Review: Application of nanotechnology for the development of microbicides

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Abstract

The vaginal route is increasingly being considered for both local and systemic delivery of drugs, especially those unsuitable for oral administration. One of the opportunities offered by this route but yet to be fully utilized is the administration of microbicides. Microbicides have an unprecedented potential for mitigating the global burden from HIV infection as heterosexual contact accounts for most of the new infections occurring in sub-Saharan Africa, the region with the highest prevalent rates. Decades of efforts and massive investment of resources into developing an ideal microbicide have resulted in disappointing outcomes, as attested by several clinical trials assessing the suitability of those formulated so far. The highly complex and multi-level biochemical interactions that must occur among the virus, host cells and the drug for transmission to be halted means that less a sophisticated approach to formulating a microbicide e.g. conventional gels, etc. may have to give way for a different formulation approach. Nanotechnology has been identified to offer prospects for fabricating structures with high capability of disrupting HIV transmission.

In this review, predominant challenges seen in microbicide development have been highlighted and possible ways of surmounting them suggested. Furthermore, formulations utilising some of these highly promising nanostructures such as liposomes, nanofibres and nanoparticles have been discussed. A perspective on how a tripartite collaboration among governments and their agencies, the pharmaceutical industry and academic scientists to facilitate the development of an ideal microbicide in a timely manner has also been deliberated briefly.

Keywords: microbicide, nanotechnology, vaginal, drug delivery, HIV transmission

1. Introduction

1.1 Vagina anatomy and adaptation for drug delivery

The human vagina has been a route for administering drugs since ancient times (Hussain and Ahsan, 2005). However, it was mainly used to deliver drugs for local effects until 1918 when it was found to be capable of systemic delivery (Macht, 1918). Since then, this route of administration has gained relevance as a viable option for drug delivery in modern medicine. Several classes of medicine are currently approved for vaginal application. In this era of increasing discovery of poorly soluble new chemical entities (NCE), protein based therapeutics and other biologics, vaginal delivery of drugs for systemic use is increasingly being considered as an alternative to oral administration (Baloglu, Bernkop-Schnürch, Karavana and Senyigit, 2009; Bassi and Kaur, 2012; Hussain and Ahsan, 2005). Furthermore, where a local effect or less invasive route of administration is desirable, vaginal delivery of a drug presents a more viable therapeutic strategy than many other routes (Fallowfield et al., 2006).

The human vagina is a fibromuscular S-shaped canal, between 6 and 12 cm long and connecting the cervix to the vulva vestibule (Neves, Palmeira-de-Oliveira, Palmeira-de-Oliveira, Rodrigues and Sarmento, 2014). The upper portion is wider and almost horizontal when in upright posture and the lower part is convex in shape (Funt, Thompson and Birch, 1978). This anatomical positioning and shape contributes to the retention of objects inserted deep into the vagina, thus making it a suitable destination for application of materials such as dosage forms and medical devices (Barnhart, Pretorius and Malamud, 2004). In addition, the increased surface area of the vagina arising from several rugae and extensive vascularisation facilitating access to major blood vessels and organs like the inferior vena cava and uterus offer immense potential for systemic drug delivery (De Ziegler, Bulletti, De Monstier and Jääskeläinen, 1997; Katz, Lentz, Lobo and Gershenson, 2007).

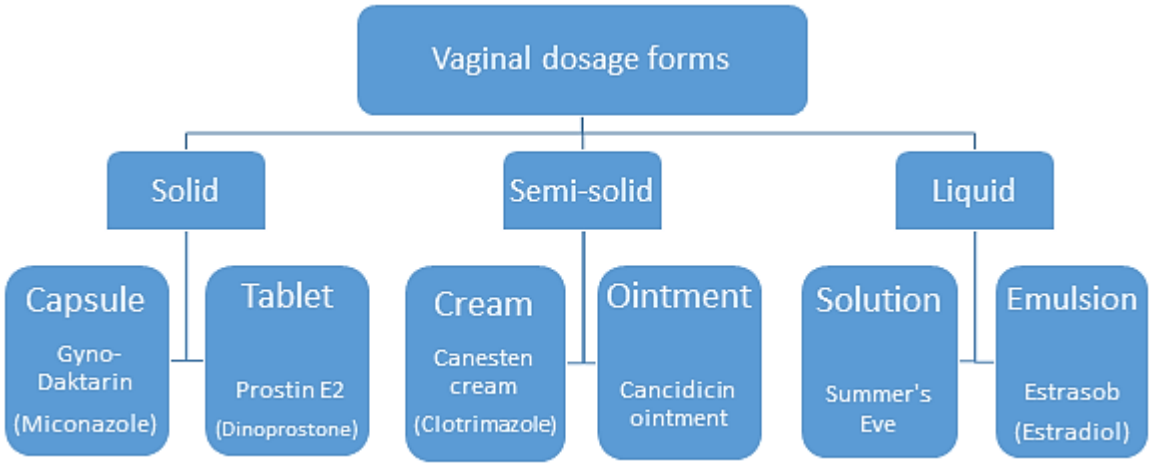


Figure. 1: Types of vaginal dosage forms presently in use.

Some advanced methods of drug delivery to the vagina has recently been reported. Coconut-oil core cationic nanocapsule of clotrimazole prepared from Eudragit® RS100 polymer has been reported to offer prolonged delivery of the antifungal drug to the vagina, offering better antifungal activity against *Candida sp.* compared to ordinary clotrimazole creams (Santos et al., 2014). In another example, Paclitaxel delivered as mucus-penetrating nanoparticles made form poly(lactic- co -glycolic acid) was reported as being more effective in suppressing tumour growth and prolonging the median survival in animal models (Yang et al., 2014).

Apart from the possibility of being useful for the systemic delivery of some drugs, the vaginal route offer prospects for other drug delivery strategies.

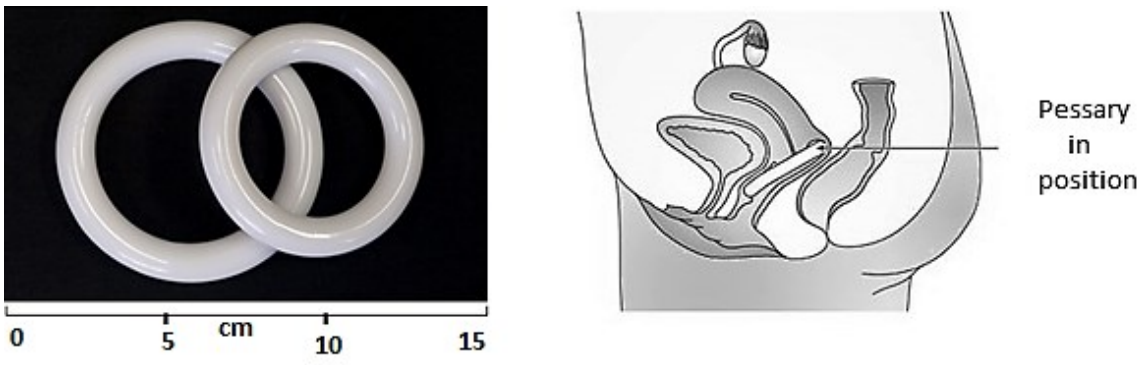


Figure 2: Silicone elastomer vaginal ring and an inserted ring in a female reproductive tract (Schopflin, Laudahn, Muhe, Hartmann and Windt, 1977)

For instance the vaginal structure and environment can be adapted for long term delivery of some medications, e.g. contraceptives, where strict adherence is required for the desired effect. Vaginal rings (Schopflin, Laudahn, Muhe, Hartmann and Windt, 1977) for delivering contraceptives over several months have been shown to maintain constant serum levels of the drug, thus offering an effective solution to problems often arising from nonadherence to oral contraceptives such as through missed dose (Potter, Oakley, de Leon-Wong and Cañamar, 1996). This also takes away the daily burden of taking pills orally and therefore a more convenient approach. Adverse drug effects such as gastrointestinal disturbances, typically occurring after oral administration of a drug can be mitigated by delivering via the vaginal route. For instance, gastrointestinal disturbances resulting from the oral administration of bromocriptine were drastically reduced when give vaginally, in addition to improved bioavailability (Vermesh, Fossum and Kletzky, 1988). Vaginal route is also particularly helpful for systemic delivery of drug susceptible to extensive hepatic metabolism or when intestinal absorption capacity is impaired and also helpful for avoiding possible drug-drug or drug-food interactions in the

gastrointestinal system (Tozer, 1996). In a more recent study, vaginal delivery of subunit vaccines was confirmed to induce better immunity when compared to rectal or intranasal delivery (Lowry, 2015)

Notwithstanding the promising prospects of optimized pharmacotherapy offered by this route, there remains challenges. Perhaps the first and most obvious disadvantage of vagina as a route of drug administration is its gender specificity (Neves et al., 2014). Drug delivery through the vagina is only possible for women. The other challenges associated with vaginal delivery of drug mainly involves cultural perceptions about insertions into the vagina, perceived interference with personal hygiene and possible interference with coitus in sexually active women. Likely local irritations and widely varying pharmacokinetics following vaginal administration of drugs also pose some challenges in this route of administration (Srikrishna and Cardozo, 2013).

1.2 HIV transmission and strategies for prevention

Heterosexual intercourse is among the most common routes for HIV transmission and prevention remains the most effective way of containing the growing epidemic (De Cock, Jaffe and Curran, 2012; Weller and Davis, 2003). In sub-Saharan Africa, where new HIV infections are nearly two-thirds of all global incidences, heterosexual intercourse has been identified as the main epidemic's driving force (UNAIDS, 2008). Considering the impact of heterosexual transmission of HIV, and the fact that prevention still remains the most effective strategy for confronting this disease, an ideal microbicide delivered vaginally has been identified to have immense potential for reducing new HIV infections by preventing heterosexual transmission (Balzarini and Van Damme, 2007).

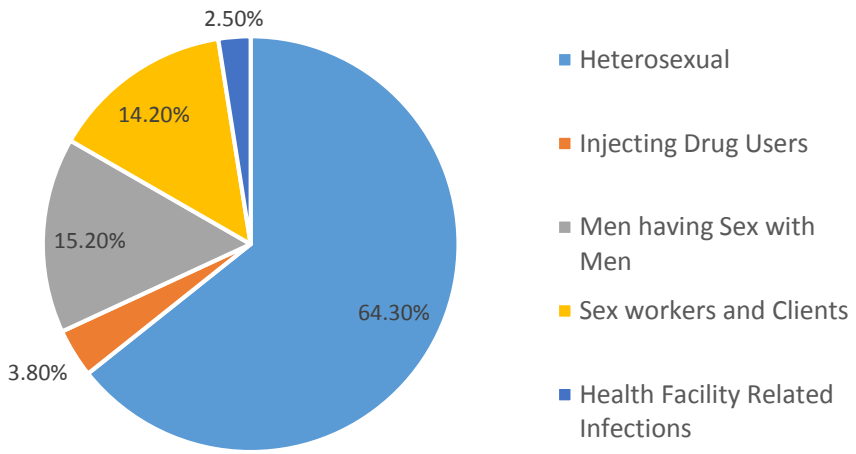


Figure 3: Sources of HIV transmission in Kenya, a sub-Saharan African country where heterosexual transmission accounts for more than half of all HIV infections (Gelmon, 2009)

1.3 Outline of review

In this review, the challenges, opportunities and the current state of affairs with the development of a suitable microbicide are discussed. The shift towards nanotechnology, exploring the possibility of utilising nanostructures either as carrier systems for effective delivery of highly active antiretroviral drugs into target areas for optimal performance or harnessing their inherent antiviral activity has been elaborated. Additionally, this review looks into the requirements, concepts and models which ought to be in place to ensure that laboratory based experiments are translated into clinical usefulness.

2. Microbicides

A microbicide is defined as an anti-infective formulation intended for vaginal or rectal application by individuals to protect themselves and their sexual partners against HIV and other sexually transmitted infections. Microbicides are typically self-administered topical preparations (Garg, Nuttall and Romano, 2009; Stone, 2010). An effective and affordable microbicide formulation is increasingly being considered by global health experts as crucial to a strategy for reduction of HIV transmission.

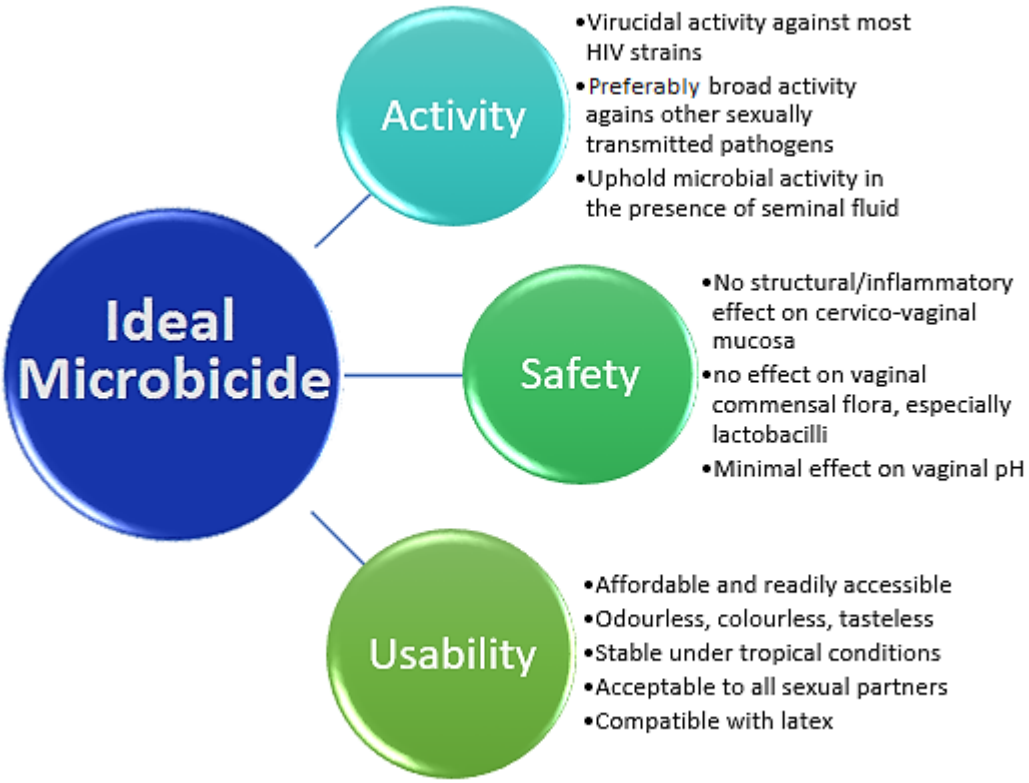


Figure 4: Characteristics of an ideal microbicide (Balzarini and Van Damme, 2007; Singh, Garg, Rath and Goyal, 2014)

Whereas other preventive strategies such as condom use have been in existence for many decades, microbicide use is considered as the most effective approach to HIV prevention where the power and choice to initiate its use totally depend on women, thus empowering them to protect themselves and their partners. For a global-wide implementation of a preventive scheme based on microbicide usage, an availability of a wide range of formulations to meet the diverse expectations of different populations with widely varying socio-cultural attitudes to sex is vital. Outcomes from trials of microbicide formulations developed over the last decade have not been too encouraging (Grant et al., 2008). Issues relating to the performance and safety of conventional formulations like gels, physical presentation and acceptability are among the tremendous challenges encountered in our quest to develop an ideal microbicide.

It is now clear that an effective product against HIV transmission, more so a kind that could be applied externally and at the discretion of women is urgently needed if we are to contain the threat posed by new HIV infections. Therefore investigating a product already licenced for vaginal application with a potential for disrupting the integrity of the viral structure upon contact appeared to be a prudent and faster route to obtaining a microbicide. A fast-track approach led to extensive trials on Nonoxynol-9, a surfactant capable of disrupting the membrane structure of spermatozoa and with similar effects on HIV lipid membrane and many other pathogens that are sexually transmitted (Stone, 2002). The hopes with Nonoxynol-9 as possible transmission barrier to HIV was halted when a review of numerous trials by WHO, particularly on the potential benefits and safety, concluded that this surfactant was not a good candidate for the topical prevention of HIV (WHO, 2002). It was observed that frequent application of Nonoxynol-9 specifically resulted in adverse effects including irritation, inflammation, tissue infiltration by immune cells and massive disruption of the vaginal flora (Beer et al., 2006). This actually resulted in a higher rate of infection among women on this trial product than the placebo group (Van Damme et al., 2002). This conclusion has heightened the urgency for a suitable topical medication against HIV transmission. The intensified search for this preventive medication has taken a wide and varied approach. It has been reported that though no product is commercially available yet, over 40 candidates are being investigated with a significant proportion in clinical trials (Li, Zaveri, Ziegler and Hayes, 2013). The active pharmaceutical ingredients (API) employed include, nucleoside reverse transcriptase inhibitors (NRTIs) non-nucleoside reverse transcriptase inhibitors NNRTIs and genetically engineered inhibitor-expressing bacteria (Klasse, Shattock and Moore, 2006). Much as there appear to be several active ingredients and material effective for topically preventing transmission of HIV, the complexity of the mucosa environment through which most infections are transmitted i.e. the vaginal and/or rectal region and processes involved for the virus to move through several layers to infect target cells means a herculean formulation challenge lies between humans and a microbicide effective in combating HIV transmission.

2.1 HIV transmission and microbicide activity

There are still some uncertainties and dissenting views among scientists on activities leading to HIV transmission, i.e. details pertaining to cells and receptors in the genital environment necessary for transmission to occur, the influence of vaginal biological factors on transmission and the inherent host activities that protect against or facilitate processes leading to the viral infection (Dhawan and Mayer, 2006; Shattock and Moore, 2003). In recent times however, there has been a considerable increase in our understanding of viral-host interaction underlying HIV transmission, especially through the mucosa, thus equipping us with the needed information for developing better strategies against the spread of HIV infections (Shaw and Hunter, 2012; Tebit, Ndembi, Weinberg and Quiñones-Mateu, 2012; Wu, 2008).

There are multiple infection pathways through which HIV transmission occur. For instance in the genital tract, the virus can proceed through the several layered stratified vaginal epithelium or the single layer cervical columnar epithelium layer (Pope and Haase, 2003). Transcytosis along a vesicular pathways has been established as one of the main mechanism by which HIV-1 virus cross intact barriers to infect host cells beneath the tissue (Bomsel, 1997).

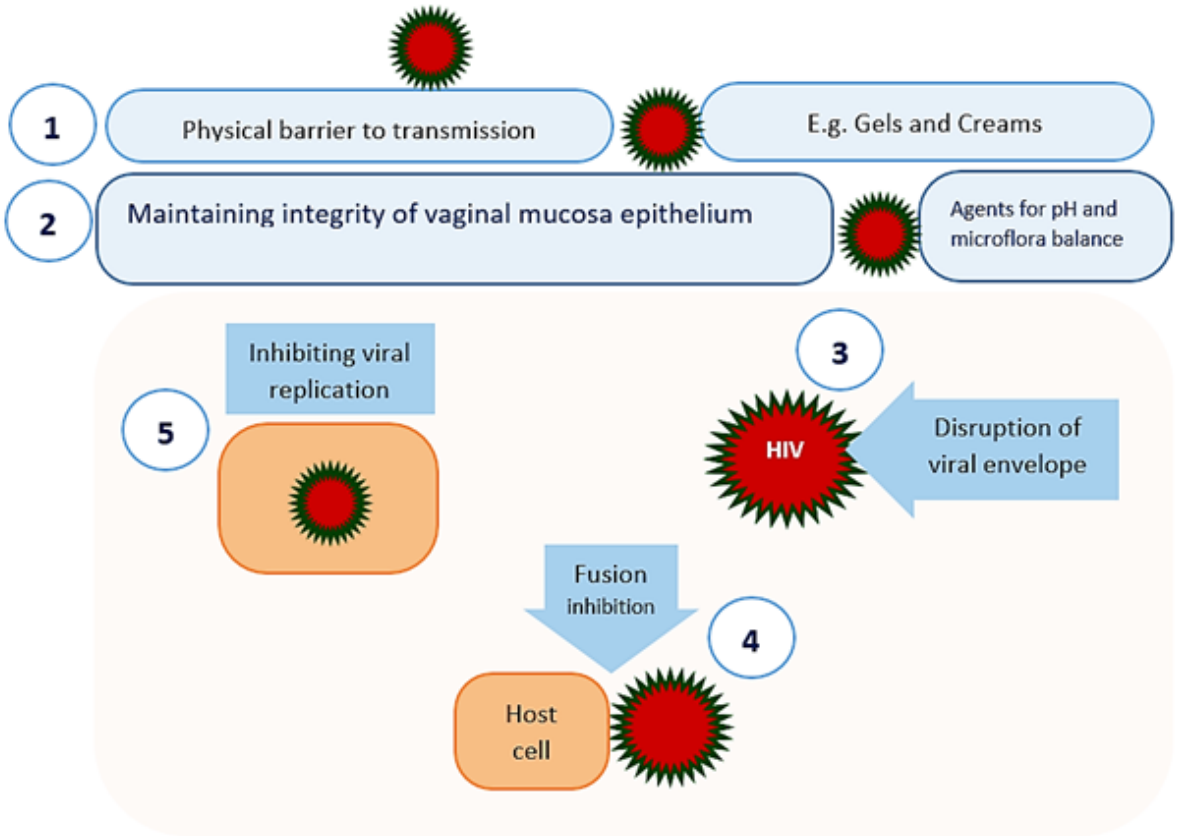


Figure 5: Potential target sites and mode of action for microbicides in preventing HIV transmission.

It is now know that viral infections including HIV occur through two main transmission modes – as cell free virion from an infected cell that moves through the extracellular environment by diffusion to encounter and infect a target cell or by direct cell-to-cell infection after a cell infected with the virus encounters a target cell (Anderson, 2014). It has been established through in vitro studies that cell-to-cell transmission of HIV can be up to or sometimes even more than 1000 times more efficient than cell-free transmission (Chen, Hübner, Spinelli and Chen, 2007; Mazurov, Ilinskaya, Heidecker, Lloyd and Derse, 2010). Once in the sub-mucosa area, the virion or virus can then infect CD4+ T-cells, macrophages and dendritic cells by attaching its envelope gp120 protein to surface proteins on these cells (Patterson et al., 1998; Veazey, Marx and Lackner, 2003).Subsequently, replication cycle of the virus in host cells occurs. An ideal microbicide would therefore be expected to combine activities against transcytosis, attachment and intracellular replication and hence a combination of different drugs may have to be administered to offer an effective and wide spectrum of protection.

The various stages of HIV transmission, up until viral replication in host cells, i.e. viral passage through various epithelia in the genital area, fusion of virus with host cells, viral replication, are principally the targets for microbicides currently in development or trial. Currently available active agents to be developed into microbicides and their respective sites and modes of action, as summarized in table 1, come in such diverse characteristics and hence require different formulation approaches to ensure their applicability and efficacy.

Table1: Target sites, mode of action and agents for the prevention of HIV transmission (Balzarini and Van Damme, 2007)

Area of activity	Mechanism of action	Microbicide agent
Mucosa environment e.g. vaginal, rectal	Direct inactivation of virus	Detergents or surfactants
	Maintaining natural vaginal environment for innate immunity against infections	Lactobacilli, buffering agents
	Physical barrier to transmission	Topical formulations e.g. gels
Tissue/cell surface	Preventing virus from entering target cells by interfering with host cell receptor/virus interactions	Monoclonal antibodies Small molecule inhibitors
Virus surface	HIV entry inhibition by targeting viral envelope fusion onto host cells	Polyanions Carbohydrate binding agents Monoclonal antibodies
Inside the host cell	Interfering with viral replication cycle. Inhibition of virus-encoded reverse transcriptase (RT) or integrase	Nucleotide RT inhibitor Non-nucleoside RT inhibitor

Table 2 shows some of the formulations presently in clinical trial. There are still opportunities to enrich the pipeline of effective microbicides and hence the advocacy for considering interventions from nanotechnology to offer some solutions to the myriad of challenges encountered in creating these systems.

Table 2: Some recent microbicide formulations clinical trials [Source: www.ipmglobal.org; http://www.mtnstopshiv.org/]

Trial Name	Phase	Start Date	Countries	Population	Candidate(s)	Status	Expected Completion Date
FACTS 002 An adolescent safety study designed to test the safety and acceptability of tenofovir gel in 16- and 17-year-old South African young women	II	July 1, 2015	South Africa	Women	1% Tenofovir gel	Ongoing	December 2016
A13-128 Safety of the TFV/LNG[a], and TFV-only intravaginal rings pharmacokinetics of TFV and LNG acceptability of intravaginal rings	I	November 30, 2014	United States of America, Dominican Republic	Women	TFV ring, TFV/LNG ring	Completed	November 2015
MTN 017 Assess the safety, acceptability, systemic and local absorption, and adherence of reduced glycerine tenofovir gel applied rectally	II	September 30, 2013	Thailand, South Africa, United States of America, Puerto Rico, Peru	Transgender, HIV negative, MSM [c]	Reduced Glycerine 1% Tenofovir Gel, TDF/FTC [b] (Truvada)	Completed	June 2016
IPM 027 (The Ring Study) To assess the safety and efficacy of a silicone elastomer vaginal matrix ring	III	April 30, 2012	South Africa, Uganda	Women	Dapivirine Ring	Ongoing	December 2016

[a] TFV/LNG = Tenofovir / levonorgestrel; [b] TDF/FTC =Tenofovir disoproxil / emtricitabine; c MSM = Men who have sex with men

2.2 Formulation of microbicides

The foremost challenge encountered in microbicide formulation is to get a drug capable of navigating the complex tissue of the cervico-vaginal lumen to provide effective protection. In order to reduce the possibility of HIV disseminating beyond microbicide's reach and action, a formulation with substantial mucosal permeating action is crucial (Hladik and Doncel, 2010). Therefore materials with structural

characteristics flexible enough to be adapted for far reaching impact will be crucial to design of an effective microbicide. Furthermore, the routine physico-chemical compatibilities between active ingredients and excipients needs to be established. Of particular importance is the drug stability in aqueous state, as the site of application, the vaginal environment is predominantly aqueous (Garg et al., 2009). Combination therapy is well accepted in managing HIV/AIDS infections, as it has been confirmed to offer better treatment outcomes than monotherapy (Lange, 1995; Moore and Chaisson, 1999). Therefore, a strategy which combines multiple active ingredients may be worth considering for development of microbicides. In addition to monitoring compatibility issues that may arise from interaction among the different active ingredients combined, reliable models and methods ought to be designed to effectively assess likely additive, synergistic or antagonistic interactions that may occur. Another challenge an effective microbicide formulation ought to withstand is the rapid pH changes that occur in the cervico-vaginal environment during intercourse. There is an immediate transition from pH approximately 4.0 to around 7.0 following release of semen. For effective protection against HIV transmission, there ought to be a microbicide robust enough to remain effective through rapid environmental changes, as there are strong indications that significant departure from the normal pH levels in this environment can affect the efficacy of most microbicides (Neurath, Strick and Li, 2006). In order to achieve such a robust formulation, a reliable model capable of establishing that efficacy and performance of a microbicide seen at the *in vitro* stage of formulation development can be realized *in vivo,* particularly in an environment combining seminal fluid, cervico-vaginal secretions and mucus which is typical of challenging situations where microbicides are likely to lose their efficacy (Turpin, 2011). Finally, the formulation strategy would have to address the critical question of product acceptability as an outstanding formulation would require high acceptance for it to ensure usage. High acceptability will guarantee appreciable level of adherence. With regard to dosage forms for vaginal application, there is a precedent that can inform decisions to arrive at a product likely to be well patronized (Vermani and Garg, 2000). Ease of applicability, dose frequency, physical state and appearance of drug are some of the key factors that determine how well these formulations are accepted.

2.3 Acceptability of microbicides

A compelling reason for advocating microbicide use as an effective strategy and the future for HIV transmission reduction is the fact that its use is largely woman initiated and therefore an outstanding opportunity to empower women with more options for safer sex. For instance in a recent survey on microbicide adherence, it was confirmed that disclosure of microbicide use by women to male partners significantly improved adherence thus establishing the role of male partners in successful implementation of a microbicide strategy (Mngadi et al., 2014). Beyond these considerations, factors

generally dictating acceptability of any drug formulation i.e. ease and convenience of use, dosage frequency, anticipated adverse reaction etc. ought to be factored into the pre-formulation planning.

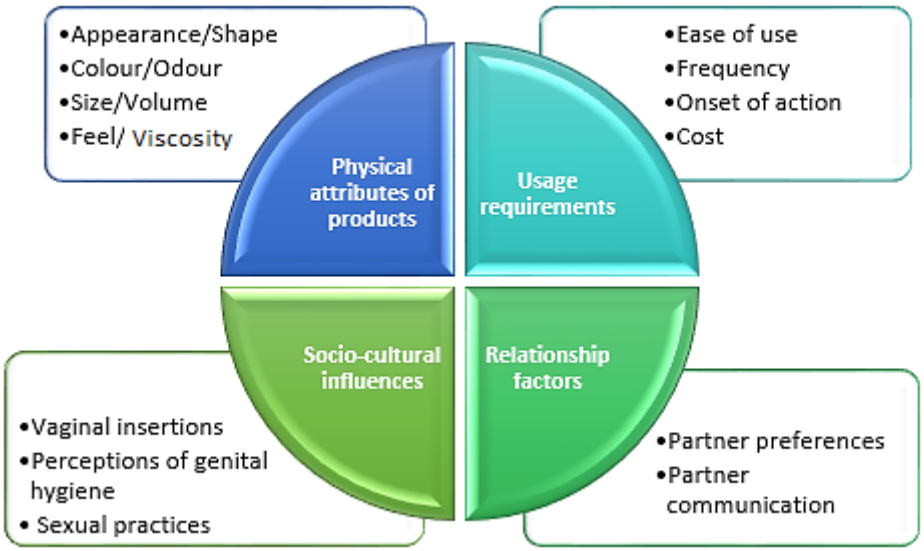


Figure 6: Factors that can influence microbicide acceptability (Balzarini and Van Damme, 2007; Singh et al., 2014)

Furthermore, as with most products relating to sexual lifestyle, the demographics and socio-cultural influences among the target market for a particular microbicide formulation ought to be accommodated in order to get a product with wide acceptability. In a study assessing acceptance rates in populations with different cultural influences, it was found that women's perception of subjects like vaginal cleanliness e.g. leakages associated with menstruation and negative or positive associations with vaginal douches resulted in different perception and acceptability of the same gel formulation in different groups (Giguere et al., 2012)

The numerous requirements to be met in order to produce a microbicide formulation for wider acceptability imply that several options including a variety of materials for fabrication is needed for different microbicide forms suiting different populations. An emerging class of materials, nanostructures, due to their high degree of structural flexibility and surface properties, when optimized to suite could be a positive addition to the stock of materials for an effective microbicide formulation. In populations with negative perception on vaginal leakage, for instance, where gel formulations are unlikely to be widely accepted and used, nanofibre could be an alternative material for forming dosage forms capable of addressing issues of leakage. Further suggestions and reasons why nanomaterials may drive the future of microbicide formulation are expounded.

2.4 Present state of microbicide development

The majority of microbicide which has been or currently in clinical trials (some examples shown in table 2) are conventional semi-solid formulations, particularly in the gels designed to effectively deliver a single dose of an antiviral agent at a time (Di Fabio et al., 2003; Ndesendo et al., 2008; Stone, 2002; Van Herrewege et al., 2004). Several of these formulations have failed to demonstrate adequate efficacy, safety and tolerability, thus prompting a re-evaluation of the current development paradigm (Karim and Baxter, 2014; Hendrix, Cao and Fuchs, 2009). Indeed the focus on formulating an effective microbicide for the prevention of HIV transmission is gradually expanding from earlier concepts that centred on gel and cream formulations to include such forms as films and fast dissolving solids (Rohan, Devlin and Yang, 2014). However, there is still more room for improving upon the existing range of vaginal dosage forms intended for the delivery of microbicides. Some of the key challenges identified during trial of these formulations, including disruption or inflammation of mucosal epithelium, effect of pH conditions and proteolytic enzyme action in the genital tract, inadequate delivery of active drug to target sites due to poor retention and inconvenience associated with use frequency of application are likely to be surmounted through strategies utilising nanotechnology applications (D'Cruz and Uckun, 2014).

Further into this review, opportunities presented by nanotechnology, i.e. the possibility of utilising the unique physical and structural properties of nanostructures such as nanofibres, liposomes, dendrimers and nanoparticles for addressing some of the specific issues seen in the current stock of microbicides are discussed. Nanotech inspired approaches to formulating drugs and medical devices has proved to be successful in some therapeutic areas like cancer management. When extensively researched and adequately applied, these same technologies could be resourceful in the development of the next generation of microbicides.

3. Microbicide development through nanotechnology

An effective microbicide is not yet available for use and despite tremendous efforts and resources invested in the development of an ideal microbicide for over three decades, outcomes from both preclinical and clinical trials on existing formulations have been overall quite disappointing (Karim, 2010). The approach to developing microbicides, up until recently has been mainly by conventional formulation methods such as gel formulation and this appear to contribute to the numerous issues identified in the current pipeline of microbicides (Garg et al., 2010). Many of the challenges seen in current formulations being considered, such as inadequacy in targeting and delivering active drug into sites required for drug action and stability of drug in formulations could be surmounted by exploring options in nanotechnology, an area capable of delivering better targeting, flexibility for combination

therapy and more desirable pharmacokinetic profiles, as seen for instance in the area of cancer management (Sharma and Garg, 2010; Singh et al., 2014).

The antiretroviral drug tenofovir, for instance could be delivered as nanofibre based dosage form with enhanced surface properties for adequate mucosal contact to facilitate sustained release over extended periods. These nanofibres may be generated from a homogeneous mixture of the active drug at required concentration and polymers capable of yielding desired physical and release properties in a safe solvent system. The resulting fibres could offer many possibilities of formulating solid dosage forms in varying geometric forms to make them fit for purpose.

A variety of polymers are known to act against viral transmission on their own. For instance some anionic polymers are thought to adhere to viral envelopes through their negative charges and thereby blocking viral entry into target cells (Balzarini and Van Damme, 2007). Though higher concentrations of these polymers are required for this antiviral activity to occur, the extremely low general toxicity of these materials imply that employing optimal formulation methods and conditions could help realize the therapeutic benefits of these materials. A careful selection and combination of polymers from a wide range of polyanionic compounds are likely to work in synergy with antiviral agents like tenofovir for potentiated activity against transmission of HIV. Specifically, sulphated polysaccharides such as dextrin, dextran and cellulose sulphates, aliphatic and aromatic carboxylates such as carbomer 974P and some aryl sulphonates are all anionic polymers whose antiviral potential have long been established (Howett and Kuhl, 2005; Pauwels and De Clercq, 1996). Some polyanionic dendrimers are also known to have some inherent antiviral activity (Ross and George, 1995). Carboxylated fullerene-based dendrimers for instance have been shown to offer antiviral activities by inhibiting viral protease and reverse transcriptase in acutely HIV infected primary human lymphocytes (Schinazi, Brettreich and Hirsch, 2001).

Through nanotechnology, microbicide formulations capable of combining the therapeutic effect of their carrier material such as those made of polyanionic materials and an active drug such as tenofovir, a nucleotide reverse transcriptase inhibitors which works by disrupting intracellular viral replication could be developed to offer adequate protection against HIV infection. Microbicides combining multiple therapeutic agents are confirmed to be safer and more effective in preventing HIV transmission (Dang et al., 2014; Gantlett, Weber and Sattentau, 2007; Vacas-Córdoba et al., 2014). Studies have confirmed the synergistic benefit of combined antiviral action occurring at different stages of the HIV replication simultaneously in the form of multiple-fold transmission reduction and appreciable potency even at lower doses which improved the safety profile of these combinations (Liu, Lu, Neurath and Jiang, 2005; Pirrone, Thakkar, Jacobson, Wigdahl and Krebs, 2011; Sepúlveda-Crespo et al., 2014). Nanotechnology inspired formulations including liposomes, dendrimers, nanofibres and nanoparticles are known for their performance, versatility and flexibility and therefore offering immense

opportunities for tailoring their shapes and structures for specific drug delivery functions (Venkataraman et al., 2011). Nanotechnology could also be instrumental in the development of microbicides with optimal release profiles and whose activities are derived from multiple active ingredients (combination therapy).

3.1 Prospects for combination therapy

Nanotechnology is widely being used in the design of novel systems capable of delivering multiple drugs from a single unit (Parhi, Mohanty and Sahoo, 2012; Tang, Lei, Guo and Huang, 2010). These new technologies are being used to create platforms for effective combination pharmacotherapy, especially in the area of cancer management. Some of these nanotech strategies that has been so valuable in other areas of pharmacotherapy could be adapted for the development of practically useful microbicides. A microbicide, for example addressing multiple issues such as maintaining ideal environment in the vagina and at the same time preventing viral infection at different stages of an invasion would be a breakthrough. There are possibilities to include adjuvant into microbicide formulations in order to achieve multi-level protection against HIV infection. Maintaining a suitable level of acidity (pH) in the cervico-vaginal environment for instance is known to be crucial for immunity against HIV and other pathogens (Balzarini and Van Damme, 2007; Olmsted et al., 2005). However, during sex, when microbicides is expected to offer protection against HIV transmission, the slightly alkaline semen is capable of diminishing this level of acidity and thereby significantly reducing this natural defence mechanism (Baron, Singh, Chopra, Coppenhaver and Pan, 2000). Furthermore, a microbicide formulated to be effective in a particular pH environment could lose some activity when a sudden change in pH occurs. The co-administration of an adjuvant with an appreciable buffering capacity could potentiate the overall protection conferred by the microbicide. The unique structure of dendrimers, for instance, could be developed to contain functional groups capable of acting as pH buffers within its highly branched network alongside conjugates of its peripheral molecules with antiviral groups could form the base unit of an effective microbicide. Nanofibre based dosage form made from several discrete units of fibre each encapsulating either an API or an adjuvant such as pH buffer could also make a desirable microbicide. Co-application of probiotics with microbicides have been suggested to help re-acidify and maintain the commensal vaginal flora for optimal protection and indeed some buffering agent formulations such as BufferGel (ReProtect; Baltimore, MD, USA) are already in clinical trials for vaginal defence mechanism (Balzarini and Van Damme, 2007; Olmsted et al., 2005).

3.2 Modulating drug release

Nanotechnology continues to make a significant contribution to the development of new and more effective drug delivery systems (Shi, Votruba, Farokhzad and Langer, 2010). Microbicides developed through nanotechnology could benefit from improved solubility of hydrophobic drugs, controlled release of drugs and better immunogenicity and safety profile.

The fact that a wide range of material can be used in developing these nanocarriers offers opportunities for extensive manipulations to obtain a microbicide formulation with a desired drug release kinetics. In systems utilising nanofibres for instance, there have and continue to be numerous studies exploring their prospects of modulating drug release. (Singh et al., 2014; Son, Kim and Yoo, 2014; Verreck et al., 2003). Known to degrade in biological systems slowly, polycaprolactone for instance, has been used to achieve slow and steady release of tetracycline over periods up to 200 hours (Karuppuswamy, Reddy Venugopal, Navaneethan, Luwang Laiva and Ramakrishna, 2015). Getting the release kinetics of drug from microbicide is crucial to the overall usefulness and acceptability. Depending on the prevalent socio-cultural attitude towards sex, microbicide use may not be widely accepted if it ought to be applied shortly before an encounter as the presence of a male partner could discourage this. In other situations, prolonged microbicide action may be desirable as multiple sexual encounters over lengthy periods are likely but frequent application of an intervention impractical. In these two likely scenarios, extended release microbicide formulations would be required for effective protection against HIV infection.

Polyethylene oxide (PEO) and polycaprolactone (PCL) are polymers readily available and routinely used in various applications including drug delivery. Whereas PEO is soluble in water and yields fibres which are even more rapidly soluble in aqueous media (Row, Sheskey and Quinn, 2009) PCL slowly degrades, usually by hydrolysis throughout the polymer matrix after water has gradually penetrated the entire polymer bulk (Woodruff and Hutmacher, 2010). Using an appropriate solvent system, suitable amounts of PCL could be used to modulate drug release from PEO nanofibres by modifying their overall solubility in aqueous media. Microbicides based on slowly degrading nanofibres could be a good balance between ultra-long acting vaginal rings and rapidly eliminated gel based formulations. The possibility of making nanofibres from different polymers in order to confer some specific characteristic to the fibres have been demonstrated (Brako, Raimi-Abraham, Mahalingam, Craig and Edirisinghe, 2015).

Dendrimer complexes and conjugates have been used to optimize drug release in a number of formulations (Nanjwade, Bechra, Derkar, Manvi and Nanjwade, 2009). A dendritic formulation of Flurbiprofen was also used to achieve extended release of the drug, allowing drug release over periods up to three times more than what is usually seen in free drug formulations (Asthana, Chauhan, Diwan and Jain, 2005). Drug release from gel formulations has been prolonged and made to obey zero-order

kinetics by first encapsulating the active ingredient in liposomes (Glavas-Dodov, Goracinova, Mladenovska and Fredro-Kumbaradzi, 2002).

3.3 Nanostructures currently being explored for development of microbicides

3.3.1 Nanofibre based microbicide

A nanofibre typically has two similar external dimensions (making up the cross-sectional area) within the nanoscale, and the third dimension, usually the length, significantly larger (Glavas-Dodov et al., 2002). Nanofibres are currently produced by widely varying methods including molecular self-assembly, thermally induced phase separation and fibre spinning (Luo, Stoyanov, Stride, Pelan and Edirisinghe, 2012). Of all these methods, fibre spinning, a term used to describe the various methods of fibre formation by extrusion through a spinneret appears to be the most widely used method. Electrospinning and centrifugal spinning are two of fibre spinning methods commonly used. Notwithstanding, novel gyration methods such as pressurised gyration are coming to the forefront as this has demonstrated potential of upscaling production to meet commercial demands for various applications (Brako, Raimi-Abraham, Mahalingam, Craig and Edirisinghe, 2015; Mahalingam and Edirisinghe, 2013; Xu et al., 2015)

Nanofibres, depending on constituent materials and conditions of fabrication could exhibit widely varying morphology and structural properties. However, features such as high surface area to volume ratio and well defined porosity are typical of nanofibres and have often been reasons why they are desirable for drug delivery applications (Pillay et al., 2013). High surface area per unit mass of nanofibres could be harnessed to overcome solubility issues seen in many APIs e.g. ibuprofen, usually by combining them with hydrophilic materials of nanofibres (Williams et al., 2012). In addition, controlling the matrix properties such as fibre diameter and porosity by manipulating fabrication parameters allows for the incorporation of delicate molecules such as proteins, antibodies and DNA into nanofibre constructs such as meshes for site-specific delivery in the body (Pillay et al., 2013). Significantly higher encapsulation efficiency is attainable when active ingredients are incorporated into nanofibres (Liao, Chew and Leong, 2006; Xie and Wang, 2006). Achieving drug loading in excess of 90% offers the possibility of designing highly efficient delivery systems with fewer additives and excipients. This ultimately enhances the safety profile of the formulation since unwanted materials, which have to be metabolized and eliminated are already in minimal quantities. Drug delivery systems utilising polymeric nanofibres as basic units can be designed as a multi-compartment assembly delivering different active drugs from a single unit. Basically, multiple APIs may be encapsulated in different nanofibres and combined to be presented as one unit. The multi-targeted approach is a viable option since the individual encapsulations prevent the active ingredients from interacting among themselves regardless of their proximities, until onset of drug action. Finally, the potential of

modulating drug release from nanofibre systems by varying their material constituents makes these structures attractive for drug delivery (Kenawy, Abdel-Hay, El-Newehy and Wnek, 2009). Considering the wide array of suitable materials available for making nanofibres, several possible combinations may be selected for a specific release kinetic desired. The starting point for most nanofibre production requires the materials to be in solution or melt. Therefore most materials, especially polymers, once converted to the suitable liquid state and such properties as viscosity and surface tension optimized may be transformed into nanofibres by the appropriate method. This feature of making nanofibres enable the accommodation of a wide range of materials thereby enhancing the prospects of manipulating release kinetics through choice of materials.

Some of these exceptional qualities of nanofibres, if harnessed, effectively could be useful for the design of microbicide highly suitable for preventing HIV transmission. The final geometry of microbicide developed from nanofibres will be crucial both to their acceptability and drug delivery performance. Dosage forms intended for insertion into the vagina vary in shape from globular or ovoid such as round or long oval through modified conical shapes like bullet to cylindrical forms including tampons (Hanan and Durgin, 2014). A correlation between shape of vaginal suppositories and willingness to try or use the product has been established (Li, Zaveri, Ziegler and Hayes, 2013).

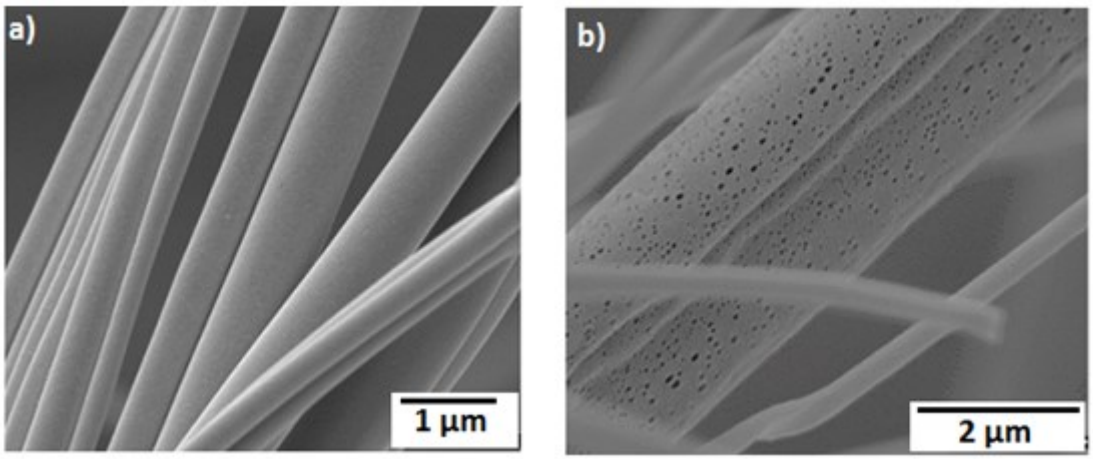


Figure 7: a) Nanofibres produced by pressurized gyration method (Mahalingam and Edirisinghe, 2013) and b) revealing porosity that can be utilized for optimal drug delivery

Presenting the shape of a nanofibre based microbicide suppository in a shape widely accepted by anticipated users is therefore fundamental to the overall success of any such microbicide. A few microbicides formulated entirely from nanofibres have been developed and trialled with some encouraging outcomes reported. Maraviroc eluting nanofibres made from polyethylene oxide and poly lactic acid polymers have been demonstrated to be affective against HIV-1 in vitro (Ball, Krogstad,

Chaowanachan and Woodrow, 2012). Furthermore, a nanofibre based delivery system capable of drug loading of up to 28 wt. % and hence able to deliver high doses of the potent antiretroviral maraviroc rapidly has been developed (Ball and Woodrow, 2014). This formulation is reported to be a superior alternative to previous gel formulations of the same antiretroviral, where the drug appears either insoluble or when made to be completely dissolved, appear too bulky, at minimum drug loading of 3.3 wt. % required to inhibit any retroviral activity (Malcolm et al., 2013). Scientist behind these antiviral eluting nanofibres (Ball and Woodrow, 2014) have been quoted as suggesting the development of nanofibre based tampons offering protection against HIV transmission when applied shortly before intercourse (Huffington Post, 2014). When successfully developed, trialled and confirmed as effective for preventing HIV transmission, this would confirm the utility of nanofibres as alternate approach to microbicide formulation. Additionally, it will inspire further research into the relatively unexplored area of harnessing the immense benefits of nanofibre structures for the superior performance of systems capable of more efficient delivery of drugs through mucosa membranes in general.

3.3.2 Polymeric nanoparticles for microbicides

Nanoparticle for drug delivery has been broadly defined to include particulate systems with mean diameter between 50 and 1000nm (zur Mühlen, Schwarz and Mehnert, 1998). Polymeric nanoparticles (NP) are typically the result of spontaneous self-assembling of amphiphilic polymers when dispersed in an aqueous medium (Uchegbu, Schätzlein, Cheng and Lalatsa, 2013). The self-assembly is usually driven by methods such as probe sonication, micro-fluidisation and high pressure homogenisation. The therapeutic agents in NPs could be dissolved, encapsulated within or adsorbed onto the constituent polymer matrix.

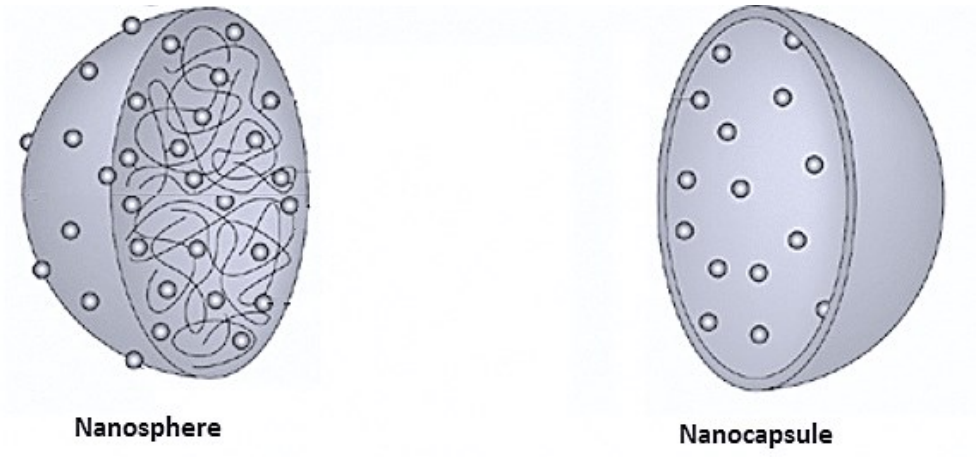


Figure 8: A schematic illustration of a) nanosphere showing how drug (small spheres) is dispersed throughout the polymer matrix and b) nanocapsule in which drug is confined in a reservoir bound by single membrane.

Depending on the processing method utilized, the resulting structure could either be nanosphere in which the active agent is dispersed throughout a polymeric matrix system in the particle or as a nanocapsule in which the drug exist in a vesicular reservoir enclosed by single polymeric membrane (Letchford and Burt, 2007). Nanoparticles, due to their ability to overcome physiological barriers while delivering drug molecules to specific cells or tissue compartments either by passive or ligand mediated movements make them a highly versatile class of drug delivery systems (Mallipeddi and Rohan, 2010). NPs have demonstrated promising prospects for superior drug delivery, especially in the area of cancer pharmacotherapy (Prabhu, Patravale and Joshi, 2015) and this novel approach could be extended into the area of HIV prevention. Targeted delivery of microbicide drugs into vaginal tissues through the use of nanoparticles has recently been developed to overcome issues such as drug movement across mucosa barriers, physicochemical stability, solubility, and immunogenic response typically associated with conventional hydrogel formulations (Rohan and Sassi, 2009). If microbicides are to offer the necessary protection against HIV infection, it will be crucial for the active components of the formulation to navigate the many barriers in order to reach the specific site for optimal antiviral activity. Nanoparticles, when employed as carrier systems could aid the delivery of active drug to these sites. Surface engineered dapirivine-loaded polycaprolactone nanoparticles have been demonstrated to have the potential to facilitate movement of the antiviral drug across mucosa barriers in the cervico-vaginal region (das Neves et al., 2012). Furthermore, microbicide formulations delivering active drug through nanoparticles have been confirmed to exhibit better antiviral activity due to increased intracellular drug delivery facilitated by better cellular uptake of the drug loaded nanoparticles (das Neves et al., 2012).

In addition to enhancing antiviral activity through more efficient drug transport across barriers and better cellular uptake, nanoparticles can be instrumental in improving the acceptability and use of microbicides when their potential to protect active agents from untimely metabolism are applied to prolonging microbicidal activities. A poly (D,L-lactide-co-glycolide) nanoparticle delivering the highly potent anti-HIV protein PSC-RANTES was shown to offer superior antiviral activity over an extended period, thus confirming the prospects of achieving longer acting microbicide action when active drugs are presented in nanoparticle carrier systems (Ham, Cost, Sassi, Dezzutti and Rohan, 2009). Some pioneering and innovative work in utilising nanoparticles to interfere with HIV infectivity is currently underway. Melittin, a cytolytic peptide component of bee venom, incorporated into shells of perfluorocarbon nanoparticles has been proven to inhibit HIV infectivity by disrupting lipid viral envelopes and at the same time being safe and actually therapeutic for host cells (Hood, Jallouk, Campbell, Ratner and Wickline, 2013; Jallouk et al., 2014). Chitosan nanoparticles containing tenofovir with exceptional mucoadhesive properties are also being developed to target mucosa reservoirs of HIV in areas such as the vaginal epithelial tissues (Meng, Sturgis and Youan, 2011) Some of these positive developments strengthen arguments that nanotechnology offers immense opportunities for developing ideal microbicides in the near future.

3.3.3 Dendrimers for microbicides

Dendrimers are highly branched and usually symmetrical three dimensional structures with a well-defined architecture where peripheral groups are joined to the core by branching units (Murugavel, 2014). They are classified as supramolecular (Astruc, Boisselier and Ornelas, 2010) in that they are typically a chemical system made up of a discrete number of assembled subunits rather than a single unit (Lehn, 1988, 1995). Dendritic molecules can be visualized as repetitive layers of multifunctional blocks of a protected and unprotected scheme of complimentary monomers typically resulting in a fractal-like tree in which each incorporated layer serves as a platform for the successive layer (Tomalia and Cheng, 2012). The first completely characterized repetitively branched and polyfuctional molecule was generated by Vogtle et al. in 1978 from a protocol based on cycles of nucleophile amine addition to electron-poor cyanoalkene, followed by reduction of the cyano groups which in turn yields new amine moieties for further reactions (Buhleier, Wehner and Vögtle, 1978). Several other contributions like Vogtle's, particularly those form the research groups of Newkome, Denkwalter and Tomalia (Denkewalter, Kolc and Lukasavage, 1982; Newkome, Yao, Baker and Gupta, 1985; Tomalia et al., 1985) build upon experiments setting out some fundamental principles of molecular organisation pioneered by P.J Flory in the middle of the twentieth century (Flory, 1942)

Extensive research into the possible applications of dendrimers in diverse areas within the biological and medical fields is currently ongoing (Caron et al., 2010; Mintzer and Grinstaff, 2011; Nasibullah, Hassan, Ahmad, Khan and Rahman, 2013; Noriega-Luna et al., 2014). Some properties of dendrimers including their nanoscopic size and uniformity, flexible molecular structure and presence of multiple peripheral functional groups to facilitate conjugate formation with a wide range of drug molecules make dendrimers highly suited for targeted delivery of drugs (Kesharwani, Jain and Jain, 2014), and hence the rigorous investigations into their potential as drug delivery systems, especially in the area of cancer pharmacotherapy and delivery of macromolecules (Brannon-Peppas and Blanchette, 2012; Cheng, Zhao, Li and Xu, 2011; Lim and Simanek, 2012; Parhi et al., 2012; Parveen, Misra and Sahoo, 2012). Furthermore, the unique architecture of dendritic molecules, especially that of its interior with enough void volume makes it ideal for doping with a wide range of molecules for desirable functioning (Tomalia and Cheng, 2012). Presently, drug-dendrimer conjugates of anticancer drugs including Fluorouracil, Methotrexate, Doxorubicin, Paclitaxel, Camptotecin and a few more are being investigated for improved physical attributes and performance. In addition to anticancer drugs, several other classes of medicines including anti-inflammatory and antimicrobial agents are being investigated for presentation as drug-dendrimer units (Tomalia and Cheng, 2012).

The attractive characteristics of dendrimers are being utilized for the development of safer and more efficient microbicides. First of all, dendrimers by themselves can be inherently antiretroviral when some functional groups capable of interfering with viral adhesion to cells are incorporated onto their surface

during synthesis (Jiménez et al., 2012). Some functional groups designed to confer anti-HIV activity on certain classes of dendrimers are shown in Table 3. Proteins present on surface of viruses bind multiple carbohydrates on target host cells during invasion (Gajbhiye, Palanirajan, Tekade and Jain, 2009). Groups similar to these carbohydrates on host cell surfaces can be incorporated onto dendrimers peripheries to act as preferred receptors for invading viruses thus sparing host cells and therefore preventing infection. These have been successfully tested with some influenza viruses and the principle can be explored for the synthesis of dendrimers to be used as microbicides (Roy, 1996; Tsvetkov et al., 2002).

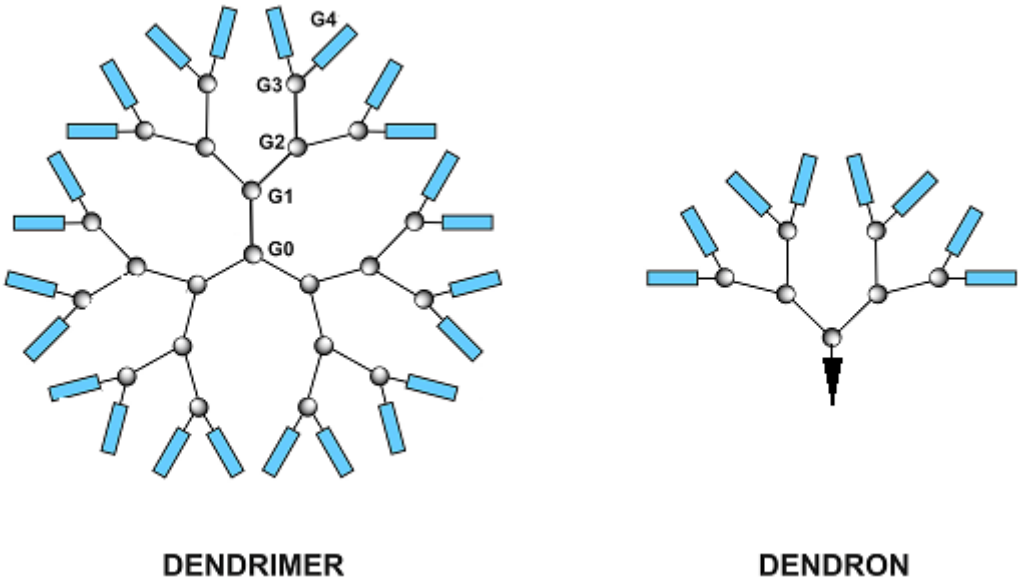
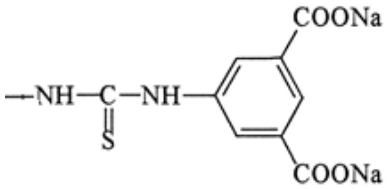
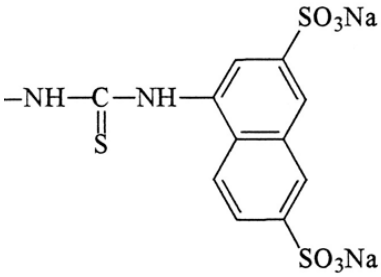
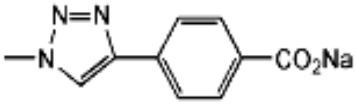
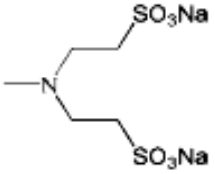


Figure 9: Schematic illustration of dendrimer showing the core (G_0) and branching points (G_1 – G_4) which serve as platforms for either expanding the network of molecules with more basic units (dendron) or with different functional groups for a specific activity (Illustration based on design by Oleg Lukin; obtained from Wikimedia Commons)

In addition to preventing viral binding to host cells, polyanionic dendrimers have been shown to affect the life cycle of viruses, including HIV (Tephly, 1991). Carboxylated fullerene-based dendrimers for instance is known to inhibit viral protease and reverse transcriptase in acutely HIV infected primary human lymphocytes (Yoshida et al., 2013). The molecular structure of dendrimers, apart from allowing the incorporation of specific functional groups to confer antiviral capabilities also facilitates the conjugation of other antiretroviral compound for possible multi-targeted activities against the transmission of viruses. Two of the most widely studied antiretroviral for microbicide development, tenofovir (TFV) and maraviroc (MVR) are reported to have been successfully conjugated onto polyanionic carbosilane dendrimers in separate formulations (Sepúlveda-Crespo, Gómez, De La Mata, Jiménez and Muñoz-Fernández, 2015). Formulations combining two different dendrimers to be used as

microbicides have also been reported (Sepúlveda-Crespo et al., 2014). In both dendrimer-dendrimer and dendrimer-drug combinations, where lower overall concentrations offered greater antiviral activity than usually seen in monotherapies, a case for using a combination formulation to offer optimal activity from low doses for minimal incidence of toxicity and emergence of resistant viral strain has firmly been established (das Neves et al., 2012).

Table 3: Chemical groups used in modification of some classical dendrimers for enhanced antiviral activity against HIV

Dendrimer type [Code name of specific compound and reference]	**Functional group delivering anti-HIV activity**	**Site and mode of anti-HIV activity**
Polyamidoamine (PAMAM) [BRI2932 (Witvrouw et al., 2000)]		Attaches to gp120 to inhibit binding to MT-4 cells
Polyamidoamine (PAMAM) [BRI6195 (Witvrouw et al., 2000)]		Attaches to gp120 to inhibit binding to MT-4 cells and capable of permeating host cell to inhibit reverse transcriptase and integrase activities during replication
Gallic acid-triethylene glycol (GATG) [[G1]-CO2Na (Doménech et al., 2010)]		Complexes with C-terminal domain of viral capsid protein which results in disruption of capsid assembly for maturation of HIV type 1
Carbosilane [2G-S16 (Chonco et al., 2012)]		Combines with both gp120 on viral surface and CD4 on host cells to disrupt fusion of virus onto host cell

Irritations, possible epithelial injury and inflammation from local application of microbicides have emerged as serious concerns (Beer et al., 2006; Buckheit and Buckheit Jr., 2012). It is therefore encouraging to learn that many dendrimer-based microbicides currently in development appear to be less irritant to the mucosa environment where their application is intended. Following the formulation and evaluation of polyanionic carbosilane dendrimers G3-S16 and G2-NF1, it was observed that in addition to blocking the entry of HIV into target host cell, these dendrimers protected the epithelial layer from cell disruption. Furthermore, these dendrimers did not induce any inflammatory cytokines or caused an irritation or vaginal lesion upon application (Córdoba et al., 2013).

A similar observation of biocompatibility and encouraging antiviral activity was recorded when water-soluble anionic carbosilane dendrimer (2G-S16) was studied (Chonco et al., 2012) confirming the consistent safety profile among these kind of dendrimers when employed as microbicides. The activity and safety profiles of dendrimer based formulations has so far been promising. The nano-range microbicide which is furthest in the development phases, dendrimer based Vivagel® (Starpharma, Melbourne, Australia) performed well on safety and efficacy at preliminary animal testing and progressing into human trials (Roy et al., 2015). Phase I clinical trials have been completed with some favourable general outcomes though further progression seems to have been halted because of excessive inflammation and damage to epithelial tissue (McGowan et al., 2011; Moscicki et al., 2012). It has been observed though that current nanotechnology being explored for microbicide formulation is shifting from utilising inherent antiviral activity of the nanostructures to using them as systems to deliver highly active antiretroviral drugs (das Neves, Amiji, Bahia and Sarmento, 2010). Therefore the prospects of utilising the flexible structure of dendrimers to deliver active drug for prevention of HIV transmission remains positive and worth considering.

As outcomes from clinical trials on conventional formulations of microbicides has largely been negative (Grant et al., 2008), shifting our focus onto nanostructures such as dendrimers in pursuit of an ideal microbicide seem to be a step in the right direction.

3.3.4 Liposomal microbicide

Liposomes, from a Greek root word meaning ‘fat body’ is a multi-layered phospholipid structure with a hollow core, the inner portion usually made of a polar phosphate group and the outer consisting of one or more bilayers of natural or synthetic lipids (Watwe and Bellare, 1995). Liposomes made of natural phospholipids are physiologically inert, weakly immunogenic and of low toxicity (Immordino, Dosio and Cattel, 2006). Furthermore, due to their combined hydrophilic and lipophilic nature, a wide range of drugs with different lipophilicities can be effectively encapsulated within liposomes, the highly hydrophilic ones staying in the polar compartment, the lipophilic ones in the lipid layers and those with intermediate partition coefficients easily apportioning between the polar and lipid portions of the

liposome (Gulati, Grover, Singh and Singh, 1998). The applicability of liposomes have been further enhanced recently due to a steady progression from conventional liposomes to a new generation of liposomes developed through modulation of lipid constituents, size and charge adjustments and surface modification (Torchilin, 2005). Structurally, the lipid microenvironment of some newer generations of liposomes, the so-called lipid raft achievable from peculiar lipid composition utilising glycosphingolipids (GSLs), sphingomyelins and cholesterols make them capable of serving as platforms of membrane with associated activities such as signal transduction, cell adhesion and lipid/protein organisation, thus increasing their appeal for biomedical applications (Anderson and Jacobson, 2002; de Gassart, Geminard, Fevrier, Raposo and Vidal, 2003; Helms and Zurzolo, 2004)

These physical characteristics of liposomes and their widening applicability due to their continuous improvement make them very attractive for consideration as delivery systems, especially in cases where drug solubility is an issue. With regards to microbicide activity against HIV transmission in the vagina, some potential challenges are anticipated. In order to inhibit activities of viruses that have broken through the physical barrier provided by microbes, active drug, ideally in nano delivery systems, capable of matching the ease with which viruses travel through epithelial layers to infect cells would be required to inhibit viral activity either at point of entry or within tissues (Pope and Haase, 2003; Vanić and Škalko-Basnet, 2013). Furthermore, the carrier system delivering the active drug ought to have minimal interference with vaginal flora and pH, minimal irritation to the mucosa as well as being effective in protecting the drug from sudden changes in the vaginal environment e.g. vaginal fluids due to arousal and release of semen (Vanić and Škalko-Basnet, 2013).

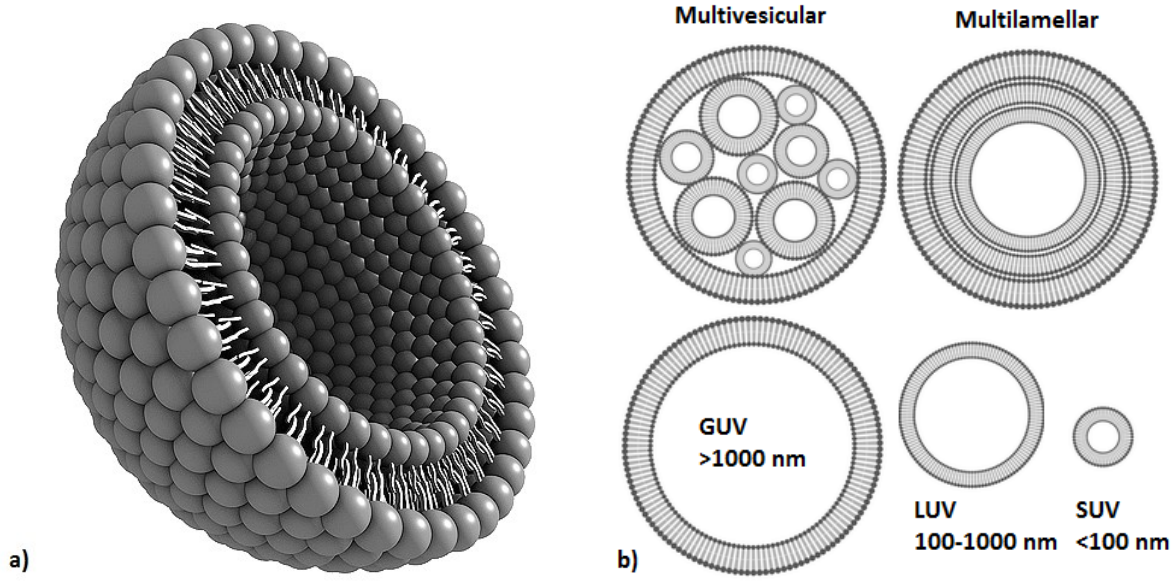


Figure 10: Schematic representation of a) Liposome showing assembly of phospholipids in a bilayer that yields both aqueous and lipid compartments within the structure and b) various forms of lamellar and sizes; small unilamellar vesicles (SUV), large unilamellar vesicles (LUV), giant unilamellar vesicles (GUV), multilamella and multivesicular (van Swaay, 2013)

Studies looking into the potential of liposomes as delivery systems for microbicides and capable of meeting these requirements have reported some optimistic outcomes indicating that these nanostructures could be the future of effective microbicides. The membrane-like characteristics of liposomes are also thought to have a potential of fusion with viron material, thus giving these structures some possibility of interfering with HIV transmission by competing with host membrane for uptake of the virus. Several liposomal membranes based on their lipid composition have been assessed for the potential of fusion with HIV-1 virus and found to be in the order cardiolipin (CL) > > phosphatidylinositol > CL/dioleoylphosphatidylcholine (DOPC) (3: 7), phosphatidic acid > phosphatidylserine (PS), PS/cholesterol (2:1) > PS/PC (1:1), PS/phosphatidylethanolamine (1:1) > DOPC, erythrocyte ghosts (Larsen et al., 1993; Malavia et al., 2011).

In a study evaluating a liposome formulation of MC-1220, a highly potent and selective non-nucleoside reverse transcriptase inhibitor (NNRTI) on the prevention of HIV transmission through the vagina of non-human primate models (Caron et al., 2010), it was found that formulating MC-1220 in a liposomal gel allowed high amount of drug loading in a small volume of formulation, thus solving some of the bioavailability issues typically seen in conventional gel formulations (Loftsson and Masson, 2001). In addition, the liposomal formulations of the NNRTI were seen to be less irritating to mucosa tissues. Finally and most importantly, it was observed that the liposomal formulation offered some protection against viral transmission and in fact reduced the viral load in infected models.

Another work that investigated the feasibility of liposomes for use as microbicides (Wang et al., 2012) utilized octylglycerol (OG), a synthetic lipid derived from human breastmilk and has been shown to destabilize viral envelopes and therefore a potential microbicide (Isaacs, 2001; Isaacs and Thormar, 1991; Skinner et al., 2010). In this study, liposomes were produced from combinations of OG and phosphatidyl choline in ratios that ensured in vitro antiviral activity and at the same time sparing the natural vaginal flora. Activities of the liposomes were compared to two conventional gel formulations.

3.3.5 RNA interference (RNAi) as strategy for microbicidal action

RNA interference (RNAi) is described as a post-translational and post-transcriptional inhibition of gene expression typically brought about by destruction of specific RNA molecules and this biological process has been demonstrated as capable of preventing HIV transcription, thus having some promising prospects in the prevention of viruses infecting host cells (Lee et al., 2002; Zhang et al., 2006). With regards to preventing HIV transmission through microbicidal action in the female reproductive mucosa, some pioneering work utilising small-interfering RNA (siRNA) densely packed into biodegradable polymer nanoparticles have been used to bring about silencing of endogenous genes in the genital track, ultimately resulting in protection against challenge from the infectious pathogens (Woodrow et al., 2009). In another study detailing the impact of siRNA on viral transmission, vaginal instillation of

siRNA targeting Herpes Simplex virus 2 (HSV2), an important cofactor in HIV transmission was confirmed to reduce overall lethal viral challenge in mice thus suggesting siRNAs as an important and a suitable component of microbicide formulation (Palliser et al., 2006). Outcomes from these studies and several others have established RNA interference firmly as a strategy for preventing viral infections and hence siRNAs as valuable components for the development of future microbicides. However, a massive challenge remains with delivery of siRNAs as these structures are highly unstable in serums and delivery across cell membranes. Delivery strategies showing promise so far, such as liposomal, viral or nanoparticle delivery are heavily reliant on nanotechnology and therefore adds to the compelling case being made for utilising nanotechnology for developing the next generation of microbicides (Nguyen, Menocal, Harborth and Fruehauf, 2008). The future of RNA interference as a strategy for disease cure and prevention is promising and this is attested by the vibrant pharmaceutical companies' involvement in research currently ongoing in this field, especially with regards to delivery of these nucleic acids

3.4 Current challenges with nanotechnology

Findings from critical examination of literature regarding microbicide development supports the need for a different approach to this venture if better outcomes than we have seen in the last few decades is desired. And this review has so far made a strong case for utilising tools presented by nanotechnology as various studies continue to demonstrate the possibility of deriving more effective microbicides when nanostructures are employed in their delivery. Notwithstanding the numerous benefits we stand to gain along this path, there are some critical challenges presently confronting the use of nanostructures for therapeutic interventions. The first and perhaps the most important of these concerns is the biosafety of nanoparticles. Indeed materials with widely varying degradation times and chemical compositions are already being either used or currently being employed as nanostructures for pharmacotherapy. But since these have only been recently used, knowledge about their long term effect on consumers are lacking (Jiang, Kim, Rutka and Chan, 2007). Furthermore, methods used in assessing toxicological hazards in conventional medicines may not necessarily be useful for nanostructures as this class of therapeutics may have entirely different surface and physico-chemical properties dictated by their extremely small size (De Jong and Borm, 2008). However, more recent work on generation of nanofibers has been directed at uncovering processes which can be scaled up in a manufacturing sense (Brako, Raimi-Abraham, Mahalingam, Craig and Edirisinghe, 2015)

The second of the most relevant challenges facing the development of nanotech based therapies is our current inability to produce commercial quantities of these fantastic nanostructures that have been demonstrated to positively impact the future of microbicide. Most of the nanotech based development strategies outlined in this review are still largely functional only on a laboratory scale and bridging the

gap in order to produce commercial quantities to consolidate gains so far made remain elusive (Beyer et al., 2015).

For the revolution in microbicide development by nanotechnology to be sustainable, these two key challenges – biosafety and upscaling of production challenges ought to be addressed in efforts to move any obstacles that may impede progress in the near future. Fortunately, a strong and rapidly emerging field of study and research, nanotoxicology promises to deliver fit-for-purpose tools and concepts in assessing the safety of therapeutic nanostructures and mitigating their immediate and long term impact among consumers (Shvedova, Pietroiusti and Kagan, 2016). With respect to producing commercial quantities of nanostructures for drug delivery, it is our hope and recommendation that as much efforts as dedicated to designing prototypes of these structures will be invested in finding various means of bridging the gap currently existing between laboratory and industrial level quantities to facilitate the movement of these innovative products into market.

4. Perspective

For over three decades, substantial challenges yet to be surmounted has kept humans from developing an ideal microbicide capable of preventing HIV transmission in the safest possible manner. For a microbicide to effectively deliver the protection expected, highly complex multi-level biochemical interaction among the host, virus and the drug would have to occur. Some details in these interaction remain unknown but recent efforts in establishing a framework for advancing knowledge required for developing microbicides has improved our understanding of the subject and has actually been translated into practical methodological approaches that are bringing us closer to achieving a suitable microbicide (Hendrix et al., 2009).

This review has looked into some of the obstacles encountered in developing a suitable microbicide, mainly from the formulation point of view. A strong case has been made for taking on new formulation approaches such us exploring the many options available in the area of nanotechnology. Nanotechnology has brought about extensive improvement in some therapeutic areas like cancer management and replicating some of these success stories in the area of HIV prevention is very likely considering preliminary outcomes from projects looking into formulating microbicides using nanotechnology concepts.

Aside formulation challenges, microbicide development appear to have been negatively affected by the lack of political enablement and incentives that otherwise would have made this an attractive venture for the big pharmaceutical establishments to get involved (Grant et al., 2008). It has been argued that no major pharmaceutical company as yet has been involved in efforts to develop microbicides and most probably due to the lack of liability protection including government supported insurance which is

typically made available for pharmaceutical organisations involved in the development of preventive medications such as vaccines (Grant et al., 2008). Sadly, this has left the development of microbicides to nongovernmental organisations, some governmental agencies and academic scientists who rarely have the necessary experience in drug development. Therefore moving into the future of microbicide development, new policies guaranteeing incentives and protection for investors in the developing of microbicides could attract the needed expertise into this area and ensure a timely development of an ideal microbicide.

Output from microbicide development, considering the amount of effort, resources and time invested in the venture to date, leaves much to be desired. A key reason for this poor output is failure in translating scientifically sound concepts into products useful in the real world. It must be appreciated that not every scientific concept will do well in real life as there are some present challenges practically unsurmountable (Klasse, Shattock and Moore, 2006). Therefore in order to improve the success rate of microbicide development, a robust model capable of subjecting concepts and drug candidates to rigorous preclinical assessment and offering an effective screening in order to accurately predict concepts capable of working in the real world ought to be developed. The normal preclinical protocols for pre-assessment of conventional formulations may not be enough for predicting the success of microbicide formulation considering the complex mix of requirements, including efficacy, safety, tolerability and acceptability needed for a microbicide to function properly. Such a robust model for predicting microbicide development success would be useful for managing the already inadequate time, resources and expertise needed to bring about an ideal microbicide.

By way of conclusion, there are diverse views on the best route to obtaining a microbicide which is safe and convenient to use, effective in protecting users from HIV infection and affordable enough to allow usage in order to bring about the wider public health gains they are intended for. But what all stakeholders agree is continuing efforts and even stepping up our commitment in developing an accessible, affordable and effective microbicide which could drive a preventive strategy to ensure a drastic reduction in new HIV infections.

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