



Recent trends and advances in microbe-based drug delivery systems

Pravin Shende¹ · Vasavi Basarkar¹

Received: 21 December 2018 / Accepted: 22 July 2019 / Published online: 2 August 2019
© Springer Nature Switzerland AG 2019

Abstract

Since more than a decade, pharmaceutical researchers endeavor to develop an effective, safe and target-specific drug delivery system to potentiate the therapeutic actions and reduce the side effects. The conventional drug delivery systems (DDSs) show the improvement in the lifestyle of the patients suffering from non-communicable diseases, autoimmune diseases but sometimes, drug resistance developed during the treatment is a major concern for clinicians to find an alternative and more advanced transport systems. Advancements in drug delivery facilitate the development of active carrier for targeted action with improved pharmacokinetic behavior. This review article focuses on microbe-based drug delivery systems to provide safe, non-toxic, site-specific targeted action with lesser side effects. Pharmaceutical researchers play a vital part in microbe-based drug delivery systems as a therapeutic agent and carrier. The properties of microorganisms like self-propulsion, in-situ production of therapeutics, penetration into the tumor cells, increase in immunity, etc. are of interest for development of highly effective delivery carrier. *Lactococcus lactis* is therapeutically helpful in Inflammatory Bowel Disease (IBD) and is under investigation of phase I clinical trial. Moreover, bacteria, anti-cancer oncolytic viruses, viral vectors (gene therapy) and viral immunotherapy are the attractive areas of biotechnological research. Virus acts as a distinctive candidate for imaging of tumor and accumulation of active in tumor.

Keywords Bacteria · Tumoricidal · Attenuated · Virosomes · Biomolecules

Introduction

Around 70% of global deaths are caused due to non-communicable diseases like cancer, cardiovascular diseases (CVD), diabetes, chronic lungs diseases, etc. The conventional drug therapy (tablets, capsules and pills). uses to treat or manage such life-threatening and infectious diseases but has shown limitations due to cytotoxicity, microbial resistance and adverse drug reaction. To overcome such undesirable effects, scientists have been worked on effective and alternative systems viz. novel drug delivery system, microbe-based delivery systems and gene delivery systems. Ancient literature reported that the microorganisms such as bacteria, virus and fungi-based delivery systems were employed to treat various

conditions like cancer, cardiovascular disorder, neurological disorder, etc. It also revealed that microbes are not always pathogenic but because of biological behavior, they reduce noxious and associated harmful effects. [1]

Microbes especially whole bacteria, bacterial toxin, bacteria ghost or viruses or fungi show some rationale results in delivery system. For example, bacterium like *C. novyi NT* shows the ability to penetrate and inhibit the growth of a tumor. Besides whole microorganisms, their contents also showed an immunostimulant influence. [1] The virus carrier envelopes potentially deliver drugs, biomolecules like peptides, nucleic acids and genetic material. Moreover, the rebuilt viral envelopes may be articulated carriers for macromolecules like nucleic acids, genes or drugs, known as “Virosomes” [2]. The cell wall constituents of fungi known as chitin is a latent carrier of many actives, nucleic acids, etc. This review article provides an insight on recent advances of different types of microbe-based delivery systems for treating various diseases like cancer, inflammatory bowel diseases [3]. *Bacillus* is another agent used to deliver the drugs and stable throughout the gastric environment. The characteristic bacilli spores were incorporated with curcumin and release of drug was determined the disintegration of outermost

✉ Pravin Shende
shendepravin94@gmail.com

¹ Shobhaben Pratapbhai Patel School of Pharmacy and Technology Management, SVKM's NMIMS, V. L. Mehta road, Vile Parle (W), Mumbai, India

coat in alkaline colon medium. These bacilli spores showed promising results for delivering of drug in colon cancer as compared to conventional drug. [4]

Classification of microorganisms

The microorganisms are classified on the basis of their cell structure as shown in Fig. 1 whereas biohybrid-based drug delivery system is shown in Fig. 2.

History of the microbe as a drug delivery system

Researchers observed that some types of cancer like breast and lung caused by accidental erysipelas for hospitalized patients. [5] Vautier in 1813, first stated the treatment of contagious disease using bacteria and during the treatment of cancer patients, gas gangrene infection was produced by *Clostridium perfringens*, for tumor deterioration [6–8]. In another episode, a clinician from the United States, William Coley explored live cultures of *S. pyogenes*, killed extracts of *S. pyogenes* and *Serratia marcescens* for the treatment of neck cancer of one of his patients. Subsequent studies showed that various species of bacteria like *Bifidobacterium spp.*, *L. monocytogenes*, *S. typhimurium*, *E. coli*, *Clostridium spp.* and *Mycobacterium bovis* were accumulated at the site of tumors. [9–13] However, research revealed that bacteria are not capable to abolish complete tumor but a part of malignant growth was destroyed. Though, a slight portion of the infected tissue may grow to form a large size tumor. For more effective management of cancer and other contagious diseases, bacterial drug delivery is combined with chemotherapies. Bacterial toxins showed an action in contrast to tumors like *C. novyi NT*. [13, 14]

Fungi are not directly used in the drug delivery system but their cell wall component like chitin is used as a carrier. Chitosan-based nanoparticles were prepared by double solvent evaporation technique and used for the delivery of anthracycline and doxorubicin. Hence chitosan-based drug delivery has been emerged due to mucoadhesion, penetration and biodegradation and used as different carrier systems like

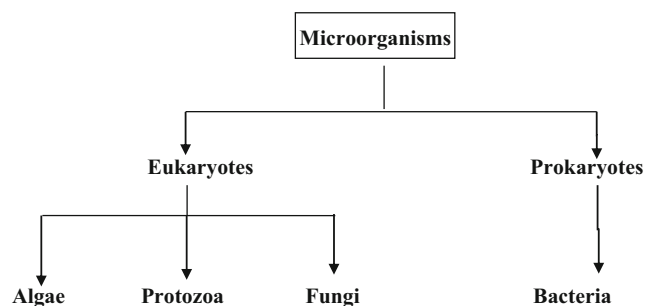


Fig. 1 Classification of microorganisms according to cell structure

nanoparticles, microparticles, etc. Algae-based drug delivery system have not been studied much, but algae-based polymer and its blend of components are used as biomaterials.

The research was further extended for targeting the tumor by genetically-engineered bacteria to decrease the toxicity and immunogenicity. Tumor cell death is mostly related to acute toxicity [15, 16] and septic shock [17, 18] whereas bacteria were manipulated and genetically modified to decrease immunogenicity [19–21]. Genetically modified bacteria used proteins, drugs, enzymes and genes for mitigation of conditions associated with gastrointestinal infection, [22, 23] diabetes disorder [24], carcinoma [14] and viral infection. [25] This method shows advantages like site-specific targeting, specific breaking up of tumor cells and interestingly gene-directed enzyme prodrug therapy.

Virosomes are biocompatible, non-toxic and biodegradable carriers and used to deliver various drugs whereas peptides and nucleic acids are delivered by viral cellular envelopes. Reconstituted viral envelopes and virosomes may be designed using vaccines as carriers for large molecules like nucleic acids, gene, antibiotics, anticancer agents and steroids. [26]

Bacterial candidature for drug delivery mechanism

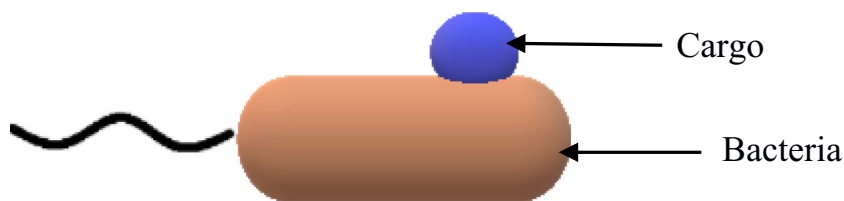
Mobility of bacteria

The different constituents of bacteria such as flagella and pili allow them to move [27] towards oxygen gradient and such property of bacteria is termed as bacterial taxis. [28] Different classes of bacteria show various taxis behavior like chemotaxis which is the mobility of bacteria in response to change in a chemical environment and phototaxis which involves mobility of bacteria with respect to change in light emission and pH taxis of bacteria. [29] Such characteristics of bacteria aid them to move towards the specific site. Unlike normal cell, cancer cells of altered chemical composition in comparison to uninfected areas induce chemotaxis property and aid them to travel towards the infected area. Similarly, bacteria are fascinated by the oxygen-deficient site and travelled to tumor area due to hypoxic condition for effective drug delivery system. [30]

Production of proteins on the site

Current treatment options using conventional dosage form cause uneven distribution of drug in the body. Some bacteria show the capability to deliver proteins only at the desired site without distributing at non-specific sites. These are produced by the bacteria only on-site and acted as a drug for effective delivery by avoiding an unfavorable environment of the stomach and functioned as a drug, prodrug and immune-stimulating agent. [31–34]

Fig. 2 Biohybrid-based drug delivery system



Bactofection

The bacterial usage for deliberately introducing naked or purified nucleic acids into eukaryotic cells is called bactofection. The bacterial plasmid combines with the gene of interest, which is absorbed by mammalian cells and transferred to the genetic material of the cell. [35] For example *Bifidobacterium* was engineered to deliver a gene to cancer cells. In another promising case, highly specific and productive endostatin gene delivery was reported with species like *Bifidobacterium longum* in liver diseases. [35–37] *Bifidobacterium infantise* herpes simplex virus was reported to possess antitumor effect in mice renal cancer cell using thymidine kinase/ganciclovir gene therapy. [38–40]. One of the most important applications of bactofection is the vaccination of DNA to produce action in contrast to several infections and tumors. This vaccine comprises of promoter, antigen and for plasmid wherein desired antigen is encoded by promotor gene of plasmid. [41, 42] DNA vaccines were delivered to macrophages by certain bacterial species like *L. monocytogenes* and *L. typhimurium* against the pathogens. [43] *Listeria* was internalized with bioengineered *E. coli* to use in breast cancer. [44]

Stimuli-responsive bacteria

Bacteria shows the capability to react with various stimuli like chemical, pH, light, temperature and also intellect a slight change in their nearby environment. Based on such properties, stimuli-responsive bacteria can be used to target drugs efficiently. [45]

Light sensitive and light generated transcription

Engineered bacteria revealed light-sensitive ion-channels which allowed to control conventional inducible promoter systems. Bacterial cells are genetically modified in response to light-sensitive ions. A bacterial protein, EL22, a light-oxygen voltage was connected to DNA in blue light and genetically-engineered in response to light-induced transcription. [45]

Magnetically-responsive bacteria

Certain bacteria synchronize in earth's magnetic field in the presence of magnetic nanocrystals [46] and respond to magnetic-stimuli called magnetotactic bacteria. In the

presence of magnetic resonance imaging (MRI) [47], the bacteria may be located and monitored beneath the impact of outer magnetic influence. This idea recommends a novel approach for crafting an accurate therapeutic agent application to infections as well as tumors. [48, 49]

Oxygen-driven targeting

Since the cancer cells bear oxygen-deficient condition, anaerobic bacteria like *Clostridium* and *Streptococcus* target the tumor [50]. *Clostridium spp.* has been modified to transport immunostimulant proteins for upsurging IL-2 facilitates anti-cancer activity [49].

Thermo- and pH-responsive delivery

Adherence of *Serratia marcescens* bacterium to microbeads showed unifacial and bifacial pH- stimulus. In general, cancerous cell is characterized by acidic condition, high temperature and deficit oxygen content [51]. Due to such distinguished properties between tumor and normal cells, bacteria are able to differentiate and identify tumor cell. These bacteria identify the elevated temperature of the cancer tissue and act like thermo-responsive drug delivery system [52]. A similar mechanism is observed in pH-responsive drug delivery, bacteria identify the acidic environment and follow pH-responsive mechanism. [53]

Bacteria for metabolic disorders

Another important application of engineered bacteria for treatment metabolic disorder is obesity. *E. coli nissle* (EcN) strains were used to express *N-acetyltransferase* which was obtained from *Arabidopsis thaliana*, this produces *N-acyl phosphatidyl ethanolamines* (NAPE's) from small intestine. By incorporating NAPE, EcN in drinking water reduced food consumption and fat gain without undesirable side effects in mice (Table 1).

Advancement in bacteria-based drug delivery system

Bacteria as tumoricidal agents

Recently, some alive strains of bacteria in the forms of weaken and genetically modified are also used in cancer treatment.

Table 1 Bacteria and their roles

Role of bacteria	Example of bacteria
Stimuli-responsive	<i>Clostridium spp.</i>
Light-sensitive	–
Magnetically-responsive	<i>Magnetotactic</i>
Oxygen-driven	<i>Clostridium, Streptococcus</i>
Thermo- and pH-responsive	<i>Serratiamarcescens</i>

Pathogenic bacteria should be avoided to use for conveying of drugs may cause toxicity using non-pathogenic bacteria. These strains show specific and direct actions on the cancer cell or can be used as a vehicle to transport anti-tumor cells at the targeted area. It was revealed that many functions of live, attenuated, nonpathogenic bacteria as an anticancer agent or as a carrier of drugs were also improved [54]. The precise altered surroundings of solid tumors are recognized and responded by some bacteria to prepare highly target-specific DDS (drug delivery systems). Some of the spores of obligate anaerobes such as *Clostridium spp.* propagate in the oxygen-deficient region of big tumors because of poor vascularization when given by parenteral route. These properties showed the oncolytic effects on cancer cell because of bacterial growth. It was exposed in animal models as well as in human patients [27]. Non-infectious strains of *Clostridium* such as ‘M55’ did not show tumor suppression [55]. BCG was used for bladder cancer and *S. typhimurium* derivative and also articulated for cancer therapy [54]. Certain species of *S. choleraesuis*, *V. cholerae*, *L. monocytogenes* and even *E. coli* [56] are currently discovered as anticancer agents.

Bacterium as a vector for drug delivery system

Bacterium like *E. coli*, *S. typhimurium* used as vectors for drug delivery system. The problems associated with bacterial drug delivery systems are the requirement of minimal dose to treat the tumor cell. Dose reduction and safe-effective dose concentration are still under investigation. To avoid such problems, genetically-engineered bacteria are considered as an alternative to provide targeted action and also to convey antitumor drugs at the desired site. [54]

Bacterial spore as DDS (drug delivery system)

It revealed probiotic properties of oral bacterial therapy were discovered by bacilli spores. These spores after administration by nasal and oral routes exhibited mucosal immune response due to the occurrence of polysaccharides and proteins on the outer surface. The spores of many anaerobic bacteria germinate, multiply, replicate and become lively in some part of the tumor in oxygen lacking areas. (e.g. *C. beijerinckii*, *C. novyi-Non Toxigene* and *C. histolyticum*) [57]. Anticancer drugs like

vinorelbine, docetaxel, mitomycin C and dolastatin-10 [14] are grouped with spores of *C. novyi – NT* for better therapeutic action. Genetic engineering increased the antitumor activity of *Clostridial* spores using prodrug converting enzymes like *cytosine deaminase* with the capability of converting 5-fluorocytosine (5-FC) to 5-fluorouracil (5-FU). This reduced local dose of antitumor drugs without affecting healthy tissues [58]. Most of the spores of bacteria such as VNP2009, a genetically engineered species of *S. typhimurium* for advanced or metastatic solid tumors [59]. Interleukin 4-PE, Interleukin-4-*Pseudomonas* exotoxin for brain enteral nervous system tumors [60] are discovered as antitumor drugs, cytotoxic peptides, and therapeutic proteins and as drug delivery vectors for gene therapy.

Bacterial toxins for tumor therapy

Bacterial toxins mostly used to destroy a tumor, at minimal concentrations modifies cellular processes and regulate cell cycles; cell proliferation, apoptosis and differentiation processes. [61]

Toxins of bacteria bind to the antigen present on the tumor superficial portion of tumor such as *Diphtheria* toxin (DT) and *Pseudomonas* exotoxin suppress protein synthesis. [61] Some bacterial toxins action was observed because of the antigens which were bound to the antigen existing on the superficial area of cancer cells, such as DT and *Pseudomonas* exotoxin A identified with catalytically ribosylate EF-2. It causes inhibition of production of protein, destruction of cell and initiation of apoptosis. [62–64] *C. perfringens* enterotoxin (CPE), an endotoxin, is accountable for gastroenteritis development but its ‘N’ terminal is significant for antitumor activity [65, 66]. Various kinds of DT ligands have been investigated for targeting cancer cell (e.g. Interleukin-3, Interleukin-4, Granulocyte Colony Stimulating Factor (GCSF), Transferrin (TF), EGF and vascular endothelial growth factor (VEGF) [62].

Bacterial cellular envelope

“Bacterial ghosts” are hollow, vacant and non-living envelope of gram-negative bacteria. These develop lysis-tunnel structure inside the covering of live bacterium after regulated expression of cloned bacteriophage gene E. The bacteria are not able to multiply as that of cytoplasm and certain components of DNA are expelled inside the medium but it holds all the structural, immunogenic and bio-adhesive characteristics because of the proteins present on the surface. The cell coverings are safe and may be lyophilized. [67] many of the drugs, nucleic acids, antigens, and proteins may be formulated with these cellular coverings for non-living drug delivery vehicles because of their unique cellular structures and abilities [68]. Biotinylated agents were inserted in cytoplasmic membrane of

genetically modified *Escherichia coli* NM 522 bacterial ghost [69]. One of the first leak-proof bacterial ghosts concept of *E. coli* NM 522 was synthesized by Paukneret in 2003. Sealing agents like membrane vesicles were used in the occurrence of Ca^{++} ions to avoid the discharge from this bacterial ghost. [70] Moreover, the formulated BGs of *Mannheimia hemolytica* with the anticancer agent doxorubicin, when administered systemically, showed a slow release of drug. [71] It was revealed that bacterial ghost concept of drug delivery can be explored as a convenient agent to transfer of active ingredient to treat ocular superficial disorder [72].

Bacterial governed enzyme prodrug therapy

The idea of prodrug emerges due to the undesirable side effects of microbial drug delivery systems. The prodrug undergoes biotransformation and forms effective pharmacological molecule in the cancer cell [73] when administered parenterally. 5-Fluorocytosine (5 FC) is converted to 5-Fluorouracil (5 FU) in presence of enzyme cytosine deaminase (CD) and microbes like *C. sporogenes*. It has been observed with nitroreductase (NR) which converts the prodrug CB 1954 to a DNA cross-linking agent. Use of active exogenous enzyme for specific delivery to cancerous cell [74, 75] was studied with CD expressed in *Clostridium acetobutylicum*. Another efficacious prodrug enzyme therapy ensured by studies with enzyme cytosine deaminase in hypoxic tumor condition with transfected *B. longum* by enzyme pBLES100 -S-eCD. [76]

Bio-hybrid bacteria for drug delivery

Superior levels of performance may be accomplished by combining the microbial cells with non-living materials like microparticles, nanoparticles or microbots. [77] For instance, the use of distant magnetic steering of a fabricated magnetic microbody may help bacteria to travel towards the target. This is possible when the attachment of fabricated microbot-specific chemical stimulus and movement of microbes are closed to the intended targeted site [78]. The delivery of microparticles or nanoparticles of therapeutic agents were targeted by bioengineered bacteria inside the cells as carriers. [79] They penetrate mammalian cells and transport small molecules, antibodies, therapeutic peptides, and DNA into the cells by employing the bacteria as carriers [79]. Various pharmaceutically inert materials like polyethylene glycol (PEG) are researched extensively to serve as a carrier due to protein binding resistance property with a bacterial cell for reduction in toxic effect and immunogenicity. [75, 80]

Bacteria as immunobiological response modifiers

Immunotherapeutic strategy involves utilization of immune system to kill tumor cells. Enhancement of antigenicity of

carcinogenic cells [81] using bacteria is a new idea of the immuno therapeutic concept. Avogadri et al. showed some fascinating outcomes with an attenuated strain of *S. typhimurium* invasion of melanoma cells which evicted the occurrence of antigenic elements of bacterial genesis. Surprisingly, fascinating outcomes were seen with intratumor *Salmonella* injection given to the tumor-bearing mice after vaccinating it with *S. typhimurium* [60]. Inflammatory response was triggered by the production of cytokines like IL-6, MIP-2, G-CSF and TIMP-1 and by attracting inflammatory cell, and destructing tumor cells after the systemic route of drug delivery of *C. novyi-NT* spores. Clinical trials in phase I was conducted with spores of *C. novyi-NT* spores and antimicrotubuli agent [81]. The use of Lm-LLO-E7 is progressed into clinical trials for cervical cancer therapy [82] as a cancer immunotherapeutic mediator.

Virus-based drug delivery system

Virotherapy

One of the applications of biotechnology is virotherapy, where viruses are used as therapeutic agents for the treatment of conditions like cancer and metabolic disorders. Anticancer oncolytic viruses, viral vectors for gene therapy and viral immunotherapy are considered as the three sub-divisions of virotherapy. Cancer patients who were vaccinated earlier and further suffered from non-relevant viral infection showed effectiveness with virotherapy [83] TNF and interferons are generated by body's immune system during viral infection; on the other hand, tumor cells are targeted by oncolytic virotherapy. In 1940s and 1950s, the usage of viruses was evaluated by various animal models for the treatment of tumors [84] whereas human clinical trials were conducted on oncolytic viruses [85]. An oncolytic virus known as RIGVIR (Riga virus) was developed and registered in 2004 by the Institute of Microbiology in Latvia [86]. Research studies revealed that the death rate of IB-IIC tumor patients was reduced by 4.3–6.59 folds with RIGVIR. [87] Researchers from Hebrew University successfully extracted slight different version of Newcastle Virus to target-specific tumor cells. [88]

Viral immunotherapy

Trovax is an innovative immunotherapeutic treatment by Oxford Biomedica where pox-virus exhibited the tumor antigen 5 T4 instigated immune response to various cancers. [89]

Virus-like particles (VLPs)

Alike to the virus, VLPs are devoid of viral genetic materials and generally considered as safe. These particles are usually

formed from constituents of virus-like viral envelope and capsid-congregate. These particles may be obtained from various virus families like *Parvoviridae* and *Flaviviridae*. VLPs can be used as carriers for genes and other therapeutic agents. [90] In vitro studies revealed that VLPs effectively target cancer cells [91] because of their higher penetrability and detainment effect. VLPs can be explored as drug delivery tumor imaging because of their accumulation property at the tumor site. [92]

Vaccines

Immunity against only one strain of microbe is provided by a single vaccine whereas multivalent vaccines, which showed immunity against multiple strains of microorganism. VLPs can be formulated in the form of vaccines. Similar to strong B cell and T-cells reactions, the surface proteins of VLPs also respond for an immune response [93]. Vaccines for hepatitis and papillomavirus infection used VLPs for FDA approval. [94, 95]

Virosomes

Virosomes are restructured viral envelopes are used to deliver vaccines as well as acted as carrier for large molecules like nucleic acids, genes or drugs. Virosomal technology may be explored for drug delivery of drugs like antibiotics, anticancer, and steroids. [26] The main composition of virosomes is essentially membrane lipids and viral-spike glycoproteins with an empty shell. The first virosomes were prepared by Almeida with liposomes of purified influenza spike proteins [96]. Subsequently, several viral species including Sendai virus, [97–99] Semliki Forest virus (SFV), [100, 101] vesicular stomatitis virus (VSV), [102, 103] and *Sindbis* virus. [104] were reconstituted in viral envelopes. Proteolytic degradation of therapeutically active substances at less pH can be achieved by virosomal formulation. Because of this unique nature, virosomes may be considered as superior drug delivery compared to liposomes and proteo-liposome carrier systems. [105]

One of the approaches for formulation and development is that, active pharmaceutical ingredients may be entrapped into the aqueous interior or inside the lipid membrane to facilitate the entry of compounds into the cells. [105] Virosomes blend with endosome or with the plasma membrane, to deliver these compounds into the cytoplasm of host cell. [106] This unique feature showed encouraging results and also enhances the application of virosomes technology. The drug delivery of virosomes is achieved by various routes like topical, oral and transdermal. The controlled release of the virus may be achieved by using implants in which they can be incorporated for prolonged delivery. [107]

Monoclonal antibody-based virus drug delivery

The on-site action of oncolytic medicaments using virosomal vehicle has been demonstrated currently by two distinctive methods: in the initial method, the superficial portion of virosomes carrying an antitumor drug (e.g. doxorubicin) cross-linked to a monoclonal antibody which bind precisely to tumor-related antigens when administered systemically to cancerous tissue whereas in the later method, the virosomes also complexed to ligands and further wrapped the surface receptors on the targeted cells. [97, 105] Different viruses used for drug delivery are listed below:

CCMV (cowpea chlorotic mottle virus)

CCMV (family *Bromoviridae*) shows a unique property to undergo a reversible pH-dependent swelling. This property enables them to gather and again separate in-vitro to dislodge the viral genes and then infix the functional species. CCMV is specifically beneficial in encapsulation of negatively charged active materials.

CPMV (cowpea mosaic virus)

CPMV belongs to family *chromoviridae* and is under research because of its high stability in nanocarriers. This virus is isolated from black-eyed pea plant leaves and stable over extreme conditions of temperature, pH and in many organic solvents.

RCNMV (red clover necrotic mosaic virus)

RCNMV belongs to family *Tombusviridae* and is useful to carry substances and to change its surface structures. It forms small pores to allow entry of the ions and also enables in packaging.

Routes of administration of microbe-based drug delivery

Depending on the nature of the drug, intended location and ease of administration, the route of microbe-based drug delivery is mentioned below:

Intratumor injection

Zhao et al. found a significant effect of tumor inhibition with intratumoral injection of *S. typhimurium* without harmful effects like uneven distribution of drug in the body [108]. Direct injection into the central nervous system is also considered as another method [109, 110] where promising results were obtained for tumor-targeting bacteria [111] with less systemic toxicity and higher efficiency.

Oral administration

Effectiveness for colitis therapy was established by expression of immunosuppressive Intein-27 (IL-27) in bioengineered *L. lactis* when administered orally in mice in comparison to parenteral route. Investigation of type I diabetes by genetically modified GAB-65 and IL-10 have been reported by oral drug delivery of *L. C. difficile* diarrhoea vaccination. [112] Expression of EspB antigen [113] for hemolytic-uremic syndrome was triggered by *E. coli*. Ulcerative colitis was treated [114] with genetically engineered *B. longum*, expressing α -melanocyte-stimulating hormone, vaccine counter to Hepatitis C virus [115], and IL-12 anti-inflammatory cytokine by oral route [116]. *S. typhimurium*, [117, 118] *E. coli* [119, 120] as well as *L. casei* [121] are some of the strains of the bacteria were under research via oral route. One of the effective ways to deliver the medicament in cancer patients is intravenous or systemic routes showed higher blood supply than the surrounding tissue [122]. Some studies also revealed that the in vivo experiments by various strains of bacterium like *Bifidobacterium bifidum* [123] and *S. typhimurium* [124–126] are not affected by systemic administration.

Intranasal injection

Genetically engineered strains of *Streptococcus gordonii* with antigen expression of *Mycobacterium tuberculosis* was delivered through intranasal route to sensitize CD4⁺ and CD8⁺ T-cells and vaccinate [127] against *Neisseria meningitidis* which is one of the important reasons for meningitis. Administration of *Lactobacillus pentosus* by intranasal route activates the immune response of the respiratory system.

Advantages of microbe-based drug delivery system

- i. Modified bacteria cause colonization of intestinal lumen which specifically conveys protective cytokines, growth factors and competitive inhibitors at the desired site, which also help in penetration of recombinant molecules in the inflamed area.
- ii. Carriers used for target-specific drug delivery avoid systemic toxicity, minimize host-immune responses to vectors and maximize local therapeutic concentrations of the actives.
- iii. Economical treatment for chronic diseases like IBD can be achieved by expanding the idea of microbial drug delivery, by replication of the microbes specifically to colonize in the intestine.
- iv. To avoid the resistance of tumor cells to the anticancer agents, the bacterial therapy combined with cytotoxic agents are explored in the form of bacterial cellular

envelope encapsulated with doxorubicin for the treatment of breast cancer.

Disadvantages of microbe-based drug delivery system

- i. Dose associated toxicity and severity of systemic infection need to be explained
- ii. Combination of chemotherapy is suggested with bacteria-based delivery as the microbes are not utilized key portions of the cancer tissue (i.e. partial tumor lysis) [54]
- iii. DNA mutation is another possibility which may letdown the therapy.
- iv. Safety and regulatory concerns are complicated.

Use of bacteria in drug delivery

1. *Clostridium novyi NT* is under investigation for its unique properties. This bacterium shows the capability to permeate and destruct the tissues, and also boost the host immune system. The extended application includes the treatment of hypoxic tumor conditions.
2. *Lactococcus lactis*, another class of bacteria is examined for inflammation related to colitis. TNF is responsible for these inflammatory symptoms of colitis. IL-10 is an agent that balances and decreases the inflammation related to colitis. *Lactococcus* is engineered in a specific way to produce IL-10.

Toxicity associated with microbe-based DDS

In application of microbes-based drug delivery, the use of live bacteria showed many hazardous reactions due to discharge of intracellular contents, endotoxins of microbes and chemicals like induction of inflammatory responses.

The main problem associated with microbial based drug-delivery systems is to alter dose-dependent toxicity. The foremost complications associated with microbial DDS which may change the therapeutic efficacy. Hypersensitivity reactions and quick removal of bacteria [128] were reported and dependent on the immune response at higher bacterial concentrations. In spite of the removal of toxic genes [129], residual toxicity has been observed. Therefore, the bacteria must not interfere with the patient's immune response, easily removed after treatment and in poor host response. [130] For a complete summary on toxicity research, different administrative recommendations such as 'Guidance for industry concentrations for developmental toxicity studies for preventive and therapeutic vaccines for infectious disease indications' by US FDA [131] and ICH guideline: Guidance on testing of

genotoxicity studies and interpretation of data for pharmaceuticals intended for human use [132] and immunotoxicity studies for human pharmaceuticals [132] are approved.

Regulatory concerns

Drug delivery using microbes is a diversified system compared to conventional route due to either live or vegetative state and are capable of self-propagating within tumor cells. The biggest challenge with microbial drug delivery is its distinctive regulatory requirements with additional safety, toxicity and manufacturing technology. Guidelines and recommendations of genetic engineering techniques like recombinant DNA technologies used for these agents are provided by regulatory authorities like USFDA and the Office of Biotechnology Activities (OBA) at the National Institutes of Health [133] has carried out a detailed analysis in this regard and instructive guidelines by USFDA like [134] are also available. “Guidance for Industry: determining the need for and content of environmental assessments for gene therapies, vectored vaccines, and related recombinant viral or microbial products”.

Conclusions

Microbe-based drug delivery has become one of the promising delivery systems for many diseases like cancer, inflammatory bowel diseases. The main problem associated with conventional drug delivery system is the resistance developed by tumor cells. So as to overcome this problem, microbial drug therapy in combination with an anti-cancer agent play important role in drug delivery system. Toxins and spores are the bacterial products and helpful candidates to treat carcinogenic cells. Moreover, other strategies to treat tumor cells include bacterial ghosts, microbots and bactofection. Even if microbial therapy shows favorable applications, toxicity, dose and therapeutic efficacy still remain a problem. Furthermore, advance development and research in this field is required. Another novel approach is virotherapy, which includes using the virus, virosomes, viral particles, to convey molecules, nucleic acids, biological actives, etc. However, some parameters need to be studied such as toxicity and compatibility of the virus with molecules.

Bacteria show promising results because of their exploited properties like penetration in the tumor, increase in immunity, etc. Bacteria in drug delivery are extensively under research and achieved success in preclinical trails as well as in clinical trials. Other than bacteria, viruses are equally proved to be an interesting candidate for drug delivery due to accumulation in tumor and tumor imaging. A novel approach of microbes-based drug delivery with reduced toxicity and side effects will surely be futuristic advanced carrier to active improve patient's health.

Authors contributions Dr. Pravin Shende is involved in constructing, planning and organizing the manuscript.

Ms. Vasavi Basarkar is involved in literature search and writing of manuscript.

Compliance with ethical standards

Conflict of interest Authors declare no conflict of interest.

References

1. Chang WW, Lee C. Salmonella as an innovative therapeutic anti-tumor agent. *J Mol Sci*. 2014;15:14546–4.
2. Bhaskar M, Sanib B. Virosomes: a novel strategy for drug Delivery and targeting. *Bio-Pharm International Supplements*. 2011;24:12–3.
3. Kumar A, Kumar A.V., Why Chitosan? From properties to perspective of mucosal drug delivery, *J Biological Macromolecules*. 2016; (S0141–8130) (16) 30465–2.
4. Yin L. Bacillus spore-based oral carriers loading curcumin for the therapy of colon cancer. *J Control Release*. 2017;S0168-3659(17): 31079–9.
5. Nauts H, The beneficial effects of bacterial infections on host resistance to cancer: End result in 449 cases, *Cancer research institute monograph no. 8, New York, USA; 1980; (2)*.
6. Barbe S, Mellaert V, Anne J. The use of clostridial spores for cancer treatment. *J Appl Microbiol*. 2006;101(3):571–8.
7. Richardson MA, Ramirez T, Russell NC, Moye LA. Coley toxins immunotherapy: a retrospective review. *Altern Ther Health Med*. 1999;5(3):42.
8. Zacharski LR, Sukhatme VP. Coley's toxin revisited: immunotherapy or plasminogen activator therapy of cancer. *J Thromb Haemost*. 2005;3(3):424–7.
9. Gravekamp C, Paterson Y. Harnecing *Listeria monocytogenes* to target tumors. *Cancer Biol Ther*. 2010;9:257–65.
10. Forbes NS. Perspectives. Engineering the perfect (bacterial) cancer therapy. *Nat Rev Cancer*. 2010;10:785–94.
11. Hoffman HR, Zhao M. Methods for the development of tumor targeting bacteria. *Expert Opinion. J Drug Discov*. 2014;(9):741–50.
12. Minton NP. Clostridia in cancer therapy. *Nat Re Microbiol*. 2003;1:237–42.
13. Carswell EP, Old LJ, Kassel RL, Green S, Williamson, an endotoxin-induced serum factor that causes necrosis of tumors. *Proc Natl Acad Sci*. 1975;72:3666–70.
14. Dang LH, Bettgowda C, Huso DL, Kinzler K, Vogelstein WB. Combination bacteriolytic therapy for the treatment of experimental tumors. *Proc Natl Acad Sci*. 2001;98(26):15155–60.
15. Gericke D, Engelbart K. Oncolysis by clostridia. II. Experiments on a tumor spectrum, 1208. *Cancer Res*. 1964;24:217–21.
16. Thiele EH, Arison RN, Boxer G. E., Oncolysis by clostridia. IV. Effect of nonpathogenic 1210 clostridial spores. *Cancer Res*. 1964;24:234–8.
17. Bone RC. Toward an epidemiology and natural history of SIRS (systemic inflammatory response syndrome). *JAMA*. 1992;268: 3452–5.
18. Dinarello CA, Gelfand JA, Wolff SM. Anticytokine strategies in the treatment of the 1214 systemic inflammatory response syndrome. *JAMA*. 1993;269:1829–35.
19. Jr Somerville JE, Cassiano LBB, Cunningham MD, Darveau RP. A novel 1216 *Escherichia coli* lipid A mutant that produces an anti-inflammatory lipopolysaccharide, 1217 *J. Clin Investig*. 1996;97: 359–65.

20. Khan S.A., Everest, P. Servos S, Foxwell N, Zahringer U, et al. 1219 Dougan, I.G. Charles, D.J. Maskell, A lethal role for lipid, A in Salmonella infections, Mol.1220 Microbiol. 1998; 29, 571–579.
21. Low KB, Ittensohn M, Le T, Platt J, Sodi S, AmossMAsh O, et al. Lipid a mutant Salmonella with suppressed virulence and TNF α induction retain tumor-targeting in vivo. Nat Biotechnol. 1999;17: 37–41.
22. Foline B, Dessein R, Marceau M, Poiret S, Chamaillard M, Pot B, et al. 1226 prevention and treatment of colitis with Lactococcus lactis secreting the immunomodulatory Yersinia LcrV protein. Gastroenterology. 2007;133:862–74.
23. Steidler L, Hans W, Schotte L, Neiryck S, Obermeier F, Falk W, et al. 1229 treatment of murine colitis by Lactococcus lactis secreting interleukin-10. Science. 2000;289:1352–5.
24. Duan FF, Liu JH, March JC. Engineered commensal bacteria reprogram intestinal cells into glucose-responsive insulin-secreting cells for the treatment of diabetes. Diabetes. 2015;64(5):1794–803.
25. Liu X, et al. Engineered vaginal lactobacillus strain for mucosal delivery of the human immunodeficiency virus inhibitor cyanovirin-N, Antimicrob. Agents Chemother. 50. 2006; 3250–3259.
26. Mazumder B., Bhattacharya S., Virosomes: a novel strategy for drug delivery and targeting. The science and business of biopharmaceuticals, Biochem Pharmacol. 2001; Jan.02, 24.
27. A.W. Paton, Morona R and Paton J.C, Bioengineered microbes in disease therapy, Trends Mol Med. 2012; (18) 7, 417–425.
28. Goldstein R.A., Soyer O. S., Evolution of taxis responses in virtual bacteria: non-adaptive dynamics, PLoS Comput Biol 2008; 23; 4 (5).
29. Taylor BL, Zhulin IB, Johnson MS. Aerotaxis and other energy-sensing behavior in bacteria. Annu Rev Microbiol. 1999;53:103–28.
30. Taniguchi S., Shimatani Y., Fujimori M., Tumor-targeting therapy using gene-engineered anaerobic-nonpathogenic *Bifidobacterium longum*, Methods Mol Biol 2016; 1409,1375 49–60.
31. Seavey MM, Pan ZK, Maciag PC, Wallecha A, Rivera S, Paterson Y, et al. A novel human Her-2/neu chimeric molecule expressed by *Listeria monocytogenes* can elicit potent HLA-A2 restricted CD8-positive T-cell responses and impact the growth and spread of Her-2/neu-positive breast tumors. Clin Cancer Res. 2009;15: 924–32.
32. Theys J, Pennington O, Dubois L, Anlezark G, Vaughan T, Mengesha A, et al. Repeated cycles of Clostridium-directed enzyme prodrug therapy result in sustained antitumor effects in vivo. Br J Cancer. 2006;95:1212–9.
33. Du Z.Q. Wang J.Y., A novel lumazine synthase molecule from Brucella significantly promotes the immune-stimulation effects of antigenic protein, Genet. Mol. Res. 14. 2015; 13084–13095.
34. Friend DR, Chang GW. A colon-specific drug-delivery system based on drug glycosides and the glycosidases of colonic bacteria. J Med Chem. 1984;27:261–6.
35. Fu GF, Li X, Hou YY, Fan YR, Liu WH, Xu GX. Bifidobacterium longum as an oral delivery system of endostatin for gene therapy on solid liver cancer. Cancer Gene Ther. 2005;12:133–40.
36. Yazawa K, et al. Bifidobacterium longum as a delivery system for gene therapy of chemically induced rat mammary tumors. Breast Cancer Res Treat. 2001;66:165–70.
37. Lee, B., P. Thiyagarajan, R.E. Winans, X. Li, Z. Niu, Q. Wang, Effect of interfacial interaction on the cross-sectional morphology of tobacco mosaic virus using GISAXS, Langmuir 23. 2007; 11157–11163.
38. Xiao X. The antitumor effect of suicide gene therapy using Bifidobacterium infantise-mediated herpes simplex virus thymidine kinase/ganciclovir in a nude mice model of renal cell carcinoma, Urology 84. 2014; 84(4), 982.
39. Jiang L. Proteomic analysis of bladder cancer by iTRAQ after Bifidobacterium infantis-mediated HSV-TK/GCV suicide gene treatment. Biol Chem. 2013;394:1333–42.
40. Yin X. Bifidobacterium infantis-mediated HSV-TK/GCV suicide gene therapy induces both extrinsic and intrinsic apoptosis in a rat model of bladder cancer. Cancer Gene Ther. 2013;20:77–81.
41. Yin X, Yu B, Tang Z, He B, Xiao X. Bifidobacterium infantis-mediated HSV-TK GCV suicide gene therapy induces both extrinsic and intrinsic apoptosis in a rat model of bladder cancer. Cancer Gene Ther. 2013;20:77–81.
42. Okuda K, Wada Y, Shimada M. Recent developments in preclinical DNA vaccination. Vaccines (Basel). 2014;2:89–106.
43. Gentschev, I., G. Dietrich, Spreng S., Kolb-Maurer, Brinkmann V, Grode L, Hess J, Kaufmann S.H.E, Goebel E, Recombinant attenuated bacteria for the delivery of subunit vaccines, Vaccine 19. 2001; 2621–2628.
44. Radford KJ, Higgins DE, Pasquini S, Cheadle EJ, Carta LA. A recombinant *E. coli* vaccine to promote MHC class I-dependent antigen presentation: Application to cancer immunotherapy. Gene Ther. 2002;9:1455–63.
45. Storz G., Hengge, R., Bacterial Stress Responses, 2nd ed. American Society for Microbiology Press. 2010.
46. Faivre D. Magnetotactic bacteria and magnetosomes. Chem Rev. 2008;108:4875–98.
47. Felfoul, O., Martel S., Assessment of navigation control strategy for magnetotactic bacteria in microchannel: Toward targeting solid tumors, Biomed Microdevices 15. 2013; 1015–1024.
48. Martel S. Bacterial microsystems and microrobots. Biomed Microdevices. 2012;14:1033–45.
49. Chen CY, Song T. Construction of a microrobot system using magnetotactic bacteria for the separation of staphylococcus aureus. Biomed Microdevices. 2014;16:761–70.
50. Brown J. M., Wilson W.R., Exploiting tumor hypoxia in cancer treatment, Natl Rev 1620. 2004; Cancer 4, 437–447.
51. Zhang X, Lin Y, Gillies RJ. Tumor pH and its measurement. J Nucl Med. 2010;51:1167–70.
52. Kluger M, Rothenburg B. Fever and reduced iron: their interaction as a host defense response to bacterial infection, Science 203. 1979; 374–376.
53. Zhuan, G.J., Wright Carlsen R., Sitti M., pH-taxis of biohybrid microsystems, Sci. Report. 2015; 5, 11403.
54. Patyar S, Joshi R, Byrav DS, Das P. Bacteria in cancer therapy: a novel experimental strategy. J Biomed Sci. 2010;17(1):21.
55. Carey R, Holland J, Whang H, Neter E, Bryant B. Clostridial oncolysis in man, Eur. J. Cancer. 1967; 3:37, 46.
56. Bermudes D, Zheng L, King IC. Live bacteria as anticancer agents and tumor-selective protein delivery vectors. Curr Opin Drug DiscovDevel. 2002;5(2):194–9.
57. Malmgren RA, Flanigan CC. Localization of the vegetative form of Clostridium tetani in mouse tumors following intravenous spore administration. Cancer Res. 1955;15:473–8.
58. Liu S, Minton N, Giaccia A, Brown J. Anticancer efficacy of systemically delivered anaerobic bacteria as gene therapy vectors targeting tumor hypoxia/necrosis. Gene Ther. 2002;9(4):291–6.
59. King I, Ittersson M, Bermudes D. Tumor-targeted *Salmonella typhimurium* overexpressing cytosine deaminase: a novel, tumor-selective therapy. Methods Mol Biol. 2009;542:649–59.
60. Avogadri F, Martinoli C, Petrovska L, Chiodoni C, Transidico P, Bronte V, et al. Cancer immunotherapy based on killing of Salmonella-infected tumor cells. Cancer Res. 2005;65(9):3920–7.
61. Sabzehali F, Azimi H, Goudarzi M. Bacteria as a vehicle in cancer therapy and drug delivery. J of Paramedical Sciences. 2017; (8), 1 52–59.

62. Frankel AE, Rossi P, Kuzel TM, Foss F. Diphtheria fusion protein therapy of chemo resistant malignancies. *Curr Cancer Drug Targets*. 2002;(1):19–36.
63. Falnes PO, Ariansen S, Sandvig K, Olsnes S. Requirement for prolonged action in the cytosol for optimal protein synthesis inhibition by diphtheria toxin. *J Biol Chem*. 2005;275(6):4363–8.
64. Pastan I., Targeted therapy of cancer with recombinant Immunotoxins *Bio chimica et Biophysica Acta (BBA)-Rev Cancer*. 1997; 1333(2) C1-C6.
65. Kokai JF, McClane BA. Determination of functional regions of *Clostridium perfringens* enterotoxin through deletion analysis. *Clin Infect Dis*. 1997;25:S165–7.
66. Kokai JF, Benton K, Wieckowski EU, McClane BA. Identification of a *Clostridium perfringens* enterotoxin region required for large complex formation and cytotoxicity by random mutagenesis. *Infect Immun*. 1999;67:5634–41.
67. Langemann T, Koller VJ, Muhammad A, Kudela P, Mayr UB, Lubitz W. The bacterial ghost platform system: production and applications. *Bioengineered Bugs*. 2010;1:326–36.
68. Tabrizi C.A. et al, Bacterial ghosts — biological particles as delivery systems for antigens, nucleic acids and drugs, *Curr. Opin. Biotechnol*. 2004; 15, 530–537, (2004).
69. Huter V, Szostak MP, Prethaler GJ. Bacterial ghosts as drug carrier and targeting vehicles. *J Control Release*. 1999;61:51–63.
70. Paukner S, Kohl G, Jalava K, Lubitz W, sealed bacterial ghosts—novel targeting vehicles for advanced drug delivery of water-soluble substances. *J Drug Target*. 2003;11:151–61.
71. Paukner S, Kohl G, Lubitz W, bacterial ghosts as novel advanced drug delivery systems: anti proliferative activity of loaded doxorubicin in human Caco-2 cells. *J Control Release*. 2004;94:63–74.
72. Stein E. et al, In vitro and in vivo uptake study of *Escherichia coli* Nissle 1917 bacterial ghosts: cell-based delivery system to target ocular surface diseases, *Invest. Ophthalmol*. 2013; *Vis. Sci*. 54 6326–6333.
73. Mengesha, In: *Clostridia: Molecular Biology in the Post-genomic Era*. Bruggemann H, Gottschalk G, editor. Caister Academic Press; *Clostridia in Anti-tumor Therapy*. 2009.
74. Theys J, Landuyt W, Nuyts S, van Mellaert L, van Oosterom A, Lambin P, et al. Specific targeting of cytosine deaminase solid tumors by engineered *Clostridium acetobutylicum*. *Cancer Gene Ther*. 2001;8:294–7.
75. Rabanel JM, Hildgen P, Banquy X. Assessment of PEG on polymeric particles surface, a key step in drug carrier translation. *J Control Release*. 2014;185:71–87.
76. Fujimori M, Amano J, Taniguchi S The genus *Bifidobacterium* for cancer gene therapy *Curr Opin Drug Discov Devel*. 2003;5:200–3.
77. Carlsen R.W., Sitti M., Bio-hybrid cell-based actuators for microsystems, *Small* 10. 2012; 1250 3831–3851.
78. Kim D, Liu A, Diller E, Sitti M, chemotactic steering of bacteria propelled microbeads, 1717 *biomed. Microdevices*. 2012;14: 1009–17.
79. Akin D, Sturgis J, Ragheb K, Sherman D, Burkholder K. Bacteria-mediated delivery of nanoparticles and cargo into cells. *Nat Nanotechnol*. 2007;2:441–9.
80. Kolate A, Baradia D, Patil S, Vhora I., PEG—a versatile conjugating ligand for drugs and drug delivery systems. *J Control Release*. 2014;192:67–81.
81. Xu J, et al. Combination of immunotherapy with anaerobic bacteria for immunogene therapy of solid tumours. *Gene Ther Mol Biol*. 2009;13:36–52.
82. Wood LM, Guirnalda PD, Seavey MM, Paterson Y. Cancer immunotherapy using *Listeria monocytogenes* and listerial virulence factors. *Immunol Res*. 2008;(42):233–45.
83. Kelly RE. History of oncolytic viruses: genesis to genetic engineering. *Mol Ther*. 2007;15(4):651–9.
84. Moore AE. The destructive effect of the virus of Russian Far East encephalitis on the transplantable mouse sarcoma 180. *Cancer*. 1949;2(3):525–34.
85. Clinical virotherapy: four historically significant clinical trials.
86. Simona D, Stréle L, Proboka G, Auziņš J, Pēteris A, Björn J, et al. Adapted ECHO-7 virus Riggvir immunotherapy (oncolytic virotherapy) prolongs survival in melanoma patients after surgical excision of the tumour in a retrospective study. *Melanoma Res*. 2015;25(5):421–6.
87. Viruses: The new cancer hunters. *Isra Cast (News article)*. March 1, 2016.
88. Amato RJ, Hawkins RE, Kaufman HL, Thompson JA, Tomczak P. Vaccination of metastatic renal cancer patients with MVA-5T4: a randomized, double-blind, placebo- controlled phase III study. *Clin Cancer Res*. 2010;16(22):5539–47.
89. Bayer ME, Blumberg BS, Werner B. Particles associated with Australia antigen in the sera of patients with leukaemia, Down's syndrome and hepatitis. *Nature*. 1968;218(5146):1057–9.
90. Petry H, Goldmann C, Ast O, Lüke W, "the use of virus-like particles for gene transfer". *Curr Opin Mol Ther*. 2003;5(5):524–8.
91. Galaway FA, Stockley PG. MS2 virus like particles: A robust, semisynthetic targeted drug delivery platform. *Mol Pharm*. 2013;10:59–68.
92. Kovacs EW, et al. Dual-surface-modified bacteriophage MS2 as an ideal scaffold for a viral capsid-based drug delivery system. *Bioconjug Chem*. 2007;18:1140–7.
93. Akahata W., Yang Z. Y, Andersen H, et al. "a VL vaccine for epidemic chikungunya virus protects non-human primates against infection". *Nat Med* 2010; 16 (3), 334–338.
94. Zhang X, Xin L, Li S, Fang M, Zhang J, Xia N, et al. Lessons learned from successful human vaccines: delineating key epitopes by dissecting the capsid proteins. *Human Vacc Immunother*. 2015;11(5):1277–92.
95. Shende P, Waghchaure M. Combined vaccines for prophylaxis of infectious conditions. *Artif Cells Nanomed Biotechnol*. 2019;47(1):696–705.
96. Almeida JD, Brand CM, Edwards DC. T.D. Heath, Formation of virosomes from influenza subunits and liposomes. *Lancet*. 1975;2: 899–901.
97. Bagai S, Puri A. Hemagglutinin- neuraminidase enhances F protein-mediated membrane fusion of reconstituted Sendai virus envelopes with cells. *J Virol*. 1993;67:3312–8.
98. Uchida T, Kim J. Reconstitution of lipid vesicles associated with HVJ (Sendai virus) spikes. Purification and some properties of vesicles containing nontoxic fragment a of diphtheria toxin. *J Cell Biol*. 1979;80:10–20.
99. Vainstein A, Hershkovitz M, Israel S, Rabin S, Loyter A. A new method for reconstitution of highly fusogenic Sendai virus envelopes. *Biochim Biophys Acta*. 1984;773:181–8.
100. Helenius A, Sarvas M, Simons K. Asymmetric and symmetric membrane reconstitution by detergent elimination. Studies with Semliki-Forest-virus spike glycoprotein and penicillinase from the membrane of *Bacillus licheniformis* *Eur J Biochem*. 1981;116:27–35.
101. Helenius A, Fries E, Kartenbeck J. Reconstitution of Semliki Forest virus membrane. *J Cell Biol*. 1977;75:866–80.
102. Metsikkö K, Simons K. Reconstitution of the fusogenic activity of vesicular stomatitis virus. *EMBO J*. 1986;5:3429–35.
103. Petri WA, Wagner RR. Reconstitution into liposomes of the glycoprotein of vesicular stomatitis virus by detergent dialysis. *J Biol Chem*. 1979;(254):4313–6.
104. Scheule RK. Novel preparation of functional Sindbis virosomes. *Biochem*. 1986;25:4223–32.
105. Cusi MG. Applications of influenza virosomes as a delivery system. *Human Vaccines*. 2006;2:1–7.

106. Daemen T de Mare A, Bungener L, de Jonge J, Huckriede A, Wilschut J. Virosomes for antigen and DNA delivery. *Adv Drug Deliv Rev.* 2005;57:451–63.
107. Felnerova D, Viret JF, Glück R, Moser C. Liposomes and virosomes as delivery systems for antigens, nucleic acids and drugs. *Curr Opin Biotechnol.* 2004;15:518–29.
108. Zhao S, Penman M, Hoffman RM. Tumor-targeting bacterial therapy with amino acid auxotrophs of GFP-expressing *Salmonella typhimurium*. *Proc Natl Acad Sci U S A.* 2005;102:755–60.
109. Rosenberg GA. Neurological diseases in relation to the blood–brain barrier. *J Cereb Blood Flow Metab.* 2012;32:1139–51.
110. Van Sorge N. M., Doran K.S., Defense at the border: the blood–brain barrier versus bacterial foreigners, *Future Microbiol* 7, 2012; 383–394.
111. Zwagerman N.T., Friedlander R. M, Monaco E.A., Intratumoral *Clostridium novyi* as a potential treatment for solid necrotic brain tumors, *Neurosurgery* 75, 2014; N17–N18.
112. Guo S.G., Yan W.W, Mc Donough S.P., Lin N.F., et al The recombinant *Lactococcus lactis* oral vaccine induces protection against *C. difficile* spore challenge in a mouse model, *Vaccine* 33. 2015; 1586–1595.
113. Ahmed B, Loos M, Vanrompay D, Cox E. Oral immunization with *Lactococcus lactis*-expressing EspB induces protective immune responses against *Escherichia coli* O157:H7 in a murine model of colonization. *Vaccine.* 2014;32:3909–16.
114. Wei P, et al. Oral delivery of *Bifidobacterium longum* expressing alpha-melanocyte-stimulating hormone to combat ulcerative colitis. *J Med Microbiol.* 2016;65(2):160–8.
115. Takei S, et al Oral administration of genetically modified *Bifidobacterium* displaying HCV-NS3 multi-epitope fusion protein could induce an HCV- NS3-specific systemic immune response in mice, *Vaccine* 32, 2014; 3066–3074.
116. Yu ZJ, Huang Z, Sao CW, Huang YJ, Zhang F, Yang J, et al. *Bifidobacterium* as an oral delivery carrier of interleukin-12 for the treatment of Coxsackie virus B3-induced myocarditis in the Balb/c mice. *Int Immunopharmacol.* 2012;12:125–30.
117. Ning JF, Zhu W, Xu JP, Zheng CY, Meng XL. Oral delivery of DNA vaccine encoding VP28 against white spot syndrome virus in crayfish by attenuated *Salmonella typhimurium*. *Vaccine.* 2009;27:1127–35.
118. Chen G. et al, Oral delivery of tumor-targeting *Salmonella* exhibits promising therapeutic efficacy and low toxicity, *Cancer Sci.* 2009; 100, 2437–2443, (2009).
119. Castagliuolo I, et al Engineered *E. coli* delivers therapeutic genes to the colonic mucosa, *Gene Ther.* 2005; 12, 1070–1078.
120. Grillot-Courvalin C, et al Fruehauf, Development of a therapeutic RNAi delivery system using nonpathogenic bacteria expressing inv and hly: trans kingdom RNA interference (tkRNAi), *Hum. Gene Ther.* 2009; 20, 670.
121. Ivory K. et al, Oral delivery of *Lactobacillus casei* Shirota modifies allergen-induced immune responses in allergic rhinitis, *Clin. Exp. Allergy* 38. 2008; 1282–1289.
122. Lee CH. Engineering bacteria toward tumor targeting for cancer treatment: current state and perspectives. *Appl Microbiol Biotechnol.* 2012;93:517–23.
123. Liu SC, Minton NP, Giaccia AJ, Brown JM. Anticancer efficacy of systemically delivered anaerobic bacteria as gene therapy vectors targeting tumor hypoxia/necrosis. *Gene Ther.* 2002;9:291–6.
124. Ganai Set al, In tumors *Salmonella* migrate away from vasculature toward the transition zone and induce apoptosis, *Cancer Gene Ther.* 2011; 18, 457–466.
125. Loeffler M. Et al reed, inhibition of tumor growth using *Salmonella* expressing Fas ligand. *J Natl Cancer Inst.* 2008;100: 1113–6.
126. Yam, Met al Hoffman, monotherapy with a tumor-targeting mutant of *S. typhimurium* inhibits liver metastasis in a mouse model of pancreatic cancer, *J Surg Res* 2010; 164, 248–255.
127. Ciabattini, A. et al, Primary activation of antigen- specific naive CD4 (+) and CD8 (+) T cells following intranasal vaccination with recombinant bacteria, *Infect. Immun.* 76. 2008; 5817–5825.
128. Palffy F. Bacteria in gene therapy: bactofection versus alternative gene therapy. *Gene Ther.* 2006;13:101–5.
129. Malmgren RA, Flanigan CC. Localization of the vegetative form of *Clostridium tetani* in mouse tumors following intravenous spore administration. *Cancer Res.* 1955;15:473–8.
130. Hosseinidoust Z. Bioengineered and biohybrid bacteria-based systems for drug delivery. *Adv Drug Deliv Rev.* 2016;106:27–44.
131. Guidance for Industry: Considerations for developmental toxicity studies for preventive and therapeutic vaccines for infectious disease indications. 2006.
132. ICH guideline on Guidance on genotoxicity testing and data interpretation for pharmaceuticals intended for human use. 2011.
133. Husain S.R., Han J, Au P, Shannon K, Puri K. R, Gene therapy for cancer: regulatory considerations for approval. *Cancer Gene Ther.* 2015; (22) 554–563.
134. Guidance for Industry: Design and Analysis of Shedding Studies for Virus or Bacteria-based Gene Therapy and Oncolytic Products. 2015; (73).

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.