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REVIEW ARTICLE

Emerging Trends and Potential Prospects in Vaginal Drug Delivery

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Abstract: The vagina is an essential part of the female reproductive system and offers many potential benefits over conventional drug delivery, including a large surface area for drug absorption, relatively low enzymatic activity, avoiding first-pass effects, and ease of administration. The vaginal mucosal cavity is an effective route for administering therapeutic agents that are intended both for local and systemic administration. The present review provides a comprehensive overview of recent trends and developments in vaginal drug delivery. Marketed formulations and products under clinical study are also reviewed. Various novel vaginal delivery systems have been studied in recent years as effective tools for delivering a range of therapeutic agents to the vagina. These systems offer numerous benefits, including sustained delivery, improved bioavailability, effective permeation, and higher efficacy. The recent focus of the scientific community is on the development of safe and efficient drug delivery systems, such as nanoparticles, microparticles, vesicular systems, vaginal rings, microneedles, *etc.*, for vaginal application. Various factors, such as the physicochemical properties of the drugs, the volume and composition of the vaginal fluid, the pH of the vaginal fluid, the thickness of the vaginal epithelium, and the influence of sexual intercourse may influence the release of drugs from the delivery system and subsequent absorption from the vaginal route. To date, only a limited number of *in vivo* studies on novel vaginal DDS have been reported. Additionally, drug release kinetics under varying vaginal environments is also not well understood. More research is needed to ensure the suitability, biocompatibility, and therapeutic effectiveness of novel DDS for vaginal delivery. Although numerous strategies and interventions have been developed, clinical translation of these systems remains a challenge. The toxicity of the carrier system is also an important consideration for future clinical applications.

Keywords: Vagina, vaginal drug delivery system, nanocarriers, mucoadhesive vaginal formulation, vaginal gel, vaginal rings.

1. INTRODUCTION

In recent decades, mucosal drug delivery has attracted considerable interest in the administration of drugs, which are poorly absorbed after oral delivery [1]. In mucosal drug delivery, drugs are administered through moist cavities, such as the lining of the mouth, vagina, and bladder. The vagina as a route for drug delivery has been extensively explored as it offers several potential benefits for drug delivery, such as large surface area, avoidance of hepatic first-pass metabolism, low enzymatic activity, and high permeability to various therapeutic agents [2, 3]. Due to the presence of an extensive network of blood vessels, the vagina is an attractive route of drug delivery for both local and systemic effects. Due to the avoidance of the hepatic first-pass effect, the vagina serves as an effective route for the delivery of hormonal contraceptives and probiotics [4]. A complex interaction of vaginal microbiota, microbial by-products, estrogens, and host factors keeps the vaginal ecosystem in a finely balanced state. The vagina is generally resistant to infection because of the acidity of the vagina and the thick protective epithelium [5].

The vaginal route has historically been used to treat local genital problems, such as infections, vaginitis, labour induction, and prevention. Traditionally, solutions, gels, creams, foams, tablets, and suppositories have been used as vaginal DDS. These vaginal dosage forms are associated with several drawbacks, including leakage, messiness, and short residence time, which lead to reduced patient compliance and therapeutic efficacy. Several novel DDS, such as liposomes, microemulsions, Nanoparticles (NPs), Microparticles (MPs), bio-adhesive gels, bio-adhesive films, bio-adhesive tablets, vaginal rings, microneedles, *etc.*, have been investigated for vaginal application in recent years [3-6] (Fig. **1**). Some key advantages of novel systems for vaginal delivery include better mucoadhesiveness, sustained action, and modified

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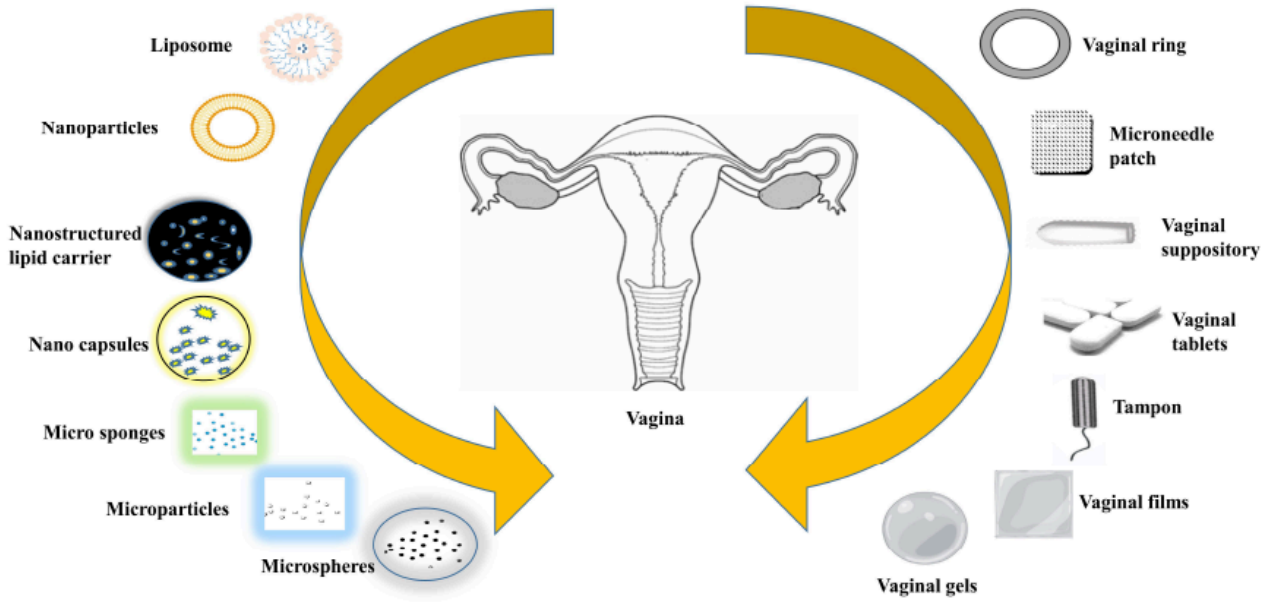

Fig. (1). Conventional and novel vaginal drug delivery systems. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

drug profiles [7]. Several novel vaginal DDS containing a wide range of pharmacologically active chemicals, such as antibacterial, antivirals, antifungals, antiprotozoals, labour inducers, spermicidal agents, and sex hormones, have been investigated for vaginal administration [8]. Compared to conventional dosage forms, bioadhesive vaginal DDS have significant advantages as they can be easily localized to the application area and thus improve the bioavailability of drugs [9]. Recently, several delivery systems for vaginal delivery of microbicides have been developed to prevent HIV and other sexually transmitted diseases [10]. The vaginal cavity can also be used to target various therapeutic agents in the uterus. The vaginal administration of chemotherapeutic drugs for the treatment of all cancer has also been investigated [11]. Temperature and pH-responsive systems have also been developed to improve the efficacy of medications administered through the vaginal route [12].

Effective delivery of drugs through the vagina remains a challenging pharmaceutical problem. The quest for new, successful local therapies with good adhesive properties that can release active agents for an extended period has attracted significant attention among the scientific community [13, 14]. Vagina has distinctive features in terms of vaginal secretions, pH, enzyme activity, and microflora which can influence formulation spreading and retention as well as absorption and drug release in the vagina. Products and devices which are vaginally delivered may cause significant irritation of the vaginal epithelium that can increase susceptibility to infection and other diseases. Therefore, the composition of the vehicle and its potential effects on the vagina must be taken into consideration while designing various systems for vaginal drug delivery [15, 16]. Dissolution is the rate-limiting step for systemic absorption of drugs from vaginal formulations due to the small volume of vaginal fluid. Drug absorption in the vaginal route occurs in two steps: drug dissolution in the vaginal space and permeability across the membrane. As a result, factors influencing drug dissolution and permeability may influence the absorption profile of vaginal DDS. To prevent discomfort, solid dosage formulations should preferably dissolve in the vaginal canal shortly after the insertion. Several physiological factors, including the volume and composition of the vaginal fluid, the pH of the vaginal fluid, the thickness of the vaginal epithelium, and the influence of sexual intercourse, may influence the release of drugs from vaginal DDS. The presence of highly viscous cervical mucus may hinder drug absorption, and increased fluid volume can result in the removal of the drug from the vaginal cavity and reduce subsequent absorption. Enzymatic activity is an essential component that may impact drug absorption and the long-term stability of intravaginal DDS. The human vaginal system has less enzymatic activity than the gastrointestinal tract, resulting in the reduced breakdown of protein and peptide drugs in the vagina. However, the presence of membrane-bound aminopeptidase could be a primary reason for the poor bioavailability of vaginally administered protein and peptide drugs. The contact time can be increased by adding mucoadhesive polymers to the formulation [2]. The rate of absorption *via* passive diffusion can be increased by increasing drug concentration in the vaginal fluid. However, high local drug concentration can cause severe localized irritation or other adverse drug reactions. Some physicochemical characteristics of drugs that may affect vaginal drug absorption include molecular weight, ionization, lipophilicity, surface charge, and chemical composition [17]. These issues must be taken into consideration during the development and evaluation of vaginal DDS.

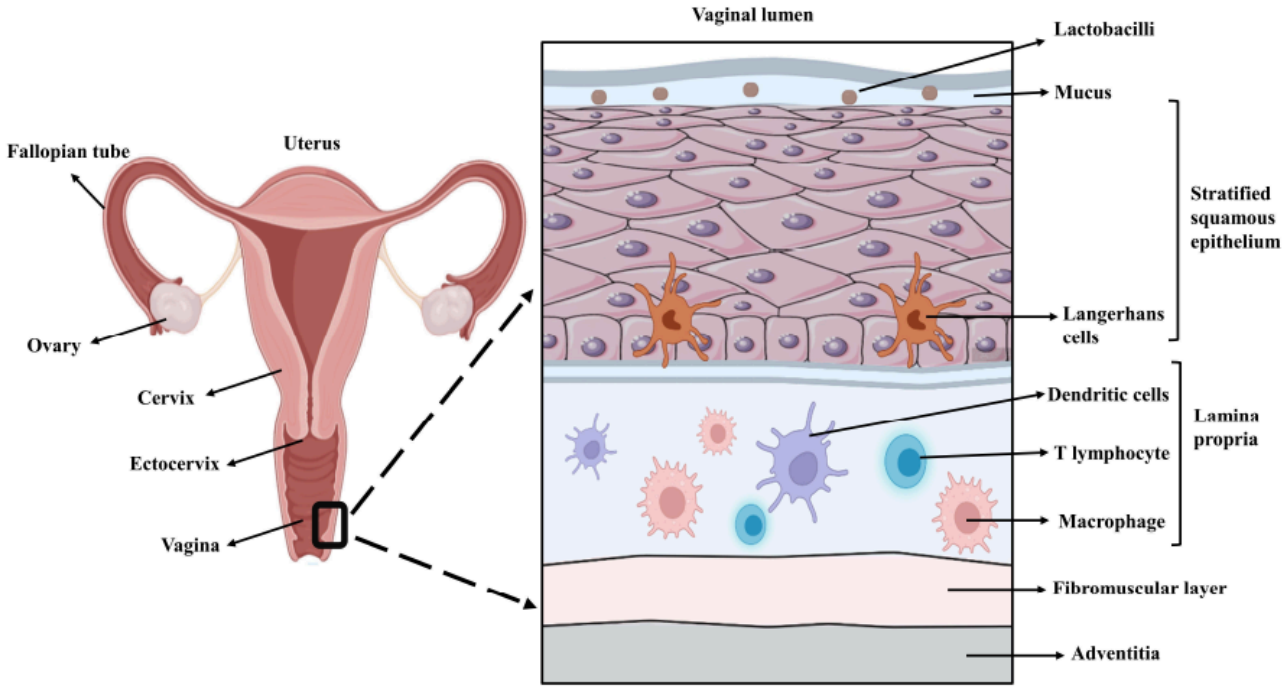

Fig. (2). Anatomy and history of vagina. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Despite several advantages, there are some misconceptions, particularly among patients, related to drug delivery *via* the vaginal route. Issues related to personal hygiene, the influence of sexual intercourse, gender specificity, and local irritation of some drugs are some of the barriers to this form of therapy. This review will provide an overview of the latest advances in vaginal DDS for the treatment of various diseases with an introduction to vaginal anatomy and histology, immunology of the vagina, and vaginal infections. Marketed formulations and products under clinical study are also reviewed.

2. ANATOMY AND HISTOLOGY OF VAGINA

The anatomical and histological description of the vagina is shown in Fig. (**2**). The vagina is a slightly s-shaped fibromuscular canal that is 5 and 10 cm long and extends from the cervix to the vulva. The vaginal wall is composed of an outer epithelial layer underlain by lamina propria, muscularis, and the tunica adventitia [18, 19]. The wall of the vagina has a tunica mucosa, consisting of a stratified squamous epithelium with numerous folds referred to as rugae. The rugae give support and distensibility and provide enlarged surface area to the vaginal wall. The thickness of the mucosa varies depending upon the environmental conditions and hormonal activities [20]. The lamina propria is the second layer, which is made up of connective tissues. It consists of elastic fibers and cells, such as macrophages, mast cells, neutrophils, eosinophils, lymphocytes, *etc.* The lamina propria also contains a network of nerve fibers, arteries, blood vessels, and lymphatic vessels. It is believed that the drugs enter the systemic circulation *via* the blood vessels of the lamina propria. The muscularis consists of smooth muscle bundles arranged into an outer longitudinal and inner circular layer and provides excellent elasticity to the vagina. The adventitia has a vast plexus of blood vessels and contains elastic fibers, which also contribute to the overall distensibility of the vagina [21]. The network of blood vessels that supply blood to the vagina includes the vaginal artery, uterine artery, and internal pudendal artery [22]. Blood from the vagina reaches the peripheral circulation through a vaginal venous plexus, draining largely into the internal iliac veins. Hence, drugs do not undergo first-pass effects after absorption through the vagina [23]. This makes the vagina a useful site for the systemic administration of the therapeutic agent.

3. IMMUNOLOGY OF VAGINA

The vagina and ectocervix have a stratified layer of cells that consists of multiple layers of squamous epithelial cells, which act as a mechanical barrier and immunological mediator against pathogens [24]. The vaginal epithelia and submucosa are localized by lymphocytes and innate leukocytes, but the recruitment of T and B antigen-specific cells is restricted to the vagina [25]. In case of infection, both innate leukocytes and epithelial cells produce interferons, cytokines, and chemokines against recognized pathogens [26, 27]. Viral pathogens are processed by sub-epithelial dendritic cells and presented to T-cells, which induce the homing of the effector T-cell to the vaginal mucosa [28]. The epithelial layer can also play a role in ensuring the success of antiretroviral therapy for HIV containment as epithelial cells of the female reproductive tract and underlying fibroblasts may deliver and store antiretrovirals, facilitating long-term protection of vaginal $CD4^+$ T-cells from viral infection [29]. Several studies suggested that the immune response in this region, and thus disease susceptibility, varies throughout the menstrual cycle [30]. The levels of progesterone (Pg) and estrogen also help to regulate the menstrual cycle and immune response in humans [31, 32].

The lower female reproductive tract contains four major subsets of antigen-presenting cells, *i.e.*, intraepithelial Lang-

erhans cells, macrophages, lamina propria, $CD14^-$ dendritic cells, and $CD14^+$ dendritic cells. Antigen-presenting cells are mobilized to the draining lymph nodes after infection to prime T-cells. $CD14^-$ dendritic cells and Langerhans cells are directed towards Th2 cell activation and regulatory functions. Macrophages and $CD14^+$ dendritic cells, on the other hand, resemble innate cells, which respond to pathogens through toll-like receptors and help to prime Th1 responses [33]. In response to coculture with HIV-like particles, neutrophils have been shown to release neutrophil extracellular traps, which contribute to virus inactivation [34]. HIV-1 DNA has been found in HIV-infected women's $CD14^+$ dendritic cells and Langerhans cells [35]. MAIT cells (mucosal-associated invariant T-cells) have also been reported to play a role in local defense against sexually transmitted infections by producing Interleukin (IL)-17 and IL-22, suggesting a potential role in protection against infections [36]. Natural killer cells in the vaginal mucosa play a significant role in limiting viral infections, and their deficiency increases the risk of herpes virus infection and the occurrence of cervical cancer caused by the Human Papillomavirus (HPV) [37]. It has also been proved that IgG antibodies in the vaginal mucosa can trap Herpes Simplex Virus (HSV)-1 in the mucus and prevents vaginal infections [38]. After viral clearance, the aggregates of $CD4^+$ and $CD8^+$ T-cells, B-cells, dendritic cells, and macrophages may survive for months, potentially providing long-term defense against reinfection. The population of commensal bacterial species in the vagina also helps to protect against pathogens.

4. VAGINAL INFECTIONS

Several infections, primarily caused by pathogenic bacteria, fungi, viruses, and parasites, may affect the vagina. There are also a number of other risk factors related to hormonal imbalance, hygiene, and lifestyle-related habits that can cause inflammation or infection in the vagina. Local intravaginal formulations and condoms remain the most reliable form of protection from these infections. However, the development of vaccines could certainly be an effective strategy for the treatment of vaginal infections. Vaginal infections are more common and more difficult to treat in HIV-positive women. The healthy vagina is colonized with lactobacilli, which produce hydrogen peroxide and convert glycogen to lactic acid. *Lactobacillus spp.* dominate the vaginal ecosystem of healthy women and protect them from urogenital infections by maintaining a low pH (4.5) [39, 40]. The loss or reduction in colonizing *Lactobacillus spp.* causes an increase in the vaginal pH, favoring the growth of commensal anaerobes. Bacterial vaginosis (BV), vulvovaginal candidosis (VVC), trichomoniasis, HPV infection, and Chlamydia infection are the most common vaginal infections [41-43]. BV and TV infections are both widespread and are associated with an increased risk of sexual transmission of HIV [44]. BV is a clinical condition characterized by inflammation of the vagina (also known as vaginitis), which occurs due to an increase in the growth of endogenous bacteria, typically *Gardnerella vaginalis* and anaerobic bacteria, for example, *Atopobium vaginae*, *Prevotella* spp., *Porphyromonas* spp*., Mobiluncus* spp*., Porphyromonas* spp., *and Peptostreptococcus* spp. [41, 42, 45]. Some of the treatment options available for BV include oral metronidazole, oral clindamycin, oral tinidazole, metronidazole gel, and clindamycin cream.

VVC is also a common type of vaginal infection caused primarily by the fungus *Candida albicans*, but it may also be caused by non-*C. albicans* species, such as *Candida glabrata*. VVC affects a large number of women globally, and it is estimated that about 70% of all women are affected at least once in their lifetime [46]. VVC is characterized by vaginal itching (pruritus), pain, discharge, and swelling [47, 48]. VVC can be managed with topical or oral antifungal agents, such as fluconazole, clotrimazole, miconazole, tioconazole, butoconazole, and terconazole. Trichomoniasis (or trich) is a prevalent sexually transmitted infection among women caused by the protozoan parasite *Trichomonas vaginalis* [49]. Trichomoniasis is characterized by vulvar irritation, foul-smelling vaginal discharge with pH > 4.5, and painful urination [50]. The treatment options for trichomoniasis include oral metronidazole and tinidazole. Topical formulations are not prescribed because they are usually insufficient for total disease eradication, which can lead to reduced therapeutic efficacy compared to an oral formulation. HPV is also a common sexually transmitted infection that could infect the vagina. Infected patients generally develop symptoms, such as fever, myalgias, and swollen lymph nodes. Patients with long-term infection are more susceptible to complications, such as cancer [42, 43]. The recommended antiretroviral drugs for HPV treatment are Tenofovir (TFV), Dapivirine (DPV), ritonavir, maraviroc, *etc*.

Chlamydia infection, caused by the bacterium *Chlamydia trachomatis*, is another common sexually transmitted infection. In women, the common symptoms of Chlamydia infection include painful urination and vaginal discharge. The infection may spread to the upper genital tract causing pelvic inflammatory disease, which may lead to infertility or ectopic pregnancy in the future [51]. Chlamydia infection can be treated with antibiotics like azithromycin, doxycycline, ofloxacin, or levofloxacin. Gonorrhea infection is a sexually transmitted disease caused by *Neisseria gonorrhoeae*. Burning sensation during urination, vaginal discharge, vaginal bleeding during periods, and pelvic pain are the most common signs of gonorrhea infection [52]. Antibiotics used to treat gonorrhea infections are ceftriaxone and azithromycin. HSV-1 and HSV-2 (herpes simplex viruses 1 and 2) are double-stranded DNA viruses that cause viral infection in the majority of people. HSV-1 (which causes cold sores) and HSV-2 (which causes the majority of genital herpes) are both prevalent and infectious. Blisters on the vulva or around the vaginal opening are the common symptoms of HSV infection [53]. Treatment usually consists of antiviral drugs that interfere with viral replication, reduce the physical severity of outbreak-associated lesions, and decrease the chance of transmission to others. Various novel vaginal drug delivery systems, such as microparticles, nanoparticles vesicular systems, bio adhesive films, vaginal pessaries, and vaginal rings, have been reported for improved antibacterial antifungal and antiviral activity.

5. VAGINAL DDS

For vaginal applications, a variety of topical dosage forms have been developed, including gels, lotions, creams,

Table 1. Commercially available vaginal products.

Drugs	Marketed Polymer-based Vaginal Preparations			
	(Product)	Dosage form	Indication	Company
Miconazole nitrate	Monistat®	Cream	Fungal infection	Johnson & Johnson
Terconazole	Terazole®	Cream	Fungal infection	Janssen Pharmaceuticals
Butoconazole	Gynazole®	Cream	Fungal infection	Sigma-Pharma
Terconazole	Fougera®	Cream	Fungal infection	Fougera Pharmaceuticals
Progesterone	Prochieve®	Gel	Infertility, secondary Amenorrhoea	Fleet Laboratories
Nonoxynol-9	Gynol II®	Gel	Contraceptive	McNeil-PPC, Inc
2-Naphthalene sulfonic acid	PRO 2000/5®	Gel	Contraceptive	Endo Pharmaceuticals
Dinoprostone	Prostin E2®	Gel	Induction of labor	Pharmacia
Chlorhexidine	Clomirex®	Gel	Bacterial infection	Mipharm Spa
Metronidazole	MetroGel Vaginal®	Gel	*Bacterial vaginosis*	3M Pharmaceuticals
Oxyquinoline sulfate	Aci-Jel®	Gel	Restoration and maintenance of vaginal acidity	Care Pharmaceuticals
Nonoxynol-9	Conceptrol®	Gel	Contraceptive	McNeil-PPC, Inc
SPL7013	Vivagel®	Gel	Treatment of sexually transmitted diseases	Starpharma
Progesterone	Progering®	Vaginal ring	Contraceptive	Andromaco Laboratorios
17β-estradiol-3-acetate	Femring®	Vaginal ring	Estrogen replacement therapy; 3 months	Warner Chilcott UK Ltd.
Etonogestrel and ethinyl estradiol	Ornibel®	Vaginal ring	Contraceptive	Exeltis Healthcare SA
Etonogestrel and ethinyl estradio	Nuvaring®	vaginal ring	Contraceptive	Organon USA Inc
Estradiol	Estring®	Vaginal ring	Vaginal atrophy	Novo Nordisk
Segesterone acetate and ethinyl estradiol	Annovera®	Vaginal ring	Hormonal contraceptive	TherapeuticsMD Inc
Etonogestrel and ethinyl estradiol	Ornibel®	Vaginal ring	Hormonal contraceptive	Exeltis Healthcare
Estradiol	Vagifem®	Tablet	Atrophic vaginitis	Novo Nordisk
Clindamycin and clotrimazole	Clingen®	Suppositories	Fungal infection	Glenmark Pharmaceuticals
Nonoxynol-9	VCF®	Film	Contraceptive	Apothecus Pharmaceutical

Mucoadhesion can be defined as a state in which two components, of which one is of biological origin are held together for extended periods of time by the help of interfacial forces. Generally speaking, Bioadhesion is a term that broadly includes adhesive interactions with any biological or biologically derived substances, and Mucoadhesion is used when the bond is formed on a mucosal surface.

suppositories, pessaries, tablets, and creams. However, due to low drug penetration and rapid removal from the vaginal canal, these dosage forms have been associated with numerous disadvantages. Novel vaginal DDS are an excellent alternative to these dosage forms and are designed to attain desirable biodistribution, bio-adhesion, retention, and release characteristics [54]. The development of controlled delivery systems that can provide a long-term therapeutic concentration of drugs after administration as a single dose has been given considerable attention. Mucoadhesive drug delivery through the vaginal route is considered highly suitable for local drug delivery or contraception [2]. Mucoadhesion refers to the phenomenon in which synthetic and natural macromolecules adhere to the biological tissue of the body for a prolonged duration. Mucoadhesive Drug Delivery Systems (DDS) can be designed to enhance the residence time of drugs at a particular site and control the drug release behavior [55]. The release of drugs from mucoadhesive vaginal DDS is presented in Fig. (**3**). For controlled drug delivery to mucosal surfaces, a variety of biodegradable and biocompatible polymers have been approved by the Food and Drug Administration and other agencies [17, 56]. Different

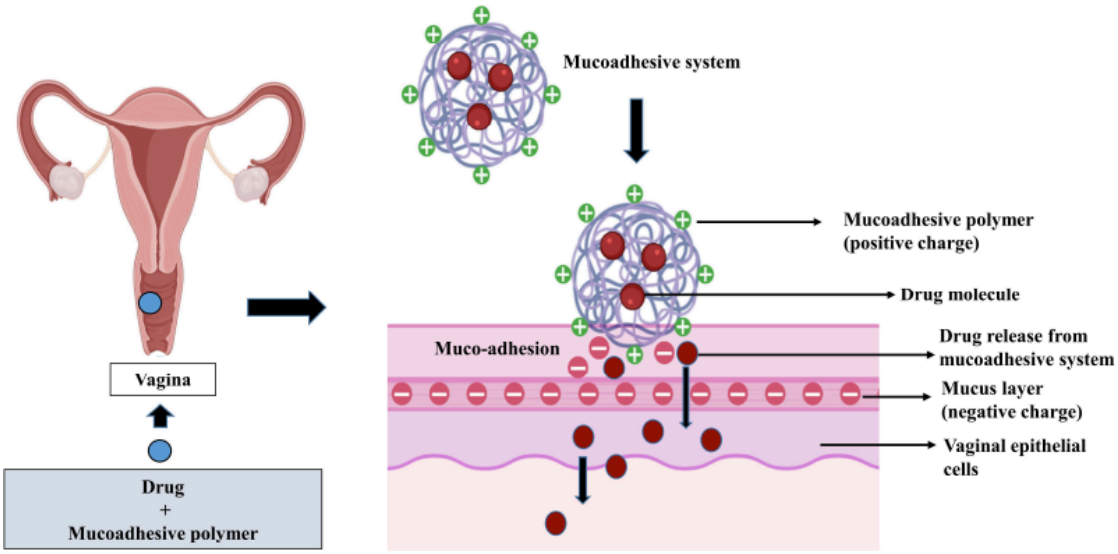

Fig. (3). Mucoadhesive vaginal drug delivery system. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

vaginal DDS developed for the treatment of various diseases are discussed below.

5.1. Micro and Nanoparticle-Based DDS for Vaginal Applications

In recent years, micro-and NPs based DDS have received considerable attention for the delivery of the drug to the vagina because of their applications in the targeted release of drugs at a particular site (tissue/organ), controlled drug release, and improved drug stability [25]. MPs and NPs not only differ in size but also affect a wide range of parameters, such as the surface area to volume ratio, drug loading, and drug release [57]. Size is most relevant as it turns out that, unlike NPs, MPs are usually unable to cross the sequential biological barriers and thus skip phagocytosis [58]. Various micro-and NPs based vaginal DDS are presented in Table **2** and Fig. (**1**).

Several researchers have used mucoadhesive polymers like chitosan, poly(lactic-co-glycolic acid) (PLGA), alginate, polyethylene glycol (PEG), gelatin, poloxamers, *etc*. as an additive in the development of various micro and NPs systems based DDS [59]. The mucoadhesive behavior of the system aims at increasing drug retention in the vaginal mucosa and the release of the drug for an extended period. For instance, Albertini and group formulated mucoadhesive MPs loaded with econazole nitrate (EN) for vaginal delivery [60]. They prepared different formulations of mucoadhesive MPs by the spray-congealing method using Gelucire 53/10 (a hydrophilic lipid matrix) as a carrier and mucoadhesive polymers like sodium carboxymethylcellulose, poloxamer, and chitosan**.** The results showed that non-aggregated microspheres with sizes ranging from 100 to 355 µm exhibited high percent yields (> 90 percent, w/w). The poloxamers improved the solubility of the MPs, as well as their bioavailability and mucoadhesive strength to vaginal mucosal tissue**.** Furthermore, poloxamers/Gelucire® based MPs inhibited the growth of *Candida albicans.* An antifungal microparticulate therapeutic system containing nystatin was developed by Martin-Villena *et al.* using emulsification/internal gelation method [61]. Three types of MPs, *i.e.*, alginate MPs, poloxamer 407 MPs, and chitosan-coated alginate MPs, were prepared, with mean particle sizes ranging from 36.088 µm to 56.146 µm. Poloxamer 407 coated MPs had a lower encapsulation efficiency than chitosan and alginate-coated MPs. Release studies showed the best kinetic parameters for poloxamer 407 coated MPs. All types of MPs exhibited excellent mucoadhesive properties to the vaginal mucosa. These microparticulate systems also effectively inhibited the growth of *Candida albicans*, signifying their potential clinical use.

Another study reports the development of chitosan-alginate microspheres of cefixime for local treatment of urogenital infections [62]. The prepared formulations showed a long permanence of the loaded drug for more than 2 hours**.** Microspheres loaded with 30 mg/mL drug showed the best water uptake and release rate. Microbiological studies showed a relationship between the rate of cefixime release by microphases and decreased *Escherichia coli* viability. Microspheres could also be used as effective drug carriers to the desired site. pH-responsive mucoadhesive microspheres based on polymethacrylate salt have been reported by Zhang *et al.* for vaginal delivery of an HIV microbicide, TFV [63]. The prepared microspheres had an average size of 4.73 µm. The optimized formulation had a drug loading and encapsulation efficiency of 2% (w/w) and 88.7%, respectively. These microspheres quickly responded to the change in pH and released over 90% of the drugs within 60 min. Furthermore, these microspheres showed excellent mucoadhesive properties and were non-cytotoxic to vaginal epithelial cells**.** Szekalska *et al.* used a spray-drying technique to prepare posaconazole-loaded fucoidan/gelatin microparticles and evaluated the effect of the gelatin addition on the formulation parameters. The addition of gelatin in microparticles improved swelling capacity and mucoadhesiveness, provided sustained drug release, and increased antifungal efficacy of microparticles against *Candida* spp. strains [64].

Table 2. Micro and nanoparticle-based vaginal drug delivery systems.

Therapeutic Drug	Intended Use	Dosage Form	Chemical Composition	Refs.
Dapivirine	HIV infection	Polymeric Nanoparticles	Polyethylene oxide, cetyl trimethylammonium bromide, and sodium lauryl sulfate coated poly (ε-caprolactone) nanoparticles	[59]
Econazole Nitrate	Vaginal fungal infection	Microspheres	Gelucire 53/10, chitosan, sodium carboxymethylcellulose, and poloxamers (Lutrol F68 and F127)	[60]
Nystatin	Vaginal fungal infection	Microparticles	Chitosan, poloxamer 407, alginate	[61]
Cefixime	Urogenital infections	Microspheres	Chitosan-alginate	[62]
Tenofovir	HIV infection	Microspheres	Eudragit RS-100, Methacrylic acid-methyl methacrylate, sodium hydroxide	[63]
Posaconazole	Vaginal fungal infection	Microparticles	Fucoidan, Gelatin	[64]
Miconazole	Vaginal fungal infection	Microsponges gel	Eudragit RS 100, Polyvinyl alcohol, Carbopol 940	[65]
Miconazole	Vaginal candidiasis	Nanoparticles	Chitosan, pentasodium tripolyphosphate, acetic acid	[66]
Clotrimazole	Vaginal fungal infection	Nanoparticles	PLGA, Polyvinyl alcohol	[67]

Microsponges that consist of porous microspheres have also been reported to improve vaginal retention and antifungal activity of miconazole nitrate (MN). Salah *et al.* formulated miconazole microsponges using Eudragit RS100 by employing the quasi-emulsion method [65]. The prepared microsponges had an average particle size of 78.2 μm and an entrapment efficiency of 92.9%. The prepared miconazole microsponges were incorporated into a Carbopol gel. The prepared formulation exhibited controlled release characteristics, which could reduce the local side effects of the drug. Furthermore, the formulation was found to be more effective in the treatment of *Candida* infection in rats than the marketed formulation.

Apart from microparticulate systems, different nanosystems have also been reported for effective vaginal drug delivery. Poly(ε-caprolactone) NPs containing DPV were formulated and tested for their capability to mediate permeability and retention in cell monolayers of pig rectal and vaginal mucosa [17]. It was found that the NPs coated with poly (ethylene oxide) reduced the permeability of the drug across monolayers and tissues, whereas modification of nanosystems with cetyltrimethylammonium bromide (CTAB) improved drug diffusion. NPs coated with sodium lauryl sulfate (SLS) exhibited no significant effect on the permeability of the drug. The prepared NPs increased the retention of the drug in monolayer/tissue compared to unformulated DPV. Moreover, the findings in this study revealed that poly (ethylene oxide) coated NPs decreased the *in vivo* toxicity of DPV (but not *ex vivo*), whereas NPs coated with SLS and CTAB showed higher toxicity. Amaral *et al.* developed MN-loaded polymeric NPs of chitosan and tested them *in vivo* using a murine VVC model [66]. The average particle size of the prepared formulation was found to be 207.3 nm. The prepared NPs were shown to have comparable therapeutic efficacy to the commercial formulation despite having a seven-fold lower antifungal content. The downregulation of IL-10 expression may be responsible for the increased therapeutic efficacy of MN. The development of PLGA NPs and PLGA NPs modified with chitosan has been reported for the controlled release of CTZ [67]. The average size of CTZ-PLGA-NPs and CTZ-PLGA-Chitosan NPs were found to be 382.9 and 491.7 nm, with entrapment efficiencies of 86.1 and 68.9%, respectively. The chitosan-modified PLGA NPs displayed higher positive zeta potential and larger size than unmodified NPs. *In vitro* release kinetic studies revealed that more than 98% of the drug was released from the formulations after 18 days. Moreover, incorporating chitosan onto the surface of NPs improved the mucoadhesive and antifungal properties of the NPs against *Candida albicans*.

5.2. Vesicular DDS for Vaginal Applications

The vesicular delivery systems are well-ordered assemblies comprising one or more concentric lipid bilayers formed by the self-assembly of amphiphilic building blocks in water [68]. Such systems have been reported for improved drug permeation across the vaginal membrane or drug targeting at a specific site in the female reproductive tract [69]. A variety of vesicular delivery systems, such as liposomes, ethosomes, niosomes, *etc*., have been reported for delivering different drugs. Some of these systems are summarized in Table **3**.

Liposomes have been extensively investigated as a carrier system for the treatment of vaginal diseases. A mucoadhesive liposomal ciclopirox olamine gel system using a 2% w/w Carbopol® 974P gel base for the treatment of vaginal fungal infections was developed by Karimunnisa *et al.* [70]. The formulated liposomal gel remained stable at the vaginal pH, and the ciclopirox olamine-loaded gel demonstrated a sustained release of 58.75% after 24 hours. The liposomal gel showed good mucoadhesion to sheep vaginal tissue and had good antifungal activity. Vanic and group prepared deformable propylene glycol-containing liposomes incorporated with CTZ or Metronidazole [71]. These liposomes were further incorporated into carbopol hydrogels to obtain the appropriate viscosity for vaginal application. The prepared liposomes released from the hydrogel system

Table 3. Vesicular drug delivery system for vaginal delivery.

Therapeutic Drug	Intended Use	Dosage Form	Chemical Composition	Refs.
Ciclopirox olamine	Vaginal fungal infection	Liposomal gel	Cholesterol, Diacetyl phosphate and terbium chloride, disodium EDTA, Phospholipon® 90H	[70]
Metronidazole and Clotrimazole	Vaginal delivery	Liposomal Hydrogel	EPC and egg phosphatidylglycerol-sodium, Carbopol 974P NF.	[71]
Curcumin	Vaginal delivery	Liposomes	Chitosan® Lipoid S 100, Carbopol® 974P NF	[72]
Clotrimazole	Vaginal fungal infection	Liposomes	Lipoid S 100, Chitosan, lactic acid, potassium hydroxide, propylene glycol	[73]
Insulin	Vaginal delivery	Niosomes	Crystalline porcine insulin, Span 40 and 60, dicetylphosphate, and cholesterol	[74]
Clotrimazole	Vaginal fungal infection	Liposomal, niosomal gel	Egg phospholipids, Span40, dicetylphosphate and cholesterol	[75]
Progesterone	Polycystic ovary syndrome (PCOS)	Nanosized Transethosomes	Phosphatidylcholine, Tween80, propylene glycol, Carbopol 974, hydroxyl Propyl Methylcellulose, and sodium alginate	[76]
Metronidazole	Vaginal delivery	Ethosomes	Cholesterol, polyethylene glycol, Phospholipon 90 H	[77]

more quickly than conventional liposomes. Furthermore, the prepared formulation showed sustained and diffusion-based drug release. In another study, Berginc and the group formulated chitosan and Carbopol coated curcumin (Cur) loaded liposomes [72]. These polymers enabled considerably higher (Cur) permeability through the isolated and artificial bovine mucus compared to control. The coating of these liposomes with bioadhesive polymers increased the mucoadhesive property. Similarly, CTZ-loaded liposomes coated with chitosan have been reported by Jøraholmen *et al.,* The prepared liposomes were in a size range of 100-200 nm [73]. The *ex vivo* penetration experiments were then conducted on the vaginal tissue of pregnant sheep. The results revealed that such systems increase the retention of CTZ in tissues and reduce its penetration compared to the control. Mucin studies revealed that coating these liposomes with a lower concentration of chitosan increases the mucoadhesive property of the system in comparison to coating with a greater concentration.

Ning *et al.* prepared niosomes using Span 60 and Span 40 for the vaginal delivery of insulin [74]. The average particle size of Span 60 niosomes and Span 40 niosomes were 259.7nm and 242.5nm, and their entrapment efficiency was 28.82% and 26.68%, respectively. The prepared niosomes had an enhancing effect on the vaginal delivery of insulin**.** However, no significant difference in pharmacological bioavailability was observed compared to the administration of insulin *via* the subcutaneous route in rats. Liposomes/niosomes-based delivery systems of antifungal drug CTZ have been reported for local vaginal therapy. The systems were prepared by employing the lipid hydration method and then incorporated into 2% carbopol gel [75]. The prepared vesicular gel system provided sustained drug release into the simulated vaginal fluid for 24 hours. The prepared formulation did not affect the morphology of vaginal tissues in rats at 24 hours post-dose and showed enhanced antifungal activity.

Transethosomes have a bilayered structure that allows for the encapsulation of both lipophilic and hydrophilic drugs with greater permeation efficiency than traditional liposomes. Salem *et al.* developed Pg-loaded vesicular transethosomes, which were incorporated into the mucoadhesive gel for luteal phase support in polycystic ovary syndrome [76]. The optimized formulation was then clinically studied for the therapy of polycystic ovary syndrome. The optimized formulation had a mean particle size ranging from 133.3 to 349.5nm, and entrapment efficiency ranged from 87.93 to 97.05%. The results indicated a substantial increase in the serum pg level, endometrial thickness, and pregnancy rate. Metronidazole-loaded pH-responsive ethosomes have also been developed for vaginal delivery [77]. The average size of the prepared vesicles was 179.9 nm. The entrapment efficiency and loading capacity of the prepared systems were found to be 50.31% and 39.89 %, respectively**.** In contrast to the aqueous dispersion, the prepared ethosomal gel demonstrated strong potential for sustained delivery under *in vitro* conditions.

5.3. Gel-Based DDS for Vaginal Application

Gels are semisolid preparations in which a liquid phase is entrapped within a three-dimensional polymeric matrix of synthetic or natural origin [78]. These types of systems have been formulated and investigated as delivery systems for labor induction, microbicides, contraceptives, and vehicles for various drug carriers [79]. The gel can offer many advantages to other vaginal DDS, such as prolonged residence time, higher bioavailability, safety, and economic saving [80]. Some of the mucoadhesive gel-based vaginal DDS are shown in Table **4**.

Table 4. Mucoadhesive gel-based vaginal drug delivery system.

Therapeutic Drug	Intended Use	Dosage Form	Chemical Composition	Refs.
Secnidazole	Vaginal fungal infection	*In situ* gel	Carbopol 940, Carrageenan, Hydroxy Propyl Methylcellulose, Hydroxy Ethyl Cellulose Hyrdoxy propyl Cellulose	[82]
Cyclodextrin, Voriconazole	Vaginal candidiasis	Thermosensitive *in situ* gel	Hydroxy Propyl-βcyclodextrin, Poloxamer407, Poloxamer 188, Polycarbophil, Hydroxyethylcellulose, Hydroxypropyl methylcellulose	[83]
Tenofovir	HIV infection	Vaginal bigels	Guar gum, sesame oil, Span® 60, Tween 60	[84]
Clotrimazole	Vaginal fungal infection	Thermosensitive gel	Poloxamers (P407, P188), Polycarbophil	[85]
Econazole nitrate	Vaginal fungal infection	*In situ* gel	Poloxamer 407 and Poloxamer 188	[86]
Sildenafil	Vaginal delivery	*In situ* thermo-responsive gels	Pluronic® F-127, hydroxyethylcellulose, Pluronic® F-68, Sodium alginate, Sodium chloride	[87]
Doxorubicin, iron (II) gluconate dehydrate	Cervical cancer	*In situ* forming and pH-responsive hydrogel	Chitosan, dimethyl sulfoxide (DMSO), glycidol, acetic acid, sodium periodate, sodium hydroxide	[88]
Ciprofloxacin	Bacterial vaginosis	pH-sensitive micellar hydrogel films	Gelatin, sodium dodecyl sulfate, sodium alginate	[89]
Tetrahydrocurcumin	Prophylaxis of HIV/AIDS	O/W microemulsion-based gel	Labrafac, Transcutol, Peceol, Capryol 90, Lauroglycol 90, Labrafil M 2125 CS, Tween 80, Span 20, PEG 400, and oleic acid	[90]
Miconazole nitrate	Vaginal fungal infection	Solid lipid microparticles Microgel	Hydrogenated palm oil, super-refined sunseed oil, polyethylene glycol, Polycarbophil	[91]
Clotrimazole	Vaginal fungal infection	Nanocapsules	Eudragit RS 100, Span80, Tween 80, Pemulen, Pullulan	[92]
Clotrimazole	Vaginal fungal infection	Nanostructured lipid carrier hydrogels	Poloxamer P407, Poloxamer P188, Carbopol 974P.	[93]

Several gel-based DDS have been investigated for vaginal application in recent years. In postmenopausal women, a vaginal gel containing sea buckthorn oil, 18-glycyrrhetic acid, aloe vera, hyaluronic acid, and glycogen has been shown to relieve symptoms of vulvovaginal atrophy and enhance sexual function [81]. An aerosol-based *in situ* gel-forming system has also been reported for the controlled release of the anti-protozoal drug secnidazole [82]. The *in-situ* gel formulations were prepared by using different concentrations of carbopol 940, cellulose polymers, and carrageenan and were further converted to foam spray by using sodium lauryl sulfate (SLS) as the foaming agent. It was observed that the formulations containing 0.45% of carbopol 940 with hydroxypropyl cellulose were found to be more efficient for the gelation process and released less than 50% of the drug in simulated vaginal fluid. Deshkar and co-workers prepared an *in situ* gel of voriconazole by forming a stable inclusion complex with Hydroxypropyl-β-cyclodextrin (HP-β-CD) [83]. The effect of different mucoadhesive polymers on the formulations was also studied. Optimized hydroxypropyl methylcellulose (HPMC) demonstrated exceptional gelling ability at 31.7 °C, excellent mucoadhesion, and showed 56.2 % of drug release within 8 h. Moreover, the optimized formulation displayed enhanced drug uptake in vaginal tissues in contrast to *the in-situ* gel without HP-β-CD and voriconazole dispersion. Freeze-dried bioadhesive vaginal bigels of TFV for controlled drug release have also been developed [84]. The authors prepared different systems containing various proportions of sesame oil and guar gum hydrogel, using Tween®60 and Span®60 as surfactants. Cytotoxicity studies revealed that the drug and excipients did not show toxicity at the concentrations tested. The system containing Tween®60 and Span®60 and a minimum proportion of guar gum hydrogel/sesame oil displayed maximum adhesion capacity and consistency. The system containing Tween®60 and Span®60 and a minimum proportion of guar gum hydrogel/sesame oil displayed maximum adhesion capacity, low swelling grade, and controlled release characteristics**.** Several vaginal gel formulations with mucoadhesive, thermosensitive, and pH-responsive properties have been investigated for vaginal drug delivery. Chang *et al.* prepared CTZ encapsulated Mucoadhesive Thermosensitive Gels (MTGs) for the treatment of Vaginal Candidiasis (VC). Several formulations of MTGs were prepared using polymers, such as polycarbophil and poloxamers [85]. The authors found that MTG enhanced the mucoadhesive property but reduced the syringe ability of the gels. Moreover, the antifungal activity exerted by CTZ against *Candida albicans* induced vaginitis in female rats was considerably enhanced after vaginal administration of MTG. Another study reports the preparation of EN-loaded *in situ* gels using thermosensitive polymers like poloxamer 188 and poloxamer 407 [86]. Formulations containing a mixture of poloxamers 407 and 188 in a 20:10 ratio exhibited typical gel-type me-

chanical spectra at 37°C, demonstrating the highest cohesiveness, adhesiveness, and hardness and were shown to be effective for the treatment of vaginal candidiasis.

In recent years, sildenafil has shown remarkable outcomes in the treatment of infertility in women. However, its clinical effectiveness is limited due to the lack of appropriate vaginal pharmaceutical preparation and the associated side effects after oral administration. To treat endometrial thinning resulting due to the use of clomiphene citrate in women, Soliman *et al.* prepared in *situ* thermo-responsive gel for the vaginal delivery of sildenafil using several grades of Pluronic® [87]. Different concentrations of mucoadhesive polymers, such as sodium alginate and hydroxyethyl cellulose, were incorporated into the gels**.** Results indicated that the gelation temperature decreased with increasing Pluronic® F-127 concentration, which was modulated by the addition of Pluronic® PF-68 within the satisfactory range of 28-37 °C. Findings of the clinical studies in women revealed that the prepared formulation considerably increased endometrial thickness and uterine blood flow without showing any adverse effects. Variations in vaginal pH due to infection can have a major effect on a formulation's efficacy. pH-responsive systems have also been exploited for the vaginal delivery of therapeutic agents. Jalalvandi E *et al.* developed a hydrogel-based pH-sensitive drug delivery system using chitosan [88]. They prepared different types of hydrogels loaded with a non-hormonal spermicide, iron (II) gluconate dehydrate, and doxorubicin hydrochloride. The prepared hydrogels degraded more rapidly at low pH. The hydrogels having less cross-linking density displayed a quicker degradation rate and more gelation time compared to hydrogels with greater cross-linking density. Moreover, these hydrogels exhibited no toxic effect on mesenchymal stem cells (>80% viability) when exposed to gel 5% and gel 3% for 48 hours. In a similar study, Adnan *et al.* prepared ciprofloxacin-loaded pH-sensitive micellar hydrogel films using gelatin/sodium dodecyl sulfate/sodium alginate polymers. The prepared film showed improved ciprofloxacin loading and controlled drug delivery in the vaginal pH condition [89].

Nanogels and microgels have proven to be versatile and effective delivery platforms for vaginal drug delivery. To improve solubility, bioadhesion, and residence time in the vaginal mucosa, Mirani *et al.* formulated tetrahydro curcumin (THC)-loaded o/w microemulsion-based gel [90]. Under *in vitro* conditions, the formulation released more than 90% of the drug after 12 hours. *In vitro,* anti-HIV activity studies on HIV-1 infected TZM-bl cells showed that the prepared formulation was four times more effective than plain THC (IC_{50} = 3.639 μM). Kenechukwu *et al.* prepared mucoadhesive microgels by using polycarbophil as a bioadhesive polymer for the effective treatment of deep-seated VVC [91]. Matrices composed of super-refined sunseed oil and hydrogenated palm oil (without or with PEG-4000) were used to prepare solid lipid MPs, which were then utilized to formulate mucoadhesive microgels containing MN**.** The formulation showed a drug content of 88% and a mean particle size in the range of 7.77-9.93 μm. It was observed that the use of PEG-4000 increased the amorphicity of the phyto lipid mixture. The prepared PEGylated mucoadhesive microgel formulation considerably prolonged the release of the drug for up to 12 h as compared to non-PEGylated mucoadhesive microgels**.** Furthermore, the formulations were stable at 40±2°C for 6 months, showed excellent mucoadhesive properties, and were histopathologically safe. Results also indicated that PEGylated mucoadhesive microgels caused a higher reduction in *Candida albicans* load (86.06%) in comparison to Daktarin® (75.0%) and MN-encapsulated polymeric hydrogels (47.74%) in rats.

A nanocapsule hydrogel formulation of CTZ was developed and evaluated for its mucoadhesive potential and penetration ability through cow vaginal tissue [92]. The polymers used for the preparation of the formulation were Pullulan and Pemulen® TR1. Pullulan 3% was essential to enhance the adhesive potency on the animal mucosa. The CTZ nanocapsules had a mean size of 137nm, a zeta potential of 15.7mV, and a slightly acidic pH of 5.09, which was ideal for vaginal administration. The hydrogel formulations showed a controlled release of CTZ (20.14 ± 2.33 μg/cm^2 after 8 h), demonstrating the potential of nanocapsules in controlling drug release. The prepared hydrogel could limit the CTZ permeation through the vaginal mucosa, which might be considered suitable for the treatment of superficial vaginal infections. Another study examined the effectiveness of CTZ nanostructured lipid carriers (CTZ-NLC) based poloxamer hydrogel as a potential system for the treatment of fungal vaginal infections. The prepared systems showed excellent thermo-gelling properties [93]. The hydrogel sustained its distinctive thermo-responsive character even after the addition of simulated vaginal fluid. Findings of the toxicological studies revealed that CTZ-NLC-gel at a dose of 1 mg/mL showed a low toxicity profile in HeLa cells with cell vitality of 77.2%. The antifungal activity of the prepared CTZ-NLC gel against *Candida albicans* was 4-fold more active than the marketed product.

5.4. Film-Based DDS for Vaginal Application

Over the last years, the scientific community has shown a great deal of interest in delivering pharmaceutical products through intravaginal films. Vaginal films are solid dosage forms that dissolve after exposure to vaginal fluids. Vaginal films as a mode of drug delivery have gained wider acceptance by women as they are unlikely to be associated with leakage and messiness. Vaginal films offer some superior properties like the ease of application, higher drug stability and retention time, and drug stability [94]. VCF®, a dissolving vaginal lubricant film containing the spermicide Nonoxynol-9, is a commercially available contraceptive [95, 96]. Some of these systems are summarized in Table **5**.

Several film-based DDS for vaginal application have been developed in recent years for the treatment of microbial infections in the vagina. Jalil *et al.* synthesized novel polymeric excipients forming mucoadhesive films for the treatment of vaginal infections [97]. For the study, gellan gum was conjugated with 2-(2-Amino ethyl disulfonyl) nicotinic acid. The film showed enhanced adhesion and considerable antimicrobial efficacy on the mucosal surface and provided a sustained release of metronidazole in 3h. EN-loaded Gelucire®-solid dispersion-based films have recently been documented to have good adhesiveness and anti-candida activity and could be a promising formulation for VC treatment [98]. After incubation for 24 hours in the acidic solution, the Gelucire®-based films showed good structural integrity. Ge-

Table 5. Film-based drug delivery systems for vaginal application.

Therapeutic Agent	Intended Use	Dosage Form	Chemical Composition	Refs.
Pyrimidinedione	HIV infection	Film	Polyvinyl alcohol, Glycerin, polyethylene glycol, hydroxypropyl methylcellulose, propylene Glycol	[94]
Dapivirine	HIV infection	Film	Propylene glycol, polyvinyl alcohol, hydroxypropyl methylcellulose	[96]
Metronidazole	Vaginal candidiasis	Film	Gellan gum, dimethyl sulfoxide sodium hydroxide, carbonate, sodium bicarbonate, dimethyl sulfaoxide, potassium dihydrogen phosphate	[97]
Econazole nitrate	Vaginal candidiasis	Film	Gelatin, polyvinyl caprolactam-polyvinyl acetate, polyethylene glycol, polyvinylpyrrolidone	[98]
Tioconazole	Vaginal candidiasis	Film	PEG 400, hydroxypropyl methylcellulose	[99]
Clindamycin phosphate	Bacterial vaginosis	Film	Hydroxypropyl methylcellulose, Xanthan gum, polyethylene glycol 400	[100]
Nystatin	Vulvovaginal candidiasis	Film	Fenugreek Gum, glycerin, monochloroacetic acid	[101]
Tenofovir and disoproxil fumarate	HIV infection	Film	Polyvinyl alcohol, polyethylene glycol, Eudragit® L 100	[102]
Tenofovir	HIV infection	Film	PLGA, poloxamer 407, hydroxypropyl methylcellulose, glycerin, polyvinyl alcohol	[103]
Dapivirine	HIV infection	Film	Hydroxyethylcellulose	[104]
Dapivirine and tenofovir	HIV infection	Film	Polyvinyl alcohol	[105]
UAMC01398	HIV infection	Film	Hydroxypropyl methylcellulose E5 and polyethylene glycol 400,	[106]
Dapivirine and Levonorgestrel	HIV infection and unintended pregnancy	Film	Polyethylene glycol 8000, Hydroxypropyl methylcellulose, Polyvinyl alcohol 4-88	[107]

lucire®-SD-based films showed a comparable release profile to gelatin films, with the drug release being enhanced in the first 5 hours and the EC release being controlled over time**.** Similarly, a vaginal film of tioconazole was prepared using polymers, such as HPMC and chitosan, for the treatment of VC [99]. The films showed similar mechanical properties and adhesiveness. Time-kill analysis indicated that the prepared films were more effective than the conventional tioconazole and pure drug against Candida species. It was observed that the films based only on chitosan demonstrated a certain degree of cytotoxicity (35 to 54 % reduction in cell viability after 24 h). However, the formulation based on chitosan and HPMC along with a plasticizer (PEG 400) showed the lowest swelling, good antimicrobial activity, and did not cause considerable hemolytic and cytotoxic effects.

A bioadhesive film prepared using the solvent evaporation method for vaginal delivery of clindamycin phosphate has been reported [100]. The prepared film was non-cytotoxic toward HeLa cells at a concentration up to 500 µg and retained for up to 8 hours in the vaginal mucosa. Another study reports the preparation of nystatin-loaded films using a carboxymethyl derivative of fenugreek gum for the treatment of VVC [101]. It was observed that polymer films containing 2% v/v glycerol demonstrated excellent properties *in vitro*. The formulation released 100% drug in about five hours and was found to be nontoxic to the vaginal mucosa. Furthermore, *in vivo* studies confirmed the antifungal properties of the prepared formulation. Prevention of transmission of HIV infection remains a significant challenge, and topical pre-proliferation prophylaxis using microbicides may help solve the problem. Film-based DDS have been documented to deliver antiretroviral drugs efficiently to mucosal tissues. Cautela *et al.* formulated films containing emtricitabine and tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC) loaded Eudragit® L 100 NPs [102]. The prepared NPs showed a mean diameter of 680 nm with a drug loading of 2.5% for FTC and 5.4% for TDF. The incorporation of TDF/FTC-loaded NPs into double-layered films delayed the drug release from the formulation. Furthermore, MTT assays and lactate dehydrogenase release studies using CaSki and HeLa cell lines confirmed the safety of all film types. Cunha- Reis *et al.* developed a fast-dissolving polymeric film containing NPs for the vaginal delivery of efavirenz (EFV) and TFV [103]. EFV-loaded PLGA NPs, along with free TFV, were loaded into fast dissolving films. *In vitro,* drug release experiments revealed that nearly all TFVs were released within 10 minutes, likely due to the rapid disintegration time seen across all films. The release of EFV from NPs in the film showed approximately 40% of the drug released within one hour, followed by another 20% for up to 24 hours. When NPs were associated with the film, they were better retained *in vivo* in both vagi-

nal lavages and tissue. Pharmacokinetics studies showed that vaginal drug levels were reduced post-administration immediately. However, NPs in the film could still increase EFV drug concentrations**.** *In vivo* studies in mice confirmed the safety of the prepared formulation for vaginal administration with no major genital histological changes. Moreover, no significant alterations in cytokine/chemokine profiles were observed.

Bunge and co-workers developed a vaginal film and gel for the delivery of DPV [104]. It was observed that healthy HIV-negative women randomized to gel and film products had a higher concentration of DPV in the vaginal and cervical tissues than in plasma. The concentrations of the drug in the tissues were found to be higher in the gel users compared to women randomized to film. Both formulations delivered a sufficient amount of drug at concentrations sufficient to prevent HIV infection in the *ex vivo* challenge assay. The film was more comfortable and showed less leakage, but many women found it more challenging to insert it than gel. Akil *et al.* developed a polymeric film for the codelivery of TFV and DPV [105]. It was observed that administration of DPV and TFV *via* a polymeric film has a significant effect on the tissue accumulation of DPV. Moreover, the findings of the study confirmed the capability of the prepared films to deliver TFV and DPV to human ectocervical tissue. Grammen *et al.* designed a vaginal film with a novel non-nucleoside reverse transcriptase inhibitor UAMC01398, which is presently under examination for use as an anti-HIV microbicide [106]. Different ratios of PEG400 and HPMC were used for the preparation of UAMC01398 films by the solvent evaporation method. The film with 70% HPMC and 30% PEG400 was chosen for further study because of its translucent appearance, softness, and flexibility. The formulation was stable over a period of one month, fast-dissolving and safe for lactobacilli and epithelial cells. The prepared film showed good penetration across epithelial cell layers both *in vitro* and *in vivo* and could be a good alternative to gel formulations for vaginal microbicide delivery. DPV and Levonorgestrel (LNG) loaded thiomer-containing bioadhesive vaginal films have been developed to deliver both DPV and LNG to prevent HIV infection and unintended pregnancy [107]. Pigtailed macaques were used to study the pharmacokinetics of single entity and combination LNG/DPV bioadhesive films. Both single entity and combination films were able to produce sustained drug release *in vivo*. Compared to the single entity film, the combination LNG/DPV film had lower local tissue clearance for DPV and higher plasma concentration for LNG.

5.5. siRNA-based Drug Delivery for Vaginal Applications

Several studies have reported the incorporation of siRNAs into NPs for therapeutic delivery at the mucosal surfaces [108]. siRNA can be used to suppress viral replication by targeting viral or host genes [109]. Successful siRNA-based drug delivery can be achieved using liposomes, lipoplexes, microemulsions, and polymeric NPs as delivery vectors. Polymer NPs are effective delivery agents for inhibiting HSV *in vivo*. In this regard, Steinbach *et al.* formulated PLGA-based siRNA NPs for intravaginal administration using a double-emulsion solvent evaporation method [110]. In this study, nectin-1 (an HSV receptor) and siRNA targeting UL28 (an HSV protein necessary for viral DNA cleavage and packaging) were bound to spermidine (cationic polyamines) to form polyplexes before being mixed with PLGA. *In vivo* tests revealed that the delivery of PLGA NPs by intravaginal administration increased the survival of mice infected with HSV-2 from 9 days to >28 days. A study conducted by Palliser and the group revealed that the intravaginal administration of siRNAs in a transfection lipid could protect mice from HSV-2 infection [111]. The siRNA targeted the herpes HSV-2 gene and significantly reduced the viral load in cell culture models after topical application. Woodrow *et al.* developed siRNA-PLGA NPs for mucosal vaginal delivery [112]. The gene expression silencing was observed for 14 days in the vaginal tract. The study showed no cytotoxic effect in Hep G2 hepatocytes and HeLa cervical carcinoma. NPs encapsulated with siRNA for targeting dendritic cells have also been documented for HIV prevention. Furthermore, the siRNA-NP-Antibody complex has been reported to knock down a synaptosome-associated 23-kDa protein (SNAP-23), suggesting a promising platform for preventing HIV infection in females [113]. The research on vaginal siRNA drug delivery focuses on siRNA stability and its effectiveness in the vaginal tract.

5.6. Vaginal Suppositories

Vaginal suppositories (or ovules) are used for the treatment of local infections, vaginal atrophy, and contraception. The composition of vaginal suppositories is based on a water-miscible base (polyethylene glycol), water-soluble base (glycerol-gelatin base), or lipid-based base (synthetic triglyceride mixtures) [114]. After insertion in the vagina, vaginal suppositories dissolve because of their hygroscopicity. Excipients, such as surfactants and preservatives, can be added to the suppository formulation to improve its action. Some of the suppository-based vaginal DDS are shown in Table **6**.

Probiotics could be a safer and more efficient alternative to antibiotics for the restoration of the healthy vaginal microbiota. However, the oral administration of probiotics may not have a significant impact on maintaining and restoring vaginal microbiota [115]. Vaginal suppositories can be a suitable alternative to deliver probiotics to the vagina. Verdenelli *et al.* investigated whether the vaginal administration of vaginal suppositories containing *Lactobacillus* strains leads to colonization and change in pH [116]. The study was conducted on 35 healthy women who received probiotic suppositories daily for seven days. It was observed that the probiotic vaginal suppositories showed no side effects and were well tolerated. Rodrigues *et al.* prepared vaginal suppositories to deliver *Lactobacillus acidophilus* [117]. The suppositories were prepared using excipients, such as polyethylene glycol 4000/400 and Witepsol H12. Results demonstrated that polyethylene glycol 4000/400 and Witepsol H12 showed no toxicity in selected vaginal cell lines with CC50 values greater than 10%. The formulated vaginal suppositories had a uniform and mild texture and showed a sustained *in vitro* release profile of Lactobacillus acidophilus**.** Vaginal suppositories could be a promising system to deliver a variety of antimicrobial agents to the vagina. Di Vito *et al.* prepared tea tree oil-loaded vaginal suppositories and investigated their *in vivo* microbicidal activity against Candida species [118]. It was found that the prepared

Table 6. Suppositories for vaginal delivery.

Therapeutic Agent	Intended Use	Dosage Form	Chemical Composition	Refs.
Probiotic Lactobacillus	Bacterial vaginosis	Suppositories	SYNBIO® (Lactobacillus rhamnosus IMC 501® and Lactobacillus paracasei IMC 502®)	[116]
Lactobacillus acidophilus	Vaginal delivery	Suppositories	Lactic acid bacillus, polyethylene glycol 400, PEG 4000, mineral oil	[117]
Tea Tree Oil	Vaginal candidiasis	Suppositories	Tween 80, gelatin	[118]
Tenofovir	HIV infection	Suppositories	Carrageenan, sodium hydroxide, potassium chloride	[119]
Vardenafil	Complementary treatment to boost *in vitro* fertilization process	Suppositories	Polyethylene glycol 400 and 4000, Witepsol H15 and Suppocire NA50, Na alginate, Tween 80, glycerin, and gelatin	[120]
SHetA2 heteroarotinoid	Human papillomavirus	Suppositories	Cocoa butter, polyethylene glycols 400, 3350	[121]
SUPPOCIRE®	Human papillomavirus	Suppositories	Polyethylenimine, Tween 80	[122]

suppositories exhibited fungicidal activity against all strains of Candida species and showed a selective fungicidal action. Zaveri and the group prepared TFV-loaded vaginal suppositories using carrageenan. It was observed that approximately half of the drug dissociated from the suppository within the first two hours, regardless of the volume and type of medium (semen, simulated fluid) and vaginal simulant fluid) [119].

Another study reports the preparation of vaginal suppositories *of* vardenafil to enhance its systemic bioavailability [120]. The vaginal suppositories were prepared using lipophilic bases, such as Suppocire NA50 and Witepsol H15. Glycerogelatin and polyethylene glycol 4000/400 were used as hydrophilic bases. It was observed that the inclusion of bioadhesive polymers like alginate significantly sustained the release of the drug from suppository bases. The findings of the organ biodistribution study revealed higher C_{max} and AUC_{0-4} of vardenafil in the uterus after intravaginal administration of suppositories compared to administration of vardenafil suspension by oral route. Furthermore, the *in vivo* activity of vardenafil following intravaginal administration was found to be better than oral administration. Vaginal suppositories have also been investigated for the delivery of anticancer agents. In 2018, Mahjabeen *et al.* developed an optimized vaginal suppository formulation for the delivery of SHetA2, a novel anticancer agent for the treatment of cervical dysplasia [121]. The formulation consisted of 5% Kolliphor and cocoa butter as a base. It was observed that after administration of suppository in mice, the concentrations of SHetA2 in the cervix were significantly higher than the SHetA2 therapeutic concentration. The advancement of gene therapy targeting HPV could pave the way for successful cervical cancer treatment. In this regard, Ren *et al.* developed a new polyethyleneimine (PEI) based vaginal suppository consisting of DNA-PEI complexes and SUPPOCIRE® (a fatty base) [122]. The findings of the *in vivo* and *in vitro* sectional immunofluorescence studies confirmed the delivery efficiency of the prepared vaginal suppository. The presence of SUPPOCIRE® contributed to the robust release of complexes from the suppository. The fluorescence quenching studies involving transgenic mice confirmed the targeting potential of the suppository for gene delivery.

5.7. Vaginal Rings

Vaginal rings or intravaginal rings are small, circular, soft, and flexible polymeric *drug* delivery devices that are inserted into the vagina for sustained and controlled drug delivery. Vaginal rings are mainly used for contraception purposes. Some of the commercially available vaginal ring products are NuvaRing® (contraceptive vaginal ring), Estring® (estradiol releasing ring), Femring® (estradiol acetate releasing ring), and Progering® (containing Pg). Vaginal rings do not interfere with sexual intercourse and do not need to be administered daily. They provide continuous delivery of the drugs for prolonged durations of time [13]. Vaginal rings are mainly prepared using elastomeric polymers like silicone. In recent years, several other polymers like styrene-butadiene and ethylene-vinyl acetate copolymer have also been tested. These copolymers offer several advantages over silicone elastomeric polymers, such as the absence of curing chemistry, versatile properties, and continuous and flexible processing *via* co-extrusion. Some of these systems are shown in Table **7**. In recent years, many researchers have investigated the use of a vaginal ring for the prevention of HIV infection and contraception purposes. Koutsamanis *et al.* prepared estradiol-loaded vaginal rings using ethylene-vinyl acetate copolymers for delivery of local and systemic hormone replacement therapy and contraception purposes [123]. It was found that high estradiol loading, high ethylene-vinyl acetate content of the polymer, and low membrane thickness could result in high estradiol release.

In another study, Saxena and the group prepared intravaginal rings comprised of non-biodegradable hydrogel and bio soluble acacia gum to provide sustained release of antiretroviral HIV *microbicides* [124]. They incorporated different combinations of reverse transcriptase inhibitors and Boc-lysinated betulonic acid (an anti-HIV agent) into the vaginal rings. The release rates of antiretroviral *microbicides* from the vaginal rings were sustained at concentrations higher than the minimal effective concentration for HIV inhibi-

Table 7. Drug-releasing vaginal rings.

Therapeutic Drug	Intended Use	Dosage Form	Chemical Composition	Refs.
Estradiol	Hormone replacement therapy and contraception	Vaginal ring	Ethylene-vinyl acetate, sodium acetate trihydrate, glacial acetic acid, sodium lauryl sulfate	[123]
Zidovudine (ZDV)	HIV infection	Vaginal ring	Acacia gum, 2-hydroxyethyl methacrylate, and sodium methacrylate	[124]
Maraviroc and dapivirine	HIV infection	Vaginal ring	Silicone elastomer	[125]
Tenofovir, disoproxil fumarate	HIV infection	Vaginal ring	Elastomer, sodium acetate, sodium chloride	[126]
Maraviroc and CMPD167	HIV infection	Vaginal ring	Silicone elastomer	[127]
5P12-RANTES	HIV infection	Vaginal ring	Hydroxypropyl methylcellulose, acetonitrile, and trifluoroacetic acid	[128]
Progesterone	HIV infection	Vaginal ring	PEG 4000, polylactic acid polycaprolactone, Tween 80	[129]
Levonorgestrel	Contraception	Vaginal ring	Silicone elastomer	[130]
Disulfiram	Cervical cancer	Vaginal ring	Polyethylene vinyl acetate, Elvax 150 and 40, silicone MED8-6382	[131]

tion. It was observed that Boc-lysinated betulonic acid inhibited more than 90% of HIV infection in H9 cells and showed minimal effects on healthy cells. Chen *et al.* prepared vaginal rings containing maraviroc and DPV alone or in combination for vaginal delivery [125]. The vaginal rings were found to be safe and well-tolerated. The tissue concentrations of DPV were found to be higher than plasma concentrations, whereas maraviroc concentrations were only observed in cervical, vaginal fluid and not in plasma. The single drug-containing vaginal ring exhibited a more stable pharmacokinetic profile. In addition, DPV showed a concentration-dependent inhibitory effect on HIV-1 infection in cervical tissue.

Smith *et al.* developed an intravaginal ring containing the prodrug TDF that delivered the drug for one month [126]. The ring fully protected pigtailed macaques from vaginal simian-human immunodeficiency virus (SHIV) challenge for four months. Malcolm *et al.* carried out a study to investigate the *in vivo* release profile of maraviroc and CMPD167 (CCR5 Inhibitors) from vaginal rings [127]. The prepared matrix-type silicone elastomer vaginal rings showed a sustained release profile of both inhibitors for 28 days. Findings of the pharmacokinetic studies on rhesus macaques showed that these agents were present in the vaginal tissue and vaginal fluid at concentrations higher than the 50% inhibitory concentrations for SHIV inhibition in macaque lymphocytes. Moreover, the plasma concentrations of both compounds were found to be low. The pretreatment of macaques with Depo-Provera resulted in a significant reduction in the vaginal fluid and tissue concentrations of these compounds, while plasma levels increased after pretreatment with Depo Provera®. McBride *et al.* prepared vaginal rings containing the antiretroviral agent 5P12-RANTES, an experimental chemokine analog for the prevention of HIV [128]. The vaginal rings were prepared using silicone elastomer and thermoplastic polymers. *In vivo* release and stability testing studies confirmed the effectiveness of the prepared rings. Furthermore, pharmacokinetic studies in a sheep model indicated that the rings provided sustained concentrations of the antiretroviral agent in vaginal fluid. A fused deposition modeling printer was used by Fu J *et al.* to prepare 3D printed "O," "Y," or "M"-shaped vaginal rings loaded with progesterone (Pg) [129]. It was observed that the "O" shaped vaginal ring had higher dissolution in comparison to the "Y" and "M" shaped vaginal rings because of its large surface area/volume ratio. Moreover, the vaginal rings demonstrated a sustained release of Pg for more than seven days. Huang *et al.* carried out a study to determine whether a vaginal ring containing ulipristal acetate could inhibit ovulation [130]. The study was performed on fifty-five healthy women with regular ovulation. Results suggested that ovulation suppression was observed in 81.8% of treatment cycles with low and high doses. Few cases of excessive bleeding were reported at the end of 24 weeks of treatment. In addition, administering a single dose of LNG every 12 weeks reduced the endometrial thickness and prevented heavy bleeding. A disulfiram-loaded intravaginal ring for the localized delivery of the drug to the cervix was developed by Boyd *et al.* The release of disulfiram from the rings followed a diffusion-controlled release profile, and the drug was released at levels higher than the IC_{50} value (124.3 nM) for the HeLa cervical cancer cell lines [131]. It was observed that the processing temperature of the rings could considerably affect the physical state of the drug inside the rings, which may sequentially influence the *in vitro* release of the disulfiram into the release media.

5.8. Tampons

Intravaginal tampons are made up of cellulose or cotton and are widely used for the absorption of menstrual discharge. Some of the commercially available brands of vaginal tampons are Kotex®, Tampax®, Lola®, *etc.* Due to their tendency to absorb large amounts of fluid, they are recommended for the absorption of extensive vaginal discharge in women infected with Trichomonas. Many researchers have also investigated the technical feasibility of intravaginal delivery drugs, probiotics [132], and their efficacy in detecting high-risk HPV [133]. Vaginal tampons containing freeze-dried probiotics are also available in the market to balance vaginal pH levels and restore the normal microbiota during menstruation. Some of the researchers have also evaluated

their tendency to maintain vaginal pH by the delivery of probiotics, citric acid, and lactic acid. Brzezinski and co-workers tested the release of citric acid and lactic acid from tampons during menstruation [132]. The study was conducted on healthy women, one group used regular tampons, and the other group used novel tampons for two consecutive menstrual cycles. Tampons with citric acid and lactic acid demonstrated the ability to lower high pH during menstruation, which may reduce the risk of BV in women. Tampons have also been investigated to deliver Maraviroc 39-and azido-39-deoxythymidine (AZT) that can prevent women from contracting HIV [134]. The drug-eluting fibers were fabricated by using the electrospinning process. The researchers found that these tampons can deliver agents that can inhibit both HIV and sperm *in vitro*. The lactobacilli tampons loaded with a mixture of freeze-dried *L. casei var. rhamnosus, L. fermentum,* and *L. gasseri.* have been investigated for the treatment of BV [135]. However, lactobacilli-containing tampons had no effect on the cure rate of treatment of BV for one menstruation. In another study, Tijana Ristic *et al.* found that specific functionalization of tampons using chitosan NPs improved antibacterial efficacy against pathogen microorganisms without causing cytotoxicity [136].

5.9. Microneedles for Delivery of Drugs and Vaccines by Vaginal Route

Microneedle devices are made of micron-sized needles, which are evenly arranged on the surface of a small patch [137]. Several types of microneedles have been investigated for delivering the drug or vaccine into the epidermis, such as solid, coated, dissolving, and hollow microneedles [138]. Solid microneedles create pores in the stratum corneum, and the formulation is then topically applied to that area. The vaccine or drug can also be coated onto the surface of the microneedles (coated microneedles) or can be incorporated into the water-soluble matrix (dissolving microneedles) and a hollow core inside the needle (hollow microneedles) [139, 140]. Microneedles have been investigated as a novel vaccine delivery platform to the vaginal mucosa. Wang *et al.* used different types of multifunctional liposomes, stealth lipid A-liposomes, and mannosylated lipid A-liposomes, encapsulated with a model Ag and NH_4HCO_3, for the fabrication of microneedle array (proSMMA) [141]. The fabricated microneedle array dissolved rapidly upon rehydration by tissue fluids. Vaccination of mice with proSMMAs by application *of a vaginal mucosa patch* elicited robust Ag-specific cellular and humoral immunity in both mucosal and systemic systems. Furthermore, the Ag delivered by these multifunctional liposomes was also displayed by Ag-presenting cells *via* MHC-I cross-presentation due to lysosome escape and ROS (reactive oxygen species) stimulation, which resulted in the release of NH_4HCO_3 and $NH_4^{+/}NH_3$ from liposomes, leading to Th1/Th2 type response, which was further promoted *via* activation of TLR4 by liposomal lipid A. The large-scale production of proSMMAs is still challenging due to the complex product development process and instable loading of NH_4HCO_3 in vesicles. In another study, to improve immune inducing efficacy, Wang and co-workers fabricated trivalent aluminum ions (Al^{3+}) based cochleates (ACOs) using a dissolvable microneedle array [142]. After the application of patches onto mouse vaginal mucosa, the microneedle array implanted vaccines in mucosal tissues, circumventing the loss of ingredients caused by mucus or fluids, which established robust cellular and humoral immunity in both mucosal and systemic levels. The findings of the study revealed that the unique structure of ACOs allowed the vaccine to enter the Ag-presenting cells favoring MHC-I antigen presentation. ACOs were engulfed into cellular endo-lysosomes *via* receptor-mediated endocytosis, wherein the ACOs disassembled to release Al^{3+}, engendering lysosome escape of Ags. Microneedle-based DDS have also been explored for the delivery of antiretroviral drugs for the prevention of HIV. Mccrudden *et al.* investigated the potential of dissolving microarray patches loaded with long-acting rilpivirine nanosuspension formulation for vaginal delivery [143]. The prepared dissolved microarray patches could penetrate a synthetic vaginal skin model, and the microneedles retained their shape after the dragging process. The findings of the *in vivo* studies on rats revealed that the mean plasma concentration of rilpivirine was comparable to that attained in the intramuscular control cohort (116.5 *vs*. 118.9 ng/mL). Long-acting rilpivirine was also detected systemically in the lymph nodes and vaginal tissue of rats, which confirmed their applicability for vaginal delivery.

6. VACCINATION BY VAGINAL AND OTHER ROUTES OF ADMINISTRATION

Several studies have compared the potential of nasal, oral, rectal, and vaginal vaccination in producing a specific antibody response. Vaginal administration has been found superior to both rectal and oral vaccination in stimulating strong antibody responses in vaginal and cervical secretions [144, 145]. Johansson *et al.* conducted a study on 21 volunteers who were vaccinated vaginally and nasally with mucosal cholera toxin B (CTB) subunit [146]. It was found that both nasal and vaginal vaccinations induced strong CTB-specific immunoglobulin (IgA) and IgG antibody responses in serum in most of the volunteers. Vaginal vaccination in volunteers on days 10 and 24 in the menstrual cycle showed a 58-fold increase in IgA and a 16-fold increase in IgG response in the cervix. Nasal vaccination demonstrated a 35-fold increase in IgA in vaginal secretions, whereas vaginal vaccination showed only a 5-fold increase in IgA. Thus, a combination of vaginal and nasal vaccinations could be the ideal vaccination approach for stimulating specific antibody responses in the genital tract. In another study, Kozlowski and the group compared different mucosal immunization routes for induction of mucosal and systemic antibodies using CTB and cholera vaccine containing killed vibrios [147]. Four groups of women were immunized by the rectal, nasal, and vaginal routes during the luteal ($V\text{-}LP_{imm}$) or follicular ($V\text{-}FP_{imm}$) menstrual cycle phase. The results indicated that nasal immunization elicited the greatest levels of CTB-specific IgG in serum and was superior to $V\text{-}FP_{imm}$ for producing CTB-specific IgA in rectal secretions. Nasal immunization, $V\text{-}FP_{imm}$, and $V\text{-}LP_{imm}$ produced comparable CTB-specific IgA responses. However, only $V\text{-}FP_{imm}$ produced cervical IgA2-specific antibodies to the bacterial LPS vaccine component. Pettini *et al.* studied *in vivo* T-cell priming after immunization by the vaginal route in hormone-synchronized mice with CpG oligodeoxynucleotide and ovalbumin [148]. It was found that vaginal immunization can prime antigen-specific CD4(+) T-cells and stimulate their dissemination from draining lymph nodes to distal lymphoid

Table 8. Clinical trials with vaginal drug delivery systems.

Formulation	Clinical trial Phase	Trial Identifier	Condition/Disease	Number of Participants	Refs.
TOL-463 (a boric acid-based vaginal anti-infective with antibiofilm activity)	Phase II	NCT02866227	Vulvovaginal Candidiasis and Bacterial	106	[149]
Hyalofemme cream (hyaluronate cream)	Not applicable	NCT03981458	Atrophic vaginitis	40	[150]
Acidform gel	Phase I	NCT02693418	Bacterial vaginosis	100	[150]
Estradiol vaginal cream 0.01%	Phase III	NCT03294538	Atrophic vaginitis	663	[150]
Mucoadhesive gels containing a propolis standardized extract (EPP-AF), with 1 and 2% of propolis	Phase II	NCT03024502	Vulvovaginal candidiasis	90	[150]
5% Monolaurin gel	Phase II	NCT02709005	Bacterial vaginitis	109	[150]
VeraCept®	Phase III	NCT03785366	Contraceptive	41	[150]
VivaGel®	Phase I	NCT01577537	HIV infection	251	[151]
CAPRISA/ 1%Tenofovir gel	Phase IIb	NCT01691768	HIV infection	372	[152]
Dapivirine vaginal ring	Not Applicable	NCT01539226	HIV infection	1959	[153]
Dapivirine vaginal ring	Phase 3	NCT02862171	HIV infection	1959	[154]
Dapivirine vaginal ring	Phase 2	NCT02028338	HIV infection	96	[155]

organs, similar to that observed after immunization by nasal route. T-cell activation at the mucosal site is considered vital for developing vaccination strategies.

7. CLINICAL STUDIES ON VAGINAL FORMULATIONS

Vaginal drug delivery has several complexities, and intense research is on its way. Both academic and industrial organizations are working to develop safer and more effective formulations. Various clinical studies have been carried out for vaginal DDS. These are listed in Table **8**. In 2016, Marrazzo *et al.* investigated the efficacy and safety of TOL-463 (a boric acid-based vaginal anti-infective with antibiofilm activity) for the treatment of VVC and BV [149]. The study was conducted on 106 participants (53 with BV, 36 VVC, and 17 both). TOL-463, particularly in vaginal insert dosage form, was found to be safe and effective in treating VVC and BV. Another study investigated the potential of hyaluronate cream (Hyalofemme) for the therapy of atrophic vaginitis in women [150]. It was observed that hyaluronate cream reduced antibiotic use after treatment. Mcgowan and co-workers conducted a study designed to assess the safety and efficacy of VivaGel® (a dendrimer-based topical microbicide). The study was conducted on 61 women (aged between 18-24 years) who received VivaGel® twice daily for 14 successive days. They observed that VivaGel® was mostly well-tolerated, but higher incidences of genital adverse events were observed compared to the hydroxyethyl cellulose placebo gel [151].

Matthews *et al.* investigated the adherence of 1% TFV vaginal gel among women with pregnancy compared to women without pregnancy. A study conducted on 863 women revealed that women with pregnancy had less adherence to 1% TFV vaginal gel than women without pregnancies [152]. The effectiveness and safety of DPV-loaded vaginal rings for the prevention of HIV infection in healthy women between 18 to 45 years of age have been reported [153]. The participants in this study used vaginal rings every four weeks for up to 2 years. The findings of the study revealed that the prevalence of HIV-1 infection was lower in the DPV-treated group in contrast to the placebo-treated group. In a phase 3 trial, a vaginal ring containing 25 mg of DPV showed a favorable safety profile and a 31% reduction in the risk of HIV-1 infection [154]. The safety and acceptability of DPV vaginal rings were also assessed in sexually active females aged between 15-17 years. The majority of the participants reported no discomfort due to the vaginal ring [155].

CONCLUSION

The vaginal mucosal cavity is an excellent route for the administration of therapeutic agents intended both for local and systemic delivery. To overcome the limitations associated with the conventional dosage forms, much of the emphasis of the researchers has been directed towards the development of novel drug delivery for improving the therapeutic efficacy by increasing the residence time, sustaining release, and permeation through the vaginal epithelium. Several novel DDS have been recently explored as an efficient method to deliver various therapeutic agents like peptides, hormones, and siRNA. Application to the vaginal environment always faces the issue of leakiness due to gravity, which is a major challenge in designing the novel DDS for vaginal application. Moreover, variation in the physicochemical properties, including molecular weight, ionization, lipophilicity, surface charge, and chemical composition, can affect vaginal drug absorption. Thus, surface modification of delivery systems using mucoadhesive polymers and fabrication with specific surface potential are the commonly used

approaches to prolong vaginal residence time, thus ensuring better patient compliance. Mucoadhesive polymers have been studied most extensively by scientists in the development of various vaginal DDS owing to their mucoadhesive, permeation-enhancing, and controlled release properties. Several thermal and pH-responsive systems have also been recently developed for improving the efficacy of drugs applied through the vaginal route. More research is needed to ensure the suitability, biocompatibility, and therapeutic effectiveness of novel DDS for vaginal delivery. To date, only a limited number of *in vivo* studies on novel vaginal DDS have been reported. Additionally, drug release kinetics under varying vaginal environments is also not well understood. Furthermore, changes in the vaginal environment during different stages of a woman's life suggest the need for further research since more personalized regimens could provide successful treatment for vaginal problems. Although numerous strategies and interventions have been developed, clinical translation of these systems remains a challenge. The toxicity of the carrier system is also an important consideration for future clinical applications.

LIST OF ABBREVIATIONS

NPs = Nanoparticles

MPs = Microparticles

AUTHORS' CONTRIBUTION

Deepak N. Kapoor and Kamal Dua conceptualized the idea and structure of the manuscript. Shikha Mahant, Himanshu Gandhi, Ridhima Wadhwa, and Abhishek Kumar Sharma collected the data and reviewed the scientific literature. All authors wrote different sections of the manuscript. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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