European Journal of Pharmaceutics and Biopharmaceutics xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Nano-carrier systems: Strategies to overcome the mucus gel barrier

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ARTICLE INFO

Article history:
Received 29 September 2014
Revised 23 January 2015
Accepted in revised form 26 January 2015
Available online xxxx

Keywords:
Mucus barrier
Mucus penetration
Nanocarriers
Innovative strategies

ABSTRACT

The present review provides an overview of nanotechnology-based strategies to overcome various mucus gel barriers including the intestinal, nasal, ocular, vaginal, buccal and pulmonary mucus layer without destroying them. It focuses on the one hand on strategies to improve the mucus permeation behavior of particles and on the other hand on systems avoiding the back-diffusion of particles out of the mucus gel layer. Nanocarriers with improved mucus permeation behavior either exhibit a high density of positive and negative charges, bearing mucolytic enzymes such as papain and bromelain on their surface or display a slippery surface due to PEG-ylation. Furthermore, self-nanoemulsifying-drug-delivery-systems (SNEDDS) turned out to exhibit comparatively high mucus permeating properties. Strategies in order to avoid back-diffusion are based on thiolated polymers reacting to a higher extent with cysteine subunits of the mucus at pH 7 in deeper mucus regions than at pH 5 being prevalent in luminal mucus regions of the intestinal and vaginal mucosa. Furthermore, particles changing their zeta potential from negative to positive once they have reached the epithelium seem to be promising carriers. The summarized knowledge should provide a good starting point for further developments in this field.

1. Introduction

Incorporation of drugs and genes in nanocarriers offers the potential for localized and sustained delivery to mucosal tissues [1,2]. The luminal surface of mucosal tissues, however, is protected by a layer of highly viscoelastic and adhesive mucus. This mucus protects mucosal surfaces in the human body such as the gastrointestinal tract, the vagina, the lung, the eye and various others. Many particulate drug delivery systems get caught in these mucus layers by steric hindrances or adhesion processes and are subsequently removed from the mucosa by the mucus turn over being in the range of seconds up to several hours depending on anatomical site. Trapped particles, with diffusivities in mucus several-thousand-fold lower than in water do not efficiently reach the deeper mucus layers that are cleared much more slowly. The efficacy of nanoparticle delivery systems is thereby strongly limited. For sustained or targeted drug delivery to mucosal surfaces, nanocarriers must quickly permeate the mucus gel layer – a long standing challenge in drug delivery [3]. The development of particulate carrier systems overcoming the mucus gel layer as soon as possible and reaching the absorption membrane is therefore of common interest.

So far pursued strategies aiming to overcome the mucus gel barrier, however, are mainly based on the breakdown of the mucus gel layer over almost the entire mucosal tissue being from a toxicological point of view highly problematic as the mucus layer has a substantial protective function. More recent strategies are therefore focused on nanocarrier systems capable of permeating the mucus without or only to a very limited extent destroying it. Generally mucus permeating particles can be divided into active and passive systems. Passive systems try to avoid as many interactions of particles with mucus as feasible. The likely most promising systems are particles exhibiting a slippery surface and self-nanoemulsifying-drug-delivery-systems (SNEDDS). In contrast, active systems interact with the mucus making it leakier for particles. These systems are mainly based on disulfide bridge breaking agents and on proteolytic enzymes. Strategies to avoid a back-diffusion of particles out of the mucus gel layer are also active systems and are so far based on thiomers and zeta potential changing systems. In the following these novel strategies are explained and highlighted in more detail.

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http://dx.doi.org/10.1016/j.ejpb.2015.01.022
0939-6411/

2. Strategies to overcome the mucus gel barrier

2.1. Slippery surface strategy

Unmodified polystyrene nanoparticles (NPs) were shown to penetrate, to certain extent human cervical mucus, whereas carboxyl-modified polystyrene nanoparticles (NPs) of the same size were completely immobilized in the mucus gel layer. These results provide evidence for the essential role of surface chemistry on the mucus permeation behavior of nanoparticles. Taking lessons from nature, the rapid transport of specific viruses, which have evolved over thousands of years to infect mucosal tissues, is of particular interest. Many viruses such as the Norwalk or polio virus are capable of diffusing through a mucus layer as fast as in water. They are densely coated with both positively and negatively charged groups leading to a densely charged yet net neutral surface. The average distance between charged groups on the surface of polio virus is, for instance, just 5 Å. The high surface charge density of the particulate virus likely creates a hydrophilic surface that decreases its hydrophobic interactions with mucus and, thus, minimizes the virus entrapment in the mucus layer. Accordingly, a high and equal density of positive and negative surface charges may facilitate the efficient particle transport through a mucus layer by minimizing the electrostatic interactions with mucus [1].

Following this strategy the combination of high charge density anionic and cationic polymers formulated to polyelectrolyte complexes should lead to "slippery" nanoparticulate systems. With respect to polyelectrolyte complexes, Laffleur and coworkers prepared neutral polyacrylic acid (PAA)/polyallylamine (PAM) polyelectrolyte complexes with a diffusion efficiency in intestinal mucus 2.5- and 18-fold higher than PAM and PAA NPs, respectively [4]. In another study, Pereira de Sousa et al., developed NPs with highly densely charged surface by combining chitosan (CS) with chondroitin sulfate (ChS) showing higher diffusion ability in intestinal mucus as compared to PLGA NPs [5]. Therapeutic macromolecules could be associated with polyelectrolyte complexes via ionic complexation of charged biomolecules with anionic or cationic polymers.

Moreover, an uncharged surface may also be considered as muco-inert provided it is sufficiently hydrophilic with a low hydrogen bonding capability [1]. In this respect, Wang and coworkers [6] modified the surface of polystyrene nanoparticles with polyethylene glycol (PEG) of various molecular weights (e.g., 2, 5, 10 kDa). It was shown that low PEG MW and high PEG surface coverage are both required for rapid mucus penetration of coated nanoparticles. More specifically, it was revealed that there exists a critical MW between 5 and 10 kDa where dense PEG coatings transition from being mucoinert to mucoadhesive.

Furthermore, Tang et al. [7] developed biodegradable poly (sebacic acid) (PSA)-PEG NPs. These nanoparticles were found to diffuse in fresh undiluted human cervicovaginal mucus (CVM) with just a 12-fold lower speed than in pure water. In another study, Cu and Saltzman [8,9] demonstrated that the surface modification of PLGA particles with PEG can improve their diffusion in mucus depending on PEG MW and density being in agreement with Yu et al. [9]. Xu and coworkers prepared mucus-penetrating PLGA–PEG NPs exhibiting dense brush PEG coatings on their surface using low MW emulsifiers [10]. In addition, Mert et al. formulated PLGA NPs using Vitamin E conjugated to 5 kDa PEG as surfactant [11]. It was shown that these NPs rapidly penetrate human CVM. Furthermore, Yang et al. produced mucus penetrating particles (i.e., PLGA, poly(ε-caprolactone) (PCL), polystyrene (PS)) via non-covalent coating with Pluronic F127 (i.e., triblock copolymer of PEG–poly(propylene oxide)–PEG) resulting in a comparatively strong improvement in the mucus penetrating properties as shown in Table 1. Therapeutic peptides can be effectively incorporated in the PEGylated NPs during their preparation process (e.g., double emulsion, precipitation, etc.).

Table 1
Characteristics of uncoated and F127-coated NPs and ratios of the average diffusion coefficient in CVM (D_m) to that in water (D_w). Table obtained from Yang et al. [3].

Formulation	Diameter (nm)	ζ-Potential (mV)	D_w/D_m
PLGA	110 ± 4	−50 ± 2	3800
PLGA/F127	138 ± 2	−5 ± 2	10
PCL	122 ± 2	−6 ± 2	2400
PCL/F127	135 ± 5	−1 ± 1	6
PS	194 ± 6	−46 ± 1	4000
PS/F127	216 ± 2	−4 ± 1	4

2.2. The SNEDDS strategy

Self-nanoemulsifying drug delivery systems (SNEDDS) are isotropic mixtures of oil, surfactant and cosurfactant spontaneously forming an O/W nanoemulsion upon mixing with water. Due to the hydrophobic surface of the formed nanodroplets their interactions with the mucus layer should be low. In addition the small size (i.e., ⩽50 nm) of the nanodroplets in combination with their shape deformation ability could allow their diffusion through mucus gel layers of small mesh sizes. Friedl and co-workers developed various fluorescently labeled SNEDDS formulations and evaluated their diffusion behavior through an intestinal mucus layer. As illustrated in Fig. 1, the mucus permeability of the developed formulations was found to be size-dependent as a permeation of 70.3% was observed for SNEDDS with droplet diameters equal to 12 nm (Formulation 2) compared to a permeation of only 8.3% observed for droplet diameters equal to 455.5 nm (Formulation 19), respectively. In addition, the composition of the formulation excipients was found to affect the permeation of SNEDDS through mucus. For example, Cremophor RH 40 and triacetin were identified as promising excipients for SNEDDS to improve their mucus permeating properties [12].

In another study, various SNEDDS formulations of similar droplet sizes (e.g., 30–40 nm) were prepared. It was shown that the change of a single excipient (i.e., the use of Labrafil M1944CS as oil instead of Lauroglycol FCC) in combination with the oil/surfactant/cosurfactant weight ratios could strongly influence the mucus

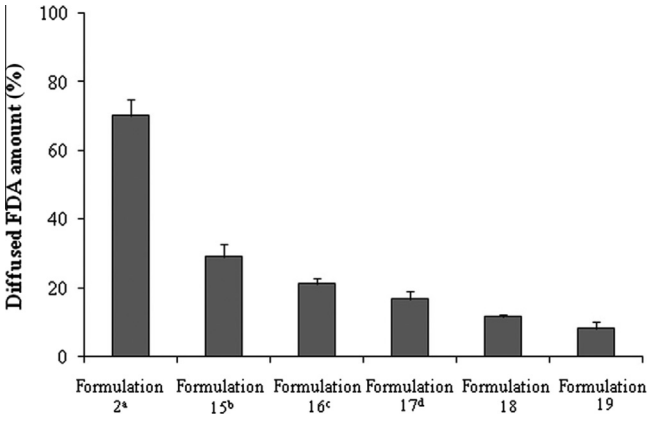

Fig. 1. An overview of diffused amount of model drug FDA through mucus layer as a function of SNEDDS droplet size. Indicated values are means of at least three experiments ± SD. ([a]Formulation 2 differs from formulation 15–19, $p < 0.001$. [b]Formulation 15 differs from formulations 16, $p < 0.05$; 17, $p < 0.01$; and 18 + 19, $p < 0.001$. [c]Formulation 16 differs from formulations 18, $p < 0.01$ and 19, $p < 0.001$. [d]Formulation 17 differs from formulation 19, $p < 0.05$). Adopted from Friedl et al. [12].