Chapter

# Nanofibers in Mucosal Drug and Vaccine Delivery

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## Abstract

Successful mucosal administration and delivery of drugs still pose a great challenge. However, the possibility to deliver not only small drug molecules but also macromolecular drugs and nanoparticles via mucosal surfaces represents a great opportunity. Rapid onset of drug action, avoidance of first-pass metabolism, and high immunocompetence of mucosa are some of the important features for mucosal drug and vaccine delivery. The use of mucoadhesive drug delivery systems, systems with fast dissolving properties, and nanomaterials with mucus penetration properties are examples of successful strategies to achieve effective mucosal drug and vaccine delivery. Non-keratinized mucosa of the oral cavity, the nasal and vaginal mucosa represent favorable sites of drug administration. Polymer nanofibers have attracted much attention because of remarkable characteristics such as a large surface area to volume ratio and high porosity. Nanofibers have been extensively used for different biomedical applications including wound dressing, tissue engineering, and drug delivery. Among their fabrication methods, the introduction of electrospinning technique was an important step toward achieving the goal of large scale industrial production of nanofiber-based drug delivery systems used in mucosal applications. This chapter provides an overview on all aspects of mucosal drug and vaccine delivery using nanofibers.

**Keywords:** nanofibers, electrospinning, transmucosal drug delivery, mucoadhesive formulation, mucus penetration nanoparticles, first-pass metabolism, mucosal vaccines

## 1. Introduction

Mucosal drug delivery is an alternative method of systemic drug delivery that offers numerous benefits over/parenteral and oral administration. Mucosal surfaces, particularly oral, nasal, and vaginal, have been widely explored for systemic delivery of drugs. Drugs that are absorbed via mucosal surfaces directly enter the systemic circulation and bypass the gastrointestinal tract including first-pass metabolism in the liver. Rapid onset of drug action is another advantage of oral, nasal, and vaginal mucosae drug administration. A lot of efforts have been devoted to the discovery of efficient delivery of vaccine antigens to mucosal sites that enhance uptake by local antigen-presenting cells in order to generate protective mucosal immune responses. Potent mucosal adjuvants and suitable mucosal vaccine delivery systems are crucial steps on the way to effective mucosal vaccines.

On the other hand, several challenges need to be overcome to successfully deliver drug molecules. Poor water solubility of numerous drugs, macromolecular nature of newly developed biologically active molecules, including therapeutic peptides, proteins, and nucleic acids, are some examples of challenging features that need to be overcome by developing new delivery approaches, devices, and dosage forms. Mucoadhesive drug formulations, rapidly disintegrating formulations, and formulations with mucus penetration properties enabling mucosal delivery of such therapeutic molecules have made great progress over the last decade.

Anatomical and physiological functions of different types of mucosa need to be taken into consideration when formulating new drug delivery systems. These need to respect the flexibility of mucosal surfaces, the flow of body fluids such as saliva or vaginal fluid, ciliary movement on the nasal mucosa, presence of mucosal absorption barriers, including the mucus layer and keratinization of some surfaces, etc.

Nanofibers represent an interesting opportunity to tackle some of the difficulties in mucosal drug delivery. Nanofibers represent an almost universal platform the properties of which can be tailored according to specific demands in terms of their composition as well as surface modifications. Advanced features of nanofibers include possible mucoadhesive properties, fast disintegration, controlled drug release, formulation of small drugs of different nature, and formulation of therapeutic macromolecules. These properties can help solve problems with mucosal delivery of poorly water-soluble drugs, drugs with fast liver metabolism, therapeutic proteins, and antigens for mucosal immunization. Taken together, formulations based on nanofibers intended for mucosal applications represent a new trend in drug delivery.

## 2. Histology and barrier properties of mucosal surfaces

Histology, physiology, and barrier functions of mucosal surfaces play a critical role in mucosal drug and vaccine delivery. All aspects have to be taken into consideration when designing a mucosal drug delivery system. Also, appropriate drug candidates for mucosal delivery have to be selected, not only with respect to physical-chemical properties of drug molecule, but also anatomical and physiological aspects of the intended site of administration and their deep knowledge are essential for successful delivery of drugs and vaccines.

### 2.1 Histology of mucosal surfaces

#### *2.1.1 Oral mucosa*

The mucosa of oral cavity is divided into the buccal, sublingual, gingival, palatal, and labial regions. The mucosa of each region is of specific histological and functional characteristics. Oral mucosa consists of three layers: a stratified squamous epithelium, composed of several cell layers, below which lies the basement membrane, and finally the connective tissue divided into the lamina propria and submucosa, which comprise a number of vascular capillaries [1]. Drugs absorbed via the oromucosal route of administration are absorbed through these capillaries and gain access to the systemic circulation [2, 3].

Three major types of epithelium located in different regions of the oral cavity differ in the degree of keratinization, namely masticatory, specialized, and lining mucosa. The masticatory epithelium is keratinized (100–200-μm thick) and covers the gingival region and the hard palate. The specialized epithelium is stratified,

keratinized, and covers the dorsal surface of the tongue. The lining mucosa covers buccal and sublingual regions of the oral cavity. The epithelial layer of the buccal and sublingual mucosa is non-keratinized, with variation in thickness [4]. The lining mucosa exhibits high permeability for different drugs, and thus is an interesting site for drug administration.

The oral epithelium is covered by a 70–100-μm thick film of saliva, the secretion from salivary glands. The daily production of saliva secreted into the oral cavity is between 0.5 and 2 L. Continuous production of saliva significantly impacts drug residence time after administration within the oral cavity, a phenomenon known as saliva washout [5].

Mucus is the intercellular ground matrix secreted by the sublingual and salivary glands, which is bound to the apical cell surface and acts as a protective layer for the cells below [6]. It is also a viscoelastic hydrogel consisting of the water-insoluble glycoproteins, water, and small quantities of different proteins, enzymes, electrolytes, and nucleic acids. The mucus layer carries a negative charge due to a high content of sialic acid and forms a strongly cohesive gel structure that binds to the epithelial cells.

The mucus layer varies in thickness from 40 to 300 μm. The mucus layer plays a critical role in the function of different mucoadhesive drug delivery systems which work on the principle of mucoadhesion, and thus prolong the dosage form retention time at the site of administration [7].

### *2.1.2 Nasal mucosa*

The total surface area available in the human nasal mucosa is estimated to be about 180 $cm^2$. Most of the surface is covered with highly vascularized respiratory mucosa and only a small surface area of the nasal cavity is covered by olfactory mucosa. Due to the presence of microvilli on the apical cell surface, the effective surface area for drug absorption is relatively high [8]. The nasal vestibule is lined by stratified squamous epithelium which gradually transitions in the valve region with a ciliated, pseudostratified, columnar epithelium characterized by presence of mucus-secreting goblet cells. Mucus lies over the epithelium as a protective layer and the mucociliary apparatus filters the air. Mucus is secreted at a flow rate of 5 mm/min and this fast renewal rate means that particles are eliminated from the nasal cavity in less than 20 min. The nasal mucosa is about 2–4-mm thick and composed of two distinct layers: periciliary layer and superficial layer [5, 9].

Enzymes and peptidases contained in the mucus, its constant secretion, and nasal clearance mechanisms significantly reduce the ability of drugs to penetrate through the epithelium [10]. Studies have shown that rapid systemic delivery of topically applied drugs can be achieved after intranasal administration due to the permeable properties of the respiratory epithelia and highly vascularized nature of the adjacent submucosa.

Immunologically specialized region localized at the gateway of the respiratory and alimentary tract consists of the palatine tonsils, nasopharyngeal tonsils (adenoid), lingual tonsils, and tubal tonsils and is designated as Waldeyer's ring or nasal-associated lymphoid tissue (NALT). It represents a site of intimate contact between exogenous antigens and the host aerodigestive tract. With exception of adenoids and tubal tonsils covered by pseudostratified ciliated columnar epithelium (respiratory epithelium) the palatine and lingual tonsil are covered by stratified non-keratinized squamous epithelium with many invaginations allowing the enhanced exposure of foreign antigens to the underlying cryptal lymphatic tissue. Therefore, nasal mucosa is one of intensively explored sites for vaccine administration.

*2.1.3 Vaginal mucosa*

The vaginal mucosa is composed of four histological layers: the stratified squamous epithelium (with underlying basement membrane), the elastic lamina propria (a dense connective tissue layer which projects papillae into the overlying epithelium), the fibromuscular layer comprising two layers of smooth muscle, and the tunica adventitia, consisting of areolar connective tissue [5].

The vaginal epithelium is composed of non-cornified, stratified squamous cells. Several layers of cuboidal cells are close to the basement membrane and become flattened as they move toward the luminal surface. The thickness of vaginal epithelium is around 200–300 μm, and is influenced by physiological factors, e.g., variability in estrogen concentration [11].

Vaginal surface is coated with a layer of cervicovaginal fluid. The fluid contains secretions from the cervix in the form of mucins and secretions from the Bartholin's and Skene's glands, endometrial fluid, and fluid transuded from the vascular bed of the vaginal tissue. It also contains a large number of squamous epithelial cells, enzymes, proteins, carbohydrates, and amino acids. The acidic vaginal environment is maintained by production of lactic acid by lactobacilli but the exact pH value is influenced by the presence of cervical mucus, the amount of vaginal fluid, infections, and other factors. Cervical mucus forms a semipermeable viscoelastic barrier at the vaginal surface. High constant production of cervicovaginal fluid influences the properties of different drug delivery systems, the residence time after administration as well as the rate of drug release and absorption [12].

**2.2 Barrier functions of mucosal surfaces**

*2.2.1 Oral mucosa*

The rate of drug absorption following oromucosal administration is influenced by the permeability of the buccal and sublingual mucosa, physical-chemical properties of the delivered drug, and other factors, namely the presence and properties of mucus, saliva production, movement of the oral tissues during speaking, food and drink intake, etc. The mucus layer is the main natural barrier of mucosa against penetration of different pathogens and foreign particles. One the other hand, mucus layer is one of the main absorption barriers to a variety of drugs, including nanoparticle-based drugs and vaccine formulations [5].

Drug permeability through the oral cavity mucosa represents a major limiting factor in transmucosal drug delivery. Mechanically stressed areas are keratinized and impermeable to water, which makes such areas unfavorable for drug delivery. On the other hand, more permeable non-keratinized buccal and sublingual epithelia make such regions of the oral cavity attractive sites for drug delivery and a great number of active ingredients are currently being explored in terms of transmucosal drug delivery [13].

One of the main permeability barriers of oral mucosa is represented by the presence of a layer of extracellular lipidic material coming from membrane-coating granules (MCGs). They are present in both keratinized and non-keratinized epithelia, but the composition of lipidic material is different between the two types [14].

Passive diffusion of drugs and drug carriers through mucosal surfaces is generally considered the primary mechanism responsible for the transport of drugs across the oral mucosa [15]. However, active transport mechanisms are connected to the activity of different types of immune cells widely present in oral mucosal tissues, especially in sublingual and buccal regions. Such immune cells, mainly different types of dendritic cells, act as an immunological barrier between the body

and foreign stimuli (including pathogens and potential allergens), mainly coming to the body with food and drink. These cells are responsible for tolerogenic, or opposite reaction to such stimuli.

Another barrier to drug permeability across oromucosal surfaces is enzymatic degradation. Saliva contains some enzymes that are able to metabolize some peptide and protein therapeutics. This fact leads to reduced bioavailability of protein-based therapeutics after oromucosal administration.

The flow of saliva makes it difficult for a drug delivery system to retain the released drug at the site of administration and, therefore, mucoadhesive drug formulations are the preferred dosage form for oromucosal drug delivery [5].

### *2.2.2 Nasal mucosa*

The main barrier functions of nasal mucosa include the role of the mucus layer and the process of mucociliary clearance. For systemically acting drugs, the mucus layer acts as a diffusion barrier. The rate of the diffusion through the mucus layer is dependent on several factors involving thickness and viscosity of the mucus, and drug physical-chemical properties. The thickness of the layer of mucus in the nasal cavity is a few microns and, therefore, in contrast to other mucosal surfaces, it does not represent a substantial diffusion barrier. The rate of mucociliary clearance influences the contact time of epithelia and a drug delivery formulation and also the drug molecules themselves [9].

Transport across epithelial barriers with respect to the nasal route, and drug permeation via the paracellular route are limited because of the presence of tight junctions. Therefore, passive diffusion via the transcellular route is the main transport pathway across the nasal epithelium [16].

Physical-chemical factors that influence nasal drug absorption are the molecular weight and lipophilicity. Polar and ionized molecules show poor permeability. On the other hand, drugs with systemic action currently administered by the intranasal route are of low molecular weight and lipophilic nature. Moreover, some therapeutic peptides, e.g., salmon calcitonin and oxytocin, also exhibit some level of systemic nasal absorption [5].

### *2.2.3 Vaginal mucosa*

Properties of the vaginal epithelium such as thickness and barrier functions are depending on several factors, including age and hormonal activity [12]. The above mentioned factors affect both vaginal fluid production and the amount of enzymes contained in the fluid. The surface of vaginal epithelium contains a variety of enzymes which can metabolize the delivered drugs, especially therapeutic peptides and proteins. The present proteases are the main barrier for absorption of such drugs into the blood as they are degraded before they cross the epithelium and can reach systemic circulation. Cervicovaginal fluid acts as a dissolution medium that enables transfer of drugs from the dosage form into the tissue. It has also been claimed that drugs can directly be transferred from dosage form to epithelial tissues. This means that a portion of the released drug molecules can overcome the fluid compartment. The pH in the vagina is around 4, thus strongly acidic under normal conditions. These conditions can lead to rapid drug degradation.

Systemic mucosal delivery through vaginal epithelium has several advantages, including ease of administration, avoidance of drug liver first-pass metabolism, relatively low metabolic activity, high permeability, prolonged retention, and the potential for sustained release from drug delivery formulations with controlled release properties. Important physical-chemical properties of drugs delivered through

vaginal mucosa include drug solubility in the vaginal fluid, tissue permeability, chemical stability of drugs under certain pH conditions, and drug lipophilicity [5].

### 2.3 Mucosal vaccines

Mucosal surfaces, such as those of the respiratory, gastrointestinal, and genital tracts, act as the first line of defense against environmental pathogens. Although immunization by mucosally applied vaccines has had a long history with numerous micro-organisms and a variety of administration routes, only a few such vaccines are currently used in human medicine. Nevertheless, these examples (e.g., poliomyelitis, influenza, cholera, *Salmonella typhi*) clearly demonstrate the validity of this strategy. There are many reasons for the development of mucosal vaccines as an attractive alternative to those administered by systemic routes. Most importantly, protective immune responses can be induced at the relevant mucosal sites of pathogen entry by mucosal delivery of vaccines, and thus the enormous potential of immunity in mucosal tissues and their associated secretory glands remains to be exploited in vaccinology. Better understanding of mucosal immune system together with new nanomaterials, including nanofibers and engineering of recombinant antigens, enhanced a long-lasting effort to develop mucosal vaccines capable of effectively inducing both mucosal and systemic immune responses, thereby resulting in two layers of host protection.

Mucosal vaccination, in contrast to other routes of vaccine administration, is of particular interest since it can enhance immune responses, mainly secretory IgA, which defends the portal of entry of various infectious pathogens [17]. Since the route of vaccine administration has a significant effect on the resultant immune response, much effort has been made to explore novel mucosal vaccine delivery routes, briefly described in this chapter.

The noninvasive needle-free vaccine delivery mode (nonparenteral routes of application) has become a global priority, both to eliminate the risk of improper and unsafe needle use and to simplify vaccination procedures. Development of alternative vaccine delivery methods, including mucosal routes, becomes a prominent field of vaccine research and represents a challenge for new biocompatible materials, especially nanomaterials. The vaccine administration route significantly affects immune responses regarding the intensity and quality (class-specified Ig, TH1/TH2 balance, anergy).

Furthermore, for vaccination campaigns organized to stop epidemics of mucosally transmitted infections, mass immunization by mucosal routes is likely to be more practicable and less expensive than immunization by systemic routes. In addition, many parents already hesitate to subject their young children to repeated needle sticks. The factors like reduced cost of mucosal vaccines must be taken into consideration. Economy of mucosal vaccines is based on both production/distribution and application levels. The purity of mucosally delivered vaccines, including endotoxin contamination, is less critical than for injectable vaccines. Finally, mucosal vaccine delivery does not require sterile syringes and needles or personnel trained in their use and disposal, although spray devices or other applicators may be needed for intranasal and other routes of administration. From this point of view, sublingual application of vaccines represents the easiest and the most favorable modes.

Nevertheless, there are some drawbacks in terms of mucosal vaccination, particularly the uncertainty of the amount of effectively delivered antigen following its mucosal administration. Another problem is the limited uptake of intact protein and polysaccharide antigens at mucosal surfaces. Moreover, potential for degradation of antigens, especially in the gastrointestinal tract following oral

administration is another issue. Therefore, as compared to oromucosal administration, significantly higher doses of vaccine antigens must be administered orally to induce measurable immune responses. To overcome such difficulties, various particulate antigen delivery systems have been designed.

Successful development of future mucosal vaccines is based on three basic pillars critical for inducing the effective immune response. These pillars are adjuvants, application/delivery systems, and antigens.

Adjuvants represent one of the key issues in all vaccine development. The identification and development of appropriate mucosal adjuvants to enhance the desired aspects of the immune response against the antigens represent a special challenge because adjuvants for mucosal application involve requirements different from those for parenteral use. On the other hand, this affords greater opportunities for discovery by exploiting the growing understanding of the mechanisms whereby mucosal pathogens and commensals interact with the immune system. Cholera toxin (CT) and related heat-labile enterotoxins such as *Escherichia coli* labile toxin (LT) are classic examples of mucosal adjuvants for their ability to break immune tolerance [18]. A major concern for human application has been to separate the toxicity of these molecules from their adjuvant properties. Different approaches were used based on targeted mutations in the enzymatically active site of the A subunit, the selection of various types of toxins including mutants that have different ganglioside (receptor)-binding B subunits and the use of nontoxic B subunits alone or coupled to vaccine antigens. Intranasal administration has generated interest and concern with the finding that CT and LT can undergo retrograde migration along neurons, potentially reaching the brain via the olfactory nerve, and thus leading to neurological pathology. An intranasal influenza vaccine which contained a low dose of LT as an adjuvant was introduced in Switzerland, but it was withdrawn due to suspicion of causing Bell's palsy in some recipients [19]. Facial nerve palsy was another side effect demonstrated after intranasal application of a flu vaccine. As an alternative route, sublingual vaccination has been demonstrated to achieve similar immune responses but without the risk of retrograde transmission to the brain [20].

Other molecules derived from microorganisms and falling into the category of pathogen associated molecular pattern (PAMP) have also been shown to have immunomodulatory properties. Ligand for Toll-like receptors, NOD1/2 receptors, and inductors of inflammasomes are examples of such molecules (e.g., monophosphoryl lipid A, "CpG" oligodeoxynucleotides, muramyl glycopeptides, flagellin). Nevertheless, none of these has been investigated as extensively as the heat-labile enterotoxins.

Delivery systems for mucosal immunization are functionally related to adjuvants for mucosal vaccines as well as to antigen formulations. The principal task for delivery systems is to keep antigen on mucosal surface and to enhance its penetration into submucosal tissue to be accessible in sufficient amount to immune cells, especially to dendritic cells. Various gels and films are available for buccal application of drugs, while sprays or droplets are used for intranasal application of influenza vaccines. Oral application of vaccines is achieved mainly by various capsules containing antigens or simply by administration of the vaccine on a spoon (polio).

While some formulations for nasal and oral delivery of vaccines are available, suitable delivery systems for sublingual vaccination are at their door step of development. In this chapter, we describe the mucoadhesive nanofiber-based film fulfilling the role of delivery system for sublingual immunization. This system is compatible with antigens formulated as proteoliposomes and immunostimulating complexes (ISCOMs), virus and virus-like particles, bacteria and bacterial ghosts, plasmid DNA, polymeric nanoparticles, and a simple free antigen. Such formulations also allow the sustained release of vaccine antigens and provide some

protection against removal from the site of application by saliva flow and tongue movement. Also, enhancers of mucosal penetration can be combined with antigens and mucosal adjuvants, which make the mucoadhesive nanofiber-based films a universal platform for mucosal sublingual vaccination. The important aspect of the mucoadhesive nanofiber-based film is its suitability for industrial production as the crucial factor of pharmacoeconomy [21].

In conclusion, sublingual immunization represents the route of a big hidden potential for development of effective, cheap, and safe vaccines for prevention of infectious diseases as well as the treatment of allergy.

### 2.4 Penetration enhancers

Penetration enhancers, sometimes also referred to as chemical enhancers, are functional pharmaceutical excipients that possess the ability to modify the physical-chemical properties of mucosal barriers. The reason for co-administration of penetration enhancers is to enhance penetration of drugs through different barriers of mucosa including mucus layer, extracellular lipid layer originating from MCGs, enzymatic barrier, and others. The target compartment of the delivered drug can differ according to the mode of its action, e.g., blood circulation in systemic drug delivery, specialized immune cells present in epithelium or submucosal tissue in vaccine delivery and local cell therapy, etc. Penetration enhancers can potentially facilitate the systemic absorption of a wide range of drugs, including large therapeutic molecules such as polypeptides, proteins, nucleic acids [22], and therapeutic nanoparticles.

Different surfactants, bile salts (deoxycholic, ursocholic, and taurocholic acids), ethylenediaminetetraacetic acid (EDTA), fatty acids, and amino acids are the most frequently explored chemicals used in mucosal enhancing of drug permeability, and thus its bioavailability [23, 24]. An important concern related to penetration enhancers is the capacity of adjacent tissues to tolerate the effects of penetration enhancers.

Penetration enhancers act usually by a combination of different mechanisms. Some penetration enhancers are amphipathic in nature, and thus associate and influence bilayers of the cell membranes, increase the fluidity and permeability of membranes and finally promote transcellular transport. Penetration enhancers can interact with tight junctions between epithelial cells that cause facilitation of paracellular drug transport. Another group of penetration enhancers, known as mucolytic agents (e.g., acetylcysteine) influence the integrity of the mucus layer.

Formulation of drugs into nanofibers, especially nanofibers with mucoadhesive properties, enhances drug absorption via mucosal surfaces in general. Moreover, combination of nanofiber-based formulations and chemical enhancers can increase penetration of drug molecules, or nanoparticle-based drug and vaccine delivery systems. Nanofibers themselves have extraordinary capacity to combine different types of chemicals including combination of drugs and penetration enhancers, whether using electrospinning technique for nanofiber production, or another technique. As an example, mucosal penetration of model mucus penetration poly(lactic-co-glycolic) acid (PLGA) nanoparticles from nanofiber-based mucoadhesive film into porcine sublingual and buccal mucosa was enhanced using sodium deoxycholate as penetration enhancer [21].

## 3. Transmucosal delivery of drugs and vaccines

Different parts of the human body are covered by mucosa with different features and barrier properties for drug delivery. Some areas are more accessible than

others. In principle, there are two ways of mucosal drug delivery—local delivery, e.g., antimicrobials, anti-inflammatory drugs, etc., and systemic drug delivery. Whereas many indications for drugs intended for local treatment exist and include all mucosal surfaces, systemic drug delivery is a completely different task and only a few parts of mucosa are suitable sites for systemic drug administration and absorption. Moreover, only several drugs with suitable physical-chemical properties have been administered via transmucosal delivery. On the other hand, mucosal drug absorption has enormous potential, and different strategies including penetration enhancers, nanoparticle-based drugs, and mucoadhesive drug formulations have been employed.

Because of easy accessibility, high vascularization and a relatively low thickness, oral mucosa, especially its sublingual and buccal parts, is a preferred site of systemic drug administration. Transmucosal delivery of certain drugs can result in a rapid onset of their action, thus having the potential to replace the injection administration of some specific drug molecules [25]. Oromucosal delivery of nitroglycerin brings many benefits for patients, and today, nitroglycerin is one of the most frequent drug molecules delivered via oral mucosa. Exploration of cardiovascular drugs (e.g., captopril, verapamil) has shown promising results. Oral transmucosal delivery of analgesics has attracted substantial attention. As an example, oromucosally administered fentanyl is designed for rapid noninvasive delivery of analgesia for severe pain treatment.

Oromucosal delivery of sedatives and hypnotics has shown favorable results with clinical advantages over other routes of administration. Another example is drugs for erectile dysfunction and transmucosal formulations of hormones, e.g., testosterone and estrogen. Transmucosal spray formulation of insulin (Oral-lyn®, Generex) is a great example of the potential of oral mucosa for systemic macromolecular drug delivery.

Transmucosal nasal drug delivery is another interesting site for systemic drug delivery. The considerable blood supply of nasal mucosa provides efficient systemic drug absorption and enables direct access to the systemic circulation for drugs. In nasal drug delivery, limited nasal capacity often results in partial swallowing of the instilled drug as the instilled volumes exceed the limited capacity of the nasal mucosa. Therefore, the administered dose of the drug is partially swallowed, and drug absorption is in part transmucosal, in part gastrointestinal. Formulation of drugs into different mucoadhesive dosage forms and nasal inserts can be beneficial in this regard. Several drugs have been successfully administered including active ingredients with hormonal activity (salmon calcitonin, oxytocin, desmopressin) and small drugs (e.g., sumatriptan, zolmitriptan, dihydroergotamine).

As nasal mucosa is the first barrier which must be conquered by pathogens, nasal mucosa is very immunocompetent. Many studies have shown that even small amounts of antigen can elicit a strong protective response. Intranasal administration, similarly to oromucosal, seems to be the best strategy for barrier vaccinations following the outbreak of highly infectious diseases, because less erudite persons (e.g., nurses) can provide mass vaccination. FluMist® (US) and Fluenz® (Europe) are examples of live attenuated influenza vaccines.

The vaginal mucosa offers many advantages as a site for drug delivery, including easy access, prolonged residence time interval of drug availability, avoidance of first-pass metabolism, and relatively low activity of proteases and other enzymes. The vagina has a rich vascularization and a large surface area due to the folds in the mucosa (rugae) making it ideal for high absorption of drug molecules. Administration of drugs through vaginal mucosa represents an interesting alternative to oral administration for drugs treating osteoporosis, hormone replacement therapy, contraception, infections, and others. Mucoadhesive polymers are often

used in formulations for vaginal drug delivery systems to prolong retention time of drug delivery systems [26].

Mucosal immune responses in the genital tract can be induced by the administration of antigen to mucosal surfaces. IgA antibodies in the vaginal tract are essential as a first line defense against pathogens that enter the body through vaginal mucosa. Immune responses of various types of vaginally administered vaccines have been investigated. Both vaginal and serum IgA and IgG levels have been enhanced following vaginal vaccine administration [27].

The nasal mucosa vaccination induces preferentially mucosa-associated immune responses. There are several vaccination approaches to induce both mucosal and systemic immune responses (antibodies and cell-mediated immunity), for example by heterologous immunization by systemic followed by mucosal routes. Combining of parenteral and mucosal administration of antigen is required because parenteral vaccines are notoriously inefficient for stimulating immune responses in mucosal tissues; and on the other hand, mucosal vaccination, particularly that administered by oral route to subjects without antecedent contact with vaccination antigen leads to induction of the "oral tolerance" phenomenon consisting in induction of IgA-mediated immune responses in mucosal compartments but dominantly cell-mediated antigen-specific tolerance in systemic compartment. In contrast, sublingual (SL) vaccination represents a route effectively stimulating both systemic and mucosal antibody- and cell-mediated immune responses. Sublingual mucosa is the place of vaccine administration which in contrast to other mucosally effective intranasal routes does not elicit neurotoxic effects as demonstrated for example with inactivated influenza virus administered SL together with a mucosal adjuvant which did not migrate to or replicate in the nerve system [28]. Furthermore, sublingual mucosa may be useful as a delivery site for mucosal vaccines because the sublingual epithelium harbors a dense lattice of dendritic cells (DC), and that using mucosal adjuvants mobilizes DCs within the sublingual epithelium. These cells migrate to the above mentioned proximal draining lymph nodes (submaxillary and superficial cervical lymph nodes), on uptake of the sublingual vaccine antigens. It is important that sublingual immunization induces antigen-specific immune responses in the female reproductive tract in addition to the respiratory tract and oral/nasal cavity [29]. Another mucosal administration route, orogastric route, can induce strong mucosal responses, especially secretory IgA in the small intestine, proximal colon, and mammary and salivary glands but it is poorly efficient for disseminating these responses to the distal segments of the gut and to the respiratory and reproductive tracts. Moreover, orogastric immunization requires substantially more antigen application because of intensive degradation. In addition to orogastric, nasal, and sublingual vaccines, transcutaneous immunizations are now part of a new generation of mucosal vaccines [30].

## 4. Nanofibers in mucosal drug delivery applications

### 4.1 Mucoadhesion and mucoadhesive properties of nanofibers

Mucoadhesion is defined as adhesion between a mucosal surface and a surface of another material. Mucoadhesive dosage forms have recently attracted much attention from pharmaceutical research as well as from pharmaceutical industry due to substantial improvements in mucosal drug delivery. Increasing the residence time of drug formulations at the site of administration automatically leads to much more effective transmucosal drug delivery, drug bioavailability, and results in increased therapeutic efficiency, thus lowering the drug dose needed [13]. Instead of small

drug molecules, mucoadhesive formulations enable delivery of therapeutic biologicals such as peptides, proteins, antibodies, and nucleic acids through a variety of routes of administration such as oromucosal, ocular, nasal, and vaginal. Moreover, mucosal delivery of nanoparticle-based therapeutic formulations can be achieved by materials with mucoadhesive properties, mucus penetration formulations, and their combinations. Taste masking properties are of importance for mucoadhesive formulations intended for oromucosal administration.

Nanofibers made up of mucoadhesive polymers exhibit one of new trends in mucosal drug delivery. Due to their extremely large surface area, unique surface topology, and porosity, nanofibers are known to significantly improve the adhesiveness of the mucoadhesive drug delivery systems utilizing nanofibers for their construction. Architecture of nanofibers significantly intensifies the intimate contact between the nanofiber-based products and mucosal surface, and high drug concentration at the site of administration is achieved (**Figure 1**). Moreover, their ability to enhance drug solubility makes nanofibers an almost ideal platform for transmucosal drug delivery.

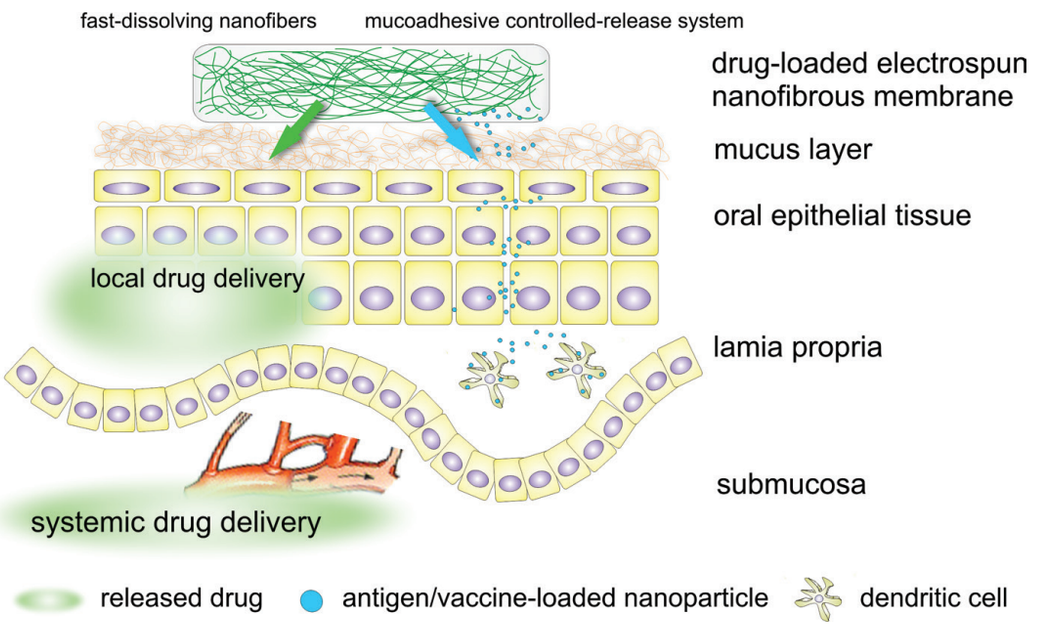


**Figure 1.**
*Schematic representation of drug and vaccine delivery after mucosal administration of nanofiber-based drug delivery system.*

The use of mucoadhesive polymers is conditioned by their ability to form nanofibers. A variety of factors affect the ability of polymers to form nanofibers as well as the mucoadhesive properties of polymers, including molecular weight, chain flexibility, hydrogen bonding capacity, cross-linking density, charge and degree of ionization of a polymer, concentration, and hydration (swelling) of a polymer [3].

These are reasons why only a few mucoadhesive polymers have been tested for nanofiber-based drug delivery system formulations. Chitosan (cationic polymer), hyaluronic acid, sodium alginate, sodium carboxymehylcellulose (anionic polymers), different cellulose derivatives, poly(ethylene oxide), and polyvinylpyrrolidone (nonionic polymers) are excellent examples of conventional mucoadhesive materials utilized for construction of nanofiber-based mucoadhesive drug delivery systems. A group of thiolated polymers, e.g., thiolated chitosan, are representatives of next-generation mucoadhesive materials [3, 13]. Mucoadhesive nanofibrous membrane made of chitosan/PEO is visualized in **Figure 2** as an example.

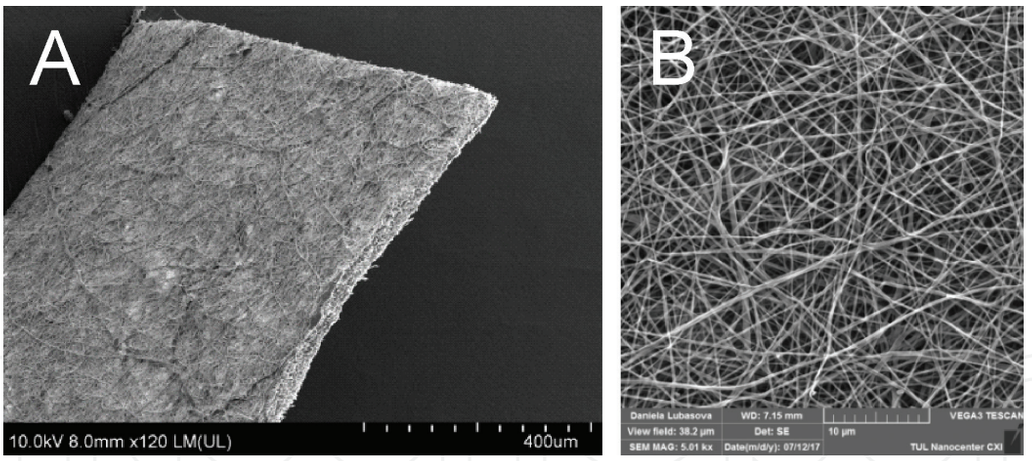


**Figure 2.**
*Electrospun nanofibrous membrane fabricated using Nanospider technology (A) and detail of mucoadhesive chitosan/PEO nanofibers (B).*

### *4.1.1 Chitosan*

Chitosan is a linear polysaccharide composed of randomly distributed D-glucosamine and N-acetyl-D-glucosamine obtained by deacetylation of chitin (**Figure 3**). Because of the broad chemistry of chitosan, which covers different degrees of deacetylation, a range of molecular weights and different distribution of the acetyl groups along the polymeric chain, chitosans can provide a number of physical-chemical as well as biological properties. Mucoadhesion of chitosan occurs due to the electrostatic interactions of amino groups of chitosan and the sialic groups of mucin in the mucus layer.

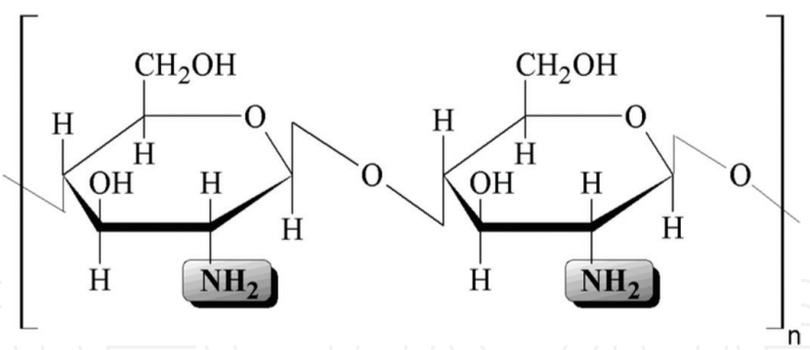


**Figure 3.**
*Structural formula of chitosan.*

Several studies have explored mucoadhesive properties of chitosan-based nanofibers. As an example, Lancina et al. have produced chitosan-based nanofiber mats capable of delivering insulin via the buccal mucosa. Chitosan was electrospun into nanofibers using poly(ethylene oxide) (PEO) as a carrier molecule. Insulin release rates were determined and showed no reduction in bioactivity due to electrospinning. Buccal permeation of insulin was significantly facilitated as compared to free insulin. Taken together, this work demonstrates that chitosan-based nanofibers have the potential to serve as a transbuccal insulin delivery vehicle [31]. In another study, mucoadhesive fibers of zein/chitosan have been prepared by electrospinning to study the encapsulation efficiency and release of tocopherol. The addition of the acidic chitosan solution to the zein containing tocopherol has improved the mucoadhesive properties of the final composite nanofibers [32]. Mucoadhesive hybrid

electrospun chitosan/phospholipid nanofibers intended for drug-delivery applications were produced by Mendes et al. [33]. Nanofibrous membranes intended for local delivery of an antimicrobial agent in combination with poly(hexamethylene biguanide) hydrochloride were produced by electrospinning of chitosan/PEO solution. Inhibition of bacterial growth for both *Escherichia coli* and *Staphylococcus aureus* were achieved using nanofiberous membranes [34].

### *4.1.2 Cellulose derivatives*

Cellulose (**Figure 4**) mucoadhesive derivatives are a wide group of pharmaceutical excipients and cover both nonionic polymers, including hydroxypropylmethylcellulose (HPMC), hydroxyethylcellulose (HEC), hydroxypropylcellulose (HPC), methylcellulose (MC), and anionic derivatives, e.g., carboxymethylcellulose (CMC).

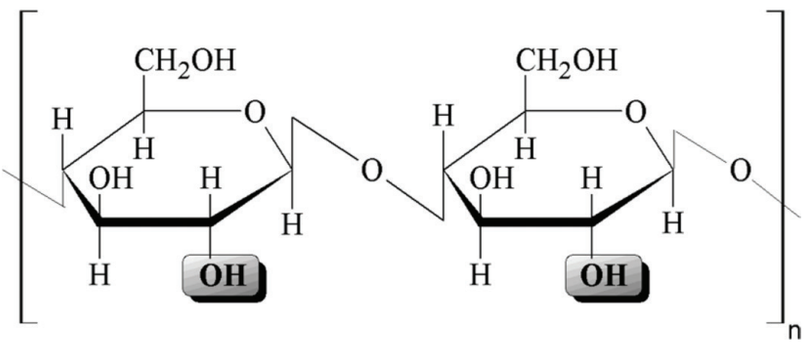


**Figure 4.**
*Structural formula of cellulose.*

The aim of the study exploring carboxymethylcellulose as a mucoadhesive agent for nanofiber formation was to develop a progesterone-loaded mucoadhesive system for vaginal application with sustained release. Presently, two dosage form options are being considered: direct compression of nanofibers into tablets for vaginal insertion and winding bundles of the fiber into a miniature tampon [35, 36].

In another work, nanofiber-based indomethacin films were prepared using different grades of methylcellulose, polyvinylpyrrolidone, and Tween® 80 by electrospinning. The addition of Tween® 80 to polyvinylpyrrolidone formulations significantly improved their wettability. Moreover, nanofiber-based patches containing methylcellulose and Tween® 80 were found to exhibit the highest permeation of indomethacin across porcine mucosa without significantly affecting the ultrastructure of the oral mucosa [37].

El-Newehy et al. demonstrated the preparation of HPC-based nanofibers. They found that the thermal stability and mechanical properties of nanofiber mats were dramatically enhanced with the addition of HPC to polyvinyl acetate or polyvinylpyrrolidone. The in vitro sustained release of an incorporated model drug, diclofenac sodium, was controlled when loaded into electrospun nanofibers of HPC with either PVA or PVP [38].

### *4.1.3 Poly(ethylene oxide)*

Mucoadhesive glutamine-loaded poly(ethylene oxide) (**Figure 5**) electrospun nanofibers were prepared by Tort et al. The effect of different polyelectrolytes on resultant properties of nanofibers was observed. 85% of the drug was released from the nanofibers after 4 h in simulated saliva solution suggesting that glutamine-loaded nanofibers have potential as an oromucosal drug delivery system [39].

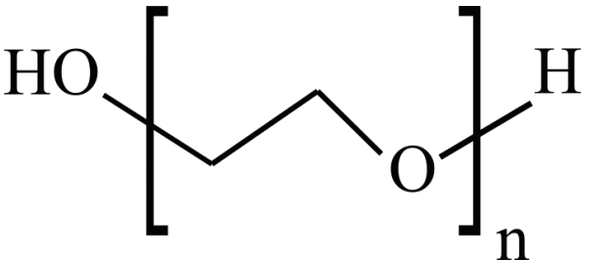


**Figure 5.**
*Structural formula of poly(ethylene oxide).*

Nanofiber-based local drug delivery system may be suitable for the treatment of cervical cancer. A pilot study by Zong et al. was carried out to examine the efficacy of cisplatin-loaded poly(ethylene oxide)/polylactide composite electrospun nanofibers as a local chemotherapy system against cervical cancer in mice via vaginal implantation. They have shown that a better balance between antitumor efficacy and systemic safety was achieved in a group of animals treated with nanofiber formulation as compared to i.v. injection group using an equal drug dose. Therefore, electrospun nanofibers present a promising approach to the local drug delivery via vaginal mucosa against cervical cancer [40].

*4.1.4 Thiolated chitosan*

Thiolated polymers are obtained by the addition of conjugated sulfhydryl groups. Thiolation of chitosan increases their mucoadhesive properties due to formation of disulfide bridges with cysteine domains of glycoproteins of the mucus. Moreover, chitosan and thiolated chitosan possess antiprotease activity due to their affinity to divalent cations, which are co-factors for proteases. All these characteristics make thiolated chitosan a promising material for mucosal administration of drugs, peptides, and proteins [13].

Leila Behbood et al. developed mucoadhesive nanofibers made up of thiolated chitosan as a drug delivery system for tetracycline and triamcinolone. Chitosan was modified via the immobilization of thiol groups from L-cysteine as a mucoadhesive reagent. Maximal mucoadhesion of nanofibers was observed at the pH value of 6. Release studies demonstrated that a sustained release of both drugs continued up to 48 h. The drug delivery system represented a novel tool for the improvement of therapeutic efficacy of various drugs that are poorly absorbed from different parts of the gastrointestinal tract. It was also shown to be an efficient system for treatment of oral ulceration [41].

The aim of the study performed by Samprasit et al. [42] was to fabricate mucoadhesive electrospun nanofiber mats containing α-mangostin for the maintenance of oral hygiene and reduction of the bacterial growth. Thiolated chitosan blended with polyvinyl alcohol was selected as the mucoadhesive polymer. The results of this study suggest that α-mangostin-loaded mucoadhesive electrospun nanofiber mats may be a promising material for the prevention of dental caries.

## 4.2 Transmucosal delivery of poorly soluble drugs using nanofibers

Many drugs are highly hydrophobic with poor water solubility and the number of poorly water-soluble drug candidates selected for development is rapidly increasing. It results in low oral absorption of these drugs as the absorption and bioavailability are limited by their poor solubility or slow dissolution in the gastrointestinal tract. This represents a major challenge for the pharmaceutical industry and novel

formulation approaches are required. Several strategies including particle size reduction, micellization, salt formation, complexation, and solid dispersions have been developed to increase the oral absorption of such drugs. Solid dispersion is defined as the dispersion of one or more drugs in an inert matrix in the solid state. Simple eutectic mixtures, solid solutions, glass solutions of suspensions, and amorphous precipitates of a drug in a crystalline carrier are examples of solid dispersions [43]. Due to difficulties occurring during conventional methods of drug formulation, the applicability of solid dispersion systems has remained limited. Electrospun nanofibers provide a novel approach to improve the dissolution rate of even poorly water-soluble drugs, and thus might minimize the limitations of oral and oromucosal drug bioavailability [44].

As an example, maraviroc, an anti-HIV drug intended for intravaginal administration, was electrospun as solid dispersion made from either polyvinylpyrrolidone or poly(ethylene oxide) nanofibers or microfibers. In the study, the role of drug loading, distribution and crystallinity in determining drug release rates into aqueous media was investigated. It was shown that water-soluble electrospun materials can rapidly release maraviroc upon contact with moisture and that drug delivery is fast [45].

Salt formation improves drug solubility. However, drugs administered onto mucosal surfaces are effectively absorbed through mucosal surfaces if they are in the unionized form. Therefore, this strategy of enhanced drug dissolution is not advantageous for mucosal drug delivery.

The rate of dissolution of drugs formulated into particles is increased with their increasing surface area and decreasing particle size. Technologies used to decrease drug particle size to sub-micrometer range are being frequently applied to poorly water-soluble drug product development. Electrospinning is one of the technologies that can produce uniform nanosized polymeric nanofibers with drugs loaded into their structure. The release rates, and thus bioavailability of nanofiber-formulated drugs, are enhanced compared to those from the original drug substance [46].

Oral mucosa provides an interesting site of drug administration and absorption including poorly water-soluble drugs. However, they are not usually suitable for the formulation into classical oromucosal drug delivery systems. The formulation of nanofibers represents one possible route to achieve effective drug absorption via mucosal surfaces. Potrč et al. formulated polycaprolactone (PCL) nanofibers intended for oromucosal delivery of poorly water-soluble drugs. In this study, two model drugs, ibuprofen and carvedilol, with similar lipophilic properties, but differing in their molecular weights were chosen, and their influence on the nanofiber's physical properties and drug release profiles were investigated. The aim of the study was to establish a correlation between the drug's properties and the release characteristics of a PCL nanofiber-based delivery system. The results obtained in this study have shown that electrospinning can be used for the fabrication of drug-loaded PCL nanofibers with a high percentage of API embedded in them. The formulation of poorly water-soluble drugs into polycaprolactone-based nanofibers significantly increases their dissolution rate. However, the release rate of drugs from nanofibers is drug-dependent. Electrospinning was shown to be a very promising approach to the formulation of poorly water-soluble drugs in order to enhance their release and enable oromucosal administration [46].

### 4.3 Transmucosal delivery of macromolecules and nanoparticle-based vaccines using nanofibers

Mucosal surfaces are the most convenient routes for drug delivery to systemic circulation. However, transmucosal transport of macromolecular drugs such as

peptides and proteins is much less effective as compared to low molecular weight drugs. Several strategies exploiting permeation enhancers, nanoparticulate carriers, nanofibers, and their combinations represent a promising strategy to facilitate transmucosal transport of macromolecules.

Recently, nanofiber-based mucoadhesive films have been invented for oromucosal administration of nanocarriers used for delivery of drugs and vaccines (**Figure 6**). The mucoadhesive film consists of an electrospun nanofibrous reservoir layer, a mucoadhesive film layer, and a protective backing layer. The mucoadhesive layer made of HPMC and Carbopol 934P polymers is responsible for tight adhesion of the whole system to the oral mucosa after application. The electrospun nanofibrous reservoir layer is intended to act as a reservoir for polymeric and lipid-based nanoparticles, liposomes, virosomes, virus-like particles, dendrimers and the like, plus macromolecular drugs, antigens and/or allergens. The extremely large surface area of nanofibrous reservoir layers allows high levels of nanoparticle loading. Nanoparticles can either be reversibly adsorbed to the surface of nanofibers or they can be deposited in the pores between the nanofibers. After mucosal application, nanofibrous reservoir layers are intended to promote prolonged release of nanoparticles into the submucosal tissue. Reversible adsorption of model nanoparticles as well as sufficient mucoadhesive properties was demonstrated. This novel system appears appropriate for the use in oral mucosa, especially for sublingual and buccal tissues [21].

Another example of novel multi-layered fibrous mucoadhesive film is based on self-assembled liposomes that are formed directly from nanofibrous layer after contacting with water. The idea came from a method of liposome preparation based on electrospinning technology. PVP was used as a nanofiber-forming matrix and phospholipid as liposome-forming molecules [47]. The membrane has been developed to improve the bioavailability of carvedilol. The whole system consists of an electrospun layer, an adhesive layer made of mucoadhesive film and a backing layer, similarly as previously described by Masek et al. [21]. Mucoadhesive film was formed using HPMC and CMC polymers and the standard solvent casting method. In general, this drug delivery system offered a novel platform for potential buccal delivery of drugs with a high first-pass effect.

One example for all macromolecular drugs is insulin. Insulin is a protein which is made of two polypeptide chains and it is not completely soluble in water. Many efforts have been made to find appropriate noninvasive routes of administration, including oral, pulmonary, rectal, oromucosal, and nasal. Although a certain degree of success exploiting all routes of mucosal administration of insulin was achieved, oromucosal, namely buccal and sublingual, delivery of insulin brings several

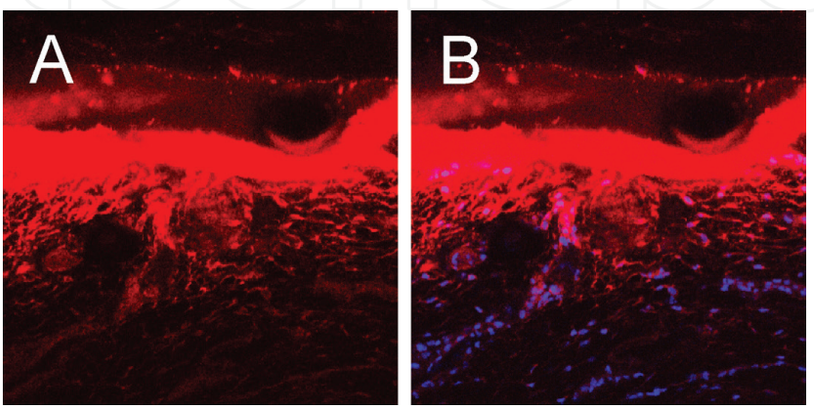


**Figure 6.**
*Transmucosal penetration of model fluorescently labeled PLGA-PEG nanoparticles after ex vivo porcine mucosal administration using nanofibers. (A) PLGA-PEG nanoparticles (red) and (B) PLGA-PEG nanoparticles (red) plus nuclei (blue).*

advantages. A number of attempts have been made to improve buccal insulin absorption by adding absorption enhancers or to modify the lipophilicity of insulin.

It is important to note that buccal and sublingual delivery of macromolecules including insulin using rodents as an animal model are of no value, because oral mucosa of rodents is highly keratinized. Therefore, the permeability for macromolecules and nanoparticles is negligible, while that of humans is quite high in the non-keratinized areas (sublingual and buccal). It means that the only animal models that can be of use when studying the human permeability of oral mucosa for macromolecules is pigs or dogs.

Several drug delivery systems have been tested for oromucosal insulin delivery, including sprays, mucoadhesive gels, and mucoadhesive films.

Transmucosal delivery of insulin via oral mucosa represents a novel approach. As an example, Sharma et al. have prepared an electrospun nanofiber-based membrane containing insulin molecules within the nanofiber structure. The solubility of insulin increases have been enhanced after formulation into polyvinyl alcohol (PVA) nanofibers as PVA itself is a surfactant and hence increases the solubility of insulin in the polymer solution. The release of insulin from a nanofiber membrane followed controlled release, and in vivo experiments confirmed high transmucosal delivery effectivity. Insulin release exhibits first order kinetics followed by an initial burst release necessary to produce the desired therapeutic activity. Furthermore, extremely high encapsulation efficacy of 99% of insulin indicates that nanofiber-based delivery system serves as an ideal carrier for the delivery of insulin via the sublingual route [48].

### 4.4 Fast-dissolving nanofiber-based drug delivery systems

Fast-dissolving drug delivery systems (FDDS) represent advanced formulations intended for oromucosal administration. FDDS are characterized by excellent flexibility and comfort for patients. The efficacy of drugs and rapid onset of their action are improved as FDDS dissolve within a minute in the oral cavity after the contact with saliva without the need of water for administration. FDDS are beneficial especially in pediatric and geriatric patients. It is also useful for delivery of drugs with local action.

Li et al. have fabricated nanofiber-based FDDS by electrospinning using PVA as the nanofiber-forming polymer and drug carrier. Caffeine and riboflavin were used as the model drugs. They found that drug release was completed in a burst manner. 100% of caffeine and 40% of riboflavin was dissolved within 60 s from the PVA nanofibrous matrices [49].

Isosorbide dinitrate-polyvinylpyrrolidone electrospun nanofibers were formulated and explored as a potentially sublingual membrane by Chen et al. The composition was favorable for the fabrication of the sublingual membrane as the dissolution was completed at 120 s. The pharmacokinetic study in rats demonstrated that the electrospinning fiber membrane had a higher $C_{max}$ and lower $T_{max}$ compared to the reference preparation [50].

Quan et al. demonstrated the concept of nanofiber-based FDDS also for poorly water-soluble drugs. In the study, feruloyl-oleyl-glycerol was used as a model drug and polyvinylpyrrolidone (PVP) K90 as a filament-forming polymer [51].

### 4.5 Mucosal administration sites of nanofiber-based drug delivery systems

Different mucosal sites of administration are a suitable target for nanofiber-based drug delivery systems, including oromucosal, nasal, vaginal, and ocular

mucosa. Nanofiber-based drug delivery systems are used for both systemic and local drug administration.

*4.5.1 Nanofibers in oromucosal drug delivery*

Nanofiber membranes intended for oromucosal administration possess different properties according to the need of the desired indication and drug administered. The oromucosal site of administration, especially sublingual and buccal regions, are the most explored mucosal surfaces for drug delivery using the nanofiber-based system (**Figure 7**). The applications include fast-dissolving nanofiber-based formulations, mucoadhesive nanofibers, nanofiber-based formulations of poorly water-soluble drugs and, finally, nanofibers for delivery of different mucosal vaccines. Small drug molecules, macromolecules as therapeutic proteins, peptides, nucleic acids, and antigens are examples of the explored nanofiber-based systems intended for oromucosal administration. As description of these applications is broad, they are divided into relevant subchapters.

*4.5.2 Nanofibers in vaginal drug delivery*

Mucoadhesive nanofiber-based drug delivery systems are investigated for vaginal drug delivery. A wide range of materials have been explored for their fabrication into nanofibers. However, the local environment of vaginal surface has to be taken into the account when designing nanofiber-based vaginal drug delivery systems. Especially, the low pH values around pH 4.0 ± 0.5 make the difference as compared to other mucosal surfaces. As an example, progesterone-loaded drug delivery nanofiber constructs are described in Chapter 4.1.2.

*4.5.3 Nanofibers in nasal drug delivery*

Supramolecular peptide nanofibers have been explored as nasal formulation for vaccines and immunotherapy. Si et al. performed a study eliciting the immune response without the use of adjuvants and without measurable inflammation. Peptides comprise an epitope from influenza polymerase and the Q11 self-assembly domain formed nanofibers which were taken up by dendritic cells in lung-draining mediastinal lymph nodes after intranasal immunization. Nanofibers administered onto nasal mucosa elicited higher antigen-specific CD8+ T cell responses in the lung-draining lymph nodes as compared to subcutaneous immunizations, while retaining the noninflammatory character of the materials as opposed to other delivery sites. Influenza vaccines that can be administered intranasally or by other needle-free delivery routes have potential advantages over injected formulations

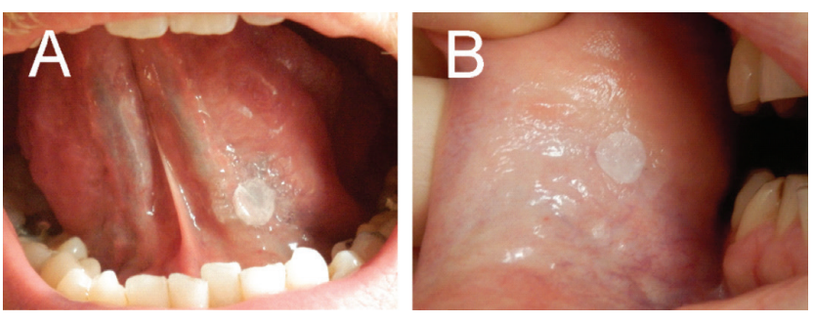


**Figure 7.**
*Application of multi-layered mucoadhesive film with nanofibrous reservoir layer to sublingual (A) and buccal (B) mucosa.*

in terms of patient compliance, cost, and ease of global distribution. It means that peptide nanofibers represent an interesting strategy for noninvasive influenza vaccines [52].

#### *4.5.4 Nanofibers in ocular drug delivery*

Ocular inserts are drug-impregnated formulations which can be placed onto ocular mucosa. Ocular inserts have been frequently used for reducing the frequency of administration, and, therefore, a controlled release profile is desired.

The objective of the study made by Mirzaeei et al. was to produce the electrospun nanofibers used as ophthalmic inserts. Triamcinolone acetonide was incorporated into a chitosan nanofiber-based ocular insert. This formulation increased the contact time between the drug and the conjunctival tissue, and thus decreased the number of administrations needed. This work showed that the concept of nanofibers in ophthalmic drug delivery is feasible [53].

## 5. Method of preparation and characterization of nanofibers for mucosal drug delivery

### 5.1 Fabrication of nanofibers for mucosal drug delivery

Nanofibers can be fabricated by several different techniques including drawing [54], phase separation [55], nanofiber seeding [56], template synthesis [57], self-assembly [58], etc. These techniques, on the other hand, allow neither control of nanofiber diameter nor continuous nanofiber production. Moreover, such techniques can only be used with specific polymers. On the contrary, electrospinning [59] is a resourceful and cost-effective technique that can be used to synthesize continuous nanofibers from numerous polymers and efficiently control their diameter.

Specifically, nanofibers produced by electrospinning (electrospun nanofibers) may be prepared from soluble polymers or from polymer solutions modified with additives such as particles, antimicrobial agents, or enzymes. Thanks to these additives, electrospun nanofibers may have desired properties. Therefore, electrospinning has gained a remarkable popularity in various disciplines boosting a recent steep rise in numbers of scientific publications.

Technically, electrospinning is a process that uses a strong electrical field to draw a polymer fluid into fine filaments. A typical electrospinning setup only requires a high voltage power supply, a syringe, a flat tip needle, and a conducting collector. When a polymer solution is charged with a high voltage, electrostatic force draws the fluid into a liquid jet (**Figure 8A**). Finally, solvent evaporation from the filaments results in solid nanofibers. In most cases, as-spun fibers deposit randomly on the electrode collector forming a nonwoven nanofiber mat. The basic equipment can be modified for various applications such as dual needle syringe (to make blended fibers), rotating collectors, etc.

Nanospider technology is a modern electrospinning technology for industrial-scale production of nanofibrous material without nozzles, needles, or spinnerets. Nanospider technology uses simply shaped electrodes covered by polymer solution (**Figure 8B**). It results in a mechanically simple technology with no parts that can be easily clogged (in comparison to needle-type electrospinning). Proven by an industrial operation, Nanospider technology provides high efficiency, outstanding fiber diameter, and web uniformity.

By electrospinning process it is possible to produce continuous nanofibers from a wide range of polymers. However, there are several parameters affecting the fiber

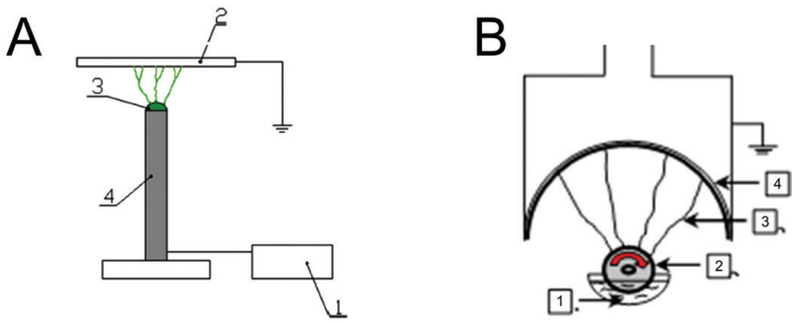


**Figure 8.**
*Schematic representation of an electrospinning process (A): (1) a high voltage supply, (2) a grounded collector of nanofibers, (3) a polymer solution, and (4) a positive electrode. Schematic representation of Nanospider technology (B): (1) a polymer solution, (2) a rotating electrode with a high voltage supply, (3) created nanofibers, and (4) a grounded collector of nanofibers.*

morphology and properties of electrospun nanofibers. The whole process can be controlled by four important characteristics: (i) process parameters such as voltage, spinning distance, flow rate, or collecting plate, (ii) systemic and (iii) solution parameters which affected concentration, conductivity, or surface tension of a polymer solution, and (iv) physical parameters such as humidity, temperature, or air velocity. All mentioned parameters are major factors affecting the fiber morphology and web properties. Because these variables interrelate, a small change in either of these variables can have a significant impact on nanofiber morphology or even the electrospinning process altogether. Solvents or their mixtures used for dissolving of polymer have a direct impact on the electrospinning process and morphology of the resulting nanofibers. Laboratory experience has shown that a solvent that creates mostly 80–99 wt% of polymer solution has a dominant impact. Solvents primarily determine (i) conformation of dissolved macromolecular chains, (ii) easiness of charging the surface layer, (iii) cohesion of the polymer solution due to the surface tension forces, and (iv) the rate of solidification of the liquid jet during evaporation of the solvent.

### 5.2 Materials suitable for nanofiber-based mucosal drug delivery systems

Nanofiber-based mucosal drug delivery systems cannot be in general electrospun from any polymer as they require specific properties. As examples of very interesting materials for nanofiber-based mucosal drug delivery systems, the following can be mentioned: biopolymers such as gelatin [60], chitosan [31], collagen [61], cellulose [62], silk fibroin [63], hyaluronic acid [64], polylactic acid [65], or polycaprolactone [46].

In addition to specific materials used for production of nanofiber mucosal drug delivery systems they also require surface modification of nanofibers. After the functionalization of a nanofiber surface, drugs might be bound or conjugated to nanofiber surfaces. In such a way, the release of drugs would be attenuated, and the functionality of the surface-immobilized biomolecules could be preserved. This strategy is usually applied in order to overcome the issue of initial burst release as well as short release time. The most used surface modifications are: (i) plasma treatment, (ii) wet chemical method, or (iii) co-electrospinning of active agents.

Different sources of plasma (e.g., oxygen, argon, ammonia, air) used for treatment of nanofibers can create different functional groups (such as carboxyl or amine groups) on the nanofiber surface. This kind of chemical groups may interact with particular drugs and create covalent bonds. However, if a target biomolecule is chemically bound onto the nanofiber surface, it would hardly be released.

Therefore, this technique is more suitable for drugs, where a slow and prolonged release of the agent is required. Plasma treatment can also change hydrophilicity and hydrophobicity of nanofibers.

Wet chemical method allows changing the wettability of nanofibers under acidic or basic conditions. Surface of nanofibers deep in mesh can also be modified by the wet chemical method. Plasma treatment is, on the contrary, more suitable for flat materials.

By co-electrospinning of active agents, it is possible to directly expose biological functional agents on the surface of nanofibers. Conjugating the biomolecules (DNA, growth factors, or enzymes) to the fiber surfaces allows their slow release into a nearby tissue significantly preserving the functionality of biomolecules.

Functionalization of the nanofiber surface enables loading of drugs. There are many methods how to load them. The most popular and used techniques are: (i) physical adsorption, (ii) nanoparticle assembly method, (iii) layer by layer method, and (iv) chemical immobilization.

In the case of physical adsorption, there is no need for nanofiber functionalization after electrospinning. The fiber web is simply immersed into a solution containing drugs and dried afterward. The same method can be used in the case of nanoparticles containing biological agents. Chemical immobilization requires functionalized surface of nanofibers by the plasma treatment or chemical wet method. Afterward, functional groups on the surface of nanofibers chemically react with added drugs and create covalent bonds. By the multilayer method, it is possible to produce a nanofiber sandwich with different properties on both surfaces. After electrospinning of one layer with drugs added during the electrospinning process, a sandwich with another nanofiber layer without drugs can be created.

## 5.3 Methods for characterization of nanofibers for mucosal drug delivery

Biomedical applications of nanofibers such as the mucosal drug delivery system put special requirements on the three-dimensional electrospun materials. Besides the biocompatibility, the morphology of nanofibers is one of the most important attributes. The specific surface area, volume, and the size of the pores have considerable effect on the loading capacity of drugs. The following methods are used to characterize electrospun materials for mucosal drug delivery systems.

Imaging methods are used for evaluation of nanofiber structure. Imaging methods involve scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM). By SEM and TEM, it is possible to evaluate the nanofiber orientation, nanofiber diameter, and the morphology of nanofibers, which do not only affect the mechanical properties of electrospun materials, but also play a key role in the loading capacity of drugs in the mucosal drug delivery systems. Imaging methods also allow visualization of the morphology of nanofibers at various points of an electrospun material.

Loading of drugs must be controlled during the assessment of biological properties and this ability is significantly affected by the physical properties such as pore size and volume of electrospun material. The surface area and the porosity could be measured by mercury porosimetry or by Brunauer-Emmett-Teller (BET) surface area analysis. A pore size distribution is one of the most often presented results of mercury porosimetry. However, the mercury porosimetry can produce a misleading result due to the mechanical deformation of the nanofibers [66]. To overcome this issue, BET measurements are used to measure the specific surface area value and distribution of pores.

Besides the morphology of nanofibers, the chemical composition is an important attribute for materials applicable in the mucosal drug delivery systems. The

Fourier-transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC) [67], and differential thermal analysis (DTA) are essential methods for measuring the chemical composition. These methods allow detection of abundance of each polymer in the final product. FTIR indicates degradation of nanofibers (for biodegradable materials) as well as it may show their bioactivity. The bioactivity is detected by infrared spectra obtained via FTIR that identifies the functional chemical groups. The hydrophilic/hydrophobic character of electrospun materials influences the loading capacity of nanofibers as well. To determine the degree of hydrophilicity, contact angle measurement is one of the most used methods.

The last most important and crucial characteristic of nanofibers is the release of drugs from electrospun materials [68]. As it was mentioned above, slow or fast release of target drugs might be changed by a different surface functionalization of nanofibers. For this purpose, a dissolution testing apparatus with UV-Vis spectrophotometer is essential to control the release profile.

## 6. Conclusions and future directions

Different parts of the human body are covered by mucosa with different features, barrier properties for drug delivery, and also with different accessibility. Formulation of drugs into nanofibers represents one of the new trends in mucosal drug delivery. Due to their extremely large surface area, unique surface topology and porosity, nanofiber-based drug delivery systems enable transmucosal delivery of poorly water-soluble drugs, macromolecules, nanoparticles, and vaccine delivery carriers.

Extraordinary flexibility of nanofibers enables us to follow unique anatomical specialities of mucosal surfaces, and hence helps to overcome different absorption barriers of mucosal sites. Moreover, the flexibility of nanofibers helps to significantly increase the comfort of nanofiber-based drug delivery formulations for patients.

Mucoadhesive nanofibers with drug-controlled release properties and nanofibers with extremely fast-dissolving properties are examples of a great variety of nanofiber-based materials and also examples of a variety of drug delivery system properties advantageous for mucosal administration. Different mucosal sites of administration, including sublingual, buccal, nasal, vaginal, and ocular mucosa, are suitable targets for nanofiber-based drug delivery systems. Mucosal surfaces, as a portal of entry of various infectious pathogens, naturally possess great potential for induction of defensive immune responses against such pathogens. Nanofiber-based delivery platforms, owning their unique properties, may play an important role in formulation of antigens into next-generation vaccine delivery systems intended for mucosal administration.

Technologies of electrospinning, such as Nanospider technology, are modern electrospinning technologies enabling cost-effective industrial-scale production of nanofibrous materials, among others, suitable for mucosal drug delivery applications.

Combinations of nanofiber-based formulations and chemical enhancers have a great potential to increase penetration of drug molecules and nanoparticle-based drug and vaccine delivery systems. In conclusion, nanofibers represent a new emerging trend in formulation of drug and vaccine delivery systems for mucosal administration.

## Acknowledgements

This work was supported by the Ministry of Education, Youth, and Sports OPVVV project “CEREBIT” CZ.02.1.01/0.0/0.0/16_025/0007397 (MR,JT); OPVVV

project "FIT" CZ.02.1.01/0.0/0.0/15_003/0000495 (JT); the Ministry of Health CZ AZV-ČR 15-32198A (MR, JT), the project MZE0002716202, RO0518 of the Czech Ministry of Agriculture (JT).

## Conflict of interest

There are no conflicts to declare.



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