Chapter 17
Nanomedicine in the Development of Drugs for Poverty-Related Diseases

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Abbreviations

ACTs	Artemisinin-based combination therapies
ADME	Absorption, distribution, metabolism and excretion
ARV	Antiretroviral
AUC	Area under the curve
C_{max}	Maximum plasma concentration
CYP	Cytochrome P450
ESE	Emulsion-solvent-evaporation
ESSE	Emulsion-solvent-surfactant-evaporation
ETB	Ethambutol
HIV	Human immunodeficiency virus
INH	Isoniazid
IV	Intravenous
MIC	Minimum inhibitory concentration
NTDs	Neglected tropical diseases
PBCA	Poly(butyl-2-cyanoacrylate)
PCL	Polycaprolactone
PEG	Polyethylene glycol
PK	Pharmacokinetics
PLGA	Poly(D,L-lactic-co-glycolic acid)
PRDs	Poverty-related diseases
PZA	Pyrazinamide
RES	Reticuloendothelial system

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RECG	Reverse-emulsion-cationic-gelification
RESCG	Reverse-emulsion-surfactant-cationic-gelification
RIF	Rifampicin
R&D	Research and development
TB	Tuberculosis

17.1 Introduction

Nanotechnology is a multidisciplinary field covering the design, manipulation, characterisation, production and application of structures, devices and systems at nanometer scale (1–500-nm-size range) which, at this size range, presents with unique or superior physicochemical properties. This scale represents the size of atoms, molecules and macromolecules [1]. Nanomedicine is the application of nanotechnology in medical sciences for imaging, diagnosis, drug delivery (nanocarriers) and therapeutics used for treating and preventing disease.

Nanomedicine has gained ground over the past several years as can be observed from the increase in the number of nanopharmaceutical patents to over 1,000 by the year 2008 [2]. Nanomedicine-based drug delivery systems offer a tool for expanding current drug markets as they can facilitate reformulation of classical drugs and failed leads resulting in improved half-life, controlled release over short or long durations and highly specific site-targeted delivery of therapeutic compounds. Examples of nanocarriers utilised in nanomedicine include nano-capsules, liposomes, dendrimers, gold nanoparticles, polymeric micelles, nanogels and solid lipid nanoparticles, among others. This technology has successfully revolutionised therapies for diseases like cancer with a number of nanomedicine products for cancer, such as Doxil® (liposome) and Abraxane® (albumin-bound nanoparticles), already on the market [3]. The current growth in this field is mainly due to the advances in nanoscience in better approaches of molecular assembly and the design of more controlled and efficient nanomaterial.

The field of drug development experiences very low success rates with regard to drugs that enter the market. These shortfalls are due to factors such as toxicity of the therapeutic compounds, poor solubility leading to lowered bioavailability and thus reduced efficacy. These challenges are even more pronounced in poverty-related diseases (PRDs), such as tuberculosis (TB), malaria and human immunodeficiency virus (HIV). The annual global death toll of HIV/AIDS, malaria and TB approaches 6 million people. According to the World Health Organisation (WHO) 2010 Global TB report, one third of the world's population is currently infected with *Mycobacterium tuberculosis (M.tb)* and an estimated 1.7 million people died from TB in 2009 with the highest number of deaths occurring in Africa [4]. It has been reported that malaria remains one of the world's most prevalent infectious diseases. Forty percent of the world's population is at risk of infection, and in 2009, there were an estimated 225 million cases of malaria reported worldwide and an estimated 781,000 deaths [5]. Sub-Saharan Africa still bears a large share of the global HIV burden with the highest number of people living with HIV, new HIV infections,

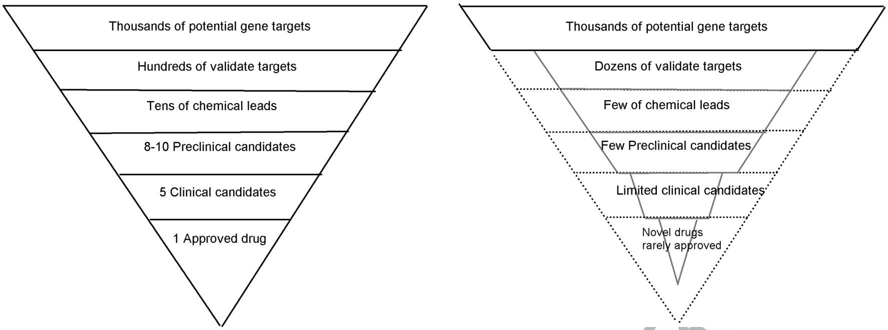

Fig. 17.1 Funding for PRD drug development does not span the whole drug development process in comparison to funding for drug development in the developed world

AIDS-related deaths and the highest adult HIV prevalence [6]. In addition, due to the weakening of the immune system by HIV/AIDS, coinfection with other diseases such as TB, malaria and leishmaniasis is beginning to gain attention. Apart from HIV, malaria and TB, neglected tropical diseases (NTDs) such as leishmaniasis also affect more than one billion people, primarily low-income populations living in tropical and subtropical climates. Visceral leishmaniasis is usually fatal in the absence of treatment [7], and there are an estimated 500,000 new cases of visceral leishmaniasis annually affecting mostly South East Asia and East Africa.

Although effective therapeutic regimens against these diseases are available, treatment failure due to poor adherence (which in turn leads to the emergence of drug-resistant strains) remains a challenge. Many of the drugs require high doses and high-dose frequency due to poor bioavailability, hence the long treatment durations and associated negative side effects. These in turn lead to poorer treatment outcomes and increased cost of treatment. In addition to these drug-related challenges, drug discovery and development research in these PRDs is not at a scale that corresponds with the impact of these diseases in the developing world [8].

The field of drug development for PRDs could benefit greatly from nanomedicine in terms of addressing the aforementioned shortfalls such as poor solubility and limited bioavailability. However, nanomedicine has not been widely applied to transform therapies for PRDs with only a few groups in Africa [9], including the authors of this chapter (DST/CSIR Nanomedicine Platform) [10–12], exploring the application of the technology for PRDs. The CSIR group as well as a group at the University of the Witwatersrand, South Africa, is investigating sustained-release nanodrug delivery systems that will enable anti-TB drugs to be administered at lower doses [9, 12].

Although statistics indicate an urgent need for the development of novel or better drugs, the investment in the research and development (R&D) of these drugs is not significant (Fig. 17.1). Pharmaceutical companies have lagged in the discovery of drugs for the diseases of the developing world due to the cost of the R&D, the risk involved and the time-consuming nature of this field. This is exemplified by a simple comparison of the global TB drug pipeline and the Novartis cancer drug pipeline (Fig. 17.2) where there are only 2 compounds in phase III for TB [13] and

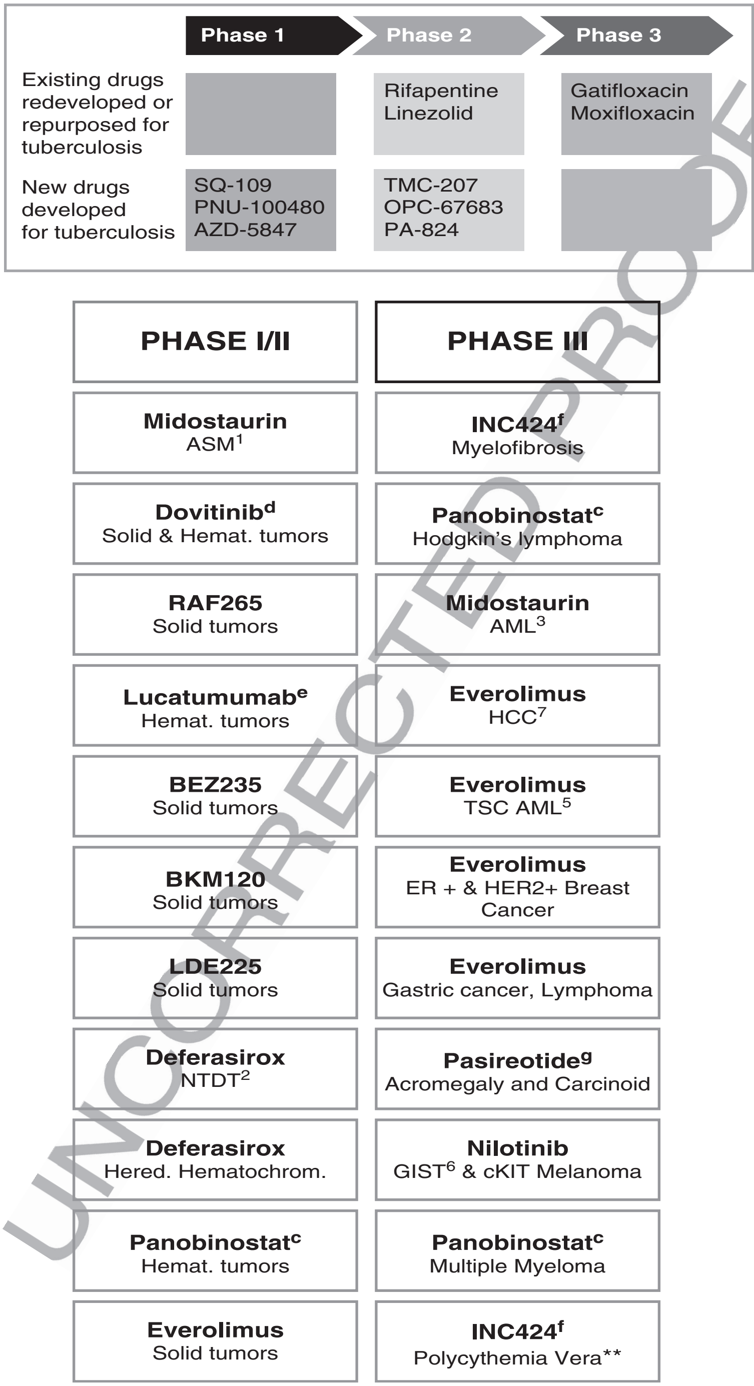

Fig. 17.2 The global TB drug development (**a**) pipeline is less promising than the Novartis oncology pipeline (**b**)

11 for cancer [14]. In the case of NTDs which, unlike HIV, malaria and TB, do not spread widely to high-income countries, there is even less incentive to industry to invest in developing new or better products for a market with low returns. Thus, for drug discovery and development for PRDs, where minimal returns if any can be expected, new approaches such as nanotechnology have to be explored.

To address the challenges in the treatment of PRDs, the investigation into nanomedicine by African researchers has revealed promising approaches for improving treatment of TB. Basic research in nanomedicine for malaria, leishmaniasis, HIV/AIDS and schistosomiasis is also being carried out, but no one is seriously developing a product in this regard.

17.2 Pharmacokinetics in Drug Development and Benefits of Nanomedicine

Pharmacokinetics (PK) is the science that describes the processes of bodily absorption, distribution, metabolism and excretion (ADME) of compounds and medicines. In drug development, PK parameters are required to determine route of administration and dose regimen.

Absorption describes the movement of molecules from the site of administration to the systemic circulation. Distribution is the movement from systemic circulation to extravascular sites. Metabolism is the enzymatic biotransformation of the molecules, and excretion is the passive or active transport of molecules into, e.g. bile and urine [15].

The oral route of drug administration is preferred due to its convenience and cost-effectiveness. However, to be absorbed into the systemic circulation and reach its target site, a drug must be able to cross cell membranes. In fact, each of the ADME processes involves passage of compounds across cell membranes. Several routes may be utilised depending on the physicochemical properties of the compound. Generally, lipophilic compounds are rapidly absorbed because they distribute into the cell membranes of epithelia via the passive transcellular route. Hydrophilic compounds are absorbed more slowly due to their poor distribution into cell membranes. Such compounds are, therefore, more likely to be transported by carrier-mediated pathways.

The bioavailability is the fraction of an administered dose of drug that reaches the systemic circulation. When administered intravenously, the bioavailability is 100%. When administered by other routes such as orally, the drug must first be absorbed in the intestine, which may be limited by efflux transporters such as P-glycoprotein in the intestinal epithelium. As the drug passes through the liver and intestine, metabolism mainly by the cytochrome P450 (CYP) family of enzymes (first-pass metabolism) and further excretion may take place thus reducing bioavailability.

Nanomedicine offers an alternative to address PK-related shortfalls in drug development, and the following sections will discuss the properties that make them advantageous as emerging therapies.

17.2.1 Factors Affecting Drug Development for PRDs

Poor PK is a major cause of PRD treatment failure due to the inability to achieve effective drug levels (poor solubility and intestinal permeability leading to poor bioavailability for orally administered drugs), production of toxic effects (poor elimination or levels above therapeutic levels) and drug interactions. For example, zalcitabine, an antiretroviral (ARV) drug, was discontinued due to adverse side effects and drug interactions [16]. The ultimate result is poor patient compliance which in turn leads to emergence of resistance. The small number of current drugs for PRDs is inadequate to address these treatment challenges, and development of new drugs is high on the agenda.

Drug discovery and development are long and complex, more so for PRDs which in addition to being pharmacologically active must meet the following criteria: oral administration with good bioavailability, well tolerated with minimal side effects and short treatment course [17]. A look at the PRD drug development pipeline reveals that there are too few compounds in clinical development with 10 for TB [13] and 17 for malaria [18] and even fewer for NTDs [19]. It is well known that the majority of compounds entering clinical testing do not make it to market due to poor PK, poor efficacy, side effects and toxicity [20]. The clinical success rate for infectious diseases has been estimated at 15% with a failure rate of about 60% at phase II [20]. Therefore, the need to strengthen the pipeline for PRDs to ensure that new products emerge requires a range of solutions. Strategies to increase the development of new treatments include re-optimising the use of current drugs, repurposing drugs used to treat other diseases, exploring natural resources and modifying existing drugs [18]. This chapter will endeavour to show the advantage of including nanomedicine in drug development programmes. The modification of existing drugs using nanomedicine has revolutionised treatment of diseases such as cancer but has not been extensively applied to PRDs. Doxil® and Abraxane® are two of several nanomedicine-based cancer therapies already on the market. Doxil® is a liposomal formulation of the anthracycline drug doxorubicin. It is used to treat cancer in AIDS-related Kaposi sarcoma and multiple myeloma. Its advantages over free doxorubicin are greater efficacy and lower cardiotoxicity due to altered PK [3]. Abraxane® consists of the anticancer drug paclitaxel bound to human albumin nanoparticles which confers it with a longer circulation half-life [3].

17.2.2 *Pharmacokinetics of Nanomedicines*

Nanotechnology-based therapies can lead to improved half-life, controlled release over short or long durations and highly specific site-targeted delivery of therapeutic compounds. This section will explain how nanomedicine can attain these improvements.

Nanopharmacokinetics [21] is distinct from pharmacokinetics of small molecules. The latter depends mainly on diffusion and transport (through blood) or metabolism as outlined in Sect. 17.2.1. However, nanopharmacokinetics is defined by physiological processes undergone by nanomaterials such as cellular recognition, opsonisation, adhesion, lymphatic transport and uptake processes such as phagocytosis [21]. The reduction in blood concentrations of nanomaterials might be related to movement into tissue from which further excretion does not occur. Indeed, many nanomaterials tend to accumulate in the liver and to be sequestered in the reticulo-endothelial system (RES) or bound to tissue proteins. In addition, nanomaterials may be transported through lymphatic pathways which must be taken into account in pharmacokinetic analysis based on blood sampling. However, this altered pharmacokinetics at the nanoscale means that nanomedicines present pharmaceutic improvement as drug delivery systems as they can:

- Improve drug stability ex vivo (long shelf life) and in vivo (protection from first-pass metabolism) [22, 23]
- Have a high carrying capacity (ability to encapsulate large quantities of drug molecules) [23]
- Incorporate hydrophilic and hydrophobic substances [23]
- Increase drug dissolution rate, leading to enhanced absorption and bioavailability [24]
- Target to specific tissues due to selective uptake by those tissues [3]
- Reduce clearance to increase drug half-life for a prolonged pharmacological effect [3]
- Present the capacity to be formulated for the purpose of controlled release [25], therefore posing the possibility to reduce dose frequency and subsequent dose-related side effects [26]
- Be actively targeted to a specific site by functionalising the nanoparticle surface with specific molecules or ligands such as monoclonal antibodies, RNA/DNA aptamers or peptides to enhance binding and interactions with specific receptors which are expressed by the cell populations at the diseased site [27] and thus reduce toxicity

The protection from first-pass metabolism is an important factor in enhancing systemic bioavailability. However, in terms of intracellular PK, targeting with specific ligands further enhances the intracellular bioavailability due to enhanced drug delivery directly into target cells [24].

17.2.2.1 Physicochemical Factors Influencing PK of Nanocarriers

When material is at a nanometre size range, it acquires unique physical and chemical properties. Specifically, the physicochemical properties attributed to the effectiveness of nanocarriers include the nano-sized range, surface properties and relative hydrophobicity.

Size

The sub-micron size of nanoparticles offers a number of distinct advantages, e.g. the ability to reach virtually all tissues in the body, particularly for particles less than 100 nm in size [28]. Desai et al. (1997) demonstrated that 100-nm-size nanoparticles showed 2.5-fold greater uptake compared to 1 µm and sixfold higher uptake compared to 10 µm microparticles in Caco-2 cell line [29]. This aspect of intracellular uptake is more so critical for intracellular pathogens such as infectious diseases, where the drug needs to act intracellularly. Thus, by nanoencapsulating the drug, one can attain intracellular delivery of drugs. Furthermore, these particles can cross barriers that in general make it difficult for conventional therapeutic compounds to reach the target. Reports on nanoparticles crossing the blood-brain barrier (BBB), the stomach epithelium and even the skin have been presented [30]. In addition, orally administered nanoparticles can enter the lymphatic system through intestinal Peyer's patches, followed by uptake via M cells.

Surface Properties AU2

The surface charge in nanoparticles reflects the electrical potential of particles and is influenced by the chemical composition of the particle and the medium in which it is dispersed. A positive surface charge which can be attained by attaching positively charged polymers such as chitosan on the surface of nanoparticles enhances attachment to the negatively charged cellular membrane, thus improving cellular uptake. Chitosan-based or chitosan-coated particles have been reported to efficiently be taken up by cells and also cross cellular barriers such as the BBB. This is as function of chitosan opening the tight junctions between cells and thus facilitates transcellular particle transport [31]. The surface charge in nanoparticles reflects the electrical potential of particles and is influenced by the chemical composition of the particle and the medium in which it is dispersed. In the case of drug delivery, opsonisation, a process that involves the adsorption of proteins particularly of the complement system, to any foreign material, is also influenced by zeta potential. These proteins make the particle more susceptible to phagocytosis and thus leading to their clearance from the body. To circumvent this effect, various groups have coated the particles with hydrophilic polymers, such as polyethylene glycol (PEG), Pluronics etc., thus affecting both the surface charge and hydrophobicity of the particles and therefore increasing the circulation time of the particles in the blood and in turn prolonging the release of the drugs from the particles [32, 33]. Thus, minimising opsonisation via changing the surface charge is important for controlled-release formulations. In addition, by coating the polymeric particles with hydrophilic polymers, the half-life of the drugs can be improved and thus their efficacy. This approach can reduce the dose and dose frequency of many effective but poorly soluble drugs and thus in turn minimise the adverse side effect since ~~less~~ doses will be administered. Furthermore, nano-sized particles have a larger surface area due to the fact that a decrease in particle size results in an increase in surface-to-volume ratio and that size is inversely proportional to specific surface area. This larger surface area allows for a higher loading of the drug, thus leading to a reduction in the dose administered [34].

Hydrophobicity

Aqueous solubility, gastrointestinal permeability and low first-pass metabolism are important for high oral bioavailability. Nano-based drug delivery systems can increase drug dissolution rate, leading to enhanced absorption and bioavailability [24]. A combination of both particle surface charge and increased hydrophobicity of the material has been reported to improve gastrointestinal uptake in case of oral delivery. Hydrophobicity also plays a role in the drug release profile by impacting the kinetics of the degradation of the polymeric shell. Mittal et al. (2007) reported that by changing the hydrophobicity of a nanocarrier, the structure/composition of the polymer/copolymer or the molecular weight, the polymer degradation and thus the drug release mechanism and/or duration are impacted [35]. Nanoparticles have the advantage of improving the solubility of drugs, particularly for the very hydrophilic or poorly soluble drugs which in most cases are not easy to formulate and have poor bioavailability. By encapsulating these drugs into polymeric particles, which are coated with hydrophilic polymers, the solubility of the drugs can be greatly enhanced, in turn improving the bioavailability of the drug. Kondo et al. (1993) documented an increase in bioavailability as a result of a 10-fold reduction in particle size, which is a result of an increase in surface area and consequently an increase in dissolution rate [34].

17.2.3 Functional Nanocarriers Used in Drug Delivery

A drug delivery system is defined as a formulation or a device that enables the introduction of a therapeutic substance in the body and improves its efficacy and safety by controlling the rate, time and location of release of drugs in the body. Nanotechnology has been increasingly used in drug delivery for nanoencapsulation of medicinal drugs (nanomedicine) [36]. Several nanocarrier devices (Table 17.1, Fig. 17.3) have been used for nanodrug delivery applications. The nanocarriers may be further modified for active disease targeting by functionalizing the surface with

Table 17.1 Nanotechnology-based drug delivery systems

Nanocarrier	Characteristics
Liposomes	Self-assembling spherical, closed colloidal structures composed of phospholipid bilayers that surround a central aqueous space [3]
Polymeric micelles	Supramolecular assembly of amphiphilic block copolymers or polymer-lipid based conjugates [37–39]
Dendrimers	Globular repeatedly branched macromolecules exhibiting controlled patterns of branching with multiple arms extending from central core [40]
Solid lipid nanoparticles	Particulate systems made from lipids where melted lipids are dispersed in an aqueous surfactant by high pressure homogenization or emulsification [41]
Polymeric nanoparticles	Solid colloidal particles existing as nanospheres (matrix structure) or nanocapsules (polymeric shell and inner liquid core). Engineered from synthetic or natural polymers. The former are essentially polyesters and poly-acids including polylactic acid (PLA), poly(D,L-lactic-co-glycolic acid) (PLGA), polycaprolactone (PCL) and poly(butyl-2-cyanoacrylate) (PBCA). The latter include oligomers that are abundant in nature such as chitosan, alginate and starch [42, 43]

ligands such as antibodies, aptamers, peptides or small molecules that recognise disease-specific antigens (Fig. 17.4). In this way, the nanoparticles become “multiple nanocarriers”. For example, a nanoparticle may be functionalised with aptamers to recognise macrophages infected with TB.

Some nanomedicine products currently on the market are summarised in Table 17.2 from which it can be noted that very little progress in the area of PRDs has been made. There is currently no nanomedicine-based product on PRDs. However, African research institutes are now initiating research in the application of nanomedicine to improve PRD therapies which shall be discussed in Sect. 17.3.

17.3 Nanomedicine Research for PRDs in Africa

The field of nanotechnology is relatively new in Africa and is not well exploited in terms of its application to the improvement of PRD therapies. The most significant progress has been made by research groups mainly in South Africa due to the expensive infrastructure the nanotechnology requires. The government of South Africa has taken nanotechnology very seriously providing all the support required as outlined in the following section. In the rest of sub-Saharan Africa, nanotechnology activities are minimal.

17.3.1 Nanomedicine Research for PRDs in South Africa

In South Africa, the national Science and Technology Ministry (the Department of Science and Technology, DST) has been the principal agency guiding

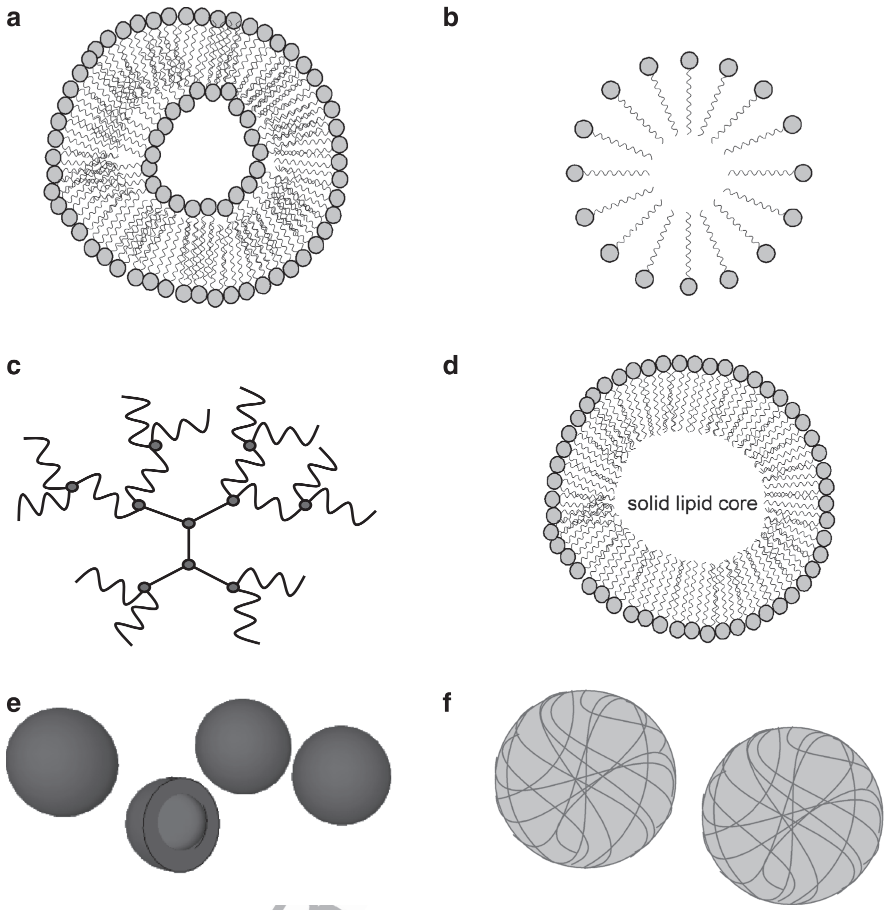

Fig. 17.3 Schematic illustration of nanotechnology-based drug delivery systems, (**a**) liposome, (**b**) polymeric micelles, (**c**) dendrimer, (**d**) solid lipid nanoparticle, (**e**) nanocapsules, (**f**) nanospheres

nanotechnology research direction and policy. In 2007, the DST launched a national nanotechnology strategy with six focus areas of high priority for the country. One of the focus areas is health with the aim of using nanomedicine to improve drug delivery systems, including traditional medicine through packaging medicine for ailments such as TB, HIV/AIDS and malaria in nanocapsules. In this regard, a nanotechnology flagship project (DST/CSIR Nanomedicine Platform) led by the authors of this chapter is being used to develop a drug delivery system for the existing TB drugs, to enhance their efficacy and to reduce dosage and dose frequency. This flagship project has now grown into a nanomedicine centre of excellence for poverty-related diseases for Africa. The centre is one of the recognised African Network for Drug Diagnostics and Innovation (ANDI) centres of excellence.

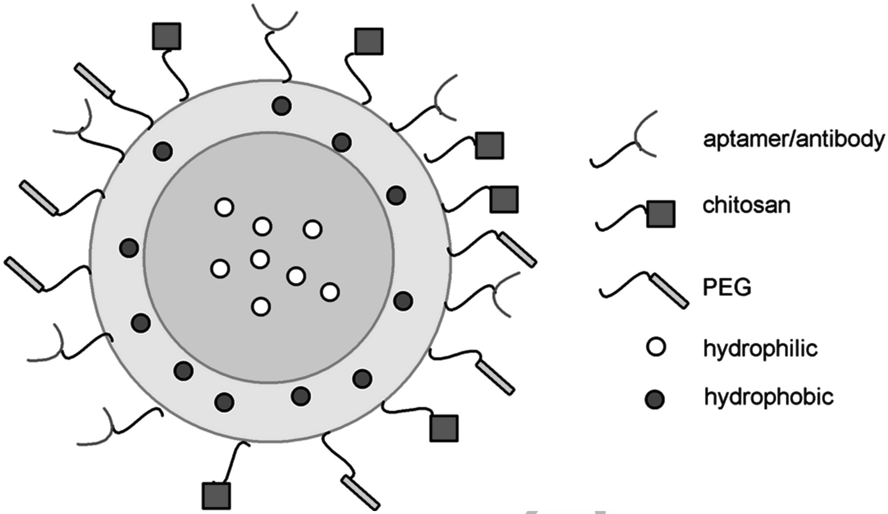

Fig. 17.4 Schematic illustration of a multifunctional nanocarrier

Other South African institutions carrying out nanomedicine research for PRDs include the University of the Witwatersrand (Wits) and North-West University. The group at Wits has also been recognised as a centre of excellence in drug delivery by ANDI.

17.3.1.1 CSIR ANDI Centre of Excellence in Nanomedicine Research

The authors of this chapter are applying nanomedicine to enhance efficacy, half-life, safety, structure and function of TB, malaria and HIV drugs. In addition, we have been spearheading several nanomedicine sensitization activities on the continent, e.g. establishing nanomedicine research programmes in Kenya, hosting international nanomedicine workshops, summer schools and lab exchange programmes.

Research in Progress for Improving TB Treatment Through Nanomedicine

We have encapsulated anti-TB drugs using a novel spray-drying technique as well as a freeze-drying technology. We will illustrate how we have managed to modify physiochemical properties of the particles and attain sustained drug release over a period of days, both in vitro and in vivo. We further indicate that our particles are taken up by cells and also that the activity of the drugs against *Mycobacterium tuberculosis* is still maintained in the process of encapsulation.

Nanoencapsulation of Anti-TB Drugs in PLGA Nanoparticles

Poly(D,L-lactic-co-glycolic acid) (PLGA) 50:50 (Mw: 45,000–75,000) nanoparticles loaded with anti-tuberculosis drug prepared using a patented multiple AU4 t2.1 **Table 17.2** Nanomedicine-based products currently on the market

	Product	Drug	Formulation	Route of administration	Application	Company
t2.3	Abraxane	Paclitaxel	Albumin-bound nanoparticles	IV injection	Metastatic breast cancer	American Biosciences (Blauvelt, NY)
t2.4	Amphocil	Amphotericin B	Lipocomplex	IV infusion	Serious fungal infections	Sequus Pharmaceuticals
t2.5	Ambisome	Amphotericin B	Liposome	IV infusion	Serious fungal infections	NeXstar Pharmaceutical (Boulder, Colorado)
t2.6	Abelcet	Amphotericin B	Lipid complex	IV infusion	Serious fungal infections	The Liposome Company (Princeton, NJ)
t2.7	DaunoXome	Daunorubicin citrate	Liposome	IV	Kaposi sarcoma in AIDS	NeXstar Pharmaceutical (Boulder, Colorado)
t2.8	Doxil	Doxorubicin	Liposome	IV injection	Kaposi sarcoma in AIDS	Sequus Pharmaceuticals
t2.9	Elestrin	Estradiol	Calcium-phosphate-based nanoparticles	Transdermal	Moderated to severe vasomotor symptoms (hot flashes) in menopausal women	BioSante (Lincolnshire, Illinois)
t2.10	Emend	Aprepitant, MK869	Nanocrystal particles	Oral	To delay nausea and vomiting	Merck/Elan(Whitehouse Sation, NJ)
t2.11	Megace ES	Megaestrol acetate	Nanocrystal particles	Oral	Anorexia, cacheixa or unexplained significant weight loss	PAR Pharmaceutical (WoodCliff Lake, NJ)
t2.12	Rapamune	Sirolimus	Nanocrystal particles	Oral	Immunosuppressant in kidney transplant patients	Wyeth/Elan (Madison, NJ)
t2.13	Tricor	Fenofibrate	Nanocrystal particles	Oral	Primary hypercholestrolemiamixed lipidemia, hypertriglyceridemia	Abbott(Abbot Park Illinois)

t2.14 *IV* intravenous, *NY* New York, *NJ* New Jersey

Table 17.3 Characterisation of nanoparticles ($n = 3$)

Drug	Type of drying	Ave size ± SD (nm)	Zeta potential (mV)
INH	Freeze-dried	210 ± 13	−14 ± 2
INH	Spray-dried	321 ± 33	+19 ± 1
RIF	Freeze-dried	280 ± 23	−10 ± 4
RIF	Spray-dried	297 ± 22	+16 ± 2

Adapted from [44]
SD standard deviation

emulsion-solvent-evaporation technique followed by freeze-drying or spray-drying. Polyvinyl alcohol (PVA) was included as a stabiliser, polyethylene glycol (PEG) to increase bloodstream residence time and chitosan as a mucoadhesive, positively charged polymer to enhance gastrointestinal uptake. Using this technique, we have successfully encapsulated all four first-line anti-TB drugs, i.e. RIF, INH, ETB and PZA, in PLGA nanoparticles for oral delivery, with an encapsulation efficiency of 50–65% for INH and RIF, 84% for PZA and 60% for ETH [12], in particles of 250–350 nm [44]. A PCT patent application has been filed (WO 2009/105792) and has already proceeded to the national phase, with the European patent granted recently.

All samples made via freeze-drying showed a negative zeta potential. The addition of chitosan to provide positive surface charge resulted in microparticles. This problem was overcome by spray-drying the double emulsion containing chitosan and PEG in the formulation as shown in Table 17.3 for INH and RIF.

The particles were relatively uniform with an average polydispersity index of 0.2, and analysis of surface morphology revealed a smooth spherical surface achieved by the addition of lactose to the formulation (Fig. 17.5) [44]. Spherical particles offer maximum volume for drug penetration, and it has been reported that spherical particles possess the right curvature allowing ~~its~~ attachment onto the cell [45] giving rise to enhanced efficiency of cell internalisation.

In Vitro and In Vivo Characterisation of PLGA Nanoparticles

The PLGA nanoparticles used to encapsulate anti-TB drugs were evaluated in vitro and in vivo with respect to cellular uptake and biodistribution. To investigate intracellular uptake, Caco-2 cells were exposed to rhodamine-labelled PLGA nanoparticles prepared in the same manner as the anti-TB drug nanoparticles. The labelled particles were taken up by Caco-2 cells and appeared to co-localise with lysosomes (Fig. 17.6) [44]. This indicates the feasibility of intracellular uptake by intestinal enterocytes in patients. In vivo, the PLGA nanoparticles were taken up by macrophages of the peritoneum when administered orally and peritoneally to female Balb/C mice [11].

The PLGA nanoparticles displayed no toxicity towards Caco-2 and HeLa cells as determined via the WST assay [10]. Subsequent to oral administration to mice, the particles remained detectable in the brain, heart, kidney, liver, lungs and spleen after 7 days, with the liver being the major organ of accumulation (Fig. 17.7). However, no pathological lesions were detected in any of the organs [10].

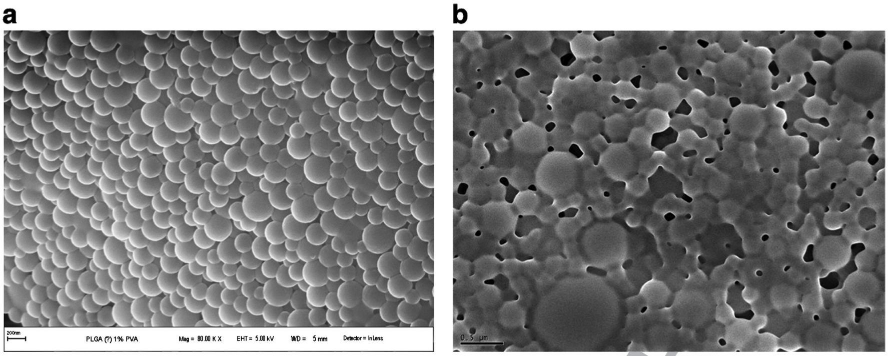

Fig. 17.5 (**a**) SEM image of spray-dried particles ~~without~~ lactose. (**b**) Spray-dried particles ~~with~~ 5% w/v lactose

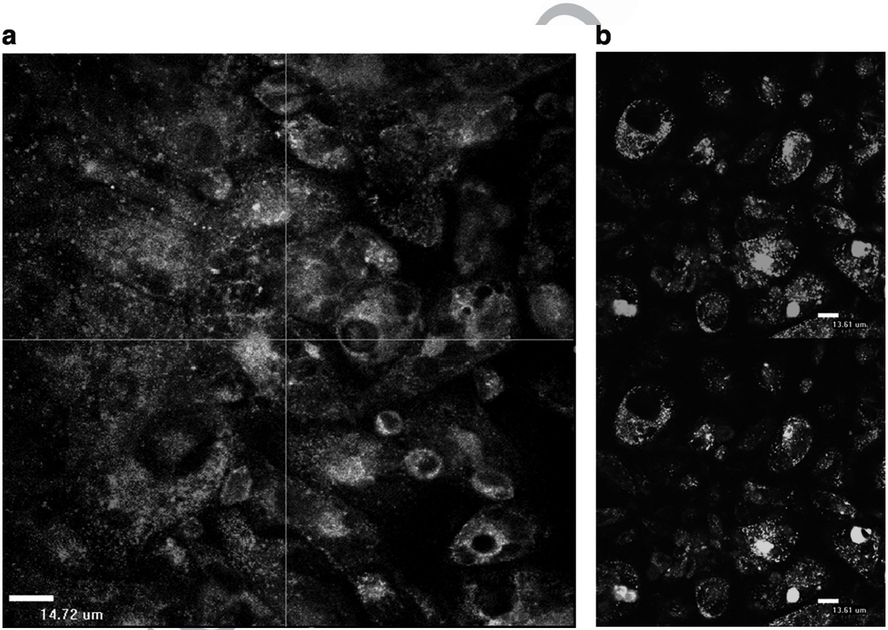

Fig. 17.6 (**a**) Indicates a Z-stack of 30 min incubation and (**b**) depicts a 60 min incubation period. Rhodamine-loaded nanoparticles co-localised with the lysosomes, as indicated by the ~~*orange* colour~~ AU5

In Vitro and In Vivo Characterisation of Nanoencapsulated Anti-TB Drugs

The nanoparticles containing anti-TB drugs were evaluated with respect to release of the drugs from the nanoparticles as well as efficacy.

In vitro release assays in phosphate-buffered saline (PBS) showed that the drugs were released in a slow manner over a period of several days preceded by an initial

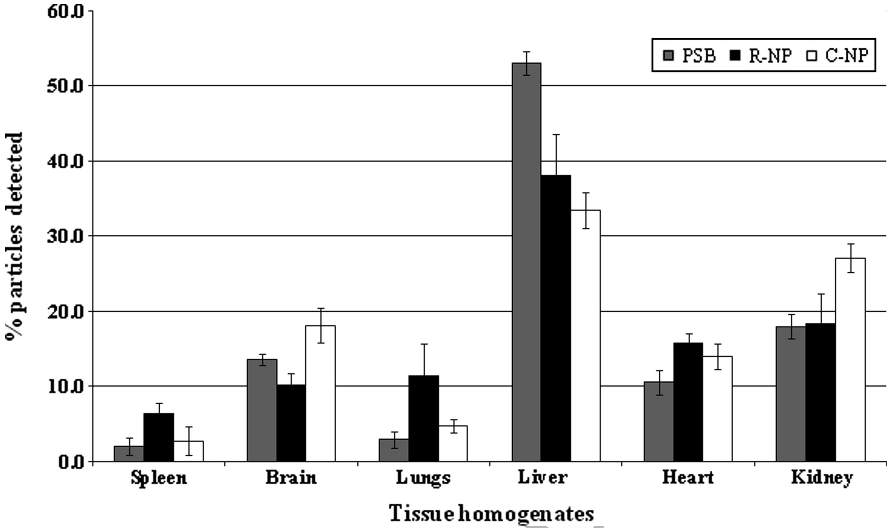

Fig. 17.7 Tissue distribution of nanoparticles after 7 days graphically represented as a measure of percentage of particles detected of the total particles. The data represent three repeats of $n = 6$; *error bars* indicate SEM. *PSB* polystyrene beads, *R-NP* rhodamine nanoparticles, *C-NP* coumarin nanoparticles

burst release. Since hydrolytic enzymes were not included in the PBS, the slow rate of nanoparticle degradation could be attributed to this factor. Faster release rates should be observed in the biological milieu with hydrolytic enzymes present.

The in vitro potency of encapsulated INH and RIF with free INH and RIF was compared using the Bactec 460 assay. The Bactec 460 assay is generally conducted to analyse the susceptibility of *M.tb* to test drugs. The efficacy of the encapsulated anti-TB drugs against $H_{37}R_V$ was comparable to the free drugs (Fig. 17.8) [44]. Therefore, the multiple emulsion spray-drying technique does not have any effect on the potency of the drugs.

When orally administered to mice, nanoparticles containing INH and RIF maintained a sustained-release profile (Fig. 17.9) over a period of at least 5 days when compared to free drugs which reached levels below the minimum inhibitory concentration (MIC) within 16 h. With the encapsulated drugs, drug concentration in plasma above the MIC level of RIF and INH was sustained for the 5 days [44].

An efficacy study in which equal doses of free anti-TB drugs were administered to TB-challenged mice once every day and encapsulated drug once every 7 days indicated comparable efficacy (unpublished data).

These are important results because they confirm the feasibility of slow release and reduced dose frequency.

Targeting of Nanoencapsulated Anti-TB Drugs

PLGA nanoparticles containing anti-TB drugs were further functionalised with mycolic acids (MAs) or nucleic acid aptamers for active targeting of *Mycobacterium tuberculosis*-infected macrophages. MA (a lipid molecule on the cell

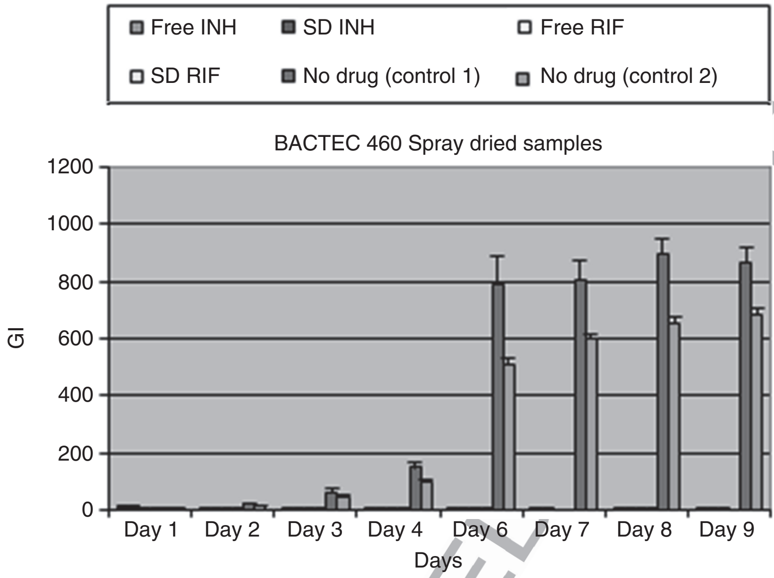

Fig. 17.8 BACTEC 460 data indicating bacterial growth index of $H_{37}R_V$ treated with encapsulated RIF and INH and unencapsulated drugs

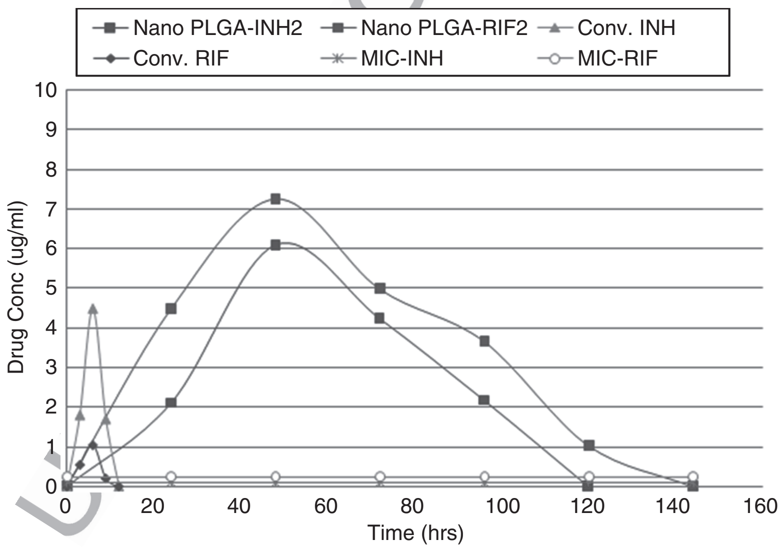

Fig. 17.9 In vivo release of free drugs versus spray-dried nanoparticles encapsulating RIF and INH and PZA

wall of *M. tuberculosis*) was explored due to its cholesteroid properties [46], and the aptamers were prepared against the mannose receptor, which is significantly over-expressed during the activation of the macrophages in the presence of *M.tb*. Intracellular uptake of the MA PLGA nanoparticles was achieved in U937 cells. However, little co-localization was observed with endocytic markers, indicating that they could be localised in the cytosol. Vesicles bearing these particles were also observed in the cell membrane of the cells [47]. Uptake of the aptamers into THP-1 cells was also observed, illustrating the feasibility of using the nucleic acid species for active targeted delivery of the encapsulated anti-TB drugs [47]. A provisional patent application titled "High Affinity Nucleic Acid Ligands to the Mannose Receptor" has already been filed on the method. The success of these two approaches of anti-TB drug targeting will greatly address the challenges of poor bioavailability, reduced efficacy and adverse side effects for diseases such as TB.

Research in Progress for Improving HIV and Malaria Treatment Through Nanomedicine

Based on the successes and experiences obtained through the research work on nanomedicine for TB, the authors have begun on nanoencapsulation of antiretroviral and antimalarial drugs. To date, efavirenz and lamivudine have been encapsulated in PCL nanoparticles with an average size of 230 nm (unpublished data). For malaria, nanocarriers are being designed to target parasites in the liver (pre-erythrocytic) and the red blood cell (erythrocytic) of the parasites transmission cycle. Prophylactic and curative measures of the chemical agents will be investigated before and after the application of drug delivery systems.

Research Strategy for Improving NTDs Using Nanomedicine

The parasites causing NTDs such as leishmaniasis and trypanosomiasis often disseminate throughout the RES, e.g. leishmaniasis in the lymph nodes [48] and schistosomiasis in the spleen [49]. Therefore, the strategy for nanomedicine for these diseases is to take advantage of the selective uptake of nanocarriers by the RES which may be further enhanced by actively targeting the nanocarriers to the parasites in the, e.g. lymphatic system.

Activities to Build Nanomedicine Research Capacity in Africa

Towards advancing nanomedicine and the benefits of the technology in Africa, the authors organised the first international sensitisation workshop on nanomedicine for infectious diseases of poverty, in South Africa on March 2011. Officially opened by the minister of the Department of Science and Technology, this workshop brought together about 90 delegates from over 20 different countries and included representatives from academia, the pharmaceutical industry, regulatory authorities, donor agencies, international organisations and policymakers, all interested in supporting the advancement of nanomedicine in Africa. The workshop comprised a panel of highly accomplished experts in various aspects of nanomedicine and drug delivery as well as experts in drug development for poverty-related diseases. Oral and poster presentations encompassed basic science through to translational efforts and addressed topics on various initiatives and funding. The 4-day workshop featured plenary lectures, invited talks and round table discussions focusing on specific tenets of nanomedicine and drug development. The fourth day was dedicated to discuss intellectual property rights and technology transfer, an aspect which must be kept in mind when developing new technologies.

Following the workshop, the authors presented a series of nanomedicine sensitisation seminars (road shows) to students and young researchers at a total of 18 institutions in Kenya, Nigeria and Ethiopia with more seminars planned for Cameroon and other African countries such as Uganda, Sudan and Tanzania. These nanomedicine road shows highlight the urgent need for more in-depth training in nanomedicine for PRDs. Accordingly, the authors are planning the first Pan-African summer school in nanomedicine for PRDs, in collaboration with leading nanomedicine experts that have nanomedicines on the market and also have experience in operating such nanomedicine schools and conferences in Europe and the USA annually, as well as African PRD experts. The school aims to bridge the gap between the sciences, health and development in Africa, by educating young African scientists on the potential of applying nanomedicine in PRD drug development research. To achieve this, the school will focus on crucial areas to build capacity in nanomedicine. Furthermore, the school will assist in establishing networks and collaborations among trainees, to ensure that every trainee can confidently enhance knowledge dissemination and skills acquisition. The school will also encourage the young scientists to bring with them any compound which has failed to reach the market due to the above-mentioned shortfalls. In this workshop, they will have the opportunity to apply different nanocarriers to address the shortfalls.

17.3.1.2 University of the Witwatersrand (Wits)

The Wits Advanced Drug Delivery Platform (WAADP) is focused on advancements in polymeric science, formulation stability and drug delivery design including nanomedicine for infectious diseases such as TB. In a recent publication, the group evaluated sustained release of INH and RIF from polymeric nanoparticles synthesised via four emulsion-based processing strategies, namely emulsion-solvent-surfactant-evaporation (ESSE) and emulsion-solvent-evaporation (ESE) approaches for PLGA nanoparticles and reverse-emulsion-cationic-gelification (RECG) and reverse-emulsion-surfactant-cationic-gelification (RESCG) approaches for alginate hydrogel nanoparticles [9]. Encapsulation efficiencies were in the range of 73–82%. The ESSE and RESCG approaches which included sorbitan monooleate as a stabiliser yielded smaller sizes of nanoparticles in the range of 200–290 nm for INH and RIF and displayed sustained release over 8 h with zero-order kinetics in vitro.

Another group at Wits, the Antiviral Gene Therapy Research Unit (AGTRU), is using nanocarriers [50] to deliver nucleic acids that are capable of silencing gene expression of viruses that are responsible for infections of serious public health importance to South Africa such as HIV infection [51].

17.3.1.3 North-West University (NWU)

The Unit for Drug Research and Development at the NWU is conducting research aimed at optimising the delivery of anti-TB and antimalarial drugs using Pheroid™ technology. Pheroid™ technology is a drug delivery system patented by the NWU which can be described as a colloidal system that contains stable, submicron- and micron-sized active pharmaceutical ingredient dispensing vehicles. Recently, entrapment of the new artemisinin derivative, artemisone, in Pheroid™ vesicles has been shown to significantly enhance the absorption of the drug. The C_{max} was improved by 90%, and the $T_{1/2}$ increased three times after oral administration in a mouse model [52]. In addition, a Pheroid™ formulation for TB drugs is currently undergoing phase I clinical trials. The CSIR and NWU research groups are now collaborating on entrapping PLGA nanoparticles in Pheroids to further improve bioavailability and achieve controlled release for TB drugs.

17.3.2 Nanomedicine Research for PRDs in the Rest of Africa

In the rest of sub-Saharan Africa where PRDs are endemic, there is little advancement in nanomedicine research for the treatment of these diseases. A few groups exist carrying out basic research into nanomedicine-based therapies, with only two identified thus far at the University of Mauritius (UOM) and American University in Cairo, focusing on PRDs.

At the 4th ANDI Conference in October 2011, the Centre for Biomedical and Biomolecular Research at the UOM presented its unpublished work focusing on engineering novel block copolymer nanomicelles for the delivery of anti-TB drugs. The group has engineered amphiphilic block copolymers based on poly(ester-ether)s, polyLysine-b-caprolactone and oligoagarose-g-polycaprolactone. They reported loading of rifampicin up to 70% and sustained drug release over 72 h. The group in Cairo is investigating nanomedicine for schistosomiasis and filariasis but has not published any data as yet.

In terms of non-PRD nanomedicine-based therapies, Prof. Wole Soboyejo at the African University of Science and Technology (Abuja, Nigeria) is working on nanoparticles for cancer detection and treatment in collaboration with Princeton University, USA (Personal communication). In Ghana, Dr. Ofori-Kwakye and

Dr. Stanley Moffat are conducting basic research in pharmaceutical nanotechnology. Dr. Moffat was recently appointed the African coordinator for USEACANI (US-Europe-Asia Pacific-Caribbean Nanotechnology Initiative).

17.4 Conclusions

The number of discovery programmes for PRDs is too low to ensure a steady stream of treatments on to the market [53]. This is mainly due to the lack of activity from the pharmaceutical industry because refinancing the high development costs will not be profitable. Only 1.3 products are expected to reach the market out of 100 entering the screening phase of drug discovery [53]. These figures indicate that there is an urgent need for new strategies, such as nanomedicine, in drug development programmes for PRDs. Nanomedicine has been successfully applied for treatment of cancer with several products already on the market. Critical properties of nanomedicine systems include protection of instable drugs, cell-adhesion properties, intracellular delivery of drugs and the ability to be surface-modified by conjugation of specific ligands, enabling targeted delivery and controlled release. Thus, nanodrug delivery systems seem to be a promising and viable strategy for improving treatment of PRDs. However, in Africa, there is minimal application of this technology for the treatment of PRDs with only a few groups in South Africa making significant progress. Therefore, serious efforts need to be focused on the exploitation of the potential of applying nanomedicine in drug development for PRDs. We believe this is one way of taking failed leads through commercialisation and ultimately bridging the 90/10 gap. To this end, the DST/CSIR nanomedicine platform is sensitising African researchers and building capacity to include nanomedicine in drug development programmes in Africa.

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