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Immunology-Guided Biomaterial's Design as Mucosal Cancer Vaccine

*Shiran Ferber, Rodrigo J. Gonzalez, Alexander M. Cryer, Ulrich H. von Andrian, and Natalie Artzi**

Cancer of mucosal tissues is a major cause of worldwide mortality for which only palliative treatments are available for patients with late-stage disease. Engineered cancer vaccines offer a promising approach for inducing antitumor immunity. The route of vaccination plays a major role in dictating the migratory pattern of lymphocytes, and thus vaccine efficacy in mucosal tissues. Parenteral immunization, specifically subcutaneous and intramuscular, is the most common vaccination route. However, this induces marginal mucosal protection in the absence of tissue-specific imprinting signals. To circumvent this, the mucosal route can be utilized, however degradative mucosal barriers must be overcome. Hence, vaccine administration route and selection of materials able to surmount transport barriers are important considerations in mucosal cancer vaccine design. Here, an overview of mucosal immunity in the context of cancer and mucosal cancer clinical trials is provided. Key considerations are described regarding the design of biomaterial-based vaccines that will afford antitumor immune protection at mucosal surfaces, despite limited knowledge surrounding mucosal vaccination, particularly aided by biomaterials and mechanistic immune–material interactions. Finally, an outlook is given of how future biomaterial-based mucosal cancer vaccines will be shaped by new discoveries in mucosal vaccinology, tumor immunology, immunotherapeutic screens, and material–immune system interplay.

1. Introduction

Mucosal cancer represents a group of malignancies derived from the epithelium and connective tissue. Several different classifications of neoplasms can fall under the remit of mucosal cancers, including cancers of the lung, head and neck, digestive tract (specifically esophagus, stomach, small intestine, colorectal), bladder, reproductive tract (predominantly uterine cervix and corpus), and a small proportion of melanomas. In the United States, lung and colorectal cancers are, respectively, the second and third most commonly diagnosed tumors in both men and women, and result in the highest and third highest number of deaths. Collectively, these malignancies comprise ≈40% of all new cancer diagnoses and are responsible for just over 50% of all cancer-related deaths,[1] which equates to about 280 000 deaths annually. Mucosal cancers also represent a significant disease burden on a global scale. Lung cancers lead the way as the most frequently diagnosed and most lethal malignancy, with colorectal cancers placed fourth and fifth in those respective categories and other mucosal cancers such as stomach and esophageal are featured in the top ten most fatal neoplasms worldwide.[2] Disquietingly, the global burden of cancer is set to increase. The World Health Organization projections of cancer mortality over the coming decades reveal that the number of deaths due to diseases such as lung, stomach, bladder, colorectal, and cervical cancer are all set to rise significantly. In 2016, ≈4.6 million deaths worldwide could be attributed to mucosal cancers, which is predicted to increase

Dr. S. Ferber, A. M. Cryer, Dr. N. Artzi
Department of Medicine
Engineering in Medicine Division
Brigham and Women's Hospital
Harvard Medical School
Boston, MA 02139, USA
E-mail: nartzi@mit.edu
Dr. S. Ferber, A. M. Cryer, Dr. N. Artzi
Institute for Medical Engineering and Science
Massachusetts Institute of Technology
Cambridge, MA 02139, USA
Dr. R. J. Gonzalez, Prof. U. H. von Andrian
Department of Immunology
Harvard Medical School
Boston, MA 02115, USA
Prof. U. H. von Andrian
The Ragon Institute of Massachusetts General Hospital
Massachusetts Institute of Technology, and Harvard
Boston, MA 02139, USA
Dr. N. Artzi
Broad Institute of Harvard and MIT
Cambridge, MA 02139, USA
Dr. N. Artzi
State Key Laboratory of Molecular Engineering of Polymers
Fudan University
Shanghai, China

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to around 5.8 million by 2030[3] reinforcing the lethality of mucosal tumors.

Cancer vaccines offer an attractive approach to attaining a robust and long-lasting antitumor immune response and could operate in a prophylactic or therapeutic setting. Cancer vaccines aim to tailor and use immune cells as living therapeutics by recruiting and activating T cells and/or natural killer (NK) cells that recognize tumor-associated and tumor-specific antigens on cancer cells and eliminate them. A successful mucosal cancer vaccine elicits cell-mediated antitumor immunity, in both mucosal and systemic compartments. In order to do so, effector cells need to be provided with both the information on how to recognize cancer cells (i.e., antigen), and how to home to the tissue in which the antigen is located. Therefore, vaccination requires imprinting, that is, molecular instructions for effector cells that guide them to specific tissues. Unfortunately, imprinting molecules are mostly unknown, and thus delivery relies on the mucosa-associated secondary lymphoid organs (SLOs), where specific tissue tropism is naturally generated.

Therapeutic cancer vaccines have been previously evaluated in mucosal cancers such as lung, head and neck, colorectal, oral, and bladder[4] but a lack of objective responses and failure to meet clinical endpoints has resulted in no Food and Drug Administration (FDA) approved mucosal cancer vaccines. Most traditional vaccination strategies have focused on enhancing immunogenicity via the parenteral route. However, T cells that have been exposed to cutaneous antigen in skin-draining lymph nodes (following the traditional intramuscular or subcutaneous route of vaccination), migrate preferentially to the skin, whereas effector cells that arise in mesenteric lymph nodes (LNs) or Peyer's patches (PPs) display a profound tropism for the small intestine.[5] Accordingly, it has been suggested that vaccine administration to mucosal surfaces (i.e., through oral, nasal, rectal or vaginal routes) elicits both mucosal and systemic immune responses,[6] whereas conventional parenteral immunization is generally a poor inducer of mucosal immunity and is therefore less effective against antigens at mucosal surfaces.[7,8] Moreover, most of the studies that have specifically compared routes of immunization suggest mucosal routes are preferable for the control of mucosal tumors (**Table 1**).

Induction of mucosal immunity against cancer however is a persistent challenge and requires a suitable delivery vehicle, able to overcome the barriers associated with traversing the mucosa and successful antigen presentation to T cells. Biomaterials can be defined as substances designed to interface with the body in order to elicit a biological response that is of clinical benefit. The versatility of biomaterials, by virtue of material type, shape, composition, and physicochemical properties may facilitate the engineering of mucosal vaccine platforms with improved efficacy. Biomaterials may be designed to (1) provide concomitant administration of antigen, adjuvant, and imprinting molecules to facilitate tumor homing, (2) overcome transport barriers associated with the structure and the physiology of mucosal surfaces, and (3) reshape the inhospitable and inaccessible tumor microenvironment to ensure long-term survival of homed T cells (e.g., checkpoint blockade, IL-12/15, CXCL9/10, etc.).[9] A range of synthetic and natural materials

Shiran Ferber received her B.Sc. in medicine and biological sciences and her Ph.D. from the Sackler School of Medicine, Tel-Aviv University, Israel. She then continued to a postdoctoral position in the Department of Chemical Engineering, working with Prof. Robert Langer to develop new vaccines against infectious diseases for developing countries. She is currently a postdoctoral associate in the Department of Engineering in Medicine at Brigham and Women's Hospital, Harvard Medical School, and at the Institute for Medical Engineering and Science at MIT. Her work focuses on immune engineering for cancer and autoimmune diseases.

Rodrigo J. Gonzalez is a postdoctoral fellow in the Department of Immunology at Harvard Medical School and received his Ph.D. in microbiology and immunology from the University of North Carolina at Chapel Hill. He studies how mucosal surfaces mount immune responses beyond the steady state with strategies that include the use and optimization of nanoparticle and other biomaterial vaccines to protect different mucosal tissues.

Natalie Artzi is an assistant professor at Brigham and Women's Hospital, Harvard Medical School. She is a principal research scientist at the Institute for Medical Engineering and Science at MIT and is an associate member of the Broad Institute of Harvard and MIT. She received her Ph.D. in chemical engineering at the Israel Institute of Technology (Technion). Her main research interests are in developing biomaterials for sensing- and therapy-applications. By studying tissue–biomaterial interactions under specific tissue microenvironments while considering biological and immunological mechanisms that are altered in the face of disease, the lab creates "personalized" materials and devices for diverse applications.

Table 1. Preclinical studies that assessed mucosal and nonmucosal routes of immunization for the control of different mucosal cancer models.

Vaccine	Model	Relevant findings	Ref.
B subunit of Shiga toxin bound to antigen. Intranasal or intramuscular administration	Orthotopic tumor of head, neck, and lung	Tumor growth was inhibited after intranasal delivery and not after intramuscular delivery.	[161]
Antigen mixed with cholera toxin. Oral, subcutaneous, or intraperitoneal administration	EL4 thymoma cell lines implanted into gastric tissue	Tumor growth suppression after oral delivery and not after subcutaneous or intraperitoneal delivery	[162]
Plasmo virus-like particles with antigen encoding plasmid. Intracheek, or intradermal delivery	Head and neck squamous cell carcinoma model with tumors implanted into the buccal mucosa	Long-term protection after intracheek delivery and not after intradermal delivery	[163]
DNA vaccine. Intramuscular delivery at sites thought to drain into LNs that also drain mucosal sites	Orthotopic cervicovaginal and oral cavity tumor	Tumor growth suppressed in vaccinated animals, except when the draining lymph nodes at the target site were surgically removed in advance.	[164]
Adjuvanted HPV polypeptide vaccine. Intranasal, intravaginal, and subcutaneous delivery	Vaginal tumor	Intranasal and subcutaneous immunization resulted in protection	[165]

may be employed as building blocks to fabricate particulate-based systems (e.g., nano/microparticles) and scaffolds to attract and/or manipulate cells and to release factors with spatiotemporal control.[10–15]

This, however, requires a better understanding of tissue–biomaterial interactions in the context of mucosal immunity, as well as key structure–function relationships to inform material design. While several studies have shown successful engineered mucosal vaccines in eliciting antibody responses,[16–18] which allows for a degree of extrapolation, such studies are lacking for induction of cell-mediated responses. Herein, we provide a brief overview of the central aspects of mucosal anatomy, physiology, and the principles that govern the generation of long-lasting protection in mucosal tissues. Moreover, we delineate the key considerations in material design as related to mucosal anatomy, physiology, and immunology. We highlight material types and forms that can be used as a function of administration route, choice of therapy, and regimen, while considering their potential translation into the clinic.

2. Mucosal Immunology: Considerations for Material-Based Vaccine Design

In the presence of the appropriate stimulus, specific immune responses can be orchestrated to trigger protection. This is one of the premises of vaccinology—providing a specific stimulus so that the immune system generates a long-lasting response to prevent (prophylactic vaccines) or ameliorate (therapeutic vaccines) disease. In spite of the substantial success of systemic vaccines, they have often proven to be suboptimal in protecting mucosal sites.[19] Remarkably, vaccination efficacy increases significantly when proper delivery takes place at mucosal sites.[20,21] Thus, the implementation of mucosal delivery for mucosal vaccines is essential. In order to engineer a proper vaccine formulation that targets mucosal sites, it is important to understand the immune components that are present in these tissues, how immune responses are triggered to generate long-lasting protection, and the barriers that hinder vaccine delivery to target cells. These aspects are discussed in this section.

2.1. The Immune Landscape in the Mucosae

For the purposes of vaccine design, mucosal sites can be divided into four main components: the epithelium, secretions that cover the epithelium (mucus), the lamina propria, and mucosa-associated lymphoid tissue (MALT) (**Figure 1**). The epithelium provides the first cellular barrier for antigens to penetrate mucosal tissues. Tissues that are more exposed to abrasion or damage, such as the lower genital tract, tend to possess stratified epithelium, while tissues where efficient exchange of materials is needed, like the intestinal tract, tend to have a simple epithelium. The composition of the epithelial layer can vary and includes cells with a high degree of specialization. **Table 2** only describes basic characteristics of the four most well-known specialized epithelial cells in the mucosa, namely, goblet cells, Paneth cells, microfold cells (M cells), and tuft cells. These cells have been studied mostly in the context of the gastrointestinal (GI) and respiratory tract and little is known about their presence in other mucosal sites.

Mucous secretions are composed of mucins, produced in the epithelium by goblet cells. In the mucosae, three main functions of mucous secretions can be highlighted: protection from desiccation, lubrication, and protection from foreign particles (e.g., microorganisms).[22,23] The latter function is relevant for cancer vaccine design using biomaterials, as mucus will vastly affect particle motility and penetrance.[22,24]

The lamina propria contains components that include lymphatic vasculature, blood vasculature, and different populations of leukocytes. The lymphatic vessels of the lamina propria serve as conduits through which soluble mucosal antigens and migratory antigen presenting cells (APCs), such as dendritic cells (DCs), reach the draining LNs. SLOs, such as LNs are sites where adaptive immune responses are initiated and where activated lymphocytes differentiate into tissue-tropic effector cells that express mucosal homing receptors. Once an effector cell has received proper "instructions" in a mucosal SLO, the cell departs via the draining lymphatics to enter the blood stream. Here, the mucosal blood microvasculature of the lamina propria is essential as the circulating effector cells must recognize and bind to local endothelial cells to home into their peripheral target tissue.

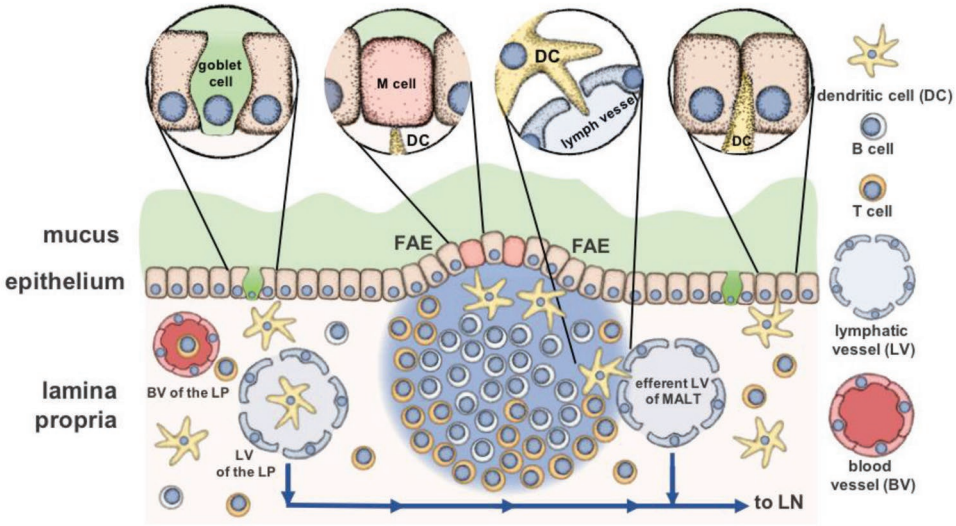

Figure 1. Mucosal tissues and key components to elicit long-lasting immune responses. Epithelial cells, covered by a layer of mucus produced by goblet cells, protect the lamina propria, where many immune components reside. DCs are found throughout the lamina propria, where they sample antigens to posteriorly migrate into lymphatic vessels (depicted in cross-section, with partially open walls). MALT, delimited by FAE (containing M cells) on the luminal side, is shown as a conglomerate of cells, that includes DCs, B cells, and T cells. This depiction of MALT shows B cells and T cells in well-defined areas, in an arrangement that resembles NALT. After priming and expansion, T cells travel through blood vessels (depicted in cross-section with red endothelial cells) to home into the mucosa. The circles at the top of the illustration depict magnified views of (from left to right) a goblet cell, an M cell with interacting with a DC, a DC entering a lymphatic vessel, and a DC interacting with the epithelium in search of luminal antigen.

In addition to these three mucosal compartments, mucosal sites contain MALT, which includes organized SLOs such as tonsils, PPs, and the appendix. Depending on its location, MALT can adopt more specific names: In the respiratory tract MALT is described as bronchiolar associated lymphatic tissue (BALT), in the GI as gut associated lymphatic tissue (GALT), etc. GALT has been studied more than any other MALT and it consists of PPs, the appendix, and isolated lymphoid follicles. In the GI, stimulation of cognate naïve T and B cells by mucosal antigens takes place at these three locations, thus referred to as inductive sites.[25] This is in contrast to effector sites (e.g., lamina propria), where effector cells play their specific role in immunity. Similar to LNs, MALT possesses B cell follicles, defined T cell zones, and high endothelial venules (HEVs), highly specialized blood vasculature that enables circulating lymphocytes to extravasate into a tissue.[25,26] MALT also contains large numbers of APCs, such as DCs and macrophages. MALT is surrounded on the mucosal luminal side by follicle-associated epithelium (FAE), where M cells reside. These cells facilitate the entrance of antigens into the mucosa. In summary, for vaccine purposes, the two most relevant characteristics of MALT are that it enables the entrance of luminal antigen into the lamina propria[26–28] and that it can facilitate the establishment of long-lasting protection in some mucosal tissues. Consequently, specific targeting of antigens and adjuvants to MALT is highly desirable for vaccine purposes.

Mucosal draining LNs are not strictly considered part of mucosal tissues but they play a key role in the establishment of mucosal immune responses. LNs are SLOs distributed throughout the body and can be connected to mucosal tissues through lymphatic vasculature. As opposed to MALT, LNs do not receive antigens directly from the lumen.[25] LNs are located deeper into the body and possess a more complex structure than MALT, as they possess a capsule (not present in MALT, with the exception of PP), and a defined cortex and medulla. In the cortex, the paracortex contains the T cell area, where the DC–T cell interactions that facilitate long-lasting protection occur.[29] Trafficking of immune cells through LNs is fundamental for the development of long-lasting protection. Immune cells enter LNs through afferent lymphatic vessels and HEV, and exit through efferent lymphatic vessels to eventually reach the systemic blood circulation.[5,30] Immune cell migration through LNs is of remarkable importance as LNs can act as inductive sites for mucosal tissues.

Table 2. Specialized epithelial cells in mucosal tissues.

Type	Function	Reported tissues	Ref.
Goblet cell	Mucin secretion, perhaps transepithelial trafficking[62]	Nasal mucosa, gastrointestinal tract, and genital tract	[166]
Paneth cell	Regulation of host–microbiota interactions through secretion of antimicrobial peptides	Small intestine	[167]
Microfold-cell (M-cell)	Transepithelial trafficking	Colon, small intestine, and nasal mucosa	[54,56,58,59,152,168]
Tuft cells	Chemosensory cells with a proposed role during parasitic infections	Colon, small intestine, and respiratory tract	[169]

2.2. Eliciting Long-Lasting Responses against Tumors

Some similarities exist between cancer vaccines and vaccines for infectious diseases. However, the design of most cancer vaccines is particularly aimed at eliciting the differentiation of tumor antigen-specific $CD8^+$ T cells into cytotoxic $CD8^+$ T cells (also known as cytotoxic effector cells or CTLs),[31] as the goal is to eliminate tumor cells. For T cell-dependent long-lasting protection to occur, APCs must be exposed to antigens. DCs excel at finding and phagocytosing antigens, mostly in the lamina propria, though some reports have described intestinal DCs to partially cross the epithelium to engulf luminal particles.[19,20] Phagocytosis of antigen by APCs can also take place in MALT, after antigen transcytosis through M cells (e.g., in PP and nasopharynx associated lymphatic tissue (NALT)), and in mucosal draining LNs after transport via afferent lymphatics. Phagocytosed antigen is processed inside the DC and antigen-derived peptides are loaded into molecules termed major histocompatibility complex (MHC) in mice, or human leukocyte antigen (HLA) in humans. Peptide-loaded MHC complexes are transported to the DC surface so peptides can be presented to T cells in complex cell-to-cell interactions. Endogenous peptides degraded in the cytosol by the proteasome are loaded into MHC class I (MHC-I) and are presented to $CD8^+$ T cells, while exogenous peptides are loaded into MHC class II (MHC-II) molecules and are presented to $CD4^+$ T cells.[32,33] Accordingly, exogenous protein or peptide antigens, provided by vaccination formulations, will primarily be processed to enter the MHC-II pathway. This represents a challenge for a cancer vaccine aiming to trigger $CD8^+$ T cell activity. In a process termed cross-presentation,[33–35] however, a small and specialized subset of DCs known as cDC1 ($CD8\alpha^+$ DCs)[36] can present antigen through the MHC-I pathway to prime $CD8^+$ T cells.[35] Consequently, this subset should be of major consideration as a target when designing cancer vaccines. For example, delivery of Fms-like tyrosine kinase 3 ligand (Flt3L) was recently demonstrated to promote accumulation of intratumoral, cross-presenting DC, resulting in tumor-specific $CD8^+$ T cell response.[37]

Interactions of DCs with naïve T cells, whose T-cell receptors recognize the peptide-MHC complex on the APC carries, aided by costimulatory molecules, result in the generation of activated antigen-specific T cells. This process, termed priming, is of great importance as the newly generated antigen-specific T cells will clonally expand and can potentially become long-lived memory cells, essential for protection.[20] Expanded antigen-specific T cells exit the LN via the draining lymphatics and ultimately enter the blood to circulate throughout the body and home to target tissues. Importantly, priming can occur in both, LNs and in MALT, although beyond the intestinal PPs,[27] BALT,[38] and the NALT,[39] MALT has not been studied in detail or reported in every mucosal tissue. The presence of tissue-specific cues during priming, for which peripheral tissue-derived migratory DC are key, favors homing to specific tissues, in a process termed imprinting.[40] Homing requires the expression of a combination of traffic molecules (e.g., $\alpha_4\beta_7$ and CCR9)[41] on T cells that bind to counter-receptors or ligands (e.g., MAdCAM-1 and CCL25) expressed by the vasculature of target tissues.[5,42] Thus, imprinting will determine the traffic molecules that the T cell will express and that will bind to ligands present in the vasculature of target tissues. This will facilitate the extravasation of the T cell into the target tissue in a process that employs complex molecular interactions with the endothelium.[22,29] After homing, CTLs can kill cells associated with cognate antigen (i.e., tumor cells) by mechanisms that include the secretion of effector cytokines and cytotoxic activity. Furthermore, for long-term protection to occur, homed effector cells must give rise to long-lived tissue-resident memory cells (T_{RM}), characterized by the expression of the markers CD69 and CD103.[43] Once in the target tissue, T_{RM} are nonrecirculating (they do not migrate beyond the tissue) and play the role of sentinels that can adopt effector functions upon recognition of antigen.[43] Thus, in the case of a mucosal cancer vaccine, it is mucosal T_{RM} who will facilitate the elimination of tumor cells.

This simplified description of how mucosal immunity occurs is shared by most T cell vaccine approaches and highlights the role of APCs in the establishment of long-lasting protection. Accordingly, APCs can be thought of as the ultimate target cells for the delivery of biomaterials.

Of note, while traditional prophylactic vaccines are administered to naïve individuals, therapeutic cancer vaccines will introduce antigens that already exist in a patient. This represents a major challenge as pre-existing antigens can trigger immune responses that are inadequate for protection. In the absence of the correct stimulatory signals, encounter with antigen can result in T cell phenotypes that correlate with dysfunction or unresponsiveness, resulting in tumor escape.[44] Indeed, the tumor microenvironment contains cells with phenotypes that correlate with anergy (induced hyporesponsiveness) and exhaustion (decreased effector function).[45] Thus, and as opposed to prophylactic vaccines, therapeutic vaccine design must take into consideration reprogramming of this inadequate response.

2.3. Key Players in the Mucosal Delivery of Biomaterials

2.3.1. The Mucus Barrier

Mucins are the key component of mucus secretions and are composed of peptides with hydrocarbon side chains decorated with glycans.[46] Mucus secretions are the first barrier a biomaterial will encounter when delivered to a mucosal surface. It is a hydrophilic viscous fluid that while allowing for the exchange of gases and nutrients, will trap and immobilize antigenic materials of certain sizes, including bacteria and particles.[22,47] While immobilized, biomaterials can be exposed to hydrolytic or enzymatic activity compromising not only the delivery platform but also its cargo.

The GI tract produces large amounts of mucus, in the range of liters per day. Mucus in the GI tract can accumulate to form a mucus blanket, which can be up to 800 μm thick in the colon.[48] This blanket is complex in structure, forming two separate layers. A more rigid inner layer is attached to the epithelium while a less dense and more fluid layer faces the lumen.[49] Thus, in the GI tract mucus builds up growing perpendicular to the epithelium and is eliminated as intestinal contents pass through. In the respiratory tract, mucus accumulates less

proficiently with movement occurring almost immediately after secretion, aided by beating cilia of the epithelium. Overall, this illustrates how mucus is continuously produced, removed, and renewed. Consequently, mucus-associated biomaterials are at risk of being shed along with feces, sputum, saliva, etc.

Mucus can interact with biomaterials through electrostatic forces due to its negative charge,[41] which originates from carboxylate and sulfate groups.[22] Because of the variability in the presence of these groups, charge can vary in different mucosal organs. Biomaterials can also interact with the mucus by adsorption due to hydrophobic interactions with lipid-coated domains as well as hydrogen bonding with glycoproteins and van der Waals forces, which all increase adhesion. For the purpose of vaccine delivery with biomaterials mucus should be considered a protective mesh. As with other mucus properties listed in this review, pore size is highly variable between organs and in the same organ between species.[22,50,51] Most mucus properties have been determined by studies on the GI tract, and to some extent the lower respiratory tract or lower female genital tract (FGT). However, chemical and physical properties of mucus might vary in other mucosal organs. In addition, the physiology of a mucosal site can vary depending on hormonal stage, age, and disease type and state.

2.3.2. The Epithelial Barrier

The epithelium represents a second barrier (after mucus) that biomaterials have to cross to deliver an antigen, mainly to APCs—the main target cells for immunization that reside in the lamina propria.

Besides modifying the physical characteristics of biomaterials (e.g., size and charge), their surface may also be modified with molecules that target the epithelium to facilitate intake and transcytosis.[52] In the intestine, two cell types of the epithelium have been considered as targets for trans-epithelial delivery: M cells and enterocytes. It is widely accepted that M cells can serve as portals for particles to cross the epithelium, perhaps with the best examples coming from microbial pathogenesis.[53,54] For example, *Salmonella typhimurium* interactions with the epithelium can occur through GP2, a protein expressed on M cells.[55] Accordingly, M cells of the GI tract[56] and the nasal mucosa[54,57] have been suggested as attractive targets for vaccine delivery. M cell-specific ligands,[58,59] such as the mouse antibody NKM 16-2-4,[60] have already been used successfully in some vaccine models.[58] In humans, clusterin and cathepsin E have been described as M cell markers in the tonsils, adenoids, PPs, appendix, and colon.[59] It has been proposed that DCs can access the lumen of a mucosal tissue by extending their dendrites through M cells[61] but very little is known about this process and whether it might occur with vaccine formulations using nano- and microscale materials.

At tissues where M cells are absent, less-specialized epithelial cells (e.g., enterocytes) are an alternative target for vaccine delivery. In addition to bacterial toxins, plant lectins and microbial adhesins have been proposed for targeting these cells.[52] Notably, the ganglioside GM1 has been modified and used in a strategy that suggests successful transcytosis through non-specialized epithelial cells in the GI and in the nasal mucosa of mice.[62] Accordingly, modified gangliosides could be conjugated to biomaterials to facilitate transcytosis through enterocytes in the GI or enterocyte-analogous cells in other mucosal surfaces.

3. Targeting Immunity to the Right Tissues

3.1. Immunization Route

As mucosal delivery is key to obtaining long-lasting mucosal protection, it is important to summarize possible mucosal routes and highlight their advantages and disadvantages (**Figure 2**). The nasal mucosa has multiple advantages as an administration route, most notably by facilitating needle-free self-administration.[63] Immune-reactive sites and specialized lymphatic tissue (i.e., NALT, in mice, and the adenoids and tonsils of the Waldeyer's ring in humans) are present in the nasal mucosa.[27,51,58] With the exception of NALT,[64] the nasal compartment is an understudied tissue and very little is known about the initiation and regulation of immunity in this site. Notwithstanding, multiple vaccine strategies have used the nasal mucosa as an inoculation site,[49,60] with an intranasal attenuated virus[65] vaccine against influenza virus already in clinical use.[66] It is important to highlight that administration of intranasal vaccines has been reported to sporadically result in facial nerve paresis (Bell's palsy)[67] and that these effects might raise skepticism in the use of intranasal routes for vaccination.

Delivery through an oral route can potentially expose the entire GI tract to a vaccine (Figure 2C). The main caveat of oral routes is that hydrolyzing enzymes or gastric acid can degrade ingested biological materials. For this reason, biomaterials delivered orally must be designed to resist the harsh environment of the stomach and upper small intestine, without compromising their ability to release cargo. Another caveat is the large area of the GI tract as any vaccine formulation is likely to be highly diluted by the time it reaches distant tissues such as the colon. This is in addition to the possibility of vaccine components, especially proteins, being absorbed and processed in the liver, rather than reaching SLOs. Lastly, and as previously mentioned, the GI mucosa is protected by large amounts of mucus that can hinder vaccine penetrance.

The FGT is of high relevance as many cancers affect this tissue, particularly the cervix. For experimental purposes in mice, vaccine delivery can take place into the vagina or directly into the uterus through a commercially available transcervical delivery device.[68] Nanomaterial-based vaccines have successfully exploited this administration route.[69] However, mucus can be present in the lower genital tract in larger amounts in comparison to the upper genital tract and can be notably altered by disease or hormones. Thus, the estrous cycle (or menstrual cycle in humans) must be taken into consideration for intrauterine or intravaginal delivery. Moreover, another aspect to consider is the possibility of undetected pregnancies.

In addition to the three mucosal administration routes described, three relevant aspects are worth mentioning regarding delivery to mucosal tissues. First, sites like the colon and female upper genital tract can be immunized following

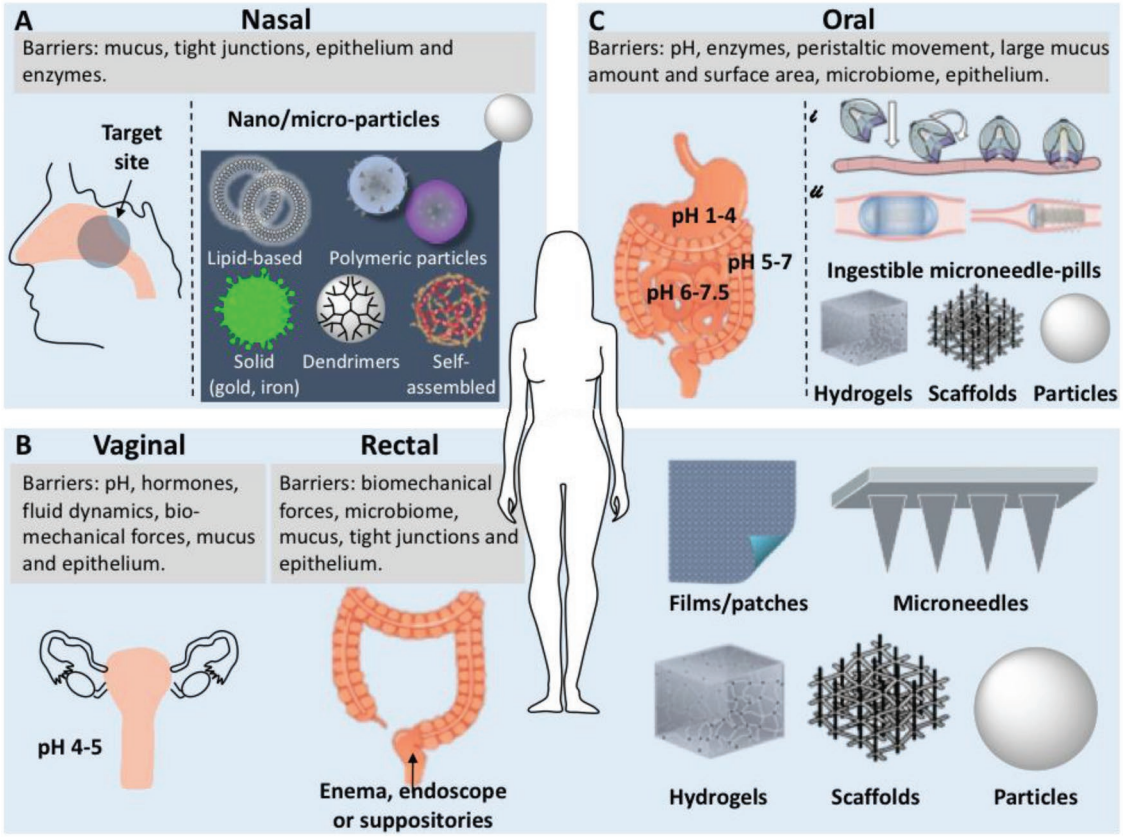

Figure 2. Key biomaterials-based platforms to overcome mucosal delivery barriers associated with each vaccination administration route—nasal, vaginal, rectal and oral delivery. A) Nasal delivery of biomaterials mainly utilizes particulate systems for delivery. These include NPs and MPs fabricated from lipids (to form nanocapsules or liposomes), polymeric particles encapsulated in (purple) or conjugated to (blue) the vaccine, solid particles, dendrimers, and self-assembled particles (like chitosan, poly(beta-amino esters) (PBAE), and polymersomes). B) Films, patches, microneedles, hydrogels, scaffolds, and NPs/MPs may be applied to the vaginal and rectal mucosa as via enema, specialized suppositories or via endoscope. C) Oral delivery devices including microneedle-based devices that directly penetrate the gastric mucosa (i) and the intestinal mucosa (ii), hydrogels, scaffolds, and NPs/MPs. i) Reproduced with permission.[124] Copyright 2019, The Authors, published by AAAS. ii) Reproduced with permission.[125] Copyright 2014, Wiley Periodicals, Inc. and the American Pharmacists Association.

vaccine delivery in the rectum and lower genital tract, respectively.[22] This is facilitated by peristaltic movements of these organs, and perhaps by simple diffusion. Second, a not fully understood phenomenon of mucosal immunity consists of the ability to imprint effector T cells to traffic to mucosal tissues distant to the site where immunization took place. For example, a vaccine against *Chlamydia trachomatis* confers $CD4^+$ T cell-dependent long-lasting protection in murine uteri after intranasal immunization.[69] In addition to the FGT, intranasal immunization has also been reported to generate protection in both the lungs and the GI tract.[21] While any given vaccine formulation might behave in a different manner in each tissue, protection of hard-to-reach mucosal tissues after immunization at a distant, yet more accessible, site is desirable and should be considered when choosing a delivery route. Third, it has been reported that subcutaneous injections of antigen and adjuvant combined with retinoic acid induces T cell homing and protection against oral *S. typhimurium* infection in the small intestine.[70] The possibility of T cells homing to mucosal tissues after subcutaneous injection by this or similar strategies could have a high impact on the implementation of mucosal cancer vaccines.

3.2. Antigen and Adjuvant

Two types of cancer-related antigens exist: those associated exclusively with tumors, termed tumor-specific antigens (TSAs); and those expressed at variable levels in healthy tissues but highly expressed in tumors, termed tumor-associated antigens (TAAs).[10,63,64,69] The advantage of TSAs over TAAs is that since they are expressed exclusively by tumor cells, they provide high specificity. Many of the proposed TSAs arise from human papilloma virus (HPV) or hepatitis B virus-associated cancers. Great success has been seen with prophylactic HPV vaccines, however these vaccines are efficient in preventing cervical cancer by targeting a pathogen and, to date, only a few pathogens are etiologically linked to cancer.[71] Neoantigens are a subclass of TSAs that are often only weakly immunogenic and that result from mutations found exclusively in tumors and not in healthy tissue. As higher neoantigen load is associated with better patient outcome, presumably as a result of a better T cell response,[31] neoantigens are considered promising candidates for cancer vaccines. However, neoantigens arise from random genomic mutations in a single tumor and thus may be unique to a

given tumor and must be individually identified and synthesized for every individual patient.

In the absence of inflammation (e.g., danger signals), exposure to antigen can result in tolerance. Adjuvants accompany antigen in vaccine formulations and can directly or indirectly provide signals that are lacking when the tissue is not disrupted, for instance by infection.[72] Common adjuvants, therefore, mimic pathogen activity by activating pattern recognition receptors, such as the Toll-like receptor (TLR), a system used by host cells to detect microbial invaders and trigger inflammation. An ideal adjuvant must trigger long-lasting responses with high efficiency so the number of vaccine administrations is reduced, in addition to showing low or no toxicity.[73] While a large number of adjuvants has been described, their efficiency is considerably reduced when applied to mucosal surfaces. Efforts to find adjuvants with higher immunogenicity than those employed for systemic vaccines resulted in the use of molecules that derive from infectious agents, albeit with high toxicity. Cholera toxin (CT) and heat-labile enterotoxin from *Escherichia coli*, for example, are highly immunogenic bacterial derivatives whose high levels of toxicity prompted the need of modifications through genetic engineering.[73] While modifications of these bacterial toxins preserve immunogenicity and reduce toxicity, adverse effects, such as facial paralysis after nasal immunization, are still reported in some human studies.[25] Other TLR ligands that have been successfully used as adjuvants for mucosal vaccines include monophosphoryl lipid A (MPL), CpG oligodeoxynucleotide, flagellin, and polyinosinic:polycytidylic acid (poly-I:C).[31,73] Other commonly used adjuvants include saponin-derived adjuvants,[74] of which ISCOMATRIX has shown induction of T cell responses in clinical trials.[75]

3.3. Testing for Vaccine Efficacy

Design of efficacious mucosal cancer vaccines using biomaterials must rely on the convergence of materials science and immunology. Therefore, it is imperative to develop assays that will provide informative data regarding in vivo vaccine efficacy, and its correlation with the physical properties of the delivery platform. This includes particle size, size distribution, stability at different pH levels and temperatures, antigen and adjuvant maximal load, antigen and adjuvant release under different conditions (e.g., pH, temperature), aggregation and solubility, storage profiles (stability at low temperatures over time and consecutive freeze–thaw cycles), as well as polymer composition.

While much information can be obtained from in vitro assays, they tend to neglect key components of both the immune system and the mucosal environment while possibly adding confounding factors. Preclinical tests for vaccine efficacy are typically done in mice and nonhuman primates to determine if the expected immune responses, including protection, occur in relevant tissues. There are many possibilities to assess T cell-specific responses after immunization.[76] A common alternative is to use mice expressing a transgenic T cell receptor that recognizes a model peptide. This implies isolating T cells from these mice for adoptive transfer into naïve mice prior to immunization. The so called OT-I and OT-II systems, respectively, provide $CD8^+$ and $CD4^+$ T cells that respond specifically to a foreign protein, ovalbumin.[77] Adoptively transferred T cells from these systems are detected in recipient mice through congenic markers using flow cytometry analysis or through microscopy if the cells are fluorescently labeled. Attention must be paid to the number of cells that are adoptively transferred to avoid exceedingly high frequencies of antigen specific T cells in recipient mice. Common parameters to measure in these systems are T cell expansion and homing to relevant tissues. Importantly, homing and the establishment of T_{RM} are parameters that cannot be routinely assessed in patients. For human trials, assessment of vaccine success is typically conducted in circulating blood. Promising results from blood tests can be misleading as they do not take into consideration immune responses in the target mucosal tissue. For this reason, ideally, biopsies should be employed to collect tissue samples and to confirm the presence of T_{RM} in patients.

Lastly, while expansion and homing are important, the ultimate goal is to detect effector T cell activity and the effect on tumor growth. For this to occur, the tumor must express the peptides that T cells react to. In the case of the OT-I and OT-II mouse systems, there is a large number of cancer models where syngeneic tumors express ovalbumin. These models include the colon carcinoma cell line MC38-OVA[78] and melanoma cell line B16-OVA. By transplanting such cells (most commonly under the skin), tumors can be induced at different time points after immunization for the assessment of phenotypic changes on T cells and tumor progression.

4. Mucosal Cancers

4.1. Clinical Trials of Mucosal Cancer Vaccines

The prevalence and mortality associated with mucosal tumors clearly highlights an unmet medical need that mucosal cancer vaccines may fulfill by complementing traditional therapeutic approaches or as an independent personalized therapy. The potential of mucosal cancer vaccines is evident by the increasing amount of preclinical literature,[31,79,80] which has laid the foundation for a large number of therapeutic vaccine strategies that are currently being clinically evaluated (**Table 3**). Cancer vaccines can be broadly categorized into tumor cell vaccines, DC vaccines, protein/peptide-based or genetic-based (DNA, RNA, viral).[80] Intramuscular and subcutaneous administration routes of soluble vaccines are the most commonly used, which is reflective of the current clinical landscape with respect to vaccination.

The mucosal route of vaccination has received little to no attention in the clinical setting. One formulation currently being investigated in a phase II clinical trial in cervical cancer patients centers around a tablet-based oral preparation of hydrolyzed TAAs derived from the blood and tumor of the patient, termed V3-Cervix (NCT03550755). The main etiological agent in cervical cancer is HPV types 16 and 18, therefore most therapeutic vaccination strategies and consequently clinical trials focus on targeting and eradicating the oncogenic virus by mounting a cell-mediated immune response using viral components. The outcome of this trial will be interesting as the route

Table 3. Vaccines for mucosal cancers under clinical investigation.

Indication[a)]	Product	Antigen[a)]	Adjuvant[a)]	Biomaterial based?	Route of administration	Phase	ClinicalTrial.gov ID
Lung cancer	Tepodi (OSE2101)	Synthetic peptides primarily derived from known TAAs		No	Intramuscular	III	NCT02654587
Lung cancer	DC vaccine	Autologous dendritic cells pulsed with allogenic NSCLC cells		No	Intradermal	II	NCT00103116
Lung cancer	P10s-PADRE	P10s carbohydrate mimetic peptide fused to pan HLA DR binding epitope	Montanide ISA-51	No	Subcutaneous	I/II	NCT02264236
Lung cancer	GEN-009	Up to 20 stimulatory peptide neoantigens	Poly ICLC	No	Subcutaneous	I/II	NCT03633110
Colon cancer	OncoVAX	Autologous tumor cells	BCG	No	Intradermal	III	NCT02448173
Colon cancer	APDC	Antigen pulsed DCs + chemotherapy		No	Intravenous	III	NCT02503150
Colorectal cancer	COREVAX-1	Autologous dendritic cells loaded with autologous tumour homogenate		No	Intradermal	II	NCT02919644
Colorectal cancer	PolyPEPI1018	Six synthetic peptides from cancer testis antigens	Montanide ISA-51	No	Subcutaneous	I/II	NCT03391232
Gastrointestinal cancers	IMU-131	Single peptide composed of 3 B-cell epitope sequences against HER2	Montanide ISA-51	No	Intramuscular	I/II	NCT02795988
Mucosal melanoma	Tyrosinase peptide	Tyrosinase, gp100 and MART1	Incomplete Freund's adjuvant or Montanide ISA-51	No	Subcutaneous	III	NCT01989572
Mucosal melanoma	LPV7	Seven long peptides based on melanoma antigens + tetanus peptide	Poly ICLC, resiquimod or Montanide ISA-51	No	Intradermal and subcutaneous	I/II	NCT02126579
Cervical cancer	V3-Cervix	Hydrolyzed tumor anitgens from blood and tumor		No	Oral	II	NCT03550755
HPV16 or 18 positive cervical cancer	GX-188E	DNA encoding the E6/E7 fusion protein combined with FLT3L		No	Intramuscular	I/II	NCT03444376
HPV16 positive oropharyngeal cancer	ISA101b	Multiple peptides that mimic E6 and E7 oncoproteins of HPV		No	Intramuscular	II	NCT03258008
Head and neck cancer	MVX-ONCO-1	Irradiated autologous tumor cells + genetically modified MVX-1 cells		No	Subcutaneous	II	NCT02999646
Urothelial and bladder cancer	PGV001	Synthetic peptides corresponding to individual neoantigens	Poly ICLC	No	Intramuscular	I	NCT03359239
Bladder cancer	RUTI	Detoxified cellular fragments of *M. tuberculosis*	BCG	Liposomes	Subcutaneous	I	NCT03191578
Lung, colon or rectal cancer	Tecemotide (L-BLP25)	Synthetic lipopeptide of MUC1	MPL	Liposomes	Intravenous	I/II/III	NCT01462513 NCT01507103 NCT00409188 NCT00960115[b)]

[a)]Abbreviations: TAA – tumor-associated antigens; DC – dendritic cells; NSCLC – nonsmall cell lung cancer; HLA DR – human leukocyte antigen DR isotype; Poly ICLC – polyinosinic–polycytidylic acid, poly-L-lysine double-stranded RNA and carboxymethylcellulose; BCG – bacillus Calmette–Guérin; HER2 – human epidermal growth factor receptor 2; MART1 – melanoma antigen recognized by T-cells 1; HPV – human papillomavirus; FLT3L – FMS-like tyrosine kinase 3 ligand; MUC1 – mucin 1; MPL – monophosphoryl lipid A; [b)]Study did not meet clinical primary or secondary endpoints.

of administration is oral rather than intramuscular as in most therapeutic vaccine clinical trials, aiming at reawakening the immune system to aberrantly proliferating tumor cells and not outright elimination of the virus.

Despite the vast number of clinical trials of therapeutic cancer vaccines, there have only been three FDA-approved therapies, Sipuleucel-T for prostate cancer, talimogene laherparepvec for advanced melanoma, and bacillus Calmette–Guérin (BCG) for urothelial cancer of the bladder, the latter of which has been approved for intravesical administration since 1990.[81] Lagging even further behind is the number of clinical trials using biomaterials for mucosal cancers, which currently stands at two (Table 3). RUTI is a liposomal formulation of detoxified cellular fragments originating from *Mycobacterium tuberculosis* and

was initially proposed as a therapy for latent tuberculosis. The mucosal response to RUTI following intravesical BCG, which is standard therapy for superficial bladder cancer, is currently being investigated in a Phase I trial (NCT03191578). Tecemotide is also a liposomal formulation comprised of cholesterol, dimyristoyl phosphatidylglycerol, and dipalmitoyl phosphatidylcholine. It contains a synthetic lipopeptide of mucin 1 which is overexpressed by several cancers and was designed for intravenous administration. Tecemotide was investigated in Phase II trials for treatment of colon and rectal cancer as well as a Phase III trial for treatment of lung cancer. However, after a Phase I/II trial in a subset of patients with lung cancer (NCT00960115), tecemotide failed to meet the primary and secondary end points and was subsequently discontinued.

Although the outcomes of mucosal cancer vaccines in clinical trials are disappointing,[4] these results can inform future investigations. The type of antigens investigated, along with any complementary adjuvants or immunotherapy arms, dosing schedules, toxicities, and route of administration may inform the future design of engineered vaccines in order to achieve the desired antitumor response in mucosal tissues. For example, several clinical trials have combined adjuvants with varied TAAs-derived synthetic peptides, autologous whole tumor cells lysate or personalized genomic vaccines, whereby the resected tumor of each individual is sequenced and synthetic peptides are identified using a computational pipeline.[82,83] Other trials have examined the therapeutic efficacy of vaccines in combination with immunotherapy, including the programmed death protein 1 (PD-1) inhibitor nivolumab, programmed death-ligand 1 (PD-L1) inhibitor atezolizumab, and utomilumab (a monoclonal antibody against CD137).[83,84] Indeed, identifying combination, synergistic therapies, and exploration of the mucosal route for vaccination may yield further progress.

4.2. Bridging the Gap in Mucosal Immunity with Biomaterials

When designed properly, biomaterials provide a molecular toolkit with which to favorably modulate the antitumor immune response. However, most preclinical studies of engineered mucosal cancer vaccine are not addressed as mucosal cancers. For example, as in **Table 4**, tumor xenografts are typically implanted subcutaneously, which does not sufficiently recapitulate the immune cascade upon vaccination in an orthotopic or a genetically modified model, and parenteral rather than mucosal route is used. Moreover, evaluating efficacy strictly by the induction of systemic immunity rather than mucosal immunity may be misleading. For example (Table 4), while subcutaneous vaccination with negatively charged silica nanospheres was successful in eliciting T cell responses,[85] if administered via mucosal routes, these would have most likely be immobilized in the mucus layer, thus releasing the soluble vaccine, which may elicit predominantly humoral response. The same applies to other examples illustrated in Table 4, including polycationic and mucoadhesive chitosan[86] and a hydrophobic alkyne functionalized 4-arm star polymer conjugate which formed microparticles.[87]

Under physiologically relevant settings, local and, by extension, distal mucosal antitumor responses can be efficiently manipulated using biomaterials. Biomaterials can: (1) protect antigens and adjuvants from mucosal degradation, (2) enable spatiotemporal delivery and control of multiple therapeutic entities, and (3) orchestrate appropriate immune cell trafficking, determining the magnitude and the nature of the immune response by virtue of the biomaterial physicochemical properties and the antibody/ligand-mediated targeting of mucosal-resident immune cells. These concepts are elegantly illustrated by the multiplicity of studies emerging with the unified aim of propelling mucosal vaccine technology forward using biomaterials.[69,88] Other than vaccine delivery vehicle, once a proper T cell response has been initiated, biomaterials may also aid to augment the antitumor response by altering the intratumoral microenvironment to ensure T cell survival and function. This aspect, however, while relevant to any cancer vaccine, is thoroughly discussed elsewhere.[89–93]

Particulate delivery systems include nanoparticles (NPs) and microparticles (MPs), which can be synthesized from a diverse range of materials and fabricated to form self-assembled particles (e.g., liposomes, emulsion, micelles, polymersomes), covalent systems (e.g., dendrimer and polymer conjugates), metallic particles (e.g., gold and silver), metal oxide particles (e.g., iron oxide), and carbon materials (e.g., fullerenes, carbon nanotubes) (Figure 2A).[72,94,95] **Table 5** summarizes some of the most common materials used to fabricate mucosal vaccines and their relevant properties to guide material choice. Antigens and adjuvants to be encapsulated within, entrapped or conjugated to the surface of the particles, thus attenuating their degradation and controlling their release. Moreover, the physicochemical properties of these particles can be rationally engineered to enable enhanced delivery to target cells and cellular compartment (**Figure 3**). Nasal delivery of antigens formulated with polyethyleneimine (PEI)[96,97] and lipid particles[98,99] has been previously shown to elicit CTL as well as antibody-mediated immune responses in genital, rectal and GI tissues. Li et al.[99] fabricated stabilized liposomes, by crosslinking the lipid headgroups, referred to as interbilayer-crosslinked multilamellar vesicles (ICMVs). By delivering ICMVs to the lung mucosa, they were able to target the high density of antigen-sampling APCs across the airway epithelium, achieve increased delivery to SLO, and thus the localization of CTL in various mucosal tissues, including the lungs, FGT, and GI. By contrast, nasal vaccination with soluble antigen/adjuvant, was poorly immunogenic. Importantly, the ICMVs-based vaccination strategy established long-lived T_{RM} in those tissues. Similarly, nasal vaccination with PEI carrying a plasmid DNA induced potent mucosal and systemic CTL responses, which resulted in the generation of T_{RM}.[97]

Another class of biomaterials that can be utilized to deliver mucosal cancer vaccines is scaffolds. These may be in the form of injectable, implantable or ingestible hydrogels, gels/foams, devices, films/patches, and microneedles. Scaffolds enable attaining localized and sustained delivery of payload in an orchestrated manner over an extended period of time (days to weeks).[90,93] Scaffolds also provide spatiotemporal control over the release of multiple immunomodulatory entities, which may ultimately lead to increased antigen presentation and an enhanced immune response. As scaffolds should be retained

Table 4. Nonexhaustive examples of biomaterial-based therapeutic cancer vaccines investigated using syngeneic mouse cancer models.

Tumor type (and model)[a]	Materials	Material properties	Antigen[a]	Adjuvant/other component[a]	Route of administration	Outcomes[a]	Strengths[a]	Weaknesses[a]	Ref.
Lung (LLC, subcutaneous xenograft)	Mesoporous silica	Negatively charged, hollow nanospheres with porous architecture, ≈200 nm diameter	LLC cell fragments	The nanospheres themselves	Subcutaneous	Increased survival after initial tumour and re-challenge with evidence of effector memory T cells	Facile particle synthesis, high loading capability and no need for adjuvants	Concerns of degradability, bioaccumulation and toxicity of silica	[85]
Lung (LLC, subcutaneous xenograft)	Ethyl vinyl acetate	Polymeric rods, controlled release of chemoattractant	Synthetic peptide corresponding to a H-2k^b-restricted epitope (MUT1)	MIP-3β	Subcutaneous	Significantly reduced tumor diameter	Early proof of concept of in situ Langerhans cell recruitment and priming using biomaterials, efficacy in multiple models	Non-biodegradable, more effective chemokines for DC attraction and immunostimulation (e.g., GM-CSF)	[170]
Lung (LLC, subcutaneous xenograft)	Poly(lactide-*co*-glycolide)	Macroporous (80-90%) polymer scaffold designed to pulse GM-CSF then sustain the release due to a high-pressure CO_2 foaming process	Tumor cell lysate	GM-CSF and CpG-ODN or Poly ICLC	Subcutaneous	Cytotoxic (CD8) T cell mediated tumor shrinkage and enhanced survival	Modular technology demonstrated by different adjuvants, biological rationale of release kinetics	Biopersistence may lead to T cell sequestration at scaffold site and may result in exhaustion, only effective with certain adjuvants.	[171]
Cervical (TC-1, transformed murine lung epithelial cells, subcutaneous xenograft)	Poly(*tert*-butyl acrylate)	Hydrophobic alkyne functionalized 4-arm star polymer conjugated with azido-peptide sequences which formed microparticles	HPV-16 E7 protein epitope	None	Subcutaneous	Improvement in survival over free peptide with adjuvant and other comparators	Single injection, increased stability compared with free peptide	Complex synthesis involving several purification steps, modification of peptide required, immunobiology not investigated	[87]
Head and neck (MOC2-E6E7, subcutaneous xenograft)	Multidomain peptides	The peptide $K_2(SL)_6K_2$ forms antiparallel β-sheet nanofibers in solution which, upon crosslinking, form an injectable hydrogel	None	Synthetic cyclic dinucleotide ML RR-S2 CDA (CDN)	Intratumoral	Sustained release of CDN upon hydrogel injection resulted in 6/10 tumor regressions and greatly improved overall survival	Single injection was able to regress tumors, well characterized delivery system.	Concentrations of CDN studied may be cytotoxic and result in aberrant STING activation, identity of inflammatory infiltrate within hydrogel not known	[172]
Head and neck (MOC2-E6E7, subcutaneous xenograft)	Mesoporous silica	High aspect ratio, injectable nanorods that spontaneously assemble to form scaffolds in situ, creating interparticle pores for cell migration	HPV-16 E7 peptide	CpG-ODN and GM-CSF	Subcutaneous	Generation of antigen specific CD8 T cell response and small to moderate enhancement of survival	Demonstration of immunoediting on vaccine efficacy, vaccine components well characterized, can actively recruit immune cells.	Tumors were able to persist in most vaccinated animals, potential toxicity and inflammation as a result of amorphous silica degradation.	[173]
Bladder (MB49 and MBT-2, orthotopic and subcutaneous xenograft)	Chitosan	Polycationic and mucoadhesive chitosan complexed with IL-12 as a high viscosity solution	None	IL-12	Intravesical	Chitosan/IL-12 complex was found to eradicate orthotopic tumors, prolong survival and generate systemic immunity.	Simple formulation, initially cured mice were protected against rechallenge due to adaptive response and induction of memory.	Translationally challenging delivery route, toxicity concerns with cytokine delivery	[86]

Table 4. Continued.

Tumor type (and model)[a)]	Materials	Material properties	Antigen[a)]	Adjuvant/other component[a)]	Route of administration	Outcomes[a)]	Strengths[a)]	Weaknesses[a)]	Ref.
Colon (CT-26, subcutaneous xenograft)	Dioleoyl lipids and hyaluronic acid	Slightly anionic multilamellar lipid-biopolymer nanoparticles, crosslinked by maleimide-thiol chemistry, ≈250 nm	Immunogenic cell death induced by mitoxantrone (no exogenous antigen)	CpG-ODN	Subcutaneous	When combined with anti-PD1, a single dose of vaccine induced complete regression of 7/9 tumors.	Highly efficacious therapy, antigen source is such that the vaccine very closely mimics the tumor cells.	Cell line variability may not generate sufficiently immunogenic antigens and may prove hard to scale-up	[174]
Colon (MC-38, subcutaneous xenograft)	Synthetic high-density lipoproteins	Nanodiscs comprised of phospholipids and apolipoprotein A1 peptide mimic, to which cysteine terminated TAA peptides were conjugated.	Tumor-specific mutated neoantigen peptides	Cholesterol modified CpG-ODN	Subcutaneous	Neoantigen vaccine combined with anti-PD1 eradicated 7/8 tumors	Potential for personalized vaccines, highly potent in combination with clinical immunotherapy	Timeline for individualized neoantigen delivery is lengthy, effect of anti PD-1 alone on MC-38 tumors not studied	[175]
Colon (CT-26, subcutaneous xenograft)	Polyphosphazenes	Weakly cationic, amphiphilic polymersomes with hydrophobic *N*,*N*-diisopropylethyleneamine and hydrophilic mPEG	None	IL-12 plasmid	Intravenous	Increased levels of serum and intratumoral IL-12 with significant reduction in tumor volume	Well tolerated formulation that appeared to induce infiltration of T cells and NKT cells	No tumor was completely eradicated, uncertainty surrounding autoimmune reactions upon transfection.	[176]
Colon (CT-26-NY-ESO-1, subcutaneous xenograft)	Carbon nanotubes	Acid-functionalized multiwalled carbon nanotubes to which antigen and adjuvant were noncovalently bound.	NY-ESO-1	CpG-ODN	Subcutaneous	Tumor specific CTL response against NY-ESO-1 expressing cells, extended survival of tumor bearing mice	Efficient uptake into DCs resulting in enhanced cross presentation of antigen, fabrication of CNTs is relatively low cost	Toxicity concerns surrounding CNTs, high biopersistence, which may result in chronic inflammation.	[177]
Colon (CT-26, subcutaneous xenograft)	Rare-earth elements	Uniform, sub-100 nm upconversion nanoparticles coated with mesoporous silica with large pore sizes	Tumor cell fragments	Merocyanine 540 (as a photosensitizer)	Subcutaneous	Combination of antigen and photodynamic therapy was able to complete abolish tumors.	Demonstration of photodynamic therapy to create antigenic pool, nanoparticles can be imaged	Biocompatibility of materials used not fully outlined, synthesis of NPs has several optimization parameters.	[178]

[a)]Abbreviations: LLC – Lewis lung carcinoma; MIP-3β – macrophage inflammatory protein-3β; DC – dendritic cell; GM-CSF – granulocyte-macrophage-colony-stimulating factor; CpG-ODN – CpG oligodeoxynucleotides; Poly ICLC – polyinosinic-polycytidylic acid, poly-L-lysine double-stranded RNA and carboxymethylcellulose; HPV – human papillomavirus; CDN – cyclic dinucleotide; STING – stimulator of interferon genes; PD-1 – programmed cell death protein 1; mPEG – monomethoxy poly(ethylene glycol); NKT cell – natural killer T cell; NY-ESO-1 – New York esophageal squamous cell carcinoma 1; CNT – carbon nanotube.

Table 5. Key properties of biomaterials when considering vaccination vectors delivered via the mucosal route.

Materials[a)]	Hydrophobicity	Resistance to acidic degradation	Biodegradation	Mucoadhesive/ Mucopenetrating	Cell penetrating	Cargo location	Charge	Form	Ref.
PLGA	Hydrophobic	Low	Hydrolysis of ester bonds	Mucoadhesive	–	Encapsulated	Anionic at neutral pH but often coated with cationic moieties for mucosal delivery	Spherical or scaffold	[104,111]
PLA	Hydrophobic	Low	Hydrolysis of ester bonds	Mucoadhesive	–	Encapsulated	Anionic	Spherical or scaffold	[179]
PCL	Hydrophobic	Degrades slowly	Preferentially degraded by lipases present at high levels in the small and large intestines	Mucoadhesive	–	Encapsulated	Neutral	Spherical or scaffold	[180,181]
PEG	Hydrophilic	Low	Oxidation and cleavage of ether bonds	Both	–	Encapsulation (particles) or entrapped (scaffolds)	Depends on functional groups	Spherical or scaffold	[181,182]
PEI	Hydrophilic	Swells due to protonation but does not actively degrade	Not degradable	Mucoadhesive	+	Complexation with polymer	Polycationic	Spherical	[181,183]
PBAE	Hydrophobic	Low	Hydrolysis of ester bonds	Mucoadhesive	+	Encapsulated	Cationic at acidic pH	Spherical	[184]
Poly(L-lysine)	Hydrophilic	Low	Enzymatic degradation	Mucoadhesive	+	Complexation or encapsulated	Cationic	Spherical or scaffold	[185]
Chitosan	Hydrophilic	Low-moderate	Lysozymes and acidic environments	Mucoadhesive	–	Entrapped	Cationic	Spherical/gel	[186]
Alginate	Hydrophilic	Moderate	Monovalent ionic exchange and oxidation	Mucoadhesive	–	Entrapped	Anionic	Spherical/ scaffold/gel	[187]
Hyaluronic acid	Hydrophilic	Low	Degradation by hyaluronidases or acids	Mucoadhesive	–	Encapsulated/ entrapped	Anionic	Spherical/ scaffold	[188]
Gelatin	Hydrophilic	Moderate	Degradation by proteases	Mucoadhesive	–	Entrapped	Neutral at physiological pH	Spherical/ scaffold/gel	[108,189]
Lipids	Both	Low	Degradation by lipases	Mucopenetrating	+	Encapsulated	Can be cationic or zwitterionic depending on lipid head group	Spherical	[107]
Metallic (Au, Ag, Pt, Fe)	Hydrophobic	High (depending on metal)	Ionic dissolution, otherwise not degraded	Mucopenetrating	–	Conjugated	Depends on surface coating	Spherical	[190]

[a)]Abbreviations: PLGA – poly(lactic-*co*-glycolic acid); PLA – poly(lactic acid); PCL – poly(caprolactone); PEG – poly(ethylene glycol); PEI – polyethylenimine; PBAE – poly(β-amino ester).

at a target site during a designated time period, incorporation of adhesive materials (e.g., chitosan and other natural polysaccharides, thiolated polymers, acrylic acid; Table 5) is essential, unless the scaffolds are designed to physically penetrate the mucosa.

Overall, biomaterials may aid in overcoming many of the limitation associated with the development of successful mucosal cancer vaccines. Composite materials are particularly interesting as the different components can be designed to pass through the different barriers.

5. Rational Biomaterial Design

The considerations and design-criteria for biomaterials as vaccine strategies were extensively discussed elsewhere.[11,89,93–95,100] Here, we aim to convey the unique considerations of biomaterial platform design in the context of mucosal surfaces, and the associated mucosal immune responses. Material design criteria for mucosal cancer vaccines is mostly derived from studies in prophylactic vaccines for infectious diseases (which mostly focus on humoral response),

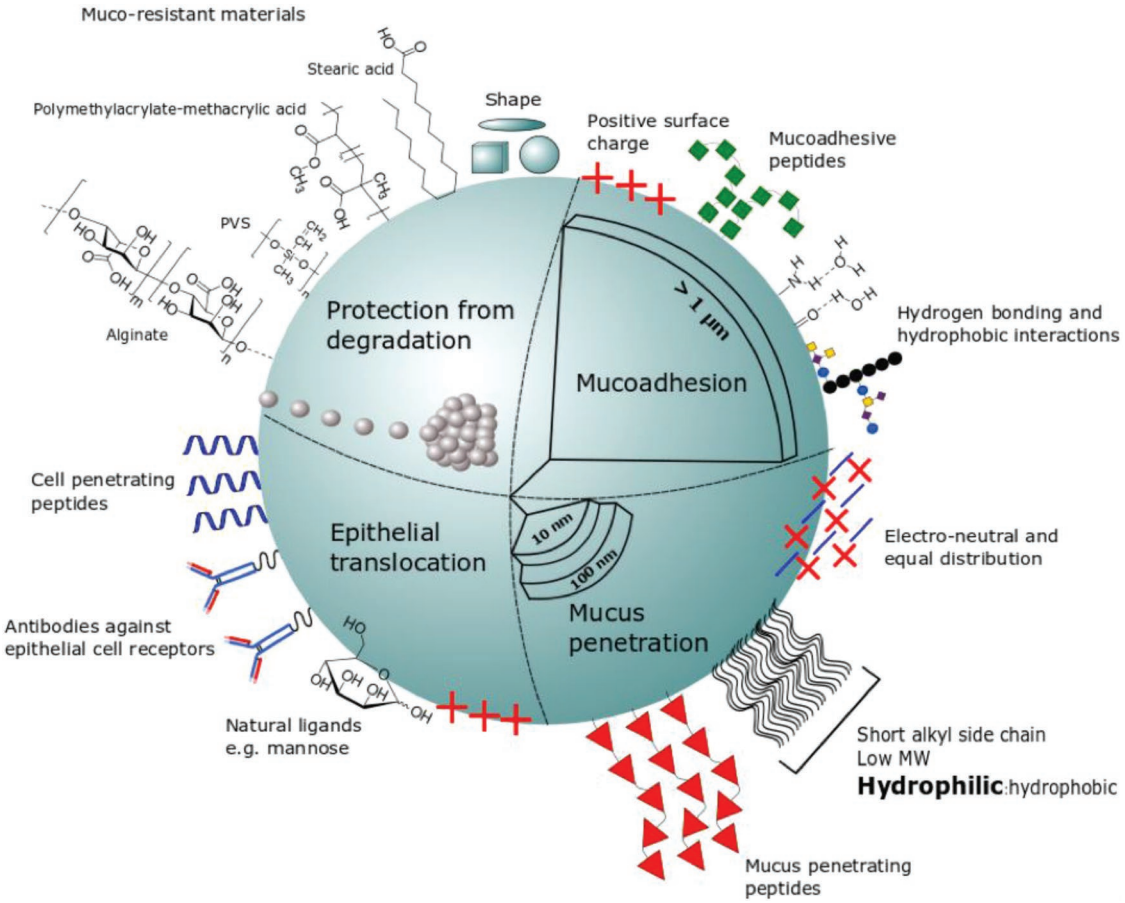

Figure 3. Nanoparticle design strategies for successful mucosal delivery. Protection from degradation: the mucosa is a degradative environment therefore materials resistant to enzymatic or acidic decomposition such as stearic acid, poly(methylacrylate-*co*-methacrylic acid), poly(vinyl siloxane) or alginate should be used to shield the encapsulated cargo from the external environment with little release until the desired site is reached. Mucoadhesion: particles are often larger than 1 µm in diameter, positively charged, and derived from materials with functional groups that can participate in hydrogen bonding, hydrophobic interactions with mucus glycoproteins or can be functionalized with mucoadhesive peptides. Mucus penetration: particles are typically much smaller, are electroneutral with few areas of positive and negative charge that are equally distributed. These particles can be decorated with mucus penetrating peptides or a dense brush-like coating of low molecular weight, hydrophilic polymers such as PEG, or amphiphilic polymers such as Pluronics. Epithelial translocation: once the mucus has been traversed, epithelial translocation can be facilitated using cell penetrating peptides, antibodies directed against epithelial cell surface receptors, or natural ligands such as mannose that can bind to CD206 on DCs. A positive surface charge also aids internalization.

from mucosal immunity in the gut, and from nonmucosal cancer vaccines. These studies provide valuable information as to the selection of antigen type and adjuvants, however, the interaction of the biomaterial with the mucus, epithelium, and the underlying APCs that will ultimately determine vaccine efficacy. Comprehensive data on the basic alterations in mucosal surfaces in the context of cancer, and on the interactions between biomaterials and cells in mucosal surfaces are lacking. The immune system is the first responder to a biomaterial in the body. It is not surprising, therefore, that the immune response to biomaterials with similar composition varies depending on the local tissue environment.[101] The divergent immune response to synthetic versus natural polymers and to different material properties (such as size, charge, shape, hydrophobicity and more), have been largely studied following subcutaneous or intramuscular implantation and in the context of tissue engineering.[102] Here, we summarize the various parameters that should be considered in the design of platforms for mucosal cancer vaccines based on evidence taken from engineered vaccines that elicit homing of $CD8^+$ T cells to mucosal surfaces.

A wide range of materials may be utilized as building blocks, and can be broadly classified as organic (lipids and synthetic or natural polymers),[94] inorganic (metals such as gold, iron, zinc or calcium, carbon, mesoporous silica) and biological (virus-like particles, proteins, peptides, and caveospheres) materials (Table 5).[10–13,72,95,103] Most common synthetic polymers include PEI, polyesters–poly(D,L-lactide-*co*-glycolide) (PLGA), poly(L-lactic acid), poly(ε-caprolactone) (PCL), polylysine, polyethers such as poly(ethylene glycol) (PEG) and triblock copolymers (poloxamers) like poly((ethylene oxide)-*b*-(propylene oxide)-*b*-(ethylene oxide)). These hold the advantage of controlled composition, which can be reproducibly processed and manufactured with a wide range of chemical and physical properties. Natural materials such as polysaccharides (e.g., chitosan, hyaluronic acid, pullulan, and alginate), collagen, gelatin, and

lipids, provide the possibility of solvent-free fabrication, high biocompatibility, active interaction with mucosal tissues, potentially an inherent adjuvant effect, and have been clinically used extensively. Historically, most common vaccine delivery platforms were based on PLGA MPs and liposomes for both systemic and mucosal vaccination.[104,105]

5.1. Mucosal Delivery

Vaccination agents, much like pathogens, must navigate the inhospitable mucosal microenvironment that is designed to resist infiltration by foreign entities. We summarize here the materials-associated consideration that must be taken under account in order to overcome the aforementioned mucosal barriers.

5.1.1. Protection from Degradation

Protein, peptide, and nucleic-acid-based vaccines may be protected from degradation by their encapsulation in biomaterials.[7,106,107] For example, encapsulation in polymers resistant to acidic degradation, such as PCL, provides protection of its cargo when passing through the acidic environment of the stomach (pH 1.5) and promotes mucosal uptake thereafter.[108] PCL is preferentially degraded by lipases, present at high levels in the small and large intestines, a potential benefit for use in controlled release delivery systems targeted to these regions.[109] Similarly, coating of chitosan-DNA vaccine polyplex with alginate, resulted in protection from degradation following oral administration and NPs accumulation in intestinal PPs with subsequent increase in antitumor CTL response.[110] Alginate is insoluble at pH 1.5 and thus self-assembles into larger particles, creating a shield from both enzymatic and acidic degradation. Zhu et al.[111] demonstrated protection of peptide antigen and TLR agonists, poly(I:C) and CpG, by their encapsulation within PLGA MPs (≥10 µm) coated with methacrylate-based Eudragit FS30D21 (poly(methyl acrylate, methyl methacrylate, methacrylic acid)). This anionic triblock polymer is pH responsive owing to its insolubility in acidic pH. Thus, following oral administration, the cargo in released in the large intestine only, where the pH is higher than 7. A potent T cell response was observed in various mucosal tissues, including the GI and cervicovaginal, as well as systemic. Protection from enzymatic degradation was demonstrated by utilizing chitosan-coated PLGA NPs to encapsulate protein antigen and the TLR7 agonist imiquimod for intranasal administration.[112] In a broad sense, any material stable at acidic pH and/or resistant to enzymatic degradation, could be used as coating for protection of antigen/adjuvant (Table 5, Figure 3).

5.1.2. Mucus Penetration

As discussed earlier, critical parameters dictating diffusion through mucus are surface charge and surface hydrophobicity of particles, and to lesser extent size (Figure 3).[14,113,114] Lessons can be drawn from the evolutionary mechanisms pathogens use to permeate through this mucosal barrier. Viruses that most efficiently navigate the mucosa have distinct surface properties. They contain few hydrophobic regions, which limit the probability of hydrophobic interactions, and their surface charge is unique, whereby areas of positive and negative charge are spatially distributed such that the overall charge is neutral. Design and modification of particles to mimic some of these properties can be achieved by coating with amphiphilic copolymers or surfactants to influence surface hydrophobicity. Decoration with materials that change from anionic to cationic when they arrive at the epithelium would not only reduce interactions within mucus, but also facilitate entry into cells. Particles engineered with a stealth layer of an inert polymer coating may serve as a trojan-horse to enhance mucosal translocation.

The most widely employed strategy to minimize mucin interactions is PEGylation, i.e., coating the particle surface with PEG.[115–120] PEGylation provides particles with hydrophilic and near neutral surface charge that prevents hydrophobic or electrostatic interactions. Evidently, PEGylated particles are less affected by mucin protonation compared to charged particles of the same size.[113] PEGylation of hydrophobic poly(sebacic acid) (PSA) NPs and anionic PLGA NPs significantly increase their translocation through cervicovaginal mucus and the hyperviscoelastic mucus of the lungs in patients with cystic fibrosis.[117,118] Other mucoinert polymers that may be used as stealth layer include hydroxyl-containing nonionic hydrophilic polymers, such as poly(2-alkyl-2-oxazolines), polysarcosine, poly(vinyl alcohol); zwitterionic polymers with sulfobetaine, carboxybetaine or phosphorylcholine as monomers and mucolytic enzymes.[121,122]

Particle–mucin interactions may be further modulated in strength by the molecular weight of the polymer used as stealth layer and its surface density. However, the specific requirements are difficult to predict. Low molecular weight PEG coating (2 kDa) was demonstrated to increase mucus translocation, while 10 kDa PEG results in mucoadhesive latex and polystyrene-based NPs.[113,120,123] By contrast, Cu and Saltzman[118] demonstrated that coating of PLGA NPs with higher molecular weight PEG (10 kDa) is more effective in preventing mucus binding compared to 2 and 5 kDa at similar coating density, without evidence of mucoadhesion. In addition, partial coating (10%) with low molecular weight PEG (2 kDa) resulted in more aggregation and mucin binding of PLGA NPs.[118] Partial coating of PLGA NPs with 10 kDa PEG diffused as efficiently as fully PEGylated NPs with 2 or 5 kDa through a glass capillary tubes loaded with fresh human cervical mucus. This is unrelated to surface charge as all PEGylated particles were neutral. These examples stress that the optimal surface parameters for mucosal diffusivity are still being elucidated and they differ for each particle type,[122] including the particles "stickiness," density of reactive groups for conjugation, surface charge, and charge distribution. These findings imply that particles with high density of reactive groups and less prone to aggregation, like hyperbranched dendrimers, would benefit from dense coating with low molecular weight PEG. However, poly(β-amino ester) NPs with low density of reactive groups, highly charged and prone to aggregation, would require coating with higher molecular weight PEG. Precise control over coating density may be achieved, for example, by using diblock copolymer systems. Tang et al.[119] fabricated 5 kDa PEG-PSA NPs

that exhibit efficient transport across the cervicovaginal mucus. Similarly, Boylan et al.[116] exhibited efficient transport across the lungs mucosa of 10 kDa PEG–polylysine diblock carrying plasmid DNA following intranasal delivery. Particles size seems to play a smaller role in the mobility within mucus once these are coated with stealth layer.[120] Surprisingly, particles larger (200 and 500 nm) than the cervicovaginal mucus mesh cut-off (<200 nm) exhibit rapid translocation when coated with low molecular weight PEG.

Alternatively, microneedles may be designed to deliver proteins directly to mucosal surfaces, via rectal application of a patch or using ingestible devices, that autonomously inserts payload into the GI mucosa.[124,125] In one example, the device, composed of PCL and stainless steel, was shaped as monomonostatic body, with a shifted center of mass and a high-curvature upper shell.[124] The shape feature enables it to align itself with the tissue of the GI tract and deploy a microneedle (Figure 2Ci). The injector unit is a mixture of 80% protein and 20% poly(ethylene oxide) compressed into a sharp conical shape, serving as the microneedle, and compressed sucrose. Once the sucrose is dissolved, a spring deploys, pushing the needle into the mucosal layer. In a different example, an ingestible microneedle capsule with a pH-responsive coating was used to deliver proteins to the lower GI tract.[125] Once the pill reaches the desired anatomical location, the coating dissolves, revealing the microneedles that can then penetrate the mucosa (Figure 2Cii). Such technologies could provide protection of the vaccine formulation from degradation and facilitate efficient penetration of a known dose through the mucosal layer.

5.1.3. Mucus Adherence

An alternative to the approach of engineering particles for mucosal translocation is to develop delivery platforms that are adhesive to mucus and/or underlying epithelial cells. Here, polymers with properties such as high molecular weight, high charge (preferably cationic) and possesses functional groups for hydrogen bond/hydrophobic interactions enable strong material–mucin interactions. Using this strategy, vaccination typically relies on the release of soluble antigens and their mucus translocation, essentially serving as a vaccination depot. Induction of T cell and antibody-mediated responses, both systemic and mucosal, were previously shown with intranasal immunization with adherent chitosan glutamate NPs.[126] Intranasal immunization with soluble antigen and parenteral immunization, however, were poor inducers of mucosal immunity. Similarly, cationic cholesteryl-pullulan nanogel (CHP) mainly remains adherent to the nasal epithelium surface, releasing antigen to the underlying tissue and DCs, while nonionic CHP was no better than the soluble antigen.[127] Alternatively, and perhaps more efficient for potent CTL response, adhesive materials may serve as a depot for mucoinert NPs, thus concentrating the vaccine to a smaller surface while optimizing mucus translocation. Such platform was fabricated by Laroui et al.[128] comprised of mucoadhesive chitosan-alginate hydrogel encapsulating 400 nm PVA-coated PLA particles.

Other materials were also shown to induce mucosal immunity through the nasal route, utilizing electrostatic interactions with polyelectrolyte MPs,[129] hydrophobic interactions with polyanhydride NPs[130] or interactions with a zwitterionic lipid particle like dilauroylphosphatidylcholine liposomes.[17] The strength of mucoadhesion is determined by the type of bond formed between the polymer and the mucin. Strong bonds comprise of ionic or electrostatic interactions with macromolecules containing numerous hydroxyl, carboxyl or amine groups (e.g., cellulose derivatives, poloxamers, alginate, Carbopol, hyaluronic acid, and chitosan) or covalent bonds (e.g., oxidized polymers) (Table 5).[14,15,131] Weaker bonds include mainly hydrophobic interactions and hydrogen bonds (with glycosylated mucin), but also van der Waals bonds. Mucoinert or weak mucoadhesive materials may be introduced with mucoadhesive properties like thiol groups (form disulfide bonds with mucin), ethyl hexyl acrylate (forms hydrophobic interaction), dihydroxyphenylalanine (DOPA; an amino acid in mussel adhesive protein), glyceryl monooleate, and lectins.[14]

A given material may possess mucoadhesive, or mucoinert properties, depending on context. For example, as mentioned above, PEG is typically used as stealth layer but was reported to obtain mucoadhesive properties as well.[132] This was presumably due to the formation of hydrogen bonds with glycosylated mucin or by forming an interpenetrating polymer network between the polymer chains and mucus. The transition cut-off from mucus penetrating to mucoadhesive of dense PEG was suggested to be between 5 and 10 kDa for some particles.[123]

5.1.4. Breaching the Epithelial Layer

The absorption of engineered vaccines to mucosal surfaces has been mainly studied in the intestine after oral delivery of NPs and MPs.[13,132,133] Under these conditions, particle penetration through the epithelium may be largely divided to transcellular and paracellular transport,[135] as well as intracellular transport via transfection of epithelial cells.[136] Particles smaller than ≈5 μm have been reported to be taken up transcellularly through endocytosis by the various mucosal phagocytotic cells, while NPs may be taken up by nonphagocytotic cells.[133,137] NP PEGylation is a useful strategy to enhancing penetration through the airway epithelial barrier and uptake by lung-resident cells, primarily pulmonary macrophages and DCs.[138] Importantly, 30 nm PEGylated pluronic-stabilized poly(propylene sulfide) NPs were carried by APCs to SLO, resulting in cross-presentation of antigens and a strong systemic and mucosal CTL responses. Particle shape also plays a role in determining its uptake by phagocytic cells, whereas particles with spherical and cylindrical shapes are generally readily taken up compared with disk-shaped particles.[139]

The paracellular pathway involves transport of particles through tight junctions between cells of 7 to 20 nm in diameter.[135,140] This pathway is most relevant for polycationic NPs, able to transiently open the tight junctions. For examples this may be achieved with PEI dendrimers and NPs composed of chitosan, poly-L-lysine and other polyamines. These materials induce a reversible increase in tight junction permeability that is associated with morphological changes in the actin cytoskeleton and with the localization of tight junctional proteins.[141] Interestingly, the anionic methylated β-cyclodextrin was also

suggested to enhance paracellular permeability of the nasal epithelium by opening of the tight junctions.[142] Polymers with highly dense polyamines surface, such as PEI, were shown to be highly efficient in delivering nucleic acid-based vaccines, but were shown to have suboptimal toxicity profile to nasal epithelium. Linking anionic β-cyclodextrin to PEI, lowers the charge density of the polyamine backbone, resulting in decreased cationic surface charge and increased nasal epithelium penetration of mRNA vaccine via intra- and paracellular pathways.[143]

Functionalizing the surface of NPs with ligands or antibodies for epithelial cell receptors, or cell-penetrating peptides may also aid in crossing the epithelium. As alluded to earlier, particles carrying M cell specific ligands are a promising strategy for mucosal vaccination. Carbohydrate- and lectin- (like *Ulex europaeus* agglutinin 1 and wheat germ agglutinin) conjugation to particles was shown to improve the efficacy of mucosal vaccines.[60,144] Similarly, conjugating polylysine particles with the reovirus protein σ1, facilitates M cell binding and vaccine delivery to NALT, resulting in enhanced CTL responses.[145]

5.1.5. Particle Size

A number of publications have highlighted that size is a key design feature for vaccine formulations, particularly for the induction of cell-mediated immunity. In a broad sense, NPs (20–200 nm) are considered more successful in the induction of cell-mediated response, while MPs encourage antibody-mediated response.[146] For example, parenteral immunization with PLGA NPs (<500 nm) was shown to induce greater CTL response compared with PLGA MPs (>2 µm),[147] while 1 µm PLGA particles elicit stronger antibody-mediated response compared to 200 and 500 nm via both subcutaneous and oral routes.[148] Another study found that parenteral immunization with 40 nm solid beads provided better CTL-mediated tumor immune protection than both smaller (20 nm) and larger particles (100 nm to 2 µm).[149] PLA MPs at a size of 2–8 µm induce stronger antibody-mediated response compared to both MPs smaller than 2 µm and greater than 10 µm, presumably as these falls into the optimal size range for transcellular transport through M cells.[150] This is mostly associated with the way APCs interact with particles to elicit cell-mediated immune responses, but in the absence of a mechanistic understanding, the contribution of size versus intrinsic material properties and administration route is unclear. Even though there is evidence that, via the parenteral route, smaller nanosized particles generate stronger cell-mediated immune responses, how this translates to mucosal surfaces is still unclear. MPs at a size and shape similar to pathogens may be more readily engulfed by phagocytic cells to enable prolonged antigen release compared with NPs.[151] Therefore, it is difficult to establish the proper size of a given platform, as it depends on polymer composition, surface modifications, as well as the nature of antigens/adjuvant and the route of administration.

In summary, biomaterials play a key role in facilitating efficient mucosal vaccination. Many exhibited potent mucosal and systemic antibody-mediated response via an engineered vaccine,[16] allowing for the extrapolation of better selection criteria for the design of a proper platform. By contrast, only limited examples were successful in eliciting strong CTL response following mucosal vaccination. Paradoxically, properties that are advantageous in the subepithelium, could be disadvantageous in the lumen or even hinder their ability to get there.[113,123] While cationic particles, for example, are readily engulfed by APCs and better localize antigens for CTL response,[152,153] they form strong mucin interaction, entrapping them in the mucus layer. This suggests that the use of composite materials may aid in achieving the desired properties. For instance, coating of liposomes with chitosan improves their stability and facilitates mucoadhesive properties by its strong interaction with mucin.[154] Also, encapsulation of PLGA NPs in an alginate-chitosan hydrogel, selectively degraded by digestive enzymes in the colon, allowed for a potent immune response at a lower dose following oral administration.[128] In addition, the effects of cancer on the local mucosal milieu must be studied to understand how the local environment may affect interactions with biomaterials. Relevant changes in affected tissues might include alterations in local pH, mucus production and density, and enzyme production. For this reason, alternative administration routes, that do not encounter barriers derived from disease, are worth considering (e.g., nasal delivery for colorectal cancer patients).

5.2. Parenteral Immunization

Intramuscular and subcutaneous immunization are the most studied routes of administration and are employed in clinical trials for both mucosal and nonmucosal cancers. Interestingly, transcutaneous immunization was suggested to provide systemic and mucosal protection, presumably through skin DCs trafficking to PPs.[155] Though this mechanism is yet to be elucidated, studying the immune microenvironment under these settings may shed light on new strategies that can be leveraged to evoke mucosal immunity via parenteral routes. As discussed earlier, parenteral immunization for mucosal immunity necessitates the administration of imprinting molecules in addition to antigen and adjuvant.[5] To date, these are only known for the small intestine and include the integrin $\alpha4\beta7$ and CCR9. Therefore, a vaccine engineered to concomitantly deliver retinoic acid, antigen, and adjuvant to peripheral draining lymph node, may enable skin DCs to partially mimic mucosal DCs, thereby potentially inducing the generation of gut-homing antigen-specific $CD8^+$ T cells.[24,156] Of note, the distribution and extent of such gut-homing $CD8^+$ T cells, as well as the generation of mucosal tissue resident T cells is yet to be investigated. Nevertheless, this strategy represents an especially intriguing approach for mucosal cancer vaccination. The discovery of additional imprinting molecules in the future, will facilitate employing this strategy to other mucosal tissues. It could be postulated that homing ligands may be utilized as targeting moieties on the surface of NPs for their extravasation from the systemic circulation into the lumen via endothelial cells. Though dilution of the vaccine might still occur, hindering its efficacy. Even in the absence of such knowledge, the ability to use materials to target the relevant SLOs would facilitate trafficking to the appropriate mucosal tissue.

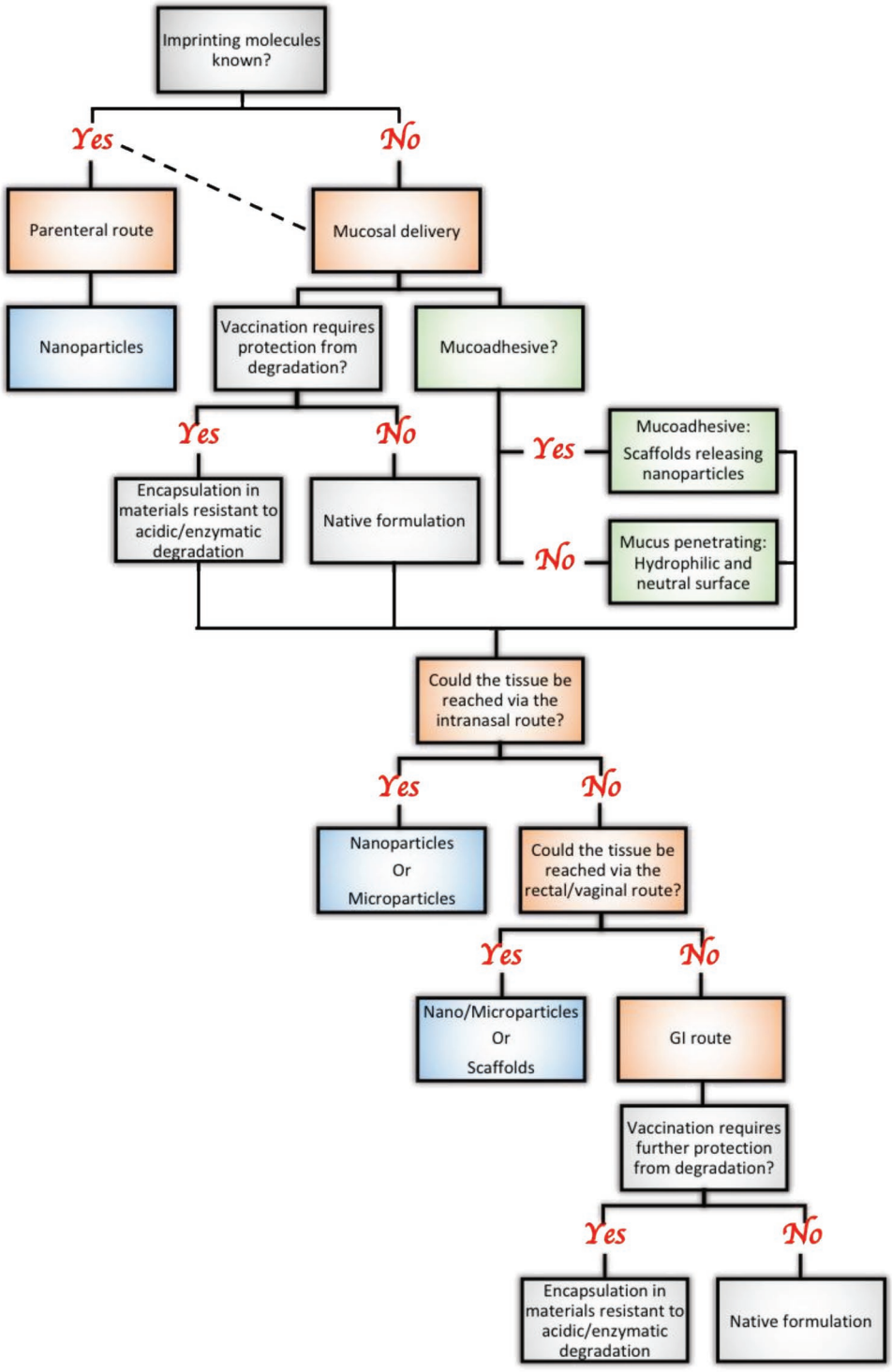

Figure 4. Simplifies decision tree to guide the choice of material and platform for engineered mucosal cancer vaccine.

We have summarized the key considerations in the engineering of a biomaterial-aided mucosal cancer vaccine (**Figure 4**) in a decision tree as a simple design tool. To first choose between parenteral and mucosal route, the main criteria is whether the imprinting molecules are known. In the case of retinoic acid and the small intestine, the most straightforward choice of a delivery platform is NP administration via subcutaneous or intramuscular immunization. NPs may be utilized as stand-alone or in convergence with a scaffold to attain sustained release. When the imprinting molecules are unknown, as in most cases, mucosal administration route can be used. An attractive approach would be intranasal delivery (Figure 2A),

since it can target effector cells to most mucosal tissues, can concentrate the vaccine at a relatively small surface, and compared to other mucosal route, has fewer physical barriers and minimal risk of degradation. However, the relatively small nasal cavity allows for the administration of particulate-based systems only (Figure 2A). Alternatively, rectal or vaginal immunization can be utilized using a wider range of delivery platforms, including microneedles, films/patches and other scaffolds, as well as particles (Figure 2B). Particles may be administered in solution using enema, while larger and more viscous platform may require designated suppositories or guidance by endoscope. Vaccination to the GI mucosa (predominantly oral delivery) encompasses the most significant barriers, including pH, enzymes, mechanical forces, and, perhaps most challenging, vaccine dilution (Figure 2C). Thus, it should be chosen when other delivery routes are indisposed. In that case, in addition to particulate-based vaccine and scaffolds, oral delivery can be used, for example with ingestible microneedle-based devices.[124,125] While protection from pH and enzymatic degradation is a key consideration for all delivery routes, it is of particular importance when the vaccination platform passes through the GI. Therefore, under these circumstances, the most important consideration is whether the vaccine requires additional protection from degradation. If the answer is yes, a biomaterial able to resist harsh pH conditions and enzymatic degradation should be utilized at the outer surface of the delivery platform (some of each summarized in Table 5), either as coating of a core polymer or for encapsulation. Next, the choice should be between mucoadhesive or mucus penetrating materials. To avoid, or at lease minimize, dilution of the vaccine throughout the surface of the mucosal tissues, mucoadhesive gels harboring mucus-penetrating NPs might be the preferred strategy. Another important consideration for core polymer choice is the type of antigen.[11,89,93–95,100] Protein or peptide antigens may be entrapped, encapsulated, adsorbed or conjugated to the vehicle. Whereas, DNA or mRNA vaccine would typically be complexed with polycationic materials (e.g., dendrimers, PBAEs, chitosan, polylysine) or encapsulated in liposomes or polymersomes.

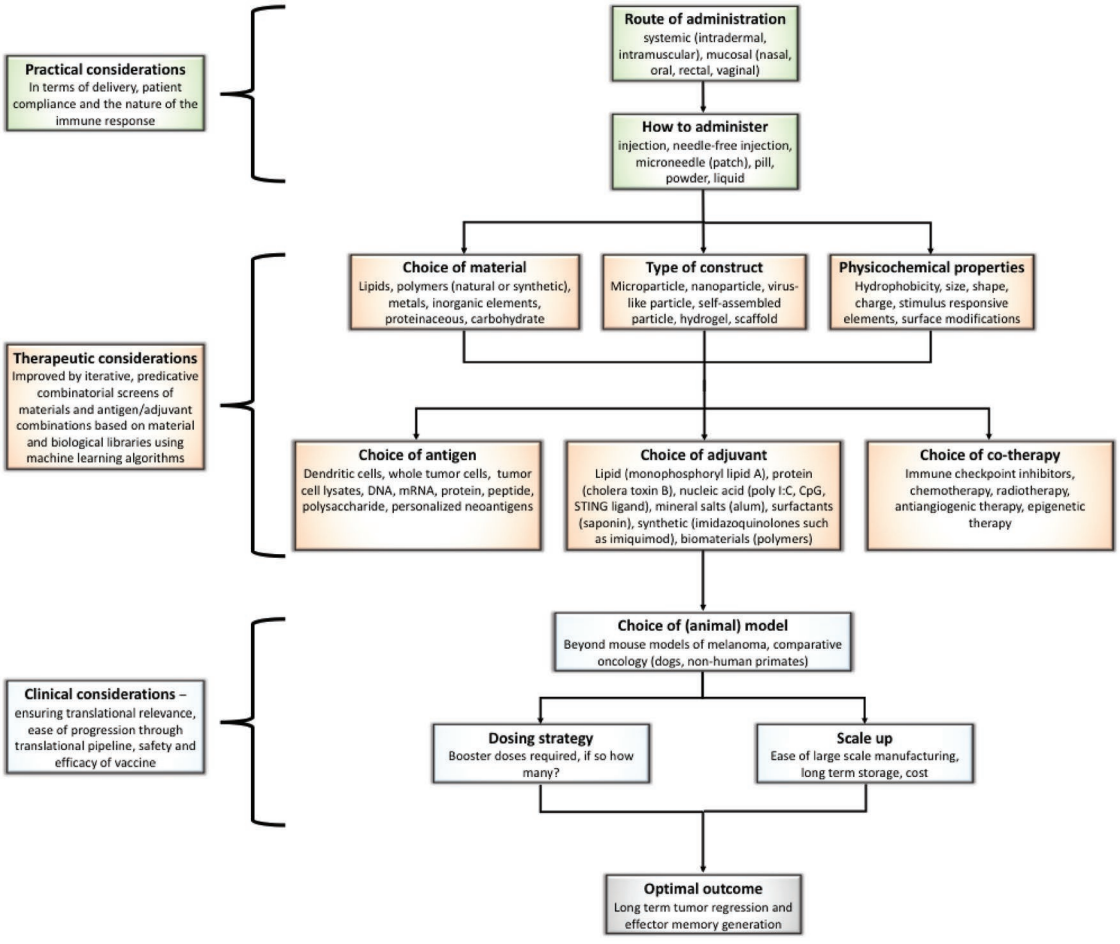

Figure 5. Key considerations of biomaterial mucosal cancer vaccines. Currently, there is no consensus or knowledge-base whereby researchers can reliably predict the full trajectory of the biomaterial-aided mucosal immune response. Though not fully understood, it has been shown that the administration route as well as the immune-formulation (biomaterial, adjuvant, antigen) can considerably alter the mucosal immune response. Furthermore, the preclinical landscape must be adjusted to consider a broader range of mucosal cancers, confounding factors, and potential for scale-up if biomaterial vaccines are to achieve their full translational potential. The magnitude and location of a cell-mediated mucosal response can be very much dictated by the highlighted factors and a methodical process examining these factors will ultimately reveal the optimal biomaterials and parameters required to induce the desired anticancer mucosal immune responses.

6. Outlook

The classical mechanisms of tumor escape partially explain the poor clinical success of mucosal cancer vaccines. Successful mucosal cancer vaccine induces humoral- and cell-mediated immune responses in both the systemic compartment and mucosal surfaces. This requires that the antigens/adjuvant will survive the harsh environmental conditions in mucosal surfaces, cross the mucus layer as well as the epithelium, while promoting cross-presentation. Vaccination studies of mucosal pathogenic microorganisms have highlighted the necessity of using mucosal routes of administration to target effector cells to mucosal tissues, which most likely play a major role in determining vaccination efficacy.

Immunization with biomaterial-based platforms has long been established as superior over soluble antigen/adjuvant delivery, particularly in a preclinical setting. Though certain guidelines have been elucidated, the requisite properties of a biomaterial that would successfully generate long-lasting protection in humans from mucosal cancers are yet to be identified. There are several aspects of therapeutic cancer vaccines that each have key parameters for consideration (**Figure 5**). From a delivery perspective, practical considerations such as tumor location will determine the route and method of administration; this is especially important as the route of administration influences the nature of the immune response.[157] Comparison of administration routes in order to achieve potent CTL responses would be insightful. The choice of material, type of construct, and the required modifications to obtain optimal physicochemical properties for the specific application will be dictated by delivery route and delivery platform, as well as the underlying mucosal and tumor biology. Combinatorial screening studies that seek to identify materials with desirable physiochemical properties when delivered via the mucosal route are desirable. Screening of antigens and adjuvants will highlight potent combinations that may reduce the propensity for immune-evasion.[158] Combination strategies, such as immune checkpoint blockade, activation of STING, depletion of immune inhibitory cells, and macrophage polarization should be investigated contemporaneously with mucosal vaccines. For studies in mucosal delivery and cancer vaccines to advance, models that better recapitulate both mucosal tumors such as orthotopic xenografts or genetically modified models, and delivery via the mucosal route will serve to better inform us of the nature of the antitumor immune response and the response induced directly by the biomaterial, which is understudied. Mechanistic studies of the communication between biomaterials and the immune system will facilitate understanding of the type and magnitude of the immune responses,[159] which can be leveraged to enhance immunotherapy and therapeutic vaccination strategies.[91] Toxicity of biomaterial-based vaccines should be closely monitored, not just as a result of the cargo but also the foreign body response to the material itself.[160] The molecular toolkit currently available and that has expanded over the past decades has facilitated development of delivery platforms that can effectively protect and present antigen and adjuvant to the mucosal immune system. Comprehensive understanding of underpinning immunological drivers, biomaterial–immune interactions, and precise properties required for efficient and reproducible mucosal navigation and elicitation of the desired response is beginning to emerge. Discoveries in these areas will expedite clinical investigation and may open up opportunities for therapeutic remediation of other immune-driven diseases and processes such as autoimmunity, wound healing, and infection.

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Conflict of Interest

The authors declare no conflict of interest.

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