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Functional PLGA NPs for Oral Drug Delivery: Recent Strategies and Developments

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Abstract: This article presents the potential of PLGA nanoparticles for the oral administration of drugs. Different strategies are used to improve oral absorption of these nanoparticles. These strategies are based on modification of nanoparticle surface properties. They can be achieved either by coating the nanoparticle surface with stabilizing hydrophilic bioadhesive polymers or surfactants, or by incorporating biodegradable copolymers containing a hydrophilic moiety. Some substances such as chitosan, vitamin E, methacrylates, lectins, lecithins, bile salts and RGD molecules are employed for this purpose. Of especial interest are nanoparticles production methods and, in order to improve oral bioavailability, the mechanism of each additive.

Keywords: Biodistribution, cellular uptake, mucoadhesion, NPs, oral bioavailability, PLGA, targeting.

INTRODUCTION

Oral administration is one of the most commonly-used routes for drug administration because of its non-invasive nature and the fact that it avoids the pain and discomfort associated with injections, as well as eliminating contamination. However, it is not feasible when the actives present unfavourable characteristics: inadequate physicochemical properties for intestinal absorption, stability or solubility problems and a clear decrease in bioavailability by first-pass hepatic effects [1]. The use of polymeric NPs presents a great opportunity to solve these problems. Compared to larger carriers, their submicron size and large specific surface area favour their absorption. Different polymers have been developed to formulate NPs: (poly(lactic acids) (PLA), poly(lactic-co-glycolic acids) (PLGA), poly(ε-caprolactone) (PCL), poly(methyl methacrylates), and poly(alkyl cyanoacrylates)) or natural polymers such as albumin, gelatin, alginate, collagen or chitosan [2, 3]. Particles comprised of the polymer poly(lactic-co-glycolic acid) (PLGA) are widely studied as therapeutic delivery vehicles [4]. Interest in PLGA is due to its biocompatibility, commercial availability in different grades, its ability to control drug release, the fact that it degrades into completely safe products (its monomers) and glass transition temperature (Tg) in the range of 45 °C–55 °C, which is above the physiological temperature of 37 °C, lending the required mechanical strength for formulation development. In fact, particles comprised of PLGA have already been approved for establishing the sustained release of leuprolide (Lupron Depot®, Abbott Lab.) and triptorelin (Trelstar®, Watson Pharm.). Similar PLGA particles also show promise as a delivery vehicle for proteins [5], siRNA [6] and for presenting antigens to dendritic cells for vaccination [7].

After oral administration, NPs (usually in suspension) are taken up and transported across the mucosal epithelium. Before reaching these locations, NPs protect the active ingredient in the gastro-intestinal (GI) tract and/or prolong the residence time of their contents on the mucous membrane. The main mechanism for drug uptake through the intestinal epithelium is passive diffusion driven by a concentration gradient. Passive diffusion can occur between cell junctions (paracellular transport) or through the cytoplasm (transcellular transport) [8]. Transcellular transport of NPs occurs through the process of transcytosis, by which particles are first internalized (endocytosis) at the cell apical plasma membrane, followed by transportation through the cell and finally, released (exocytosis) across the basolateral membrane. Endocytotic processes can be divided into receptor-mediated endocytosis (RME) and adsorptive endocytosis. RME requires the binding of specific ligands to apical cell membrane receptors in order to initiate the endocytotic process. Moreover, adsorptive endocytosis does not require any specific ligand–receptor interactions and can be initiated by the physical adsorption of particulate carriers to the cell surface through electrostatic forces (H-bonding or hydrophobic interactions) [9].

The existence of multiple drug transport pathways allows the development diverse strategies to improve the oral bioavailability of drugs encapsulated in PLGA NPs. Some focus on M cells, while others target not only M cells but all intestinal cells, especially enterocytes. Strategies to escape the clearance mechanism (NPs are rapidly recognized and uptaken as foreign objects by macrophages) and thus to

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improve the *in vivo* biodistribution are developed. Most of these approaches are based on Nanoparticle surface engineering. Modification of Nanoparticle surface properties can be achieved either by coating the Nanoparticle surface with stabilizing hydrophilic bioadhesive polymers or surfactants, or by incorporating biodegradable copolymers containing a hydrophilic moiety in the formulation. These modifications mainly change Nanoparticle zeta potential (ZP), as well as their hydrophobicity, thus influencing the formulation of colloidal stability, Nanoparticle mucoadhesion properties, and protein adsorption at their surface, and finally, oral absorption of the NPs [2].

A clear example that PLGA NPs can improve the oral bioavailability of a drug, is evidenced in the recent work by Zhang *et al.* 2012 [10]. In this work, insulin-loaded modified PLGA NPs were administered orally. In vivo pharmacodynamics assays in rat showed that PLGA NPs presented a stronger effect on plasma glucose levels, indicating that chitosan-modified PLGA NPs could enhance the intestinal absorption of insulin due to their positive charge behavior when compared to free insulin and also to non-modified-PLGA NPs. The pharmacological availability of the two kinds of NPs assayed, PLGA-NP and CS-PLGA-NP, relative to subcutaneous injection were calculated to be 7.6% and 10.5%, respectively, at an insulin dose of 15 IU/kg. On the other hand, *in vivo* transport of NPs via peroral administration assays demonstrated that NPs can efficiently protect the encapsulated drug from gastric juice and enzymatic degradation. CS-PLGA-NP has good biological activity, consistent with the conclusions drawn from pharmacodynamics studies.

The aim of this review is to summarize the challenges and opportunities of using PLGA (and its co-polymers) NPs to enhance drug transport across the intestinal barrier in order to improve oral drug bioavailability.

PLGA NPs SURFACE ENGINEERING

The main strategies employed to modify PLGA NPs and the role of each additive is described. All the examples and references included in the present work correspond to NPs that were synthesized with PLGA as their main polymeric matrix. Among the additives employed to modify the surface are polymers such as chitosan, polyethyleneglycol, methacrylates, bile salts, lecithins, RGD molecules, etc.

CHITOSAN

Chitosan is a hydrophilic biopolymer which forms films, hydrogels, scaffolds, fibers, sponges and micro- and NPs under soft conditions. Chemically, it is an aminopolysaccharide obtained by N-deacetylation of chitin from several crustacean species. In conventional pharmaceutical devices it is used as binder, disintegrant and coating material. In NPs for oral administration, chitosan has been widely examined as a potential oral absorption enhancer due to its mucoadhesive properties and its capacity to open tight junctions between epithelial cells, thus facilitating the transport of macromolecular drugs through well-organized epithelia [11]. Recently, Zheng *et al.* (2011) [12] studied the action mechanism of CS-NP on the tight junctions of Caco-2 cells. These authors have demonstrated a disruption of tight junctions by CS or CS NPs, indicated by the translocation of Z0-1 proteins and loose F-actin proteins in the plasma membrane.

Although, chitosan NPs are also presented as a possible system for oral delivery, these systems presented limited physical stability in mucosal fluids. This polymer can therefore be employed as coating material for PLGA NPs. In this case the main objective is to modify the NPs' surface charge and to improve mucoadhesion properties. Chitosan coating can be performed by simple electrostatic interaction between negative charged PLGA and positively charged chitosan [13] or via chemical modification of PLGA chains with chitosan using the carbodiimide strategy [14].

Furthermore, *in vitro* studies have demonstrated protein adsorption onto PLGA NPs due to the negative ZP values [15].

ADSORPTION BY ELECTROSTATIC INTERACTION

This problem can be avoided by coating the PLGA NPs with, among other substances, chitosan, (see Fig. **1**) which, due to its hydrophilicity, should be adsorbed better than other lipophilic polymer derivatives. Moreover, this coating process does not exert any influence on subsequent particle behavior [16].

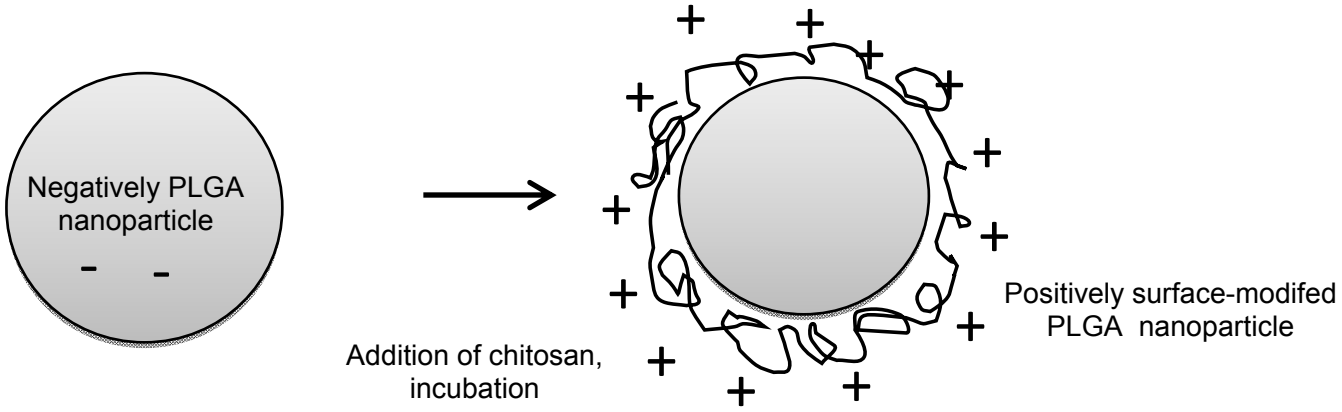

Fig. (1). Adsorption of chitosan on a negatively-charged PLGA particle.

This procedure has been successfully employed to improve oral bioavailability of peptides and proteins demonstrating its ability to enhance the penetration of large molecules across mucosal surfaces [11, 17].

In (Fig. **1**) are represented the electrostatic interaction of negative PLGA NPs with a cationic polymer, chitosan.

Tahara *et al.* (2011) [13] prepared PLGA NPs by double emulsion, $w_1/o/w_2$. The CS coating process was carried out during Nanoparticle synthesis by an electrostatic interaction, employing a blend of CS and a surfactant solution (PVA 1%). Cationic NPs able to bind DNA efficiently were obtained. The same authors prepared PLGA NPs by a simpler method, nanoprecipitaction. They compared plain *vs.* CS-coated NPs, both of which were loaded with a kappa B nuclear factor (NF-κB) for inflammatory bowel disease treatment.

In both cases the authors found that NPs coated with CS increased their size up to 50-70% due to the high molecular weight of CS and modified their ZP from slightly negative (-31.1 mV) to positive values (+11.2 mV). NPs were assayed in cell cultures and in animal models after oral administration, finding that coated NPs presented reasonably low cytotoxicity, improved stability and good interaction with inflamed mucosa.

Another possible process for PLGA NPs is to coat them with CS after the synthesis process. Once NPs are lyophilized, they are suspended in a CS buffer solution under stirring. Both strategies were assayed by Lee *et al.* (2011) [18] who prepared salmon-calcitonin-loaded PLGA for oral administration. Important differences in particle size between methods were found. The authors explained that when CS is added during particle synthesis, the increasing viscosity hampers drop formation. Meanwhile, when CS is added at the final stage, an additional coat would not increase the final size. Differences in entrapment efficiency were also found. When calcitonin-loaded PLGA particles were dipped into a CS solution, calcitonin diffuses into the solution with an acid pH. Under these conditions, calcitonin acts as a weak base (isoelectric point 10.4). On the other hand, *in vivo* studies demonstrated that particles coated during the synthesis process show a stronger, longer hypocalcemic effect. This was probably due to the embedded structure of CS molecules in the PLGA matrix.

Previously, Kawashima *et al.* (2000) [17] compared several polymers as mucoadhesive enhancers for PLGA nanospheres: poly(acrylic acid), sodium alginate and chitosan. The assays were carried out *in vitro* in everted rat intestinal sac. It was found that CS-coated nanospheres showed significantly stronger mucoadhesion. Furthermore, a direct relationship between CS molecular weight and mucoadhesion properties was also found. Although there were no specific mucoadhesion sites the following downward trend for mucoadhesion was observed: duodenum > jejunum > ileum. *In vivo* assays demonstrated, after intragastric administration, that CS-coated PLGA NPs reduced Ca level in a satisfactory manner over 12h (see Fig. **2**).

VIA CHEMICAL MODIFICATION

Chakravarthi *et al.* (2011) [14] compared the effect of CS adsorbed or chemically conjugated onto the particle surface on paclitaxel delivery in 4T1 cells. In this case, after the two coating processes no difference in particle size was observed. Lower negative ZP was found although polarity was not reversed. The authors studied the efficiency of both processes, quantifying the unincorporated amounts of CS by the ninhydrin method [19] and evaluating cellular association of the drug released. With regard to the coating process, a direct correlation of the extent of adsorption or conjugation as the initial amount of CS was observed, the conjugation process achieving 99% efficiency.

The chemical reaction between PLGA and CS occurs via the carbodiimide strategy where the formation of the amide

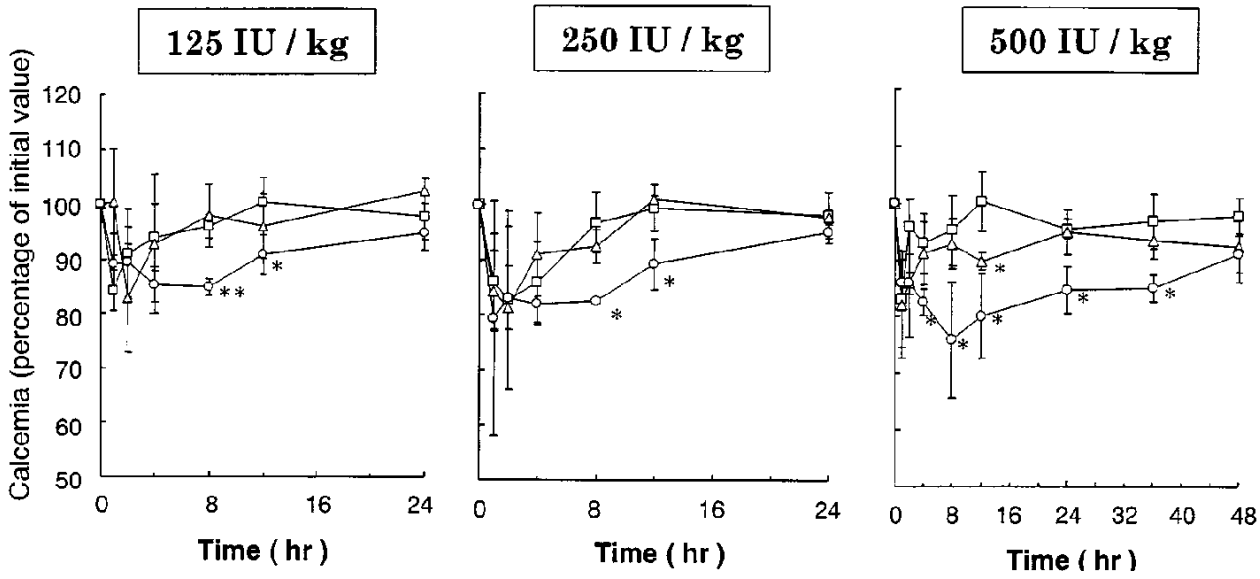

Fig. (2). Profiles of blood calcium after intragastric administration of elcatonin to fasted rats [17]: □ elcatonin solution; Δ uncoated PLGA NPs with ecaltonin; ○ CS-coated PLGA NPs with ecaltonin.

group takes place. The reaction, occurring on the surface of lyophilized particles, is catalyzed by 1-ethyl-3-(3-dimethylaminopropyl (EDC) and N-hydroxysuccinimide (NHS) is used as an additive to increase yields and decrease side reactions.

Grabovac and Bernkop-Schnürch, (2007) [20] used thiolated CS to prolong residence time on the mucosa. The surface of PLGA NPs was chemically modified with CS using the carbodiimide method described above. The remaining free amino groups of CS were then thiolated with 2-iminothiolane. This modification gives rise to the cationic amidine structure increasing the positive character of NPs which were confirmed by ZP measurements. Mean particle size increased notably (up to 3 times) and entrapment efficiencies were notably lower in coated PLGA NPs (28 *vs.* 56%). Nevertheless, *in vitro* assays on porcine small intestine demonstrated a significant improvement in mucoadhesion properties: 52% for uncoated PLGA NPs *vs.* 80% for coated PLGA NPs after 2h.

POLYETHYLENEGLYCOL (PEG)

To increase the success rate of drug targeting, it is necessary to minimize the NPs' opsonisation and prolong their *in vivo* circulation. This can be achieved by several methods. One such method is to coat NPs with hydrophilic polymers such as polyethyleneglycol (PEG). PEG is a hydrophilic, nonionic polymer that has been shown to exhibit excellent biocompatibility. PEG molecules can be added to the particles via a number of different routes including covalent bonding, mixing in during Nanoparticle preparation or surface adsorption [21].

Surface modification of NPs with PEG was found to reduce the accumulation of NPs in off-target organs such as liver and spleen [22]. This modification has favourable intrinsic physicochemical properties (e.g., high flexibility and hydrophilicity and low toxicity and immunogenicity). Moreover, this hydrophilic polymer forms a sterically stabilizing crown on the surface of the nanocarriers which protects the particles against GI degradation.

The length, shape and density of PEG chains on the Nanoparticle surface mainly affect the hydrophilicity and phagocytosis of the nanosystems [23]. When the PEG surface density is low, the configuration of PEG chains is called a "mushroom" configuration. When the density increases, the configuration is called a "brush" configuration [24]. Several studies show that the presence of PEG on Nanoparticle surfaces prevents opsonisation by complement and other serum factors [25]. It has been considered that the brush configuration would be effective at blocking or repelling opsonins than the mushroom one [26].

Much of the research conducted with these systems focuses on intravenously administered particles. However, very little information is known about the behaviour of PEGylated particles when administered orally. Semete *et al.*, 2010 [27] studied the *in vivo* uptake of CS and PEG coated PLGA NPs and the immunological response within 24h of oral and peritoneal administration. From their data, it can be suggested that PLGA particles are taken up *in vivo* by macrophages and thus intracellular delivery can be achieved.

Semete *et al.*, 2012 [13] explored the effect of PEGylation on the biodistribution of PLGA particles after oral administration. They indicated that the detection of 1% PEG coated PLGA NPs in plasma was higher than that of uncoated PLGA particles, indicating that systemic circulation time can also be increased with oral formulations. Furthermore, these results indicated that in the case of Nanoparticle formulations, *in vitro* observations cannot represent or be correlated to the NPs' *in vivo* behavior.

PEG is also believed to facilitate transport through the Peyer's patches in the GALT [28]. These authors noted that the PEG coating improves the stability of NPs in the gastrointestinal fluids and facilitates the transport of the encapsulated protein, tetanus toxoid, across the intestinal and nasal mucosae.

Therefore, another use of the PEGylated-PLGA systems is their application in the field of oral vaccinations. Garinot *et al.*, 2007 [29] produced PEGylated PLGA-based NPs displaying RGD molecules at their surface to target human M cells (see section *RGD molecules*). It was demonstrated that 30% of PEG in the formulation was sufficient to induce a shielding of the Nanoparticle surface charges (ZP =−9 mV vs. −40 mV without PEG). *In vivo* studies demonstrated that RGD-labeled NPs concentrated particularly in M cells. Moreover, ovalbumin-loaded NPs were administered orally to mice and induced an IgG response, demonstrating antigen ability after oral administration. Antigen loading in the NPs allowed the use of a small amount of antigen: 5 μg of antigen, against the 100 μg and more usually employed in assays.

VITAMIN E

Vitamin E was first employed as a formulation component in PLGA NPs in the form of D-alpha-tocopheryl polyethylene glycol 1000 succinate (Vitamin E-TPGS or TPGS), as surfactant [30]. Vitamin E TPGS is a derivative of vitamin E, consisting of a hydrophilic polar head group (tocopherol succinate) and a lipophilic alkyl tail (polyethylene glycol) resulting in amphiphilic properties. TPGS has a relatively low critical micelle concentration, 0.02 wt %, and solubilizes lipophilic compounds [31]. The goal was to improve paclitaxel (Taxol®) oral bioavailability by means of NPs, and thus avoid the use of adjuvant Cremophor EL, which causes serious side-effects. Vitamin E TPGS was incorporated during the aqueous phase of a modified solvent/evaporation technique, replacing commonly-used surfactants such as polyvinyl alcohol (PVA). This novel surfactant showed better emulsifying efficiency than PVA and an improved controlled Nanoparticle release property when blended with other biodegradable polymers. The PLGA NPs' surface appeared to be dominated by the TPGS molecules demonstrating the emulsification role of TPGS and suggested the possibility that Vitamin E-TPGS enhances both Nanoparticle adhesion to cells and their homodynamic properties in the bloodstream [30].

This TPGS-PLGA formulation was later studied in both *in vitro* and *in vivo* experiments. TPGS modification of the NPs' surface was investigated as a potential strategy to extend TPGS's half-life in plasma and to increase its cellular

uptake. Vitamin E-TPGS was meant to "mask" the NPs, enabling them to cross the gastro intestinal barrier (GI), as well as to solubilize lipophilic paclitaxel and facilitate its release from the NPs. This was successfully tested on human colon adenocarcinoma cells (HT-29) and male Sprague-Dawley rats. Paclitaxel-loaded PLGA NPs showed a higher efficiency than Taxol® in cell viability, a larger area-under-the-curve (AUC) and longer sustainable therapeutic time values than Taxol® [32]. Indeed, *in vivo* evaluation confirmed the advantages of the TPGS-emulsified PLGA Nanoparticle formulation versus Taxol® in promoting oral bioavailability. Compared to Taxol®, the therapeutic effect achieved by NPs was 9.74-fold higher and it also presented a longer sustainable therapeutic time (12.56-fold)

Further studies on TPGS-coated PLGA NPs' cell uptake by Caco-2 and HT-29 cells [33] and Hela cells [34] supported these results. More specifically, paclitaxel TPGS NPs were also tested on MCF-7 human breast cancer with similar results. The ability of NPs to prevent cytochrome P450 Nanoparticle degradation was mentioned as an important factor [35]. In addition, this paclitaxel formulation was studied regarding cardiovascular restenosis treatment. Cellular uptake of fluorescent NPs was investigated *in vitro* in coronary artery smooth muscle cells and *in vivo* in the carotid arteries of rabbits, showing higher cellular uptake and drug cytotoxicity than PVA-emulsified NPs [36].

The potential of TPGS-modified PLGA NPs as potential carriers for drug delivery to the brain was also considered. The potential health benefits of Vitamin E in curing neurological symptoms associated with vitamin E deficiency and the promising characteristics of NPs as a drug delivery system to the brain are synergized in TPGS-PGLA NPs. *In vitro* cytotoxicity of NPs was studied using PC12 cell line as an important neural cell model by the MTT assay. No obvious cytotoxicity was observed, and the increased TPGS ratio in Nanoparticle formulation led to a higher *in vitro* therapeutic effect. Nanoparticle degradation may release the highest amounts of TPGS components. These components have a synergistic activity, improving Nanoparticle efficiency [37].

Further *in vivo* studies in terms of Nanoparticle pharmacokinetics were carried on with male Sprague-Dawley rats (DS) [38]. A significant portion of drug was accumulated in the liver and spleen, which could be due to the fact that the mononuclear phagocytosis system (MPGS), resides principally in these organs. Moreover, the Nanoparticle formulation showed a relatively high drug level in the brain compared to Taxol®. This was attributed to the TPGS coating on the Nanoparticle surface. Furthermore, the TPGS inhibition effect on P-glycoprotein activity was pointed out. P-glycoprotein (P-gp), the multidrug resistance transporter, is a membrane protein, ATP-dependent drug efflux pump with increased expression in human tumors. This protein removes a large number of chemically unrelated drugs and is therefore associated with failure of chemotherapy due to drug resistance. Vitamin E TPGS functions as an inhibitor of P-gp, and it has been found to enhance cytotoxicity and micelle-solubilization permeability of several antineoplasic drugs [31] and amprenavir [39]. This surface-coating and efflux-pump-inhibition effect of TPGS may be responsible for drug access to the brain. In addition, NPs significantly increased the blood circulation time of the drug to 224.5h in comparison to Taxol®'s 22.9h after i.v. administration of the same dose, in spite of it being 350-450-nm sized. This was attributed to the TPGS surface coating's ability to reduce greatly the recognition and elimination of NPs by macrophages, and thus to protect NPs once they are in the blood system [38].

A further step towards targeted delivery was taken by using folate-decorated PLGA-Vitamin E-TPGS NPs, targeting cancer cells with folate receptor overexpression. The NPs were prepared by the solvent extraction/evaporation single emulsion method and then decorated by folate. Firstly, TPGS-COOH copolymer was synthesized by reacting TPGS, glutaric acid, DCC and DMAP in DMSO under nitrogen atmosphere the carboxylic group of glutaric acid therefore reacted with the hydroxyl-terminated TPGS via ester bond formation. NPs were prepared by the solvent extraction/ evaporation single emulsion method from the synthesized copolymer, and then decorated by folate. To perform the decoration, the surface carboxyl group of the NPs was activated by NHS and EDC. In parallel, FOL was aminated by reaction with DCC and NHS in DMSO, and later reacted with excess ethylene diamine. FOL-NH(2) was then added at TPGS-COOH/FOL/NH(2) to enable the reaction to take place. The targeting effect of folate-decorated NPs was studied *in vitro* on MCF-7 breast adenocarcinoma cells and C6 glioma cells, which have a high level of folic acid receptors on their surface. Results showed a higher cell uptake of coumarin-6-loaded NPs. This was further confirmed by the cytotoxicity of cancer cells treated with the drug formulation. The targeting effect of the folate decoration for the NPs of formulation was significant on both cell lines, and indicated that saturation may be reached by increasing the targeting molecules on the NPs' surface [40].

Furthermore, the authors also added TPGS to folate-conjugated polymer micelles PLGA-PEG-FOL to encapsulate the anticancer drug doxorubicin for targeting cancer cells rich in surface folate receptors. An increase in cytotoxicity and in drug uptake on Caco-2 cells was observed, in comparison to minimal cytotoxic enhancement on normal fibroblasts. P-gp inhibition of TPGS to reduce drug efflux was again supported by results [41].

Similar experiments were carried out for docetaxel-loaded TPGS-emulsified PLGA NPs in contrast to Taxotere®, the commercialized form of docetaxel. In this case, montmorillonite (MMT), with similar emulsifier effects to TPGS, was included in the formulation. MMT is also a detoxifier and thus may cure some of the side effects caused by the formulated drug. MMT was included in the formulation replacing, or in combination with TPGS in the aqueous phase of a modified solvent extraction/evaporation method. Cellular coumarin 6-loaded NPs uptake was tested on Caco-2 and MCF-7 cell lines, leading to higher cell viability when MMT was present. *In vivo* pharmacokinetics was also studied on male Sprague-Dawley rats, showing an extended half-life for all MMT-TPGS combinations of NPs in when compared to Taxotere® [42]. Moreover, docetaxel

was also incorporated into didodecyldimethylammonium bromide (DMAB)-modified PLGA-TPGS NPs. DMAB is a cationic surfactant that increases cell surface retention time, thus increasing the chances of particle uptake and improving oral drug bioavailability. Particle surface decoration was made by sonicating pre-weighed PLGA-TPGS NPs suspended in a DMAB solution. The surface charge of NPs was changed to positive. *In vitro*, NPs again presented cytotoxicity advantages over commercial Taxotere® against MCF-7 cells and a higher level of Caco-2 cells uptake [43]. Another combination of NP surface coating consisted of using alpha-tocopherol and ascorbic acid (vitamin C), a surfactant of antioxidant properties, by nanoprecipitation technique. NPs showed antioxidant activity and were located in the cytoplasm of HepG2 cells after *in vitro* internalization [44]. Finally, drug-loaded mucus-penetrating particles were produced, aiming at reducing the mucoadhesion of NPs and enhancing their penetration into mucus. A novel surfactant molecule for particle formulation composed of Vitamin E conjugated to 5 kDa poly(ethylene glycol) (VP5k) was used instead of Vitamin E conjugated to 1kDa PEG (Vitamin E-TPGS). VP5k-coated PLGA NPs rapidly penetrated human cervicovaginal mucus, whereas PLGA NPs coated with PVA or Vitamin TPGS were trapped [45].

METHACRYLATES (EUDRAGIT®)

Eudragit® polymers are copolymers derived from esters of acrylic and methacrylic acid whose physicochemical properties are determined by functional groups. They are available in a wide chemical variety and in numerous forms which allow tailor-made modified drug release profiles offering enteric, protective or sustained-release properties. Accordingly, they have been included as coating material for orally- administered micro- and NPs [46]. Eudragits can be incorporated as part of the polymeric matrix either as a mixture with PLGA or separately in the aqueous phase of the synthesis procedure by emulsion-solvent evaporation.

The potential mucoadhesive properties of Eudragit® RS and/or PLGA NPs have been studied for the oral delivery of low-molecular heparin (LMWH) which is not absorbed in gastrointestinal tract [47, 48]. LMWH was adsorbed onto NPs surface resulting in negatively charged particles of ~100 nm in diameter. Furthermore, blank NPs were positively charged. The authors compared the properties of both types of NPs in order to elucidate the role of surface charge. NPs mucoadhesion properties were quantitatively measured on porcine gastric mucin by a resonant mirror system. This study demonstrated that loaded NPs presented smaller maximum responses in comparison to their blank counterparts due to surface charges; in this case PLGA NPs showed the lower association coefficient (PLGA < RS/PLGA < RS). Uptake and transport studies on Caco-2 cells confirmed these results and showed that binding and transcellular transport of LMWH was higher for RS and RS/PLGA NPs than for PLGA NPs.

Cetin *et al.* (2010, 2011, 2012) [49-51] have developed PLGA NPs coated with several Eudragit® polymers or PLGA-Eudragit® blends to control the release profiles of small therapeutic molecules such as sodium diclofenac or metformin hydrochloride while for big proteins such as salmon calcitonin (sCT). These blends can increase oral bioavailability. In the latter case, efficient sCT encapsulation (69-83%) and *in vivo* hypocalcemic effect were observed for all the Nanoparticle formulations. However, in the case of pure PLGA NPs drug release was incomplete and reached only 20% after 4 weeks.

Plain PLGA NPs have also shown potential for specific accumulation in areas with inflamed tissue increasing the selectivity of local drug delivery [52]. pH-sensitive NPs using Eudragit® P-4135F have been also studied as an alternative for colon delivery by the same authors [53]. In this case, the targeting strategy is based on polymer disintegration in the distal ileum where all of the drug is released. The authors compared tacrolimus loaded-PLGA and Eudragit® P-4135F NPs for the treatment of inflammatory bowel disease in mice. They found similar results in both cases. Nevertheless, Eudragit® NPs exhibited a lack of specificity. PLGA NPs presented high selectivity in adhesion to the inflamed tissue areas increasing drug concentration specifically inside the inflamed tissue with a lower total amount of drug but with a more selective accumulation.

LECTINS

Lectins are sugar-binding proteins that are highly specific because of their sugar moieties. They play a role in biological recognition phenomena involving cells and proteins. Lectins may be disabled by specific mono- and oligosaccharides, which bind to them and prevent their attachment to cell membranes [54]. Wheat germ agglutinin (WGA), tomato lectin, gorse lectin, and concanavalin A are employed in pharmaceutical applications. WGA is the most widely-used due to its low immunogenic properties.

WGA has been exhaustively researched and it is presented as a possible mucoadhesion enhancer in oral delivery. WGA is conjugated to PLGA NPs' surface by the carbodiimide strategy (conjugation of protein amino groups to the PLGA carboxyl groups).

Mo and Lim (2005) [55] compared WGA- and BSA-conjugated PLGA NPs prepared using an emulsion solvent evaporation method and studied their uptake on A549 cells in *an vitro* model of the Type II alveolar epithelial, useful for elucidating the uptake mechanisms. Conjugation efficiency was 22-24 μg/mg and conjugated NPs were 80-150% larger in size compared to plain NPs without presenting agglomeration. All the formulations presented negative ZP values. The authors investigated the role of clathrin and caveolae in mediating the uptake; both proteins regulate the cellular uptake of materials by endocytosis. The uptake of the fWGA-PLGA NPs was a receptor-mediated endocytic process exceeding by five- to eightfold that of the fBSA-PLGA and was clearly evidenced by confocal images.

This WGA-PLGA association has been also researched for administering peptides such as thymopentin (TP5), a synthetic pentapeptide (Arg-Lys-Asp-Val-Tyr) for the treatment of autoimmune diseases [56]. This molecule was chosen as a drug model due to its very high aqueous solubility, poor membrane permeability, extensive metabolism in the GI, and an extremely short half-life of 30s.

After the WGA-PLGA conjugation by the carbodiimide strategy, NPs were synthesized by the double-emulsion method, producing particles of around 200 nm in diameter and with a negative ZP, although a significant decrease in polymer surface charge density was found when WGA was incorporated. Low encapsulation efficiencies were found in all cases (28-31%) due to the high water solubility of the drug. *In vitro* studies of NPs' interaction with pig mucin showed that the degree of interaction depends on the incubation time and on the NPs' WGA content. After oral administration to Wistar rats, levels of CD4+ and CD8+ T lymphocytes were measured. The measurements showed an increase of CD4+ / CD8+ ratio when the amounts of WGA increased from 1 μg WGA/mg NPs to 10 μg WGA/mg NPs. The authors suggested that these results demonstrated the enhanced oral uptake was related to the increase in the NPs' WGA content and also suggested that there was a protective effect against TP5 degradation.

The same authors investigated the binding efficiency and sites of WGA-PLGA NPs on rat intestinal mucosa and the biodistribution of fluorescent NPs in rat tissues after oral administration [57]. *Ex vivo* bioadhesion studies using the intestinal mucosa samples without Peyer's patches demonstrated that WGA-PLGA NPs interaction was about 1.5–4.8 times higher when compared to plain PLGA NPs. Nevertheless, when WGA-PLGA NPs were assayed using intestinal mucosa samples with Peyer's patches, no statistically significant difference was found. These results showed no specific binding of WGA-conjugated NPs to the Peyer's patches region. To clarify the WGA interaction sites, NPs were then preincubated with an excess of competing sugar, N-acetyl-D-glucosamine, prior to incubation with the intestinal mucosa. The interactions of plain NPs were not influenced by the presence of sugar, giving results which were not noticeably different from the control values ($p<0.05$). The competition studies showed that N-acetyl-Dglucosamine effectively inhibited WGA binding, which provided further evidence for the specific nature of the binding of lectins to intestinal glycoconjugates. *In vivo* biodistribution of TP5-loaded PLGA NPs with or without WGA conjugation in rat tissues were studied as a function of total dose administered per tissue weight after a single-dose oral administration. Results showed a noticeable quantity of WGA-conjugated NPs was found in each tissue. The highest amount was observed in small intestine, implying improved intestinal bioadhesion and uptake level from the GI tract.

Successful oral vaccine delivery is also possible when these systems are administered in a micrometer range via oral/aerosol [58, 59]. In this sense, Gupta *et al.* (2006) [7] developed PLGA NPs loaded with HBsAg for oral immunization against hepatitis B. Particles were conjugated to lectin from *Arachis hypogaea* (PNA) to the surface hydroxyl group of PVA via glutaraldehyde. Neeraj *et al.* (2011) [60] also encapsulated HBsAg with the same objective. In this case LTA (*Lotus tetragonolobus* from *Winged or Asparagus pea*) was used as a homing device for PLGA NPs to target M cells. LTA grafted PLGA NPs were assayed in mice and showed stronger mucosal and systemic response than plain NPs.

LECITHINS

With essential functions for humans, animals and plants, lecithin is a natural constituent of all living cells. The term "lecithin" describes the complex mixture of phospholipids and other naturally-occurring concomitant components such as triglycerides, fatty acids, sterols and glycolipids. Phospholipids are the value-providing components in lecithin and are the main components of the cell membrane and the liposomal structure. They are part of the structural lipids and their main feature is their amphiphilic or amphipathic character: part of the molecule has a hydrophilic affinity for water and the other part is lipophilic (apolar) [61]. A phospholipid is constructed of a molecule of glycerol, a phosphate group and two fatty acid chains (lipids). The phosphate group has another group coupled to it, sometimes with an electric charge (choline, serine and ethanolamine, among others). Phospholipids are classified according to their hydrophilic polar heads. The most important are: phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol [62].

In PLGA NPs synthesis, lecithins are used as surfactants due to their properties as stabilizers. Not only they decrease the drug adsorption on the polymer surface, but they also serve to increase the encapsulation efficiencies in drug delivery systems. It could be added to the polymer in the same phase (lecithin will be on the surface and inside the Nanoparticle as well as acting as a surfactant). Similarly, it could be used in a separate phase in which the polymer is added dropwise. In this case lecithin will be expected to be on the particles' surface [63]. Lecithins can also be used for the surface recognition of cells to deliver drugs to their site of action. This is because lecithins have the same structure as cell membranes' bilayer of phospholipids, these latter being the most important components. Therefore, lecithins improve the recognition and entrapment of the drug into the cell [64, 65]. Moreover, lecithins are used to decrease the hydrophobicity of some of the polymers which are employed in the formulation of NPs, such as PLGA, improving their affinity to cell membranes, ensuring a better release profile [61].

Chan *et al.* (2009) [66] used a modified nanoprecipitation method to synthesize PLGA-lecithin-PEG core shell NPs. Lecithin and PEG were dissolved in an aqueous ethanol solution to which a PLGA/acetonitrile solution was added dropwise under gentle stirring. In order to enhance the encapsulation efficiency and oral bioavailability of vincristine (VCR). Ling *et al.* (2010) [67] developed a PLGA hybrid NPs composed of a PEG shell, lecithin monolayer, and DS (dextran)-PLGA hybrid core, which integrated the structures of liposomes and biodegradable polymeric NPs. During the NPs development the lecithin was dispersed in ethanol aqueous solution (with the PEG and the VCR) and a solution of PLGA in acetonitrile was injected dropwise into the previous dispersion.

An association of a lecithin with heparin (used as an efficacious substrate for the functionalized surfaces of biomaterials) in the external layer has been also applied to the surface modification of PLGA NPs. An organic phase with the PLGA was added slowly into the aqueous phase (in

which is the lecithin, L -α-phospatidylcholine type II) under magnetic stirring (600 rpm and 25º C for 10 min) [68].

Although lecithin tends to form lamellar liquid crystals in aqueous dispersions, it has been reported to form microemulsions in combination with short-chain alcohols such as ethanol, thereby making it suitable for oral drug delivery [69]. Nevertheless, Zhu *et al.* (2007) [61] prepared poly-L-lactide acid polymer with lecithins, dissolving both in methylene chloride. The lecithin was dissolved completely at room temperature. Shi *et al.* (2009) [64] conjugated PLGA and lecithin dissolving them in methylene chloride as an oil phase in which the drug is emulsified in an aqueous solution with, gentamycine/bovine serum albumin.

The main effect in conjugating lecithins with nanosystems, particularly PLGA NPs for oral administration, is observed in both *in vitro* and *in vivo* studies. It was observed that the lipid monolayer was acting as a molecular fence and contributed to maintain the drug molecules in the hydrophobic core, as well as keeping water out of the core. This is important because the water would hydrolyze the PLGA polymer and increase erosion and drug release [66]. The lipid monolayer was a limiting factor in controlled drug release. Moreover, the NPs were well tolerated by human cell line models, HeLa and HepG2, which were used to evaluate the cytotoxicity of the nanosystems.

A high cellular uptake has been observed when the lecithin was conjugated to PLGA. This uptake was 12.4-fold higher than uptake without the association [67]. (Fig. **3**) shows the scheme of a modified lecithin-PLGA NP. It was found in cell culture tests that the modification of cellular proliferation and cell attachment on the PLGA/lecithin depended on the content of lecithin. The adhesion and proliferation of the cell cultures was enhanced significantly when the lecithin content increased (3-7 wt %) [61].

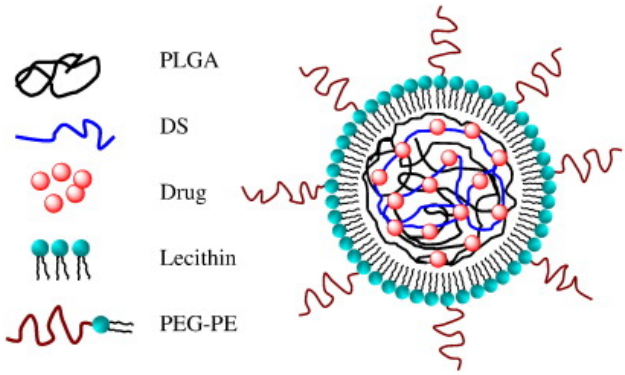

Fig. (3). Schematic illustration of a modified PLGA Nanoparticle comprising a dextran-PLGA hybrid core, a hydrophilic PEG shell, and an amphiphilic lipid monolayer on the core surface [67].

BILE SALTS

Potentially, the selective reabsorption of bile salts can be used to improve the bioavailability of NPs. Bile salts have also been shown to disrupt tight junctions in the epithelial lining, allowing for paracellular and transcellular transport pathways [70, 71]. Indeed, deoxycholic acid (DCA), one of the secondary bile acids, has been conjugated to protein and peptides for oral delivery for several purposes. Samstein *et al.* (2008) [72] described a system where PLGA particles are orally delivered in a non-covalently associated emulsion of DCA in order not to impair the therapeutic response. Lyophilized NPs loaded with rhodamine as a drug model were administered to C3H mice as a suspension in PBS or in an emulsion of DCA. *In vitro* release profiles showed a slower release at pH 2 from particles administered in a DCA emulsion. This could be advantageous because of the protection offered in the acidic environment of the stomach. However, the addition of DCA caused visible aggregation. In pharmacokinetic studies, AUC values were almost 2 times greater for NPs in DCA, demonstrating an increased bioavailability. In both cases, t_{max} was 4h but for PLGA NPs in DCA, a lower C_{max} was found which potentially avoided the side effects of rhodamine. The authors attributed the increased absorption to several factors including DCA's known ability to disrupt tight junctions and to pass through the cells via the active and passive DCA pathways. The authors also pointed out that the aggregation of the particles delayed transit time in the stomach which is translated into an increased absorption. A direct relation between particle size and oral absorption rate has been thoroughly proved [73].

Bile acids can also be incorporated into the PLGA NPs during the synthesis procedure. Jung *et al.* (2009) [74] compared the effects of increased amounts of bile salts in NPs for the oral delivery of salmon calcitonin, measuring their hypocalcemic effect in rats. Particles, 1000-240 nm in diameter, were synthetized by a double $w_1/o/w_2$ emulsion method. It was observed that increased amounts of bile salts resulted into a lower encapsulation efficiency (EE). Bile salts are good surfactants but they could not prevent the drug from the migrating into the outer water phase. EE was also strongly affected by particle size. Particles 240 nm in diameter presented the lowest EE, resulting, during *in vivo* assays, in ineffective systems. The authors reported that transcellular transport and carrier-mediated transport could be associated with these NPs, using bile salts as absorption enhancers.

Bile salts incorporation into PLGA NPs for oral administration, does not always result in improvements in oral bioavailability Gutierro *et al.* (2003) [75] orally administered BSA-loaded 1 μm PLGA particles into mice and tested several enhancers (surfactants and oil vehicles) in the serum IgG responses. The authors found that bile salts elicited a similar immune response to that obtained when they were suspension in an aqueous solution without any enhancer. This suggests that this particular enhancer is not capable of increasing the absorption of NPs antigens.

Finally it is necessary to take into account the fact that bile acids exhibit distinct biological effects and that, due to their potential toxicity, DCA levels must be maintained within a narrow effective range. DCA has been suggested as a potential tumor promoter and induces apoptosis in cancer cells [76].

RGD MOLECULES

RGD or Arginine-Glycine-Aspartic acid has been widely used in biomaterials science for stimulating cellular adhesion on surfaces via integrin receptors [77]. As M cells are present at a very low density in the gut, the specific targeting of β1 integrins in M cells could enhance particle uptake. This strategy has mainly been employed for administering oral vaccines or anticancer drugs. In this case, PEGylated PLGA NPs are needed because RGD molecules are covalently linked onto PLGA NPs surface.

Fievez *et al.* (2009) [78] assayed four ligands grafted onto ovalbumin-loaded PLGA NPs surface targeting: β1 integrin RGD as the peptidic reference, RGD peptidomimetic (RGDp), LDV derivative (LDVd) and LDV peptidomimetic (LDVp). A mannose derivative was also evaluated in order to compare the targeting of antigen-presenting cells (APC) such as the ligand of macrophages and dendritic cells. The authors studied the influence of these different ligands on 200nm-diameter PLGA NPs' transcytosis through the intestinal epithelium or on the follicle-associated epithelium of Peyer's patches and their ability to induce an immune response after oral vaccination. Mouse oral immunization was carried out after intragastric gavage and by intraduodenal administration. These *in vivo* assays revealed that all of the formulations were able to induce a cellular immune response. However, some differences were observed as a function of the ligand grafted at the Nanoparticle surface, suggesting a different process in the induction of the immune response, possibly due to a different recognition process by the APC. Results obtained after intragastric administration were, however, unreliable due to the high variability within the animal groups. LDVd-NPs clearly induced an increase in the mean total IgG serum concentration. Using intraduodenal administration, high immunization was obtained after with RGDp, LDVd and man-NPs immunization. The authors explained these results in part by the improved transport of the above formulations by the M-like cells and/or by their better internalization by macrophages compared to other formulations.

A summarized table of the action mechanisms and main advantages and disadvantages for every possible additive and/or enhancer used in PLGA NPs for oral delivery has been included in this paper (see Table **1**).

CONCLUSIONS

The advances in pharmaceutical technology endow us with the potential for developing a variety of orally-administered drug delivery nanosystems which optimize oral bioavailability. These delivery nanosystems can provide a viable solution to problems of low solubility, degradation

Table 1. Possible Additives and Bioavailability Enhancers Employed in PLGA NPs for Oral Drug Delivery.

Additive/Enhancer	Mechanism	Advantage	Disadvantage	References
Chitosan	Translocation of Z0-1 proteins and loose F-actin proteins in the plasma membrane.	Mucoadhesive properties open tight junctions.	Increased particle size.	[11, 12, 17]
PEG	The hydrophilic polymer forms a sterically stabilizing crown on the surface of the Nanoparticle.	Prevents opsonization. Reduces accumulation in off-target organs. Systemic circulation time increased.	Non-specific targeting.	[22, 25]
Vitamin E	Emulsifying properties. Surface coating Cytochrome P450 avoidance. P-glycoprotein inhibition.	Stabilizer during NPs synthesis. Improved controlled release property. Enhanced NPs adhesion to cells. Enhanced homodynamic properties. Extended half-life. Enhanced permeability.	Non-specific targeting.	[30-32, 35]
Methacrylates (Eudragit)	Mucoadhesive properties. Possibility of pH dependence	Modify controlled release property. Increases loading efficiency.	Non specific targeting.	[47, 48]
Lectins	Specific mucoadhesion to sugar moieties.	Receptor-mediated endocytic process.	Conjugation by the carbodiimide strategy.	[55, 57]
Lecithins	Surface recognition of cells.	Stabilizer during NPs synthesis. Increases the encapsulation efficiencies. Improves affinity to the cell membranes with a better release profile.	Non-specific targeting.	[61, 63-65]
Bile salts	Disrupt tight junctions. Allow paracellular and transcellular transport.	Selective reabsorption.	Potential tumor promoter.	[70, 71]
RGD molecules	Cellular adhesion via integrin receptors.	Receptor-mediated endocytic process.	Conjugation by the carbodiimide strategy.	[77, 78]

and narrow 'absorption windows'. The drugs therefore benefit from targeted delivery to an appropriate GI segment.

In this review, several major techniques based on PLGA NPs that seek to overcome these limitations are presented. Strategies are mainly focused on Nanoparticle surface modification including: (i) covering NPs with another polymer which present mucoadhesion properties, such as chitosan; (ii) linking specific molecules that bind selectively to cell membranes receptors; (iii) reducing the lipophilicity of PLGA NPs using molecules such as vitamin E which acts also as surfactant and (iv) using mixtures of polymers or PLGA derivates, such as PEGylated polymers.

These strategies provide flexible options to develop tailor-made PLGA NPs for different orally-administered drugs. These techniques can also be useful to revive the clinical efficacy of toxic drugs or facilitate the clinical transition of candidate drugs that have been deemed failures simply because of their lack of solubility.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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