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Delivery of biologics: topical administration

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Abstract

Biologics are unaffordable to a large majority of the global population because of prohibitively expensive fermentation systems, purification and the requirement for cold chain for storage and transportation. Limitations of current production and delivery systems of biologics were evident during the recent pandemic when <2.5% of vaccines produced were available to low-income countries and ~19 million doses were discarded in Africa due to lack of cold-chain infrastructure. Among FDA-approved biologics since 2015, >90% are delivered using invasive methods. While oral or topical drugs are highly preferred by patients because of their affordability and convenience, only two oral drugs have been approved by FDA since 2015. A newly launched oral biologic costs only ~3% of the average cost of injectable biologics because of the simplified regulatory approval process by elimination of prohibitively expensive fermentation, purification, cold storage/transportation. In addition, the cost of developing a new biologic injectable product (~\$2.5 billion) has been dramatically reduced through oral or topical delivery. Topical delivery has the unique advantage of targeted delivery of high concentration protein drugs, without getting diluted in circulating blood. However, only very few topical drugs have been approved by the FDA. Therefore, this review highlights recent advances in oral or topical delivery of proteins at early or advanced stages of human clinical trials using chewing gums, patches or sprays, or nucleic acid drugs directly, or in combination with, nanoparticles and offers future directions.

1. Introduction:

Despite the availability of therapeutic proteins for treatment of various metabolic, inherited, or infectious diseases for several decades, they remain mostly unaffordable for large

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Declaration of interests

Henry Daniell reports a relationship with PhylloPharma LLC that includes: equity or stocks. Henry Daniell has patent issued to University of Pennsylvania. None

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populations of low-income or developing countries. This became quite evident during the recent pandemic. The cost of production of biologics is very high due to their production in prohibitively expensive fermentation systems, high cost of purification, and cold storage/transportation. Limited cold storage/transportation infrastructure in developing countries was quite evident during the pandemic when African countries administered <50% of all coronavirus disease 2019 (COVID-19) vaccines they received, leading to disposal of ~19 million doses [1,2]. Therefore, the global rollout of COVID-19 vaccines progressed at two alarmingly different rates; among 7.51 billion administered doses, 52.1% were in developed countries but only 4.5% in low-income countries have received their first vaccine [3]. Only 2% of the African continent was vaccinated, even though it has >17% of the global population, when developed countries offered a third or even a fourth booster shot.

In this review, we compiled comprehensive data on biological drugs approved by the FDA since 2015, cost and their modes of delivery. Although intravenous, intramuscular, and subcutaneous injections are bioavailable methods to quickly deliver drug products, they are prohibitively expensive, limiting their availability to a large majority of global population. Limitations of current production and delivery of biologics were quite evident during the recent pandemic. In contrast, topical delivery of drugs offers a more affordable, less invasive approach. In this review, we evaluate advantages and disadvantages of different delivery methods, including intravenous, subcutaneous, intramuscular, and topical delivery. Recent advances in human clinical trials on topical delivery include the chewing gum with viral trap proteins to decrease self-infection and transmission of Corona or other viruses in the oral cavity [4–6], oral [7,8,9] and topical delivery of peanut protein allergens to prevent peanut allergy [10,11]. Among different drug production systems, expression of protein drugs in plant cells offers unique advantages by elimination of prohibitively expensive fermentation, purification processes, and the need for cold storage/transportation. Therefore, we evaluate recent advances in this field. In addition, we review recent advances in oral topical delivery of nucleic acid therapeutics, including the use of nanoparticles. Since oral delivery of nanoparticles for gastrointestinal and systemic applications has been covered by other excellent reviews recently [12–15], our emphasis will be on delivery to the oral cavity to match the theme of this review.

2.1 Biological drugs approved by FDA since 2015

In order to gain an understanding of recent trends in biological drugs approved by the United States (U.S.) Food and Drug Administration (FDA) since 2015 and their mode of delivery, we utilized publicly available and reliable databases at FDA, Center for Drug Evaluation Research (CEDER), and Center for Biologics Evaluation and Research (CEBER). Therefore, the chemical name, brand name, applications, and date of licensure were obtained from the Purple Book [16,17], an online database that lists all licensed biological products monitored by the FDA (CEDER). The Biological Product Patent Transparency (BPPT) requires that the FDA update the list every 30 days to add any biologics approved under section 351(k) or 351(a) of the Public Health Service (PHS) Act [16]. The National Institutes of Health (NIH) National Library of Medicine (NLM) RxNorm database was utilized as confirmation of the biological product reaching the market [18]. In line with the focus of this review, all vaccines, monoclonal antibodies and their

fragments, and diagnostics were excluded. As the Purple Book in the portable document format (pdf) is from April 2020, the Purple Book Database of Licensed Biological Products (accessed January 8, 2023) was used to access products approved from May 2020 through December 2022 [16]. Therefore, the data obtained for this review is current. Biological products that did not have a proprietary name and those voluntarily revoked were excluded from the analysis. Similarly, newly approved biological products regulated by the CEBER that received approval for their Biological License Application (BLA) in or after 2015 were also evaluated [19]. Information regarding the clinical trials mentioned in the BLAs was identified from [ClinicalTrials.gov](https://clinicaltrials.gov), a database regulated by the NIH U.S. National Library of Medicine [20]. The route of delivery of the biologics was gathered from the [drugs@FDA](https://www.fda.gov/drugs) website [21]. Additional descriptive qualities were obtained from both the NIH NLM Drug Information Portal and NIH NLM DailyMed database [22,23].

Since 2015, 89 biological products were approved by the FDA and met the inclusion criteria. In terms of the main routes of delivery that these FDA-approved biologics utilize, ~45% and 36% follow a pathway of intravenous or subcutaneous injection, respectively. The remaining 19% is split among various other routes, with less than 10% being non-invasive (Fig. 1–2, Table 1) [16,21]. Although injection is the dominant mode of protein drug delivery and saves millions of lives, there are several limitations, as discussed below. Beyond these safety concerns and lower patient compliance than other modes of drug delivery, injectable drugs are very expensive and pose a greater total (pharmacy and medical) economic burden than drugs that utilize an oral delivery pathway [24]. The loss of ~50% of vaccines during the recent pandemic in African countries due to failure of the cold chain [2] is yet another challenge for injectable drug delivery. Among many other factors, equity barriers to accessing life-saving drugs persist, especially in low-income and lower-middle-income countries (LMICS). Furthermore, those in LMICS are often living in near proximity to others, making infectious diseases more dangerous. Therefore, creating protein therapeutics that do not rely on the cold chain is an urgent priority [25], especially when considering how social inequities and social determinants of health also influence disease susceptibility and distribution [26].

2.2 Unaffordability of protein drugs

Strikingly, the cost of per capita prescription of drugs in the U.S. is the highest in the world [27]. From 2008 to 2021, the median launch prices of drugs first marketed increased by \$177,892, or by 8,411%. The interquartile range of biological product prices ranged from \$18,861 to \$288,759 during this same timeframe [28]. This median price does not include prohibitively expensive gene therapy drugs. For example, hemophilia A drug Roctavian costs \$2.9 million per patient [29] and hemophilia B Hemgenix costs \$3.5 million per patient [30]. Additionally, clinical trials can cost hundreds of millions of dollars and have an inherent risk component [31,32], which acts as a barrier to drug commercialization and contributes to the high cost of drug products. The estimated cost to develop a new biological product is ~\$2.6 billion [31,33]. As drug prices continue to rise, many countries have sought to implement a drug price transparency initiative to better maintain affordability and control prices. However, these initiatives have not guaranteed a reduction in the price of the drug [34].

The Patient Protection and Affordable Care Act of 2010 facilitates the development of biological products that are “biosimilar” to FDA-approved biologics and are given a shorter drug review and licensure process [35]. Compared to the reference biologic, biosimilar products generally are priced around 28%, 30%, and 60% lower in the European Union, Japan, and China, respectively [36]. Unfortunately, the complexity of patent disputes, regulations, and litigations has resulted in biosimilar products being withheld from the market [37,38]. The lack of availability of these more affordable drug options in the U.S. has prevented market competition [38]. This competition is necessary to lead to less expensive drug options. For example, as multiple manufacturers create generic medications, the drug is listed at a fraction of the price of the brand-name product. More specifically, the price of a drug product decreases to around 55%, 33%, and 13% of the reference product when the medications are created by two, five, and 15 generic manufacturers, respectively. When this was done for the drug metformin (small molecule), approximately a 92% decrease in drug price was observed, leading to a much more affordable and accessible product [39]. However, such significant price declines have not been achieved with protein products. When Semglee, the first FDA-approved biosimilar insulin product, was approved, it was listed at 95% of the price of Lantus [40]. Despite a predicted savings of \$44.2 billion in the U.S. from 2014 to 2024, the introduction of biosimilar products has almost had the opposite effect. For example, a biosimilar product to adalimumab resulted in a sudden and drastic 73% increase in the price of this drug [41]. Therefore, many factors have accounted for the high cost of drug products in the U.S., and urgent attention and action are required from policymakers and scientists to ensure more equitable and accessible medical treatment. The technological revolution has dramatically lowered the price of communication via phone or Skype or Internet in the past two decades, with dramatic improvements in speed and service quality. In order to achieve such affordability for biologics, new modes of protein drug production and delivery are urgently needed.

2.3 Delivery Methods

During the last eight years, only eight approved biological products that met this review’s inclusion criteria were non-invasive, and these non-invasive drugs could be administered in one of three ways: topical (includes ophthalmic), sublingual, or oral. Topical delivery was the most common (50%) of these types, followed by both sublingual (25%) and oral drug delivery pathways (25%). Of the four topically delivered biologics, only one followed the ophthalmic pathway. Furthermore, although drug delivery through the oral cavity has been a popular noninvasive method to deliver drugs since 2015, it only entered the drug delivery space in 2020. The proportion of non-invasive biologics approved to total biologics approved each year was around 6%, 0%, 17%, 8%, 0%, 10%, 20%, and 7% in 2015, 2016, 2017, 2018, 2019, 2020, 2021, and 2022, respectively. Therefore, a trend in proportionality cannot be established. Similarly, despite being safer overall and leading to higher patient compliance and satisfaction rates, the actual number of non-invasive biologics approved does not have a clear or increasing trend by year. Strikingly, since 2015, there has not been a year that more than two non-invasive biological products were approved, and the maximum proportion of non-invasive to total biologics approved in a year peaked at less than 20% in 2021. Additionally, over the past eight years, biological products approved in

2017, 2021, and 2022 showed the most variation in delivery methods, with six different delivery pathways existing among the 41 approved biologics.

Moreover, all insulin products in this review require an injection for administration, with subcutaneous injection being the most popular way to deliver the drug and intravenous being the only other delivery method. All coagulation factor complexes, drugs designed to treat cancer, and many other biologics also require an injection for drug delivery. Overall, >90% of approved biologics are invasive. Interestingly, the non-invasive biological products approved since 2015 have been centered around preventing allergies and controlling excessive bleeding during surgery. For example, of the four biologics that are administered topically, two control bleeding (Raplixa and Fibrin Sealant) and one (StratGraft) treat thermal burns. Only one biologic can be administered ophthalmically (Oxervate), and it is used to treat neurotrophic keratitis. Similarly, both biologics delivered through sublingual tablets either control allergies induced by house dust mites (Odaetra) or ragweed pollen (Ragwitek). Finally, two biologics can be administered orally, with one mitigating the effects of peanut allergies (Palforzia) and the other being an oral replacement therapy to treat sucrase deficiency (Sucraid). Strikingly, 360 capsules with peanut cells (annual dose) are < 3% (~\$2500) of the median annual price of biologics that were newly marketed between 2008 and 2021 (\$84,508) [28,42]. Therefore, as seen over the past eight years, a non-invasive method to deliver proteins has not been achieved with great success and remains highly elusive (Fig. 1–2, Table 1).

Every year, it is estimated that >16 billion injections are administered globally [43–45], with ~95% for therapeutic reasons [43]. As shown in Fig. 1–2, Table 1 [16,21], intravenous infusion (IV) is the most bioavailable and quick method [44,46] to deliver large doses of drugs [47,48] into the systemic circulation [46]. IV infusion facilitates immediate and continuous drug delivery into the circulatory system [47–49], but the dose should be titrated very carefully. In the case of insulin, recent clinical trials reveal that insulin pens (used by >95% of diabetic patients) cause hypoglycemia and related consequences, but precise delivery of insulin doses using pumps based on blood glucose levels overcomes this problem [50,51]. Unfortunately, pumps are not affordable for most diabetic patients. In the U.S., insulin pumps cost approximately \$6,000 and require an additional \$3,000 to \$6,000 every year for supplies [52]. With one-third of the global population earning <\$2 per day, insulin pumps pose high barriers to the global population at large. Insulin delivery to the peripheral circulation by injections is a major reason for hypoglycemia [53–55] but delivering insulin to the liver overcomes this challenge. Indeed, oral insulin bioencapsulated in plant cells or enteric capsules does not lead to hypoglycemia, similar to that of natural insulin released from pancreas [56,57]. Nasal delivery of insulin failed in the clinic largely due to a limited absorption surface of around 180 centimeters² [58]. Moreover, intranasal insulin has a low bioavailability of 15–25%, has an onset of action of around 20 minutes (mins), and can cause blistering, irritation to the nasal cavity, and redness [59–61]. Due to its low bioavailability, larger insulin doses are required compared to subcutaneous methods, leading to higher patient costs [62,63]. In contrast, the large mucosal area of the human small intestine (30 meters²) offers the greatest surface area for protein drug absorption and delivery [64].

The medications that are administered subcutaneously use a bolus and are delivered into the subcutis [44]. Subcutaneous injections (SC) result in a rapid onset of action and high bioavailability (80% for insulin) [58,65] but are constrained by a limited volume of delivery (maximum of 1.5 milliliters (mL)) [66,67] and intra-individual and inter-individual variation [68–70]. The rate of insulin absorption varies depending on the site of injection. A barrier to subcutaneous insulin absorption is passing through the extracellular matrix [70] without binding to matrix proteins, like type V collagen [71].

Intramuscular delivery is most commonly used [72], comprising >75% of all injections performed around the world [45,73]. In injecting a medication intramuscularly, the drug is delivered within a person's highly vascularized muscles, allowing for uniform and rapid absorption by the bloodstream and incorporation into the systemic circulation [44,72]. The onset of action for intramuscular injection (IM) delivery ranges from 5 to 10 mins [74], faster than SC, can deliver larger volumes, and avoids first-pass metabolism [72]. However, some disadvantages of IMs include (i) constriction due to small drug volumes (1–2 mL for the deltoid site and 5 mL for the quadriceps site), (ii) periostitis, (iii) bleeding, (iv) haematoma, (v) tissue necrosis, (vi) infection, (vii) contractures, (viii) abscess, (ix) nerve and vascular injury, (x) muscle fibrosis, (xi) gangrene, (xii) skin slough, and (xiii) pain [72,75–77].

Oral delivery of biologics is one of the most challenging drug delivery methods to develop but is highly preferred by patients. The first challenge is to protect protein drug digestion from acids and enzymes in the stomach. Therefore, protein drugs should be bioencapsulated in materials that could protect them from acids and enzymes. One of the drugs approved by FDA (Palforzia) (Table 1), the therapeutic protein (Ara h), was protected via bioencapsulation within peanut plant cells. Biologics bioencapsulated in plant cells are protected from the human digestive system because digestive enzymes cleave alpha linkages but plant cell wall polymers are linked by beta 1,4 –1,6 bonds [78–80]. The next challenge is the lysis of plant cells to release protein drugs in the gut lumen. This is naturally done by gut microbes because they release enzymes that cleave beta linkages of plant cell wall polymers [81,82]. The final challenge is the delivery of therapeutic proteins across the gut epithelium into the immune or circulatory system. While a few proteins like Ara h may recognize human cell receptors, most proteins require tags to cross the gut epithelium. Several such tags are developed and engineered to be cleaved off using proteases present ubiquitously during transport and confer desired therapeutic goals upon oral delivery of protein drugs [80,82–85].

3. Topical drug delivery

The non-invasive drug delivery methods that are compliant and safe for the patients are getting more attention recently. Topical delivery has the unique advantage of delivering higher doses to the target site, without getting diluted by the large volume of blood in circulation and degraded by several proteases. The topical delivery method facilitates the delivery of a mixture of proteins, which is not feasible for systemic delivery due to unfavorable immune response or potential toxicity. Proteins or peptides are known for their high specificity and potency [86] and are therefore preferred over non-specific small

molecules or chemical agents. When small molecules are unable to penetrate extracellular polymeric substances (EPS) in the dental biofilm/plaque, topical delivery of enzymes is ideal to disrupt EPS and increase access to antimicrobials and kill pathogenic microbes [87,88]. Topical drug delivery interventions such as prophylaxis measures of infections in oral cancer patients undergoing radio or chemotherapy become more important [89]. These immunocompromised patients are more prone to oral cavity infections [90] because of hyposalivation of saliva [89]. Mucositis (tissue swelling and irritation in mouth) and neutropenia (too few neutrophils) further facilitate bacterial and fungal pathogen infection in the oral cavity of these patients. Bacterial infections predominate during the early phases of a neutropenic episode, while fungal infections are more common with prolonged neutropenia [91,92]. Among the bacterial pathogens, gram-positive are more common [90], while among the fungal pathogens, *Candida albicans* (*C. albicans*) are more prominent [89]. Although small molecules are used in the clinic [89,93], lack of specificity or inability to penetrate biofilm are major challenges. Furthermore, the duration of oral rinse or mouthwash is very short and ineffective in killing pathogens protected by EPS secreted by pathogens.

Saliva contains diverse pathogenic viruses, including severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and salivary droplets, and aerosols are the prime agents for the transmission of these viruses. Pathogens in the aerosolized state can persist for a prolonged time period and could be infectious to nearby patients or healthcare providers. The use of mouth rinses before the dental procedure is recommended to reduce the viral load and possible spread of infections [94]. Unfortunately, clinical evaluation of mouth rinses did not reduce the viral load at statistically significant levels [95]. The chewing gum delivery method has been recently explored for the targeted delivery of anti-viral proteins/peptides and to enhance the duration of their delivery to neutralize oral viruses.

3.1 Chewing gum

Chewing gum production has flourished and is expected to continue growing, with many Americans chewing gum on a weekly basis [96]. Chewing gums can contain various nutrients and active pharmaceutical ingredients [97,98]; while chewing these gums, the nutrients and active drug ingredients can be absorbed by both the oral mucosa and gastrointestinal tract, providing local and systemic delivery pathways [99]. Chewing gums containing small molecules are a highly attractive therapeutic and preventative treatment option as they conveniently offer a way for patients to discreetly consume medication, have an agreeable taste, promote swift drug absorption, and support patient compliance even among young children and those with difficulties swallowing [99,100]. Moreover, chewing a piece of gum can increase a person's alertness [101] and memory [101,102], prevent dental caries [103] and dry mouth [104], improve a person's mood [101,102,105], and reduce hunger cravings [106]. To name a few, there are commercially available plant-produced small molecules, such as aspirin [107], nicotine [108], vitamin C [109], and Xylitol [110]. However, there has yet to be an effective chewing gum impregnated with target proteins for drug delivery until recently [4–6,88].

3.2 Chewing Gum History

The expanding success of the \$25 billion global chewing gum market [111] has involved over 9000 years of history and experimentation. Transitioning from chewing resin and sap directly from the tree [111–115], chewing gum was first commercialized in the mid-1800s [111,113,115] and quickly gained traction. This growing popularity [116,117] can be attributed to many factors, including (i) the creation of a chewing gum factory [113,118], (ii) the introduction of flavored chewing gums [111], (iii) the desire to patent the chewing gum [115,119,120], and eventually, (iv) the invention of the modern chewing gum [111,117]. Almost a century after chewing gum was first commercialized by John Curtis, small molecules were incorporated into chewing gums as a method of topical delivery [98]. A few decades later, sugarless chewing gums were invented to curb the onset of cavities [115]. More recently, many have sought to develop chewing gums with proteins, such as insulin, to deliver protein drugs orally. Even so, most of the protein drug has been lost due to degradation by the acids and enzymes of the stomach [121]. However, when insulin is bioencapsulated in plant cells, protein drug delivery is effective [57,122] as plant cell wall α 1,4 and α 1,6 linkages protect protein drugs from digestion [82]. The Daniell Lab has been able to successfully incorporate plant cell proteins into chewing gums to prevent dental caries and debulk the SARS-CoV-2 and Influenza viruses alike [4–6,88]. On May 31, 2022, the Daniell Lab received FDA/Investigational New Drug (IND) approval (IND 154807) and became the first to engineer a plant-based protein drug that does not require purification, can be delivered orally, and is free of the restraints posed by cold chain products. A timeline representing the milestones of chewing gum is highlighted in Figure 3 [4,5,14,88,98,111–120].

3.3 Chewing gum delivery for prevention and treatment of diseases and beyond

Plant-derived protein therapeutics offer one possible sustainable solution to combat biologic unaffordability due to its low production cost, ease to scaleup, and elimination of cold chain for storage and transportation. Most recently, vaccines against the current pandemic were produced in tobacco (Phase I- [123]; Phase III – [124]). Although this addressed cost at the early stages of production, the purification of vaccine antigen and cold chain required for storage and transportation did not significantly lower the cost of this vaccine. However, alternate approaches are currently advanced to the clinic for complete elimination of the cost of fermentation, purification, cold storage, and transportation. Several biologics expressed in chloroplasts are now advancing to the clinic: angiotensin converting enzyme 2 (ACE2) to prevent SARS-CoV-2 infection and transmission is currently in Phase I/II clinical trials [4–6] for topical drug delivery using the chewing gum containing freeze-dried plant cells. Preclinical studies are in progress for oral drug delivery through the protection of therapeutic proteins in the digestive system via bioencapsulation in plant cells [80,82,84].

Topical delivery of plant-derived pharmaceuticals is believed to be a promising candidate for anaphylactic and pre-prophylaxis or post-prophylaxis measures against food allergens and infectious diseases that are particularly incurable. Developments in these perspectives are compiled in Table 2. Recent successful completion of Phase III trials of peanut proteins to manage anaphylactic reactions in toddlers of one to three years through the topical delivery route [10] is one of the most promising efforts. The patches loaded with peanut

proteins were safe and efficacious in toddlers in inducing tolerance against peanut allergens. Peanut proteins delivered through the topical route in patches were safe and effective in preventing allergy in Phase III clinical trials [11]. It is envisaged that similar technologies could be developed against other food allergens (egg and milk allergens). Development of topical delivery interventions is highly relevant to induce tolerance against food allergens because the delivery of allergens at an early stage of life is more efficacious in tolerance inductions against food allergens, and therefore, the topical delivery method is ideal for infants. Significant efforts in topical delivery interventions have also been recognized for the prevention of sexually transmitted diseases like the acquired immunodeficiency syndrome (AIDS) and Herpes Simplex Virus (HSV) mediated diseases. Several promising anti-viral lectin molecules (carbohydrate or glycoprotein binding) like PC-6500 (Griffithsin lectin of red algae expressed in tobacco), scytovirin and cyanovirin lectins (expressed in *Oryza sativa*), engineered banana lectin, etc. are expressed recombinantly in an intention to be delivered topically through the vaginal route as an important component of the microbicides [125–129]. The safety, pharmacokinetics, and pharmacodynamics of the plant lectin griffithsin formulated in carrageenan vaginal gel have been evaluated in healthy women in a Phase I clinical trial ([NCT02875119](#)).

Several recombinantly expressed monoclonal antibodies (mAb) expressed in plants, including 2G12, Anti- α -C-C chemokine receptor type 5 (anti- α -CCR5), bNVRC01, and 2F5 (expressed in *Zea mays*) have been evaluated for passive immunization against the Human Immunodeficiency Virus (HIV) infection [130–132]. Among all explored monoclonal antibodies, Anti- α -CCR5 and 2G12 are at the drug development stage of preclinical and clinical trial Phase I, respectively. A double-blind, placebo-controlled, randomized, dose-escalation Phase I Safety Study of protein drug product 2G12 as a single vaginal administration in healthy female subjects was evaluated in 2011 ([NCT01403792](#)) but has not advanced further into the clinic. Griffithsin expressed in tobacco and anti-HSV-2 mAb expressed in soybean have been demonstrated for anti-HSV activity [129,133]. Recent developments in the topical delivery approach using chewing gums containing biologics expressed in plant cells to prevent and treat oral diseases received significant attention from the scientific community, as discussed in this section.

3.3.1 Oral diseases—Worldwide, dental caries is the most prevalent human disease [134,135], afflicting upwards of the vast majority of both adults and children [136,137] and disproportionality affecting those living in poverty [138] and people of color [134,136]. Dental caries is a microbial disease caused by a bacterial infection [135] from the transmissible bacteria *Streptococcus mutans* (*S. mutans*) [134], with the word caries deriving from the Latin word “decay” or “rot” [139]. Tooth decay typically originates at the proximal or occlusal surfaces of a tooth [134] after consumption of carbohydrates and sugars. In this, the bacterium in plaque produces acids and leads to repeated cycles of demineralization of the inorganic component of the tooth and the simultaneous breakdown of the organic part of the calcified dental tissue [140]. Moreover, in addition to dental caries, the accumulation of dental biofilm leads to periodontal and gingival disease [141–144]. The most popular method to mitigate these effects and remove plaque is by manually brushing one’s teeth,

however, human brushing habits are often highly variable and ineffective [145–147]. Therefore, additional measures to prevent dental caries are needed.

Treatment or prevention of oral diseases becomes necessary because these conditions are not only restricted to the oral cavity, but dental biofilm in advanced stages, like in the periodontal pocket, is sometimes responsible for bacteremia. It accumulates in the endothelial cells and causes cardiovascular diseases. Inflammatory responses against dental caries pathogen transfers to other organs; as a result, dental caries is a risk factor for other auto immune or inflammatory diseases, including Diabetes mellites. It is reported that *C. albicans* prevalence in children with childhood caries is related to the prevalence of *C. albicans* in mothers; this suggests that oral hygiene matters not only for the present generation, but also for the next generation. Taking into consideration topical delivery interventions of plant-derived molecules, like monoclonal antibodies, Guy's 13 SIgA/G (Secretory IgA -antibody against cell surface adhesion (antigen I/II) molecule of *S. mutans* expressed in tobacco) and Anti-FimA (fimbrial protein fimbrillin A of *Porphyromonas gingivalis*), and antimicrobial peptides (Protegrin and Retrocyclin expressed in tobacco effective against *S. mutans*) have been developed and demonstrated for efficacy [87,148,149]. The developed monoclonal antibodies and microbial peptides are targeted for single microbial species while dental biofilm or other oral complications are derived from multispecies or multi-kingdom microbial partners. Moreover, dental biofilm is a complex structure in which microbes are embedded inside the EPS matrix and are protected from the exposure of applied antimicrobial components. For the efficacy of the antimicrobial agent, the matrix needs to be first degraded.

For example, Singh et al. [88] developed recombinant enzymes dextranase, mutanase and lipase in lettuce for topical delivery as chewing gum formulations and demonstrated anti-biofilm efficacy of plant-produced molecules *in vitro* against cross-kingdom microbes (*S. mutans* and *C. albicans*). The dextranase/mutanase enzyme produced in lettuce chloroplasts efficiently degrades the EPS matrix that resulted in a dispersed bacterial colony of *S. mutans* in the biofilm. Interestingly, lipase produced in lettuce was able to inhibit the transition of *C. albicans* yeast form to filamentous form in the biofilm. Interestingly, dextranase/mutanase and lipase topical application together were able to kill both bacterial and fungal partners (killed pathogens are visualized as magenta color in the treatment while alive bacteria in green and fungus in cyan color in the untreated sample after staining with suitable dye) in the bacterial-fungal mixed kingdom biofilm (Fig. 4). In this study, authors demonstrated the suitability of freeze-dried plant powder as chewing gum formulations. The plant powder impregnated into the chewing gum released the protein in a time-dependent manner in a mechanical simulator device in conditions simulated as an oral cavity. Most importantly, proteins in the chewing gum remained stable when stored at ambient temperature for several years. Chewing gum formulations using antimicrobial peptides have also been explored for better oral health or anti-plaque potential. Two clinical trials investigated the safety of antimicrobial gum formulation (NCT02864901; NCT01877421). Efficacy was not the primary endpoint of NCT01877421 and therefore, the study was not powered for determination of a statistically significant efficacy outcome but suggested that the antimicrobial peptide could be effective but with limited sample size or short duration of 4 days [150]. The trial NCT02864901 was designed for longer durations (14, 28, 34 days)

but antimicrobial gum did not meet the anticipated efficacy. One of the key limitations of these studies would be the inability of antimicrobial peptides to penetrate the EPS secreted by colonizing microbes. Therefore, therapeutic delivery of proteins is still elusive. Pedersen et al. [151] examined the efficacy of *Lactobacillus reuteri* containing lozenges for severity of recurrent aphthous ulcer in another pilot study (NCT02976922). Although reduced severity of aphthous ulcer after daily administration of *L. reuteri* up to 90 days was observed, the improvement was not significantly better than the placebo.

3.3.2 COVID-19 pandemic—On March 11, 2020, the World Health Organization (WHO) declared COVID-19 to be a global pandemic [152], with unprecedented health and economic burdens following [153–155]. As of early June 2022, the WHO has reported over 529 million cases of COVID-19 and more than 6.5 million deaths globally. By early June of 2023, more than 238 million more cases of COVID-19 were reported, increasing the number of deaths by nearly half a million people [155]. Moreover, poor and worsening psychiatric conditions, burnout, and substance use disorder among the public and healthcare professionals alike have been exacerbated by the pandemic [156–159]. The pandemic has also induced immense economic strain, affecting globalization, trade, travel, food, agriculture, academia, healthcare, business, and tourism, ultimately instigating the crash of many financial markets [154]. The total estimated cost of the pandemic is \$16 trillion and growing [153], which is more than the 2020 gross domestic product for the entire European Union [160]. Among underserved racial, ethnic, and socioeconomic populations, these effects are even more pronounced, leading to severe global health disparities [161].

COVID-19 is a highly transmissible respiratory illness, with a transmission rate of around 2.2 [162]. Moreover, with almost all the latest COVID-19 diagnostic targets facing mutations [163], it is estimated that the annual rate of nucleotide mutation in the SARS-CoV-2 genome is approximately 6.7×10^{-4} substitutions per site [164]. Furthermore, the targets of the SARS-CoV2 nucleocapsid (N) gene probes and primers undergo most mutations [163,165,166]. Heightened rates of transmission have been observed among close-contact communities while singing [167], speaking, breathing, sneezing, and coughing [168–170], highlighting the intimate role the oral and nasal cavity play in the transmission, replication, and infection of the SARS-CoV-2 virus [171–173]. Additionally, the urgency to debulk pathogens in the oral cavity is further highlighted as there are more ACE2 receptors in the salivary glands than that in the lungs, the most common transmission pathway comes from salivary droplets, and the virus is abundant in the oral cavity during the first 10 days of infection [174].

As of May 2022, the WHO recommends social distancing, mask-wearing, hand washing, and quarantining to prevent the onset and mitigate the effects of COVID-19 [175]. However, an affordable, complete, safe, and convenient measure to decrease both the rate of transmission and reinfection is not yet available. The Daniell Laboratory has sought to engineer a novel treatment to prevent viral transmission and reinfection reliably and effectively by decreasing the viral load of the SARS-CoV-2 virus in the oral cavity and throat area, the primary sites of viral replication and infection [174,176]. To target the oral cavity, chewing gum containing anti-viral proteins expressed in plant cells served as a novel topical delivery system [4–6].

3.3.3 Recombinant CTB-ACE2 and FRIL chewing gum for topical

delivery: Recombinant therapeutic protein CTB-ACE2 (Cholera toxin B subunit – Angiotensin Converting Enzyme 2) produced in lettuce chloroplast is considered a potential drug candidate to prevent infection or reinfection of the SARS-CoV-2 virus through the oral cavity when used as a chewing gum formulation. Moreover, CTB-ACE2 administration is also helping patients to prevent secondary complications as an outcome of renin-angiotensin aldosterone dysregulation. In a study, Daniell et al. [4] found that the lettuce produced CTB-ACE2 as chewing gum formulation debulked SARS-CoV-2 in swab/saliva samples of COVID-19 patients. The debulking potential of CTB-ACE2 was confirmed experimentally by microbubble SARS-CoV-2 antigen assay (Fig. 4) and quantitative polymerase chain reaction (qPCR). When CTB-ACE2 was incubated with vesicular stomatitis virus spike (VSV-S) pseudo typed virus particle, the authors found spike-mediated entry into the Vero E6 cells was inhibited. This means that CTBACE2 formulated as chewing gum can prevent reinfection in patients through the oral route and in general transmission to others by acting as a trapping agent and inhibiting viral entry into the host cells. Interestingly, during chewing gum formulation, the CTB-ACE2 protein remained functionally active. Moreover, CTB-ACE2 chewing gum can be stored at ambient temperature for several years. As an advancement of the study, authors demonstrated the efficacy of CTBACE2 chewing gum in another SARS-CoV-2 strain Omicron [5] that is considered even more contagious. The chewing gum formulated CTB-ACE2 debulked SARS-CoV-2 strain Omicron in the nasopharyngeal (NP)/oropharyngeal (OP) swab samples. The debulking potential was confirmed by microbubble SARS-CoV-2 antigen assay (Fig. 4) and Real-time Accurate Portable Impedimetric Detection (RAPID) assay. The FDA has approved the evaluation of CTB-ACE2 chewing gum to decrease SARS-CoV-2 infection and transmission in Phase I/II clinical trials (IND 154807, [NCT05433181](#)). In addition, the authors showed the anti-viral efficacy of the Flt3 Receptor Interacting Lectin (FRIL) from lablab bean through neutralization of different strains of SARS-CoV-2 (omicron, delta strains). FRIL chewing gum also neutralized potent Influenza virus strains H1N1 and H3N2 in plaque reduction assay. Recently, another plant-derived lectin, i.e. engineered griffithsin (Q-Griffithsin, single substituted amino acid increased resistance to oxidation and enhanced stability), was evaluated for topical application as a nasal spray to prevent SARS-CoV-2 infection. The safety, acceptability, pharmacokinetics, and tolerability of Q-Griffithsin have been successfully evaluated in Phase I clinical trial ([NCT05180500](#)).

Another promising topical delivery formulation against SARS-CoV-2 is the nasal spray. One of the limitations of currently approved mAbs delivered through the parenteral route is suboptimal delivery due to the poor transportation of these large biologics across the respiratory epithelium to the airways. Therefore, monoclonal antibodies have been formulated as a nasal spray for local neutralization action when delivered topically. Monoclonal antibody SA58, identified from a large collection of broad sarbecovirus neutralizing antibodies isolated from SARS-CoV-2-vaccinated SARS convalescents [177] has been explored for post-exposure prophylaxis against COVID-19 [178,179]. In a randomized, single-blind, placebo-controlled clinical study ([NCT05667714](#)), nasal spray of SA58 was efficacious and safe in preventing symptomatic COVID-19 infection in adults who had exposure to SARS-CoV-2 within 72 hours [178]. The safety and effectiveness of

the SA58 nasal spray were evaluated in healthcare workers at high risk of SARS-CoV-2 infection (NCT05664919) [179]. Nasal spray formulated with 35B5 monoclonal antibody provided protection against different variants of concern, including Alpha, Beta, Delta, or Omicron variants in a clinical trial not registered in the federal website ([ClinicalTrials.gov](https://clinicaltrials.gov)), but registered locally (2022-005-02-KY) [180]. Nasal spray formulated with a cocktail of two monoclonal antibodies 55A8 and 58G6 neutralized SARS-CoV-2 Omicron variant BA.4/5 in a clinical trial not registered in the federal website ([ClinicalTrials.gov](https://clinicaltrials.gov)) (ChiCTR2200066525) [181].

4 Nanoparticle-based topical delivery in the oral cavity

4.1 Overview of Nanoparticles

4.1.1 Importance of nanoparticles in drug delivery—The basic principles and features described for these non-protein drugs are also applicable to proteins. Nanoparticles have garnered considerable attention in the field of drug delivery with a particular emphasis on their potential for topical administration in the oral cavity. For example, nanoparticles loaded with antifungal drugs, such as fluconazole [182] and miconazole [183,184], have been used for the topical treatment of oral candidiasis, a fungal infection of the oral cavity most associated with immunosuppression. The unique physicochemical properties of nanoparticles, characterized by their minute size and large surface area-to-volume ratio, make them highly desirable for trafficking to specific tissues and facilitating efficient drug delivery. In this section, we provide an overview of the use of nanoparticles for topical drug delivery in the oral cavity, emphasizing their pivotal role in enhancing drug stability, improving drug bioavailability, enabling targeted delivery of drugs to specific tissues, and mitigating systemic toxicity.

4.1.2 Enhanced drug stability—The significance of nanoparticles in drug delivery stems from their ability to ameliorate drug stability and augment drug bioavailability within the oral cavity, a critical factor that ensures the potency of pharmaceutical formulations. Drugs encapsulated within nanoparticles can be safeguarded against degradation from factors that complicate the oral cavity environment, such as fluctuations in pH, enzymatic degradation, and mechanical stresses. Consequently, nanoparticles can function as protective carriers that shield drugs from these deleterious factors, thereby enhancing drug stability, prolonging shelf-life, and ensuring optimal drug performance [185]. For example, although spearmint oil, which contains terpene derivatives, has antitumor activities, its *in vivo* efficacy is very low due to poor water solubility. The use of polyoxyethylene castor oil derivatives to form oil-containing nanoemulsion droplets in water resulted in significantly improved drug stability and increased cytotoxicity against oral cancer cells [186]. Chitosan nanoparticles surface-modified with alginate and poly(ethylene glycol) (PEG) nanoparticles showed enhanced adhesion to the mucosal tissue and sustained release of ovalbumin in intestinal fluid, demonstrating their potential for vaccine delivery through the oral mucosa [187]. Nanoparticles loaded with antibiotics, such as metronidazole [188] and doxycycline [189,190], have been explored for topical treatment of periodontal disease, a chronic inflammatory condition affecting the gums and supporting tissues of the teeth. These

nanoparticles can target the periodontal pockets and release antibiotics over an extended period, improving their local bioavailability and reducing the need for frequent dosing.

4.1.3 Targeted delivery of drugs to specific tissues—Nanoparticles allow for precise and targeted delivery of drugs to specific tissues in the oral cavity. Size tuning and surface modification of nanoparticles enable specific interactions with the target tissues, minimize off-target effects, and maximize therapeutic efficacy [185]. This is particularly important for the treatment of oral cancers, and nanoparticles loaded with anticancer drugs, such as paclitaxel [191], have been investigated as potential therapeutic agents. These nanoparticles can selectively accumulate in the tumor tissues and deliver the drugs directly to the cancer cells while minimizing systemic toxicity. Nanoparticles can be functionalized with ligands, such as anti-clusters of differentiation 44 (CD44), anti-epidermal growth factor receptor (EGFR), anti- $\alpha v \beta 6$, and folate, to actively target tumor cells [192]. By delivering drugs directly to the site of action, nanoparticles can significantly reduce systemic exposure to drugs, thereby lowering the risk of systemically induced adverse effects. The ability of nanoparticles to provide localized drug delivery with minimal systemic toxicity is particularly relevant for drugs with narrow therapeutic windows or high toxicity profiles [193].

4.1.4 Alternatives to nanoparticles for drug delivery to the oral cavity—

Although we have focused on the analysis of nanoparticle-based topical drug delivery in the oral cavity, there are alternative approaches that are worth mentioning, particularly microscale biomaterials such as patches, films, or microdevices. Even though these systems are not likely to penetrate the oral mucosa, they present other strengths that can still make delivery possible, such as strong adhesiveness and high loading capacity.

The use of microscale biomaterials for drug delivery to the oral cavity is another burgeoning area of research in pharmaceutical technologies. These novel delivery systems offer advantages such as controlled release, enhanced bioavailability, and targeted administration. Oral patches and films, composed of biocompatible polymers, adhere to the oral mucosa, enabling drug delivery into the bloodstream following transport across the mucosal layers, circumventing hepatic first-pass metabolism. While many engineering advances have been made to improve tolerance of these films and patches, biocompatibility issues continue to arise that lead to inflammatory immune responses and can reduce passage of active ingredients across the mucosa [194].

Various pharmacological drugs have been incorporated into oral transmucosal formations, including cardiovascular agents, sedatives, and analgesics [195]. A typical oral fast-dissolving film consists of a thin adhesive strip that is placed on the patient's tongue or any oral mucosal tissue. The strip rapidly hydrates and attaches to the site of application, which is wet by saliva [196]. The biodegradable oral films facilitate the drug delivery to the systemic circulation [195,196], which is highly beneficial for those suffering from dysphagia, repeated vomiting, hypertension, heart attack, asthma, nausea, paralysis, and mental disorders [197].

Microdevices, or microfabricated devices, can release drugs responsively, exploiting factors like pH shifts. A widely studied type of microdevices is microneedles (MNs) made of three-dimensional microstructures with microscale length (<1500 μm), designed to bypass the barrier of transdermal drug delivery. MNs can generate transient microchannels by piercing the stratum corneum, allowing for the effective delivery of drugs that are 10 to 100 times less permeable than the mucosa [198]. Another example is microcontainers (MCs), which are small devices that can be loaded with drugs and then adhered to the buccal mucosa. MCs with a chitosan coating have been shown to have good adhesion to the mucosa [199]. They can be loaded with antimicrobial peptides, which can be released over time to provide a sustained effect on the oral microbiome. These microdevice-based drug delivery systems have the potential to offer several advantages over traditional methods, such as localized and systemic drug administration, reduced side effects, and heightened therapeutic efficacy. While each of these devices may find use in specific applications, biocompatibility hurdles and patient compliance pose risks to the long-term effectiveness of these devices in many oral pharmaceutical applications [200]. Challenges encompass formulation stability, adhesion optimization, and regulatory considerations. Continued progress in materials and biotechnology will hopefully propel the advancement of these drug delivery strategies and lead to greater adoption.

4.2 Different materials and formulations of nanoparticles

Nanoparticles for topical drug delivery in the oral cavity can be prepared using various materials and formulations, each with their unique properties and advantages. In this section, we provide an overview of different types of nanoparticles commonly used for topical drug delivery in the oral cavity, including polymeric nanoparticles, lipid-based nanoparticles, dendrimers, and inorganic nanoparticles.

4.2.1 Polymeric nanoparticles—Polymeric nanoparticles are among the most extensively studied nanoparticles for topical drug delivery in the oral cavity. They include biocompatible polymers such as poly (lactic-co-glycolic acid (PLGA), polyethylene glycol (PEG), and chitosan, among others. Polymeric nanoparticles offer numerous advantages, including controlled drug release, excellent biocompatibility, and the ability to encapsulate a wide range of drugs with varying physicochemical properties. Additionally, their size and surface properties can be easily tailored to achieve desired drug delivery characteristics. For example, various polymeric nanoparticles, such as alginate, polylactic acid (PLA), PLGA, and chitosan, have been used for oral topical delivery of nystatin, an antifungal drug [201–207]. When compared with the direct administration of nystatin, polymeric nanoparticles have demonstrated high encapsulation efficiency (>70%), prolonged release, and high adhesion capacity to the oral mucosa [203]. Hydrogel nanoparticles were also developed to deliver nystatin. When tested in a rat model of oral infection, nystatin-encapsulated nanocapsular hydrogels resulted in a significantly reduced fungal count and eradication of infection [208]. Methotrexate (MTX), which is used to treat oral inflammatory conditions such as oral lichen planus, is another drug that has been investigated for oral topical delivery via nanoparticles. MTX-loaded PLGA nanoparticles showed a sustained release and improved therapeutic outcome in both rats and mice when compared with free MTX [209,210]. Chitosan microspheres were also associated with promising outcomes in terms of

their ability to provide sustained delivery of MTX [204]. Chitosan, with its positive charge, can temporarily disrupt the mucosa in the oral cavity and enhance the transport of drugs across the epithelium barrier [205].

In most cases, the bioactivity of a drug delivery system is due to the drug itself. The polymers used in these systems are typically chosen for their benign effects on non-target tissues. However, some polymers and other nanoparticle systems can be used specifically for their inherent bioactivity in situ, which can promote synergistic effects with the drug being delivered. This synergistic activity is most notable in nanoparticle-mediated cancer immunotherapy treatments. For example, anti-PD1 and anti-PD-L1 cancer immunotherapies exhibit improved outcomes when delivered in polymeric nanoparticle systems that trigger an immune response. The more rapid and elevated immune response leads to a greater influx of lymphocytes and macrophages at the target tumor site, as well as a better overall treatment response [206].

4.2.2 Lipid-based nanoparticles—Lipid-based nanoparticles (LNPs), including liposomes and solid lipid nanoparticles (SLNs), are another prominent class of nanoparticles for topical drug delivery in the oral cavity. Lipids have hydrophobic tails and hydrophilic heads, which can form monolayer micelles, bilayer vesicles, or solid matrices to encapsulate drugs. These nanoparticles offer several advantages such as high biocompatibility and biodegradability, high drug loading capacity, protection of drugs from degradation, as well as enhanced drug absorption due to their lipid nature, and controlled drug release. LNPs are associated with improved retention at the targeted sites, permeability, and enhanced antifungal activities [211]. Liposomes have also been used to deliver various therapeutics for the treatment of oral cancers, including chemotherapeutics, radiotherapeutics agents, and photosensitizers for photodynamic therapy [192]. In addition to drug delivery, LNPs can also deliver nucleic acids [212,213] making them one of the most promising nano-formulations for employing gene therapy to treat oral cancers [214]. Given the recent success of LNPs in delivering messenger ribonucleic acid (mRNA) for COVID-19 vaccines, it is likely that researchers will give them a high priority in contemplating gene therapy for oral cancers in the future. Although there are no clear gene targets for oral cancers, a recent study suggested that *PE38KDEL* may be used as a suicidal gene for treating oral squamous cell carcinoma [215].

4.2.3 Dendrimers—Dendrimers are highly branched macromolecules with a defined structure that have also emerged as a promising class of carriers for topical drug delivery in the oral cavity. Dendrimer nanoparticles are formed as unimolecular micelles with a hydrophobic interior and hydrophilic exterior. An important characterization of dendrimers is their generation, which is defined as the number of radial branching units. Dendrimers offer unique advantages because of their precisely controlled composition, size, shape, potential biodegradability, and surface functionalities, which can be customized to achieve desired drug delivery properties. Dendrimers can encapsulate hydrophobic cargoes within their branches or conjugate drugs to their surface, thus enabling controlled drug release, targeted delivery, and improved drug stability. The use of dendrimers for topical application has greatly expanded in dentistry, as studies demonstrated their ability to

stimulate remineralization in damaged enamel and dentin [216]. Based on this discovery, drugloaded dendrimers have been developed to enhance therapeutic effects. For example, dendrimers loaded with alendronate, a medication used to treat osteoporosis, can help restore tooth enamel by inducing remineralization [217]. Similarly, a triclosan-loaded dendrimer was shown to provide extended release of the antimicrobial while promoting the repair of damaged human dentin [218]. Thiolated dendrimers have also been developed to enhance mucoadhesion by forming covalent bonds with the cysteine domains of the mucin [219,220]. Cationic polyamidoamine (PAMAM) dendrimers have been shown to decrease inflammation by scavenging negatively charged cell-free deoxyribonucleic acid (DNA) [221] and consequently reduce inflammatory bone loss in cases of periodontitis [222]. This last example deviates from conventional drug delivery in that the nanoparticle exerts its therapeutic effect by removing a negative factor but not delivering a therapeutic agent. The future might be to incorporate a drug delivery function into these nanoparticles to achieve a synergistic push-pull effect for maximal therapeutic benefits.

4.2.4 Inorganic nanoparticles—Inorganic nanoparticles, such as gold, silver, and silica nanoparticles, have also gained attention for use in topical drug delivery in the oral cavity. Inorganic nanoparticles offer distinct properties, such as high stability, unique shapes, and flexibility for surface functionalization. Both gold (Au) and silver (Ag) nanoparticles have antimicrobial effects, making them potential anticaries agents. They can also serve as a synergistic enhancer to other antimicrobial drugs and biomolecules [223,224]. In addition, both Au and Ag nanoparticles have been reported to promote osteogenesis [225], which adds great therapeutic value when integrated into titanium dental implants [226] – another good example of synergistic therapeutics. Silica nanoparticles, particularly mesoporous silica nanoparticles (MSNs), have high surface areas and thus are advantageous for drug loading and enhancing therapeutic benefits. For example, although curcumin has anti-inflammatory and antitumor activities, it exhibits poor aqueous solubility and low permeability [227,228]. MSNs can serve as an effective vehicle for loading curcumin and providing a controlled and responsive release [229]. MSNs are also a versatile platform that can be functionalized for multiplexing, such as loading curcumin and silver nanoparticles simultaneously [230]. Another advantage of MSNs is their biodegradability when they contain disulfide and diselenide bonds in the silicate network [231,232].

In summary, nanoparticle-based topical delivery in the oral cavity holds great promise for the treatment of various diseases. The unique advantages of nanoparticles, namely enhanced drug stability, improved drug bioavailability, targeted drug delivery, and reduced systemic toxicity, make them attractive for this application. When comparing different types of nanoparticles, several factors should be considered, including their physicochemical properties, drug loading capacity, release kinetics, biocompatibility, and ease of fabrication. The choice of nanoparticle formulation will depend on the specific drug and therapeutic application as well as the desired drug delivery characteristics. Proper selection of nanoparticles can greatly influence the success of topical drug delivery in the oral cavity. Additional research and development in this area are necessary to optimize nanoparticle formulations for specific oral healthcare applications [186,233–235] and may lead to novel and effective therapeutic approaches for oral diseases.

4.3 Challenges and Limitations of Nanoparticle-based Delivery

Despite the promising potential of nanoparticle-based topical delivery in the oral cavity, there remain considerable challenges and limitations for translation into clinical practice. An additional burden for biologics is their delicate nature, which requires special effort to retain their bioactivity in formulation, storage, and shipping.

4.3.1 Regulatory challenges—While there are many nanoparticle-based drug delivery systems on the market, regulatory guidelines specific to nanoparticles and their use in the oral cavity may not be well-established, which can add complexity to the development process which is already time-consuming and resource-intensive [236].

4.3.2 Manufacturing challenges—The manufacturing of nanoparticles with reproducible quality and scalable production can pose challenges. The formulation and synthesis of nanoparticles need to be carefully controlled to achieve desired properties, such as size, drug loading capacity, and drug release kinetics. The scalability of nanoparticle production can also be challenging, as the methods used for laboratory-scale production may not be feasible for large-scale manufacturing. The cost-effectiveness of nanoparticle production and the stability of nanoparticles during storage also need to be considered, along with process optimization and quality control measures to ensure consistent and reliable nanoparticle production.

4.3.3 Delivery challenges—The delivery of nanoparticles to the target tissues in the oral cavity can be challenging due to the complex anatomy and physiology of the oral cavity. The oral mucosa, which serves as the primary barrier for drug penetration, can vary in thickness and permeability in different regions of the oral cavity. The presence of saliva, which can dilute and clear nanoparticles, can also affect their delivery. Therefore, optimizing the formulation and delivery methods to achieve efficient and controlled delivery of nanoparticles to the target tissues in the oral cavity is a critical challenge. Strategies such as the use of penetration enhancers, and mucoadhesive nanoparticles or encapsulation of nanoparticles in carrier systems, such as gels or films, may be employed to improve the delivery efficiency in the oral cavity. Additionally, the selection of appropriate administration techniques, such as swabbing, rinsing, or spraying, can also impact the efficacy of nanoparticle-based topical delivery and requires careful consideration.

A more focused examination of the anatomy of the physiological barriers reveals the challenges of achieving effective drug delivery across the barrier. When a drug carrier enters the oral cavity, the first major barrier is the oral mucosa layer, which includes both the sublingual and buccal mucosa. Unlike the gastrointestinal (GI) mucosa, the oral mucosa does not have the micro-villa structure, which means the adsorptive surface area is small. Also, the administration time or retention time in the oral cavity is highly limited, due to patients' behaviors, such as eating or drinking. A typical time window for drug administration at the oral mucosa is up to 5–6 hr [205]. These limitations require the drug to be highly potent. Furthermore, the tissue in the oral mucosa is much thicker than that in the GI tract. The top layer is the oral epithelium, which is mainly made of keratin. This keratinized layer provides mechanical protection, similar to the stratum corneum of the skin.

Deeper down, there is the epithelial tight junctions (ETJ), a barrier formed by epithelial cells connected by tight junctions. This barrier can block the penetration of nanoparticles [237,238]. However, studies have shown that nanoparticles with the appropriate size and surface properties can exploit paracellular routes by modulating the tight junction permeability [237,239]. The deeper layer is known as the lamina propria, which is a fibrous connective tissue layer that consists of a network of collagen and elastin fibers. This layer also contains an array of extracellular matrix proteins that rapidly opsonize foreign nanoparticles [240]. For effective passage across these two layers, nanoparticle systems must be designed with the appropriate charge, architecture, and composition to achieve both non-interactive and diffusive capabilities.

Additionally, saliva is a very dynamic environment for nanoparticle-drug systems. It has a multifaceted composition and several functions. Saliva houses several enzymes, including amylases and proteases, which are capable of enzymatic degradation of certain nanoparticles. Recent research underscores the significance of enzyme-nanoparticle interactions, which can impact nanoparticle stability and functionality. Rheologically, saliva's lubricating properties can both enhance and impede nanoparticle mobility within the oral cavity, complicating passage across oral mucosa layers. Salivary mucins, high-molecular weight glycoproteins, contribute to the formation of the mucosal barrier and can influence both nanoparticle adhesion to the mucosal surface and subsequent translocation [241].

In summary, several challenges and limitations, namely regulatory, manufacturing, and delivery challenges, need to be addressed for successful translation. Overcoming these challenges and limitations will contribute to the development of safe, effective, and reliable nanoparticle-based drug delivery systems for oral diseases, ultimately benefiting patients and advancing the field of oral healthcare. Further research and innovation in this area are warranted to unlock the full potential of nanoparticle-based topical delivery in the oral cavity and translate these technological advances into clinical practice.

5. Conclusions:

Vaccines use surface proteins of pathogenic bacteria or viruses, along with adjuvants to stimulate human immune response. Such vaccine therapeutics have been used for almost a century and they have saved millions of lives. However, the recent COVID-19 pandemic brought to light limitations of vaccine production and delivery methods. Although breakthrough innovations through mRNA vaccines saved millions of lives, they are beyond the reach of low or middle-income countries. The reason for this is their production in prohibitively expensive cell culture systems and the cost of regulatory requirements, including the number of exchanges of sterile air in fermenters and other measures to eliminate contamination. Furthermore, the requirement of cold-chain for storage and transportation is a major challenge – ~19 million mRNA vaccine doses were discarded in Africa due to lack of cold chain. Most importantly, all SARS-CoV-2 vaccines induced systemic immunity (IgG – immunoglobulin G) that conferred protection after infection but not mucosal immunity (IgA – secretory immunoglobulin A) that would have prevented transmission. This review addresses this important gap, by debulking viruses in the oral or

nasal cavity using chewing gum or nasal sprays containing viral trap proteins, or monoclonal antibodies, to prevent self-infection and transmission. This approach is not limited to oral viruses but could be used to kill pathogenic bacteria or fungi in the oral cavity.

In addition, this review examines the reasons behind the high cost of biologics production and delivery methods. Among FDA-approved biologics, >90% are invasive injectable drugs. These biologics are produced in cell cultures requiring prohibitively expensive fermentation systems and associated regulatory cost. In addition, removal of host proteins to purify biologics to >99% purity is the most expensive aspect of biologics production. After such heavy investment, biologics are highly unstable and have short shelf-life despite requirement for cold chain for their storage and transportation. Oral or topical drugs are highly preferred by patients because of their affordability and convenience, but only two oral drugs has been approved by FDA since 2015. Oral delivery of biologics reduces their cost >90% by elimination of complex and expensive production, purification and delivery systems [42]. In addition to immunotherapy to reduce antidrug antibodies from injected proteins, the oral delivery of antigens bioencapsulated in plant cells could reduce antibodies generated by gene therapy [249]. This is even more important considering the high cost of gene therapy per patient – \$2.9–3.5 million [30,42]. This also addresses the major healthcare crisis – greed in healthcare [250]. Oral delivery of functional therapeutic proteins bioencapsulated in plant cells has been reviewed recently [251,252]. Recent advances include oral delivery of ACE2 bioencapsulated in plant cells treated cardiopulmonary diseases [82], oral insulin regulated blood sugar levels like natural insulin [57] and oral growth hormones accelerated diabetic bone fracture healing [80].

Topical delivery has the unique advantage of targeted delivery of high concentrations of protein drugs, without getting diluted in circulating blood. Proteins/enzymes in chewing gum are stable for many years at ambient temperature and could be transported to remote villages around the globe. However, any new platform for drug delivery would face regulatory challenges. Surprisingly, the chewing gum containing viral trap protein was rapidly approved by the FDA (IND 154807; [NCT05433181](#)) because of extensive toxicology studies conducted and minimal systemic exposure of this drug. Topical delivery of peanut proteins in patches delivered to babies is successful in preventing allergy in Phase III clinical trials ([NCT03211247](#)). Similarly, several nasal spray formulations were evaluated in the clinic to minimize SARS-CoV-2 infection ([NCT05180500](#); [NCT05122260](#); [NCT05437029](#)). Completion of these trials will reveal future challenges in large scale production and global distribution. Elimination of cell culture, purification, and cold chain could significantly reduce the cost of protein drugs, thereby driving forward health equity. Therefore, this review highlights recent advances in topical delivery of protein or nucleic acid drugs directly, or in combination, with nanoparticles and offers future directions.

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Declaration of competing interest

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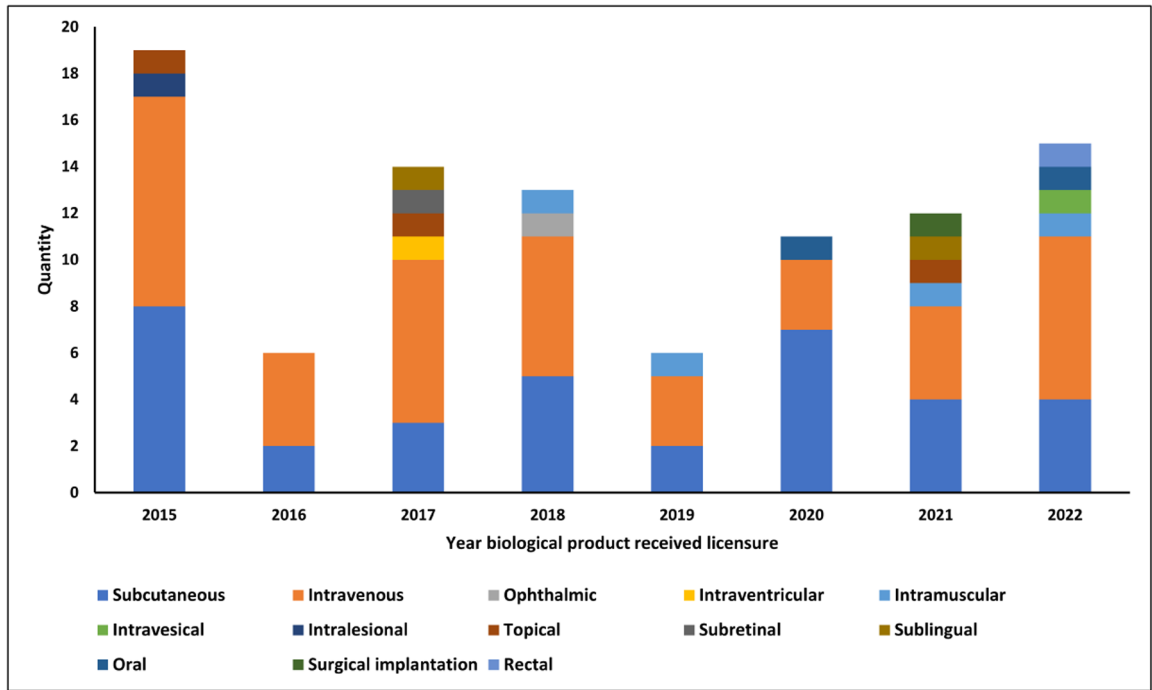


Figure 1: Drug route of administration trends from biological products approved by the FDA between 2015 and 2022 that met the inclusion criteria [16, 19].

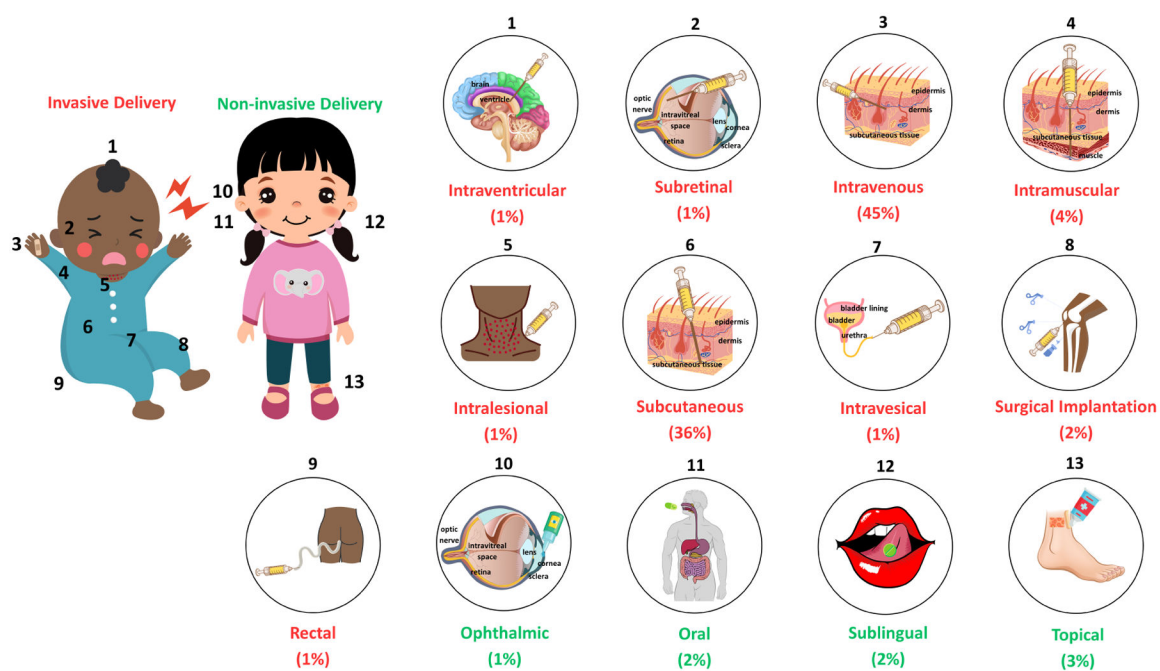


Figure 2:

Visualization of the drug delivery pathways that the 89 biologics approved by the FDA between 2015 and 2022 follow. The numbers on the children portray typical sites of administration for each respective delivery method, and all numbers are associated with a more in-depth depiction of the delivery type. The percentage below each delivery method highlights the proportion of FDA-approved biologics that can be administered through that specific pathway. The red percentage (>90% total) indicates an invasive delivery method, and the green percentage corresponds to administration techniques that do not require invasive means. Monoclonal antibodies and their fragments, diagnostics, vaccines, and biologics that were voluntarily revoked and/or did not have a proprietary name were excluded [16, 19].

Chewing Gum Milestones

The global chewing gum market is expected to reach USD 37 billion by 2023.

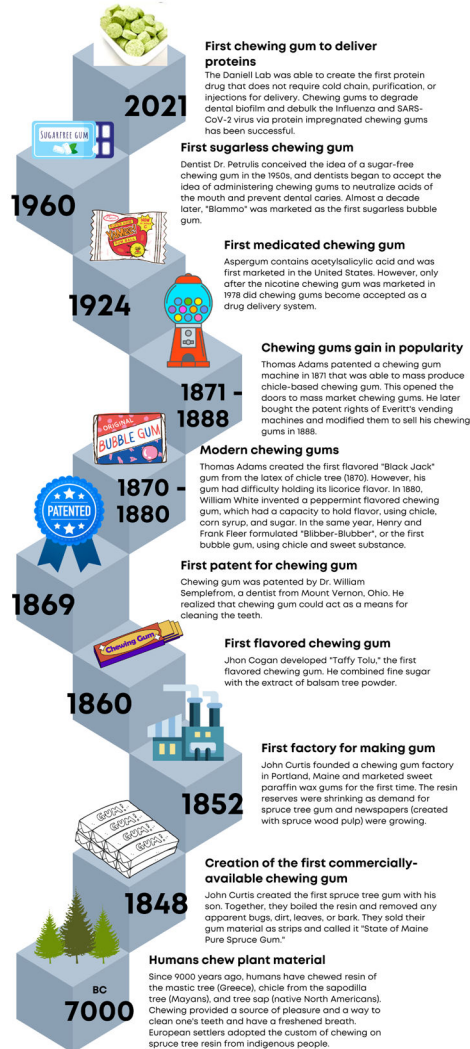


Figure 3:
 Overview of chewing gum milestones [4,5,14,88,98,111–120].

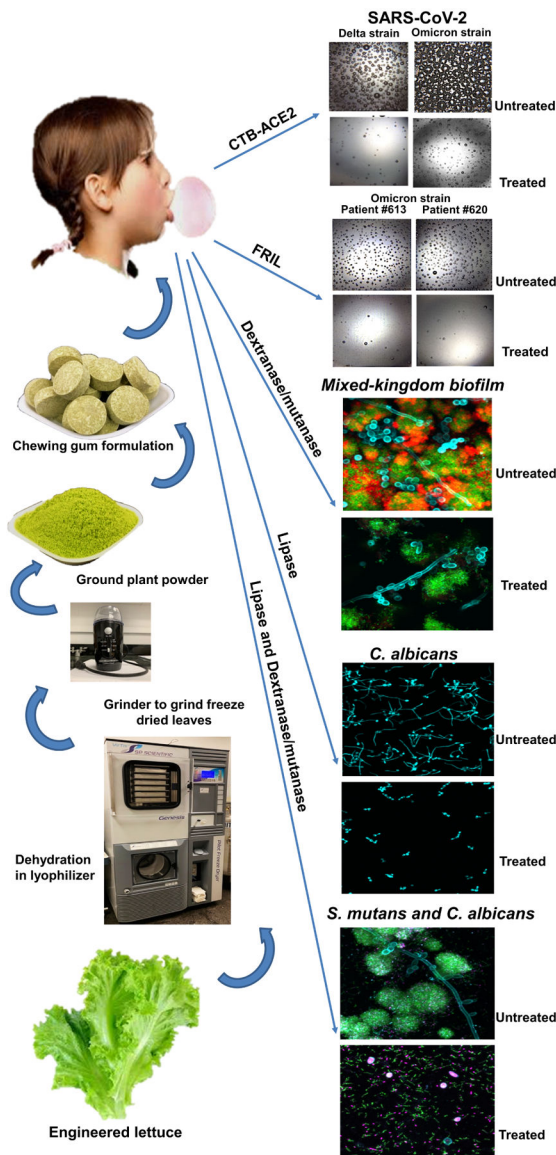


Figure 4: Demonstration of plant-made (Pm) protein therapeutics as chewing gum formulations and topical delivery to control growth of human pathogens responsible for various infectious diseases.

The engineered lettuce producing protein therapeutics is grown in a contained environment where the leaves are harvested, dehydrated in lyophilizer, and ground into plant powder. This powder is then incorporated into chewing gums for topical drug delivery (left panel). The Pm protein therapeutics impregnated in chewing gums are able to control the growth of human pathogens of different domains, such as viruses (SARS-CoV-2 Delta and Omicron strains), bacteria (*S. mutans*), and fungi (*C. albicans*) (right panel) [4,5,88].

Table 1:

Drug delivery methods of all FDA-approved biological products regulated by CEDER and CEBER since 2015 that met the review's inclusion criteria [16,21].

Proprietary Name *	Proper Name	Licensure Year	Indicated Usage
Non-invasive			
Sublingual			
Odactra	House dust mite (HDM) (<i>Dermatophagoides farinae</i> and <i>Dermatophagoides pteronyssinus</i>) allergen extract	2017	Treats allergic rhinitis induced by HDM
Ragwitek	Short ragweed pollen (SRP) allergen extract	2021	Allergic rhinitis induced by SRP
Oral			
Palforzia [243]	Peanut (<i>Arachis hypogaea</i>) allergen powder-dnfp	2020	Mitigates allergic reaction to peanuts
Sucraid [244]	Sacrosidase	2022	Treats genetically determined sucrase deficiency
Topical			
Raplixa [245]	Fibrin sealant (Human)	2015	Controls bleeding during surgery
Vistaseal [246]	Fibrin sealant (Human)	2017	Controls bleeding during surgery
StrataGraft [247]	Allogeneic cultured keratinocytes and dermal fibroblasts in murine collagensat	2021	Treats thermal burns
Ophthalmic			
Oxervate ** [248]	cenegermin-bkbj	2018	Treats neurotrophic keratitis
Invasive			
Intralesional			
Imlygic	talimogene laherparepvec	2015	Treats lesions
Intraventricular			
Brineura	cerliponase alfa	2017	Slows the loss of ambulation
Subretinal			
Luxturna	voretigene neparvovec-rzyl	2017	Treats biallelic <i>RPE65</i> mutation-associated retinal dystrophy
Implantation			
Rethymic	Allogeneic processed thymus tissue-agdc	2021	Immune reconstitution
Rectal			
Rebyota	Fecal microbiota, live - jslm	2022	Prevents the recurrence of <i>Clostridioides difficile</i> infection
Intravesical			
Adstiladrin	nadofaragene firadenovec-vncg	2022	Treats high-risk Bacillus Calmette- Guerin-unresponsive non-muscle invasive bladder cancer
Intramuscular			
Revcovi	elapegademase-lvlr	2018	Treats adenosine deaminase severe combined immune deficiency
Jeuveau	prabotulinumtoxinA-xvfs	2019	Temporarily improves appearance of glabellar lines

Proprietary Name *	Proper Name	Licensure Year	Indicated Usage
Rylaze	asparaginase erwinia chrysanthemi (recombinant)- rywn	2021	Treats acute lymphoblastic leukemia and lymphoblastic lymphoma
Daxxify	daxibotulinumtoxinA-lanm	2022	Temporarily improves appearance of glabellar lines
Subcutaneous & Intravenous			
Humalog	Insulin lispro injection	2015	Improves glycemic control
Zarxio	filgrastim-sndz	2015	Decreases the incidence of infection and expedites recovery
Fiasp	Insulin aspart injection	2017	Improves glycemic control
Admelog	Insulin lispro injection	2017	Improves glycemic control
Retacrit	epoetin alfa-epbx	2018	Treats anemia
Nivestym	filgrastim-aafi	2018	Decreases the incidence of infection and expedites recovery
Lyumjev	Insulin lispro-aabc	2020	Improves glycemic control
Subcutaneous			
Strensiq	asfotase alfa	2015	Treats hypophosphatasia
Tresiba	Insulin degludec injection	2015	Improves glycemic control
Ryzodeg 70/30	Insulin degludec and insulin aspart injection	2015	Improves glycemic control
Basaglar	Insulin glargine injection	2015	Improves glycemic control
Toujeo	Insulin glargine injection	2015	Improves glycemic control
Natpara	Parathyroid hormone	2015	Controls hypocalcemia
Xultophy 100/3.6	Insulin degludec and liraglutide injection	2016	Improves glycemic control
Soliqua 100/33	Insulin glargine and lixisenatide injection	2016	Improves glycemic control
Haegarda	C1 Esterase Inhibitor Subcutaneous [Human]	2017	Prevents Hereditary Angioedema attacks
Udenyca	pegfilgrastim-cbqv	2018	Decreases the incidence of infection
Fulphila	pegfilgrastim-jmdb	2018	Decreases the incidence of infection
Palynziq	pegvaliase-pqpz	2018	Decreases blood phenylalanine concentrations
Reblozyl	luspatercept-aamt	2019	Treats anemia
Ziextenzo	pegfilgrastim-bmez	2019	Decreases the incidence of infection
Nyvepria	pegfilgrastim-apgf	2020	Decreases incidence of infection
Semglee	Insulin glargine injection	2020	Improves glycemic control
Qwo	collagenase clostridiumhisto lyticum-aaes	2020	Treats cellulite in the buttocks
Sogroya	somapacitan-beco	2020	Treats growth hormone deficiency
Trulicity	dulaglutide	2020	Improves glycemic control and reduces risk of adverse cardiovascular events
Sogroya	somapacitan-beco	2020	Treats growth hormone deficiency
Skytrofa	lonapegsomatropin-tcgd	2021	Treats growth hormone failure
Besremi	ropeginterferon alfa-2b-njft	2021	Treats polycythemia vera
Rezvoglar	Insulin glargine-aglr	2021	Improves glycemic control
Semglee	Insulin glargine-yfgn	2021	Improve glycemic control
Fynetra	pegfilgrastim-pbbk	2022	Reduces incidence of infection

Proprietary Name *	Proper Name	Licensure Year	Indicated Usage
Stimufend	pegfilgrastim-fpgk	2022	Reduces incidence of infection
Rolvedon	eflapegrastim-xnst	2022	Reduces incidence of infection
Erelzi	etanercept-szsz	2022	Treats arthritis, plaque psoriasis, and ankylosing spondylitis
Intravenous			
Kanuma	sebelipase alfa	2015	Treats Lysosomal Acid Lipase deficiency
Vonvendi	von Willebrand factor (Recombinant)	2015	Treats and controls bleeding episodes
Adynovate	Antihemophilic Factor (Recombinant), PEGylated	2015	Treats and controls bleeding episodes
Coagadex	Coagulation Factor X (Human)	2015	Treats and controls bleeding episodes
Nuwiq	Antihemophilic Factor (Recombinant)	2015	Treats and controls bleeding episodes
Anavip	Crotalidae Immune F(ab') ₂ (Equine)	2015	Manages North American rattlesnake envenomation
Ixinity	Coagulation factor IX (Recombinant)	2015	Treats and controls bleeding episodes
Clevecord	HPC, Cord Blood	2016	Procedures involving hematopoietic progenitor cell transplantation
Afstyla	Antihemophilic Factor (Recombinant)	2016	Treats and controls bleeding episodes
Kovaltry	Antihemophilic Factor (Recombinant)	2016	Treats and controls bleeding episodes
Idelvion	Coagulation Factor IX (Recombinant), Albumin Fusion Protein	2016	Treats and controls bleeding episodes
Mepsevii	vestronidase alfa-vjbjk	2017	Treats Mucopolysaccharidosis VII
Yescarta	axicabtagene ciloleucel	2017	Treats refractory or relapsed large B-cell lymphoma
Kymriah	tisagenlecleucel	2017	Treats refractory or relapsed large B-cell lymphoma
Fibryna	Fibrinogen (Human)	2017	Treats and controls bleeding episodes
Rebinyn	Coagulation Factor IX (Recombinant), GlycoPEGylated	2017	Treats and controls bleeding episodes
Asparlas	calaspargase pegol - mknl	2018	Treats acute lymphoblastic leukemia
Elzonris	tagraxofusp-erzs	2018	Treats blastic plasmacytoid dendritic cell neoplasm
Albuminex 25%	Albumin (Human)	2018	Maintains and restores circulating blood volume
Andexxa	Coagulation factor Xa (recombinant), inactivatedzhzo	2018	Reverses apixaban and rivaroxaban
Myxredlin	Insulin human in sodium chloride injection	2019	Improves glycemic control
Zolgensma	onasemnogene abeparvovecxioi	2019	Treats spinal muscular atrophy
Esperoct	Antihemophilic factor (Recombinant), glycopegylated-exei	2019	Treats and controls bleeding episodes
Sevenfact	Coagulation Factor VIIa (Recombinant)-jncw	2020	Treats and controls bleeding episodes
Tecartus	brexucabtagene autoleucel	2020	Treats refractory or relapsed acute lymphoblastic leukemia and mantle cell lymphoma
Nexviazyme	avalglucosidase alfa-ngpt	2021	Treats Pompe disease
Abecma	idecabtagene vicleucel	2021	Treats refractory or relapsed multiple myeloma
Ryplazim	plasminogen, human-tvmh	2021	Treats plasminogen deficiency type 1
Breyanzi	lisocabtagene maraleucel	2021	Treats large B-cell lymphoma
Kimmtrak	tebentafusp-tebn	2022	Treats HLA-A *02:01 positive patients

Proprietary Name *	Proper Name	Licensure Year	Indicated Usage
Nuwiq	Antihemophilic Factor (Recombinant), rAHF	2022	Treats and controls bleeding episodes
Carvykti	ciltacabtagene autoleucel	2022	Treats refractory or relapsed multiple myeloma
Zynteglo	betibeglogene autotemcel	2022	Treats patients with β -thalassemia
Xenpozyme	olipudase alfa-rpcp	2022	Treats acid sphingomyelinase deficiency
Skysona	elivaldogene autotemcel	2022	Slows neurological dysfunction progression
Hemgenix	etranacogene dezaparvovecdrlb	2022	Treats Hemophilia B

* Vaccines, monoclonal antibodies and their fragments, diagnostics, biologics voluntarily revoked, and products that did not have a proprietary name were excluded.

** Oxervate is an ophthalmic biologic medication for topical ophthalmic usage.

Table 2:

Plant-made or synthetic peptides used as biologics for topical delivery.

Candidate molecule/ active ingredient	Class of molecule	Mode of action	Plant host	Type of expression (heterologous)	Disease/ target	Product development stage (Phase I/II/III/date/status)	Treatment regimen	Reference(s)
Peanut allergy								
AR101	Peanut-protein	Tolerance induction	Peanut	NA	Peanutallergy	Phase III (NCT03211247)	Viaskin peanut-protein-containing patches	[10]
COVID-19								
CTB-ACE2	Angiotensin converting enzyme-2	Virus trap	<i>Lactuca sativa</i>	Stable	SARS-CoV-2	Phase I/II (NCT05433181)	Chewing gum	[4,5]
FRIL	Lectin	Virus trap	<i>Lablab purpureus</i>	NA (Natural plant source)	SARS-CoV-2	--	Chewing gum	[5]
Q-GRFT	EngineeredGriffithsin (lectin)	Inhibitory activity against SARS-CoV-2	<i>N. benthamiana</i>	Transient	SARS-CoV-2	Phase I (NCT05180500; NCT05122260; NCT05437029)	Nasal spray	https://clinicaltrials.gov/study/NCT05180500?term=NCT05180500&rank=1; [242]
AIDS								
PC-6500	Griffithsin (lectin)	Inhibitory activity against HIV	<i>N. benthamiana</i>	Transient	HIV	Phase I (NCT02875119)	Griffithsin formulated in carrageena n vaginal gel	[126]
Anti-biofilm/plaque/gingivitis								
KSL-W	Antimicrobial peptide	Reducing plaque and gingivitis	NA	NA	Plaque and gingivitis	Phase I/II (NCT01877421; NCT02864901)	Topical oral formulation/ Chewing gum	https://classic.clinicaltrials.gov/ct2/show/NCT01877421; [150]
GFP-PG1	Antimicrobial peptide	Killing of <i>S. mutans</i>	<i>N. tabacum</i>	Stable	<i>S. mutans</i>		Topical oral formulation/ Chewing gum	[87]
GFP-RC101	Antimicrobial peptide	Killing of <i>S. mutans</i>	<i>N. tabacum</i>	Stable	<i>S. mutans</i>		Topical oral formulation/ Chewing gum	[87]
Lipase	Esterase enzyme	Inhibit transition of <i>C. albicans</i> yeast to filamentous form	<i>Lactuca sativa</i>	Stable	<i>C. albicans</i>		Topical oral formulation/ Chewing gum	[88]
Dextranase	Glucanase enzyme	Degrade dental biofilm matrix	<i>Lactuca sativa</i>	Stable	Biofilm matrix		Topical oral formulation/ Chewing gum	[88]
Mutanase	Glucanase enzyme	Degrade dental biofilm matrix	<i>Lactuca sativa</i>	Stable	Biofilm matrix		Topical oral formulation/ Chewing gum	[88]