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DOI:

[10.2217/nnm-2018-0029](https://doi.org/10.2217/nnm-2018-0029)

Document Version

Peer reviewed version

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Citation for published version (APA):

Hashem, L., Swedrowska, M., & Vllasaliu, D. (2018). Intestinal uptake and transport of albumin nanoparticles: potential for oral delivery. *Nanomedicine*, 13(11), 1255-1265. <https://doi.org/10.2217/nnm-2018-0029>

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Abstract

Aim: To explore the potential of albumin nanoparticles for oral drug delivery. **Methods:** sub-150 nm human serum albumin nanoparticles were fabricated via a desolvation technique. Nanoparticle cell uptake and epithelial translocation were tested in Caco-2 monolayers, while comparing with albumin solution. **Results:** data suggest epithelial transcytosis of albumin, applied in solution form, via neonatal Fc receptor (FcRn). Cell uptake of albumin nanoparticles demonstrated behaviours indicating a different cell uptake pathway compared to albumin solution. Importantly, application of equivalent concentrations of albumin solution or nanoparticles resulted in higher epithelial transport capacity of the latter, suggesting improvement of intestinal delivery via nanoformulation. **Conclusion:** This study highlights for the first time that simply-fabricated, non-toxic human serum albumin nanoparticles may find application in oral drug delivery.

Keywords

Albumin, Albumin nanoparticles, Intestinal absorption, Nanoparticle uptake, Oral delivery

Introduction

Oral administration is the ultimate drug delivery route due to convenience and patient compliance, but is currently not viable for advanced therapeutics, such as biologics or complex (e.g. targeted) nanomedicines. Both biotherapeutics and nanomedicines suffer from poor penetration across the formidable barrier of the intestinal epithelium. Considering the individual components of the overall gastrointestinal (GI) barrier to systemic drug absorption, significant progress has been made in solid dosage systems that protect the therapeutic from degradation, as well as mucus-penetrating materials. However, overcoming the formidable epithelial barrier remains the most challenging aspect of oral delivery of biologics and nanomedicines.

Current approaches to improve oral delivery typically employ 'absorption enhancers' that non-selectively disrupt and increase the intestinal permeability. However, safety concerns, such as those associated with many surfactants [1], have hindered the clinical translation of this approach in general. Key to safe and effective oral delivery of biologics and nanomedicines is the engineering of systems capable of delivering the drug selectively across the intestinal mucosa without disrupting this physiologically important barrier. This has led to efforts to explore biological transport processes as potential routes to deliver therapeutics across the intestinal barrier. These approaches typically rely on appropriate formulation of a drug, a suitable drug carrier, usually nanocarrier, and a ligand to target the system towards the biological receptor and transport process of choice. The combination of such requirements leads to complexity of systems and difficulties in achieving a compromise between bioactivity and manufacturability.

The use of albumin nanoparticles in drug delivery has recently increased significantly, since as an endogenous material, albumin has the combination of properties of an ideal drug carrier, including long half-life, serum stability, biodegradability, lack of toxicity, non-immunogenicity and water solubility [2]. Serum albumin is known for its remarkable ligand

binding capacity. Specifically, it contains eleven specific binding sites for hydrophobic compounds [3]. Many drugs and endogenous molecules are known to bind to albumin; therefore, albumin nanoparticles can serve as depot systems, with drug release achieved naturally by protease digestion. The combination of the desirable properties of albumin and the simple fabrication approach of albumin nanoparticles explains the recent focus on these systems, particularly for targeted delivery of cancer therapeutics [4, 5].

Albumin nanocarriers could present a new and simple way of achieving transcytosis-targeted nanocarriers for oral drug delivery. Because the biological transport mechanism of albumin is expressed in the intestinal epithelium, the albumin nanocarrier could serve both as a drug carrier and a 'transport-enabling' entity, bypassing the requirement for another component (e.g. transcytosis ligand). This would make the overall drug delivery system simpler and less expensive, in addition to improving drug loading and potentially drug transport properties.

This study explored whether biological transport of albumin demonstrates potential for intestinal drug delivery. The work reports on the transcytosis of albumin across differentiated Caco-2 monolayers as an *in vitro* intestinal model, while probing the biological transport mechanism of albumin. We then fabricated sub-150 nm albumin nanoparticles of low polydispersity and investigated their uptake and transport across polarised Caco-2 cell monolayers. We show that albumin nanoparticles displayed a different cell uptake and transintestinal transport behaviour to albumin in solution, suggesting a shift in the trafficking route with nanoformulation. A notably higher amount of albumin traversed Caco-2 monolayers following the application of albumin nanoparticles, as compared to equivalent concentration of albumin solution, suggesting, significantly, improvement of transintestinal delivery via nanoformulation.

Materials & methods

Materials

Fluorescein isothiocyanate (FITC)-labelled human serum albumin was purchased from Abcam (UK). Glutaraldehyde, Hank's Balanced Salt Solution (HBSS; with sodium bicarbonate, without phenol red), Dulbecco's Modified Eagle's Medium and all other reagents, unless otherwise stated, were purchased from Sigma (UK). Goat, anti-human FcRn antibody and donkey, anti-goat TRITC-IgG were obtained from Santa Cruz (USA). Prolong Gold Antifade mountant was purchased from ThermoFisher Scientific (USA). Caco-2 cells were purchased from the European Collection of Cell Cultures (ECACC) and used between passages 52-55. Transwell permeable inserts of 12 mm diameter, polycarbonate filters and 0.4 μm pore size were obtained from Costar (USA). 3-(4,5-dimethylthiazol-2-yl)-5(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) reagent, which is commercially known as 'CellTiter 96 AQueous One Solution Cell Proliferation Assay', was obtained from Promega (USA).

Fabrication of albumin nanoparticles

Albumin nanoparticles were prepared by the established desolvation technique. Briefly, albumin or FITC-albumin were dissolved in Tris-HCl (pH 6.9) to make a 1% w/v solution. The pH was then adjusted to pH 9.0 using NaOH. Desolvation of this solution was achieved by constant stirring and the dropwise addition of 0.8 ml ethanol manually using a syringe, at room temperature. After desolvation, 2 μL of 25% glutaraldehyde was added to induce particle cross-linking. The crosslinking process was performed under stirring of the suspension over a 24-hour period at room temperature.

Albumin nanoparticle characterisation

After preparation of albumin nanoparticles in water and HBSS the average particle size and polydispersity, was measured by dynamic light scattering (DLS) using a Malvern zetasizer (Malvern Instruments Ltd., Malvern, UK). The samples were diluted 1 in 20 with purified water, then measured at a temperature of 25°C and a scattering angle of 173°. Albumin

nanoparticles made in Tris-HCl at pH 9.0, 9.5 and 10.0 were characterised using the same technique; three readings were taken for each sample. To assess reproducibility of nanoparticles produced in Tris-HCl at pH 9.0 three batches of albumin nanoparticles were characterised; three readings were taken for each sample. Then to measure the stability of albumin nanoparticles in different biological buffers, the nanoparticles were diluted 1 in 20 with purified water, PBS or HBSS and three readings were taken at each time point (0, 30, 60, 90 and 120 minutes).

Cell culture

Caco-2 cells were cultured using DMEM. Cells were seeded on Transwell permeable plates at 10^5 cells/cm². Culture medium was replaced every other day. Caco-2 cells were typically cultured on permeable supports for 21–23 days, prior to their use as polarized cell layers.

For 'saturation study', 'IgG competition', 'albumin nanoparticle competition' and 'albumin nanoparticle uptake' studies, polarised cells were equilibrated in HBSS for 45 min.

Transepithelial electrical resistance (TEER) was then measured to confirm cell monolayer integrity; cells typically displayed TEER between 700-1200 Ω /cm² and those below 500 Ω /cm² were not used.

Nanoparticle toxicity

The MTS assay was employed to assess the effect of albumin nanoparticles on Caco-2 cell viability. Cells were seeded on 96-well plates at a density of 5,000 cells per well and cultured overnight in DMEM. Culture medium was removed and replaced by albumin nanoparticle suspensions diluted in HBSS at concentrations of 17.5, 35, 70, 140 and 280 μ g/ml (albumin content). Triton X-100 (1% v/v) was employed as a positive control (assumed to induce 100% cell death) and HBSS as negative control (assumed no cell death). Cells were then incubated (at 37 °C, 5% CO₂) with the samples and controls for three hours. This was followed by sample removal and cell washing with PBS. MTS solution reagent was added to

each well (20 μ l) and cells incubated (at 37 °C, 5% CO₂) for 1.5 hours. Absorbance was recorded at 490 nm using a 96-well plate reader.

FcRn expression by Caco-2 cells

Polarised Caco-2 monolayers were fixed in paraformaldehyde, washed with PBS and permeabilised by incubating with Triton X-100 (1% v/v in PBS) for approximately 10 min. Cells were washed again and incubated for 1 hour with BSA in PBS (1% w/v). Goat, anti-human FcRn primary antibody was diluted to 10 μ g/ml in 1% w/v BSA/PBS and incubated with the cells for 30-60 min at room temperature. Cells were then washed extensively and treated with donkey, anti-goat TRITC-IgG (secondary antibody, diluted according to supplier's instructions in 1% BSA/PBS) for 30-60 min. Thereafter, cells were washed extensively and filters excised and mounted on a glass slide (using a DAPI-containing ProLong[®] Gold antifade mounting medium) for confocal imaging. Control experiments were conducted where the cells were treated with the secondary antibody only. Confocal imaging was conducted using a Leica TCS SP2 system mounted on a Leica DMIRE2 inverted microscope.

Albumin uptake saturation

FITC-albumin was applied to the apical side of Caco-2 monolayers in HBSS at 8 μ g/ml, 40 μ g/ml and 200 μ g/ml for two hours. FITC-albumin uptake was measured by permeabilising the polarised Caco-2 cells using Triton X-100 (1% v/v; 10 min incubation) and determining the fluorescence intensity of the samples (quantified via a calibration curve).

Albumin and IgG permeability

Human serum FITC-albumin was applied to the apical side of Caco-2 monolayers in HBSS at 40 μ g/ml and human IgG at 1 μ g/ml. Permeability was measured by sampling the basal solution every 20 min for two hours. FITC-albumin was quantified by fluorescence. IgG was quantified by ELISA using NUNCIImmuno 96 MicroWell™ (NUNC, Denmark) plates, 1% BSA

w/v in PBS as the blocking buffer, horseradish peroxidase (HRP)-conjugated anti-human IgG (The Binding Site, UK), diluted 1:1000 in blocking buffer as the detection antibody and 3,3',5,5'-tetramethylbenzidine (TMB, Autogen Bioclear Ltd, UK) as the substrate; the reaction was stopped using 2.5 M sulphuric acid (20 μ l) and absorbance determined at 450 nm.

Albumin uptake and permeability competition studies

40 μ g/ml FITC-albumin was applied to polarised Caco-2 monolayers in combination with 100 μ g/ml IgG in HBSS. FITC-albumin permeability was measured by sampling the basal solution every 20 min for two hours. FITC-albumin uptake was measured by permeabilising the polarised Caco-2 cells using Triton X-100 (1% v/v; 10 min incubation) and quantitation by fluorescence.

FITC-albumin at 40 μ g/ml was applied to polarised Caco-2 monolayers together with 140 μ g/ml albumin nanoparticles (unlabelled) in HBSS. FITC-albumin permeability and uptake were determined as above.

Albumin nanoparticle uptake and epithelial transport

FITC-albumin nanoparticles were applied to polarised Caco-2 monolayers at 40 and 200 μ g/ml (albumin content) in HBSS. Additionally, to determine FcRn involvement in the uptake and transport process of FITC-albumin nanoparticles, they were applied at 40 μ g/ml in combination with 100 μ g/ml IgG in HBSS. Transepithelial transport was determined by regular sampling of the basal solution (every 20 min for two hours) and uptake following permeabilisation using Triton X-100 (1% v/v; 10 min incubation).

Cell uptake of FITC-albumin nanoparticles was also determined qualitatively using confocal microscopy. FITC-albumin nanoparticles were applied to polarised Caco-2 monolayers at 40 μ g/ml in HBSS for two hours. Cell monolayers were then washed with PBS and fixed using

4% w/v paraformaldehyde (10 min incubation). Cells were then stained with DAPI-containing ProLong® Gold antifade mounting medium.

Statistical analysis

Student's *t*-test was performed for comparisons of two group means, while one way analysis of variance (ANOVA) was utilised for comparison of three or more group means. *P* value of <0.05 was considered statistically significant. **** and * indicate $p < 0.0001$ and $p < 0.05$, respectively, whereas “ns” indicates nonsignificant. Statistical analysis was conducted using GraphPad Prism® Software.

Results

Nanoparticle characterisation

Albumin nanoparticles prepared in Tris-HCl (pH 9.0) were tested for stability, in terms of size, in water and biologically-relevant buffers, PBS and HBSS. Figure 1 shows albumin nanoparticle size following incubation in these conditions for up to two hours. The data show that albumin nanoparticle size remained stable with no statistically significant changes in the mean diameter over the two-hour incubation period in all conditions. The mean nanoparticle diameter at 0 hour and 2 hours in HBSS, in which nanoparticles were later applied to the cells, was 142.3 ± 1.3 nm and 146.0 ± 2.1 nm, respectively. The average polydispersity index at 0 hour and 2 hours was 0.2 ± 0.02 and 0.1 ± 0.01 , respectively.

Figure 1.

Nanoparticle toxicity

The effect of albumin nanoparticles on relative cell viability over a concentration range 17.5-280 µg/ml (albumin content) is shown in Figure 2. Albumin nanoparticle suspensions up to 140 µg/ml albumin concentration showed no significant negative effect on Caco-2 viability,

while albumin nanoparticle suspensions of 280 µg/ml exhibited a 50 (\pm 15.3%) reduction in relative cell viability (statistically significant).

Figure 2.

FcRn expression by Caco-2 cells

Red fluorescence signal due to donkey, anti-goat TRITC-IgG, was distributed in the regions around the nuclei. This fluorescence was only observed in cells incubated with both, primary and secondary antibodies (Figure 3A & B). In control cells where incubation with the primary antibody was omitted (i.e. cells were exposed to secondary only) no red fluorescence was detected (Figure 3C).

Figure 3.

Albumin uptake saturation

To assess whether albumin uptake by Caco-2 cells follows a receptor-mediated route, uptake was determined following the application of different concentrations. Figure 4 shows that albumin uptake increased modestly, from 100 ng to 114 ng per cell monolayer, as the concentration applied apically increased from 8 to 40 µg/ml. Further increase in the applied albumin concentration from 40 to 200 µg/ml did not enhance albumin uptake, with uptake levels reaching 110 ng per cell monolayer.

Figure 4.

Albumin and IgG permeability

In this set of experiments, we compared the permeability of albumin across Caco-2 monolayers with that of IgG as two substrates for the FcRn receptor. Figure 5 shows that

albumin displayed a markedly higher permeability compared to IgG, with basal levels reaching approximately 68 ng compared to IgG, which amounted to 2.7 ng after two hours.

Figure 5.

Albumin uptake and permeability competition studies

To obtain more insight into the internalisation and transport route of albumin and to determine whether albumin nanoparticles follow the same trafficking as albumin in solution, studies were conducted whereby albumin solution was applied to the cells in combination with IgG or albumin nanoparticles. The data in Figure 6A show that IgG more than halved the uptake of albumin, from 49 to 21 ng per cell monolayer ($p=0.020$). On the other hand, application of unlabelled albumin nanoparticles alongside FITC-albumin, did not suppress the uptake of the latter (statistically insignificant difference).

Considering apical-to-basal permeability of albumin, Figure 6B indicates that co-application with IgG somewhat reduced FITC-albumin permeability, particularly in the second hour of the experiment, although this effect did not reach statistical significance. Albumin nanoparticles did not influence FITC-albumin permeability across Caco-2 monolayers.

Figure 6.

Albumin nanoparticle uptake and epithelial transport

FITC-albumin nanoparticles were applied to Caco-2 monolayers at different concentrations to determine saturation and in combination with IgG in order to establish whether albumin nanoparticles are internalised via the FcRn pathway. Figure 7A indicates that FITC-albumin nanoparticle uptake was notably higher following application at 200 $\mu\text{g/ml}$ (183 ng per cell monolayer) relative to the lower concentration of 40 $\mu\text{g/ml}$ (69 ng per cell monolayer). IgG on

the other hand did not influence the internalisation of FITC-albumin nanoparticles (64 ng per cell monolayer).

Regarding FITC-albumin nanoparticle transport, Figure 7B shows that FITC-albumin nanoparticles traversed polarised Caco-2 monolayers and the rate or final amount accumulated on the basolateral side was not influenced by the applied concentration (40 versus 200 µg/ml) or co-application with IgG. Up to 380 ng of FITC-albumin, formulated as nanoparticles, permeated across the cell monolayers in two hours.

Figure 7.

Confocal microscopy analysis of cellular internalisation of FITC-albumin nanoparticles is shown in Figure 8. Strong green, FITC-associated fluorescence intensity signal can clearly be seen in the entire imaged area of the Caco-2 monolayer (Figure 8A). Furthermore, green fluorescence can be observed across the depth of the imaged cell monolayer area (shown by image orthogonal view or 'z stacks' in Figure 8B), suggesting fluorescence in the interior of the cells.

Figure 8.

Discussion

The intestinal epithelium presents a significant barrier to oral delivery of biologics and nanomedicines for systemic effect. Despite the improved understanding of mucosal barrier homeostasis and recent advances in nanotechnology, technologies that safely and effectively surmount the intestinal epithelium, enabling oral delivery of complex therapeutics, are currently lacking. Transcytosis-enabled nanosystems have been proposed by several groups, including our own, for delivery of biomolecules or nanomedicines across the

mucosal surfaces of the airways [6-8] and the intestine [9-12]. Regarding oral delivery, these strategies have demonstrated that it is feasible to transport both biotherapeutics and nanoparticles across the intestinal mucosa and into the systemic circulation after oral administration [13-15]. However, none of these have progressed beyond the research stage.

The challenge for clinical translation of nanomedicines for oral delivery is related to the complexity of the gastrointestinal system, namely the multicomponent nature of the intestinal barrier. Nanosystem delivery into the systemic circulation following ingestion requires stability in the digestive tract and penetration of additional, non-epithelial barriers, including the thick mucus layer and the basement membrane, above and below the epithelium, respectively. Assuming that all of these physiological factors have some effect on the overall nanoparticle translocation into the systemic circulation (resulting in drug loss), it is apparent that epithelial transcytosis pathway of choice should have a reasonable transport capacity if it is to present a viable strategy for the delivery of complex therapeutics across the gastrointestinal tract.

Fabrication of transcytosis-exploiting nanosystems for oral delivery requires complex, multiconstituent systems, typically composed of the drug, the nanocarrier and the ligand directing the system to the transcytosis receptor of interest. This potentially increases the complexity of the system, presenting challenges in terms of formulation and stability of the system. In this respect, albumin nanoparticles are potentially interesting system as they may serve both as drug carriers and 'transport-enabling' entities – assuming they traverse the epithelium by transcytosis in the same manner as albumin in solution – hence removing the requirement for the 'transport-enabling' ligand. This would make the overall drug delivery system simpler and potentially more efficient. However, while albumin nanoparticles have demonstrated clear utility in the delivery of hydrophobic chemotherapeutic agents [16-19], attributed to features amenable to passive targeting via the enhanced permeability and retention (EPR) effect [16], studies that fully explore the potential of albumin nanoparticles

for oral delivery are limited to the use of bovine albumin, e.g. by coating as a 'sacrificial' component to protect drug degradation or provide nanosystem stability [20]. We explored whether albumin nanoparticles may be utilised as potential nanovehicles for oral therapeutic delivery based on: i) potential targeting of albumin transcytosis in the intestinal epithelium, and ii) the small size of albumin nanoparticles, achieved via a simple fabrication approach. The issue of stability of albumin nanoparticles in the gastrointestinal environment is appreciated, but the delivery of albumin nanoparticles to the intestinal mucosa is envisaged to take place via enteric delivery technologies, which are now well established.

Albumin nanoparticles fabricated in this study displayed a size below 150 nm, low polydispersity and stability in a commonly used biological buffer, HBSS (Figure 1). Furthermore, apart from a single tested concentration, albumin nanoparticles did not exhibit toxicity towards Caco-2 cells (Figure 2).

We subsequently confirmed FcRn expression in Caco-2 monolayers (Figure 3) as this receptor system is thought to play a key role in albumin uptake and transcytosis [21]. Our findings showing FcRn presence in intestinal Caco-2 cells confirm previous reports of its expression in the intestinal epithelium [22, 23]. A series of studies focused on probing the uptake and transepithelial transport of albumin in solution. The data in Figure 4 show that albumin uptake by Caco-2 monolayers is a saturable process, hence indicating a receptor-mediated uptake. Albumin in solution form is capable of traversing the Caco-2 monolayers in a significantly more efficient manner than IgG as another FcRn ligand (68 ng versus 2.7 ng per 1.1 cm² area) in a two-hour experiment (Figure 5).

Importantly, the complete interactive interface for the FcRn-albumin interaction remains enigmatic and no *in vitro* cellular studies have so far demonstrated that FcRn can transport albumin efficiently in the presence of IgG across polarised cells [21]. Indeed, previous studies reporting on albumin transcytosis on intestinal epithelial cells are lacking. A rare and

recent study on this reported that albumin can be transcytosed across polarized T84 human intestinal epithelial cells, which express FcRn endogenously [24]. Furthermore, the rate of transcytosis was lower upon cell treatment with an antibody that blocks the albumin-binding site on FcRn, as well as for a mutant with no measurable binding to FcRn. We demonstrate for the first time that intestinal epithelial cell uptake and translocation of albumin is suppressed by co-application of IgG (Figure 6). This suggests FcRn involvement in the uptake and apical-to-basal transport of albumin by Caco-2 cells. Although FcRn binds both IgG and albumin at independent binding sites [19], FcRn-IgG interaction is likely to influence FcRn availability and engagement in albumin trafficking. Although albumin has been shown to interact with numerous cellular receptors, including glycoproteins Gp60, Gp30 and Gp18, secreted protein that is acidic and rich in cysteine (SPARC), and the cubilin complex, it has now become apparent that FcRn plays a fundamental role in homeostatic regulation of both IgG and albumin [21].

The delivery potential of albumin nanoparticles was examined in Caco-2 monolayers by determining their cell uptake and transport across the cells, as well as establishing information on uptake behaviour. The data in Figure 7 show that cell uptake of albumin nanoparticles increased with application concentration, a trend not followed by albumin in solution. Furthermore, co-application of IgG did not attenuate the uptake of albumin nanoparticles, indicating that albumin nanoparticles follow a different trafficking route to albumin solution and that this route is not dependent on FcRn. This is also confirmed by data in Figure 6, whereby application of unlabelled albumin nanoparticles did not suppress the uptake of FITC-albumin in solution. Cell uptake of FITC-albumin nanoparticles was confirmed and shown convincingly by confocal microscopy, where fluorescence intensity of nanoparticles can clearly be seen in the cell interior (Figure 8).

Comparing transport amounts between scenarios whereby albumin was applied in solution or as nanoparticles, it is apparent that a notably higher amount of albumin traverses the cell

monolayers following the application of albumin nanoparticles, as compared to albumin solution (380 ng versus 68 ng per 1.1 cm² cell monolayer area; 5.6-fold difference). This is an important finding with regards to the system's transport capacity, although it must be confirmed in vivo.

Overall, our study demonstrates the potential of albumin nanoparticles, fabricated via a simple process, as drug delivery systems capable of traversing the intestinal epithelium. Although the mechanism of intestinal transit of these systems is not clear, work showed remarkable level of internalisation of albumin nanoparticles by intestinal Caco-2 cells and more efficient apical-to-basal mass transport of albumin when formulated as nanoparticles compared to the solution form. To fully evaluate the potential of this approach for oral delivery, the stability of albumin nanoparticles in different regions of the intestinal mucosa (e.g. colon *versus* small intestine), diffusion across the intestinal mucus, potential immunogenicity and the mechanism of trafficking by intestinal epithelial cells should be studied.

In conclusion, this study is important as it highlights for the first time that simply-fabricated, non-toxic albumin nanoparticles show good uptake and permeation across the intestinal epithelial barrier and hence may find application in oral drug delivery.

Summary Points

- Human serum albumin nanoparticles, fabricated via a desolvation technique, had a diameter of approximately 150 nm
- Albumin nanoparticle cell uptake and transport were compared with albumin solution in the Caco-2 intestinal model
- Albumin nanoparticles were not toxic to Caco-2 cells at concentration below 140 µg/ml

- Caco-2 cells express neonatal Fc receptor (FcRn)
- Albumin in solution form demonstrated cell uptake and transport behaviour suggesting involvement of FcRn (process was suppressed by co-application of FcRn ligand, IgG)
- Albumin nanoparticles demonstrated behaviours indicating different cell uptake and transport pathways compared to albumin solution
- Albumin transport was higher following application of equivalent concentration of nanoparticles compared to solution
- The work suggests improvement of intestinal delivery of albumin by nanoformulation
- Simply-fabricated, non-toxic human serum albumin nanoparticles may find application in oral drug delivery of poorly absorbed drugs

References

1. Vllasaliu D, Shubber S, Fowler R, Garnett M, Alexander C, Stolnik S. Epithelial toxicity of alkylglycoside surfactants. *J Pharm Sci* 102(1), 114-125 (2013).
2. Jahanban-Esfahlan A, Dastmalchi S, Davaran S. A simple improved desolvation method for the rapid preparation of albumin nanoparticles. *Int J Biol Macromol* 91 703-709 (2016).*
This study reports on improved methodology for fabrication of albumin nanoparticles
3. Larsen MT, Kuhlmann M, Hvam ML, Howard KA. Albumin-based drug delivery: harnessing nature to cure disease. *Mol Cell Ther* 4 3 (2016).
4. Liu L, Bi Y, Zhou M *et al.* Biomimetic Human Serum Albumin Nanoparticle for Efficiently Targeting Therapy to Metastatic Breast Cancers. *ACS Appl Mater Interfaces* 9(8), 7424-7435 (2017).
5. Lomis N, Westfall S, Farahdel L, Malhotra M, Shum-Tim D, Prakash S. Human Serum Albumin Nanoparticles for Use in Cancer Drug Delivery: Process Optimization and In Vitro Characterization. *Nanomaterials (Basel)* 6(6), (2016).
6. Vllasaliu D, Alexander C, Garnett M, Eaton M, Stolnik S. Fc-mediated transport of nanoparticles across airway epithelial cell layers. *J Control Release* 158(3), 479-486 (2012).*
First study demonstrating the potential of FcRn-targeted nanoparticles for mucosal delivery
7. Fowler R, Vllasaliu D, Falcone FH *et al.* Uptake and transport of B12-conjugated nanoparticles in airway epithelium. *J Control Release* 172(1), 374-381 (2013).
8. Dumont JA, Bitonti AJ, Clark D, Evans S, Pickford M, Newman SP. Delivery of an erythropoietin-Fc fusion protein by inhalation in humans through an immunoglobulin transport pathway. *J Aerosol Med* 18(3), 294-303 (2005).
9. Fowler R, Vllasaliu D, Trillo FF *et al.* Nanoparticle transport in epithelial cells: pathway switching through bioconjugation. *Small* 9(19), 3282-3294 (2013).
10. Pridgen EM, Alexis F, Kuo TT *et al.* Transepithelial transport of Fc-targeted nanoparticles by the neonatal fc receptor for oral delivery. *Sci Transl Med* 5(213), 213ra167 (2013).*
First in vivo study demonstrating the potential of FcRn-targeted nanoparticles for oral delivery of biologics
11. Chalasani KB, Russell-Jones GJ, Jain AK, Diwan PV, Jain SK. Effective oral delivery of insulin in animal models using vitamin B12-coated dextran nanoparticles. *J Control Release* 122(2), 141-150 (2007).
12. He H, Wang P, Cai C, Yang R, Tang X. VB12-coated Gel-Core-SLN containing insulin: Another way to improve oral absorption. *Int J Pharm* 493(1-2), 451-459 (2015).
13. Pridgen EM, Alexis F, Farokhzad OC. Polymeric nanoparticle drug delivery technologies for oral delivery applications. *Expert Opin Drug Deliv* 12(9), 1459-1473 (2015).
14. Yun Y, Cho YW, Park K. Nanoparticles for oral delivery: targeted nanoparticles with peptidic ligands for oral protein delivery. *Adv Drug Deliv Rev* 65(6), 822-832 (2013).
15. Lundquist P, Artursson P. Oral absorption of peptides and nanoparticles across the human intestine: Opportunities, limitations and studies in human tissues. *Adv Drug Deliv Rev* 106(Pt B), 256-276 (2016).
16. Kim B, Seo B, Park S *et al.* Albumin nanoparticles with synergistic antitumor efficacy against metastatic lung cancers. *Colloids Surf B Biointerfaces* 158 157-166 (2017).
17. Noorani M, Azarpira N, Karimian K, Heli H. Erlotinib-loaded albumin nanoparticles: A novel injectable form of erlotinib and its in vivo efficacy against pancreatic adenocarcinoma ASPC-1 and PANC-1 cell lines. *Int J Pharm* 531(1), 299-305 (2017).
18. Sharma A, Kaur A, Jain UK, Chandra R, Madan J. Stealth recombinant human serum albumin nanoparticles conjugating 5-fluorouracil augmented drug delivery and

- cytotoxicity in human colon cancer, HT-29 cells. *Colloids Surf B Biointerfaces* 155 200-208 (2017).
19. Gou Y, Zhang Y, Zhang Z *et al.* Design of an Anticancer Copper(II) Prodrug Based on the Lys199 Residue of the Active Targeting Human Serum Albumin Nanoparticle Carrier. *Mol Pharm* 14(6), 1861-1873 (2017).
 20. Lopes M, Shrestha N, Correia A *et al.* Dual chitosan/albumin-coated alginate/dextran sulfate nanoparticles for enhanced oral delivery of insulin. *J Control Release* 232 29-41 (2016).
 21. Sand KM, Bern M, Nilsen J, Noordzij HT, Sandlie I, Andersen JT. Unraveling the Interaction between FcRn and Albumin: Opportunities for Design of Albumin-Based Therapeutics. *Front Immunol* 5 682 (2014).**
Detailed and informative account on the interaction between FcRn and Albumin
 22. Israel EJ, Taylor S, Wu Z *et al.* Expression of the neonatal Fc receptor, FcRn, on human intestinal epithelial cells. *Immunology* 92(1), 69-74 (1997).
 23. Hornby PJ, Cooper PR, Kliwinski C *et al.* Human and non-human primate intestinal FcRn expression and immunoglobulin G transcytosis. *Pharm Res* 31(4), 908-922 (2014).
 24. Bern M. Engineering of the albumin-FcRn interaction. *Centre for Immune Regulation Department of Biosciences Faculty of Mathematics and Natural Sciences* PhD (1876), (2017).**
This thesis reports on albumin transcytosis across polarized T84 human intestinal epithelial cells

Figure legends

Figure 1. Size stability of albumin nanoparticles over time in different environments. Size was characterised by dynamic light scattering (DLS) following incubation in water, phosphate buffered saline (PBS) and Hank's Balanced Salt Solution (HBSS) for different periods of time. Data shown as mean \pm SD of three separate measurements. Each individual measurement is in turn reported by the equipment as a mean of ten measurements.

Figure 2. Effect of albumin nanoparticles on the viability of Caco-2 cells, measured by the MTS assay. Albumin nanoparticles were incubated with cells for three hours. Results presented as % viability relative to controls (Hank's Balanced Salt Solution and Triton X-100) and expressed as the mean \pm SD (n=6).

Figure 3. Immunostaining for FcRn in polarised Caco-2 monolayers. A) Cells incubated with goat, anti-human FcRn (primary) antibody, followed by donkey, anti-goat TRITC-IgG (secondary antibody); i) Blue channel: cell nuclei stained with DAPI, ii) Red channel: TRITC fluorescence, and iii) Overlay image (Blue and Red channels). B) Immunostaining of cells in 3D. C) Control cells incubated with donkey, anti-goat TRITC-IgG (secondary antibody) only. DAPI: 4',6-diamidino-2-phenylindole; TRITC: Tetramethylrhodamine isothiocyanate

Figure 4. Uptake saturation of FITC-albumin by differentiated Caco-2 monolayers. FITC-albumin was applied to the apical side of Caco-2 monolayers in HBSS at 8 μ g/ml, 40 μ g/ml and 200 μ g/ml for two hours. FITC-albumin uptake was measured following the permeabilisation of cells using Triton X-100 (5% v/v; 10 min incubation) and determining the fluorescence intensity of the samples (n=3).

Figure 5. Permeability of human serum FITC-albumin ('Alb') and human immunoglobulin G (IgG) across differentiated Caco-2 monolayers. FITC-albumin was applied at 40 μ g/ml and sampled from the basal side every 20 min, whereas IgG was applied at 1 μ g/ml, with 30 min sampling period. Data shown as the mean \pm SD (n=3).

Figure 6. Albumin uptake and permeability competition studies. 40 µg/ml FITC-albumin solution was applied to polarised Caco-2 monolayers in combination with 100 µg/ml IgG or 140 µg/ml unlabelled albumin nanoparticles in HBSS. A) FITC-albumin uptake was measured by permeabilising the polarised Caco-2 cells using Triton X-100 (5% v/v; 10 min incubation) and quantitation by fluorescence. B) Apical-to-basal transport of FITC-albumin was determined by sampling the basal solution every 20 min and quantitation by fluorescence. Data shown as mean ± SD (n=3).

Figure 7. Albumin nanoparticle uptake and transport across differentiated Caco-2 monolayers. FITC-albumin nanoparticles were applied at 40 µg/ml, with or without 100 µg/ml IgG, or at 200 µg/ml (albumin content) in HBSS. A) Nanoparticle uptake was determined following permeabilisation using Triton X-100 (5% v/v; 10 min incubation) and quantitation by fluorescence (n=3). B) Transepithelial transport was determined by regular sampling of the basal solution (every 20 min). Data shown as mean ± SD (n=3).

Figure 8. Imaging of albumin nanoparticle uptake by Caco-2 cells. Confocal micrographs depicting Caco-2 monolayers treated with FITC-albumin nanoparticles, applied at 40 µg/ml. A) A single section of cell monolayer area. B) Orthogonal view showing the depth of the cell monolayer (constructed via imaging of a series of 'z-stacks'). Blue: cell nuclei stained with DAPI; green: FITC-albumin nanoparticles.

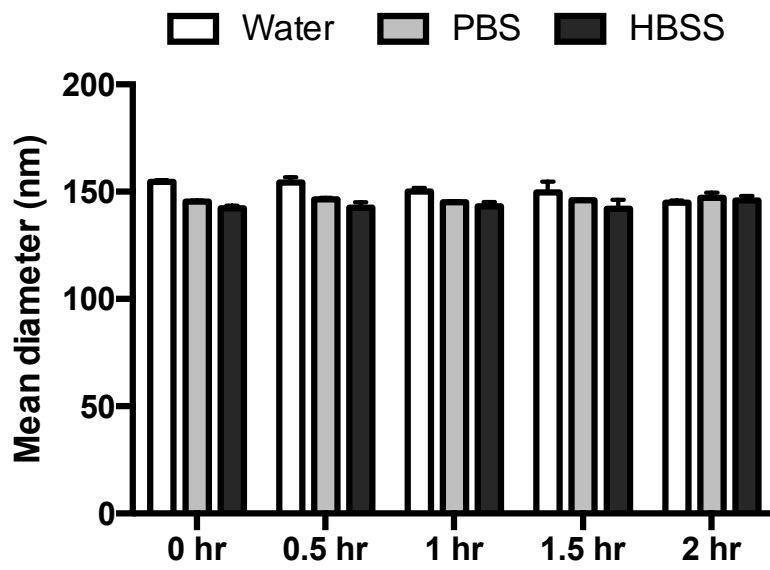


Figure 1.

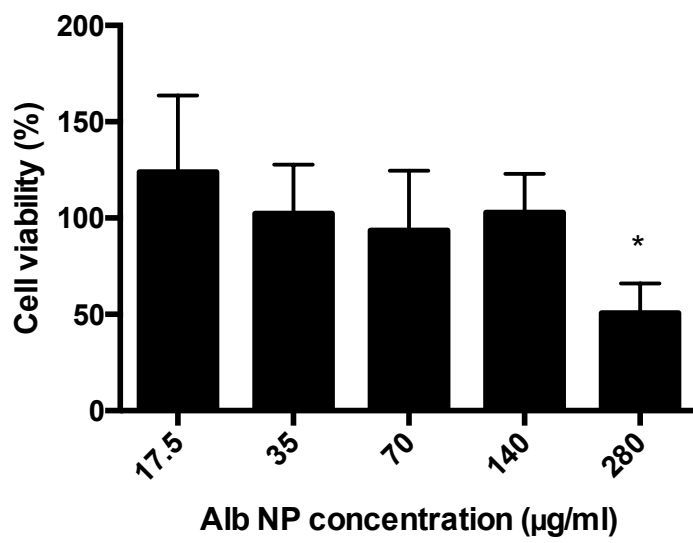


Figure 2.

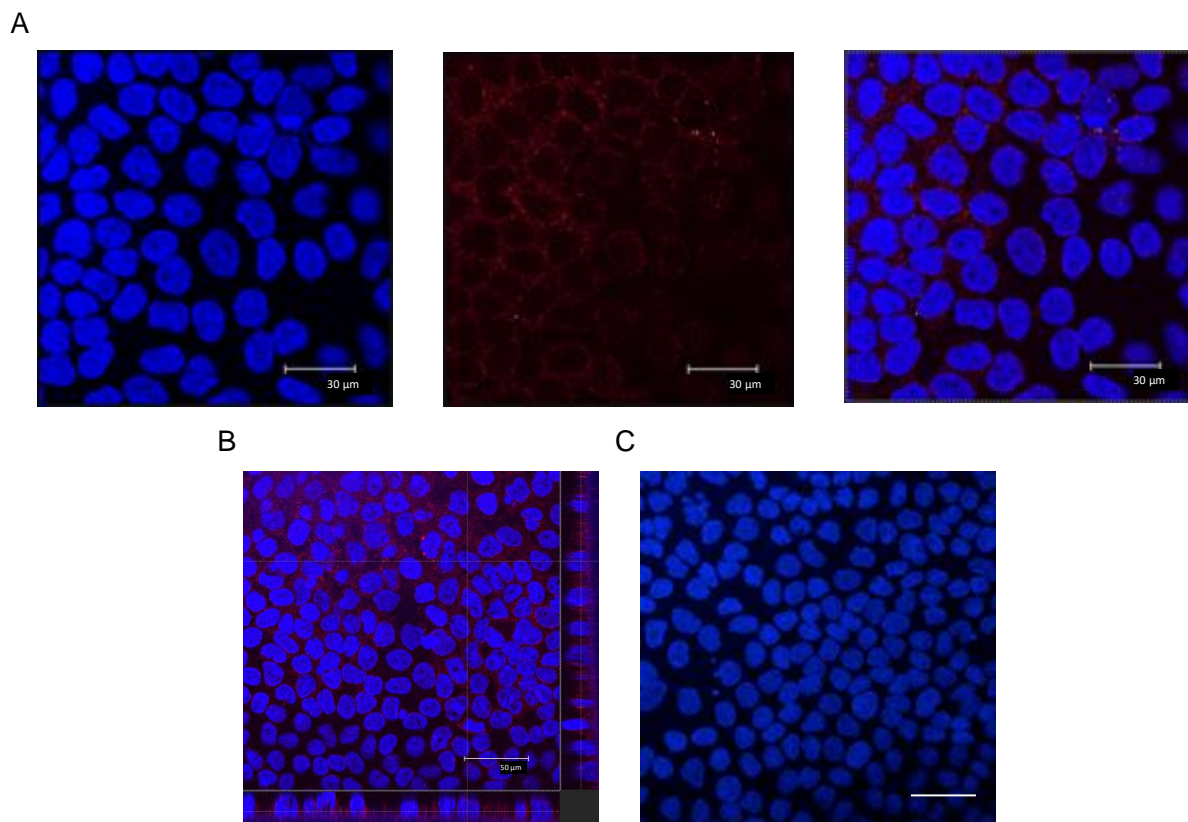


Figure 3.

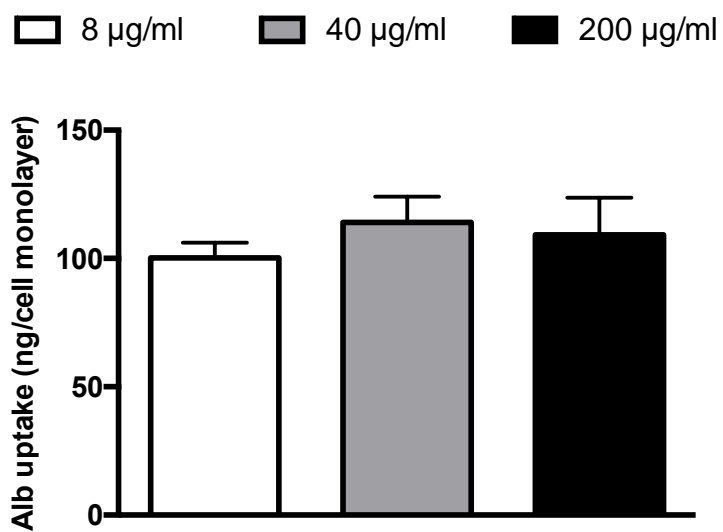


Figure 4.

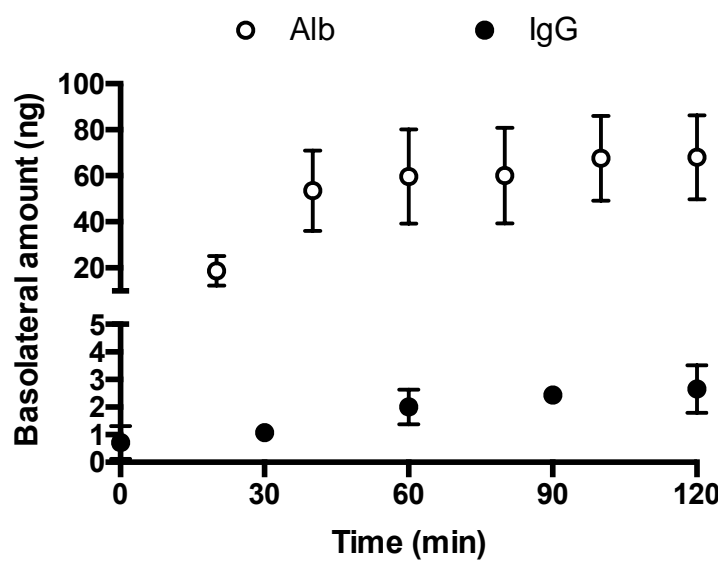
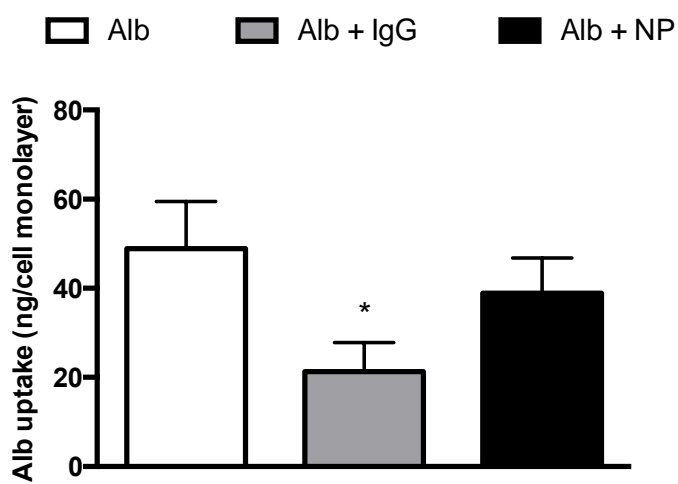


Figure 5.

A)



B)

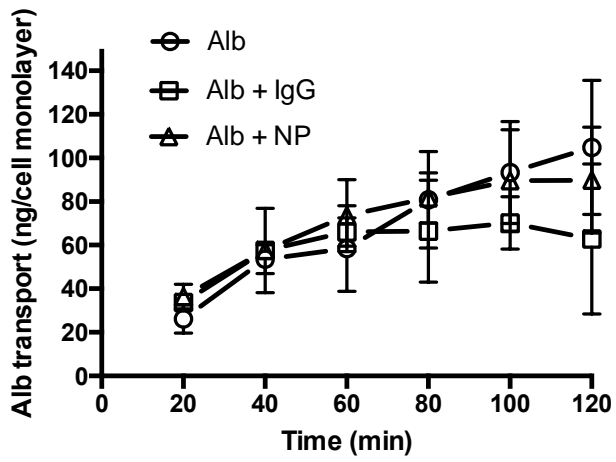
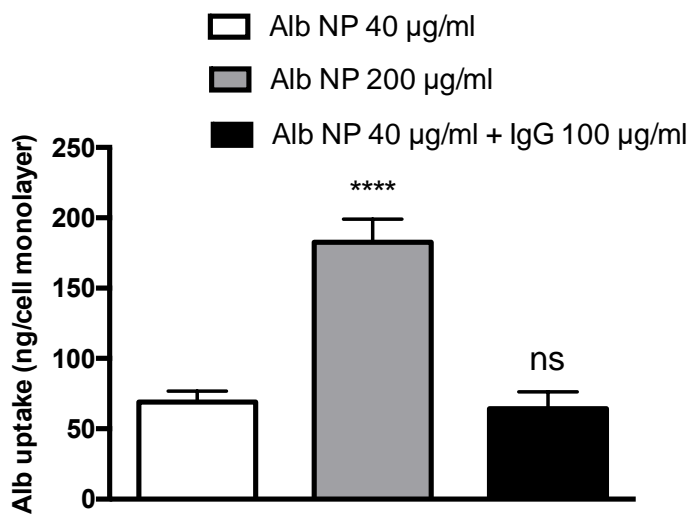


Figure 6.

A)



B)

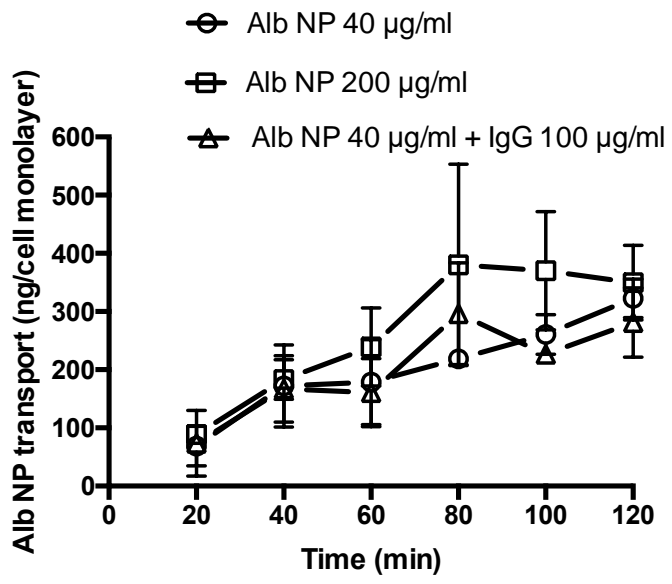


Figure 7.

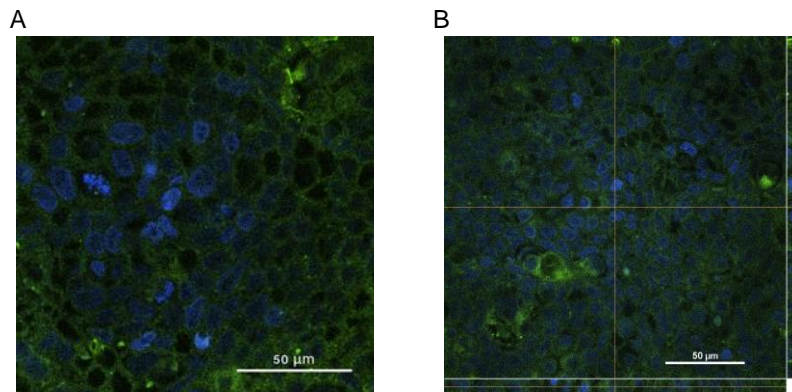


Figure 8.