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Nano-microbicides: Challenges in drug delivery, patient ethics and intellectual property in the war against HIV/AIDS☆

Lisa C. du Toit, Viness Pillay *, Yahya E. Choonara

University of the Witwatersrand, Department of Pharmacy and Pharmacology, 7 York Road, Parktown, 2193, Johannesburg, South Africa

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ABSTRACT

As we continue to be embroiled in the global battle against the human immunodeficiency virus (HIV), there has been an ongoing evolution in the understanding of the molecular mode of sexual transmission of HIV. This has gone hand-in-hand with a paradigm shift and research focus on the development of microbicides — compounds designed for vaginal (and possibly rectal) administration that are envisaged to put safe, affordable and accessible protection into the hands of women. However, an effective microbicide is not yet available; innovative approaches for the design of topical vaginal microbicides are urgently needed. The potential of the advancing field of nanomedicine has been earmarked in the increasing efforts to address the major health problems of the developing world. In this review, advances in the design of innovative microbicide nanocarriers and nano-enabled microbicides, henceforth referred to as 'nano-microbicides', are presented; elaborating on nanotechnology's role in the antiviral arena. The role of nanotechnology in the antiviral arena and the unique issues facing the generation of intellectual property relating to nano-microbicides in the ongoing global 'tug-of-war' of 'patients versus patents' are also explicated.

Contents

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* Corresponding author. Tel.: +27 11 717 2274; fax: +27 86 553 4733.
E-mail address: viness.pillay@wits.ac.za (V. Pillay).

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1. Introduction

'If they can put a man on the moon, why can't they make something we can use to protect ourselves from HIV/AIDS?'

Peer educator, Uganda, 1991 in Grant et al. [1]

In light of the fact that women bear the burden of individuals living with the human immunodeficiency virus (HIV) and those newly infected with HIV in several regions of the world according to the latest World Health Organization (WHO) update [2], advances in understanding the molecular mechanisms of HIV sexual transmission have witnessed a paradigm shift and research focus on the development of microbicides. Microbicides are compounds designed for vaginal (and possibly rectal) administration that are envisaged to put safe, affordable and accessible protection against HIV into the hands of women [3,4]. The lack of effective vaccines against pathogens that cause sexually transmitted diseases (STDs) has stimulated great interest in the development of topical microbicides as one means of curbing the epidemic of STDs [5–9]. Kenneth Mayer, a professor of Medicine and Community Health at Brown University (Providence, Rhode Island, USA), who has conducted extensive clinical research on microbicides, has reported to United Press International (a leading provider of critical information to researchers worldwide) 'It's like the tortoise and the hare whether we'll have an effective microbicide before we have a vaccine' [10].

The provision of a chemical rather than a physical barrier to HIV transmission, dictates that microbicides need not interfere with coitus, or with conception. Emerging microbicide formulations offer the promise of once-daily or even monthly application and can be complementary to existing prevention methods and to future vaccines that may control, but not prevent, HIV infection. Mathematical modelling studies estimate that a partially effective microbicide used in half of coital acts by 20% of women at risk could prevent 2.5 million HIV infections in 3 years [11]. The pragmatic design of an effective microbicide dictates an in-depth understanding of HIV and its intricacies.

An initial focus on the composition of the exterior of the HIV-1 virus demonstrates a lipid membrane interspersed with protruding glycoprotein knobs (Fig. 1). The knobs are formed by trimers comprising two subunits: the gp120 surface glycoprotein subunit, which is exposed to the exterior, and the gp41 transmembrane glycoprotein subunit, which spans the viral membrane and connects the exterior gp120 glycoprotein with the interior p17 matrix protein [12,13]. The functionality of the protruding gp120 glycoprotein knobs lies in their ability to bind to CD4 receptor sites on host cells. Numerous cellular proteins are also embedded within the viral envelope [14]. It is this highly exposed structure, i.e. the gp120 glycoprotein knob that serves as a point of exploitation for potential nanoparticle interactions. As extrapolated by Leonard and co-workers [15], the gp120 subunit has nine disulfide bonds, and three of these are located in the vicinity of the CD4 binding domain, serving as attractive sites for nanoparticle–viral interactions.

If we delve into the mechanism of HIV infection, emphasis has been placed on its incomplete elucidation; however, two critical steps are implicated:

1. binding of gp120 to the CD4 receptor site on the host cell, and
2. induction of a conformational change of gp120, resulting in exposure of new binding sites for a chemokine receptor [13].

The chemokine receptor, chemokine (C–C motif) receptor 5 (CCR5), proposedly acts as an essential cofactor for HIV entry and acquisition of infection. The lack of surface CCR5 expression in certain individuals due to mutation renders these individuals almost immune to HIV infection. Furthermore, viruses that utilize CCR5 prevail in early stages of mucosal transmission [16]. This provides an indication that mucosal transmission may selectively involve CCR5, and inevitably emanated in inhibition of CCR5 as a potential microbicide strategy for the prevention of HIV infection. Nevertheless, we must be cognisant of the fact that HIV is able to achieve or enable infection using other host cell factors available at mucosal sites. The overall importance of CCR5 for infection across mucosae is thus contentious, as well as the possibility that targeting CCR5 alone may be inadequate to prevent trans-vaginal HIV transmission [16].

Microbicides are designed to inhibit HIV infection by directly inactivating the virus or interrupting its attachment, entry, or replication [17]. Recent insights into how HIV infiltrates the genital mucosa, initial interactions with immune cells and the role of dendritic cells in transporting the virus to the lymph nodes have suggested numerous potential new targets for microbicides [18]. Data

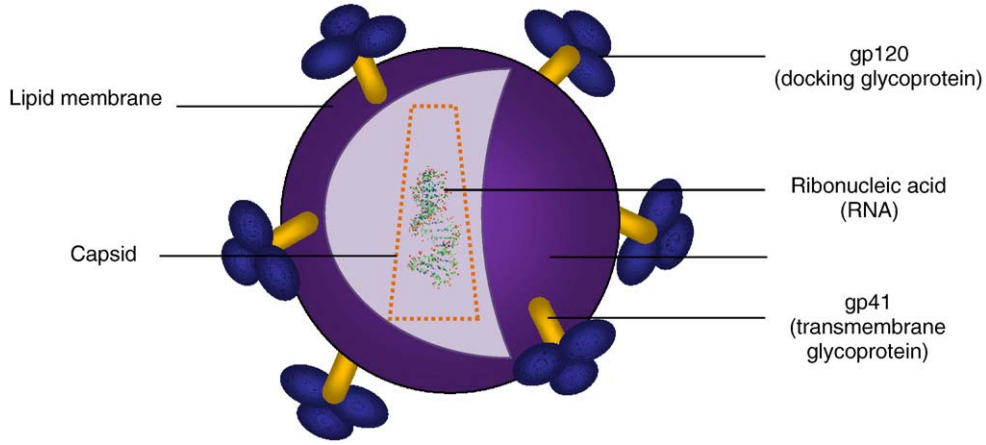

Fig. 1. Schematic of the human immunodeficiency virus.

from a study in the recent past had already referred to the safety and efficacy testing of more than 40 potential microbicidal compounds in laboratory assays *in vitro* and with *in vivo* with animal models [19]. Cognisance must be taken of these factors and innovative strategies pursued in the development of effective microbicides.

The subsequent testing of microbicide efficacy, however, presents an obstacle, as no surrogate marker or animal model is known to reliably predict the potential of the microbicide in humans. Thus large-scale clinical trials are conducted, which are a logistically challenging undertaking as thousands of non-infected, high-risk women are randomized to active or placebo microbicide groups and followed for several years to compare the rate of HIV infection in these two trial arms. Undeniably, this requires targeted efforts and significant financial investment. Several organizations have established clinical sites capable of conducting these trials according to international guidelines. At the time of Coplan and co-workers' [3] report, six candidates were currently in or about to enter Phase III studies (requiring approximately 31,000 volunteers), both the infrastructure and the ability to recruit and retain enough volunteers will be strained. Expanding the capacity for performing efficacy trials is therefore crucial for all prevention interventions [3]. Coplan and co-workers [3] emphasize the paradox that, just as support and scientific prospects for microbicide development are improving, the regulatory obstacles to testing and licensing them are growing, but possibly with due cause, that being the failed outcome of a number of microbicide clinical trials.

A number of debacles have arisen thus in the vaginal microbicide field, as conveyed by Grant et al. [1], the most recent being the outcome of the Carraguard® Phase III Efficacy Trial, conducted by the Population Council in South Africa from March 2004 to March 2007 at three different sites in South Africa. The compound in question, a sulfated polysaccharide (polyanion), failed to demonstrate efficacy against HIV-1 vaginal transmission. Concurrently, the U.K. Microbicide Development Program terminated the high-dose arm of the efficacy trial of another polyanion, PRO-2000, due to its poor potential for demonstrating efficacy [20]. Grant and co-workers [1] further emphasized the current state of mind surrounding this field saying that 'there were sighs of relief when it became clear that Carraguard® had not [actually] enhanced HIV-1 transmission rates' — this was the apparent outcome of the efficacy trial of yet another polyanion, Ushercell™ (a cellulose sulfate gel produced by Polydex Pharmaceuticals of Toronto, Canada), which resulted in the Phase III study of more than 1300 women being halted in Benin, South Africa, Uganda, and India two years ago [21]. The detergent nonoxynol-9 [22] was the first microbicide to enter the efficacy trial; enhanced HIV transmission was the result. This was purportedly also so for another detergent, SAVVY Vaginal Gel ($C_{31}G$) [23]. Unsurprisingly, the track record of microbicide products in large-scale trials is undoubtedly unfortunate [1].

The failure of polyanions is not unforeseen as these compounds have limited potency *in vitro*, particularly against the most commonly transmitted strains of HIV-1 that utilizes the chemokine receptor CCR5 for cell entry [24,25]. Moreover, evidence is now emerging that cellulose sulfate can enhance HIV-1 infection *in vitro*, particularly of CCR5-using viruses [26]. Since similar *in vitro* and *in vivo* observations of polyanion-mediated enhancement of such HIV viruses were made 15–20 years ago [27,28], the subsequent testing of polyanions in thousands of women raises concerns regarding the preclinical research that was performed on these microbicide candidates. Yet another detergent, sodium lauryl sulfate, is still being evaluated; the rationale for continuing this study is unclear. We thus need to be cognisant of past failures and focus on the next phase of microbicide development [1].

The Joint Center for Bioethics, University of Toronto, Canada (News Release, 31 March 2005) [29] gave direction to this 'next phase' and have emphasized the significant role that nanomedicine will play in addressing sickness and poverty in the global South (developing nations, primarily in the Southern Hemisphere). In a concerted effort to attain microbicidal efficacy, the promise held by drug delivery systems that can precisely control the release rates or target drugs to a specific site in the body has already witnessed positive effects on the healthcare system. With reference to the pharmaceutical industry, Mathiowitz et al. [30] have commented on the decidedly 'avant-garde' interface among the fields of polymer and material science during the last two decades. A surge in the design of intelligent and innovative delivery systems has been the result [30,31].

The Action Group on Erosion, Technology and Concentration (ETC Group), an international organization dedicated to 'the conservation and sustainable advancement of cultural and ecological diversity and human rights', expounded on this potential by highlighting two products currently under development as exemplars of nanomedicine's potential to address major health challenges in the developing world [32]. These included the promising dendrimer-based microbicide 'VivaGel™' developed by Starpharma (Melbourne, Australia). Nanoparticle preparation methods introduce a new-found flexibility, this, together with their ease of incorporation into a variety of media, incentivizes further research into this exciting avenue for tackling the Acquired Immunodeficiency Syndrome (AIDS) pandemic. However, the full potential of such systems cannot be realized without adequate consideration of the toxicity and long-term health effects that nano-engineering may incur on the patient, healthcare professional, and/or caregiver.

The scope of this review will thus delve into such nanocarriers for microbicides and nano-enabled microbicides, henceforth referred to as 'nano-microbicides', with elaboration on the role of nanotechnology in the antiviral arena. Nano-sized systems, which do not all fit neatly into the category of a 'microbicide', and whose clinical applicability is still in the early stages of discovery, but which demonstrate potential as nano-microbicides, are also described. This review also sought to tackle the unique issues facing the generation and testing of intellectual property relating to nano-microbicides, and their ultimate safety, in the ongoing global 'tug-of-war' of 'patients versus patents'.

2. The role of nanotechnology in the design of antiviral agents

2.1. Defining nanotechnology

The era of nanotechnology is upon us and this technological revolution is rapidly unfolding. Nanotechnology, an umbrella term that describes a rapidly evolving interdisciplinary field of technology based on manipulation of matter at a sub-micron scale, embraces objects, mechanisms, assemblies, and various drug delivery systems based on size scales between 1 nanometer (nm) and 100 nm [32,33].

It is becoming increasingly evident that advancement to a new covalent complexity level yields novel materials with behavior that cannot be understood by simple extrapolation of the properties of their building blocks. These advancements result in the fabrication of entirely new structures or architectures with properties that have new fundamentals and require unprecedented rationalization, conceptualization and generality [34]. The essence of this is that the 'new complexity' is not only different, but always more than the linear summation of its components' [35].

In tandem with the expansion of the general nanotechnological arena, recent years have witnessed an unprecedented growth in progressive research in the area of nanopharmaceuticals, which has been instituted with a significant promise to provide pertinent advances in the diagnosis and treatment of disease [36]. Nanopharmaceuticals encompass biologically active drug products and drug delivery systems with nanoscale assemblies (Fig. 2), which may be simple systems (e.g. nano-emulsions, nanoparticles, or polymer conjugates of proteins or drugs), or complex multi-component systems

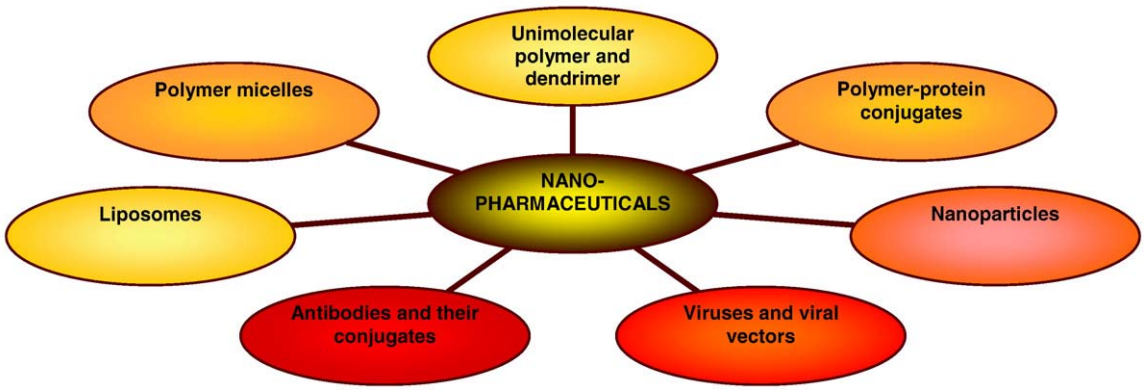

Fig. 2. The array of nanosystems encompassed by nanopharmaceuticals (adapted from: the European Medical Research Council [36]).

(containing drugs, proteins or genes, targeting ligands and signal systems for *in vitro* or *in vivo* detection). This surge in advancement and the need to acquire 'nano-knowledge' in drug delivery is driven by the challenge to implement nanotechnology to design multifunctional, structured materials that are able to target specific diseases or containing functionalities to allow transport across biological barriers. Furthermore, nanostructured scaffolds are urgently required, as exemplified in this review, to physically target treatments for local administration of therapeutics, such as within the vaginal canal. There is also a need to develop pharmaceutical formulations that can be conveniently administered to patients and that display acceptable shelf-life and overall stability [36].

In accordance with the nano-knowledge surge, three principle drug delivery goals can be derived that spurred the development of nanoscale drug delivery systems, which include: 1) more specific drug delivery and targeting; 2) greater safety and biocompatibility; and 3) faster development of new, safe medicines [36]. To achieve the aforementioned goals, diverse nanotechnological approaches (e.g. supramolecular chemistry, nanoprecipitation and nanocrystal formation, antibody technologies, *in situ* polymerization, emulsification and liposome technologies) have been applied to drug delivery and pharmaceutical research [36]. Nanotechnology thus offers a range of possibilities for healthcare and medical breakthroughs, with specific reference to the scope of this article, including targeted microbicide delivery systems, extended release vaccines, enhanced diagnostics and imaging technologies, and antimicrobial coatings [37].

Bowman and co-workers [38] have emphasized that the efficacy of nanoscale systems lies in their comparable size to proteins, and the presentation of multiple protein-binding ligands that may be effective at disrupting protein–protein interactions that drive disease pathogenesis. Mammen et al. [39] and Caruthers et al. [40] reported on the successful targeting of pathogenic biomolecule targets by multivalent linear polymers, dendrimers, proteins, and liposomes. In particular, Mammen et al. [39] elaborated on the binding capabilities of nanoscale delivery systems, namely sialic acid (SA)-coated liposomes and dendrimers, to hemagglutinin (HA) on the surface of an influenza virus with significantly enhanced affinity. The interaction of nanoscale systems and viruses can thus be seen as pivotal and is the crux spurring the design of nano-microbicides.

2.2. Nanoparticle–HIV interaction

The significant potential nanoparticles hold as agents for the prevention of HIV transmission is exemplified via observation of the nanoparticle–HIV interaction. Imaging yields important information in this regard. Imaging of the interaction of single HIV-like particles with the cell surface has been achieved employing scanning surface confocal microscopy, simultaneous recording of high-resolution topography and cell surface fluorescence to facilitate imaging of fluorescent particles in the nanometer range on fixed or live cells. It has been observed that single particles submerge into the cell membrane in pinocytic-vesicular-like invaginations [41,42].

An example of note has been the observed size-dependent interaction of silver nanoparticles with HIV-1 whereby viral attachment occurred in the range of 1–10 nm [13] and is applicable to other noble-metal nanoparticulate systems (e.g. gold nanoparticles) [43]. Preferential binding to the gp120 glycoprotein knobs of HIV is attributed to the attractive binding sites availed to the nanoparticles, as witnessed by their regular spatial arrangement, regular distance between nanoparticles, and the exposed sulfur-bearing residues of the glycoprotein knobs. There is thus clear *in vitro* demonstration of nanoparticle inhibition of HIV binding to host cells [44].

3. Nanoparticulate systems possessing antiviral activity

The concept of nano-microbicides is a progressively emerging one. The systems described henceforth do not all fit neatly into the category of a 'microbicide', and the clinical applicability of some systems are still in the early stages of discovery, but their potential to be implemented as such cannot be ignored.

3.1. Dendrimer-based microbicides

The versatility of dendrimers as a class of regularly branched macromolecules with unique structural and topological features confers them as valuable tools for exploitation by both scientists and technologists [34,45]. The term dendrimer was first proposed by Tomalia in 1985 and originates from the Greek word '*dendron*,' meaning tree; the structural shape yielding its unique attributes [46]. Dendrimers are dissimilar from conventional polymers in that they have a multi-branched, three-dimensional architecture with minimal polydispersity and high functionality. Dendrimers are archetypically comprised of three different topological components of chemical significance: 1) a poly-functional core, 2) interior layers, and 3) a multivalent surface. The poly-functional focal core can encapsulate various chemical species and exhibits unparalleled properties due to the special nano-environment surrounded by extensive dendritic branching. As an example, the core may be synthesized from ammonia or ethylenediamine. The building blocks with several interior layers are composed of repeating units (e.g., polyamidoamine [PAMAM], polyamino acids, polyphenyls, polyporphyrins, and polyethers), which creates a flexible space within the voids for the possible encapsulation of various small guest molecules. The multiple peripheral functional groups (multivalent surface) can accommodate a large number of functionalities that can interact with the external environment. Thus by altering the nature of the core and repeating units, the number of layers, and the composition of the surface layer, it is possible to synthesize a single polymeric dendrimer of defined

three-dimensional structure and size with predictable physicochemical properties, thereby defining the macroscopic properties of the dendrimer [9,47].

The biological applications of dendrimers are primarily as complexing carrier molecules. They have been shown to possess an inherent biological activity, which could be seen to have a significant contribution in the antiviral field [9,48,49]. The antiviral activity of dendrimers is attributed to the synthetic modification of dendrimer molecules such that they include functional groups in the surface layer, which are capable of forming complexes with cell or viral receptors, pivotally resulting in the disruption of normal virus–cell interactions, including the initial virus–cell binding. The primary antiviral mechanism of dendrimers thus occurs early in the infection process through blockade of the virus attachment to the cell, or interference with adsorption. However, certain compounds may possess secondary mechanisms of action [9].

In vitro activity of dendrimers has been demonstrated against the influenza virus, respiratory syncytial virus (RSV), measles virus, and HIV [50,51]. Thus the propensity of dendrimers to disrupt virus–cell binding implicates their usefulness as topical microbicides [9]. The stark resemblance between the three-dimensional architecture of dendrimers and natural bio-macromolecules, particularly proteins, has instigated 'bio-inspired' applications. One such dendrimer-based application that is significant in the field of topical microbicides is VivaGel™ (Starpharma, Melbourne, Australia), a topical microbicide, which has been granted 'Fast Track' status as an Investigational New Drug (IND) for the prevention of HIV transmission and other STDs [48].

McCarthy and co-workers [48] emphasized that subtle changes in the dendrimer design parameters, such as the type of initiator, branching unit type, dendrimer generation, linker, and surfaces, ultimately emanated in the possibility of creating diverse arrays of dendrimers with various patterns of biological activity. This is epitomized by their dendrimer-based-antiviral program that also looked at the antiviral properties of dendrimer-based polyanions [52]. Polyanions such as the synthetic sulfated carbohydrates (e.g., polystyrene sulfonate) and traditional polymer-based polyanions demonstrate inhibition of HIV and other enveloped viruses; however the naturally-occurring sulfated carbohydrates have a more complex synthetic program and limited diversity [53]. Polymer-based polyanions have a similar complexity, making alignment of the precise structural components and the observed biological activity complicated. McCarthy et al. [48] proposed the usefulness of preparation of dendrimer-based polyanions, which being 'single' molecular species, would allow the timely determination of structure–activity relationships, and specific dendrimers could be propelled into development based on the unique biological properties of well-defined molecular entities. McCarthy and co-workers [48] thus accentuate the potential held by this class of dendrimers for diversification into a wider range of polyanion structures, having various biological activities and mechanisms of cellular uptake, following in the footsteps of small-molecule medicinal chemists' drug discovery programs. This point is illustrated for the contrasting biological activity of dendrimers synthesized as their program progressed (Table 1).

Ultimately following intensive investigations, SPL7013 proved to be the easiest of the investigated dendrimers to prepare on a large scale as a single molecular species and was selected to enter formal preclinical development. It is engineered from a divalent core, a benzhydrylamine amide of L-lysine. Successive additions of four L-lysine layers lead to a dendrimer with 32 amine groups on the surface, 16α-amines and 16ε-amines from the outer L-lysine layer. The final step in the synthesis involves the last amide-bond-forming reaction to attach 32 sodium 1-(carboxymethoxy)naphthalene-3,6-disulfonate via amide linkers. The clinical drug product of SPL7013 is now called VivaGel™, a relatively simple water-based polyacrylic acid gel buffered to a pH that is physiologically compatible. Efficacy studies have since been conducted in non-human primates, where a single intravaginal dose of the clinical formulation containing 5%w/w SPL7013 protected all pig-tailed macaques from a single intravaginal infection by a strain of Simian-Human Immunodeficiency Virus (SHIV). Thereafter, an investigational new drug application (IND) was submitted to the United States Food and Drug Administration (FDA) in June 2003, being the first submission of a dendrimer-based drug to the FDA — a milestone in the quest for the design of nanotechnology-based solutions for human health conditions. VivaGel™ showed a comparable safety profile to a placebo gel in a Phase I clinical trial, and showed no absorption into the systemic circulation following intravaginal dosing. This was indicative of the desired retention in the vaginal lumen following dosing, thus allowing immediate inhibition of HIV infection [48]. The binding interaction of the dendrimer to HIV, instigated in the prevention of infection, is represented in Fig. 3.

3.2. Fullerenes as inhibitors of HIV infection

Following on from dendrimers in terms of intensity of investigation as potential nano-microbicides, are fullerenes. Also known as 'buckyballs', fullerenes originated from carbon-60 (C_{60}); a molecule of C_{60} forms a hollow sphere 1 nm in diameter. Functionalization introduces solubility to the molecule, and in the presence of hydration and various other conditions, spontaneous formation of stable aggregates within nano-dimensions (25–500 nm) occurs, known as nano-C_{60}. These structures are toxic to microorganisms, interacting with DNA, proteins and living cells [44]. The activity of nano-C_{60} depends on the properties of the fullerene core (i.e. the core is essential for anti-HIV activity), while the substituents attached at the surface of the fullerene core control and modify the biological activities of their derivatives [55].

Recently, this nano-C_{60} structure has been investigated for use as potential microbicides [56]. Marcorin and co-workers [57] synthesized

Table 1
Characteristics and anti-HIV activity of dendrimers (data extracted from: McCarthy et al. [48]).

Antiviral dendrimer compound	Branching unit	Core	Dendrimer surface	Purported mode/s of action
SPL2923	PAMAM	An ammonia	Naphthalenedisulfonic acid groups are attached by a thiourea linker	1. Inhibition of HIV attachment/fusion 2. Reverse transcriptase inhibition 3. Integrase inhibition
SPL6195	PAMAM	Ethylenediamine	Benzenedicarboxylic acid groups attached by thiourea linker	1. Inhibition of HIV attachment/fusion only
SPL7013	L-lysine	Benzhydryl amine amide of L-lysine	Sodium 1-(carboxymethoxy)naphthalene-3,6-disulfonate attached by amide linker	1. Inhibition of HIV attachment/fusion 2. Reverse transcriptase inhibition 3. Integrase inhibition
SPL7304	PAMAM	Benzhydryl amine amide of L-lysine	Sodium 1-(carboxymethoxy)naphthalene-3,6-disulfonate attached by amide linker	1. Inhibition of HIV attachment/fusion 2. Reverse transcriptase inhibition 3. Integrase inhibition
SPL7320	PPI	Benzhydryl amine amide of L-lysine	Sodium 1-(carboxymethoxy)naphthalene-3,6-disulfonate attached by amide linker	1. Inhibition of HIV attachment/fusion 2. Reverse transcriptase inhibition 3. Integrase inhibition

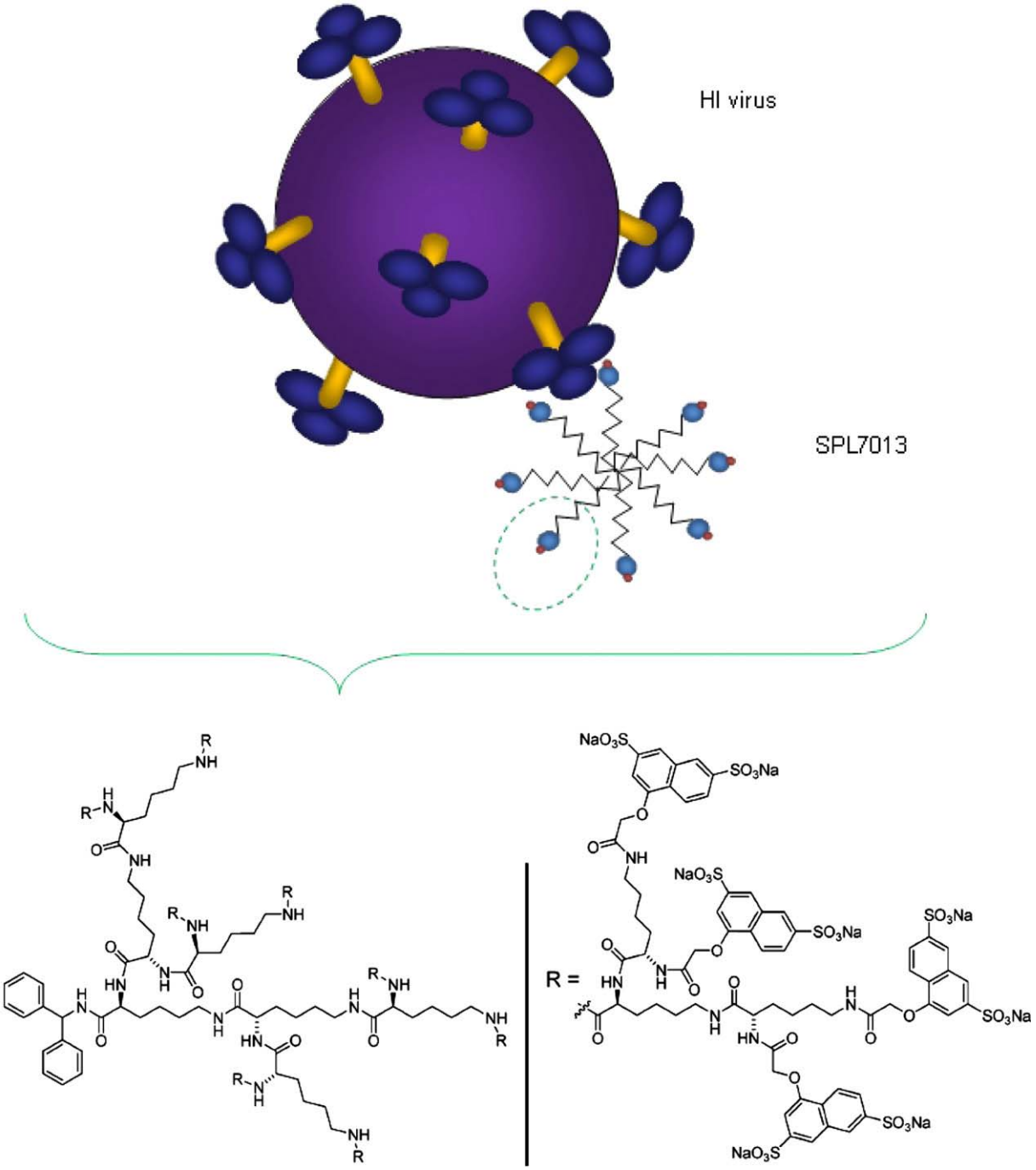

Fig. 3. A schematic depicting SPL7013, the active dendrimer in Starpharma's topical microbicide, VivaGel®, binding to surface proteins on HIV and subsequently preventing the virus from infecting human T-cells. The chemical structure of SPL7013 is also shown (adapted from: www.starpharma.com/info-centre.asp [54] and McCarthy et al. [48]).

fullerene derivatives and exploited their potential as inhibitors of HIV aspartic protease enzyme for the elucidation of a novel anti-HIV system. They defined the active region of HIV-protease as a cylindrical hydrophobic cavity (diameter ~10 Å), containing two amino acid residues, aspartate 25 and aspartate 125. Binding at these sites caused suppression of protein slicing and inhibited viral replication. The side chains (containing NH_2 or NH_3^+ groups) of the water-soluble fullerene derivatives which were synthesized underwent electrostatic and/or hydrogen bond interactions with Asp 25 and Asp 125 (Fig. 4b) [57,58].

Marchesan et al. [59] developed a series of bis-fulleropyrrolidines containing two ammonia groups. Their activity was evaluated against HIV-1 and HIV-2. They elucidated that a trans-2 arrangement of the two substituents and positive charges in proximity to the carbon cage was a prerequisite for antiviral activity; whereas bulky polar chains on a C_{60} sphere were shown to induce cytotoxicity and reduce potency, suggesting a significant steric control. These findings were considered, and patterns of substitutions that were furnished improved the action against HIV-1 and HIV-2 strains, leading to a superior selectivity of antiviral inhibition. Interestingly, the compounds only displayed inhibitory activity against HIV, with no effect on other DNA or RNA viruses. The authors could not attribute the activity against the HIV infection to a specific mechanism (i.e. HIV-protease and/or HIV-reverse transcriptase mediated).

Just prior to these findings, Mashino and co-workers [55] reported on the HIV-reverse transcriptase (HIV-RT) inhibition by fullerene derivatives. All examined fullerene derivatives were more effective

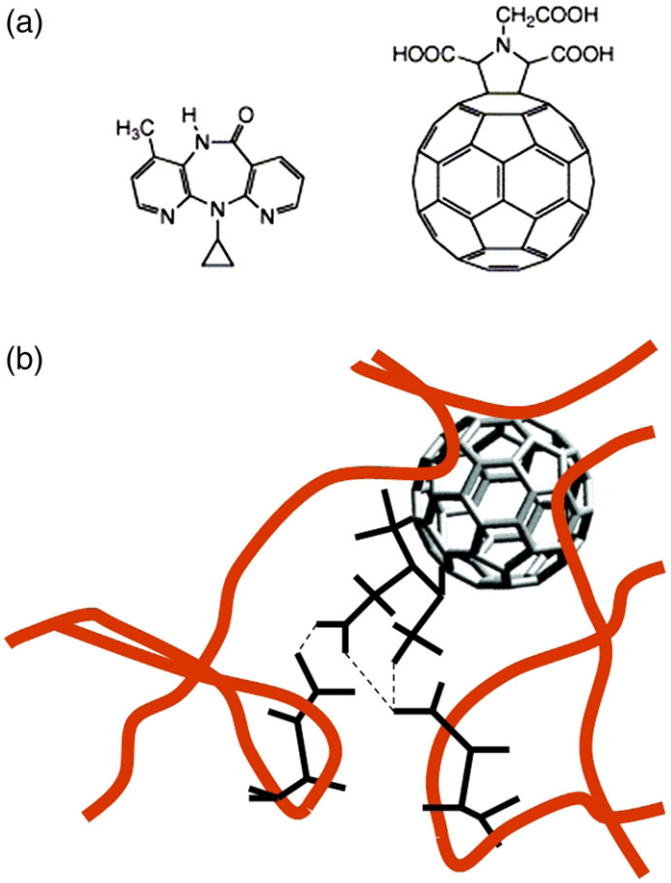

Fig. 4. (a) Comparative structures of nevirapine and the active amino acid fullerene derivative (source: Mashino et al. [55]) and (b) schematic representation of the hydrogen bond between NH_2 or NH^{3+} groups with Asp 25 and 125 (adapted from: Marcorin et al. [57]).

than the non-nucleoside analogue of the HIV-RT inhibitor, nevirapine, which is employed for HIV infection. The amino acid-type nano-C_{60} derivative (Fig. 4a) demonstrated the strongest inhibition of HIV-RT, and was considered as a potentially significant lead compound as an anti-HIV agent. Two carboxylic groups at the pyrrolidine ring were significant for inhibition in the case of the amino acid derivatives. Among the examined fullerene derivatives, cationic derivatives were more effective than others. In the case of HIV-RT inhibition, the long alkyl group had a small effect.

Two main mechanisms of action are possible for anti-HIV agents, namely inhibition of HIV-protease or inhibition of HIV-RT. With respect to the fullerenes produced, molecular modelling studies revealed that the C_{60}-core could bind within the large and highly hydrophobic substrate-binding site of HIV-protease, which was the case for a portion of the fullerene derivatives which inhibited HIV-protease [60]. Zhu and collaborators [61] undertook the elucidation of the suggested mode of action of fullerene derivatives as anti-HIV compounds through investigation of the spatial hydrophobic relationship between C_{60} and the cavity regions of HIV-protease (a homodimeric enzyme that belongs to the class of aspartic proteases) via simulations of molecular dynamics, free energy techniques and simulations of the effect of C_{60} on water content of the cavity. As an inhibitor, the C_{60} is held firmly in place due to the hydrophobic interactions between C_{60} and cavity regions, which causes the release of water, providing indirect evidence for inhibitor presence. The free energy profiles prove a connection of opening and closing of flaps in the cavity to the potential inhibitor binding [58]. Bosi and co-workers [62] reported the anti-HIV activity of fullerene derivatives, but only speculated, with lack of experimental evidence, that the mechanism of anti-HIV activity was HIV-protease inhibition. As described, Mashino et al. [55] also found inhibition of HIV-RT. The results shed further light on another possible mechanism of the anti-HIV activity of fullerene derivatives.

In general results appear promising in terms of their large surface area. However, as carbon derivatives, the toxicological definition for fullerene is still quite controversial. Recently, fullerenes have been suggested to be carcinogenic [63,64] and genotoxic, but only upon photosensitization [65].

3.3. NanoViricides™ in anti-HIV therapy

In a similar vein to Starpharma (Melbourne, Australia), it was the goal of NanoViricides, Inc. (West Haven, Connecticut, USA) [66] to merge the advantages of nanomedicine with the antiviral therapy platform. The concept of a 'NanoViricide™' was originated by NanoViricides, Inc., as a flexible nanoscale microbicide specifically targeted to a particular type of virus. Targets for this approach thus far have included H5N1 bird flu, seasonal influenza, epidemic kerato-conjunctivitis (EKC), hepatitis C, rabies, dengue fever, Ebola virus, and HIV among others. Specifically they are polymeric nano-micelles — a single polymeric chemical chain with covalently attached ligands that specify the virus target. The antiviral spectrum of the drug is determined by the specificity of the set of ligands attached to the chain, in addition to other functionally important aspects inherent in the physicochemical properties [44,65]. Active pharmaceutical ingredients are optional and can be hidden in the core of the NanoViricide™ 'missile', and purportedly reduce toxicity [44]. NanoViricides™ are designed with the goal of reducing viremia through an intricate interplay of four mechanisms that are outlined henceforth and schematically depicted in Fig. 5 [44,66]:

1. NanoViricide™ is designed to seek a specific virus type. In contrast to other approaches, a NanoViricide™ micelle is considered to recognize and bind to more than one type of binding site on the virus. Currently NanoViricide™ system enables design of a drug that binds to as many as three different sites, which is purported to enhance the mechanism of attack, and attack and is termed 'multi-specific targeting'.
2. Attachment to the virus particle.
3. Engulfment or coating of the virus particle, thereby neutralizing the virus's infectivity.
4. Destabilization and possibly dismantlement the virus particle. NanoViricides™ act as 'molecular chisels', and may also be made capable of attacking the viral genome thereby destroying the virus completely [44].

NanoViricides, Inc. recently reported that its anti-HIV drug candidates demonstrated significant therapeutic efficacy in the recently completed preliminary animal studies. The studies were performed at a Bio-Safety Level 3 Laboratory (BSL-3) facility in Boston, MA, USA. Eugene Seymour, CEO of NanoViricides, Inc., explained that the purpose of this initial study was twofold: 'To assess therapeutic efficacy and to determine the appropriate dosages of drug that should be used in the later animal study. [If] initial performance goals are met, it will be a critical first step in validating the Company's HivCide™-I as a potential treatment for HIV/AIDS' [67].

This approach is assuredly promising in the challenge towards the development of an efficacious nano-microbicide. However, a comprehensive explanation if the observed efficacy is still unclear and is attributed to the complex mechanism of action of nanomaterials when used as drugs.

3.4. Cyclodextrins as nanocarriers for anti-HIV agents

Nanocarriers have been identified as an avenue for improving the efficacy of novel anti-HIV agents. The highly potent anti-HIV agent UC781 is currently under evaluation as a topical microbicide to prevent

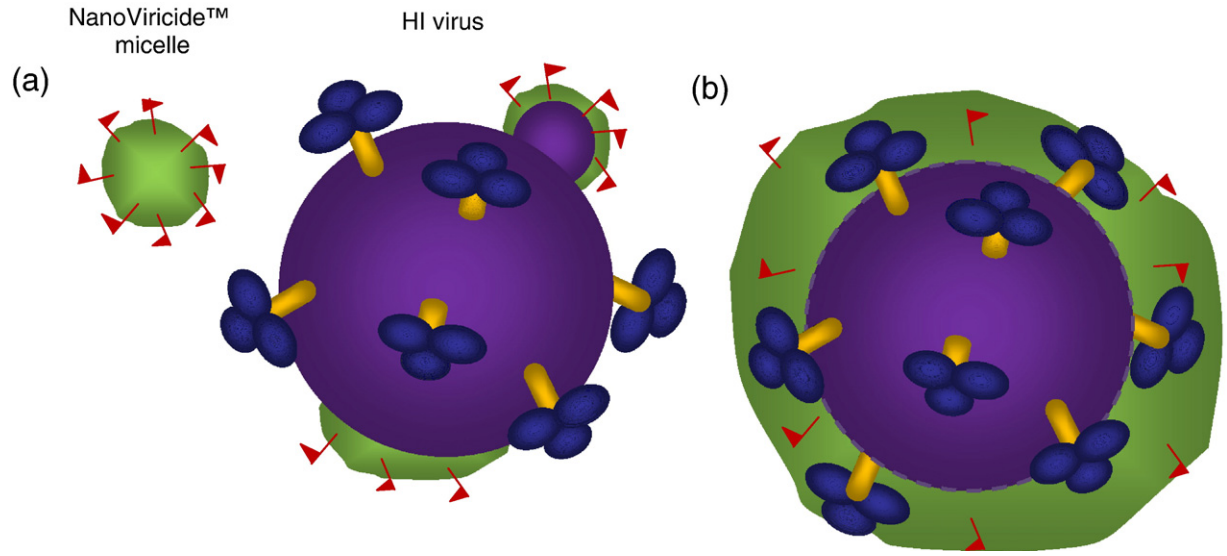

Fig. 5. Schematic depicting the mechanism of action of NanoViricides™: (a) micelle attachment to the virus at multiple points and commencement of viral engulfment (b) the flexible micelles engulf, coat, neutralize and dismantle the viral lipid coat (adapted from: http://www.nanoviricides.com/index.html [65]).

HIV transmission [68]. However, UC781 is extremely hydrophobic with poor water solubility, a property that may complicate appropriate formulation of the drug. Yang and co-workers [68] have examined the ability of several classes of cyclodextrins, β-cyclodextrin (βCD), methyl-β-cyclodextrin (MβCD), and 2-hydroxylpropyl-β-cyclodextrin (HPβCD), to enhance the aqueous solubility of UC781. The inhibitory potency of UC781 and its HPβCD inclusion complex were evaluated using an *in vitro* HIV-1 reverse transcriptase inhibition assay and the inhibitory potency of the complex was found to be 30-fold greater than that of UC781 alone. Complexation of the drug within the nanocarrier significantly enhanced the aqueous solubility and thus the inhibitory potential of the drug, which is essential for the development of a useful vaginal microbicide drug delivery system [68].

3.5. Inhibition of HIV fusion with gold nanoparticles

The anti-HIV potential of noble-metal nanoparticles has been described [13,43]. Bowman and co-workers [38] have documented the first application of small-molecule coated gold nanoparticles as effective inhibitors of HIV fusion. Their concept arose from the premise of Mammen and co-workers [39] that 'biological systems exploit multivalency in the synthesis of high-affinity ligands because they allow an organism to take advantage of an existing set of monovalent ligands without the need for evolving completely new molecules for every required function'.

The concept of 'multivalent therapeutics' is well conceived by the described system. The gold nanoparticles employed as a platform (2.0 nm diameter, mercaptobenzoic acid modified gold particles) transformed a weakly binding and biologically inactive small molecule into a multivalent conjugate that effectively inhibited HIV-1 fusion to human T-cells. Of significance is the similarity of this class of gold particles to proteins and dendrimers in terms of their atomical precision and mono-disperse nano-size [38]. The mercaptobenzoic acid-coated gold nanoparticles were conjugated to SDC-1721, a derivative of TAK-779, which is a known CCR5 antagonist (Fig. 6). Structure–activity relationship data generated for TAK-779 has shown that the quaternary ammonium salt was essential for high-affinity binding and effective inhibition of HIV fusion [69]. Since the quaternary ammonium salt imbues TAK-779 with poor pharmacological properties (e.g. significant irritation at the injection site) searches were conducted for alternate small-molecule CCR5 antagonists [69] culminating in the synthesis of SDC-1721 (a fragment of TAK-779 that lacks the quaternary ammonium salt moiety) by Bowman and co-workers [38]. Free SDC-1721 had no inhibitory effect on HIV infection; however, the (SDC-1721)-gold nanoparticle conjugate displayed activity comparable to that of TAK-779. This molecule was thus instituted to demonstrate the feasibility of conjugating a low-affinity, biologically inactive small molecule to a gold nanoparticle for the design of biologically active multivalent gold nanoparticle therapeutics. The mechanism of inhibition of viral replication was specific for viral entry [38].

A significant contribution was thus made by this study in demonstrating the first application of small-molecule coated gold nanoparticles as effective inhibitors of HIV fusion. The demonstration that therapeutically inactive monovalent small organic molecules may be converted into highly active drugs by simply conjugating them to gold nanoparticles is considered to add impetus to the discovery of effective new drug formulations.

Mintek (Pty) Ltd., (Randburg, South Africa), South Africa's national mineral research organization, aims to add value to mineral resources through technology, industrial growth and human development in a sustainable manner. They initiated the AuTEK biomedical program, which originally focused on anti-tumor drugs, but which now has broadened to include anti-HIV agents, in collaboration with seven local and six European universities [70]. A facet of the driving force for the AuTEK biomedical program rests in the development of drug delivery systems for anti-HIV agents. The early 1990s saw the evaluation of some gold drugs for activity against HIV. Results generated indicated that there might be some inhibition of HIV exhibited by gold compounds, such as sodium aurothiomalate and aurothioglucose [71]. Due to the biomedical group having a variety of gold-based drugs within their consortium, a range of these compounds has been submitted for HIV screening [71,72].

3.6. Silver nanoparticles employed as microbicide delivery systems

Silver nanoparticles exploit the microbicide properties of silver with various materials to produce effective microbicide delivery systems for the prevention of HIV transmission [73,74]. Elechiguerra and co-workers [13] focused on the interaction of silver nanoparticles with HIV-1 (Fig. 7). The concept of surface chemistry predictability of interactions with external systems has been challenged in their investigation via the development and testing of silver nanoparticles with three different surface chemistries, namely, foamy carbon, poly (N-vinyl-2-pyrrolidone) (PVP), and bovine serum albumin (BSA). Contrary to expectations, they established congruency amongst all the formulations in that only

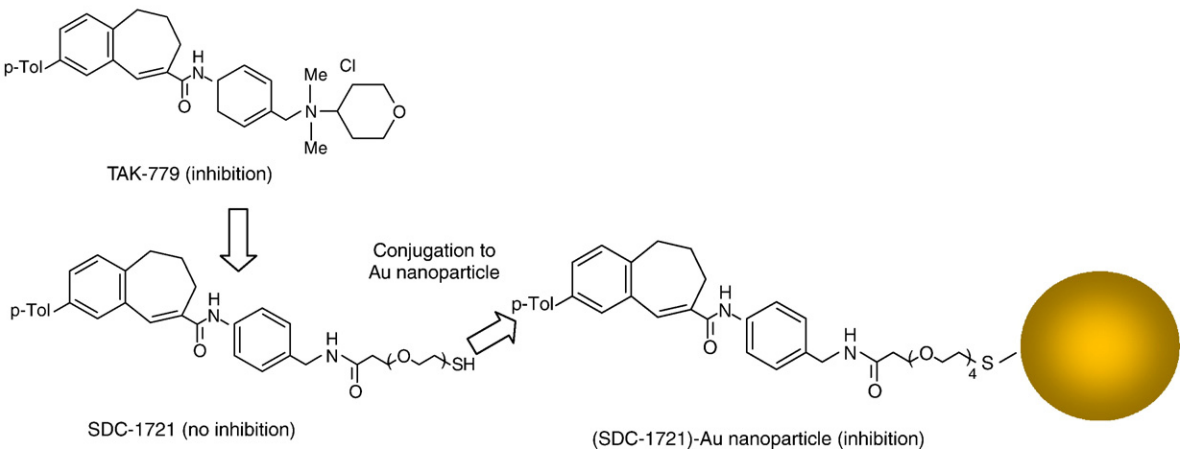

Fig. 6. Design of small-molecule (SDC-1721)-gold nanoparticles (adapted from: Bowman et al. [38]).

nanoparticles below ~10 nm attached to the viral envelope and this occurred independently of the formulations' surface chemistry. Additionally, a regular spatial arrangement with equivalent center-to-center distances between the nanoparticles bound to the virus–cell was found. They ameliorated their findings with regard to both the spatial arrangement of nanoparticles and the size dependence of interaction in terms of the HIV-1 viral envelope. These investigations ultimately provided a deeper understanding into the mode of interaction between the virus and nanoparticles. Silver nanoparticles proposedly undergo specific interaction with HIV-1 via preferential binding with the gp120 subunit of the viral envelope glycoprotein, through interaction of the silver nanoparticle with the exposed disulfide bonds of the gp120. This indicated the aforementioned probability that other noble-metal nanoparticles may also exhibit similar activity [13,43].

However, the toxicity and inhibition results differed, despite the congruency among the surface modified nanoparticles with reference to their interaction with HIV-1 [13]. The differential behavior was attributed to the capping agents employed for each nanoparticle preparation. BSA- and PVP-protected nanoparticles displayed slightly lower inhibition because the nanoparticle surface was directly bound to and encapsulated by the capping agent. Contrarily, the carbon-coated nanoparticles exhibited a greater inhibitory effect due to their essentially free surface area. The free surface nanoparticles demonstrate higher cytotoxicity because of their surface chemistry, and the carbon-coated form shows comparatively free surfaces, which is able to interact strongly with the host cells, thus increasing their toxicity [13].

3.7. Polystyrene nanospheres

The ability of nanoparticles to function as nanocarriers for novel microbicides having functionality via various routes of administration is highlighted in investigations undertaken on polystyrene nanospheres. The role of mucosal secretory IgA may be significant in the prevention of HIV-1 transmission during sexual intercourse. Substances that induce HIV-1-specific IgA antibodies have shown promise for use as prophylactic vaccines against HIV-1 infection [75]. In an attempt to develop an effective tool for the prevention of HIV-1 transmission Hayakawa and co-workers [76] designed lectin-immobilized polystyrene nanospheres (~400 nm in diameter) and investigated their HIV-1 capture activity. Concanavalin A (Con A) was immobilized on the surface of polystyrene nanospheres engaging poly(methacrylic acid) branches. Nanospheres incubated with HIV-1 suspension achieved a >3.3 log and a 2.2 log reduction of viral infectivity in HIV-1 (IIIB strain) suspension at a concentration of 2 and 0.5 mg/mL, respectively. The combination of Con A-nanosphere treatment followed by filtration with a microporous membrane was shown to remove virion-free gp120 as well as infectious viral particles from HIV-1 suspension. The mechanism of action of the nanospheres was microscopically demonstrated whereby HIV-1 virions were trapped on the surface of Con A-nanospheres with high-affinity. Con A-nanospheres were thus proposed as a potentially effective tool for the prevention of HIV-1 transmission as intravaginal immunization was shown to induce vaginal anti-HIV-1 IgA antibody in mice [76]. In a

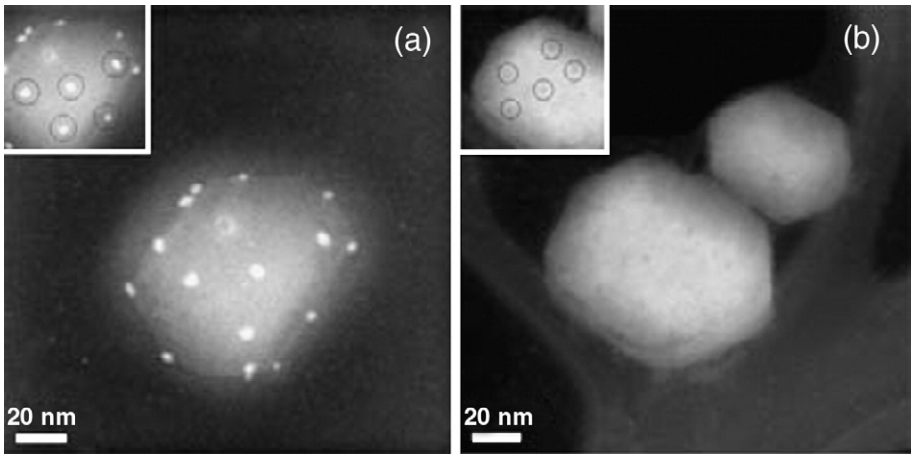

Fig. 7. High angular annular darkfield (HAADF) images of the HIV-1 cells (a) exposed to BSA conjugated silver nanoparticles (inset depicts the regular spatial arrangement between groups of three nanoparticles) and (b) without silver nanoparticle treatment (inset shows the regular spatial arrangement observed on the surface of the untreated HIV-1 virus). (Source: Elechiguerra et al. [13]).

follow-up investigation, Akagi and co-workers [75] employed various strategies for immunization with inactivated HIV-1-capturing nanospheres (HIV-NS) to induce HIV-1-specific IgA response in the mouse genital tract. HIV-NS were administered intravaginally, orally, intranasally or intraperitoneally to mice. Intranasal immunization with HIV-NS was more effective compared with other immunization routes in terms of vaginal IgA response. In addition, vaginal washes from intranasally immunized mice were capable of neutralizing HIV-1_{IIIB}. They proposed that application of HIV-NS would be practical for the promotion of HIV-1-specific IgA response by the vaginal mucosa in the mouse. The intranasal immunization route proved to be an effective immunization route in this animal model and they indicated the pursuance of this system for its potential as an HIV-1 prophylactic vaccine [75].

3.8. Bioresponsive nanoparticles for microbicide delivery and the nano-enabled gel-like molecular condom formulation

The concept of microbicide nanocarriers, incorporating an additional bio-responsive element, is an important avenue of investigation. In one embodiment of their invention, as specified in United States Patent Application No. 20070166382, Kiser and co-workers [77] described a nanoparticulate system that was designed to degrade in the presence of ejaculate, having application in the treatment or prevention of STDs, the prevention or promotion of fertility or for hormone replacement therapy. Derived from this, is their elaboration of an anti-HIV vaginal gel [78]. The term 'molecular condom' arises from the concept that the polymer construct is liquid at room temperature and, when applied intravaginally, spreads and converts to a gel that effectively coats the vaginal wall. The gel is designed to release anti-HIV bioactives upon contact with semen during sexual intercourse. It is a hydrogel sensitive to body temperature and pH, and serves as a 'smart semen-triggered vaginal microbicide delivery vehicle'. The nano-enabled system delves into the concept of bioresponsiveness in drug delivery by tailoring the physiological and mechanical requirements essential for intravaginal application. The technology is anticipated to protect both women and unborn or nursing children from HIV infection [78].

3.9. Bioadhesive nanosystems as intravaginal microbicide delivery systems

The success of novel nanocarriers may be limited by their short residence time at the site of absorption. The advantages of providing an approach for promoting intimate contact of the carrier system with the absorbing membrane are evident [79]. This has already been achieved for intravaginal microsystems by coupling bioadhesion characteristics to the microspheres for the development of bioadhesive systems, where 'bioadhesion' implicates the attachment of a synthetic or biological macromolecule to a biological tissue. An adhesive bond may form with the epithelial cell layer, the continuous mucus layer, or a combination of the two [31,79]. Although this concept has not been applied specifically to a nanoparticulate intravaginal system, its applicability with reference to such a system is evident, and the approach employed is expounded upon. The quest for such bioadhesive systems has seen the advent of newer polymers known as HYAFF, a benzyl ester of hyaluronic acid, produced by the chemical modification of hyaluronic acid, which has conferred an advantageous step forward for the intravaginal delivery of drugs from bioadhesive microspheres [80,81].

Distribution and retention time of the bioadhesive intravaginal microspheres have been investigated using gamma scintigraphy. Richardson and co-workers [82] reported the intensity and distribution of radioactivity in the genital tract after administration of technetium labelled HYAFF microspheres. Dimensions of the vaginal cavity of the *in vivo* sheep model were outlined and imaged using labelled gellan gum and the data collected subsequently were used for comparison of the distribution of radio-labelled HYAFF formulations. The retention of the bioadhesive radio-labelled microspheres based on HYAFF was enhanced compared to the pessary formulation after 12 h of administration to the vaginal tract. Due to the high biocompatibility and controllable degradation rate of HYAFF microspheres they have been used for the localized delivery of steroids, analgesics, anti-inflammatory agents and anti-infectives. This in turn has promoted their applicability in the development of safe and effective bioadhesive intravaginal systems for microbicide delivery [31,83].

3.10. Nanoparticle-loaded electrospun polymer fibers with microbicidal activity

In the search for novel nano-microbicides, crossfield applicability of nanoparticulate systems finding use in the textile industry, for example, must be given due consideration. Greiner and Rocker [84] described polyethyleneimine nanoparticle-loaded microbicidal electrospun polymer fibers for texture applications. It is the quaternized polyethyleneimine nanoparticle (PEIN) that is ultimately responsible for the microbicidal properties of the fibers. They established that the polymer fibers derived from this approach may be implemented as textiles, to produce, for example protective clothing for medical staff patients, for medical drapes and dressings. The potential of the described system as an intravaginal microbicide is, as yet, an unexplored facet.

3.11. Liposomes for intravaginal drug delivery

Nano-sized systems designed for an intravaginal mode of delivery with diverse therapeutic modalities may also possess relevance for microbicide delivery. Pavelic and co-workers [85] designed liposomal intravaginal drug delivery systems, having the propensity to deliver entrapped drugs during an extended period of time at the site of action. The phosphatidylcholine-based liposomes were prepared by two different approaches, specifically the polyol dilution approach and the pro-liposome approach, and incorporated three commonly applied drugs for the treatment of vaginal infections, namely, clotrimazole, metronidazole and chloramphenicol that were tested for *in vitro* stability. The extended residence period of the drug delivery system in the vaginal tract may perceive them to be suitable for the delivery of microbicides.

4. Nano-microbicides: the global patent versus patient debacle

With the realization that effective female controlled measures for the prevention of transmission of HIV are, in fact, a necessity in socioeconomic environments where disempowerment of women is a reality [86], the global community undoubtedly anticipates a surge in the development of microbicides and issuance of patents. Ultimately, the full impact that the realm of nanotechnology can bring to the vaginal microbicidal field is yet to be realized. Importantly, issues such as safety, affordability, and accessibility of nano-microbicides are the ultimate consideration in developing countries. A case in point: sex workers in Nigeria are now applying lime juice into their vaginas in an attempt to protect themselves from contracting HIV [32]. Yoghurt has also been drawn into the HIV prevention armamentarium [87]. Can such high-tech strategies be successfully implemented in such populations? Specifically the global South is faced with a milieu of poor living conditions, which can complicate safe and effective use of the nano-microbicide [32]. Furthermore, based on historical patterns, there is concern that political pressures to approve effective microbicides could compromise rigorous testing and, as indicated, vulnerable populations of women are already being used as guinea pigs, a precarious situation [88]. The controversial debate that ensues regarding the issuing of nano-based patents and the ultimate impact on the patient is thus a pervasive consideration to the microbicide field.

4.1. Nano-microbicide patient considerations: complexities and issues

4.1.1. Consideration of potential safety and long-term health effects of nano-microbicides on the patient

The inherent difficulty with the concept of a 'nano-microbicide' is that it combines the general concerns related to nanomedicine, with those of microbicides. Scientists implicated in their development may find themselves on thin ice in terms of satisfying interested parties of the overall safety of their intellectual property (IP). In their article, Mnyusiwalla and co-workers [89] expressed concern that the science of nanotechnology is bounding ahead, while the ethical issues lag behind, leaving the belief in some, including the US Nanoscale Science, Engineering and Technology Subcommittee (the interagency body responsible for coordination of the National Nanotechnology Initiative based in Arlington, VA, USA), that there is the danger of 'derailing' nanotechnological advances if comprehensive studies on the ethical, environmental, economic, legal, and social implications of nanotechnology is not aligned with the scientific progression of the technology. This could be severely detrimental to public-, shareholder-, and investor confidence in the nanotherapeutics industry [90].

The employment of a nanomedicine is intentional in terms of a defined and distinctive property that its size and chemistry avails [91]. The uniquely beneficial properties of engineered nanomedicines undoubtedly goes hand-in-hand with their equally unique health risks when used in medical applications and their environmental impact [92,93]. According to the Australian Department of Employment and Workplace Relations, a customer-focused organization [94], such nano-sized systems, given the novelty and variety of products, are highly reactive and mobile within the human body, and there is distortion of the diagnostic and therapeutic classifications of 'medicines' and 'medical devices'. Furthermore there are currently no effective methods of monitoring the risks of nanoparticle exposure in patients or healthcare workers. The lack of knowledge regarding the manner in which nanoparticles interact with the biochemical pathways and processes of the human body is worrisome and should spur scientists to focus on nanoparticle toxicity, characterization and exposure pathways [95]. Additionally accruing, analyzing, categorizing and characterizing safety data for individual nanotherapeutic products, i.e. nano-microbicides, could present several challenges [96,97].

The current situation has culminated in the National Institutes of Health (NIH), a part of the U.S. Department of Health and Human Services and the primary Federal agency for conducting and supporting medical research, which evaluates pertinent safety issues. These include nanoparticle pathways in the human body, the duration that nanoparticles remain in the body, nanoparticle effects on cellular and tissue functions, access of nanoparticles to the systemic circulation via dermal exposure, and unanticipated reactions *in vivo* [95].

Professor Günter Oberdörster (2007), a leading expert in nanotoxicology with regard to environmental medicine at the University of Rochester (Rochester, NY, USA), realizes the potential of nanomedicines, but expresses concern that until recently, only *in vivo* animal studies have shown proof of concept [95]. However, he believes that regulatory processes instituted by agencies such as the FDA will require the appropriate toxicity testing prior to approving any nanomedicine applications. This testing must be meticulous, though, with evaluation also undertaken in susceptible parts of the population [95]. There is thus a fine balance between obtaining sufficient and applicable toxicological data for the nano-microbicide, bearing in mind regulatory hurdles.

In addition to safety issues are the ethical implications. Professor John Weckert (2007) of the Centre for Applied Philosophy and Public Ethics (Canberra, Australia), whose research lies mainly in the areas of ethics and new technologies such as nanotechnologies, has highlighted the concerns emanating from the ethical use of nanomedicine, such as informed consent, risk assessment, toxicity, and human enhancement. Additionally, and specifically with relation to microbicides, there is a 'fine-line' between medical and non-medical uses of nanotechnology for diagnostic, therapeutic and preventive purposes. A topic of contention is whether nanotechnology should be used to make intentional changes in or to the body when the change is not medically required [94].

Thus a superior understanding of nanomaterials is required, however experience with inorganic and organic chemicals may not be directly relevant to nanomaterials, as determination of their physical and biological properties is generally via novel relationships between their size, structure, and the presence of added functional groups [91]. Linkov and co-workers [91] thereby highlighted the need for a risk assessment, having four general components: 1) hazard identification, 2) toxicity assessment, 3) exposure assessment, and 4) risk characterization. Nanomaterials can easily be identified as a potential hazard, but obstacles may be encountered in the subsequent three steps.

The nano-microbicides discussed thus far have been developed due to a number of unique properties that their size (and often chemistry) imparts. For example, the nano-sized particles may have the ability to access different tissues than larger particles, or to be tagged with specific antibodies to target specific cells. Nano-microbicides would thus have a propensity to cause side-effects on accessing different tissues, and a toxicity assessment would require knowledge of their metabolism and distribution in the body. Radio-labelling is one technique exploited for determining the distribution of a nanomedicine in a patient, which can be used to evaluate nanoparticle distribution and uptake into specific cells and tissues that is dependent on factors such as the mechanism of targeting. Consideration of the route of delivery is also pivotal (the intravaginal route being preferable for most microbicides), as is the propensity of the nanomaterial to remain localized or re-enter the systemic circulation, and its eventual use, metabolism, and elimination. These factors are of course all inherent of the nanomaterial used. Multiple variables would thus influence nano-microbicide exposure assessment [91,98,99].

A decision tool, as depicted in Fig. 8, was developed by Linkov and co-workers [91] for making efficient judgments on appropriate nanomaterials for medical applications with combinatory consideration of the medical factors and side-effects together with associated uncertainties related to selection of alternative nanomaterials and treatments. Decision analysis tools facilitate the generation and mapping of technical data as well as individual judgments into organized structures that can be linked with other technical tools from risk analysis, modelling, monitoring, and cost estimations. Decision analysis software can also provide valuable graphical techniques and visualization methods for interpretable expression of generated data.

Three basic groups of stakeholders (nanotechnology managers and decision makers, scientists and engineers, and patients, in this case, nano-microbicide users) are included, with the roles of all parties being essential in maximizing the utility of human input into the decision-making process. The result of the entire process is purportedly a comprehensive, structured process for selecting the optimal alternative with relation to the nanomedicine considering stakeholder preferences and value judgments as well as scientific modelling and risk analysis. For nanomedicines, into which the realm of nano-microbicides lies, a structured approach is important for making justifiable and transparent decisions with explicit trade-offs between societal (e.g. patient factors) and the technology implicating the nanomaterial in question. Thus, thorough consideration of the ethical, environmental, economic, legal, and social effects is a pivotal step towards the design of safe and effective nano-microbicides [89,91]. Intrinsic to this lies the issue of the testing of potential nano-microbicides, which is further elaborated on.

4.1.2. Selection of alternative models for testing of microbicide candidates

According to Grant and co-workers [1] an expanded role for non-human primate models should be implemented. There seems to be a general consensus that meaningful data can only be derived from human trials; non-human primate models have fallen to the wayside, the underlying rationale being the concern that a failed

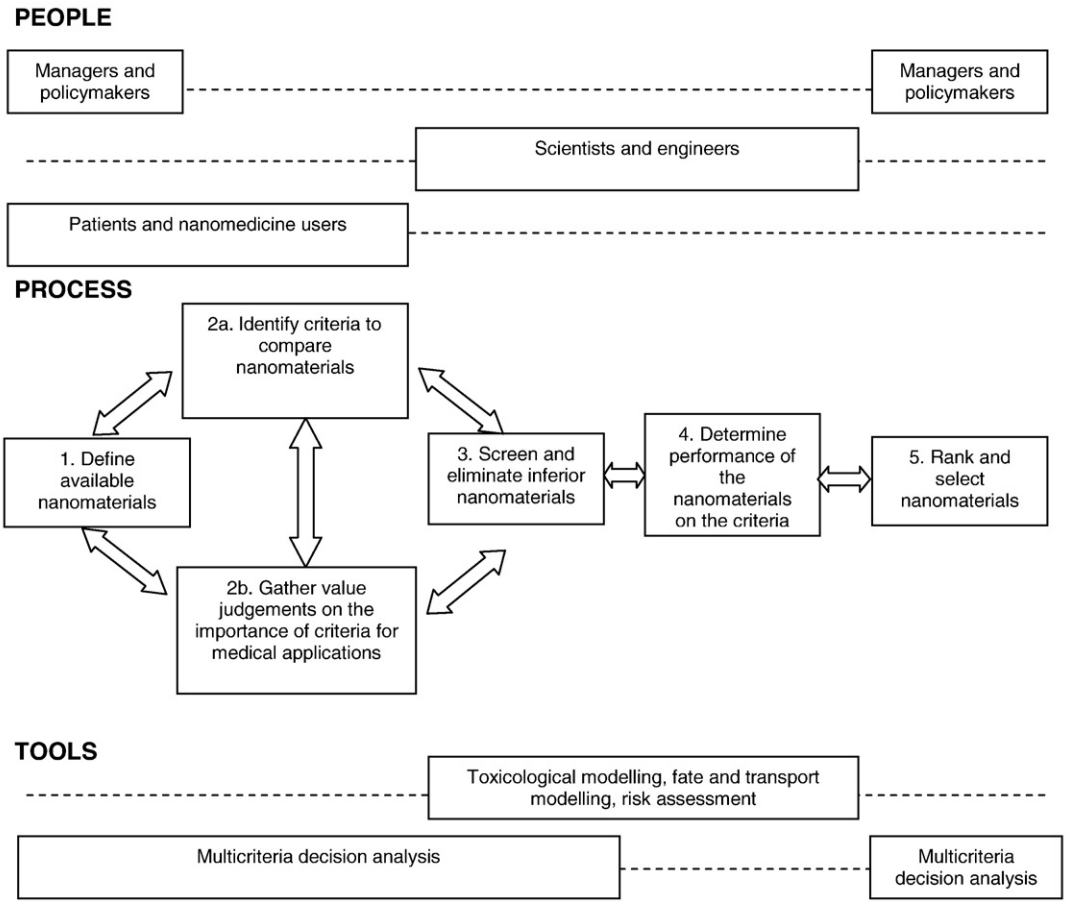

Fig. 8. A schematic diagram outlining the steps involved in the decision-making process. Blocked labels indicate direct involvement or applicability, and dotted lines indicate less direct involvement or applicability (adapted from: Linkov et al. [91]).

monkey experiment might lead to the rejection of a concept that could work in humans. Additionally, inconvenient data generated from such studies may nullify real-world applicability. It is a prerogative that any microbicide candidate being considered for human trials should demonstrate proof of both safety and efficacy in a macaque model. Although success in a macaque model does not guarantee that a compound will protect women from infection by diverse HIV-1 strains, failure to protect monkeys should be regarded as a significant indication that the compound lacks potency. The development of non-human primate challenge protocols is essential for ascertaining whether microbicide candidates might enhance susceptibility to infection. Globally applicable and standardized preclinical testing programs need to be adopted. Furthermore, Grant and co-workers [1] emphasized that 'the perceived need to conduct large-scale trials should be replaced by the real need to test only the best agents and answer the most important questions'. Within the microbicide field, there is a need for the substantial revision of its management, superior and more accountable procedures for coordinating its major programs, and a focus on the underlying science [1,3,89], which, ultimately overlaps with the goal of nanotechnological advocates. Concurrently, and often in contention with patient considerations during nano-microbicide development, are deliberations arising when patenting of the intellectual property becomes a paramount concern.

4.2. Nano-microbicide patent considerations: complexities and issues

4.2.1. Pertinent complexities regarding nanotechnology and microbicides

The amount of work that is required for the development of a drug candidate from the design stage to the attainment of regulatory approval for use as a drug in humans is undoubtedly an arduous course [66]. This task is expounded in the case of microbicides, particularly those designated with the 'nano' criterion. According to the ETC Group [32], the quest of microbicide advocates currently entails seeking support for a product category that is not yet available. Their primary goal is the expansion of research and development efforts, which is in stark contrast with the astounding rate of research that is occurring within the nanotechnology arena. There is a lack of funding for microbicide research compared to HIV/AIDS treatments and vaccines for the prevention of HIV, with current funds being provided primarily from governments and philanthropic donors, with industry interests being few and far between.

Potts and Short [100] commentary on the article 'Raising new barriers against HIV infection' (R.S. Trager, News Focus, 3 Jan., p. 39) [101] welcomed the increasing interest in the use of microbicides to fight HIV. However, they agreed with the judgment expressed in Trager's article by Zeda Rosenberg, the CEO of the new International Partnership for Microbicides (a non-profit product development partnership established in 2002 to prevent HIV transmission by accelerating the development and availability of a safe and effective microbicide for use by women in developing countries), that adherence to current strategies would culminate in the improbable approval of any microbicides before 2010. Furthermore, it was emphasized that the achievement of an epidemiologically significant level of use would add several more years to this timeline. A point that is repeatedly stressed is that the development of available, acceptable and affordable new methods for the prevention of sexual transmission of HIV is becoming a necessity in the wake of the AIDS pandemic [100]. Potential self-imposed barriers that may stand in the way of achieving this goal are the issues of patenting, confidentiality, profit motives and the goal of high

efficacy versus almost zero risk could mean that probable solutions are prematurely discarded. The developing world may be seen as poorly equipped to respond to this challenge [100].

Earlier, it was exemplified that therapeutic nanotechnologies have a powerful and revolutionary role to play in the diagnosis and treatment of disease at the molecular level. The National Science Foundation in the USA estimates that by 2015, the annual global market for nanotechnologically related goods and services will reach $1 trillion. By 2014, 16% of goods in healthcare and life sciences in terms of revenue will incorporate emerging nanotechnology, making it one of the fastest growing industries in history, and ultimately, an exceptionally large economic force [33].

In countenance to current observations in the microbicide field, we are witnessing that developed nanomedicines/nanopharmaceuticals employing the approaches described earlier in this review article have already entered routine clinical use and will soon be bombarding patent offices with their applications. There are a growing number of marketed nano-sized drug delivery systems in clinical development. These first generation successes can only be expected to act as the support on which future successes are built [36]. According to Mullally and Winn [102], close to 3000 patents were issued in the USA between 1996 and 2002 with the term 'nano' used in the patent with a considerable number having application in nanomedicine. Furthermore, when classifying nanopharmaceutical patents by disease abstracts for cited diseases, it has been shown that patenting is strongest for non-communicable diseases [103].

Our search of the United States Patent and Trademark Office (USPTO) for patents issued from January 1976 to 26 May 2009 found 18,936 patents with the term 'nano'; 749 patents with the term 'microbicide'; and only 2 patents with the terms 'nano' and 'microbicide'. Since research and development in this field is ensuing at a rapid pace, the issuance of valid enforceable patents will be the predictors of success and failure for emerging nanotechnological companies [102]. Patent lawyer Drew Harris and his colleagues [104] have explored the major issues faced by nano-biotechnology companies dealing with cross-infringing patents. Nanoparticle-based drug delivery systems may be among the first types of products to generate serious nanotechnology patent disputes as the multi-billion dollar pharmaceutical industry begins to adopt this approach to attain faster drug absorption and controlled drug release capabilities for enhancing the effectiveness of existing drug delivery systems.

Since nanotechnology is an interdisciplinary convergence of physics, chemistry, biotechnology, electronics and medicine, inventions can only be expected to be of a multidisciplinary nature. Patent offices will thus be expected to face multiple challenges in dealing with patent applications that claim inventions in this field. One of the foremost stated difficulties by law experts is the identification of examiners with sufficient knowledge and experience of the technology to examine applications [102]. Furthermore, applications need to be compartmentalized such that nanopharmaceutical applications are sent to examiners skilled in the art of drug design and delivery and should assist Patent Offices in educating examiners and develop guidelines for examining nanopharmaceutical patent applications [102].

The increasing number and complexity of applications from all technological areas as well as the lack of experienced examiners is likely to result in a backlog, and patents in nanopharmaceuticals are likely to take longer to prosecute. Delays in obtaining issued patents may impact the pace of the industry's growth, particularly the ability to secure funds for further research and development or commercialization of products. Furthermore, nanotechnology, as nanopharmaceuticals, is an emerging technology, and thus bears the risk of overbroad patents being granted, which could impede growth and innovation [33]. The fundamental nature of nanotechnology is part of the intricate challenge for effectively defining patents in this arena. Many patent applications may result from a single nanopharmaceutical invention; hence, a single patent may generate a number of products [33]. What needs to be considered is whether the nano-product, in this case the nano-microbicide, is novel as a result of its nano-size or whether, from previous patents that contain generic claims cover the traditional product concept, it is obvious and therefore not patentable. When the size of the nano-microbicide alone does create an innovative or undiscovered property, an invention may be present [33,102].

Designing a nanopharmaceutical that is efficient and safe in the biological environment with future patent-potential will require combined knowledge and expertise of synthetic and physical chemistry as well as biological chemistry. Innovators should strive for biological and medical relevance with this in mind. The future of nanopharmaceuticals is, however, still promising, and it is hoped that the surge generated in this arena could in turn fuel nano-microbicide developments. According to a report from NanoMarkets [105], an industry consulting firm based in Glen Allen, Virginia, USA, nanotechnology-enabled drug delivery systems would have generated over US$1.7 billion by now (2009) and over US$4.8 billion in 2012.

These are promising statistics, but once again, the issue of global disparity raises its head. The considerable absence from certain countries with regard to nanotechnology patenting signals that the 'nano-divide' is already here and exists just as strongly within the developing world as between the North and South. This is mainly due to considerable barriers existing for less-developed countries seeking to engage with nanotechnology research and development on a national level that would ultimately contribute to patenting [103,106]. Viewing the overall picture of health-related nanotechnology patents, control lies firmly with the industrialized countries of the North, although China is ensuring strong representation from the developing world, relative to its general patent output. There are some encouraging signs however, that certain developing countries could play a significant role in the global development of nanotechnology patents [103] and it is with anticipation that this role is urged to extend to the nano-microbicide field.

An overall sign of hope is noted with VivaGel™, which, as described earlier, is the first dendrimer-based nano-microbicide to go through the FDA regulatory approval process. The tentative advances witnessed for products such as VivaGel™ has been cited as a step in the right direction [32] for the microbicide and nanopharmaceutical fields in addressing major health challenges in the marginalized communities of the developing world, and is currently undergoing global testing in various populations, including a Kenyan trial site. In addition, their toxicological programs demonstrate adequate progress [107]. In 2005 the US NIH awarded Starpharma (Pty) Ltd., (Melbourne, Australia) US$20.3 million to support the development of VivaGel™ for the prevention of HIV. In 2006, the US NIH also funded a clinical trial to test the use of VivaGel™ in the prevention of genital herpes. Market analysts predict that should VivaGel™ demonstrate successful protection against STDs and pregnancy, it could be seen as an avid competitor with the condom market [108]. Intrinsic in Starpharma's proprietary technology is their business strategy: 'to create value from dendrimer nanotechnology by utilizing its IP through product development, licensing and partnerships' [107]. Starpharma holds the rights to three broad-based US patents in the dendrimer pharmaceutical area, and its subsidiary, Dendritic Nano-Technologies, Inc. (DNT), holds a comparatively significant number of patents on dendrimer technology.

4.2.2. Regulatory issues

It is a fact that a number of regulatory concerns could potentially stand in the path of nano-microbicide development [3]. In regions with high HIV rates, the definite lack of well-defined criteria and pathways for microbicide licensure is an undeniable obstacle. Product developers are thus currently deprived of essential guidance in terms of licensure requirements [3]. The rationale for such a discrepancy is manifold. Firstly, all microbicides currently in clinical testing are new drugs, except for tenofovir. Secondly, chronic use of topical intravaginal drugs to prevent infection is a new concept, essentially leaving regulators in the dark. Developing countries may not have sufficient resources to

complete a multifaceted review of new drug applications within their National Regulatory Authority (NRA), and thus rely heavily on product reviews by U.S. or European agencies [109]. South Africa and India have been listed as possible exceptions to this fact. The inherent challenge with this is that even though the requirements to demonstrate safety and efficacy are essential globally, the risk-benefit profiles of microbicides differ substantially between developed countries and regions where HIV infection is higher by two orders of magnitude. These differential risk-benefit profiles cannot be ignored [109].

According to Milstien and Belgharbi [109], significant steps have been taken in order to strengthen the capacity of developing country NRAs to make licensing decisions in light of inadequate resources. Certain strides have been made to remove potentially rate-limiting regulatory hurdles, which has included expanding the mission of agencies such as the FDA to evaluate the safety and efficacy of microbicides on behalf of NRAs in developing countries, but leaving risk-benefit analysis to the NRAs; fostering links between NRAs to allow for joint review of product dossiers; increasing the transparency of decision-making by developed NRAs such that rejection of licensure applications based on risk versus benefit, not safety or efficacy, does not unduly prejudice independent assessment by developing countries; provision of additional resources by donor organizations to enhance autonomous decision-making by NRAs in developing countries; and establishing an international advisory group of scientific, clinical and regulatory experts to assist NRAs in the decision-making process.

5. Conclusions

Innovative steps toward the design of safe and effective intravaginal microbicides to prevent HIV infection are urgently needed. Nanomedicine could hold the key in this respect through the introduction of desirable capabilities. This review has explored various nanosystems developed as nano-microbicides or having potential in this regard, in the hope of spurring their clinical development in this direction. The expanding clinical program for VivaGel™, if successful, would embody the significance of nanotechnology's power to generate important societal health benefits. A few promising nanotechnological developments have been initiated in developing countries (e.g. Mintek, South Africa). However, the toxicity and long-term health effects of exposure to nano-microbicides must be considered through an appropriate risk assessment. The need for clear safety and efficacy data for microbicides is essential, with cognisance of the undeniable disparity in risk-benefit profiles between developed and developing countries. In addition, it is crucial that equilibrium be established between nano-microbicide patent and patient considerations for the attainment of noteworthy advances in the fight against HIV.

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