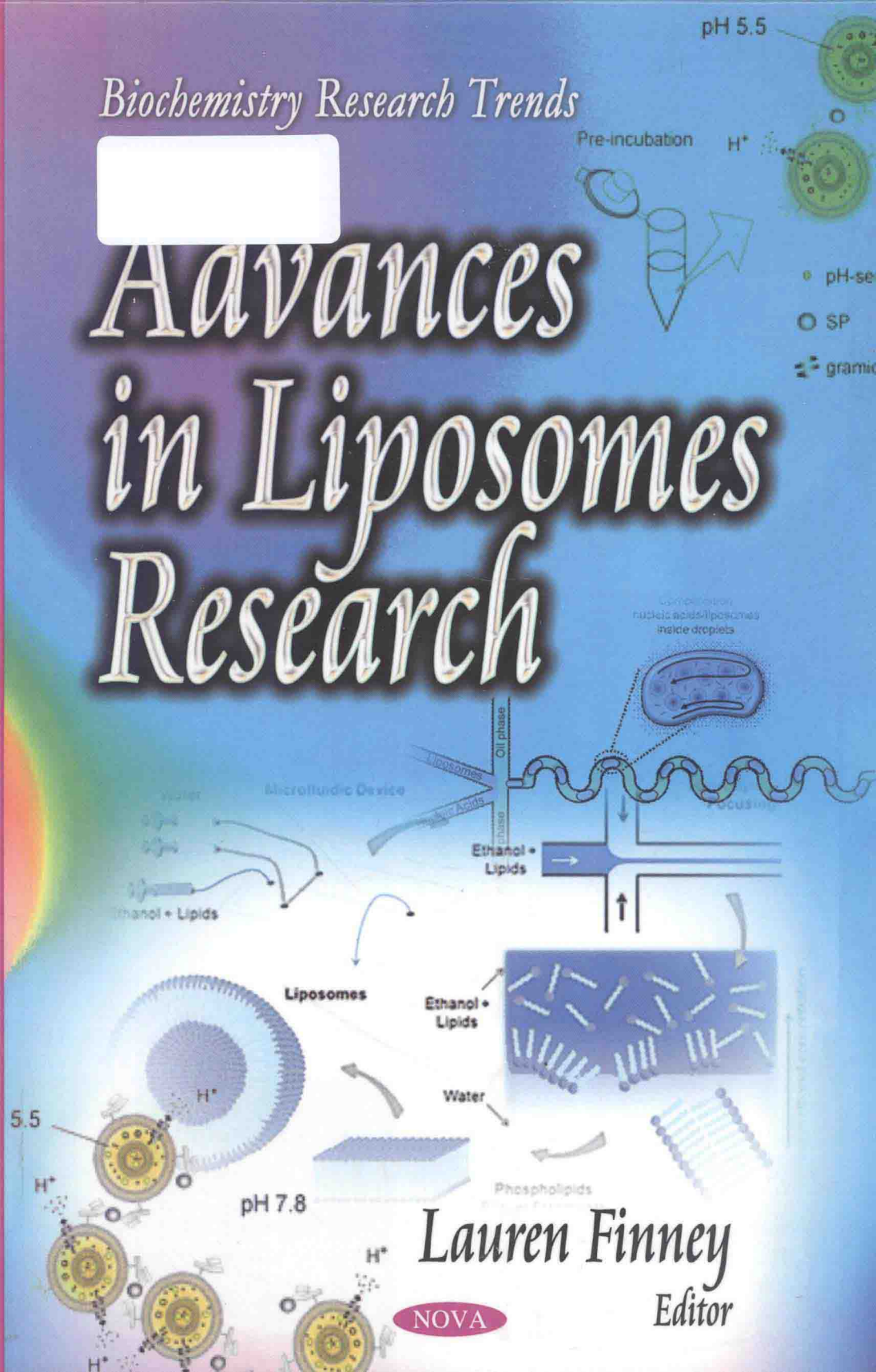
Biochemistry Research Trends

Advances in Liposomes Research



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Editor

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ADVANCES IN LIPOSOMES RESEARCH

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PREFACE

In this book the authors present current research in the study of liposomes. Topics discusses in this compilation include liposome mediated malaria vaccine development; liposomal delivery of antimicrobial agents in advances in liposome research; trends on microfluidic liposome production through hydrodynamic flow-focusing and microdroplet techniques for gene delivery applications; liposomes as important drug carriers in cancer therapy; liposome application in the veterinary field; and design of liposomes with a pH-sensitive fluorescent dye and gramicidin channels for immune-sensing.

Chapter 1 - The introduction of vaccine technology has facilitated an unprecedented multi-antigen approach to develop an effective vaccine against complex systemic inflammatory human malaria (*Plasmodium falciparum*). The capacity of multi subunit DNA vaccine encoding different stage Plasmodium antigens to induce $CD8^+$ cytotoxic T lymphocytes and interferon-responses in mice, monkeys and humans has been observed. The cytotoxic T cell responses are categorically needed against intracellular hepatic stage and humoral response with antibodies targeted against antigens from all stages of malaria parasite life cycle. As genetic vaccination is capable of eliciting both cell mediated and humoral immune responses, the key to success for any DNA vaccine is to design a vector able to serve as a safe and efficient delivery system. This has encouraged development of non-viral DNA-mediated gene transfer techniques such as liposome, virosomes, microsphere and nanoparticles. The efficient and relatively safe DNA transfection using lipoplexes makes them an appealing alternative for gene delivery. In addition, liposome entrapped DNA has been shown to enhance the potency of DNA vaccines, possibly by facilitating uptake of the plasmid by antigen-presenting cells (APC). The control of residual non-adaptive immune effecters (mainly

wandering macrophages and polymorphonuclears) by clodronate-loaded liposome in NSG immunodeficient mice further advocates their value for translational biomedical research.

Chapter 2 - Chronic and slow/non-healing wounds require extensive management to reduce the repair and recovery time. Wound dressings and devices are often designed to suit varying wound characteristics and strategically manage the complexity of different wound types. The main challenges in managing the chronic wound environment include:

- delivery of sufficient antimicrobial agent to maintain bioavailability at biocidal concentrations
- control of the quantity of wound exudate whilst promoting/ maintaining the availability of pro-healing factors
- reduction of the risk of uneven antimicrobial deposition, lowering the risk of localized toxicity
- improvement in the ease of antimicrobial and wound dressing application
- reduction of the frequency of dressing changes thus minimising patient discomfort and avoid risk opportunity for further infection

Topical administration of agents can require penetration through dead matter, purulent exudates and scar tissue as well as the dermis, which serves as the first line of defence. The dermal barrier has low permeability to large hydrophilic entities but will selectively allow permeation of small lipophilic molecules. Whilst essential for maintenance of host homeostasis, this limited permeability dramatically restricts delivery of many antimicrobial agents both to the wound surface as well as into the various layers of the dermis. These issues can be reduced with the aid of controlled release drug delivery systems such as liposomes, which can improve targeting, efficacy and the biopharmaceutical properties of the antimicrobial agent. Liposomes are biocompatible, biodegradable, lipid bilayer vesicles with a large aqueous inner-core for encapsulation and delivery of active agents. Encapsulation of antimicrobial agents in liposomes provides protection from enzymatic and immunological inactivation. Additionally, the liposome's capacity to bind water may aid moisture retention, which promotes an environment that is highly conducive to tissue repair. The capacity to transport both hydrophilic and hydrophobic materials, has allowed a wide range of pharmaceutical formulations to be incorporated into liposome vesicles. In terms of encapsulation, agents with varying lipophilicities can be sequestered within the

phospholipid bilayer (hydrophobic), entrapped in the inner core (hydrophilic), as well as in the inner and outer bilayer interface (hydrophilic) of the liposome. This ability of liposomes to encapsulate antimicrobial agents with a broad range of physicochemical properties makes them valuable in wound management applications. This chapter will examine the diverse antimicrobial payloads, including antibiotics, antifungals, natural products and essential oils, which are amenable to liposome delivery and show enhanced therapeutic outcomes. The advantages of liposome encapsulated antimicrobials are their potential to achieve effective drug delivery whilst reducing problems related to targeting, biodistribution and bioavailability of microbiocidal agents.

Chapter 3 – Recent studies on liposome production for gene delivery have aimed to obtain final formulations with physicochemical properties, like size and polydispersity in optimum ranges for biological applications without the need for post-processing steps. In this context, microfluidics emerges as a promising technology to overcome the major challenges of the pharmaceutical industry, especially for gene delivery purposes. In this field, microfluidic hydrodynamic focusing (MHF) technique and microfluidic based in droplets were used for liposomes production and to complex liposomes with nucleic acids. Essentially, MHF technique is composed by a central organic stream of lipids dispersed in alcohol hydrodynamically focused by two adjacent aqueous streams; the water-alcohol diffusion along the main microchannel forms instability regions for the phospholipids that self-assemble into vesicles. Additionally, MHF technique allows the formation of monodisperse nanosized liposomes in one-step for a variety of applications. The other technique, microfluidic based in droplets, was described as an innovative and promising technique. Basically, the droplet contains the aqueous phase with liposome and nucleic acid and it is stabilized in the oil phase by a surface active compound. The main advantage of microdroplet platform is to allow more rapid mixing, generating complex with smaller size and polydispersity, forming the aggregates inside microsized droplets in emulsions. Thus, in this chapter, we summarize recent studies and trends on microfluidics liposomes context about liposomes in the microfluidic techniques context, for production of liposomes through the MHF and microfluidic droplet techniques emphasizing future applications in the gene delivery field. We hope that the main findings of the state-of-the-art disclosed herein can be useful for rational use of microfluidics devices for liposome production for formation of nonviral gene delivery systems.

Chapter 4 – Normal human cells grow, multiply and die, by it self control process. When there is loss of this cell control it start a fast and reproducible

division that may lead to the invasion to other normal cells and tissues. This uncontrolled growth and aggressive process, in most of the cases, featuring a set of more than 100 different types of diseases called normally as cancer. Among the forms of treatment for cancer in general, chemotherapy, radiotherapy and surgery are the most acceptable and used, (alone or combined), however, early diagnosis is essential for effective healing or eradication of the diseases. These treatments do not affect only the damaged tissue and cause undesirable side effects such as hair loss, nausea and other, increased risk of infections, asthenia, intestinal obstruction and even mutilation once in some cases surgery compromise patient life, may lead to irreversible psychological damage. Therefore there is a need for new therapeutic methods that enable reduction of the tumor and restricting adverse effects to diseased tissue while protecting the healthy tissue. These could be archive by the use of specific drugs in the conventional treatments as well as with new therapeutic approaches whose goal is to reducing the tumor size and spread process and with protocol minimally invasive with restriction of adverse effects in the diseased tissue what is essential for achieving a successful outcome. Liposomes as Drug delivery system have been studied over the last 3 decades as DDS with special appeal when applied to anti-cancer therapy and to treat many other diseases. Standing out among the nanosized systems for drug delivery because they allow the incorporation of hydrophobic drugs in the lipid bilayer and hydrophilic drugs in the aqueous phase, while maintaining their physical and chemical characteristics, as well as promoting their selective distribution in tissues when incorporated efficiently to liposomes. This selective distribution in tissues is greater in the case of cancer cells, since these have high metabolism and require higher nutrient. This chapter provides a general approach with the discussion of the liposome research focusing in cancer treatment and the introduction of two new liposomal system applied in combination with a an alternative protocol know as photodynamic therapy (PDT), one of them containing cisplatin - conventional chemotherapeutic - for synergistic effect with the chloro-aluminum phthalocyanine and other containing folic acid vetorizador of the drug to treat mamarin and other visceral cancers.

Chapter 5 – In the field of veterinary medicine, liposomes are the most widespread nanotechnological tool. Already for some time, they have been applied in animal therapies to improve delivery of different drugs, comprising analgesic, antiviral, antimicrobial, antifungal, and anticancer agents. More recently, with the rise of recombinant DNA technologies, liposomes have been included in veterinary vaccine formulations to entrap antigen-coding DNAs,

siRNAs, peptides, and recombinant antigens, as well as effectors of the innate immune system, in order to elicit protective responses against viruses, bacteria and parasites. Reported applications include their use in companion and productive animals, among others horse, dog, cattle, poultry, and fish. Liposomes are generally well tolerated and, in accordance with their purpose, may be delivered through the intramuscular, subcutaneous, intravenous, ocular, and/or intranasal route to their desired target. In order to achieve a more specific tissue targeting, engineering of liposomes has also been described in the veterinary field. Besides therapeutics, liposomes have also been applied in transfection technologies and in the cryopreservation of stallion or bull semen. Use of liposomes can be limited by the high manufacturing costs of lipid synthesis or purification. Therefore, the formulation of low-cost liposomes made of non-purified lipid mixtures will open the possibility of large-scale applications in animal productive systems. This chapter presents an overview on possibilities, advantages, and perspectives of liposome employment for veterinary use.

Chapter 6 - In this review, the authors describe a liposome array for direct fluorometric immunoassay using liposomes encapsulating a pH-sensitive fluorescent dye, BCECF ([2',7'-bis(carboxyethyl)-4 or 5-carboxyfluorescein]). The method has a signal amplification system based on modulation of channel kinetics of gramicidin, which forms a nanopore in a lipid bilayer and allows permeating monovalent cations. The detection of analytes is performed without any lysis of liposomes and labeling with a fluorescent molecule. Instead, immunoreactions between analyte and $F_{ab'}$ fragment linked to liposomes are monitored though a fluorescence change of the encapsulated dye, which depends on the channel activity of gramicidin. The assay is simple, rapid and highly sensitive. The method allowed quantification of substance P (SP), neurokinin A, growth-hormone-related peptides, and streptolysin O (SLO) at sub-pg to ng level. The highly sensitive assay was applied to detection of SP and SLO in human serum by simply diluting the sample 125 times (0.8% human serum). The method has the potential of applying it as a bioanalytical technique for clinical analyses and diagnoses.

CONTENTS

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Chapter 1

LIPOSOME-MEDIATED MALARIA VACCINE DEVELOPMENT: MORE THAN A TOUR DE FORCE

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ABSTRACT

The introduction of vaccine technology has facilitated an unprecedented multi-antigen approach to develop an effective vaccine against complex systemic inflammatory human malaria (*Plasmodium falciparum*). The capacity of multi subunit DNA vaccine encoding different stage Plasmodium antigens to induce $CD8^+$ cytotoxic T

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lymphocytes and interferon-responses in mice, monkeys and humans has been observed. The cytotoxic T cell responses are categorically needed against intracellular hepatic stage and humoral response with antibodies targeted against antigens from all stages of malaria parasite life cycle. As genetic vaccination is capable of eliciting both cell mediated and humoral immune responses, the key to success for any DNA vaccine is to design a vector able to serve as a safe and efficient delivery system. This has encouraged development of non-viral DNA-mediated gene transfer techniques such as liposome, virosomes, microsphere and nanoparticles. The efficient and relatively safe DNA transfection using lipoplexes makes them an appealing alternative for gene delivery. In addition, liposome entrapped DNA has been shown to enhance the potency of DNA vaccines, possibly by facilitating uptake of the plasmid by antigen-presenting cells (APC). The control of residual non-adaptive immune effecters (mainly wandering macrophages and polymorphonuclears) by clodronate-loaded liposome in NSG immunodeficient mice further advocates their value for translational biomedical research.

1. Background: The Burden of Malaria

Malaria continues to present a major health challenge in many of the poor countries in the world, with 225 million cases leading to an estimated 781,000 deaths in 2009 [1]. Numerous efforts towards control and eradication of this disease are directed at different areas including insect vector control, vaccine development, and the discovery of new therapeutic drugs. Although, battle to control malaria has been fought on several grounds including improved methodologies of diagnosis and chemo prophylaxisas well as integrated vector control through various physical methods such as treatment with insecticide and house spraying [2], prevalence and resurgence of malaria continues to persist because of drug resistant parasites and insecticide resistant vector [3, 4]. Therefore, due to this bleak situation, the need to develop additional control measures such as malaria vaccine is both attractive and urgent. The malaria vaccine is still elusive despite of enormous and continued efforts on to develop an effective vaccine [5]

There are number of existing approaches to malaria vaccine based on attenuated sporozoite, synthetic and recombinant immunogenic peptides. These strategies have proved significant in terms of safety, duration of immunity and specificity [6-10]. As vaccines based on live, attenuated malaria parasites, are economically and technically not feasible, malaria research focuses on recombinant or synthetic subunit vaccines. The optimal vaccine

should have the ability to elicit protective immunity blocking infection, prevents pathology and blocks transmission of parasite. Therefore, combination vaccine consisting subunits from different stage of the parasite would meet all these requirements. The progress in developing a malaria vaccine is not going up to that pace as it was expected after the complete genome sequencing of *P. falciparum* [11], perhaps, in part because of the larger genetic diversity of *plasmodium* parasite. Thus identification, expression and degree of variability of candidate vaccine antigens render it more complex to understand various biological processes of parasite. The complex life cycle of malaria parasite and antigenic diversity are the barriers associated with vaccine development [12].

2. Biology of Malaria Parasite: Approaches for Effective Immune Interventions

The causative agent of malaria parasite has a complex multi-stage life cycle involving both primary (mosquito) and secondary (human) hosts in different cellular environments (intra and extracellular) in which the parasite develops. The disease in humans is caused by one or a combination of four species of Plasmodia: *P. vivax, P. falciparum, P.malariae,* and *P. ovale.* Also, in geographically limited zones of South-East Asia, the Malaysian island of Borneo in particular, infections by *P. knowlesi* as a zoonose have been known to occur [13-15]. While it remains a possibility, there does not appear to be any evidence to indicate that infections of this "fifth human malaria parasite" can be transmitted from humans to other human hosts [16] and hence they are not considered to be important in terms of public health outside these zones. Malaria parasite has a large genome of 14 chromosomes comprising 26-30 mega bases encoding around 5000-6000 proteins [17, 18]. Most of the *Plasmodium* strains have a complex life cycle that begins when a female mosquito injects sporozoites into the skin of an individual at the time of blood meal. After differentiation and passing through various forms, parasite produces thousands of merozoites that are released from the hepatocytes and rapidly invade circulating erythrocytes. The rupture of infected erythrocytes in the blood circulation release pigments initiating malaria related symptoms (Figure 1).

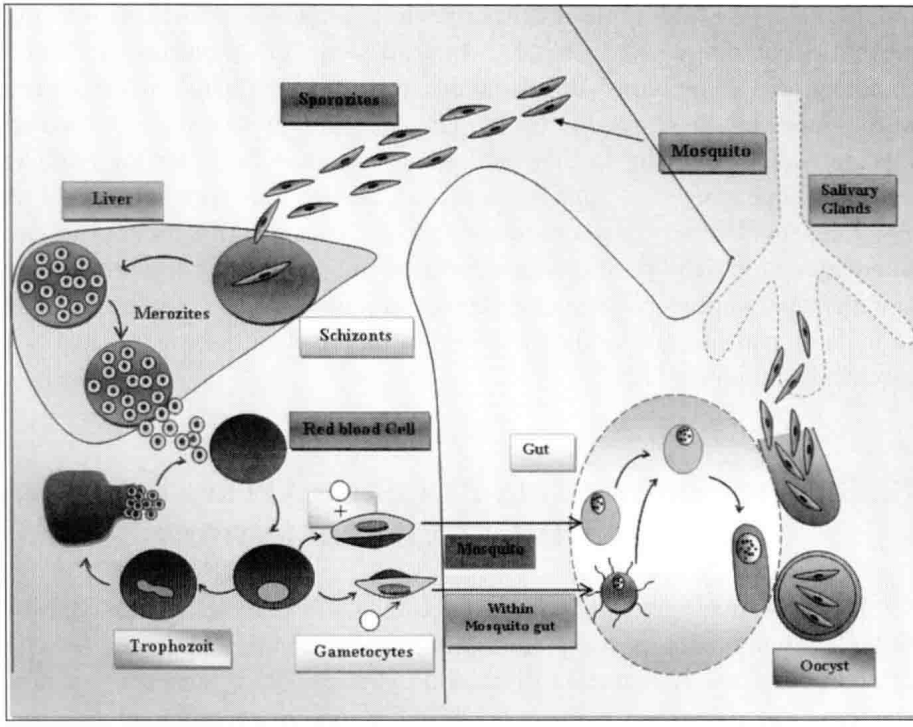

Figure 1. Life cycle of malaria parasite.

3. Existing Malaria Vaccines

Although the complexity and genome variability of the parasite hampers the development of a universal, effective and long lasting vaccine, the feasibility of malaria vaccine is supported by the several lines of evidence. The repeated exposure of malaria develops natural immunity against un-wanted clinical manifestations of infection [19]. In addition, passive transfer of antibodies and immune effectors from an immunized animal to the susceptible host induces protection against malaria infection [20].

3.1. Pre-Erythrocytic Vaccines

The pre-erythrocytic vaccine is aimed to prevent the entry of sporozoites into the hepatocytes and their development into tissue schizonts. The clinical