INAUGURAL LECTURE

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Prof Anne Grobler

Nano & micro-delivery systems: the Pheroid® story

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Nano & micro-delivery systems: the Pheroid® story

Prof Anne Grobler

DST/NWU Preclinical Drug Development Platform

1. Abstract

For a drug to have a therapeutic effect, it has to reach its site of action in sufficient quantities. The Pheroid® delivery system enhances the absorption of various pharmacological compounds and biological molecules. The resulting nano- and micro vesicles and -sponges acts as bio-transporters that can entrap hydrophilic, hydrophobic or amphiphilic compounds for biomedical and agricultural application.

The potential use of Pheroid® technology in vaccines, peptide drugs, topical products and cosmeceuticals, antimicrobial treatments and agriculture was investigated. The in vivo absorption and bioavailability, as well as the in vitro efficacy of Pheroid®-based formulations were determined. In a phase 1 bio-equivalence study, a Pheroid®-containing combination for tuberculosis was compared against the comparative market leader. For oral administration, a precursor format, the pro-Pheroid®, was developed, wherein the vesicles and/or sponges are formed post-administration. In all of these areas, the Pheroid® showed applicability: the results showed improved uptake and/or efficacy of the entrapped chemical or biological compound after administration by a number of administration routes.

Based on these in vitro and in vivo results, a number of products are currently under development and eight patents are registered in various countries to protect its applications.

Keywords: delivery system, Pheroid® technology, bio-transporter, anti-infective agents, vaccines, cosmetics, peptides, plants.

2. Introduction

The acceleration in the discovery of new therapeutic moieties (chemical, biological, genetic and radiological) has ensured that there are ample numbers of particularly efficient therapeutic agents readily available to eliminate both chronic and opportunistic diseases. Approximately 40% of new chemical entities exhibit poor and generally variable bioavailability due to their poor aqueous solubility, high hydrophobicity with limited solubility in the aqueous phases of the body and high intra-subject/inter-subject variability and lack of dose proportionality1. For such new candidate drugs to become therapeutically useful, effective delivery systems and dosage forms and formulations to improve the bioavailability of such drugs is needed.

During drug design, predominant attention is often placed on the therapeutic effectiveness achieved, while other aspects such as stability and dosage form are neglected, leading to failure of the drug. Ideally, the bioavailability, biodistribution, pharmacokinetics and the therapeutic effect of the administered moieties need to be predictable and controllable. These factors led to a demand for delivery or transporter systems capable of protecting, transporting, and selectively depositing therapeutic agents at desired sites.
The process of drug delivery encompasses a number of interdependent steps, each with its own characteristic challenges to be overcome. The dosage form is dependent on the site or mode of administration of the drug, which in turn is dependent on the desired therapy and the drug used for the therapy. For a drug to be transported to its site of action, it has to cross biological barriers. These barriers are determined by both the site of administration and the site of therapeutic action. The drug should not be deactivated, degraded or cleared during the transport processes. Once the drug has arrived at its site of action, it has to be released from the delivering biomaterial without losing its efficacy through chemical or stereo-chemical instabilities or interactions with surrounding body components.

3. Methodology

3.1 The design of delivery and transporter systems

The properties of any drug are a composite of the innate activity and properties of the compound as modulated by the formulation in which it is presented. The application of delivery and formulation technologies has not only enabled the therapeutic use of new generation drugs but has also led to the resurrection of older molecules. An understanding of physicochemical and pharmacological properties combined with formulation technologies has been applied to improve absorption (intestinal, buccal and transdermal) and to prolong therapeutic effects.

Delivery systems are used not only to improve the delivery of drugs, but also to improve their water solubility; to decrease their toxicity; to increase their permeability; to protect them from possible enzymatic degradation or hydrolysis; and/or to increase the site-specific delivery of drugs. When developing new drug delivery systems, the main objective is the ability to rationally manipulate the pharmacological profiles of drugs and their concomitant therapeutic indices. Such delivery systems may then be used to modify potential therapeutic agents.

3.2 Pheroid® technology within context

Delivery systems or bio-transporters are extensively applied in biotechnology, as well as in the pharmaceutical, the food and the cosmetics industries. The material used in the manufacturing of delivery systems or transporters should comply with a number of criteria: it should be non-toxic and non-antigenic; biocompatible and biodegradable; the delivery system and active pharmaceutical ingredient (API) should be compatible. Delivery systems and transporters should be versatile and it should be stable. Manufacturing of delivery systems should be simple, reproducible and maintainable. The manufacturing process should preferably be environmentally friendly. All these factors also determine the cost of manufacturing, which is probably the determining factor after efficacy of use.

Pheroid® technology seen within the context of drug delivery and therapy is a complex polydisperse technology, based on colloidal emulsion systems and used for the delivery of pharmaceutical and other compounds. It is composed of an organic carbon backbone of unsaturated fatty acids with some side-chain interactions that result in self-emulsifying characteristics. The resulting vesicles and sponges can be manipulated as to loading ability, mechanical resistance, permeability, size and solubility.
Studies on the Pheroid® have shown that it has several unique advantages: Pheroid® consists of biomaterial that is generally regarded as safe; the material is biodegradable and may even be therapeutic. The raw materials are inexpensive and the manufacturing process is simple and side-steps many of the difficulties generally encountered with the manufacturing of lipid-based delivery vesicles by the generation of the actual delivery vehicle at the point of origin of delivery. In addition, the manufacturing process is environmentally-friendly, using no harsh chemicals and does not contaminate water.

3.3 Pheroid® technology: from concept to product

A conscious decision was taken not to patent the Pheroid® itself but rather to patent applications. The requirements for patenting, i.e. for the generation of intellectual property (or unique knowledge) and the requirements for the development of a medicinal drug are not the same: To generate intellectual property, there has to be generally uniqueness, inventive step and the invention or knowledge must be usable, while the main requirements for the development of medicines are that the product must be effective and it must be safe. Thus if one wants to develop a patentable marketable drug the requirements are a summation of the requirements of the two processes.

The generation of a new concept, technology or platform is usually a valuable asset on the balance sheet of any institution and may be more valuable than individual products flowing from such a technology. Intellectual property (IP) is not tangible and its value lies in the fact that its owner is allowed a 20-year period within which the IP may be used unimpeded by competitors and with the advantages generated by that specific know-how. A patent is property that can be sold; it can also be licensed. The examination of a patent application is more complex and harsh than that usually associated with academia since the grant of a patent recognizes the commercial value of an invention within the context of its time. Furthermore, the grant of a patent can be opposed by outside parties on an academic basis or by parties with a commercial interest.

In developing Pheroid®-based intellectual property and medicinal products, both the criteria for patenting and for drug development had to be met: a product that is effective, safe, unique, inventive and usable. The Pheroid® consists basically of an oil phase, a water phase and a gas phase. The pro-Pheroid® contains no water phase and has no particles; macroscopically it looks like an oil phase. Pheroid® micro- and nanoparticles form spontaneously upon addition of a water phase to the pro-Pheroid® (figure 1). While this spontaneous reaction occurs, the APIs present are packaged into the particles. When the water phase is added externally, it can contain electrolytes and may be buffered.

The pro-Pheroid® system unlocks the potential of this technology for administration routes other than the topical route. It is based on the intrinsic property of hydrated membrane fatty acids to form vesicles and/or other lipid aggregates on dilution with water. Pro-Pheroid® is especially important in the case of drugs or APIs that are unstable in the presence of moisture, such as rifampicin. In the case of Pheroid®, the ingredients of the water phase contribute to the final packaging.
3.4 Various applications of the Pheroid®

3.4.1 The use of the Pheroid® in infectious disease therapy

The development of Pheroid®-based pharmaceuticals for use in combating infective organisms i.e. all compounds that either destroy or inhibit the growth of microscopic and sub-microscopic organisms; including the antimicrobial agents, the antihelmintic agents and the anti-ectoparasitic agents, was investigated. The aim was to research the potential of Pheroid® technology for the development and production of quality, accessible cost-effective medications for the treatment of non-resistant and drug resistant infectious diseases. The objectives were to develop stable anti-infective pharmaceutical preparations, a method of manufacturing and a dosage form with an improved therapeutic effect and index; and to endeavour to reduce the treatment time and side effects of current treatment regimens.

Tuberculosis (TB) was chosen as the primary model for the investigation into the enhancement of drug efficacy. It is caused by a bacterium, Mycobacterium tuberculosis, one of the Mycobacterial complex species. Tuberculosis has been infecting humans for the past 4,000 years and although the prevalence of tuberculosis has been steadily declining in developed countries, the same is not true for developing countries. Africa alone is estimated to have approximately 170 million TB patients. Various sources estimated that between 2000 and 2020...
nearly 1 billion people will be newly infected with TB, 200 million will become sick and 35 million will die. Even discounting the financial consequences of this disease for patients and their families, the cost to the healthcare systems and national economies is estimated to be US$16 billion annually – $4 billion for the costs of diagnosis and treatment and $12 billion from lost income.

The present treatment regimens for tuberculosis require extended periods of chemotherapy for at least 6 to 9 months of treatment with severe side effects, resulting in low compliance. Multidrug resistance (MDR and XDR) is also of concern. While TB may not be the main cause of mortality, in combination with HIV/AIDS its impact on mortality and morbidity is fearsome. In addition, we wanted to develop TB treatment formulations that will prevent drug-drug interactions by entrapment of some of the compounds in Pheroid®.

The API’s used in the treatment of TB are well-known, including its mechanisms of action, potential side effects and expected pharmacokinetic (PK) and pharmacodynamic (PD) parameters as well as therapeutic requirements. The treatment regimens for both drug sensitive and drug resistant TB are problematic and the number of regimes available is limited, especially in resource restricted settings.

The investigation addressed two of the three drug development stages, i.e. formulation, preclinical or clinical development and where necessary, use was made of outside expertise. The individual studies of the investigation and site of investigation are shown in table 1 below.

### Table 1: The studies performed and institutions/companies involved

<table>
<thead>
<tr>
<th>Infectious agent</th>
<th>Formulation characteristics, dosage forms</th>
<th>Preclinical</th>
<th>Clinical</th>
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<tr>
<td>Tuberculosis</td>
<td>(i) Formulation studies; (ii) Oral dosage form studies, including disintegration of capsules (iii) Membrane diffusion and release studies (iv) Stability of oral dosage form</td>
<td>(ii) <em>In vitro</em> studies at the US/MRC Centre for Molecular and Cellular Biology (ii) <em>In vivo</em> studies at MeyerZall Laboratories</td>
<td>Phase I healthy volunteer trial, including (i) Bioavailability parameters; (ii) Safety parameters; (iii) PK/PD modeling (iv) <em>In vitro/in vivo</em> correlation studies</td>
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### 3.4.2 The use of Pheroid® in vaccines

An indirect relationship exists between vaccine immunogenicity and safety. Human immune responses to peptide vaccines administered with standard adjuvants tend to be poor; hence there is an urgent need for effective vaccine adjuvants to enhance the immunogenicity and immunostimulatory properties of vaccines. Adjuvants can be broadly separated into two classes, namely immunostimulatory or -modulatory adjuvants and vaccine delivery systems. Few described adjuvants have been shown to be comparable to aluminium hydroxide-adjuvanted parenteral vaccines in terms of efficacy, safety and ease and cost of manufacturing.

The Pheroid® delivery system is *per se* an adjuvant and was investigated to determine whether it enhances vaccine efficacy; to evaluate the different routes that can be used to administer Pheroid®-based vaccines; to determine the humoral (IgG) and mucosal (IgA)
responses to Pheroid®-based vaccines in comparison to the aluminium-adjuvanted vaccines and to establish some correlates of protection of Pheroid®-based vaccines. The specific vaccines used as models in this investigation are a rabies vaccine, a diphtheria vaccine and a hepatitis B vaccine.

At least 55 000 people die from rabies annually, more than 10 million receive post-exposure rabies vaccination, whilst more than 2.5 billion people live in regions where rabies is endemic. Infection of humans from rabid animals is almost invariably fatal once signs of disease occur. Animal studies were used to measure immune responses of protection against intracerebral inoculation of a challenge virus in mice. The study was performed in concert with the then SA State Vaccine Institute, since that institute was equipped to handle the live and activated rabies virus used as antigen in the study. Various vaccine formulations, using inactivated rabies viruses as antigens, were prepared. The commercially available rabies vaccine, in which alum is used as adjuvant, acted as positive control for the Pheroid®-based vaccine, whilst phosphate buffer were used as negative control. All experiments were performed and results analysed according to the specifications of the World Health Organization (WHO).

Pheroid® was also investigated as vehicle for the nasal and oral delivery of the diphtheria toxoid (DT) as antigen. Immune responses were compared by measuring neutralizing antibodies against DT in pathogen-free experimental animals. Alum-based parenterally administered DT of equal dosage was used as reference in a well-described mouse model. The IgG titers (systemic immune response) and IgA titers (local immune response) were determined by enzyme linked immunosorbent assays (ELISA).

3.4.3 Other studies

The application of the Pheroid® to the delivery of peptide drugs and agricultural compounds were similarly investigated but due to space constraints, these studies will not be described.

4. Results

4.1 Pheroid® technology development

Pheroid® can be used to transfer molecules by a number of administration routes, including oral, nasal or transdermal routes. Pheroid® vesicles and sponges show high cell penetration characteristics and the pro-Pheroid® shows potentiating capabilities. Pheroid® does allow targeting to specific cell surface receptors for uptake by these cells. As shown in figure 3, various types of Pheroid® carriers were custom-made for different applications and compound types.
Figure 3: Different types of Pheroid® were prepared by changing components and the manufacturing process.

Pheroid® has also been shown to be stable, both in terms of shelf life and in body fluids, solving one of the main production problems of peptide drugs and gene delivery.

4.2 Various applications of the Pheroid®

4.2.1 Results on the use of the Pheroid® in infectious disease therapy

Dosage forms used were limited to suspensions and liquid gelatin capsules. Because of the incompatibility between 3 of the generally used TB drugs, two novel Pheroid® combinations for tuberculosis were formulated: Formulation Pyrftol RP (containing rifampicin and pyrazinamide) and Pyrftol IE (containing isoniazid and ethambutol) a shown figure 2.

Figure 2: Pyrftol P (green) and Pyrftol C (red) soft (left) and hard (right) gelatin capsules containing the four tuberculosis drugs. The daily dosage for an adult is 2 Pyrftol P and 3 Pyrftol C capsules. According to all efficacy and bioavailability analysis these capsules should be more effective and safer than currently available products. It is also a unique product in that rifampicin was separated from isoniazid to prevent drug-drug incompatibility.
Entrapment of tuberculosis’ drugs in Pheroid® resulted in:

- A significant increase in the drug plasma levels of the rifampicin and isoniazid despite administration of only a 60% dosage;
- Quicker absorption, with generally decreased faster response;
- Decreased dosage required for the same effect;
- Increased duration of the therapeutic window;
- Reduced side effects with prospects of better compliance.

A Pheroid®-based anti-tuberculosis product deserves investigation. The in vitro/in vivo correlations suggest that it may be possible to increase the interval between dosages and decrease the dosage and the duration of treatment may well be shortened. Such an evaluation would be the primary objective of a phase 2 trial.

Similar results were found for Pheroid®-based antimalarial and antiretroviral formulations and have led to collaborations with international players, such as the Swiss Tropical and Public Health Institute and Medicines for Malaria Venture (MMV).

4.2.2 Results on the use Pheroid® for the delivery of vaccines

The WHO requires that the antibody titre elicited by a rabies vaccine should be 2.5IU/ml. This value was easily surpassed by the Pheroid®-adjuvanted vaccine: The Pheroid® rabies vaccine showed a 9 fold increase in antibody response in comparison to the conventional commercial vaccine. This study was repeated four times with similar results.

Similarly, the Pheroid®-adjuvanted diphtheria formulations showed enhancement in vaccine efficacy. The immune responses obtained from both oral and nasal Pheroid®-based vaccines were comparable with that found for the alum-adjuvanted parenteral administration. The size of the particles used had an effect on the antibody response. The Pheroid® seems to have a dual role in vaccines; firstly as delivery system for disease specific antigens, and secondly as immuno-stimulatory adjuvant. It also complies with international requirements in terms of safety.

5. Conclusion

Results from various studies showed that the Pheroid® technology is a platform technology with potential application in infectious diseases, such as TB, malaria; chronic diseases, such as cancer, diabetes, osteoporosis, inflammation and pain; vaccines, and agriculture. Space constraints prevent results from all studies to be presented. The bioavailability and in some cases the efficacy of the active compounds in the Pheroid® was at least as good but mostly significantly better than that of the comparative medication. Entrapment in Pheroid® may protect labile drugs such as peptides against degradation. The incidence of side-effects, where evaluated, was decreased in the case of the Pheroid®-based formulations.

The results have resulted in a push for commercialization by a number of companies in South Africa (Nelesco 883 Pty Ltd), Germany (Agraforum AG), Pasture Peak Holdings (New Zealand) and Deltamune (South Africa). In addition, strong collaborations have been established with institutions such as the Swiss Tropical and Public Health Institute and Medicines for Malaria Venture.
Pheroid®-technology proved to be nearly limitless in its scope in the chemical and biological compound industry. Development of this technology should lead to substantial revenues from transfer of technology and the strengthening of the local pharmaceutical and bio-agricultural industry. While the long term goal is the commercialisation of effective and affordable pharmaceutical products for a number of application, one of the most important contributions of this project has been the mind shift of our scientists as it relates to our ability to conceptualize and develop a very high quality pharmaceutical products.

References