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The Mucosal Immune System: Modulation by Microemulsion

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1. Introduction

Microemulsion techniques are capable of delivering organic and inorganic nano-sized particles with minimal agglomeration because the reaction occurs in nano-sized domains. However, microemulsion techniques present several disadvantages, including the use of a large amount of oil and surfactant and low yield. Nevertheless, microemulsion techniques are an alternative method for synthesising several types of inorganic and organic nano-sized particles[1, 2, 3].

The use of microemulsions as vehicles for pharmaceutical preparations and drug release systems has aroused significant interest due to their potential advantages[4], which can be attributed to their ability to improve the solubility of hydrophilic and lipophilic substances and their capacity to increase the stability and absorption of drugs, allowing greater bioavailability[5].

Furthermore, microemulsions can provide sustained drug release by compartmentalising active substances within structures organised by surfactants, water and oil, allowing the slow and continuous release of drugs over a long period of time[6].

Microemulsion is a suitable technique for obtaining nanometre-sized inorganic particles with minimal agglomeration[7]. Microemulsion is the thermodynamically stable, transparent, isotropic dispersion of two immiscible liquids such as water and oil, which are stabilised by surfactant molecules at the water/oil interface. In water-in-oil microemulsions, nano-sized water droplets are dispersed in the continuous hydrocarbon phase and are surrounded by a monolayer of surfactant molecules[7]. The diameter of aqueous droplets is usually 5–20 nm[1,8]. The aqueous droplets act as a microreactor or nanoreactor, and reactions occur when droplets containing suitable reactants collide with each other. First, precursor particles of hydroxide or oxalate are formed in a microemulsion system. After drying and calcination of the precursor powder at an appropriate temperature, the desired oxide system is obtained.

A number of biological barriers are present between the application site and the place where substances exert their pharmacological effect; thus, many pharmacologically active substances fail to achieve the appropriate concentration in the target tissue, and normal tissues of the body are exposed to the effects of potentially toxic substances. The mucosal immune system is a biological barrier that allows the passage of substances under certain conditions.

Microemulsions may interact with the stratum corneum and disorganise the lipid bilayer due to the presence of surfactants. Thus, these lipids pass as a fluid in a disorderly fashion, increasing the permeability of skin. As a result, the penetration of substances is facile. The control of drug release by microemulsions is an important advantage when the active compound seeks specific targets and therapeutic action must be extended.

The mucosal barrier control is important for immunomodulatory effects. The gastrointestinal and respiratory tract are the most important sites of infection and can act as open windows that microorganisms can use for the entrance, invasion and colonisation of tissue[9]. Mucosal surfaces represent an important physical-biological barrier between internal and external *millieu*[10]. During feeding and respiration, the body comes into contact with allergens, harmful substances, and opportunistic and pathogenic microorganisms. Notwithstanding, the body-environment equilibrium is almost perfect, and the state of illness is exceptional.

Intestinal mucosa is colonised by numerous bacteria derived from more than 500 different species[11]. Despite high bacterial colonisation and frequent allergen contact, acute inflammatory and allergic reactions are rarely observed in the mucosa[12].

Gastrointestinal associated lymphoid tissue (GALT), which is composed of discrete inductive and effector sites, is able to discriminate between harmful and harmless antigens while maintaining homeostasis.

Intestinal epithelial cells are the major producers of multiple peptides and proteins with antimicrobial activity in the intestine, which are key effector molecules of innate immunity[13]. The differential expression of diverse antimicrobial proteins in the gastrointestinal tract suggests that these proteins have distinct functional niches in mucosal innate defence, allowing the pharmacological exploitation of their antimicrobial properties[14].

2. Microemulsion

In 1943,[15] Hoar and Schulman described water-in-oil microemulsions, which they referred to as transparent water-in-oil dispersions. Subsequently, these systems have been investigated by others authors,[16] who calculated the size of droplets found in microemulsions.

Microemulsion is a suitable technique for obtaining nanometre-sized inorganic particles with minimal agglomeration[29]. Oxide and carbonate nanoparticles have been successfully synthesised by microemulsion techniques[17, 18, 19, 20]. A microemulsion is a thermodynamically stable, transparent, isotropic dispersion of two immiscible liquids (such as water and oil) that are stabilised by surfactant molecules at the water/oil interface. In water-in-oil microemulsions, nano-sized water droplets are dispersed in the continuous hydrocarbon phase and are surrounded by a monolayer of surfactant molecules[21].

Microemulsions improve therapeutic efficacy, reduce the volume of the drug delivery vehicle, mimimise toxic side effects[16,22] and improve immunological response[5,23,24]. In addition to these advantages, microemulsions are easy to administer and offer several benefits for oral administration. Namely, microemulsions are ideal for the oral and nasal delivery of drugs and vaccines[25,26,27]. Microemulsion techniques are capable of delivering inorganic and organic nano-sized particles with minimal agglomeration because the reaction occurs in nano-sized domains.

However, the major disadvantage of using microemulsion formulations as vehicles for drug delivery systems is the high concentration of surfactant and cosurfactants required to create these formulations. In microemulsion formulations used for technological applications in oil recovery, nanoparticles are composed of incompatible surfactants, cosurfactants, and oils that are compatible with human physiology and are non-irritating. Phospholipids, particularly lecithin, are nontoxic alternative emulsifiers that can be biocompatible[22]. Biocompatible surfactants and cosurfactants used in pharmaceutical microemulsions are important technological challenges for the development of adequate delivery systems that can be used in pharceutical formulations and vaccines for treating diseases and improving the immune system.

3. The mucosal immune system

In general, mucosal tissues are a specialised immune network composed of inductive and effector sites. The latter include lamina propria mucosae, the stroma of exocrine glands and surface epithelia, whereas inductive sites comprise mucosa-associated lymphoid tissue (MALT) and local and regional draining lymph nodes. The histological architecture of MALT is similar to the structure of the lymph nodes; however, MALT lacks afferent lymphatics. Antigens are captured and processed directly from the mucosal luminal side through specialised follicle-associated epithelium (FAE) containing microfold or membrane (M) cells. These cells deliver antigens to MALT antigen presenting cells (APCs), which are able to stimulate naive B and T cells[11].

Intestinal mucosa is one of the primary tissues that comprise the mucosal immune system and possess the highly elaborate architecture of the intestinal system. Intestinal mucosa is constantly challenged with the considerable task of allowing the exchange of nutrients, ions and liquids across the intestinal epithelium in the presence of an enormous density of potentially harmful luminal antigens and microbes[27].

Intestinal mucosa constitutes a barrier equipped with local defence mechanisms against invading pathogens; however, the mucosa must be selectively permeable to allow the uptake of nutrients. This dual function becomes even more challenging when we consider that the intestinal lamina propria contains a large number of immune cells with potent effector functions[26]. The unique architecture of the gastrointestinal tract facilitates both of these functions. In particular, the gastrointestinal tract possesses multiple levels and an immense overall surface area, allowing maximal nutrient absorption while housing the largest number of immune cells in the body[27].

The recognition of invading microorganisms is paramount to the survival of the host, and the innate immune system has evolved as the first line of defence in the immune response. At the mucosal surface, the host physically interacts with a nonsterile environment, and the ability to detect and contain invading pathogens is regularly tested[27.28].

Gastro-intestinal associated lymphoid tissue (GALT), which is composed of discrete inductive and effector sites, is able to discriminate between harmful and harmless antigens while maintaining homeostasis. Inductive sites are organised into specialised aggregations of lymphoid follicles called Peyer's Patches (PP), while effector sites are more diffusely dispersed[28,29].

The separation of these sites limits and controls immune responses. The human gastrointestinal tract is colonised by an abundance of bacteria, which are in constant interaction with the epithelial lining, leading to an intricate balance between tolerance and immunological response. Ample evidence suggests that the abundant presence of bacteria plays a role in the maintenance of human health and the induction of chronic inflammatory diseases of the gastrointestinal tract[30].

4. Defence of innate and adaptive immunity against infections

The mucosal barrier is formed by highly adapted epithelial cells, which are interconnected with tight junctions and are covered with mucus and bactericidal peptides. As a result, only a small number of bacteria are allowed to penetrate the intestinal epithelium. Primary defects in the barrier function can trigger intestinal inflammation[13, 31]. Mucosal surfaces have an efficient anti-infective protection system, which consists of many nonspecific mechanisms such as peristaltic movements and the transportion of mucus-fimbri, mucosal enzymes and antimicrobicidal proteins.

The mucosa surface presents intestinal epithelial cells, especially paneth cells. Paneth cells are the major producers of multiple peptides and proteins with antimicrobial activity in the intestine, which are key effector molecules of innate immunity. The most abundant and diverse antimicrobial proteins are the defensins, which are highly microbicidal in vitro and are likely important in vivo. However, the physiological functions of defensins remain incompletely understood. Paneth cells also produce cathelicidin, which contributes to the mucosal defence against epithelial-adherent bacterial pathogens, helps to set a threshold for productive infection, and is expressed constitutively by neutrophils, mast cells and differentiated epithelial cells in the colon and stomach but not the small intestine[32].

In contrast, adaptive mechanisms are represented mainly by the mucosal immune system (MALT, mucosal associated lymphoid tissue). This system consists of immuno-competent cells that infiltrate the mucosae, nodules that form lymphoid structures such as Peyer's plates and its equivalent in bronchial mucosae, and regional lymph nodes such as the mesenterics[32].

Under normal conditions, human gut mucosa is infiltrated by a large number of mononuclear cells due to the continuous stimulation of luminal antigens[33]. Approximately 70% of all lymphocytes in the human body are concentrated in the intestinal intra-epithelial and subepithelial layers, and the largest pool of tissue macrophages is located in the intestinal wall[30].

Mucosal tissue possesses the largest activated B-cell system of the body and contains 80–90% of all immunoglobulin (Ig)-producing cells. The major product of these lymphocytes is secretory (S) IgA (mainly dimers) with associated J chain[18]. Polimeric IgA contains a binding site for polymeric Ig receptor (pIgR) or secretory component (SC), which is required for their active external transport through secretory epithelia. When produced by local lymphocytes, the pIgR/SC binding site depends on the covalent incorporation of the J chain into the quaternary structure of the polymer. This important differentiation appears to be sufficient functional justification for the expression of the J chain by most B-cells terminating at IgD- or IgG-producing secretory effector sites. These cells likely

represent a spin-off from sequential downstream CH switching for subsequent pIgA expression, apparently reflecting an effector B-cell clone maturational stage that is compatible with homing to these sites[11].

The differential expression of diverse antimicrobial proteins in the gastrointestinal tract suggests that they occupy distinct functional niches in mucosal innate defence, allowing the pharmacological exploitation of antimicrobial properties associated with active substances in microemulsions, which can modulate the mucosal immunological system.

5. Mucosal immune system and microemulsion

Over the past several decades, microemulsions have been used in various pharmaceutical technologies. For instance, the storage, stability, dosage, viability, side effects, controlled release, biological response and homogenous distribution of drugs in microemulsions have been explored for their potential use as drug or vaccine delivery systems [23,24].

Immunisation strategies have largely focused on the use of microemulsions. However, the efficiency and/or cost of vaccines must also be improved. In the literature, the theoretical basis of current research is to rationally design immunization methodologies for nanoparticle-based delivery systems. These methodologies may reduce the required dose and enhance the breadth and depth of protective immune responses.

In previous studies, experimental methods for the design and characterisation of nanoparticles directly from oil-in-water microemulsion precursors have been developed, and the breadth and depth of immune responses after immunisation with nanoparticles were enhanced[34, 35.36]. The intradermal administration of novel cationic nanoparticle-based DNA vaccine delivery systems using a jet injection device led to significantly enhanced Th1/Th2-balanced immune responses.

To elicit effective mucosal and systemic immune responses, a novel cationic DNA-coated nanoparticle engineered from a microemulsion precursor was modified, optimised and applied intranasally as a vaccine delivery system. DNA-coated nanoparticles significantly enhanced specific serum IgG and IgA. An enhanced splenocyte proliferative response was also observed after immunisation with pDNA-coated nanoparticles; thus, these particles may be used for immunisation via the nasal route[25] and inhalable nanoparticles may enhance immune responses[25,28].

6. Conclusion

The exact mechanism of the modification of mucosal immune response by microemulsions remains to be elucidated. However, the effect of microemulsions may be attributed to an enhancement in nanoparticle uptake by cells present in the mucosal immune system. The incorporation of antigens into nanoparticles may be a promising approach because colloidal formulations protect antigens from degrading milieu in the mucosa and facilitate their transport across barriers.

The use of nanoparticles for vaccine delivery provides beneficial effects, and good immune responses can be achieved. Although the mechanism of induction of mucosal immunity

after vaccination has not been fully elucidated, antigens associated with microemulsion nanoparticles may enhance mucosal immune responses and improve vaccination methods.

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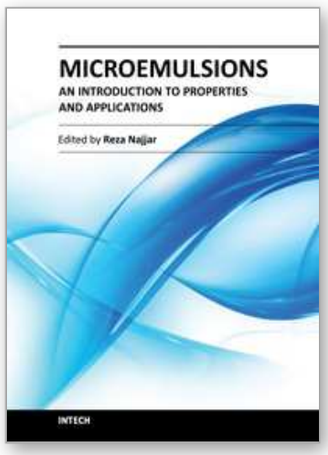

Microemulsions - An Introduction to Properties and Applications
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The rapidly increasing number of applications for microemulsions has kept this relatively old topic still at the top point of research themes. This book provides an assessment of some issues influencing the characteristics and performance of the microemulsions, as well as their main types of applications. In chapter 1 a short introduction about the background, various aspects and applications of microemulsions is given. In Part 2 some experimental and modeling investigations on microstructure and phase behavior of these systems have been discussed. The last two parts of book is devoted to discussion on different types of microemulsion's applications, namely, use in drug delivery, vaccines, oil industry, preparation of nanostructured polymeric, metallic and metal oxides materials for different applications.

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