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Polysaccharide-coated liposomal formulations for dental targeting

Sara Pistone[a,*], Morten Rykke[b], Gro Smistad[a], Marianne Hiorth[a]

[a] SiteDel Group, School of Pharmacy, University of Oslo, P. O. Box 1068, Blindern, 0316 Oslo, Norway
[b] Department of Cariology, Faculty of Dentistry, University of Oslo, Oslo, Norway

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ABSTRACT

The efficacy of treatments of oral ailments is often challenged by a low residence time of the conventional pharmaceutical formulations in the oral cavity, which could be improved by using bioadhesive formulations. This *in vitro* study investigated charged liposomes, both uncoated and coated through electrostatic deposition with polysaccharides (chitosan, alginate and pectin), as bioadhesive systems for the oral cavity. First, formulations that provided liposomes fully coated with polysaccharide were selected. Thereafter, the stability of both the uncoated and the polysaccharide-coated liposomes was investigated in artificial saliva simulating pH, ionic strength, and ionic content of natural saliva. Additionally, adsorption to hydroxyapatite (model for tooth enamel) was tested. The surface charge was of high importance for both the stability in salivary environment and bioadhesion. In artificial saliva, the negatively charged liposomes were the most stable, and the stability of the positively charged liposomes was improved through coating with a negatively charged polysaccharide. On the contrary, the positively charged liposomes were the most bioadhesive, although a moderate adsorption was recorded for the negatively charged liposomes. Based on the present results, the negatively charged liposomes seem to be the most promising formulations used as a tooth adhesive nanosystem and could as such provide improved treatment of tooth ailments.

1. Introduction

The burden of oral conditions seems to have increased worldwide in the last decades (Marcenes et al., 2013). Oral diseases are usually treated or prevented through local administration of therapeutic agents (Silk, 2014). However, conventional local treatments are often challenged by a low residence time in the oral cavity due to salivary secretion, swallowing, food intake and abrasive actions of the soft tissue. For this reason, substances that cannot adhere to oral surfaces are effective only for a limited time after application (Cummins and Creeth, 1992). However, bioadhesive formulations might improve the retention time of active substances in the oral cavity, and consequently, as such improve the efficacy of the treatments (Paderni et al., 2012).

Several types of bioadhesive delivery systems for the treatment of oral ailments have been extensively investigated (Paderni et al., 2012). In particular, bioadhesive nanosystems, such as liposomes, have been shown to be advantageous since their small size could allow to reach sites inaccessible to other types of formulations, and furthermore, can be formulated for site specific targeting (Ozak and Ozkan, 2013; Zupancic et al., 2015). The oral mucosa is the most studied adhesion site for bioadhesive formulations in the oral cavity (Paderni et al., 2012). Even though less investigated, the tooth surfaces could represent another important targeting site especially for the treatment of tooth ailments, such as tooth erosion and dental caries (Beyer et al., 2010; Lee et al., 2012; Ozak and Ozkan, 2013). Liposomal formulations could be particularly of interest for tooth targeting, since, in addition to site specific drug delivery, they could also provide physical protection through a biomimetic effect. In fact, in physiological conditions, components of saliva are selectively adsorbed onto tooth enamel surfaces, thus forming the acquired enamel pellicle, which provides protection *e.g.* against acid attacks, mineral loss and abrasive factors (Siqueira et al., 2012). Similarly, the liposomes adsorbed onto the tooth surface could provide physical tooth protection.

Little information is available in the literature regarding the adsorption of liposomes onto the tooth surface. Previous studies

Abbreviations: Alg-Lip, alginate-coated liposomes; Chit-Lip, chitosan-coated liposomes; HA, hydroxyapatite; Neg-Lip, negatively charged uncoated liposomes; PDI, polydispersity index; Pec-Lip, pectin-coated liposomes; Pos-Lip, positively charged uncoated liposomes.

* Corresponding author.
E-mail addresses: sara.pistone@farmasi.uio.no (S. Pistone), morten.rykke@odont.uio.no (M. Rykke), gro.smistad@farmasi.uio.no (G. Smistad), marianne.hiorth@farmasi.uio.no (M. Hiorth).

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indicated that the adsorption of liposomes onto hydroxyapatite (HA), the main component of the tooth enamel, is mainly based on electrostatic interactions, and it can be influenced by the presence of saliva (Nguyen et al., 2013, 2010). Moreover, saliva has previously been shown to influence also the stability of the liposomal suspensions (Nguyen et al., 2011b, 2013). This knowledge can be complemented by investigations regarding how the salivary components influence the adsorption of liposomes to HA and the liposomal stability in saliva. This information could provide the rational for the improvement of tooth adhesive formulations. Nevertheless, natural saliva is an extremely complex and variable fluid (Lentner, 1981; Nunes et al., 2015). Therefore, the understanding of the specific influence of the salivary constituents can be facilitated by using artificial saliva with a relatively simple composition and salivary components at known concentrations, as in previous studies regarding other nanosystems (Pistone et al., 2017; Sarkar et al., 2009).

The application of liposomes as drug carriers is well known and their coating with specific molecules has been exploited for improving their properties. For example, coating of liposomes with polymers have been reported to increase the bioadhesive capacity (Filipović-Grčić et al., 2001; Takeuchi et al., 2003), modulate the release and the entrapment efficiency of drugs (Filipović-Grčić et al., 2001), as well as improve the stability of the system by preventing liposomal oxidation or aggregation (Mady et al., 2009; Meland et al., 2014). In addition, the influence of pectin coatings on tooth adhesion has previously been studied (Nguyen et al., 2013), and has been suggested to improve the strength of adhesion between the liposomes and the tooth enamel. Charged polysaccharides, such as chitosan, pectin and alginate, could be a rational choice for the coating of liposomes for oral applications in virtue of their biocompatibility, bioadhesive and HA adhesive properties (Beyer et al., 2010; Lee et al., 2012).

On this basis, in the present study liposomal coatings consisting of chitosan, pectin and alginate were investigated. Liposomes coated with charged polysaccharides can be prepared through ionic complexation between charged liposomes and an oppositely charged polysaccharide. In order to obtain stable coated liposomes, only polysaccharide concentrations in a specific range can be used (McClements, 2005), which are generally determined through empirical studies. While chitosan-coated liposomes have been widely studied (Chun et al., 2013; Filipović-Grčić et al., 2001; Guo et al., 2003; Laye et al., 2008; Mady et al., 2009; Quemeneur et al., 2010; Takeuchi et al., 2003), pectin- and especially alginate-coated liposomes have received limited attention (Alund et al., 2013; Klemetsrud et al., 2013; Nguyen et al., 2011a, 2013; Sriamornsak et al., 2008).

The aim of the present study was to investigate the preparation and the characteristics of liposomes coated with chitosan, pectin, or alginate. Thereafter, the stability of the liposomal formulations was tested in an artificial saliva containing only salivary electrolytes in physiological concentrations (Gal et al., 2001). Furthermore, the formulations were tested for *in vitro* adsorption onto HA in the presence of either phosphate buffer or artificial saliva.

2. Materials and methods

2.1. Materials

The polysaccharides and lipids used in the study are listed in Table 1. The pectin and the alginate were purified prior to utilization as previously described (Nguyen et al., 2011a; Pistone et al., 2015). The chloroform used for the liposome preparation was of analytical grade. A Millipore Milli-Q system with 0.22 μm Millipak® 40 filter (Millipore™, Ireland) was used to purify the water. Triton X-100 (t-Octylphenoxypolyethoxyethanol) was obtained from Sigma-Aldrich GmbH (Germany). Spray-dried HA powder with homogeneous particle diameter of 10.0 ± 2.0 μm was purchased from Fluidinova (Portugal).

2.2. Preparation of the liposomes

The liposomes were prepared according to the thin-film method as previously described (Nguyen et al., 2011a). In short, the lipid film was formed through rotary evaporation of a lipid solution in chloroform at 40 °C (Heidolph W 2001 rotavapor, Heidolph Instruments GmbH & Co. KG, Germany). The lipid solution contained 89 mol% of egg-PC; 1 mol% of NBD-PC, a fatty acid labeled fluorescent phospholipid; and 10 mol% of the charged lipids, egg-PG or DOTAP, for the preparation of negatively and positively charged liposomes, respectively. In order to remove thoroughly the chloroform, the film was freeze dried overnight (Christ Alpha 2–4 freeze drier, Christ, Germany). The film was then hydrated with a 5 mM phosphate buffer (pH 6.8) for two hours at room temperature, and stored overnight in the refrigerator. The size reduction was obtained by extrusion (Lipex extruder, Lipex Biomembranes Inc., Canada) repeated ten times through two-stacked 200 nm polycarbonate membranes (Nucleopore®, Costar Corp., USA) at room temperature. The samples were stored overnight in the refrigerator under nitrogen atmosphere, to avoid oxidation of the lipids, before characterization, coating, and further experiments. The lipid concentration in the final preparations was 3 mM.

2.3. Coating of the liposomes with polysaccharides

The coating of the charged liposomes was achieved through electrostatic deposition by mixing the liposomal dispersion with a solution of oppositely charged polysaccharide. The liposomes were coated by dripping 2 ml of liposomal dispersion into 8 ml of polysaccharide solution (prepared in phosphate buffer 5 mM, pH 6.8) in a 10 ml vial (2.2 cm diameter), under constant magnetic stirring (cylindrical stirring bar 12 × 2.5 mm) at about 480 rpm. The addition of the liposomal dispersion was performed using a peristaltic pump at a constant flow of 1.7 ml min^{-1}. The samples were stirred for ten minutes and characterized after storage overnight in the refrigerator under nitrogen atmosphere. The lipid concentration in the final preparations was 0.6 mM. Both uncoated and coated liposomes were prepared at least in triplicate. All the liposomal dispersions were diluted to a concentration of 0.3 mM with a 5 mM phosphate buffer immediately prior to characterization (the uncoated liposomes were diluted 1:10 and the polysaccharide coated liposomes 1:2).

2.4. Characterization of the liposomes

2.4.1. Size determination

A Zetasizer Nano ZS (ZEN3600, Malvern Instruments Ltd., UK) with a red light laser (λ = 633 nm) was used for determination of the size through dynamic light scattering (DLS) at 25 °C. A backscattered detection was used at a scattering angle of 173°. The refractive index and the viscosity of pure water at 25 °C were employed as constant parameters in the calculations. The autocorrelation function, obtained through the fluctuations of the intensity of the scattered light, was fitted by the Zetasizer software (version 7.11) with the general purpose fitting method. The Stockes-Einstein equation was used to calculate the hydrodynamic diameters of the liposomes. The software provided the mean size (z-average) and the polydispersity index (PDI). The measurement was carried out after temperature equilibration. The size and PDI obtained for each batch were the average of three measurements on the same sample aliquot.