccharides producing, within the sessile

bacterial populations, new conditions that increase antibiotic tolerance, making these microorganisms more organized and resistant if compared to planktonic bacteria [5] [19] [20].

These microorganisms have developed some defense systems so they could establish a microbial ecosystem, with three of these systems deemed essential for biofilm formation and development of caries: the capacity of 1) adhering to the tooth surface, 2) producing acids and 3) withstanding this environment without damage [21]. Regarding the adhesion process, three major groups of antigens (Ags) associated with the surface of these microorganisms participate in the process of adhesion and accumulation of *S. mutans* biofilm: the adhesin antigen I/II (Ag I/II), glycosyltransferases (GTFs) and glucan-binding proteins (GBP) [22].

The glycosyltransferases produced by *S. mutans* are able to synthesize extracellular (ECPs) and intracellular (ICPs) polysaccharides from sucrose from the diet. In the absence of carbohydrates, these ICPs are metabolized to produce energy. The ECPs allow the aggregation of *S. mutans* to other oral streptococci, which seems to occur through interaction with glucan-binding proteins associated with cell surface. Four Gtfs have been identified in *S. mutans*, GtfA, GtfB, GtfC and GtfD. Some of these proteins may be secreted or attached to cell surfaces (GtfA, GtfB and GtfD) or covalently bind to the cell wall (GtfC) [22].

The *S. mutans* also have a number of sugar transport systems, including high-affinity phosphoenolpyruvate-phosphotransferase systems (PEP-PTS), capable of capturing sugar when present in low concentrations in the oral environment. This property makes *S. mutans* an extremely resistant bacteria, able to adapt to environments scarce in carbohydrates [1].

3. Caries Control and Search for New Antimicrobials

Currently, the best means of preventing the initiation and controlling caries progression and, consequently the loss of hard dental tissue, is still the use of mechanical forms (toothbrushes and dental floss) to disrupt the biofilm of the dental surfaces and chemical substances (mouthwash and toothpaste) to kill the bacteria embedded in the biofilm [23] array.

Effective antimicrobials against microorganisms responsible for development of caries can play a significant role in disease prevention [24]. However, they are not prescribed and used on a large scale for this purpose at risk of developing adverse effects, selecting bacteria resistant to certain antibiotics and causing teeth staining [25].

Attention in recent years have turned to research on natural antimicrobials that may be effective against oral bacteria, but without being detrimental to the host [26]. Some studies conducted trials to evaluate the antimicrobial activity of extracts from various plants against pathogens related to caries aimed at developing an effective natural antimicrobial [27] [28].

Natural products with antibiofilm effect [29] are also a promising line of research. In addition, nanoscale systems, due to their adjustable size, that increase interactions with biological systems at molecular level, also contributed to the improvement of innovative therapies related to the biofilm diseases [30] [31].

Another approach considered effective in controlling dental caries would be the change in diet, eliminating refined sugars (sucrose and fructose) of food and promoting the consumption of protein, complex carbohydrates and lipids [23]. However, it is not expected to occur easily cultural changes in the diet worldwide to prevent tooth decay. Thus, innovative approaches to the treatment of dental caries are required [32].

Among the possible new approaches, an interesting one would be blocking or impairing the Quorum Sensing system, a communication system of microorganisms based on the emission of stimuli and responses dependent on population density. Many members of the genus *Streptococcus* that cause infections in humans use this system [33]. Other approaches would be blocking the ability of *S. mutans* to produce acid and survive in this extreme environment of acidity and/or its ability to interact with other cells to form biofilms [34].

Some research has sought a way to prevent tooth decay with the aid of immunology and host response in the search for a possible anti-caries vaccine [10] [22]. Furthermore, many studies on the molecular level have also been conducted in order to inhibit cell growth of *S. mutans* [35] [36].

The metabolism of *S. mutans* has also been extensively studied, mainly biochemical and physiological adaptations that allow this microorganism to produce acids and survive at low pH [37] [38]. Research suggests that the ability to tolerate the acid is due in part to a membrane-bound protein called F-ATPase that extrudes proton H⁺ from the cell, preventing decreased intracellular pH and consequent damage to sensitive enzymes to the acid, DNA and various proteins [39] [40].

The development of bacterial resistance in a large number of microorganisms has become the least effective

current antimicrobial, which justifies the need for design and development of new drugs. Some essential bacterial proteins have been identified as potential drug targets because they are essential for survival of microbial cell, highly conserved in a spectrum of clinically relevant, missing, or radically different species in humans, besides having their biochemistry elucidated [41]. In the case of microorganisms embedded in oral biofilms, the most appropriate would keep pathogenic *S. mutans* living in the biofilm, but less virulent [42].

Various strategies have been used to predict drug targets, among the main ones are highlighted: 1) those that seek to analyze the targets of known therapeutic drugs, based on the level of sequence homology or domains in proteomes [43] [44], in search of potential new drug targets in protein families, and 2) those based on the 3D structures of binding sites on protein surfaces, with the aim of identifying those sites which can bind with reasonable affinity to certain compounds [45] [46]. This latter method is limited due to the little availability of 3D structures, besides it can not be applied to genomic scale.

The data quality on drug targets restricts the predictive power of the models, so that multiple versions of lists of drug targets have been proposed [44] [47]-[49] based on different criteria for their selection. Possible reasons for many versions include: the definition of the drug target is difficult and arbitrary [50]; poor understanding of the drug pharmacology hinders the assignment of each drug to a target; some targets are multimeric protein complexes where the same subunits can come unite in different combinations to form different targets [44] [48].

The wrong selection of targets in preclinical research stages may result in the failure of the newfound drug in the market. Thus, prediction of proteins as drug targets can be used to direct novel therapies, reducing the experimental time and cost during development of drugs and making more reliable the disease treatment [16].

Still, the steps of discovery, development and registration of a drug requires time and investment, being of great interest to identify as early as possible the agents that are probably less promising, allowing a concentrated effort into compounds that are more likely to hit the market. The discovery and planning of new drugs within the area of Rational Drug Design Based on Structure (RDBS), the understanding of molecular mechanisms of receptor-ligand recognition, besides being one of the major challenges in molecular biology, it is one of the central aspects for its success [51].

4. Potential Microbial Targets and Strategic Targets in *S. mutans*

Recent advances in molecular biology technologies have significantly increased the ability for discovery of new antibacterial targets and quickly predict their spectrum and selectivity. Most bacterial targets evaluated to develop drugs include: quorum sensing biosynthesis; signal transduction systems of two components; cell division machinery; the shikimate pathway; biosynthesis of isoprenoid and biosynthesis of fatty acids [41].

The *Quorum Sensing* systems are also important determinants of morphology and communication when the bacteria grow as aggregates in biofilms [52]. When there is increased density of bacteria in biofilms, they respond by inducing or suppressing the expression of groups of genes. These *Quorum Sensing* systems play a critical role in the control of many metabolic processes in the cell, including bacterial virulence. For this reason, they are considered attractive targets for new antibacterial drugs [53].

The signal transduction systems of two components are the primary means to coordinate bacterial responses to environmental changes as well as in some plants, fungi, protozoa, archaea. These systems have an excellent target for drugs due to the 1) significant homology shared among different genera of bacteria, particularly those where amino acid residues are located close to the active sites, 2) bacteria use this system to regulate the expression of virulence factors required for their survival *in vivo*, and 3) bacteria contain many two-component systems, and at least one of them is important for *in vitro* growth [41].

The bacterium division machinery comprises seven or more essential proteins conserved in the majority of microorganisms. It is considered an attractive target due to its essential role in cell division of prokaryotes, its conservation in bacteria, its absence in the mitochondria of eukaryotes, its evolutionary distance of tubulin and its atomic structure and chemical activity known [41] [54].

Other bacterial target that can be used is the shikimic acid pathway (aromatic biosynthetic pathway), due to its preservation in bacteria, fungi, plants and some parasites, and absence in mammals. This pathway affects the conversion of two simple products of carbohydrate metabolism: phosphoenolpyruvate and erythrose 4-phosphate in chorismate, which is a precursor for biosynthesis of a variety of important aromatic metabolites. The enzymes of the shikimate pathway are an excellent target for the design of new antibacterial agents [55].

Isoprenoids are known to have an invaluable role in various biological processes such as cell wall biosynthe-

sis, electron transport, light capture in photosynthesis, membrane's lipid structure and signaling. Isopentenyl diphosphate (IPP) and its isomer, dimethylallyl diphosphate (DMAPP) act as a universal precursor for the biosynthesis of isoprenoids. IPP and DMAPP reactions are catalyzed by the enzyme isopentenyl diphosphate isomerase. The disruption of these processes by blocking of enzymes related to isoprenoid biosynthesis and catabolism is essential for the rational development of potential therapeutic targets [41].

The fatty acid synthesis (FAS), required for construction of membrane phospholipids in living organisms, comprises a repeating cycle of reactions involving condensation, reduction, dehydration and subsequent reduction of carbon-carbon bonds. More evolved eukaryotes perform these reactions through a multifunctional protein (type I pathway). Whereas in bacteria, plant chloroplasts and *P. falciparum*, each reaction is catalyzed by discrete enzymes (type II pathway), allowing selective inhibition [56]. Most of these enzymes are essential for bacterial viability, making it therefore targets for research for new antimicrobials.

Despite little knowledge about the expression of genes during biofilm formation of *Streptococcus mutans*, research has committed effort in comparing laboratory planktonic strains with those organized in biofilms [57]; however, there is little information about the differential gene expression in biofilm formation in clinical strains, since these strains can survive under severe conditions involving multiple bacterial agents, as seen in the oral cavity, leading to expression of several genes involved in biofilm formation. In contrast, laboratory strains may not require these same genes when cultured under mild and planktonic conditions and may lose the ability to express them [58].

Previous studies have indicated the role of sucrose and glucosyltransferases in the biofilm formation of *S. mutans* [59]. Other studies reported the existence of several genes associated with genetic competence [34], regulatory functions, combined with one or more genes, including *ccpA* and *brpA* [60] and *luxS* [61]. These genes have also been shown function as putative regulators of the two-component response system [34], which are involved in biofilm formation. DNA microarrays have been used to monitor the global expression profiles of genes in response to different stimuli [57], such as heat shock and other stresses [62], *Quorum Sensing* [63], anaerobic metabolism [64], sporulation [65] and biofilm formation [57].

Due to the complete genome sequencing of *S. mutans* UA159 [66], genes associated with virulence and/or survival have been identified, and their protein expression considered ideal microbial targets, preferably important proteins for bacteria virulence (exo-enzymes, proteases, other extracellular and surface proteins); avoiding the performance on targets related to this microorganism survival (transport mechanisms, cell division, cell wall synthesis, metabolic pathways, and regulation and signaling in mobile genetic elements). The goal would be to keep the bacteria alive in the biofilm, but less virulent [67].

In *S. mutans* organized in biofilms, proteins related to its metabolism and important for its survival would not be good targets once they would cause cell death, favoring the growth of other perhaps more pathogenic. Future antimicrobial agents that will have a chance of success would be those with seemingly conflicting requirements of maintaining the biofilm in compatible levels with the oral health, but without disrupting the beneficial properties of the resident oral microbiota [67].

One of the biggest challenges for development of modern drugs directed at resident microorganisms in biofilms is to keep the organism alive, but less virulent. This purpose would be possible by means of a probiotic approach, interference during the biofilm formation or disruption of bacterial communication networks [42].

The bacterial adaptation, while modifying the environmental conditions, occurs at different levels, including the multicellular level (cell aggregation and biofilm formation). These processes are modulated by several response regulators, and the distribution patterns of these regulators are generally conserved in the different microbial strains [68]. Thus, compounds that could interact with these response regulators are ideal candidate drugs to reduce the multi-species biofilm [69].

It has been observed that the inactivation of any component of the *Quorum Sensing* intercellular communication system seems to influence the early stages of biofilm formation [34]. These pathways can be selected as targets for controlling the cariogenic biofilms, so that the blocking of genes that encode protein components of this signaling system results in the formation of an abnormal biofilm [33]. The ability to prevent or confuse the *Quorum Sensing* system can block the expression of virulence genes and biofilm formation. However, this knowledge was not applied for clinical use so far [70].

The products of some genes of Quorum Sensing signaling system (*comAB*, *comX* and *comCDE*) are very important for communication of *S. mutans* in biofilm, so that the loss of these proteins can reduce biofilm formation. In this context, it was demonstrated by means of computer modeling techniques and molecular docking that

a derivative of quinic acid (quinic acid) can act as ComA inhibitor [71].

Some natural and synthetic molecular agents showed moderate efficacy against cariogenic bacteria, but no clinical trial was conducted. Overall, combined preclinical and clinical approaches to the rational design of drugs are rare. Since the targets of antimicrobial agents are protein binding sites, the protein structure is required for the rational design of drugs [72] [73]. The recent explosion of sequencing technology has made available the corresponding sequences for all genes of *S. mutans* and several other biofilm bacteria. Thus, the prediction of protein structures from its sequence presented a new opportunity for rational drug design with multi-targets in multi-species [73].

The computational drug design has greater chances of success when it has multiple targets in a microorganism. If a compound can inhibit multiple proteins, it is likely that it inhibits definitely at least one. The identification of multiple protein targets in *S. mutans* will allow the creation of useful pathways for development of new treatments for tooth decay pathways. Thus, despite being interesting the concept of having *S. mutans* as target, the multispecies therapy is essential, since other species present in the biofilm also contribute to the initiation and development of dental caries [73].

5. Computational Detection of Molecular Targets for Drug Development

Bacteria are single-celled organisms with a pretty small genome; however, they show marked variations in their cell architectures, metabolic properties and phenotypic characteristics. In order to show such diversity of phenotypic and ecological characteristics, the bacteria need to have extremely dynamic genomes, involving processes such as acquisitions, deletions and rearrangements of genes [74]. The ability of bacteria to accept and express genetic material transmitted not only by direct descent, but also through external sources, is one of the most prominent areas in recent times within the experimental molecular genetics and biotechnology [75].

The nucleotide sequences originated from other species of organisms that no antecedents are called genomic islands and may differ from the genome as a whole, because such genes may have their features in sequence, as the number of G + C, the use of synonymous codons, frequency of dinucleotides and use of amino acids that are beyond the pattern that genome has [76]. It is known that genomic islands may also be associated with tRNA genes and the so-called direct repetitions and even with mobile genetic elements such as transposons and integrase, besides the insertion sequences. Although the presence of all these features is required, a set of them can mean that there exist genes that originate in the event of horizontal transfer [76].

The horizontal transfers of genes can be studied using tools of bioinformatics based on independent- and dependent-homology methodologies. Detection of these genomic regions can collaborate in the study on the benefits of the presence of these regions to the holders of the various possible forms, such as regarding the emergence of new metabolic pathways, development of virulence, antibiotic resistance and providing the ability to colonize new environments and occupying other niches [77].

Researchers can use surveys about the genomic islands still to explain the pathogenicity of a particular microorganism. Furthermore, the development of drugs, vaccines and antibiotics by pharmaceutical companies is already influenced by the research of genes derived from horizontal transfer [78].

[79] made use of dependent homology methodology when searching proteins of unknown functions that could be the result of events of side gene transfer and could be involved in antibiotic resistance in *S. mutans*. Thus, 500 of the 1600 proteins of the organism were detected without any function described and one of them with very few homologs in the protein data banks already described, the SMU.440. From the studies on structural bioinformatics, once crystallized the protein and defined its three-dimensional structure, it was concluded that SMU.440 could be indeed involved in antibiotic resistance.

5.1. Bioinformatics Tools for Studies on Drug Development

In the work of [79] described above, although the *a priori* lack of protein function from its linear sequence, once determined experimentally the three-dimensional structure and studies on its structure, it was determined its important role in resistance to antibiotics.

The knowledge of the structures of macromolecular targets or ligand-receptor complexes allows the use of strategies for drug design based on the receptor structure (SBDD, *structure-based drug design*). In contrast, when the chosen target structure is not known, methods of drug design based on ligand structure (LBDD, *ligand-based drug design*) may be used, exploring the properties and characteristics of bioactive ligands series

[11] [12].

Methods based on strategies for drug design and receptor structure involve the need of using homology modeling that arises when one has to determine the structure of a protein of which only its sequence is known by using its alignment with a homologous protein from which the structure is known [80] or molecular docking, which is one of the major SBDD strategies, consisting of predicting the bioactive conformation of a small molecule (ligand) in the binding site of a target protein, followed by the assessment and classification of the binding mode proposed [81].

5.2. Drug Design Based on the Receiver Structure

There are two key points in molecular docking programs: the search for the “best” conformation resulting from the formation of the protein-ligand complex and the calculation of the energy free from this association, or its affinity constant [81].

The search algorithm should investigate the energy hyper surface seeking the global minimum free energy. To do so, the receiver is usually considered a rigid structure, and the search algorithm explores the different positions for the ligand in the active site region by using for that translational, rotational and conformational degrees of freedom (for flexible ligands) [82].

At the molecular docking, the ligand’s flexibility treatment is commonplace in current methods, and its performance drops drastically with increased conformational degrees of freedom (ie, increased number of torsional chemical bonds) of the ligand [83]. Some methods have tried to add flexibility to the receiver taking into account the torsional degrees of freedom of the amino acids side chains in the protein active site [84], or considering various conformations of protein obtained from different crystal structures (*i.e.* where the same protein is complexed with different ligands) or obtained from molecular dynamics calculations [85] [86].

Simulations of molecular dynamics (MD) provide information about the molecular motion, thus enabling the detection of biologically relevant movements, during the process of molecular docking or even as in the active site of a protein. It is a technique commonly used after the determination of the three-dimensional structure of molecules by molecular modeling. Thus, it is expected an adequation of the molecules system to the simulated environment in which it is inserted, seeking the position of atoms in a minimum amount of free energy [87].

Molecular dynamics can be carried out by Molecular Mechanics that is based on classical mechanics and considers the interactions among the cores of molecules. Or it can be performed through the use of Quantum Mechanics. In this case, the *ab initio* and/or semi-empirical methods are used. The choice between these approaches depends on the properties that one aims at evaluating, the desired accuracy and computational capacity available for calculations [87].

Methods using classical force fields (also known as molecular mechanics methods) ignore the movements of electrons and calculate the energy of the system as a function only of nuclei positions. Molecular mechanics is therefore normally used for performing calculations in systems containing a large number of atoms [88].

In some cases, force fields can provide answers that are as accurate as those obtained by performing high-level quantum mechanics calculations at a greatly reduced computational time. However, molecular mechanics cannot obviously determine properties that depend on the electron distribution in a molecule. The classical force fields are based on simple models of interactions within a system with contributions from the removal of bonds, opening and closing angles and rotations of simple bonds [88].

The force fields commonly used in molecular docking programs and molecular dynamics/mechanics are GROMOS [89], AMBER [90] [91], CHARMM [92] and MMFF94 [93]-[97] and used in molecular dynamics programs like GROMACS [98], CHARMM [99] and AMBER [87]. The Gaussian [100] GAMESS [101] and SPARTAN [102] programs perform quantum mechanical calculations, commonly suitable for small systems.

There are also programs that allow the combination of methods of quantum mechanics and molecular mechanics (QM/MM), and the active site or binding site is treated by an *ab initio* density functional theory and semi-empirical potentials, while the rest of the system is calculated using force fields in molecular mechanics [87]. Typically, methods that use molecular mechanics already offer the possibility to also use this combination of methods.

5.3. Drug Design Based on the Ligand Structure

Modern strategies used in the rational design of new compounds-prototypes are based on the physiological ap-

proach. This approach enables to design a chemical structure of the new molecule based on the previous definition of therapeutic action mechanism, *i.e.* the biomacromolecule to which the new drug will bind to change a particular biochemical pathway.

Knowing the three-dimensional molecular structure of the chosen therapeutic target, particularly the region responsible for chemical interaction, it is possible to identify a compound capable of binding to that active site and changing its properties by using computational molecular modeling techniques. The rational design of this molecule is carried out by using molecular simplification techniques (reduction of a compound structural complexity), molecular hybridization (obtaining new chemical structure from parts of two or more different bioactive substances) and bioisosterism (replacement of structure parts of a bioactive substance by another or others with similar electronic behavior), besides the use of chemical intuition of an experienced researcher to propose new changes.

A new computational approach has been mainly used by industrial research laboratories, where there is the search for compounds prototypes in databases containing the description of a wide variety of natural and synthetic compounds, pure or combined, for performing bioassays. The goal is to discover active compounds prototypes that can, when assessed experimentally, present a micromolar receptor-ligand affinity (μM), *i.e.* active at a concentration of one millionth of a mole per liter, or nanomolar (nM), active in a concentration of one billionth of a mole per liter.

In addition to this filtering methodology *in silico*, this search is carried out by applying the “Rule of Five”, where molecules that violate one or more rules can be eliminated; a set of empirical rules and decision-based systems are also applied. This filtering can be used as positive and negative selection and it has been increasingly complemented by algorithms that predict physicochemical properties, ability to penetrate the blood-brain barrier, low toxicity, among others [103].

Still, for being performed *in vivo* filtering, where the exclusion of certain molecules happens after applied some experimental tests such as cytotoxicity or certain cytokinetic properties [104]. After the discovery of compound-prototype desired, a congener series (similar compounds with small structural variations) should be built and evaluated pharmacologically to guide the optimization of the future drug. This step is critical because it represents the pursuit of better characterization of the therapeutic efficacy of the drug candidate.

Finally, it should be then performed step tests for the validation of the selected ligand, determining whether this is indeed the ideal molecule for obtaining the expected result with the evaluation of ligand’s pharmacokinetic properties related to its path in the body since its absorption, distribution and elimination.

6. Conclusions

Despite dental caries being a simple prevention disease through frequent oral hygiene habits, its high prevalence in the global population still indicates the need for developing effective preventive methods to control the disease. Since *Streptococcus mutans* is considered as the main etiological agent of dental caries, more studies are needed to better understand its functioning and performance and, its virulence mechanisms in biofilm formation, in order to find new alternatives against this microorganism in the ear future and therefore, against this pathology.

Chemical control of biofilm by reducing the virulence or even death of these micro-organisms seems an interesting alternative in this endeavor; but it is important to note that the use of broad-spectrum antimicrobials results in the risk of inhibiting or killing beneficial bacteria and modifying the normal microbiota, which may create clinical problems such as the development of opportunistic pathogens, especially in dental biofilm. Therefore, it is worth mentioning that all efforts should be directed towards the appropriate use of these bacterial agents.

In order to better understand and simulate these mechanisms, computational methodologies have become crucial components of many programs used in pharmaceutical production. Thus, this computational approach will allow the testing to expedite the discovery and use of new chemicals that may decrease virulence of cariogenic microorganisms present in dental biofilms.

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