Expert Opinion

The performance of nanocarriers for transmucosal drug delivery

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Most of the newly designed drug molecules are characterised by low solubility in aqueous medium, low permeability through biological membranes and/or an insufficient stability in the biological environment. Fundamental studies have provided proof-of-concept of the potential of particulate nanocarriers for overcoming these unsuitable properties. For example, it is known that polymeric nanosystems may enhance transmucosal transport of drugs with poor penetration capacities while preserving their biological activity. Moreover, in recent years it has become clear that through an appropriate selection of the nanosystem components it is possible to enhance its affinity for the mucosa and, hence, the residence time of the drug in contact with the absorptive epithelium. These properties, combined with a suitably tailored release profile can markedly increase the efficacy of pharmaceuticals. Overall, the properties that have been identified as critical for the performance of these delivery systems are particle size, surface charge and surface chemical composition. These properties are known to affect the physical and chemical stability of the nanoparticles in the biological environment as well as their ability to interact (unspecific bioadhesion, receptor-mediated interaction and so on) and, eventually, overcome biological barriers. The present article aims to critically review the latest advances in this area and to provide some insights into these complex issues. Thus, herein the most widely investigated transmucosal drug delivery nanosystems and their most promising applications are reported.

Keywords: chitosan, mucosal delivery, nanoparticles, peptide delivery, poly(lactic acid), protein delivery, surface modification, vaccine delivery

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1. Introduction

The use of delivery approaches in order to achieve simple, tolerable and patient-compliant administration of drugs with undesired physicochemical and biopharmaceutical properties has been a main objective of many research institutions and pharmaceutical companies. The compelling need of new pharmaceutical carriers in order to meet the demands of drugs is now best exemplified by the new generation of biopharmaceuticals and gene medicines [1]. However, low molecular weight drugs of strong hydrophobic character may also benefit from the new particulate drug delivery approaches. Among the different modalities of drug administration, the delivery through mucosal surfaces is the most attractive but also the most challenging method for drug delivery [2,3].

The simplest way for improving the transmucosal delivery of drugs has been based on the use of chemicals that reduce the intrinsic resistance of the physiological barriers (i.e., by the use of enzyme inhibitors or permeation enhancers). However, in this straightforward approach it should be taken into account that these types of chemicals might bring sanitary risks, such as infections, caused by the reduced protective capacity of the mucosa [4]. Alternatively, the use of drug

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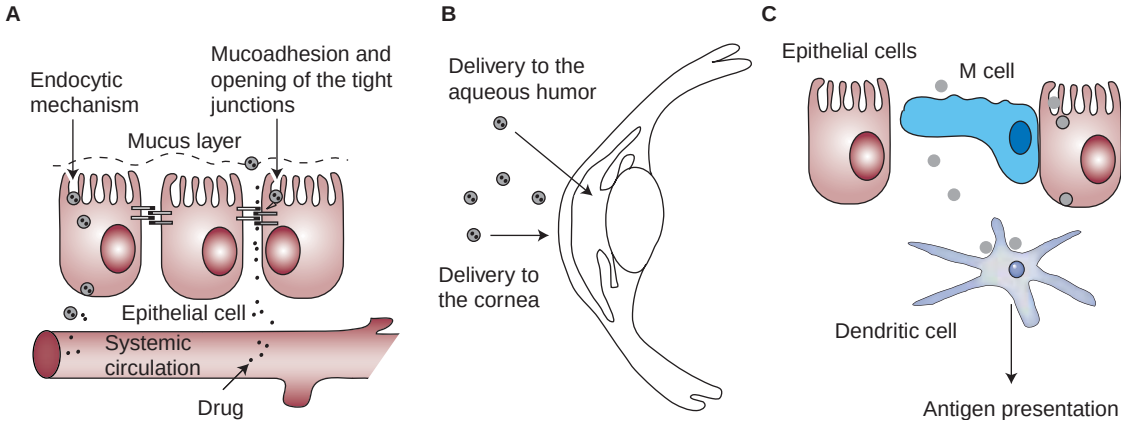

Figure 1. Schematic examples of possible mucosal applications of nanocarriers: systemic drug delivery (A), local delivery (B) and delivery to the immune cells (C).

nanocarriers that are able to increase drug absorption by carrying the drug across the epithelium, or by reducing the resistance of the epithelium to drug transport in a localised area, does hold great promise [5].

The idea of designing nanocarriers for improving the transmucosal transport of drugs has been highly motivated by the evidence of the transport of nanosized matter across mucosal barriers [6]. Efforts in this line have been directed at achieving high surface-volume ratios of nanosized matter in order to maximise biomaterial–mucosal interactions. An additional recent challenge in this area has been the search for suitable nanostructured biomaterials intended to optimise their interaction and/or transport across mucosal surfaces [7]. Moreover, there is a tendency to increase the complexity of these systems by making them biologically reactive. This means that the nanocarrier would be provided with a capacity to interact and overcome the biological barrier due to the use of bioadhesive molecules, promoters of drug absorption, presenting specific ligands for active particle uptake and so on [8-11].

Most of these particle-based transmucosal delivery approaches have only been explored at the academic and preclinical level. However, there are a few examples of industrial developments that have been successful. These include insulin microparticles (their size is the micron range) intended for pulmonary delivery (Nektar Therapeutics), nanometric drug–chemical complexes for oral administration of macromolecules (Eligen®; Emisphere Technologies, Inc.) or drug nanosuspensions for oral delivery of poorly soluble drugs (NanoCrystal™; Elan) [301-303]. Several other nanometric drug delivery devices are currently in clinical trials. However, despite the evident potential of some specific delivery approaches, there is still some controversy regarding the true potential of the increasing number of nanoparticulate drug delivery systems found in the literature.

This review is intended to present an overview of the most relevant advances made within the last few years regarding the use of nanostructured carriers for transmucosal drug delivery.

2. Main applications of nanosystems for transmucosal delivery

Among the transmucosal drug delivery routes, this paper particularly concentrates on those intended to deliver drugs and antigens to the systemic circulation (oral and nasal) or the immune system, as well as those intended to improve local drug delivery; more specifically, ocular drug delivery (Figure 1).

2.1 Nanosystems for transmucosal drug delivery to the systemic circulation

The delivery of poorly absorbed drugs to the systemic circulation continues to be the focus of attention. The two major fronts of this area are i) the transmucosal administration of drugs whose bioavailability is partially impaired by their poor-solubility properties and, ii) the transmucosal delivery of large hydrophilic molecules whose bioavailability is limited by their degradation and poor penetration across the mucosal barriers.

2.1.1 Nanosystems for transmucosal delivery of poorly soluble drugs to the systemic circulation

Nanoparticle technology has found a niche of interest in the formulation of drugs whose bioavailability is limited by their low solubility. The traditional method of formulating these drugs requires the use of co-solvents that allow their solubilisation. This approach presents several drawbacks among which toxicity problems related to this co-solvent is the most prominent [12]. Alternatively, complexing agents such as natural cyclodextrins and their derivatives have been proposed as delivery agents for these molecules. Indeed, some cyclodextrin derivatives show excellent toxicological profiles that make them

Table 1. Nanocarrier systems for the delivery of low solubility and low permeability drugs to the systemic circulation.

Nanocarrier system	Drug	Administration route	Ref.
Low solubility drugs (class II drugs)			
Drug–surfactant nanosuspension	Danazol	Oral	[16]
Drug–surfactant nanosuspension	301029	Oral	[17]
Drug–surfactant nanosuspension	Budesonide	Pulmonary	[18]
Low solubility–low permeability drugs (class IV)			
Coated drug nanosuspensions	Ciclosporin A	Oral	[19]
Eudragit® nanoparticles	Ciclosporin A	Oral	[20]
Polycaprolactone nanoparticles	Ciclosporin A	Oral	[21]
Gliadin nanoparticles	Carbazol	Oral	[22]

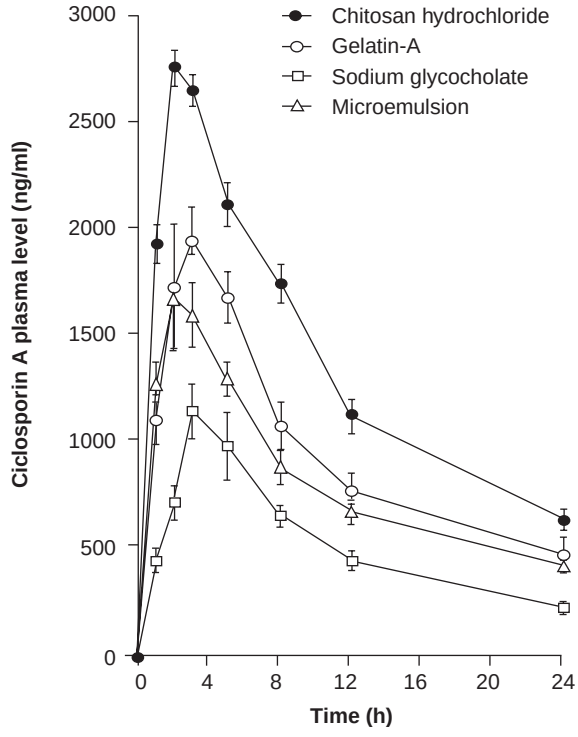

Figure 2. Mean plasmatic levels of ciclosporin A following oral administration of the drug microemulsion (Neoral®; Novartis), or different nanoparticles formulated with sodium glycocholate, gelatin-A or chitosan hydrochloride (n = 6). Reproduced with permission from EL-SHABOURI MH: Positively charged nanoparticles for improving the oral bioavailability of cyclosporin-A. *Int. J. Pharm.* (2002) **249**(1-2):101-108 [19].

attractive choices as formulation agents [13]. Nonetheless, this approach often fails because of the high concentration of the complexing agent that is required to achieve the desired drug concentration. To circumvent this limitation, the formation of drug nanosuspensions or drug encapsulation in lipophilic nanocarriers (nanoemulsions, lipid nanoparticles and so on) represent new alternatives for this delivery challenge (Table 1).

Drug nanosuspensions are systems formed by nanosized suspensions of drug that are stabilised by surfactants [14]. These drug–surfactant nanostructures have several interesting features: they present satisfactory stability during storage, they allow the administration of drugs in high concentrations without the need of harsh excipients, and they increase drug solubility rates due to their high intrinsic surface area [15]. These features usually lead to a considerable increase in the oral bioavailability of lipophilic drugs. For instance, in a pioneer paper, Liversidge *et al.* achieved a > 16-fold increase in the bioavailability of orally administered danazol when the drug particle size was reduced from 10 µm to the nanometric range [16]. Recent studies performed with a thiadiazole derivative have further evidenced the potential of drug nanosuspensions for enhancing the bioavailability of lipophilic drugs [17]. In a similar fashion, pulmonary-administered budesonide doubled its maximal plasmatic concentration and reduced its absorption time when changing drug particle size from 4.5 µm to the nanometric range (75 – 300 nm) [18].

Although drug nanosuspensions may represent a good choice for drugs whose bioavailability is limited by their low solubility, this formulation approach may not be the best option for drugs with low-solubility and low-permeability characteristics simultaneously. For these drugs (i.e., class IV drugs), the use of carriers that combine the capacity of loading these hydrophobic drugs with the potential to enhance their transmucosal transport represents a more promising alternative.

Bearing this idea in mind, El-Shabouri prepared ciclosporin A nanoparticles by nanoprecipitating this drug in association with different materials: sodium glycocholate (anionic), gelatin-A or chitosan (CS) (both cationic) [19]. Despite the similarities in particle size between the three different formulations (100 – 150 nm), pharmacokinetic studies in beagle dogs showed very pronounced differences on their performance (Figure 2). Indeed, formulations modified with anionic sodium glycocholate showed a reduced relative bioavailability (64%) when compared with the commercial Neoral® (ciclosporin; Novartis) microemulsion. In contrast, the relative bioavailability increased by up to 118% for the gelatin-A-coated nanocarriers and up to 173% for the

CS-modified systems. These results indicate that the nature of the coating material around the nanoparticles is critical for the success of a transmucosal formulation. It is also interesting to note that the two positively charged formulations (gelatin-A and CS) behaved quite differently. The favourable behaviour of CS has been attributed to its inherent characteristics, such as its mucoadhesive character and its permeation-enhancing properties [19].

In another work, cationic Eudragit® RS and Eudragit® RL (Rohn Pharma) nanoparticles were also evaluated for the oral delivery of ciclosporin A. These formulations led to 25 – 27% of relative bioavailability as compared with the Neoral microemulsion and 5 – 6% of absolute bioavailability [20]. Although these results are not strictly comparable with those reported by El-Shabouri [19], they underline the importance of the nanocarrier composition and the characteristics of the nanoparticulate formulation on its capacity to increase the absorption of ciclosporin A.

A different nanocarrier that was explored for the oral administration of ciclosporin A was made of a polyester (i.e., poly[ε-caprolactone] [PECL]). Following the oral administration of PECL nanoparticles containing ciclosporin A, a significant increase in the drug levels was observed in a variety of tissues, as compared with those achieved for the control formulation, together with a clear immunosuppressant effect. Interestingly, these increased levels did not result in an enhancement of the nephrotoxicity characteristic of this drug [21].

It is also worth mentioning an interesting new system, consisting of gliadin nanoparticles, which has shown potential for enhancing the intestinal absorption of poorly soluble drugs. As mentioned for CS, gliadin is a protein that exhibits mucoadhesive properties and a high affinity for the upper intestinal regions. This favoured interaction has been the explanation for the capacity of these nanocarriers to enhance the oral bioavailability of carbazole (49% bioavailability versus 39% of an oral drug–surfactant solution) [22].

Alonso *et al.* have contributed to the efforts aimed at designing carriers for low solubility drugs, with particular emphasis on class IV molecules (low solubility, low permeability), by developing new delivery systems that combine the ability of CS to enhance drug permeability with specific solubilisation strategies. A delivery approach was based on the use of submicrometric emulsions that were coated with CS in order to implement mucoadhesive properties in the carrier [23]. In this case, the drug was dissolved in the inner oily phase and surrounded by CS. Alternatively, a new approach for the delivery of hydrophobic drugs, which combines drug–cyclodextrin complexes with CS nanoparticles was investigated [24]. The formation of the complexes allowed this research group to markedly enhance the capacity of CS nanoparticles to load hydrophobic drugs. For instance, the entrapment of furosemide into the nanoparticles was increased eightfold when it was previously complexed with cyclodextrins. Interestingly, despite this increased loading capacity, the incorporation of cyclodextrins into the nanoparticles did not significantly affect the size or the positive zeta potential of CS nanoparticles [24]. Consequently, it could be expected that these carriers would maintain the favourable properties of CS nanoparticles and that they could be an attractive carrier for the transmucosal delivery of class IV drugs. Nevertheless, despite these promising features, the efficacy of these nanoparticles for enhancing the intestinal absorption of hydrophobic drugs remains to be investigated.

2.1.2 *Nanosystems for transmucosal delivery of hydrophilic macromolecules to the systemic circulation*

The idea of using nanoparticles as carriers for oral delivery of hydrophilic macromolecules has been motivated by the systematic work of Florence *et al.* on the transport of nanosized matter across the intestinal mucosa [6]. This idea has been reinforced by the group of Couvreur *et al.* who reported the first piece of evidence of the performance of polyalkylcyanoacrylate nanocapsules as carriers for the intestinal delivery of insulin [25]. These initial discoveries have stimulated a significant amount of research work oriented to the design of sophisticated nanocarriers specifically adapted to different modalities of administration. In this section, the latest advances in the transmucosal delivery of hydrophilic macromolecules will be covered, focusing mainly on nasal and oral administration. The authors are aware of the potential of the pulmonary route for the delivery of peptides; however, it should be clarified that most of the work in this field has been performed with microparticles rather than with nanoparticles.

The advances made with nanosystems for nasal and intestinal macromolecular drug delivery will be classified according to the nature of the polymer material use for particle formation (Table 2).

2.1.2.1 *Chitosan-based nanosystems for macromolecular drug delivery*

As indicated in Section 2.1.1, due to their mucoadhesive character CS-derived nanocarriers are particularly suited for transmucosal delivery applications. This has been the subject of a recent review in the context of oral delivery [26] and, thus, will only be briefly summarised here. The first proof-of-concept of the potential of CS nanoparticles for trasmucosal delivery of peptides was reported in 1999 [27]. More specifically, insulin-loaded nanoparticles were administered nasally to conscious normoglycaemic rabbits and the glucose levels were monitored. The results showed a 40% reduction in the serum glucose levels, this level being significantly lower than those corresponding to the controls. These initial results have been further corroborated in diabetic rats (manuscript in preparation). Moreover, these latter experiments revealed that the incorporation of PEG in the nanoparticles suspension helped to enhance the therapeutic efficacy of CS nanoparticles.

The same type of nanoparticles, prepared by ionic gelation [28], has also been tested for the oral administration of insulin. The results showed a prolonged hypoglycaemic effect and a maximum reduction in the serum glucose levels of 40% [29,30].

Table 2. Nanocarriers systems for the delivery of hydrophilic macromolecules to the systemic circulation or to the immune cells.

Nanocarrier system	Drug	Administration route	Effects	Ref.
PACA nanoparticles	Insulin	Oral	Systemic	[25]
CS nanoparticles	Insulin, pDNA*, ovalbumin, tetanus toxoid*	Oral, nasal	Systemic, immunological	[27,38,90,91]
CS-poloxamer nanoparticles	Insulin	Oral	Systemic	[29,30]
CS-PEG nanoparticles	Insulin, pDNA*, diphtheria toxoid*	Oral, nasal	Systemic, immunological	[28]
CS-glucomannan nanoparticles	Insulin	Oral	Systemic	[32]
CS nanoparticles with enteric coating	Insulin	Oral	Systemic	[31]
CS nanocapsules	Calcitonin	Oral	Systemic	[26]
CS-coated lipid nanoparticles	Calcitonin	Oral	Systemic	[33]
CS-coated liposomes	Insulin, calcitonin	Oral, intragastric	Systemic	[34-37]
CS-coated PLGA nanoparticles	Calcitonin, tetanus toxoid*	Oral, nasal	Systemic, immunological	[38,49]
PLGA nanoparticles	Albumin*, influenza epitope*	Oral, nasal	Immunological	[100,101]
PLA-PEG nanoparticles	Tetanus toxoid*, pDNA*	Oral, nasal	Immunological	[38,102,103]
PLGA:polyoxyethylene blend nanoparticles	pDNA*	Nasal	Immunological	[48]
PLGA:Eudragit® nanoparticles	Heparin	Oral	Systemic	[53]
PLGA:polyanhydride nanoparticles	Insulin	Oral	Systemic	[50]
Polyanhydride nanoparticles	Insulin, pDNA,	Oral	Systemic	[58]
PVA-grafted-PLGA nanoparticles	Tetanus toxoid*	Nasal	Immunological	[108]

*Antigenic molecules.

CS: Chitosan; PACA: Polyalkylcyanoacrylate; pDNA: Plasmid DNA; PEG: Poly(ethylene glycol); PLA: Poly(lactic acid); PLGA: Poly(lactic-co-glycolic acid); PVA: Polyvinyl-alcohol.

The *in vivo* results were variable depending on the type of formulation, thus suggesting the necessity to conveniently optimise these nanocarriers in order to obtain the whole potential benefit. Other authors have designed CS nanoparticles with an enteric coating, suggesting that this protective coating could enhance the efficacy of the nanocarrier [31]. However, the pharmacological results that were observed were within the same range as those obtained by other authors for the corresponding particles without enteric coating.

Another CS-based approach for oral insulin delivery has been a nanocarrier that combines CS and Konjac glucomannan. The idea behind this design is that bioadhesion of the nanocarrier could be further enhanced by the presence of glucomannan, due to its specific interaction with the mannose receptors in the intestinal cells. These nanoparticles, administered orally to normoglycaemic rats, were able to produce delayed and sustained decreases in plasma glucose levels for up to 24 h. This delayed pharmacological effect has been attributed to the nanostructure of the carrier, which is able to release the associated peptide in a controlled manner [32].

A different category of CS-based nanocarriers for transmucosal delivery of macromolecules is the one consisting of a nanocore coated by CS. Nanocapsules, composed of an oily reservoir (Miglyol 812®; Omya Peralta GmbH, Universal Preserv-A-Chem, Inc.) and a CS shell, belong to this category. These nanocapsules have been evaluated for their ability to favour the nasal and intestinal absorption of salmon calcitonin. The results have shown a significant pharmacological effect of salmon calcitonin when associated to CS nanocapsules [26]. Equally positive results (Figure 3) have been observed for CS-coated solid lipid nanoparticles that were administered orally [33].

In agreement with the results observed for CS-coated lipid cores, CS-coated liposomes have also exhibited an interesting behaviour as oral peptide (i.e., insulin) delivery systems [34,35]. More specifically, these liposomes have shown the capacity to induce a significant reduction of glycaemia following oral administration to rats. The same type of formulation has also been successful for the oral delivery of salmon calcitionin [36,37].

Finally, with regard to the potential application of CS-coated systems for the delivery of macromolecules, the interest of CS-coated poly(lactic acid) (PLA) nanoparticles for the nasal delivery of proteins should be mentioned [38].

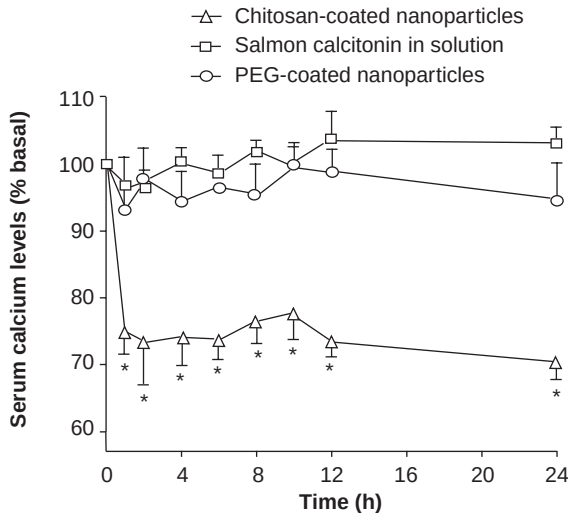

Figure 3. Serum calcium levels alter oral administration to conscious rats of salmon calcitonin in solution, or associated to PEG-coated nanoparticles or chitosan-coated nanoparticles (mean ± standard deviation, n = 6). Reproduced with permission from GARCIA-FUENTES M *et al.*: *Eur. J. Pharm. Sci.* (2005) **25**(1):133-143 [33].

*Significant differences from the control calcitonin solution ($\alpha < 0.01$).
PEG: Poly(ethylene glycol).

However, these specific systems will be described in more detail in the following section.

2.1.2.2 PLA- and PLGA-based nanosystems for macromolecular drug delivery

Controlled-release biodegradable polyesters such as PLA and PLGA have been the most investigated materials for drug delivery application, including nanosized carrier systems for hydrophilic macromolecules [9,39]. However, over the last few years it has become clear that these very popular carriers could be further improved in terms of i) protection of the encapsulated biologically active molecules, ii) their stability in contact with biological fluids and, most importantly within the context of this review, iii) their interaction and penetration capacity with biological membranes such as mucosal surfaces. This improvement has recently been the focus of several research reports [38-42].

Considering the fact that the above-described limitations are, at least in part, related to the hydrophobic character of the polyesters, it has been proposed that the modification of the carriers with hydrophilic materials could further improve the performance of the carriers. Without doubt, the most successful hydrophilic polymers for such a modification have been poly(ethylene oxide) (PEO), also named PEG, and its derivatives, such as PEO-poly(propilene oxide) block copolymers (i.e., poloxamers or poloxamines).

The first reports on the potential of PLA-PEG nanoparticles for improving the transmucosal transport of large proteins either administered nasally or orally were published at the end of the 1990s [43,44]. The explanation for the improved performance of PLA-PEG particles compared with the classical PLA nanoparticles was found in the increased stability of these nanocarriers in biological fluids [38,44]. It was suggested that the greater stability could result in a greater transport of the particles across the mucosal barriers, as was later observed by confocal microscopy [45]. This improved transport could also explain the ability of these nanoparticles to enhance the absorption of the encapsulated model protein (i.e., tetanus toxoid [TT]). Indeed, the blood circulation levels of radioactive TT were 10- and 5-fold of those achieved for PLA nanoparticles following nasal and oral administration, respectively [38]. More recent studies have also led to the conclusion that this carrier function of the nanoparticles is predominately dependent on their size and PEG surface density. A small size (200 nm) and a high PEG density were identified as critical factors for an efficient transmucosal transport (Figure 4) [46].

Similarly good results have been obtained with a newly developed system consisting of intimate blend nanostructures of PLGA with different PEO derivatives (i.e., poloxamers and poloxamines). These systems were designed with the aim of stabilising delicate macromolecules inside the polymeric nanoparticles [42,47]. However, recent studies have pointed out the additional advantage of these nanocarriers related to their ability to overcome mucosal surfaces [48]. So far, the performance of these nanostructures as nasal carriers has been mostly investigated for antigenic proteins and plasmid DNA. Consequently, these specific aspects will be discussed in detail in the following section, which is dedicated to transmucosal antigen delivery to the immune cells.

Another hydrophilic polymer that has been proposed for improving the performance of PLGA nanoparticles is CS. In this case, the idea was to confer bioadhesive properties to the nanocarrier. However, the results of the work of Alonso *et al.* have also shown that this CS coating prevents the aggregation of PLGA nanoparticles in the presence of proteins (i.e., lysozyme) [38]. Moreover, it has been observed that CS-coated PLGA nanoparticles have a greater capacity to transport a large protein through the mucosa than the uncoated PLA nanoparticles [38]. These results agree with those obtained by Kawashima *et al.* who described the benefit of the CS coating around PLGA nanoparticles in terms of improving the intestinal absorption of calcitonin [49].

The use of polyanhydrides as bioadhesive additives for PLGA particles has also been reported. Particles consisting of blends of PLGA and fumaric anhydride oligomers were more efficient as oral insulin carriers than the corresponding unmodified PLGA particles. Indeed, this formulation reached a 11.4% of pharmacological bioavailability relative to an intraperitoneal zinc–insulin injection [50]. Recent works have also described the synthesis of polyester–polyanhydride copolymers, and their use for micro/nanoparticle formation, indicating their potential application for the delivery of plasmid DNA [51,52]. However, so far, the efficacy of these gene delivery carriers has only been evaluated *in vitro*.

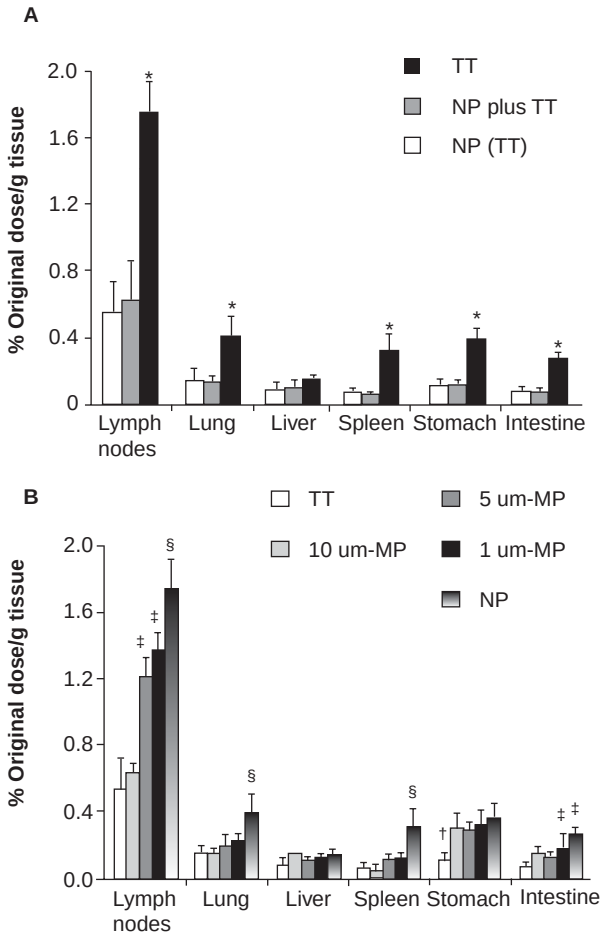

Figure 4. A. ^{125}I-TT tissue levels following nasal administration of the free antigen (TT), a physical mixture with the nanoparticles (NP plus TT) and encapsulated TT (NP [TT]), to conscious rats. B. ^{125}I-TT tissue levels following nasal administration of the free antigen, or encapsulated in poly(lactic acid)-poly(ethylene glycol) particles of different size. Adapted with permission from VILA A *et al.*: *Int. J. Pharm.* (2005) **292**(1-2):45-52 [46].

*Significant differences ($p < 0.05$) of the encapsulated toxoid with respect to TT and NP plus TT. ‡Significant differences to the solution, the 10 µm particles and the 5 µm particles. §Significant differences ($p < 0.05$) with respect to the other groups.
MP: Microparticle; NP: Nanoparticle; TT: Tetanus toxoid.

PLGA copolymers have also been blended with polyacrylic polymers such as Eudragit RL or Eudragit RS with the final goal of delivering heparin by the oral route. Interestingly, the encapsulation efficiency of heparin in these blended nanoparticles was significantly improved compared with that of PLGA nanoparticles. Moreover, the bioavailability of heparin following oral administration to rabbits of these nanocarriers was very high, ranging between 9 and 23% depending on the particle composition [53]. Unfortunately, the specific role of the acrylic polymers on the *in vivo* performance of the nanocarrier could not be elucidated because the PLGA control carriers were not evaluated *in vivo*, due to the low heparin loading capacity of the control PLGA particles. Irrespective of the specific role of each component of the nanoparticles, these results emphasise the possibilities of further improving the potential of PLGA nanoparticles as transmucosal delivery carriers.

2.1.2.3 Polyacrylic derivatives as nanocarriers for transmucosal macromolecular delivery

Other polyacrylic acid derivatives, different to Eudragits, such as poly(methacrylic acid-g-ethyleneglycol) have also been used in the development of transmucosal nanocarriers. The rationale behind the design of poly(methacrylic acid-g-ethyleneglycol) nanoparticles is the mucoadhesive character of this copolymer, together with its capacity to transiently open tight junctions in the absorptive epithelium. This hypothetical mechanism has been corroborated in cell culture models [54,55]. Although no *in vivo* data are yet available for this promising nanosystem, studies performed with larger particulate carriers have already indicated the potential of these polymers for oral peptide (insulin) delivery [56,57].

2.1.2.4 Polyanhydride-based nanocarriers for transmucosal macromolecular delivery

Besides the use of blends and copolymers of polyanhydrides and PLGA as described in Section 2.1.2.2, nanoparticles made of solely polyanhydrides have also been studied as carriers for intestinal delivery of drugs with very different physicochemical properties and molecular size (i.e., dicumarol, insulin, plasmid DNA) [58]. This early work, which had a great deal of impact on the transmucosal drug delivery field, showed the capacity of these particles to cross biological membranes and to increase the absorption of the associated compounds. More specifically, this work showed that orally administered insulin-loaded poly(fumaric acid-co-sebacic acid) nanoparticles were able to protect normoglycaemic rats from a subcutaneous glucose load. In addition, this work reported the efficiency of polyanhydride nanoparticles as oral gene delivery carriers.

Another strategy that has been very actively investigated relies on the use of specific targeting moieties (e.g., lectins, invasins and so on) in order to enhance the selective binding and uptake of particles to the mucosa. Although this technology has been, until now, mostly investigated in nonbiodegradable model nanoparticles [59], very recent reports have indicated its potential for the design of new polyanhydride-based nanocarriers [60,61]. This can be illustrated by the use of Gantrez® AN (methyl vinyl ether-maleic anhydride copolymer; International Speciality Products) nanoparticles containing flagellin from *Salmonella enteritidis*. These carriers display an important bioadhesive character and a tissue distribution similar to the colonisation profile of *S. enteritidis*. Unfortunately, there is still very little information regarding the beneficial effects of these targeted polyanhydride formulations when loaded with macromolecules [61].

2.2 Transmucosal drug delivery for local treatment: drug delivery to the ocular surface

As well as the attention given to the transmucosal delivery to the systemic circulation, in the past few years there have also

Table 3. Nanocarrier systems for topical ocular drug delivery.

Nanocarrier system	Drug	Ref.
PACA nanoparticles	Pilocarpine, amikacine, progesterone	[64-66]
PECL nanocapsules	Metipranolol, carteolol	[68,69,73]
CS- coated PECL nanoparticles	Marker*	[72]
PEG-PECL nanoparticles	Marker*	[72]
CS nanoparticles	Ciclosporin A	[74]
CS nanocapsules	Indometacin	[73]
PEG-PECA nanoparticles	Acyclovir	[67]
PLA-PEG nanoparticles	Acyclovir	[71]
CS-hyaluronate nanoparticles	pDNA	[76-78]

*Mechanistic studies.
CS: Chitosan; PACA: Polyalkylcyanoacrylate; pDNA: Plasmid DNA; PECA: Poly(ethyl-2-cyanoacrylate); PECL: Poly(ε-caprolactone); PEG: Poly (ethylene glycol).

been important advances in the area of transmucosal drug delivery for local therapeutics. Among the different mucosal surfaces for local drug administration, the ocular surface is considered to be one of the clinical targets that could significantly benefit from the development of an efficient drug carrier. In fact, it is well known that some ocular therapies are being greatly limited by the critical biological barriers of this modality of administration. These barriers include i) the intensive drainage of the eye surface, which makes the retention of the drug very difficult, ii) the highly organised multilayered structure, which restricts the drug penetration to the target site, and iii) the highly vascularised conjunctiva, which facilitates the systemic absorption [62].

The design of drug carriers that target the outer regions of the eye (i.e., the conjunctiva, corneal tissue and aqueous humor) is presently considered as a promising approach towards increasing the drug surface retention, as well as enhanced ocular penetration. This has been the specific subject of a recent review chapter [63]. Therefore, in the next few sections the authors intend to briefly analyse the evolution in the design of these nanocarriers (Table 3).

2.2.1 *PACA-based nanosystems for ocular drug delivery*

The pioneering work in this field was carried out in the 1980s for polyacrylcyanoacrylate (PACA) nanoparticles. These authors showed, for the first time, that these nanoparticles could be retained in the precorneal area and, consequently, they suggested their potential as reservoires for sustained drug release [64]. This retention of the carriers at the ocular surface was the explanation for the increased drug concentration in the cornea, and for the enhanced and/or prolonged pharmacological effect reported for a number of drugs [65,66].

Recently, it has been indicated that the efficacy of PACA nanosystems can be further improved by providing them with a PEG coating. More specifically, the authors of this work found that PEG-poly(ethyl-2-cyanoacrylate) nanoparticles have a much greater capacity for the transport of the antiviral drug acyclovir across the cornea than the corresponding drug-loaded nanocarriers without the hydrophilic PEG segments [67]. Consequently, this work suggests the possibility of further enhancing the performance of ocular nanocarriers using the pegylation approach.

2.2.2 *PECL-based nanosystems for ocular drug delivery*

Over the 1990s, the attention in this specific field was significantly driven from PACA nanoparticles to polyester-based nanosystems. In particular, PECL nanocapsules were found to be efficient carriers for increasing the ocular penetration of drugs across the cornea [68,69]. This carrier capacity was attributed to their ability to interact with the ocular mucosa and enter the corneal epithelium, as shown by confocal microscopy [70].

As observed for PACA nanoparticles, the performance of polyester (PLA or PECL) nanocapsules could be further improved thanks to the polymer pegylation [71]. Mechanistic confocal microscopy studies have evidenced that PEG-coated PECL nanocapsules tend to penetrate the corneal epithelium to a greater extent and more deeply than those that are non-pegylated [72]. Therefore, the conclusion is that, as in the case of PACA, the coating of polyester nanosystems with PEG has a positive effect on the efficiency of these carriers for the transport of drugs across the corneal barrier.

2.2.3 *Chitosan-based nanosystems for ocular drug delivery*

An alternative to the PEG coating has been found in the use of hydrophilic bioadhesive materials, such as CS. In fact, bioadhesive drug delivery nanosystems are particularly attractive for this goal, as they exhibit an intimate interaction with the protective ocular mucosa. This advantageous effect has been described for CS-coated nanocapsules [73] and, more recently, for CS nanoparticles [74]. Indeed, confocal microscopy analysis of the *in vitro* and *in vivo* interaction of these nanocarriers with the ocular mucosa has shown their capacity to adhere to, and even enter, the superficial layers of the conjunctival and corneal epithelia [75]. This mechanism has been proposed to explain the efficacy of both CS-coated nanocapsules and CS nanoparticles for improving the ocular penetration of indometacin [73] and ciclosporin A [74], respectively. The specific details of these studies are described in a previous review of the potential of CS for ocular drug delivery [41].

As a step further, Alonso and colleagues have recently developed a new ocular drug carrier consisting of a nanomatrix of CS and hyaluronic acid [201]. The idea of incorporating hyaluronic acid in the nanoparticles structure comes from the known presence of hyaluronic acid receptors (CD44) in the corneal and conjunctival epithelial cells.

Therefore, the purpose was to optimise the performance of the nanocarriers due to a receptor-specific interaction. As expected, the experiments that were performed in cell culture studies (conjunctival and corneal cell lines), and also *in vivo* (rabbits), have revealed the capacity of these nanoparticles to enter the cells and the specific role of the CD44 receptor in this uptake. Moreover, and in agreement with this mechanistic information, these nanocarriers have shown a great efficiency as gene delivery systems to the corneal and conjunctival cells [76,77]. Finally, these novel ocular nanocarriers have exhibited very good tolerance and a low toxicity, both *in vitro* and *in vivo* [78].

2.3 Transmucosal vaccine delivery to the immune system

The interest in the field of the transmucosal delivery of antigenic peptides and antigen encoding genes arises from the fact that mucosal surfaces represent the major site of entry for many pathogens. Indeed, it is widely accepted that the best strategy for combating these pathogens is the development of an efficient local defence at the starting point of the infection [79,80]. In contrast to injected antigens, mucosal vaccine administration can induce both systemic and mucosal immune responses, including immunity even at mucosal surfaces different from the administration site [81,82]. Besides its unique possibility for the induction of combined and suitable immune responses, the transmucosal route could also offer simplified, cost-effective administration protocols and better patient compliance as compared with parenteral vaccination [79,83].

The most accessible routes for mucosal vaccination are the nasal, pulmonary and oral routes. Although the oral route is the most attractive for delivering drugs to the systemic circulation, the nasal route is now considered to be particularly attractive for vaccination purposes. This attractiveness relies on a number of features. For example, the nasal mucosa presents a greater permeability and lower enzymatic activity than the gastrointestinal compartment [84]. In addition, due to their defensive role, the nasal and the upper airway epithelia are characterised by the presence of a massive number of dendritic cells, which are known as the most potent antigen-presenting cells [85].

The majority of vaccines are large and are delicate biomacromolecules (i.e., antigenic peptides/proteins and plasmid DNA), which can be easily degraded and poorly transported across mucosal surfaces. Consequently, their incorporation in a transmucosal nanocarrier could be very positive in terms of protection and facilitated transport through the mucosal barrier. However, due to the very specific organisation and function of the immune system, a successful carrier should also meet some additional requirements. More precisely, apart from protective and carrier roles, it is also necessary that the delivery system provides a suitable way for antigen recognition by the immunocompetent cells. This represents a key factor in the adequate processing and presentation of the antigen and the subsequent development of an immune response [86]. In this sense, and as indicated in previous review articles [80,87,88], the design of optimised nanoparticulate vaccine carriers offers a promising future for transmucosal vaccination.

From the early 1990s, microparticulate vaccine delivery systems have received much attention, due to the high phagocytic activity of antigen-presenting cells that readily ingest particulates in the low micrometric size range [89]. However, as the knowledge in this specific area has evolved, there has been a growing interest in the design of submicrometric systems for antigen delivery. Within the context of mucosal administration, the idea behind this interest is that nanocarriers could offer superior penetration capacities compared with micrometric formulations. In addition, it is expected that the rational design of nanostructures could improve their interactions with the target cells and their internalisation through phagocytic/endocytic processes.

Due to their demonstrated potential in drug delivery applications, polysaccharide- and polyester-based nanocarriers have been the most intensively investigated platforms as candidates for transmucosal vaccine delivery. The potential of these types of nanoparticles has been extensively revised in [87]. Therefore, herein the authors emphasise the critical information that is paving the way of the novel transmucosal vaccination approaches.

2.3.1 *Chitosan-based nanosystems for vaccine delivery*

CS-based antigen delivery systems include CS nanoparticles, CS-coated nanoemulsions (also called nanocapsules) and, in the specific case of genetic vaccines, electrostatic assemblies with plasmid DNA. The first reports of the potential of CS nanoparticles for antigen delivery were published only a few years ago [38,90]. These original reports showed that intranasally administered CS nanoparticles carrying the model antigen TT were able to elicit high and increasing levels of both serum and secretory immune responses for prolonged periods (Figure 5). Other subsequent studies carried out with ovalbumin encapsulated into CS nanoparticles and CS-coated nanoemulsions also corroborated the efficacy of these nanocarriers in terms of antibody production [91].

Knowing the positive role of the polymer pegylation on the performance of transmucosal drug delivery systems, Alonso *et al.* recently decided to explore the possible benefits of this modification on CS-based vaccine nanocarriers. Therefore, a comparative study of the efficacy of CS nanoparticles and CS-PEG nanoparticles as carriers for diphtheria toxoid was performed. The results, in terms of IgG and IgA responses, were very positive for both types of nanocarriers. However, the response was significantly more pronounced for the pegylated nanocarriers (manuscript in preparation).

As previously mentioned, in the field of genetic vaccination (i.e., administration of DNA/RNA sequences encoding antigenic proteins), electrostatic polymer-nucleic acid assemblies have also gained considerable attention. The presence of positively charged primary amine groups give CS the potential to interact ionically with the negatively charged nucleic acids (e.g., plasmid DNA), thereby allowing the formation of electrostatic complexes following mixing [92]. During the last few years,

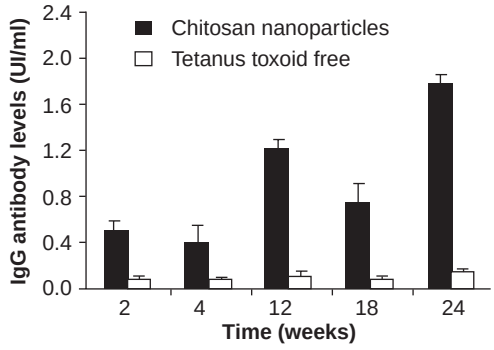

Figure 5. IgG antibody levels following intranasal administration of tetanus toxoid entrapped in chitosan nanoparticles or in phosphate-buffered saline solution to mice (mean ± standard deviation, n = 6 – 9). Reproduced with permission from VILA A, SANCHEZ A, TOBIO M, CALVO P, ALONSO MJ: Design of biodegradable particles for protein delivery.: *J. Control. Release* (2002) **78**(1-3):15-24 [38].

several reports have been published on the ability of CS-based complexes to deliver and enhance the expression of antigen-encoding plasmid DNA. More importantly, these complexes led to successful transfection levels *in vivo*, following nasal, oral and pulmonary administration [93-97].

Recently, the promising characteristics of CS–DNA complexes and the excellent capacity of CS and CS-PEG nanoparticles for antigen delivery were combined by the adjustment of the ionic gelation process for the encapsulation of plasmid DNA. Indeed, this new method results in more specific interactions and renders better stability to DNA and to the delivery system itself (manuscript in preparation). In addition, this technique also allows the coencapsulation of other molecules that may facilitate the extremely complex intracellular trafficking of the genes of interest, which remains a crucial factor in the success of genetic vaccines.

Analogously to protein antigen delivery, nasal immunisation with a β-galactosidase encoding gene encapsulated into CS and CS-PEG nanoparticles showed significantly higher IgG levels than the DNA control solution. The IgG titres, generated as a consequence of the expression of the β-galactosidase protein, were similar for the non-pegylated and pegylated carriers; however, the pegylated carriers provided a more prolonged systemic immune response (manuscript in preparation).

Altogether, these studies clearly indicate the potential of CS-based nanosystems for the efficient transmucosal delivery of both protein and DNA vaccines, eliciting specific, significant and prolonged immune responses against the encapsulated antigens. They also underline the possibility of further increasing this potential using pegylated CS.

2.3.2 PLA- and PLGA-based nanosystems for vaccine delivery

The application of polyesters such as PLA and PLGA in the field of vaccine delivery commenced at the beginning of the 1990s and was greatly supported by institutions such as the WHO. PLGA microparticles were originally proposed for vaccine delivery due to their biodegradability and their capacity for controlling the release of antigens for long periods of time. Therefore, the primary goal was to design single-dose vaccine formulations [98,99]. Unfortunately, these initial microspheres, which were composed of only PLGA, soon showed important limitations that were related to the instability of the encapsulated vaccine in the course of the polymer degradation. As a consequence, the objective in this area over the last decade has been the design of antigen stabilisation approaches within PLGA microspheres [89]. Within the context of transmucosal vaccination, it was thought that the reduction of the size down to the nanometric range (PLGA nanoparticles) could enhance their performance as transmucosal carriers. In fact, a number of articles have been published showing the capacity of PLGA nanoparticles to induce high immune responses against the associated antigen (bovine serum albumin, ovalbumin) following either nasal [100] or oral administration [101]. Overall, the results of these studies have underlined the importance of the size on the efficacy of PLGA particles as carriers for transmucosal vaccination. However, it was soon found that this potential of PLGA nanoparticles could be further improved by making them more stable in biological fluids. In fact, it has been observed that PLGA nanoparticles have a tendency to aggregate in mucosal fluids, a behaviour that may logically affect their capacity to interact and overcome mucosal barriers (as detailed in Section 2.1.2.2).

Taking into account these limitations, the research that has been carried out in the last few years has been addressed towards overcoming the poor stability of the particles in biological fluids and the poor stability of the encapsulated antigen. A successful, recently identified method is based on the use of hydrophilic polymers such as PEG, poloxamers and poly(vinyl alcohol) (PVA), either covalently linked or physically blended with PLGA. For example, advanced nanocarriers have been developed from PLA-PEG block copolymers, PLGA:PEO blends and PVA-graft-PLGA branched polymers.

As indicated in Section 2.1.2.2, the first evidence of the positive role of a PEG coating in terms of improving the nasal transport of TT encapsulated into PEG-PLA nanoparticles was shown in 1998 [43]. Some years later the same nanoparticles were assayed for their efficacy to elicit an immune response following nasal administration to mice. The results showed high and prolonged immune responses, both at the mucosal and systemic levels, the corresponding IgA and IgG antibody titres being significantly higher than those elicited by PLA nanoparticles [102,103].

Furthermore, PLA-PEG nanoparticles have also been evaluated regarding their potential for the delivery of genetic vaccines. In this case, the serum IgG levels were monitored following the nasal administration of a β-galactosidase encoding plasmid DNA (pCMV-BGal). At 1-month postadministration,

the systemic immune response generated by the encapsulated plasmid was significantly stronger than that elicited by the naked plasmid DNA [103].

Similar results were obtained very recently with PLGA:PEO blend nanostructures [48]. More specifically, the nanoparticles consisting of PLGA:poloxamer 188 blends led to IgG immune responses, which were significantly higher than those corresponding to the control pCMV-BGal solution. In addition, the results of this work showed that the performance of the carriers varied depending on the hydrophilic/hydrophobic character of their matrix compositions [48]. In the case of the PLGA:poloxamer 188 blend nanoparticles, their success could be attributed on one hand to the facilitated transport observed for these nanoparticles across the nasal mucosa and, on the other hand, to the enhanced lymphatic access that has already been described for poloxamer-coated nanostructures [104].

Another interesting approach for the modification of polyesters has been their grafting to a PVA backbone, forming a branched polymer structure. Similarly to the PEG-based approaches, the PVA provides a hydrophilic nature for the polymer; the degree of the hydrophilia being easily adjustable according to the length of the PLGA chains and the grafting ratios [105]. Further modifications of this new class of polymers include the replacement of the PVA backbone by sulfobutylated PVA [106] or the inclusion of diethylaminoethylamine, diethylaminopropylamine or dimethyl-aminopropylamine to render a net positive charge to the polymer structure [107]. Sulfobutylated PVA-graft-PLGA nanoparticles have been evaluated for the delivery of TT by the oral and nasal route. Following nasal administration, a significant increase of IgG and IgA levels were detected for the particle-associated antigen with respect to the control formulation. The results of the immune response that were obtained following oral administration were satisfactory, although the levels were lower than those observed following nasal administration [108]. In addition, in a different report, the authors suggested that higher degrees of sulfobutylation facilitate the cellular uptake of the particle [109]; however, this finding remains to be confirmed *in vivo*. Similarly, the positively charged amine-modified branch PVA-g-PLGA polymers have shown good DNA association capacities and satisfactory *in vitro* transfection results [40,107]. However, these novel carriers still need to be evaluated *in vivo* in order to assess their potential for genetic vaccination.

3. Conclusions

In this manuscript the advances in the most prominent applications of drug nanocarries for transmucosal drug delivery have been reviewed. These applications include the oral/nasal systemic delivery of hydrophilic macromolecules (drugs and antigens) and also local drug delivery to the eye surface. Numerous findings suggest the great potential of nanosized drug delivery systems and their promising future from a clinical and industrial perspective. However, rational design of these carriers should take into consideration the necessity to optimise the characteristics of the system for the desired application. Overall, the results from the authors and several others suggest that the use of carrier systems based on, or modified with, hydrophilic polymers such as polysaccharides (CS, glucomanan, hyaluronic acid and so on) PVA, and PEO and its derivatives present a feasible way of enhancing interactions with the mucosa and penetration through biological barriers.

4. Expert opinion

The first pieces of evidence of the potential of nanoparticles for transmucosal drug delivery were published almost two decades ago, specifically for systemic oral and ocular drug delivery. Since then, they have gained increasing attention as compared with other traditional approaches for the delivery of problematic drugs, such as absorption enhancers. The results from a number of successful proof-of-concept studies provide sound reasons for bringing this technology into the spotlight. Nevertheless, there are still numerous challenges that are lying ahead before nanocarriers can reach widespread clinical application.

Most limitations that are related to the use of nanocarriers arise from the poor understanding of their exact mechanism of action and their possible toxicity. For example, the beneficial effects of a particular composition are often attributed to different mechanisms of action by different authors (e.g., transcellular transport of particles versus simple adhesion to the mucus layer). The lack of critical knowledge has commonly led to very simplistic considerations about the way these systems behave in biological systems. It is evident that many studies have ignored existing barriers or potential processes such as i) particle aggregation in biological fluids, ii) enzymatic degradation of the delivery system, iii) limited transport through the mucus layer, iv) inadequate drug-release profile in relation with the uptake kinetics of the carrier, and v) slow transit from the lymph nodes to the blood and so on. In this regard, the biological and pharmaceutical considerations that should be addressed for the rational design of a transmucosal carrier have been summarised in a recent review [3]. It is this limited understanding of the biopharmaceutics of a newly designed system that accounts for several failures, particularly in the clinical setting when carriers sometimes do not perform accordingly to what was expected from previous animal experimentation.

These very simplistic considerations have also led to misconceptions about the optimal characteristics that are required for a transmucosal nanocarrier. Although the traditional hypothesis has been that hydrophobic carriers are expected to be taken up at a higher extent, results from the authors' laboratory and many others have shown better therapeutic potential for some hydrophilically coated carriers. For instance, it has been found that PEG can improve particle stability and also particle transport through some epithelia [38,44]. Particle coating with some cationic materials (most prominently CS) have also resulted in

better efficacy of many drug carriers, which can be attributed to multiple factors such as improved stability, enhanced adhesion to the mucus, improved diffusion through the mucus layer and higher particle transport through the cell membrane [38,110,111]. Thus, it is also plausible that specific surface modifications of these nanostructures can lead to optimised nanocarriers in the near future. However, when making this prediction, it is important to keep in mind that those hydrophilic polymer-coated carriers cannot be seen as simple nanocontainers, whose behaviour is independent of the drug loaded into them. In contrast, they should be seen as nanomedicines that have to be conveniently optimised for each specific compound and modality of administration.

The oral and nasal routes are probably those that will benefit the most from new drug delivery carriers. However, the positive results that were observed for the topical ocular route of administration also offer a very optimistic perspective for this specific application. Similarly, there are other modalities of administration for which nanocarriers have not been extensively investigated at the academic level; however, they could potentially become a niche market for novel therapies. An example of this may be vaginal administration.

With respect to the target drugs to be associated to transmucosal nanocarriers, it is obvious that the therapeutic benefits will be especially important for drug molecules with great difficulties in overcoming mucosal barriers. These are molecules that are poorly soluble, poorly permeable or highly degradable. Among those, a critical impact could be anticipated for therapeutic macromolecules, vaccines and nucleic acid-based drugs.

Overall, the information accumulated until now in this field indicates that the promise of nanomedicines for overcoming mucosal barriers are now starting to be fulfilled. Alonso *et al.* believe that recent advances in the design of drug delivery systems, as well as an improved comprehension of the behaviour of these carriers in the biological environment, will lead to a new generation of nanomedicines that are capable to simplify and make more effective actual and future therapies.

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