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Transport of stearic acid-based solid lipid nanoparticles (SLNs) into human epithelial cells

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ABSTRACT

Development of drug delivery systems, as much as the drug molecule itself, is an important consideration for improving drug absorption and bioavailability. The mechanisms by which drug carriers enter target cells can differ depending on their size, surface properties and components. Solid lipid nanoparticles (SLNs) have gained an increased attention in recent years and are the drug carriers of interest in this paper. They are known to breach the cell-membrane barrier and have been actively sought to transport biomolecules. Previous studies by our group, and also other groups, provided an extensive characterization of SLNs. However, few studies have investigated the uptake of SLNs and these have had limited mechanistic focus. The aim of this work was to investigate the pathway of uptake of SLNs by human epithelial cells i.e., lung A549 and cervical HeLa cells. To the best of our knowledge, this is first study that investigates the cellular uptake of SLNs by human epithelial cells. The mechanism of cellular uptake was deciphered using pharmacologic inhibitors (sucrose, potassium-free buffer, filipin and cytochalasin B). Imaging techniques and flow assisted cell sorting (FACS) were used to assess the cellular uptake of SLNs loaded with rhodamine 123 as a fluorescent probe. This study provided evidence that the cellular uptake of SLNs was energy-dependent, and the endocytosis of SLNs was mainly dependent on clathrin-mediated mechanisms. The establishment of entry mechanism of SLNs is of fundamental importance for future facilitation of SLNs as biological or drug carriers.

1. Introduction

Solid lipid nanoparticles (SLNs) have been studied and developed extensively as promising drug carriers in the recent past [1]. Their unique properties of small particle size, large specific surface area, solid nature, particle shape and surface chemistry have generated enormous enthusiasm and anticipation regarding pharmaceutical applications. SLNs can combine the advantages of other traditional colloidal carriers such as liposomes, emulsions, polymeric nanoparticles and micelles, and at the same time reduce their associated shortcomings [2,3]. Some of the attractive features of SLNs include better protection of incorporated drug molecules from the external biological environment, better physicochemical stability, controlled drug release and their use to target specific sites in the body [4,5].

In our previous study, we reported a novel "one-pot" microwave-assisted microemulsion process to produce SLNs [6]. This study indicated that the microwave-assisted procedure, at least for stearic acid based SLNs, resulted in improved physicochemical characteristics over conventional SLNs and also indicated that the SLNs could successfully encapsulate the drug tetracycline. It is not clear, however, how universal that drug uptake is, nor how well, or by what mechanisms, these microwave-assisted, drug encapsulated, SLNs will be taken up by human cells.

Traversal through numerous biological barriers is one of the major challenges in drug development and delivery [7]. For example, the epithelial tissue that is extensively distributed in the gastrointestinal tract (GIT) forms the biological barrier that dictates the passage of drug molecules across the cell membrane when administered orally. Drugs with limited aqueous solubility

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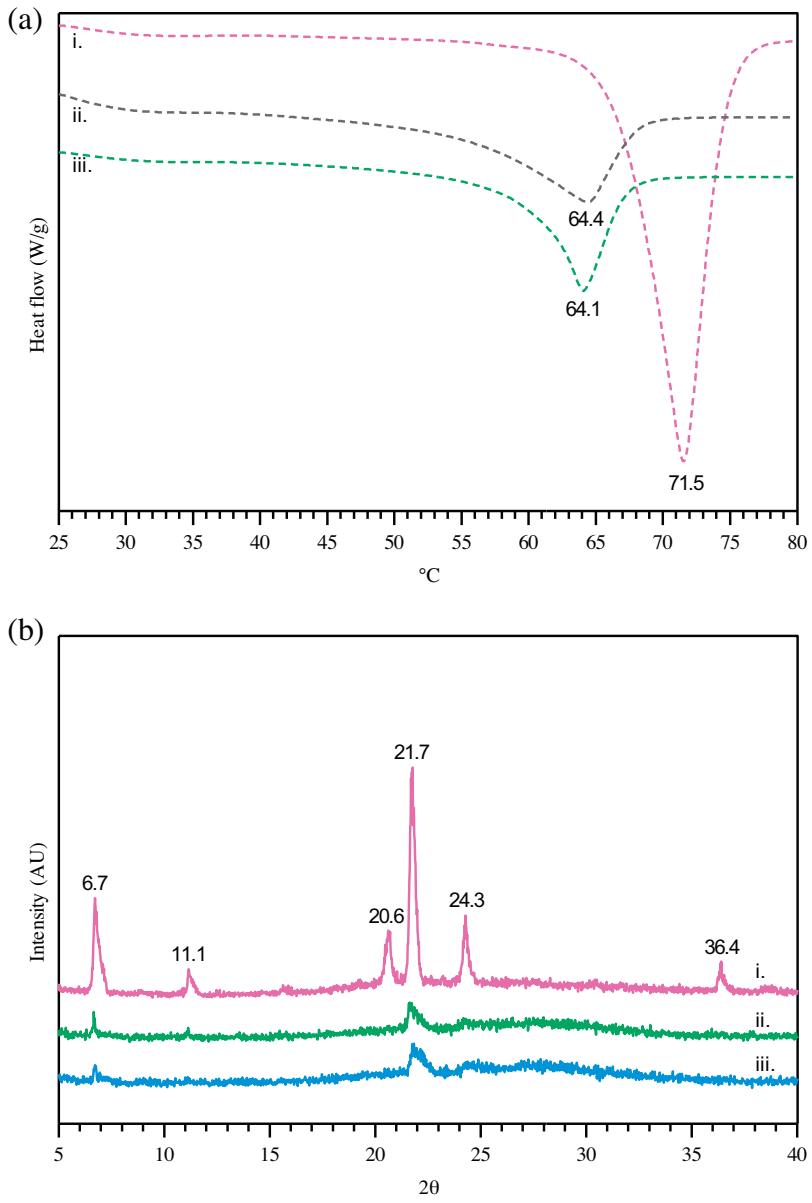

Fig. 1. Crystallinity of SLNs. (a) Differential scanning calorimetry (DSC) curves and (b) X-ray diffraction (XRD) of (i) bulk stearic acid, (ii) unloaded SLNs and (iii) fluorescent SLNs.

and/or membrane permeability [8] may find it difficult to cross this membrane and thus their bioavailability and therapeutic efficacy is reduced.

The cell membrane is a biological barrier that segregates the intracellular milieu (cytoplasm) from the extracellular milieu. It is a selectively permeable membrane that regulates the transport of exogenous materials into the cells through "cellular uptake", which is one of the many mechanisms operative in the human body to maintain intracellular homeostasis [9]. The application of nanostructured drug carriers, such as SLNs, is looked upon as potentially one of the most potent approaches to overcome these barrier difficulties [10].

The growing body of evidence suggests that cellular uptake of nanomaterials depends on (1) physicochemical characteristics of the nanomaterial (such as particle structure, particle shape, particle size and surface charge, particle hydrophobicity and coating material), (2) interaction of surface moieties on the nanomaterials with the cells (i.e., nanomaterial–cell interactions) and (3) endocytic machinery in different cell types [11].

Previous studies have investigated the cellular uptake of SLNs into different cell types [3,12–14]. Few studies, however, have investigated the mechanisms of SLN entry into human cells [15–17] and these have not necessarily involved the epithelial cell lines of interest here. Moreover, none of these have involved uptake of stearic acid-based SLNs produced by the "one-pot" (microwave-assisted) procedure used here.

Chai et al. [18] reported that SLN uptake in Madin-Darby canine kidney (MDCK) epithelial cells was through endocytosis and mediated via lipid raft- and/or clathrin-dependent pathways. The MDCK cells are highly polarized and usually devoid of any caveolae on the apical surface [11], thus do not test for the role of caveolae. Not only is the expression of receptors in MDCK different to that in human cells [19], but there are also mechanistic differences across human and canine cells that suggest differences on nanoparticle uptake in these cells [20,21]. Delgado et al. [17] have investigated the SLN uptake in human retinal pigmented and embryonic kidney epithelial cells, but the SLNs (complexed with DNA and protamine) involved are intrinsically different to the SLNs prepared in this