Nanoparticle compositions

Field of the invention

[0001] The present invention relates to nanoparticle compositions. The present invention also relates to methods for preparing nanoparticle compositions. The present invention further relates to methods of using the nanoparticle compositions.

Cross-reference to related application

[0002] This application claims priority from Australian provisional patent application no. 2024901063 the entire contents of which is hereby incorporated by reference in its entirety.

Background of the invention

[0003] Nanoparticles, which have been applied for various purposes in the field of diagnosis and treatment, exhibit new physical and chemical properties different from bulk materials *in vivo* due to their nanoscale size. Many studies have been conducted on nanoparticles, and efforts have been made to develop nanoparticles that exhibit optimal properties suitable for medical use by adjusting the particles' composition, shape, size and properties as appropriate to the purpose.

[0004] One particular application of nanoparticles includes developing biocompatible nanoparticles that have suitable properties for *in vivo* applications. These properties include but are not limited to: hydrophilic surface chemistries that minimise aggregation and non-specific binding *in vivo*, use of non-toxic materials, and particle sizes amenable to *in vivo* applications. A further development of biocompatible nanoparticles is conjugating biomolecules to biocompatible nanoparticles to provide nanoparticle – biomolecule conjugates which may effectively provide a vehicle to deliver biomolecules to a target site *in vivo*.

[0005] Quantum dot nanoparticles having a size range of about 1-20 nm hold particular promise for *in vivo* applications due to their particle size, which is optimal to minimise rapid renal clearance and maximise circulation time. However, to date, there has been limited product development of quantum dots in nanomedicines due to challenges with developing biocompatible quantum dots that are non-toxic, stable, selectively target specific cells or tissues, and avoid off-target effects.

[0006] There still remains significant limitations with developing biocompatible nanoparticles, in particular biocompatible quantum dots, including developing a platform nanoparticle technology that may be suitable to deliver a range of different therapeutic agents to target sites *in vivo*, including delivering therapeutic agents that are not orally bioavailable via an oral route of administration.

[0007] The present invention aims to alleviate at least one of the deficiencies in the prior art.

[0008] Reference to any prior art in the specification is not an acknowledgment or suggestion that this prior art forms part of the common general knowledge in any jurisdiction or that this prior art could reasonably be expected to be understood, regarded as relevant, and/or combined with other pieces of prior art by a skilled person in the art.

Summary of the invention

[0009] In one aspect, the present invention provides a quantum dot – polymer composition comprising:

- a quantum dot, and
- a polymer shell,

wherein the polymer shell comprises a first polymer layer comprising an endosomal disruptive polymer, and a second polymer layer comprising a biopolymer, wherein the biopolymer is suitable for oral administration.

[0010] Preferably, the endosomal disruptive polymer is selected from the group consisting of: polyethylene imine; poly(arginine); poly(lysine); poly(histidine); poly-[2-{(2-aminoethyl)amino}-ethyl-aspartamide] (pAsp(DET)); a block co-polymer of poly(ethylene glycol) (PEG) and poly(arginine); a block co-polymer of PEG and poly(lysine); a block co-polymer of PEG and poly{N—[N-(2-aminoethyl)-2-aminoethyl]aspartamide} (PEG-pAsp(DET)); Poly-l-lysine (PLL); DET functionalised polyphenol (-)-epi-gallocatechin gallate (EGCG); Poly(amidoamine) dendrimers; amine functionalised dextran; Poly(histidine-arginine) 6-modified chitosan; PEG-PCL-PEI; a polymer comprising a backbone including poly-aspartic acid, glutamic acid, or a combination thereof, functionalised with diamine, triamine, lysine, histidine, arginine, or a combination

thereof; and combinations thereof. More preferably, the endosomal disruptive polymer is pAsp(DET).

[0011] Preferably, the biopolymer is selected from the group consisting of: Poly(N-isopropylacrylamide) (PNIPAM); Polyacrylamide (PAM); Poly(acrylic acid); Polymethacrylate and other acrylic polymers; hyaluronic acid; heparin; chondroitin sulfate; chitosan; polyglutamate; poly-lysine; poly-histidine; poly (glutamic acid); poly-aspartic acid; N-acetylgalactosamine; glucose; sucrose; maltose; fructose; galactose; gelatin; and combinations thereof.

[0012] More preferably, the biopolymer comprises a block co-polymer of chitosan and glucose (CS/GS). Preferably, the CS/GS is formed from deacetylated chitosan, preferably about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85% deacetylated chitosan, most preferably about 75% deacetylated chitosan. Preferably, the CS/GS is formed from deacetylated chitosan, preferably no more than about 50%, no more than about 55%, no more than about 60%, no more than about 65%, no more than about 70%, no more than about 75%, no more than about 80%, no more than about 85% deacetylated chitosan, most preferably no more than about 75% deacetylated chitosan. Preferably, the CS/GS comprises about 50%, about 45%, about 40%, about 35%, about 30%, about 25%, about 20%, about 15% acetyl groups. Preferably, the CS/GS comprises at least about 50%, at least about 45%, at least about 40%, at least about 35%, at least about 30%, at least about 25%, at least about 20%, at least about 15% acetyl groups, most preferably at least about 25% acetyl groups.

[0013] In a preferred embodiment, the biopolymer may be conjugated to the endosomal disruptive polymer.

[0014] In a preferred embodiment, the endosomal disruptive polymer may be conjugated to the quantum dot. Preferably, the endosomal disruptive polymer may be conjugated to a surface of the quantum dot, a surface ligand of the quantum dot, or a combination thereof. Preferably the endosomal disruptive polymer may be conjugated to a surface ligand of the quantum dot.

[0015] In a preferred embodiment, the quantum dot is formed from a material which comprises or consists of: carbon, silver, gold, platinum, aluminium, palladium, copper,

cobalt, indium, zinc, nickel, silicon, and combinations thereof. In a particularly preferred embodiment, the quantum dot is formed from: carbon or Ag_2S.

[0016] Preferably the quantum dot has an average diameter of about 1 nm to about 10 nm, including any value or range therein, including about 1 nm, about 2 nm, about 3 nm, about 4 nm, about 5 nm, about 6 nm, about 7 nm, about 8 nm about 9 nm, and about 10 nm. Preferably, the quantum dot has an average diameter of less than about 20 nm, preferably less than about 15 nm, more preferably less than about 10 nm. Most preferably, the quantum dot has an average diameter of about 5 nm to about 7 nm. Preferably, the quantum dot size distribution is about +/- 20%, +/- 15%, +/- 10%, or +/- 5%.

[0017] Preferably, the quantum dot – polymer composition has a diameter of between about 5 nm and about 50 nm including any value or range therein, such as between about 5 nm and 30 nm, about 10 nm and 30 nm, about 20 nm and 30 nm, about 20 nm, 21 nm, 22 nm, 23 nm, 24 nm, 25 nm, 26 nm, 27 nm, 28 nm, 29 nm, and 30 nm.

[0018] Preferably, the quantum dot – polymer composition has a hydrodynamic diameter of between about 50 nm and about 400 nm, such as between about 100 nm and 300 nm, or between about 100 nm and 250 nm, including any value or range therein, including about 100 nm, 110 nm, 120 nm, 130 nm, 140 nm, 150 nm, 160 nm, 170 nm, 180 nm, 190 nm, 200 nm, 210 nm, 220 nm, 230 nm, 240 nm, and 250 nm. Preferably, the size distribution of the quantum dot polymer composition hydrodynamic diameter is about +/- 20%, +/- 15%, +/- 10%, or +/- 5%.

[0019] In one embodiment, the quantum dot – polymer composition may further comprise a target molecule. Preferably, the target molecule is conjugated to the biopolymer.

[0020] Preferably, the target molecule is selected from the group consisting of: a peptide, polypeptide, antibody, lipid, hyaluronic acid, and combinations thereof. Preferably, the target molecule is a polypeptide. More preferably, the target molecule is cross-linked serum albumin, most preferably formaldehyde treated serum albumin (FSA). The serum albumin may be derived from any suitable source, including but not limited to: bovine, human, and recombinant.

[0021] In a particularly preferred embodiment, the present invention provides a quantum dot – polymer – target molecule composition comprising:

- a quantum dot,
- a polymer shell, and
- a target molecule,

wherein the polymer shell comprises a first polymer layer comprising an endosomal disruptive polymer, and a second polymer layer comprising a biopolymer, wherein the biopolymer is suitable for oral administration,

wherein the endosomal disruptive polymer is conjugated to the quantum dot and the biopolymer,

wherein the biopolymer is further conjugated to the target molecule.

[0022] In one embodiment, the quantum dot – polymer composition, optionally including a target molecule, may further comprise a therapeutic agent.

[0023] Preferably, the therapeutic may be conjugated to the quantum dot, the endosomal disruptive ligand, or a combination thereof. Wherein the therapeutic agent is conjugated to the quantum dot, preferably the therapeutic agent may be conjugated to a surface of the quantum dot, a surface ligand of the quantum dot, or a combination thereof.

[0024] In a preferred embodiment, the therapeutic agent may be selected from the group consisting of: protein, peptide, DNA, RNA, microRNA, siRNA, CRISPR-Cas9, and combinations thereof. In a particularly preferred embodiment, the therapeutic agent is a protein or fragment thereof, siRNA, or CRISPR-Cas9.

[0025] In a particularly preferred embodiment, the present invention provides a quantum dot – therapeutic agent - polymer – target molecule composition comprising:

- a quantum dot,
- a therapeutic agent,
- a polymer shell, and

- a target molecule,

wherein the polymer shell comprises a first polymer layer comprising an endosomal disruptive polymer, and a second polymer layer comprising a biopolymer, wherein the biopolymer is suitable for oral administration,

wherein the therapeutic agent is conjugated to the quantum dot, the endosomal disruptive polymer, or a combination thereof, preferably a combination thereof,

wherein the endosomal disruptive polymer is conjugated to the quantum dot, the therapeutic agent, the biopolymer, or a combination thereof, preferably at least to the therapeutic agent and the biopolymer,

wherein the biopolymer is conjugated to the endosomal disruptive polymer and the target molecule.

[0026] In another aspect, there is provided a method of preparing quantum dot – polymer compositions as described herein.

[0027] In one embodiment, the method comprises the steps of:

- conjugating a quantum dot comprising hydrophilic surface ligands to an endosomal disruptive polymer to provide a quantum dot – endosomal disruptive polymer conjugate;

- conjugating the quantum dot – endosomal disruptive polymer to a biopolymer to provide a quantum dot – polymer composition.

[0028] Preferably the biopolymer is conjugated to the endosomal disruptive polymer by an imine, a hemiaminate, or a combination thereof. This reaction may be performed at a pH of less than about 6.0, less than about 5.9, 5.8, 5.7, 5.6, 5.5, 5.4, 5.3, 5.2, 5.1, 5.0, 4.9, 4.8, 4.7, 4.6, 4.5, 4.4, 4.3, 4.2, 4.1, 4.0, 3.9, 3.8, 3.7, 3.6, 3.5, 3.4, 3.3, 3.2, 3.1, or 3.0. Preferably the reaction is performed at a pH of no more than about 6.0, no more than about 5.9, 5.8, 5.7, 5.6, 5.5, 5.4, 5.3, 5.2, 5.1, or 5.0. Preferably, the reaction is performed at a pH of about 3.0 to about 6.0, including any value or range therein, including 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, and 6.0.

[0029] In one embodiment, the method further comprises the step of:

- conjugating a target molecule to the biopolymer to provide a quantum dot – polymer – target molecule composition.

[0030] In another embodiment, there is provided a method comprising the steps of:

- conjugating a quantum dot to a therapeutic agent to provide a quantum dot – therapeutic agent conjugate;
- conjugating an endosomal disruptive polymer to the quantum dot – therapeutic agent conjugate to provide a quantum dot – therapeutic agent – endosomal disruptive polymer conjugate;
- conjugating the quantum dot – therapeutic agent – endosomal disruptive polymer to a biopolymer to provide a quantum dot – therapeutic agent – polymer composition.

[0031] In one embodiment, the method further comprises the step of:

- conjugating a target molecule to the biopolymer to provide a quantum dot – therapeutic agent - polymer – target molecule composition.

[0032] In another aspect there is provided a method of preventing or treating an autoimmune disease or condition in a subject in need thereof, the method comprising administering to the subject a therapeutic amount of a quantum dot – polymer composition, optionally comprising a target molecule, as described herein.

[0033] In another aspect there is provided a method of preventing or treating an autoimmune disease or condition in a subject in need thereof, the method comprising administering to the subject a therapeutic amount of a quantum dot – therapeutic agent - polymer composition, optionally comprising a target molecule, as described herein.

[0034] In another aspect there is provided a method of treating insufficient endogenous peptide production in a subject in need thereof, the method comprising administering to the subject a therapeutic amount of a quantum dot – therapeutic agent – polymer composition, optionally comprising a target molecule, as described herein.

[0035] In another aspect there is provided a method of treating a subject suffering from a condition, wherein the condition is treatable with administration of a therapeutic exogenous peptide or protein, the method comprising administering to the subject a

therapeutic amount of a quantum dot – therapeutic agent – polymer composition, optionally comprising a target molecule, as described herein.

[0036] In any aspect, the composition may be administered to the subject orally.

[0037] In another aspect, there is provided a method of delivering a therapeutic agent to an organ or tissue of a subject, the method comprising orally administering a quantum dot – therapeutic agent – polymer composition, optionally comprising a target molecule, as described herein to the subject, wherein the organ or tissue is selected from the liver, pancreas, intestine, small bowel or kidneys.

[0038] Further aspects of the present invention and further embodiments of the aspects described in the preceding paragraphs will become apparent from the following description, given by way of example and with reference to the accompanying drawings.

Brief description of the drawings

[0039] **Figure 1:** Characterisation of Ag_2S QD nano-BITS and QD nano-insulin with either a Ag_2S quantum dot core or carbon quantum dot core**.** (**A**) Hydrodynamic diameter (HD), (**B**) zeta potential and (**C**) poly dispersity index (PDI) data were collected at 0.1 mg/mL at pH 7. (**D**) TEM data was collected at 0.1 mg/mL at pH 7. Ag_2S QD nano-BITS refers to Ag_2S QD-Pasp(DET)-CS/GS-FSA. Ag_2S QD nano-insulin refers to Ag_2S QD-insulin-Pasp(DET)-CS/GS-FSA or CQD nano-insulin refers to carbon QD-insulin-Pasp(DET)-CS/GS-FSA.

[0040] **Figure 2:** Fourier-transform infrared spectroscopy (FTIR) of Ag_2S nano-BITS **(A)** and Ag_2S nano-insulin **(B).**

[0041] **Figure 3:** Fourier-transform infrared spectroscopy (FTIR) of different targeting proteins and modifications to CS/GS for the Ag_2S QD nano-insulin and Ag_2S QD nano-BITS formulations.

[0042] **Figure 4:** Scanning electron microscopy (SEM) micrographs of dried Ag_2S QD nano-insulin**.** Micrographs were collected at x 5,000 (**A-B**), x 10,000 (**C-D**) and x 20,000 (**E-F**).

[0043] **Figure 5.** Transmission electron microscopy (TEM) micrographs of Ag_2S QD nano-insulin. Samples were negatively stained. Micrographs were collected at x 110,000 (**A**) and x 400,000 (**B-F**).

[0044] **Figure 6.** Transmission electron microscopy (TEM) micrographs of carbon QD nano-insulin. Samples were negatively stained. Micrographs were collected at x 400,000 (**A-B**) and x 560,000 (**C-D**).

[0045] **Figure 7:** Development of hyperglycaemia in female non-obese diabetic (NOD) mice treated with Ag_2S QD nano-insulin**.**

[0046] **Figure 8:** EC_{50} of diabetes development in female non-obese diabetic (NOD) mice treated with Ag_2S QD nano-insulin**,** markers show the EC_{50} of anti-CD3 treated mice (32 weeks) and control mice (19 weeks).

[0047] **Figure 9:** Development of hyperglycaemia in female non-obese diabetic (NOD) mice treated with QD nano-insulin with a Ag_2S or carbon quantum dot cores.

[0048] **Figure 10:** Effects of Ag_2S QD nano-insulin on auto insulin antibody in female NOD mice. Data shows mean ± SD, ** $P < 0.01$. Nano-insulin refers to Ag_2S QD-insulin-Pasp(DET)-CS/GS-FSA. Nano-insulin without Pasp(DET) refers to Ag_2S QD-insulin-CS/GS-FSA. Nano-insulin without CS/GS refers to Ag_2S QD-insulin-Pasp(DET)- FSA.

[0049] **Figure 11:** Effects of Ag_2S QD nano-insulin treatment on splenocytes T cell subsets: (**A**) CD3, CD4, (**B**) CD25, (**C**) Foxp3, and (**D**) CD8. Data shows mean ± SD, * $P < 0.05$.

[0050] **Figure 12:** Effects of Ag_2S QD nano-insulin treatment on liver non parenchymal and immune cells: (**A-B**) dendritic cells (DCs), (**C-D**) macrophages, (**E-F**) Kupffer cells (KCs), (**G-H**) monocyte derived macrophages (MoMFs), and (**I-K**) liver sinusoidal endothelial cells (LSECs). Data shows mean ± SD (n=3), * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

[0051] **Figure 13:** Biodistribution of Ag_2S QD nano-(^{14}C)-insulin over 24 hours. Pharmacokinetic (PK) data from (**A**) blood and (**B**) faeces collected between 0-24 hours; (**C-F**) ^{14}C distribution data from major organs after mice were euthanized at 24 hours.

[0052] **Figure 14:** Effects of chronic Ag_2S QD nano-insulin treatment on splenocytes T cell subsets: (**A**) CD3, (**B**) CD4, (**C**) CD25, Foxp3 and (**D**) CD8. Data shows mean ± SD.

[0053] **Figure 15:** Effects of Ag_2S QD nano-insulin on insulitis in female NOD mice: (**A**) nano-insulin (2 IU/kg), (**B**) nano-insulin (20 IU/kg) from weeks 4-40 or anti-CD3 (5x 5 µg/kg/day), (**C**) histological evaluation. Data shows mean ± SD.

[0054] **Figure 16:** Development of hyperglycaemia in female non-obese diabetic (NOD) mice treated with Ag_2S QD nano-BITS, nano-insulin-GAD and nano-insulin-GAD without the targeting protein. Nano-Ins-FSA refers to Ag_2S QD-insulin-Pasp(DET)-CS/GS-FSA. Nano-BITS-FSA refers to Ag_2S QD-Pasp(DET)-CS/GS-FSA. Nano-Ins-Gad refers to Ag_2S QD-insulin-GAD65-Pasp(DET)-CS/GS. Nano-Ins-Gad-FSA refers to Ag_2S QD-insulin GAD65-Pasp(DET)-CS/GS-FSA.

[0055] **Figure 17:** Changes in dendritic cell MHC-II A-I^{g7} expression following 24 hour treatments *in vivo* and *in vitro*: **(A)** Ag_2S QD nano-BITS or Ag_2S QD nano-insulin (50 IU/kg), **(B)** I-A^{g7} for liver non-parenchymal cells with individual nano-insulin constituents, **(C)** I-A^{g7} for cells treated with Ag_2S QD nano-insulin with different targeting proteins, **(D)** cells treated with Ag_2S QD nano-insulin and Ag_2S QD nano-BITS with modified CS/GS.

[0056] **Figure 18:** Changes in Kupffer cell MHC-II A-I^{g7} expression following 24 hour treatments *in vivo* and *in vitro*: **(A)** Ag_2S QD nano-BITS or Ag_2S QD nano-insulin (50 IU/kg), **(B)** I-A^{g7} for liver non-parenchymal cells with individual Ag_2S QD nano-insulin constituents, **(C)** I-A^{g7} for cells treated with Ag_2S QD nano-insulin with different targeting proteins, **(D)** cells treated with Ag_2S QD nano-insulin and Ag_2S QD nano-BITS with modified CS/GS.

[0057] **Figure 19:** Changes in liver sinusoidal endothelial cells (LSECs) MHC-II A-I^{g7} expression following 24 hour treatments *in vivo* and *in vitro*: **(A)** Ag_2S QD nano-BITS or Ag_2S QD nano-insulin (50 IU/kg), **(B)** I-A^{g7} for liver non-parenchymal cells with individual Ag_2S QD nano-insulin constituents, **(C)** I-A^{g7} for cells treated with Ag_2S QD nano-insulin with different targeting proteins, **(D)** cells treated with Ag_2S QD nano-insulin and Ag_2S QD nano-BITS with modified CS/GS.

[0058] **Figure 20:** Changes in macrophage MHC-II A-I^{g7} expression following 24 hour treatments *in vivo* and *in vitro*: **(A)** Ag_2S QD nano-BITS or Ag_2S QD nano-insulin (50

IU/kg), **(B)** I-A^{g7} for liver non-parenchymal cells with individual Ag_2S QD nano-insulin constituents, **(C)** I-A^{g7} for cells treated with Ag_2S QD nano-insulin with different targeting proteins, **(D)** cells treated with Ag_2S QD nano-insulin and Ag_2S QD nano-BITS with modified CS/GS.

[0059] Figure 21: Effects of Ag_2S QD nano-siRNA of GAPDH siRNA following 4 hour *in vitro* and 24 hour *in vivo* treatments**: (A)** hepatocyces, **(B)** liver sinusoidal endothelial cells, **(C)** mice with single gavage 15 nM, 24 hour. Ag_2S QD nano-siRNA refers to Ag_2S QD-siRNA-Pasp(DET)-CS/GS-FSA.

[0060] Figure 22: Biodistribution of Ag_2S QD nano-(^{14}C)-Cas9 over 24 hrs. ^{14}C distribution data from major organs after mice were euthanized at 24 hours. Ag_2S QD nano-(^{14}C)-Cas9 refers to Ag_2S QD-(^{14}C)-Cas9-Pasp(DET)-CS/GS-FSA.

[0061] Figure 23: Confocal microscopy of GFP tagged CRISPR/Cas9 with and without Ag_2S QD attachment in Sk-Hep1 cells. **(A)** control (untreated) cells and **(B)** control treatments of free Cas9 RNP without and **(C)** with conjugation to a guide RNA sequence. **(D)** Ag_2S QD conjugated to Cas9 without and **(E)** with conjugation to a guide RNA sequence. Ag_2S QD conjugated to Cas9 without guide RNA refers to Ag_2S QD-Cas9-Pasp(DET)-CS/GS-FSA. Ag_2S QD conjugated to Cas9 with guide RNA refers to Ag_2S QD-Cas9-guide RNA-Pasp(DET)-CS/GS-FSA.

[0062] Figure 24: Effects of Ag_2S QD-CD98-siRNA treatment on CD98 gene expression in healthy Bl6 mice 24 hours post 0.02 mg/kg treatment in liver sinusoid endothelial cells (LSECs), hepatocytes (Hep), splenocytes (Spleen), and payers patches (PP). Ag_2S QD-CD98-siRNA refers to Ag_2S QD-CD98-siRNA-Pasp(DET)-CS/GS-FSA.

[0063] Figure 25: Effects of Ag_2S QD nano-insulin treatment in non-human primates (NHPs). NHPs were treated for 28 days with Ag_2S QD nano-insulin (9 mg/d 28 d) with a washout period of 28 days. Effects on haematological markers were assessed: haemoglobin, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean cell volume (MCV), pact cell volume (PCV), platelets, red cell count (RCC), white cell count (WCC), neutrophils, lymphocytes, eosinophils, and basophils.

[0064] **Figure 26**: Effects of Ag_2S QD nano-insulin treatment in non-human primates (NHPs). NHPs were treated for 28 days with Ag_2S QD nano-insulin (9 mg/d 28 d) with a washout period of 28 days. Effects on biomarkers of regulatory T cells in peripheral blood: **(A)** Foxp3+ of CD3+ CD4+ cells and **(B)** CD62L+ of CD3+ CD4+ FoxP3+ cells. Effects on dendritic cell populations in peripheral blood: **(C)** plasmocytoid dendritic cells (pDC), **(D)** type 1 conventional dendritic cells (cDC1) and **(E)** type 2 conventional dendritic cells (cDC2).

[0065] **Figure 27:** Effects of Ag_2S QD nano-insulin treatment on non-obese diabetic (NOD) mice. NOD mice were treated for 28 days with Ag_2S QD nano-insulin. EA2 refers to Ag_2S QD nano-insulin. Effects on biomarkers of regulatory T cells: **(A)** combined Ki67+ of Tregs, **(B)** combined effector Tregs of Foxp3+ cells, and **(C)** combined central memory-like Tregs of Foxp3+ cells.

[0066] **Figure 28:** Effects of Ag_2S QD nano-insulin treatment on non-obese diabetic (NOD) mice. NOD mice were treated for 28 days with Ag_2S QD nano-insulin. EA2 refers to Ag_2S QD nano-insulin. Effects on biomarkers of CD4 and CD8 cells: **(A)** combined naïve CD8+ cells, **(B)** combined naïve CD4+ cells, **(C)** combined Ki67+ of CD4+ cells, and **(D)** combined effector memory (EM) of CD4+ cells.

[0067] **Figure 29:** Effects of Ag_2S QD nano-insulin treatment on blood glucose levels in fasting NHPs in an insulin tolerance test (ITT). **(A)** blood glucose in male NHPs administered with control, 1 IU/kg Ag_2S QD nano-insulin, or 10 IU/kg Ag_2S QD nano-insulin. **(B)** AUC of blood glucose curves in A. **(C)** blood glucose in female NHPs administered with control, 1 IU/kg Ag_2S QD nano-insulin, or 10 IU/kg Ag_2S QD nano-insulin. **(D)** AUC of blood glucose curves in C. **(E)** Comparison of blood glucose in NHPs treated with control, 10 IU/kg oral insulin, or 10 IU/kg Ag_2S QD nano-insulin.

[0068] **Figure 30:** ATR-FTIR spectrum of **(A)** peanut oil, **(B)** BSA and **(C)** Ag_2S QD nano-peanut (referred to as EA3PN). Ag_2S QD nano-peanut refers to Ag_2S QD-Ara h 1-Ara h 3-Pasp(DET)-CS/GS-FSA.

[0069] **Figure 31:** Effect of Ag_2S QD nano-peanut and omalizumab on IgE levels across sexes. Mice were treated with control, 3 dosages of Ag_2S QD nano-peanut or omalizumab. Peanut specific IgE in females: (**A**) baseline, **(B)** 60 minutes following treatment, **(C)** 24 hours following treatment, (**D**) Combined data (A-C). Peanut specific

IgE in males: (**E**) baseline, (**F**) 60 minutes following treatment, **(G)** 24 hours following treatment, **(H)** Combined data (E-G). PPC refers to "post-peanut challenge".

[0070] **Figure 32:** Effect of Ag_2S QD nano-peanut and omalizumab on IgG2a levels across sexes. Mice were treated with control, 3 dosages of Ag_2S QD nano-peanut or omalizumab. Peanut specific IgG2a in females: (**A**) baseline, **(B)** 60 minutes following treatment, **(C)** 24 hours following treatment, (**D**) Combined data (A-C). Peanut specific IgG2a in males: (**E**) baseline, (**F**) 60 minutes following treatment, **(G)** 24 hours following treatment, **(H)** Combined data (E-G). PPC refers to "post-peanut challenge".

[0071] **Figure 33:** Effect of Ag_2S QD nano-peanut and omalizumab on IL-10 levels across sexes. Mice were treated with control, 3 dosages of Ag_2S QD nano-peanut or omalizumab. Peanut specific IL-10 in females: (**A**) baseline, **(B)** 60 minutes following treatment, **(C)** 24 hours following treatment, (**D**) Combined data (A-C). Peanut specific IL-10 in males: (**E**) baseline, (**F**) 60 minutes following treatment, **(G)** 24 hours following treatment, **(H)** Combined data (E-G). PPC refers to "post-peanut challenge.

[0072] **Figure 34:** Effect of Ag_2S QD on GFP endocytosis in SK-HEP1 cells. Cells were treated with control, GFP, Ag_2S QD-GFP, Ag_2S QD -GFP-Pasp(DET), Ag_2S QD-GFP-Pasp(DET)-CS/GS, Ag_2S QD-GFP-Pasp(DET)-Cs/GS-FSA.

Detailed description of the embodiments

[0073] It will be understood that the invention disclosed and defined in this specification extends to all alternative combinations of two or more of the individual features mentioned or evident from the text or drawings. All of these different combinations constitute various alternative aspects of the invention.

[0074] Reference will now be made in detail to certain embodiments of the invention. While the invention will be described in conjunction with the embodiments, it will be understood that the intention is not to limit the invention to those embodiments. On the contrary, the invention is intended to cover all alternatives, modifications, and equivalents, which may be included within the scope of the present invention as defined by the claims.

[0075] It will be understood that the invention disclosed and defined in this specification extends to all alternative combinations of two or more of the individual

features mentioned or evident from the text. All of these different combinations constitute various alternative aspects of the invention.

[0076] All of the patents and publications referred to herein are incorporated by reference in their entirety.

Definitions

[0077] For the purposes of interpreting this specification, terms used in the singular will also include the plural and vice versa.

[0078] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which the present disclosure belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, preferred methods and materials are described. For the purposes of the present disclosure, the following terms are defined below.

[0079] The articles “a” and “an” are used herein to refer to one or to more than one (ie to at least one) of the grammatical object of the article. By way of example, “a reactive group” means one reactive group or more than one reactive group.

[0080] As used herein, the term “and/or”, eg, “X and/or Y” will be understood to mean either “X and Y” or “X or Y” and shall be taken to provide explicit support for both meanings or for either meaning.

[0081] As used herein, the term “about” refers to a quantity, value, dimension, size, or amount that varies by as much as 10%, 5%, 1% or 0.1 % to a reference quantity, value, dimension, size, or amount.

[0082] Throughout the present disclosure, various aspects of the disclosure can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosure. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as

individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

[0083] The term "nanoparticle" as used herein refers to a particle characterized by a longest dimension of about 1 nm to about 1000 nm, or about 1 nm to about 500 nm, or about 1 nm to about 100 nm, or about 1 nm to about 50 nm, or about 1 nm to about 20 nm, or about 1 nm to about 10 nm. Nanoparticles can be globular or in the shape of spheres, platelets, rods, wires, disks, or prisms.

[0084] Nanoparticles typically have organic or inorganic material, or a mixture thereof, bound to or associated with the particle surface. As defined herein, the terms "surface ligand", "surface coating," "stabilizing agent," and "capping agent" are used interchangeably and refer to an adsorbed or chemically bonded monolayer of organic molecules, inorganic molecules, or mixtures thereof, at the surface of the particle(s).

[0085] Herein, nanoparticle "size" or "size range" or "size distribution", refers to the average longest dimension of a plurality of nanoparticles that falls within the specified range. "Longest dimension" is defined herein as the measurement of a nanoparticle from end to end along the major axis of the projection. The "longest dimension" of a particle will depend on the shape of the particle. For example, for particles that are roughly or substantially spherical, the longest dimension will be a diameter of the particle.

[0086] The term "quantum dot" as used herein refers to a substantially spherical nanoparticulate material with an average size distribution of approximately less than about 100 nm, less than about 50 nm, less than about 20 nm, or less than about 10 nm. Such quantum dots also generally exhibit optical and/or electronic properties that are distinct from particles formed of the same material but of a larger size.

[0087] The term "hydrodynamic diameter" as used herein refers to the degree of arrangement of the water molecules close to the surface when the conjugate is dispersed in water. It is defined as the diameter of a perfect solid sphere that would exhibit the same hydrodynamic friction as the conjugate when dispersed in water. In other words, as the surface of the conjugates described herein have an electrostatic charge, the water molecules adjacent to the surface would be arranged and associated with the surface by electrostatic forces to provide a particle essentially a defined water layer shell, and hence

with a hydrodynamic diameter equivalent to a solid particle without such water association.

[0088] The term "conjugate" as used herein refers to an arrangement whereby two or more species and/or structures are in association. For example, a quantum dot of the present invention may be associated with an insulin protein chain to form a quantum dot-insulin conjugate. Such association may take any suitable chemical form, so long as the two or more conjugated elements are found in close proximity. "Conjugation" may include association by at least one of a covalent bond, an ionic bond, a van der Waals interaction, dipole-dipole interactions, and/or a hydrogen bond.

[0089] The term "polymer" as used herein refers to a molecule or a macromolecule formed by linking monomers with covalent bonds. The monomers may be the same or they may be different. The monomers may be formed into repeating subunits of monomers (eg polyethylene) or they may have a non-repeating sequence (eg a protein). The term "polymer" is understood to encompass polymers of both a synthetic origin and a biological origin.

[0090] The term "biopolymer" as used herein refers to a polymer chain of biological origin, whereby the polymer is produced by a biological system, and/or in which a polymer chain is formed from monomer moieties which have a biological origin.

[0091] The term "biopolymer suitable for oral delivery" as used herein refers to a biopolymer that may be used to formulate oral drug delivery systems. Such polymers are typically capable of protecting a therapeutic agent from the harsh conditions of the gastrointestinal tract, facilitate absorption, or a combination thereof. Biopolymers suitable for oral delivery are typically biocompatible and biodegradable (non-toxic, non-immunogenic, and able to be broken down by the body's natural processes), pH sensitive or responsive (ie stable in the acidic environment of the stomach and release the therapeutic agent in the more neutral environment of the intestines), controlled release (can provide sustained or targeted release of the therapeutic agent), or combination thereof.

[0092] The term "endosomal disruptive polymer" as used herein refers to a polymer that is capable of disrupting the endosomal membrane. Endosomal disruptive polymers may comprise pH sensitive and/or membrane active components that can destablise

the endosomal membrane under slightly acidic conditions found within endosomes, such as proton-sponge groups and lipophilic moieties.

[0093] The term "peptide" or "polypeptide" as used herein refers to a short chain of amino acids, connected in sequence by peptide bonds, usually between about 2 and about 50 amino acids in length. The term "protein" as used herein refers to a chain of amino acids which are longer in length than peptides, ie, about 51 amino acids or more in length.

[0094] The term "subject" as used herein refers to any human or non-human animal to be treated. Accordingly, compositions of the present invention may be suitable for human treatment, and they may also be suitable for veterinary treatment of non-human manilas, including companion animals such as cats and dogs, or farm animals, such as pigs, horses, sheep and cattle. A "subject" herein is preferably a human subject. It will be understood that the terms "subject" and "individual" are interchangeable in relation to an individual requiring administration of the aqueous formulation of the present disclosure.

[0095] The term "administration", or variations thereof, including but not limited to "administer" or "administering", as used herein refers to providing a subject with a therapeutic composition.

[0096] The term "oral", with reference to administration of compositions of the present invention, refers to delivery to the mouth of the subject to whom the composition is being administered, with the expectation that most, if not all, of the composition is absorbed in the gastrointestinal tract. The term "oral" in the context of the present invention is not intended to refer to transdermal absorption across the mucosal membranes.

[0097] As used herein, the terms "treatment" or "treating" of a subject include the application or administration of a conjugate (or pharmaceutical composition) described herein to a subject with the purpose of delaying, slowing, stabilizing, curing, healing, alleviating, relieving, altering, remedying, less worsening, ameliorating, improving, or affecting the disease or condition, the symptom of the disease or condition, or the risk of (or susceptibility to) the disease or condition. The term "treating" refers to any indication of success in the treatment or amelioration of an injury, pathology or condition, including

any objective or subjective parameter such as abatement; remission; lessening of the rate of worsening; lessening severity of the disease; stabilization, diminishing of symptoms or making the injury, pathology or condition more tolerable to the subject; slowing in the rate of degeneration or decline; making the final point of degeneration less debilitating; or improving a subject's physical or mental well-being.

[0098] As used herein, the term "prevention" or "preventing" are intended to refer to at least the reduction of likelihood of the risk of (or susceptibility to) acquiring a disease or disorder (i.e., causing at least one of the clinical symptoms of the disease not to develop in a patient that may be exposed to or predisposed to the disease but does not yet experience or display symptoms of the disease). Biological and physiological parameters for identifying such patients are provided herein and are known in the art.

[0099] The term "blood glucose" as used herein refers to the amount of glucose circulating in the blood of the circulatory system of a subject. Accordingly, the related terms "lowering blood glucose" or "decreasing blood glucose" and the like should be understood to refer to less glucose circulating in the blood of the subject compared to an earlier time, which may be before administration of a treatment. Likewise, terms such as "raising blood glucose" or "increasing blood glucose" and the like should be understood to refer to more glucose circulating in the blood of the subject compared to an earlier time.

[0100] The terms "hyperglycaemia" and "hyperglycaemic" and the like refers to when the blood glucose level of a subject is higher than the normal range for blood glucose in a healthy subject. Similarly, the terms "hypoglycaemia" and "hypoglycaemic" and the like refers to when the blood glucose level of a subject is lower than the normal range for blood glucose in a healthy subject.

[0101] As used herein, unless the context requires otherwise, the term "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

[0102] As used herein the term "consisting essentially of" means to the exclusion of other additional components purposefully added, or only the following recited elements

are intended to be present. Additional components that are in the defined composition or device that are not intentionally present are acceptable.

[0103] The use of the terms "adding", "contacting" or "mixing" does not limit the order, method or how the materials being added are combined, unless indicated otherwise. For instance, "adding A to B" may also describe "adding B to A". Furthermore, "adding A and B to C" may also describe the various other combinations such as "adding A to B and C", "adding A and C to B", "adding B to A and C", "adding B and C to A", and "adding C to A and B".

[0104] The inventors previously developed quantum dot insulin conjugates comprising a biopolymer (WO 2021/142516). The biopolymer may be selected from the group consisting of heparin, gelatin, hyaluronic acid, chitosan, galactose, glucose or a combination thereof. The biopolymer was shown to protect the protein or peptide of the conjugate during transit through the gastrointestinal tract or specifically target the hepatocytes in the liver. In this way, the inventors were able to effectively deliver insulin, which is not orally bioavailable, to its target site, the liver, in oral dosage form.

[0105] Herein, the inventors have developed an improved quantum dot biopolymer composition that comprises an endosomal disruptive polymer. The endosomal disruptive polymer increases the cellular uptake and efficacy of the quantum dot composition. The inventors have surprisingly shown that quantum dot polymer compositions that comprise a biopolymer and an endosomal disruptive polymer, but which do not include a therapeutic agent (such as insulin, siRNA or CRISPR-Cas9) provide a therapeutic effect. Without wishing to be bound by theory, it is thought that the quantum dot polymer compositions that do not include a therapeutic agent provide a therapeutic effect by increasing the expression of MHCII on antigen presenting cells (APCs). Further, quantum dot polymer compositions that do not include a therapeutic agent may non-specifically bind proteins *in vivo*, including auto-antibodies and antigens, which are effectively delivered to APCs via endocytosis mechanisms thereby providing a therapeutic auto-immune response. The inventors have also shown that quantum dot polymer compositions described herein that include a therapeutic agent (such as insulin or siRNA) provide improved therapeutic efficacy compared to quantum dot insulin conjugates of the prior art that only include a biopolymer, and no endosomal disruptive polymer. As described herein, preparing quantum dot polymer compositions comprising an endosomal disruptive polymer and a biopolymer is non-trivial given that suitable

endosomal disruptive polymers and suitable biopolymers include reactive groups that require specific conditions to react to form a polymer shell bilayer.

Quantum dots

[0106] The compositions used in the present invention comprise a nanoparticle. The nanoparticle may be a quantum dot. By definition, a quantum dot is a nanoparticle of relatively small size, up to about 50-100 nm in diameter, but which displays electronic and/or optical properties (or optoelectronic properties as used interchangeably herein) that are different compared to particles formed from the same matter but of larger size to the bulk material. An example of the optical properties of quantum dots that may be observed is photoluminescence, whereby a wavelength in the ultraviolet portion of the electromagnetic spectrum is absorbed by the quantum dot, exciting an electron into a higher energy level which then degrades to a lower energy electron shell, releasing a quantum of energy which is observed as a wavelength in the visible portion of the electromagnetic spectrum. An example of the electronic properties of quantum dots that may be observed is superconductivity. These optoelectronic properties of quantum dots may be dependent on particle size and the materials used in their construction. In the context of the present invention, the optoelectronic properties of quantum dots may be used diagnostically to determine the location of the conjugate in different tissues of the subject following administration of a composition comprising these conjugates with suitable imaging techniques.

[0107] The quantum dot may be formed from any suitable material. By “suitable”, it is meant that the quantum dot comprises a material, or materials, which are capable of forming a particle small enough to be characterised as a quantum dot, which display the optoelectronic properties characteristic of a quantum dot, and which are capable of associating with a therapeutic agent and/or an endosomal disruptive polymer, either on the native particle surface, via native surface ligands, or after functionalisation. By “functionalisation”, it is meant that the surface or native surface ligand is changed following a chemical reaction. As they are for therapeutic administration to a subject, the quantum dots of the present invention must also be non-toxic to the subject and well-tolerated. As they may be for treatment of a chronic condition, preferably they are substantially eliminated soon after therapy (ie do not accumulate in the body, tissues or cells of the subject). In this regard, “substantial” elimination may mean that greater than about 75% of the quantum dots that are administered are eliminated from the subject in

a given time, including greater than about 80%, greater than about 85%, greater than about 90%, greater than about 95%. Preferably, greater than about 75% of the quantum dots are eliminated from the subject within 48 hours, within 24 hours, or within 12 hours after administration.

[0108] The quantum dots may be core-type quantum dots that are formed from a single material, such as carbon (carbon quantum dots (CQD)), or chalcogenides (eg selenides, sulphides or tellurides) of non-heavy metals, such as zinc or silver.

[0109] The quantum dots may be core-shell quantum dots that are formed from at least two different materials, whereby a layer of a higher band gap semiconducting material is coated onto a core. The shell layer may comprise a non-toxic material. A common example of a core-shell quantum is a shell layer that consists of ZnS applied onto a core comprising CdSe, although this material would not be suitable for use in the present invention due to the presence of cadmium, a known toxic heavy metal.

[0110] The quantum dots may be alloyed quantum dots, whereby two or more semiconducting materials are combined by alloying. Alloyed quantum dots may result in properties distinct from the bulk properties of either of the constituent materials.

[0111] In a preferred embodiment, the quantum dot is formed from a material which comprises or consists of: carbon, silver, gold, platinum, aluminium, palladium, copper, cobalt, indium, zinc, nickel, silicon, and combinations thereof. In a particularly preferred embodiment, the quantum dot is formed from: carbon or Ag_2S.

[0112] The quantum dots may be crystalline.

[0113] The quantum dots of the present invention are generally defined herein as particles with a diameter of less than about 100 nm, less than about 50 nm, less than about 20 nm, or less than about 10 nm. Accordingly, each quantum dot disclosed herein may have a diameter of between about 1 nm and about 20 nm, or between about 1 nm and 5 nm, 5 nm and 10 nm, 10 nm and 20 nm, 5 nm and 15 nm, 1 nm and 15 nm, or 5 nm and 20 nm, eg, they may have an average diameter of about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 nm. As will be discussed below in further detail, this upper size limit is due to the restrictions inherent in the processes of absorption through the intestinal wall and endocytosis in the hepatocytes of the liver, which are believed to be steps involved in the therapeutic effects provided by the

present invention, rather than any inherent limit placed on the material itself. Accordingly, quantum dots larger than 20 nm, up to 50 nm or up to 100 nm, eg, average diameters of up to 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 50 or 100 nm may be suitable for use in the present invention.

[0114] Preferably the quantum dot has an average diameter of about 1 nm to about 10 nm, including any value or range therein, including about 1 nm, about 2 nm, about 3 nm, about 4 nm, about 5 nm, about 6 nm, about 7 nm, about 8 nm about 9 nm, and about 10 nm. Preferably, the quantum dot has an average diameter of less than about 20 nm, preferably less than about 15 nm, more preferably less than about 10 nm. Most preferably, the quantum dot has an average diameter of about 5 nm to about 7 nm. Preferably, the quantum dot size distribution is about +/- 20%, +/- 15%, +/- 10%, or +/- 5%.

[0115] The quantum dot comprises surface ligands. As used herein, "surface ligands" refers to a monolayer of ligands disposed on a surface of the quantum dot. The surface ligands minimise particle aggregation. The surface ligands may comprise an organic molecule, an inorganic molecule, or a combination thereof. Preferably, the surface ligands are hydrophilic. The surface ligands may be any suitable hydrophilic molecule or polymer that is capable of dispersing the quantum dot in an aqueous solution, including but not limited to water and biological buffers, to form a stable solution-phase colloid.

[0116] Preferably, the surface ligands are coordinated to the surface of the quantum dot by at least one of a covalent bond, an ionic bond, a van der Waals interaction, dipole-dipole interactions, and/or a hydrogen bond. The surface ligands may comprise a first functional group coordinated to the quantum dot surface, including but not limited to: thiol, amine, carboxyl, or hydroxyl. The surface ligands may be mono- or multi-dentate ligands, comprising one or multiple first functional groups coordinated to the quantum dot surface.

[0117] The surface ligands may comprise a second functional group. The second functional group may impart hydrophilicity to the surface ligand. For example, the second functional group may comprise a terminal polar functional group including but not limited to: amine, carboxyl, or hydroxyl. The second functional group may be capable of forming an amide or ester. Preferably, the second functional group is an amine or carboxyl, preferably a carboxyl. The second functional group may be used as

a chemical linkage to other inorganic, organic or biological material. The second functional group may be polymerisable and can be used to form a polymer around the quantum dot.

[0118] The first functional group and the second functional group of the surface ligand may be the same or different.

[0119] The surface ligand may be a native surface ligand, a modified native surface ligand, an exchange surface ligand, or a combination thereof.

[0120] Native surface ligand refers to ligands disposed on a surface of the quantum dot arising from formation of the quantum dot. Native surface ligands can be the solvent that the quantum dot preparation is undertaken in, or a ligand that is added to the solvent that the quantum dot preparation is undertaken in. In one embodiment, carbon quantum dots may be synthesised using citric acid to provide carbon quantum dots comprising citric acid as a native surface ligand.

[0121] A native surface ligand may be modified to change or introduce a second functional group. For example, carbon quantum dots comprising citric acid as a native surface ligand include a carboxylic acid second functional group. The carboxylic acid functional group may be modified to provide an amine second functional group.

[0122] The native surface ligand may be replaced with an exchange surface ligand. For example, Ag_2S quantum dots may comprise a hydrophobic ligand as a native surface ligand, such as an alkyl thiolate. The hydrophobic native surface ligand may be exchanged with a hydrophilic surface ligand, such as a carboxylic acid alkyl thiolate. The exchange surface ligand replaces at least a portion of the native surface ligands coordinated to the quantum dot surface. In some embodiments, substantially all of the native surface ligands may be removed from the surface of the quantum dot such that substantially all of the available surface may coordinate with the exchange surface ligands.

Silver sulphide quantum dots

[0123] In one embodiment, the quantum dots of the present invention may consist of or comprise Ag_2S. Quantum dots that consist of Ag_2S are well known in the field of nanoparticles. They have been shown to have low or no toxicity to mammals and may

also have near-infrared fluorescence. Commonly, they are prepared by using a self-assembly method which results in Ag_2S quantum dots that have a hydrophobic coating, however the Ag_2S quantum dots of the present invention may be made by any suitable method. Such hydrophobic Ag_2S quantum dots are commonly functionalised to have a hydrophilic coating. This functionalisation may be carried out with use of any suitable method to obtain a hydrophilic coating. In one example, the hydrophilic reagent used may be a mercapto- or thiol-containing reagent which, when incubated with the hydrophobic quantum dots in suitable conditions, results in a hydrophilic surface chemistry on the Ag_2S quantum dots. The surface of the Ag_2S quantum dot may be completely or substantially covered in polar functional groups, such as for example carboxyl, hydroxyl, thiol or amino functional groups. Functionalisation to form a hydrophilic surface on the Ag_2S quantum dot usually occurs in a polar solvent. The resulting hydrophilic Ag_2S quantum dots are then usually stable, do not aggregate together and can be associated with one or more polymers, optionally including a therapeutic agent and/or a targeting molecule, to form a composition suitable for therapy.

[0124] In one embodiment, the quantum dot consists of or comprises Ag_2S comprising hydrophilic surface ligands. The hydrophilic surface ligands comprise polar functional groups. Preferably, the polar functional groups are selected from the group consisting of: amine, carboxyl, or hydroxyl, more preferably the polar functional group is a carboxyl.

[0125] In one embodiment, the quantum dot consists of or comprises Ag_2S comprising hydrophilic surface ligands, wherein the hydrophilic surface ligand comprises:

– a first functional group coordinated to the quantum dot surface, preferably wherein the first functional group is selected from the group consisting of: thiol, amine, carboxyl, or hydroxyl, more preferably wherein the first functional group is a thiol; and

– a second functional group capable of forming a chemical linkage to other inorganic, organic or biological material, such as a therapeutic agent, an endosomal disruptive polymer, or a combination thereof, preferably wherein the second functional group is selected from the group consisting of: amine, carboxyl, or hydroxyl, more preferably wherein the second functional group is a carboxyl. The second functional group of the

hydrophilic surface ligand is also referred to herein as a polar functional group of the hydrophilic surface ligand.

Carbon quantum dots

[0126] In one embodiment, the quantum dots of the present invention may consist of or comprise carbon. Carbon quantum dots (CQD) are well known in the field of nanoparticles. They have been shown to have low or no toxicity to mammals and may also fluoresce in the visible region of the electromagnetic spectrum. Commonly, they are prepared by using a single-step pyrolysis using citric acid and branched polyethyleneimine (PEI) which results in carbon quantum dots that have a hydrophilic coating, however the carbon quantum dots of the present invention may be made by any suitable method. The surface of the carbon quantum dot may be completely or substantially covered in polar functional groups. The surface of the carbon quantum dot may be functionalised to introduce additional functional groups such as for example hydroxyl, thiol or amino functional groups. Functionalisation usually occurs in a polar solvent. The resulting hydrophilic carbon quantum dots are usually stable, do not aggregate together and can be associated with one or more polymers, optionally including a therapeutic agent and/or a targeting molecule, to form a composition suitable for therapy.

[0127] In one embodiment, the quantum dot consists of or comprises carbon quantum dots comprising hydrophilic surface ligands. The hydrophilic surface ligands comprise polar functional groups. Preferably, the polar functional groups are selected from the group consisting of: amine, carboxyl, or hydroxyl, more preferably the polar functional group is a carboxyl.

[0128] In one embodiment, the quantum dot consists of or comprises carbon quantum dots comprising hydrophilic surface ligands, wherein the hydrophilic surface ligand comprises:

– a first functional group coordinated to the quantum dot surface, preferably wherein the first functional group is selected from the group consisting of: thiol, amine, carboxyl, or hydroxyl, more preferably wherein the first functional group is a carboxyl; and

– a second functional group capable of forming a chemical linkage to other inorganic, organic or biological material, such as a therapeutic agent, an endosomal disruptive

polymer, or a combination thereof, preferably wherein the second functional group is selected from the group consisting of: amine, carboxyl, or hydroxyl, more preferably wherein the second functional group is a carboxyl. The second functional group of the hydrophilic surface ligand is also referred to herein as a polar functional group of the hydrophilic surface ligand.

Quantum dot – polymer composition

[0129] The quantum dot compositions used in the present invention comprise a polymer shell. The inventors have surprisingly found that quantum dots encapsulated in a polymer shell, wherein the polymer shell comprises (i) an endosomal disruptive polymer and (ii) a biopolymer suitable for oral administration, can enter the circulatory system of the subject after oral administration and are therapeutically effective. In an exemplary embodiment, the inventors have shown that the quantum dot – polymer composition may delay and inhibit development of diabetes. The quantum dot – polymer composition may optionally comprise a therapeutic agent, a targeting molecule, or a combination thereof.

[0130] In one embodiment, there is provided a quantum dot – polymer composition comprising:

- a quantum dot, and
- a polymer shell

wherein the polymer shell comprises a first polymer layer comprising an endosomal disruptive polymer, and a second polymer layer comprising a biopolymer, wherein the biopolymer is suitable for oral administration.

[0131] The polymer shell may at least partially coat the quantum dot, or it may substantially coat the quantum dot. Preferably, the polymer shell substantially coats the quantum dot.

[0132] In a preferred embodiment, the biopolymer may be conjugated to the endosomal disruptive polymer.

[0133] In a preferred embodiment, the endosomal disruptive polymer may be conjugated to the quantum dot. Preferably, the endosomal disruptive polymer may be

conjugated to a surface of the quantum dot, a surface ligand of the quantum dot, or a combination thereof. Preferably the endosomal disruptive polymer may be conjugated to a surface ligand of the quantum dot.

[0134] The endosomal disruptive polymer is a cationic polymer comprising:

- a first complementary reactive functional group and a second complementary reactive functional group, wherein:
    - the first complementary reactive functional group is capable of forming a bond with: the polar functional group of the surface ligand, a reactive functional group of a therapeutic agent, or a combination thereof; and
    - the second complementary reactive functional group is capable of forming a bond with a reactive functional group of the biopolymer.

[0135] In a preferred embodiment, the first and second complementary reactive functional groups of the endosomal disruptive polymer are independently capable of forming an amide, an imine, a hemiaminate, or a combination thereof. Preferably, the first and second complementary reactive functional groups of the endosomal disruptive polymer are independently an amine, preferably a primary amine or a secondary amine.

[0136] The first complementary reactive functional group and the second complementary reactive functional group of the endosomal disruptive polymer may be the same or different, preferably the same.

[0137] In one embodiment, the endosomal disruptive polymer may be selected from the group consisting of: polyethylene imine; poly(arginine); poly(lysine); poly(histidine); poly-[2-{(2-aminoethyl)amino}-ethyl-aspartamide] (pAsp(DET)); a block co-polymer of poly(ethylene glycol) (PEG) and poly(arginine); a block co-polymer of PEG and poly(lysine); a block co-polymer of PEG and poly{N—[N-(2-aminoethyl)-2-aminoethyl]aspartamide} (PEG-pAsp(DET)); Poly-l-lysine (PLL); DET functionalised polyphenol (-)-epi-gallocatechin gallate (EGCG); Poly(amidoamine) dendrimers; amine functionalised dextran; Poly(histidine-arginine) 6-modified chitosan; PEG-PCL-PEI; a polymer comprising a backbone including poly-aspartic acid, glutamic acid, or a combination thereof, functionalised with diamine, triamine, lysine, histidine, arginine, or a combination thereof; and combinations thereof.

[0138] A person skilled in the art will understand how to identify an endosomal disruptive polymer that includes complementary reactive functional groups capable of forming an amide, an imine, a hemiaminate, or a combination thereof, such as an amine group. Further a skilled person will understand how to functionalise a polymer to include complementary reactive functional groups to form an endosomal disruptive polymer suitable for the invention described herein. For example, dextran may be functionalised to include an amine group using pyridinium p-toulenesulfonate (PPTS), which also functions as a catalyst for the polymerisation of dextran.

[0139] In a particularly preferred embodiment, the biopolymer may protect the quantum dot – polymer composition from the acid environment and digestive enzymes of the gastrointestinal tract. For example, biopolymers such as chitosan or galactose or glucose, or a combination of these, may result in the protection of the quantum dot – polymer composition from gastrointestinal pH and digestive enzymes.

[0140] The biopolymer comprises a first reactive functional group capable of forming a bond with the second complementary reactive functional group of the endosomal disruptive polymer. The bond may be selected from: covalent, hydrogen, and electrostatic interaction. Preferably, the bond is a covalent bond. Preferably, the first reactive functional group of the biopolymer is capable of forming an imine, a hemiaminate, or a combination thereof. Preferably, the first reactive functional group of the biopolymer is an acetyl.

[0141] The biopolymer may comprise a second reactive functional group capable of forming a bond with a reactive functional group of a target molecule. The biopolymer may be functionalised to provide the second reactive functional group. Preferably, the biopolymer is functionalised after the quantum dot – polymer shell composition has been formed. The second reactive functional group may be capable of forming an amide or ester. Preferably the second reactive functional group of the biopolymer is a carboxyl or an amine, preferably a carboxyl.

[0142] The biopolymer may be selected from the group consisting of: Poly(N-isopropylacrylamide) (PNIPAM); Polyacrylamide (PAM); Poly(acrylic acid); Polymethacrylate and other acrylic polymers; hyaluronic acid; heparin; chondroitin sulfate; chitosan; polyglutamate; poly-lysine; poly-histidine; poly (glutamic acid); poly-

aspartic acid; N-acetylgalactosamine; glucose; sucrose; maltose; fructose; galactose; gelatin; and combinations thereof.

[0143] In a preferred embodiment, the biopolymer comprises a block co-polymer of chitosan and glucose (CS/GS). Preferably, the CS/GS is formed from deacetylated chitosan, preferably about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85% deacetylated chitosan, most preferably about 75% deacetylated chitosan. Preferably, the CS/GS is formed from deacetylated chitosan, preferably no more than about 50%, no more than about 55%, no more than about 60%, no more than about 65%, no more than about 70%, no more than about 75%, no more than about 80%, no more than about 85% deacetylated chitosan, most preferably no more than about 75% deacetylated chitosan. Preferably, the CS/GS comprises about 50%, about 45%, about 40%, about 35%, about 30%, about 25%, about 20%, about 15% acetyl groups. Preferably, the CS/GS comprises at least about 50%, at least about 45%, at least about 40%, at least about 35%, at least about 30%, at least about 25%, at least about 20%, at least about 15% acetyl groups, most preferably at least about 25% acetyl groups.

[0144] These biopolymers, whilst selected for their barrier properties, may also affect the hydrodynamic radius of the composition and hence potentially affect the uptake of these coated quantum dots by the cells of the liver. The surface chemistry of the QDs, either with or without a biopolymer coating, can be analysed via FTIR to determine which groups are present (see Figure 2).

[0145] The endosomal disruptive polymer and the biopolymer may be applied to the quantum dot by any suitable process. By way of example, in one such method, the endosomal disruptive polymer attachment may be performed by the formation of an amide bridge between carboxylic acid groups on the QDs, preferably on the QD surface ligands, and a primary amine group on the endosomal disruptive polymer. For example, this reaction may be performed in the presence of N- hydroxysuccinimide (NHS) or its water-soluble analog (Sulfa-NHS) and 1-Ethyl-3-(3- dimethylaminopropyl)carbodiimide (EDC). This reaction requires changes in pH that can be promoted by the addition of HCl and NaOH solutions. Optionally, this method may also use additional linker(s), such as adipic acid dihydrazide.

[0146] The biopolymer attachment may be performed by the formation of an imine between a primary amine group on the endosomal disruptive polymer and an acetyl group on the biopolymer. Preferably, the biopolymer attachment is performed at a pH of less than about 6.0, less than about 5.9, 5.8, 5.7, 5.6, 5.5, 5.4, 5.3, 5.2, 5.1, 5.0, 4.9, 4.8, 4.7, 4.6, 4.5, 4.4, 4.3, 4.2, 4.1, 4.0, 3.9, 3.8, 3.7, 3.6, 3.5, 3.4, 3.3, 3.2, 3.1, or 3.0. Preferably the biopolymer attachment is performed at a pH of no more than about 6.0, no more than about 5.9, 5.8, 5.7, 5.6, 5.5, 5.4, 5.3, 5.2, 5.1, or 5.0. Preferably, the biopolymer attachment is performed at a pH of about 3.0 to about 6.0, including any value or range therein, including 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, and 6.0.

[0147] Following formation of the quantum dot – polymer composition, the biopolymer may be functionalised to provide the second reactive functional group of the biopolymer. For example, free or uncoupled acetyl groups may be oxidised to provide carboxyl functional groups. The second functional group may be used as a chemical linkage to other inorganic, organic or biological material, preferably a target molecule.

[0148] The thickness of the polymer shell, not including interactions at its surface with water molecules, may be between about 5 nm and about 50 nm including any value or range therein, such as between about 5 nm and 30 nm, about 10 nm and 30 nm, about 20 nm and 30 nm, about 20 nm, 21 nm, 22 nm, 23 nm, 24 nm, 25 nm, 26 nm, 27 nm, 28 nm, 29 nm, and 30 nm.

[0149] The resulting hydrodynamic diameter of the quantum dot – polymer composition, may be between about 50 nm and about 400 nm, such as between about 100 nm and 300 nm, or between 100 nm and 250 nm, 100 nm, 110 nm, 120 nm, 130 nm, 140 nm, 150 nm, 160 nm, 170 nm, 180 nm, 190 nm, 200 nm, 210 nm, 220 nm, 230 nm, 240 nm, and 250 nm. Preferably, the size distribution of the quantum dot – polymer composition hydrodynamic diameter is about +/- 20%, +/- 15%, +/- 10%, or +/- 5%.

Target molecule

[0150] The quantum dot – polymer composition may further comprise a target molecule. As used herein, the term “target molecule” refers to a molecule, or a fragment of a molecule, that has the ability to target a particular tissue. Preferably, the target molecule is a biological molecule.

[0151] In a preferred embodiment, the target molecule is conjugated to the biopolymer.

[0152] The target molecule comprises a functional group (such as an amine group of an amino acid residue) that is capable of reacting with the second functional reactive group on the biopolymer to form a covalent link between the target molecule and the quantum dot – polymer composition via the biopolymer.

[0153] In one embodiment, the target molecule may be a peptide (eg a targeting peptide that is used to target a specific cell type, for example hepatocytes). In another embodiment, the target molecule may be a polypeptide such as a protein (eg a transport protein such as transferrin), or an albumin (eg serum albumin). In another embodiment, the target molecule may be an antibody. The target molecule may be selected from the group consisting of: a peptide, polypeptide, antibody, lipid, hyaluronic acid, and combinations thereof. Preferably, the target molecule is a polypeptide. More preferably, the target molecule is cross-linked serum albumin. Cross-linked serum albumin may include, but is not limited to, acetylated serum albumin and formaldehyde treated serum album (FSA). In a particularly preferred embodiment, the target molecule is FSA. The serum albumin may be derived from any suitable source, including but not limited to: bovine, human, and recombinant.

[0154] In a particularly preferred embodiment, the present invention provides a quantum dot – polymer – target molecule composition comprising:

- a quantum dot,
- a polymer shell, and
- a target molecule,

wherein the polymer shell comprises a first polymer layer comprising an endosomal disruptive polymer, and a second polymer layer comprising a biopolymer, wherein the biopolymer is suitable for oral administration,

wherein the endosomal disruptive polymer is conjugated to the quantum dot and the biopolymer,

wherein the biopolymer is further conjugated to the target molecule.

Therapeutic agent

[0155] The quantum dot – polymer composition, optionally including a target molecule, may further comprise a therapeutic agent.

[0156] The therapeutic agent may be any suitable therapeutic agent. The therapeutic agent may be a small molecule drug or a macromolecular drug. In some embodiments, the therapeutic agent may comprise a small molecule drug. The small molecule drug(s) may be any suitable organic compound having a low molecular weight (less than about 900 daltons) and that can regulate a biological process to treat a particular disease or condition. In some embodiments, the therapeutic agent may comprise a macromolecular drug. Macromolecular drugs useful in the invention include large molecules (molecular weight more than about 900 daltons) such as proteins, polysaccharides and nucleic acids and that can regulate a biological process to treat a particular disease or condition.

[0157] In a preferred embodiment, the therapeutic agent is conjugated to the quantum dot, the endosomal disruptive ligand, or a combination thereof. Wherein the therapeutic agent is conjugated to the quantum dot, preferably the therapeutic agent may be conjugated to a surface of the quantum dot, a surface ligand of the quantum dot, or a combination thereof.

[0158] The therapeutic agent comprises a first functional group (such as an amine group of an amino acid residue) that is capable of reacting with the polar functional group of the surface ligand of the quantum dot to form a covalent link between the therapeutic agent and the quantum dot to form a quantum dot – therapeutic agent conjugate.

[0159] The therapeutic agent may comprise a second functional group (such as carboxyl group of an amino acid residue) that is capable of reacting with the first complementary reactive functional group of the endosomal disruptive polymer to form a covalent link between the quantum dot – therapeutic agent conjugate and the endosomal disruptive polymer. Alternatively, or in addition, the endosomal disruptive polymer may form a covalent link with the polar functional group of the surface ligand of the quantum dot.

[0160] In a preferred embodiment, the therapeutic agent may be selected from the group consisting of: protein, peptide, DNA, RNA, microRNA, siRNA, CRISPR-Cas9, and combinations thereof. In a particularly preferred embodiment, the therapeutic agent is a protein or fragment thereof, siRNA, or CRISPR-Cas9.

[0161] In a particularly preferred embodiment, the therapeutic agent may be insulin or a fragment thereof.

[0162] In another particularly preferred embodiment, the therapeutic agent may be a peanut allergen or a fragment thereof. In one embodiment, the peanut allergen may be Ara h 1 or a fragment thereof, Ara h 3 or a fragment thereof, or combinations thereof. Preferably, the peanut allergen is a combination of Ara h 1 and Ara h 3.

[0163] In a particularly preferred embodiment, the present invention provides a quantum dot – therapeutic agent – polymer – target molecule composition comprising:

- a quantum dot,
- a therapeutic agent,
- a polymer shell, and
- a target molecule,

wherein the polymer shell comprises a first polymer layer comprising an endosomal disruptive polymer, and a second polymer layer comprising a biopolymer, wherein the biopolymer is suitable for oral administration,

wherein the therapeutic agent is conjugated to the quantum dot, the endosomal disruptive polymer, or a combination thereof, preferably a combination thereof;

wherein the endosomal disruptive polymer is conjugated to the quantum dot, the therapeutic agent, the biopolymer, or a combination thereof, preferably at least to the therapeutic agent and the biopolymer,

wherein the biopolymer is conjugated to the endosomal disruptive polymer and the target molecule.

[0164] In a preferred embodiment, the therapeutic agent is conjugated to the quantum dot and the endosomal disruptive polymer. The endosomal disruptive polymer is further conjugated to the quantum dot, the biopolymer, or a combination thereof, preferably the endosomal disruptive polymer is further conjugated at least to the biopolymer. The biopolymer is further conjugated to the target molecule.

[0165] In the above description and examples provided below, insulin, peanut allergen, siRNA and Cas9 have been used to demonstrate the effectiveness of attachment of a protein-, peptide-, and siRNA based therapeutic with quantum dots of the present invention, in order to provide a dosage form of a protein-, peptide- or siRNA based therapeutic which maintains effectiveness when administered orally to a subject. However, a skilled person would readily expect that, by using the same techniques described herein, other therapeutic agents that are effective therapeutically, but not orally bioavailable, may also be suitable for conjugation with a quantum dot for oral administration. In particular, proteins, peptides and nucleic acids that are effective when delivered directly to the liver, intestine, small bowel, pancreas, kidneys or other organs of the gastrointestinal tract of the subject, yet are not currently orally bioavailable, may also be able to be delivered to a subject in an oral dosage form using the present invention.

[0166] Proteins and peptides suitable for conjugation with the quantum dots described herein may be any therapeutically-active protein or peptide, up to about 200 kilodaltons (kDa) in size, eg, it may be about or up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 50, 60, 70, 80, 90, 10, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 kDa in size. In order to attach the protein or peptide to the QD surface via EDC/NHS, they also require a primary amine group available. Non-limiting examples of proteins and peptides of this size and comprising a primary amine suitable for conjugation with quantum dots for oral administration, as described herein, may be, for example, insulin (e.g., 51 amino acids; about 6 kDa) and analogues or derivatives thereof, growth hormone (e.g., 191 amino acids; about 22 kDa), glucagon-like peptide-1 (GLP-1) agonists such as liraglutide (30 amino acids; about 4 kDa) and exenatide (39 amino acids; about 4 kDa), glucagon-like peptide-2 (GLP-2) agonists such as apraglutide (33 amino acids; about 4 kDa), platelet derived growth factor (PDGF) beta receptor modulators such as BOT191 (Fibroferon) (about 9 kDa), or integrin alpha-4/beta-7 antagonists such as PN-10943, vasopressin,

interleukins (less than 30 kDa in size), enkephalins, endorphins and the like, Cas 9 (about 160 kDa), peanut allergen such as Ara h 1, Ara h 3 or a combination thereof (about 60 kDa to about 120 kDa). These therapeutically effective proteins and peptides above may be used in the prevention and/or treatment of type I or type II diabetes (insulin and GLP-1 agonists); obesity (liraglutide); growth hormone deficiency, particularly in subjects in old age (growth hormone); diabetic nephropathy, liver fibrosis, NASH or renal fibrosis (BOT191); hypersensitivities such as peanut allergy; celiac disease and other gastrointestinal diseases (apraglutide); or inflammatory bowel disease or ulcerative colitis (PN-10943). The above peptides and proteins are only examples of current therapeutics that are not currently orally bioavailable but would be advantageous to a subject to be able to self-administer orally.

Insulin

[0167] Insulin is a peptide hormone produced by and secreted from the beta, or islet of Langerhans, cells found in the pancreas of a healthy subject. In a healthy subject, insulin secretion usually occurs in response to a high blood glucose level in a subject, acting to cause the cells of the subject to take up glucose out of the blood system, with excess glucose being either polymerised and stored as glycogen in the liver and muscles, or converted into fatty acids for storage in the adipose tissues as fat.

[0168] Structurally, insulin is a dimer of two chains linked via disulfide bonds, the dimer referred to herein is an insulin molecule. As would be understood by the skilled person, the resulting quaternary structure is important for the activity of insulin to bind to the insulin receptors embedded in the membranes of cells, with any disruption of the disulfide bonds or cleavage of the peptide chains likely to result in little to no activity. As insulin administration is currently the only therapy available to prevent and treat type I diabetes and is also a major treatment option for people with type II diabetes mellitus, the administration of insulin in a manner that maintains the integrity of the quaternary structure of the insulin molecule is important.

[0169] Commonly, the insulin that is currently used in the treatment of humans is human insulin that is produced by use of recombinant DNA technologies. Insulin from other species, such as from pigs, has also been used previously to treat humans. However, the amino acid sequence and resulting structure of insulin differs slightly between species. For example, porcine-origin insulin differs by one amino acid residue

compared to the human-origin sequence, and bovine-origin insulin differs by three amino acid residues compared to the human-origin sequence. These slight differences mean that the use of insulin harvested from another species is unlikely to be as effective as insulin produced by cells of the same species. However, cross-species administration can still be effective in therapy. For instance, before recombinant DNA techniques were available, insulin was regularly harvested from pigs for use in human therapy, due to the closeness of the two amino acid sequences. Insulin derivatives are also available with varying lengths of activity. For instance, insulin is available as a long-acting analogue (such as glargine insulin or detemir insulin), an intermediate-acting analogue (such as isophane insulin or Neutral Protamine Hagedorn insulin), or a fast-acting analogue (such as insulin aspart, insulin lispro or insulin glulisine).

[0170] Accordingly, the present invention is not limited to the use of an insulin molecule of any particular amino acid sequence or species of origin. By ''species of origin'', it is meant that the insulin used herein has the same sequence as that produced naturally by healthy members of that origin species. The ''species of origin'' does not need to be the same as the subject species.

[0171] The insulin may be of a sequence that is the same or similar to the native sequence of the subject species, or it may be a different species with a quaternary protein structure that is effective in treatment of the subject. The amino acid sequence is preferably native (ie as produced by the cells of the subject species), but it may also be chemically modified, so long as any such modification does not significantly affect the efficacy of the insulin in the subject after administration.

[0172] The insulin used in the present invention may be produced by any suitable method. For example, it may be made, collected and purified from use of recombinant DNA technology or it may be harvested from an animal or a cultured collection of mammal cells that express insulin or it may be made by chemical synthesis. It may be produced as a salt. It may be used herein as a pharmaceutically acceptable salt of insulin. It may be a long-acting, intermediate-acting, or short-acting insulin derivative.

Peanut allergens

[0173] Ara h 1 is a major peanut allergen that belongs to the vicilin family of seed storage proteins. It is a trimeric protein with a molecular weight of around 63 kDa. Ara h

1 is highly abundant in peanuts, comprising up to 12-16% of the total soluble protein content. The protein is resistant to digestion by proteases, which contributes to its allergenicity, as it can remain intact and available for recognition by the immune system.

[0174] Ara h 3 is another major peanut allergen that belongs to the legumin family of seed storage proteins. It is a hexameric protein with a molecular weight of around 60 kDa. Ara h 3 is also highly abundant in peanuts, making up 12-16% of the total soluble protein. Like Ara h 1, Ara h 3 is resistant to proteolytic digestion, which enhances its ability to sensitise and elicit allergic responses in susceptible individuals.

[0175] Both Ara h 1 and Ara h 3 contain multiple linear and conformational epitopes that are recognised by IgE antibodies from peanut-allergic individuals. The presence of these allergenic proteins in peanuts is a major concern for people with peanut allergies, as even small amounts can trigger potentially life-threatening reactions.

[0176] Administration of Ara h 1 and/or Ara h 3 may be useful to treat and/or prevent a condition in a subject, wherein the condition is characterised by an aberrant, unwanted or otherwise inappropriate immune response to Ara h 1 and/or Ara h 3. The condition may be a hypersensitivity to peanuts or tree nuts which contain Ara h 1 and/or Ara h 3. The tree nuts may be hazelnuts, almonds, or Brazil nuts.

[0177] The method may desensitise or induce immunological tolerance to Ara h 1 and/or Ara h 3.

[0178] The Ara h 1 and/or Ara h 3 may be derived from peanuts or tree nuts, or produced by use of recombinant DNA technologies. It may be produced as a salt. It may be used herein as a pharmaceutically acceptable salt of Ara h 1 and/or Ara h 3.

Methods of preparation

[0179] The present invention also provides methods for preparing quantum dot – polymer compositions as described herein.

[0180] In one embodiment, the method comprises the steps of:

– contacting the quantum dot with the endosomal disruptive polymer to form a quantum dot – endosomal disruptive polymer composition.

– contacting the quantum dot – endosomal disruptive polymer composition with the biopolymer to form the composition.

[0181] In one embodiment, the method comprises the steps of:

- conjugating a quantum dot comprising hydrophilic surface ligands to an endosomal disruptive polymer to provide a quantum dot – endosomal disruptive polymer conjugate;

- conjugating the quantum dot – endosomal disruptive polymer to a biopolymer to provide a quantum dot – polymer composition.

[0182] The endosomal disruptive polymer may conjugate to a polar functional group of the hydrophilic surface ligand via a first complementary reactive functional group. The polar functional group of the hydrophilic surface ligand may be selected from the group consisting of: amine, carboxyl, or hydroxyl, more preferably carboxyl. The first complementary reactive functional group of the endosomal disruptive polymer may be an amine. Preferably the endosomal disruptive polymer is conjugated to the hydrophilic surface ligand by an amide. This reaction may be performed in the presence of N-hydroxysuccinimide (NHS) or its water-soluble analog (Sulfa-NHS) and 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC). This reaction requires changes in pH that can be promoted by the addition of HCl and NaOH solutions. Optionally, this method may also use additional linker(s), such as adipic acid dihydrazide.

[0183] The biopolymer may conjugate to a second complementary reactive functional group of the endosomal disruptive polymer via a first reactive functional group of the biopolymer. The second complementary reactive functional group of the endosomal disruptive polymer may be an amine. The first reactive functional group of the biopolymer may be an acetyl. Preferably the biopolymer is conjugated to the endosomal disruptive polymer by an imine, a hemiaminate, or a combination thereof. This reaction may be performed at a pH of less than about 6.0, less than about 5.9, 5.8, 5.7, 5.6, 5.5, 5.4, 5.3, 5.2, 5.1, 5.0, 4.9, 4.8, 4.7, 4.6, 4.5, 4.4, 4.3, 4.2, 4.1, 4.0, 3.9, 3.8, 3.7, 3.6, 3.5, 3.4, 3.3, 3.2, 3.1, or 3.0. Preferably the reaction is performed at a pH of no more than about 6.0, no more than about 5.9, 5.8, 5.7, 5.6, 5.5, 5.4, 5.3, 5.2, 5.1, or 5.0. Preferably, the reaction is performed at a pH of about 3.0 to about 6.0, including any

value or range therein, including 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, and 6.0.

[0184] In one embodiment, the method further comprises the step of:

- functionalising the biopolymer to provide a second reactive functional group; and
- conjugating a target molecule to the biopolymer via the second reactive functional group of the biopolymer to provide a quantum dot – polymer – target molecule composition.

[0185] In a preferred embodiment, the second reactive functional group of the biopolymer is formed by functionalising the first reactive functional group of the biopolymer. In a particularly preferred embodiment, the first reactive functional group is an acetyl that is functionalised via oxidation to provide a carboxyl as the second reactive functional group.

[0186] The target molecule may conjugate to the second reactive functional group of the biopolymer via a first reactive functional group of the target molecule that is complementary to the second reactive functional group of the biopolymer. Preferably, the second reactive functional group of the biopolymer is a carboxyl. Preferably, the first reactive functional group of the target molecule is an amine. Preferably, the target molecule is conjugated to the biopolymer by an amide or ester. This reaction may be performed in the presence of N- hydroxysuccinimide (NHS) or its water-soluble analog (Sulfa-NHS) and 1-Ethyl-3-(3- dimethylaminopropyl)carbodiimide (EDC). This reaction requires changes in pH that can be promoted by the addition of HCl and NaOH solutions. Optionally, this method may also use additional linker(s), such as adipic acid dihydrazide.

[0187] In another embodiment, there is provided a method comprising the steps of:

- conjugating a quantum dot comprising hydrophilic surface ligands to a therapeutic agent to provide a quantum dot – therapeutic agent conjugate;
- conjugating an endosomal disruptive polymer to the quantum dot – therapeutic agent conjugate to provide a quantum dot – therapeutic agent – endosomal disruptive polymer conjugate;

- conjugating the quantum dot – therapeutic agent – endosomal disruptive polymer to a biopolymer to provide a quantum dot – therapeutic agent – polymer composition.

[0188] The therapeutic agent may conjugate to the polar functional group of the hydrophilic surface ligand via a first functional group. The polar functional group of the hydrophilic surface ligand may be selected from the group consisting of: amine, carboxyl, or hydroxyl, more preferably carboxyl. The first functional group of the therapeutic agent may be an amine or carboxyl, preferably amine. Preferably the therapeutic agent is conjugated to the hydrophilic surface ligand by an amide or ester. This reaction may be performed in the presence of N- hydroxysuccinimide (NHS) or its water-soluble analog (Sulfa-NHS) and 1-Ethyl-3-(3- dimethylaminopropyl)carbodiimide (EDC). This reaction requires changes in pH that can be promoted by the addition of HCl and NaOH solutions. Optionally, this method may also use additional linker(s), such as adipic acid dihydrazide.

[0189] The endosomal disruptive polymer may conjugate to the polar functional group of the hydrophilic surface ligand, a second reactive functional group of the therapeutic agent, or a combination thereof, via a first complementary reactive functional group.

[0190] In one embodiment, the method further comprises the step of:

- functionalising the biopolymer to provide a second reactive functional group; and

- conjugating a target molecule to the biopolymer via the second reactive functional group of the biopolymer to provide a quantum dot – therapeutic agent – polymer – target molecule composition.

[0191] The solvent that the coupling reaction takes place in may be polar or it may be non- polar, depending on the chemistry of the quantum dot surface and reagents involved. The solvent may be water. The solvent may be a protic polar organic solvent, such as methanol, ethanol, butanol, or propanol. The solvent may be an aprotic polar organic solvent, such as acetone, acetonitrile or N,N-dimethylformamide (DMF).

[0192] When the solvent is water, salts may be added or be present so as to approximate biological osmolarity and maintain the integrity of the therapeutic agent and/or targeting molecule. The conjugation reaction may be carried out in a saline

environment, whereby the saline is water with dissolved salts such as NaCl and KCl present. The saline environment may estimate plasma osmolarity of between about 300 and 312 mOsm/L. A buffer may also be added to the water or saline to ensure the pH of the reaction mixture does not cause the denaturation of the therapeutic agent and/or targeting molecule during the conjugation reaction. The buffer may maintain a biological pH of between about pH 6 and about pH 8. The buffer may be, for example, a phosphate buffer, a Tris buffer, a citrate buffer or a glycine buffer, or any other suitable buffer. A buffer is not generally required in an organic solvent phase, as hydronium ions are not usually present, therefore pH is not a factor.

[0193] The temperature that the reaction occurs at may be mild in any step that includes the therapeutic agent and/or targeting molecule as a reagent. The temperature may be between about 1 °C or about 40 °C, or it may be between 1 °C and 20 °C, 10 °C and 30 °C, 5 °C and 35 °C, 20 °C and 40 °C, 15 °C and 25 °C or 25 °C and 35 °C, e.g., it may be at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39 or 40 °C. It may be at a room temperature. The temperature may be consistent throughout the reaction, with variation of between 1 and 5 °C between maximum and minimum temperatures, or it may vary throughout, such as on a heating gradient of a cooling gradient. It would be expected that the minimum temperature was greater than about 1°C, and the maximum temperature is less than about 40 °C, to avoid damage to the therapeutic agent and/or targeting molecule that may be caused by freezing (in a low-osmotic water solvent) or heat-induced denaturation respectively. If the reaction is carried out sequentially, the reaction step that includes only the quantum dots and the coupling agent may occur at a higher temperature, such as between 1 °C and 100 °C, or between 10 °C and 50 °C, 25 °C and 75 °C, 15 °C and 85 °C, 35 °C and 65 °C, 70 °C and 90 °C, or 50 °C and 100 °C, e.g., it may be at 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 °C.

[0194] Each reaction step in the reaction may occur over a time of between about 5 minutes and about 10 hours, or between about 5 minutes and 5 hours, 1 hour and 6 hours, 2 hours and 8 hours, 5 hours and 10 hours, or 30 minutes and 3 hours, e.g., 5 minutes, 10 minutes, 15 minutes, 20 minutes, 25 minutes, 30 minutes, 35 minutes, 40 minutes, 45 minutes, 50 minutes, 55 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 5

hours, 6 hours, 7 hours, 8 hours, 9 hours or 10 hours. It may be left for a long enough time to ensure that the reagents are completely or substantially completely reacted.

Pharmaceutical compositions

[0195] The present invention also provides a pharmaceutical composition comprising the quantum dot – polymer compositions described herein or prepared by the method described herein, and a pharmaceutically acceptable diluent, excipient or carrier.

[0196] The pharmaceutical composition described herein may be administered, or formulated for administration by, any route described herein. As used herein, the term "administered" means administration of a therapeutically effective dose of the compound described herein to the subject. As used herein, the term "formulated for administration" means a therapeutically effective dose of the compound described herein is formulated in such a way that is suitable for the route of administration. In preferred embodiments, the pharmaceutical composition is administered orally, topically, by nasal administration or parenterally, more preferably orally. In other preferred embodiments, the pharmaceutical composition is formulated for oral administration, topical administration, nasal administration or parenteral administration, more preferably for oral administration. Most preferably, the pharmaceutical composition is formulated for oral administration.

[0197] Suitable oral forms include, for example, tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. Within yet other embodiments, compositions provided herein may be formulated as a lyophilisate.

[0198] The various dosage units are each preferably provided as a discrete dosage tablet, capsules, lozenge, dragee, gum, or other type of solid formulation. Capsules may encapsulate a powder, liquid, or gel. The solid formulation may be swallowed, or may be of a suckable or chewable type (either frangible or gum-like). The present invention contemplates dosage unit retaining devices other than blister packs; for example, packages such as bottles, tubes, canisters, packets. The dosage units may further include conventional excipients well-known in pharmaceutical formulation practice, such as binding agents, gellants, fillers, tableting lubricants, disintegrants, surfactants, and colorants; and for suckable or chewable formulations.

[0199] Compositions intended for oral use may further comprise one or more components such as sweetening agents, flavouring agents, colouring agents and/or preserving agents in order to provide appealing and palatable preparations. Tablets contain the active ingredient in admixture with physiologically acceptable excipients that are suitable for the manufacture of tablets. Such excipients include, for example, inert diluents such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate, granulating and disintegrating agents such as corn starch or alginic acid, binding agents such as starch, gelatine or acacia, and lubricating agents such as magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monosterate or glyceryl distearate may be employed.

[0200] Formulations for oral use may also be presented as hard gelatine capsules wherein the active ingredient is mixed with an inert solid diluent such as calcium carbonate, calcium phosphate or kaolin, or as soft gelatine capsules wherein the active ingredient is mixed with water or an oil medium such as peanut oil, liquid paraffin or olive oil.

[0201] Aqueous suspensions contain the active ingredient(s) in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients include suspending agents such as sodium carboxymethylcellulose, methylcellulose, hydropropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia, and dispersing or wetting agents such as naturally-occurring phosphatides (for example, lecithin), condensation products of an alkylene oxide with fatty acids such as polyoxyethylene stearate, condensation products of ethylene oxide with long chain aliphatic alcohols such as heptadecaethyleneoxycetanol, condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol mono-oleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides such as polyethylene sorbitan monooleate. Aqueous suspensions may also comprise one or more preservatives, for example ethyl, or n-propyl p-hydroxybenzoate, one or more colouring agents, one or more flavouring agents, and one or more sweetening agents, such as sucrose or saccharin.

[0202] Oily suspensions may be formulated by suspending the active ingredients in a vegetable oil such as arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent such as beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and/or flavouring agents may be added to provide palatable oral preparations. Such suspensions may be preserved by the addition of an antioxidant such as ascorbic acid.

[0203] Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, such as sweetening, flavouring and colouring agents, may also be present.

[0204] Pharmaceutical compositions may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil such as olive oil or arachis oil, a mineral oil such as liquid paraffin, or a mixture thereof. Suitable emulsifying agents include naturally-occurring gums such as gum acacia or gum tragacanth, naturally-occurring phosphatides such as soy bean lecithin, and esters or partial esters derived from fatty acids and hexitol, anhydrides such as sorbitan monoleate, and condensation products of partial esters derived from fatty acids and hexitol with ethylene oxide such as polyoxyethylene sorbitan monoleate. An emulsion may also comprise one or more sweetening and/or flavouring agents.

[0205] Syrups and elixirs may be formulated with sweetening agents, such as glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also comprise one or more demulcents, preservatives, flavouring agents and/or colouring agents.

[0206] A composition may further include one or more components adapted to improve the stability or effectiveness of the applied formulation, such as stabilizing agents, suspending agents, emulsifying agents, viscosity adjusters, gelling agents, preservatives, antioxidants, skin penetration enhancers, moisturizers and sustained release materials. Examples of such components are described in Martindale – The Extra Pharmacopoeia (Pharmaceutical Press, London 1993) and Martin (ed.), Remington's Pharmaceutical Sciences. Formulations may comprise microcapsules,

such as hydroxymethylcellulose or gelatine-microcapsules, liposomes, albumin microspheres, microemulsions, nanoparticles or nanocapsules.

[0207] Preservatives include, but are not limited to, antimicrobials such as methylparaben, propylparaben, sorbic acid, benzoic acid, and formaldehyde, as well as physical stabilizers and antioxidants such as vitamin E, sodium ascorbate/ascorbic acid and propyl gallate. Suitable moisturizers include, but are not limited to, lactic acid and other hydroxy acids and their salts, glycerine, propylene glycol, and butylene glycol. Suitable emollients include lanolin alcohol, lanolin, lanolin derivatives, cholesterol, petrolatum, isostearyl neopentanoate and mineral oils. Suitable fragrances and colours include, but are not limited to, FD&C Red No. 40 and FD&C Yellow No. 5. Other suitable additional ingredients that may be included in a topical formulation include, but are not limited to, abrasives, absorbents, anticaking agents, antifoaming agents, antistatic agents, astringents (such as witch hazel), alcohol and herbal extracts such as chamomile extract, binders/excipients, buffering agents, chelating agents, film forming agents, conditioning agents, propellants, opacifying agents, pH adjusters and protectants.

[0208] A pharmaceutical composition may be formulated as inhaled formulations, including sprays, mists, or aerosols. For inhalation formulations, the composition or combination provided herein may be delivered via any inhalation methods known to a person skilled in the art. Such inhalation methods and devices include, but are not limited to, metered dose inhalers with propellants such as CFC or HFA or propellants that are physiologically and environmentally acceptable. Other suitable devices are breath operated inhalers, multidose dry powder inhalers and aerosol nebulizers. Aerosol formulations for use in the subject method typically include propellants, surfactants and co-solvents and may be filled into conventional aerosol containers that are closed by a suitable metering valve.

[0209] Inhalant compositions may comprise liquid or powdered compositions containing the active ingredient that are suitable for nebulization and intrabronchial use, or aerosol compositions administered via an aerosol unit dispensing metered doses. Suitable liquid compositions comprise the active ingredient in an aqueous, pharmaceutically acceptable inhalant solvent such as isotonic saline or bacteriostatic water. The solutions are administered by means of a pump or squeeze-actuated nebulized spray dispenser, or by any other conventional means for causing or enabling

the requisite dosage amount of the liquid composition to be inhaled into the patient's lungs. Suitable formulations, wherein the carrier is a liquid, for administration, as for example, a nasal spray or as nasal drops, include aqueous or oily solutions of the active ingredient.

[0210] Pharmaceutical compositions may be formulated as sustained release formulations such as a capsule that creates a slow release of modulator following administration. Such formulations may generally be prepared using well-known technology and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Carriers for use within such formulations are biocompatible, and may also be biodegradable. Preferably, the formulation provides a relatively constant level of modulator release. The amount of modulator contained within a sustained release formulation depends upon, for example, the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

[0211] It will be understood that the specific dose level for any particular patient will depend upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, and rate of excretion, drug combination (i.e. other drugs being used to treat the patient), and the severity of the particular disorder undergoing therapy.

Therapy

[0212] The quantum dot – polymer compositions described herein, ie quantum dot – polymer compositions optionally including a targeting molecule, but without a therapeutic agent (also referred to herein as “nano-BITS”), have been shown to have therapeutic efficacy. Further, the quantum dot – therapeutic agent – polymer compositions, optionally including a targeting molecule, (exemplified by the nano insulin, nano-siRNA, nano-Cas9, and nano-peanut allergen compositions described herein), have been shown to be useful as a drug delivery platform for targeted drug delivery.

[0213] It will be appreciated that the compositions may be suitable for use in preventing and/or treating any disease or condition for which immune therapy is used as a treatment. The application or intended use of the composition may be tailored based on the therapeutic agent selected for use in the composition.

[0214] In one aspect there is provided a method of preventing or treating an auto-immune disease or condition in a subject in need thereof, the method comprising administering to the subject a therapeutic amount of a quantum dot – polymer composition, optionally comprising a therapeutic agent and/or a target molecule, as described herein.

[0215] In one aspect there is provided use of a therapeutic amount of a quantum dot – polymer composition, optionally comprising a therapeutic agent and/or a target molecule, as described herein, for preventing or treating an auto-immune disease or condition in a subject in need thereof.

[0216] In another aspect, there is provided use of a quantum dot – polymer composition, optionally comprising a therapeutic agent and/or a target molecule, as described herein in the preparation of a medicament for preventing or treating an auto-immune disease or condition in a subject in need thereof.

[0217] The auto-immune disease or condition may be selected from, but is not limited to: Parkinson's disease, and Type I-IV hypersensitivities.

[0218] In one embodiment, the auto-immune disease or condition is mediated by or responsive to regulatory T cells, Th1 cells, or a combination thereof. Preferably, a therapeutic effect is achieved by inducing regulatory T cells, Th1 cells, or a combination thereof.

[0219] In one embodiment, the auto-immune disease or condition is not mediated by or responsive to Th2 cells. Preferably, a therapeutic effect is achieved without inducing Th2 cells.

[0220] Preferably, the auto-immune disease or condition is a type I – IV hypersensitivity. Examples of type I hypersensitivity auto-immune diseases or conditions include, but are not limited to: hay fever, asthma, hives, angioedema, food allergies, and posterior uveitis. Examples of type II hypersensitivity auto-immune diseases or conditions include, but are not limited to: autoimmune hemolytic anemia, goodpasture's syndrome, myasthenia gravis, Graves' disease, and pemphigus vulgaris. Examples of type III hypersensitivity auto-immune diseases or conditions include, but are not limited to: systemic lupus erythematosus (SLE), and rheumatoid arthritis. Examples of type IV hypersensitivity auto-immune diseases or conditions include, but

are not limited to: type 1 diabetes mellitus, multiple sclerosis, Hashimoto's thyroiditis, contact dermatitis, Crohn's disease and ulcerative colitis, autoimmune hepatitis, celiac disease, and crescentic glomerulonephritis. Preferably, the Type I-IV hypersensitivity is a Type I or a Type IV hypersensitivity. More preferably, the Type I-IV hypersensitivity is selected from: type I diabetes, peanut allergy and inflammatory bowel disease.

[0221] The method or use may further include a step of assessing a subject's response to treatment to inform whether to continue with treatment, and/or change dosage.

[0222] In one embodiment, there is provided a method of evaluating a subject's response to treatment of an auto-immune disease or condition with a quantum dot – polymer composition, optionally comprising a therapeutic agent and/or a target molecule, as described herein, the method comprising:

- providing a subject who has received or is receiving a quantum dot – polymer composition, optionally comprising a therapeutic agent and/or a target molecule, as described herein;
- measuring or determining the level of regulatory T cells with CD26L expression in the subject;
- determining that the subject is responding to treatment when the level of regulatory T cells with CD26L expression is higher than the level in a reference data set in the form of one or more subjects who have the auto-immune disease or condition and have not received the treatment; or
- determining the subject is not responding to treatment when the level of regulatory T cells with CD26L expression is the same or lower than the level in the reference data set.

[0223] In another embodiment, there is provided a method of evaluating a subject's response to treatment of an auto-immune disease or condition with a quantum dot – polymer composition, optionally comprising a therapeutic agent and/or a target molecule, as described herein, the method comprising:

- providing a subject who has received or is receiving a quantum dot – polymer composition, optionally comprising a therapeutic agent and/or a target molecule, as described herein;

- measuring or determining the level of regulatory T cells with CD26L expression in the subject;

- determining that the subject is responding to treatment when the level of regulatory T cells with CD26L expression is the same or higher than the level in a reference data set in the form of one or more subjects who do not have the auto-immune disease or condition; or

- determining the subject is not responding to treatment when the level of regulatory T cells with CD26L expression is lower than the level in the reference data set.

[0224] In one embodiment, there is provided a method of evaluating a subject's response to treatment of an auto-immune disease or condition with a quantum dot – polymer composition, optionally comprising a therapeutic agent and/or a target molecule, as described herein, the method comprising:

- providing a subject who has received or is receiving a quantum dot – polymer composition, optionally comprising a therapeutic agent and/or a target molecule, as described herein;

- measuring or determining the level of regulatory T cells with CD26L expression in the subject;

- determining that the subject is responding to treatment when the level of regulatory T cells with CD26L expression is the same or higher than the level in a reference data set in the form of one or more subjects who have the auto-immune disease or condition and have received the treatment; or

- determining the subject is not responding to treatment when the level of regulatory T cells with CD26L expression is the lower than the level in the reference data set.

[0225] In any embodiment, if the subject is determined to not be responding to the treatment, the method may further comprise administering an additional dose of the treatment to the subject. This additional dose may be the same as the initial dosage. Alternatively, a higher dosage of the treatment may be administered to the subject.

[0226] In another aspect there is provided a method of treating insufficient endogenous peptide production in a subject in need thereof, the method comprising administering to the subject a therapeutic amount of a quantum dot – therapeutic agent – polymer composition, optionally comprising a target molecule, as described herein.

[0227] In another aspect there is provided use of a therapeutic amount of a quantum dot – therapeutic agent – polymer composition, optionally comprising a target molecule, as described herein, for treating insufficient endogenous peptide production in a subject in need thereof.

[0228] In another aspect, there is provided use of a quantum dot – therapeutic agent – polymer composition, optionally comprising a target molecule, as described herein in the preparation of a medicament for treating insufficient endogenous peptide production in a subject in need thereof.

[0229] In another aspect there is provided a method of treating a subject suffering from a condition, wherein the condition is treatable with administration of a therapeutic exogenous peptide or protein, the method comprising administering to the subject a therapeutic amount of a quantum dot – therapeutic agent – polymer composition, optionally comprising a target molecule, as described herein.

[0230] In another aspect there is provided use of a therapeutic amount of a quantum dot – therapeutic agent – polymer composition, optionally comprising a target molecule, as described herein, for treating a condition treatable with administration of a therapeutic exogenous peptide or protein in a subject in need thereof.

[0231] In another aspect, there is provided use of a quantum dot – therapeutic agent – polymer composition, optionally comprising a target molecule, as described herein in the preparation of a medicament for treating a condition treatable with administration of a therapeutic exogenous peptide or protein in a subject in need thereof.

[0232] In any aspect, the composition may be administered to the subject orally, or formulated for oral administration.

[0233] In another aspect, there is provided a method of delivering a therapeutic agent to an organ or tissue of a subject, the method comprising orally administering a quantum dot – therapeutic agent – polymer composition, optionally comprising a target molecule, as described herein to the subject, wherein the organ or tissue is selected from the liver, pancreas, intestine, small bowel or kidneys.

[0234] In another aspect there is provided use of a therapeutic amount of a quantum dot – therapeutic agent – polymer composition, optionally comprising a target molecule, as described herein, for delivering the therapeutic agent to an organ or tissue of a subject by oral administration, wherein the organ or tissue is selected from the liver, pancreas, intestine, small bowel or kidneys.

[0235] In another aspect, there is provided use of a quantum dot – therapeutic agent – polymer composition, optionally comprising a target molecule, as described herein in the preparation of a medicament for delivering the therapeutic agent to an organ or tissue of a subject, wherein the medicament is formulated for oral administration, and the organ or tissue is selected from the liver, pancreas, intestine, small bowel or kidneys.

[0236] Preferably, the method of delivering a therapeutic agent to an organ or tissue of a subject minimises non-specific uptake of the therapeutic agent. Non-specific uptake of the therapeutic agent refers to indiscriminate absorption or accumulation of a therapeutic agent in tissues or cells that are not the intended target. More preferably, the method minimises non-specific uptake of the therapeutic agent by hepatocytes, immune cells, or a combination thereof. In such embodiments, preferably the therapeutic agent is at least about 60 kDa – at least about 200 kDa, including any value or range therein, including but not limited to at least 60 kDa, 70 kDa, 80 kDa, 90 kDa, 100 kDa, 110 kDa, 120 kDa, 130 kDa, 140 kDa, 150 kDa, 160 kDa, 170 kDa, 180 kDa, 190 kDa, 200 kDa. Therapeutic agents that are less than 60 kDa may be modified to have a size of 60 kDa or more. A skilled person would understand how to modify a therapeutic agent to increase its size, for example by conjugation to polymers or macromolecules, conjugation to larger protein domains.

Diabetes

[0237] The compositions described herein, comprising a quantum dot and insulin, may be used in the prevention and/or treatment of type I or type II diabetes in a subject in need thereof. As described above, insulin is the most effective therapy for insulin-dependent diabetes.

[0238] Type I diabetes is a chronic autoimmune condition characterised by the progressive destruction of insulin-producing beta cells in the pancreas. This destruction leads to the development of three distinct stages of the disease:

Stage I: Asymptomatic individuals at risk of developing diabetes, characterised by lower C-peptide levels and higher blood sugar levels, indicating early beta cell dysfunction;

Stage II: Individuals who are autoantibody-positive, indicating the presence of an autoimmune response, but without significant beta cell mass destruction;

Stage III: Overt type I diabetes, where the majority of beta cell mass has been destroyed, resulting in the need for exogenous insulin administration.

[0239] Once the beta cell mass is significantly destroyed in Stage III, the disease has progressed to a point where therapeutic interventions become less effective.

[0240] The inventors have surprisingly found that the quantum dot – insulin – polymer conjugates described herein induce immune tolerance and minimise further destruction of the remaining beta cell mass.

[0241] The quantum dot – insulin – polymer conjugates described herein are useful to halt the progression of type I diabetes, and to prevent or slow the transition from Stage I to Stage II, and/or Stage II to Stage III.

[0242] The quantum dot – insulin – polymer conjugates described herein may also be useful in treating individuals diagnosed with Stage III, type I diabetes that have received or are receiving islet or pancreas transplantation.

[0243] In one aspect, there is provided a method of preventing or treating type I diabetes in a subject in need thereof, by administering a quantum dot – insulin - polymer conjugate described herein to the subject. The method minimises or slows beta cell mass destruction, induces immunological tolerance to insulin, or a combination thereof.

[0244] The method may induce regulatory T cells, Th1 cells, or a combination thereof. Preferably, the method does not induce Th2 cells.

[0245] Preferably, the method minimises non-specific uptake of insulin by hepatocytes, immune cells, or a combination thereof.

[0246] In contrast, type II diabetes is often associated with insulin resistance and a gradual decline in beta cell function, leading to the need for exogenous insulin administration in the end stages of the disease, a condition known as "insulinitis."

[0247] In another aspect, there is provided a method of treating end-stage type II diabetes (insulinitis) in a subject in need thereof, by administering a quantum dot – insulin - polymer conjugate described herein to the subject.

[0248] Without being bound to theory, it is believed that the quantum dot-insulin – polymer compositions are bound to the cell surface and engulfed by cells of the body of the subject, preferably the liver and more preferably the hepatocytes. Whilst there is a preference for the quantum dot-insulin compositions to target the liver, they may also be found in other organs, such as the small bowel shortly after oral administration, or in the kidney, spleen and pancreas. The quantum dot-insulin compositions specifically target the hepatocytes, thereby reducing systemic hyperinsulinemia and associated adverse effects. It is envisioned that these compositions can be administered to the subject by any suitable means that results in the compositions entering the bloodstream of the subject and contact with cells that metabolise the compositions and release the insulin. For instance, the compositions may be administered by subcutaneous injection, similarly to the common route of administration of insulin known in the art, although oral administration is preferred.

[0249] One particularly advantageous and surprising route of administration for the delivery of these compositions is via oral administration. An oral dosage form of insulin would avoid the problems known with subcutaneous injection of insulin (and other proteins and peptides), such as poor compliance by subjects and a build-up of scar tissue at common injection sites making further injections difficult. A composition in an oral dosage farm would be expected to increase compliance and reduce unwanted side effects related to subcutaneous administration. By ''oral administration'', it is meant that the subject takes the composition orally by swallowing a composition comprising the

conjugates for absorption in the gastrointestinal tract, rather than absorption across oral membranes such as the sublingual mucosa.

[0250] A composition for oral dosing of insulin comprises a quantum dot-insulin – polymer composition, optionally with a target molecule. The composition may also include pharmaceutically acceptable excipients, such as diluents (e.g., lactose, dextrin, silicates, magnesium salts, or calcium salts), binders (e.g., starches, cellulose, cellulose derivatives, or sugar alcohols), disintegrants (e.g., starch, cellulose derivatives or alginates), glidants (e.g., colloidal anhydrous silica and other silica compounds), preservatives (e.g., sodium benzoate, EDTA, sorbic acid or parabens), antioxidants (e.g., BHA, BHT, tocopherol acetate) and lubricants (e.g., steric acid and salts thereof). An oral dosage form may be in any suitable physical form, such as for example a tablet, a capsule, a powder, a suspension or a solution. Compositions of a quantum dot and insulin are capable of providing active insulin to the bloodstream of a subject, following oral administration, without the addition of additional protective excipients. Accordingly, without being bound by theory, it is believed that conjugation of the insulin to the quantum dot disrupts the action of proteases and acids in the digestive tract, reducing degradation of the insulin before absorption, leading to the delivery of active insulin to the liver of the subject. It is envisioned that the same protective effect would be applied to other proteins and peptides conjugated with a quantum dot.

[0251] It is envisioned that a suitable pharmaceutical composition would deliver the composition to the intestinal tract of the subject, from where they are absorbed through the lumen of the small intestine and into the capillaries of the small intestine. Accordingly, the hydrodynamic size of the composition, comprising a quantum dot, insulin, and a polymer shell, and optionally a target molecule, should be of a small enough size to allow rapid absorption of the composition from the intestine and into the intestinal capillaries.

[0252] Oral administration and hence an intestinal route of absorption is further advantageous and superior over other methods of absorption or administration such as injection or transdermal administration, as the blood of the small intestine capillaries proceeds directly to the liver for first-pass metabolism. Advantageously, it is preferred that the compositions are metabolised in the liver, rather than in any other cells or organs of the subject. Insulin release from the liver more closely represents natural

release of insulin in a healthy subject, compared to parenteral administration of an active form of insulin, which is the current preferred therapy.

[0253] Another advantage of oral administration of insulin is that the insulin when conjugated to a quantum dot and coated in a polymer shell, may be further protected from the harsh conditions of the digestive tract by the biopolymer, and/or absorption through the intestinal wall may be improved, depending on the characteristics of the biopolymer.

[0254] Another advantage of oral administration is that the compositions may be restrained from, or limited from, entering the systemic system of the subject.

[0255] In another aspect, there is provided use of a composition comprising a quantum dot and insulin as described herein for the manufacture of a medicament for the prevention and/or treatment of type I and type II diabetes. The medicament may be formulated for oral administration.

[0256] The prevention and/or treatment may include administration of a composition of the present invention, whereby insulin as released into the blood stream of the subject after metabolism of the conjugates by the liver. By ''metabolism'' it is meant that cleavage of the composition, so as to form at least one insulin molecule and a quantum dot, occurs in the cell with the insulin being released into the blood stream and the quantum dot excreted via the biliary system. Administration may be by any suitable route, such as parenteral administration, for example subcutaneous injection, or it may be by oral administration, or it may be by transdermal administration. Preferably, the route of administration is by oral administration. The composition may include pharmaceutically acceptable excipients that are suitable for, and hence dependent on, the route of administration. For example, suitable excipients for oral administration may not be suitable of parenteral administration.

[0257] The dose of insulin provided by the compositions may be the same as, or higher than, the dose of insulin provided by conventional parenteral therapy. For humans, the standard dose of insulin is 1 International Unit (IU), which is defined as 0.0347 mg. The dose of insulin administered to the subject may be between about 0.1 IU/kg and about 100 IU/kg, or it may be between 0.1 IU/kg and 15 IU/kg, 0.5 IU/kg and 20 IU/kg, 1 IU/kg and 10 IU/kg, 5 IU/kg and 25 IU/kg, 15 IU/kg and 30 IU/kg, 20 IU/kg

and 50 IU/kg, 25 IU/kg and 75 IU/kg, 40 IU/kg and 80 IU/kg, 30 IU/kg and 70 IU/kg, 10 IU/kg and 90 IU/kg, 1 IU/kg and 99 IU/kg or 2.5 IU/kg and 12 IU/kg, e.g., about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.5, 2, 2.5,3,4,5,6, 7,8,9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 IU/kg. The dose of oral QD- insulin composition required to achieve a therapeutic effect may be the same or higher than the dose required to achieve a therapeutic effect of subcutaneous insulin (SC-insulin). The ratio of therapeutic dose of SC-insulin to oral QD-insulin may be between about 1:1 and about 1:50, or it may be between about 1:5 and about 1:40, or between about 1:10 and 1:25, or it may be 1:1, 1:2, 1:3, 1:4, 1;5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:11, 1:12, 1:13, 1:14, 1:15, 1:16, 1:17, 1:18, 1:19, 1:20, 1:21, 1:22, 1:23, 1:24, 1:25, 1:26, 1:27, 1:28. 1:29, 1:30, 1:31, 1:32, 1:33, 1:34, 1:35, 1:36, 1:37, 1:38, 1:39, 1:40, 1:41, 1:42, 1:43, 1:44, 1:45, 1:46, 1:47, 1:48,. 1:49 or 1:50.

Peanut allergy

[0258] The compositions described herein, comprising a quantum dot and Ara h 1 and Ara h 3, may be used in the prevention and/or treatment of peanut allergy in a subject in need thereof.

[0259] The quantum dot – Ara h 1-Ara h 3 – polymer conjugates described herein may be useful to treat and/or prevent a condition in a subject, wherein the condition is characterised by an aberrant, unwanted or otherwise inappropriate immune response to Ara h 1 and/or Ara h 3.

[0260] In one aspect, there is provided a method of preventing or treating a condition characterised by an aberrant, unwanted or otherwise inappropriate immune response to Ara h 1 and/or Ara h 3 in a subject in need thereof, by administering a quantum dot – Ara h 1-Ara h 3 - polymer conjugate described herein to the subject.

[0261] The condition may be a hypersensitivity to peanuts or tree nuts which contain Ara h 1 and/or Ara h 3. The tree nuts may be hazelnuts, almonds, or Brazil nuts.

[0262] The method may desensitise or induce immunological tolerance to Ara h 1 and/or Ara h 3.

[0263] The method may induce regulatory T cells, Th1 cells, or a combination thereof. Preferably, the method does not induce Th2 cells.

[0264] Preferably, the method minimises non-specific uptake of Ara h 1 and/or Ara h 3 by hepatocytes, immune cells, or a combination thereof.

[0265] In the above description and examples provided below, insulin, peanut allergen, siRNA and Cas9 have been used to demonstrate the effectiveness of attachment of a protein-, peptide-or siRNA- based therapeutic with quantum dots of the present invention, in order to provide a dosage form of a protein-, peptide- or siRNA-based therapeutic which maintains effectiveness when administered orally to a subject. However, a skilled person would readily expect that, by using the same techniques described herein, other therapeutic agents that are effective therapeutically, but not orally bioavailable, may also be suitable for conjugation with a quantum dot for oral administration. In particular, proteins and peptides that are effective when delivered directly to the liver, small bowel, pancreas, kidneys or other organs of the gastrointestinal tract of the subject, yet are not currently orally bioavailable, may also be able to be delivered to a subject in an oral dosage form using the present invention.

[0266] Proteins and peptides suitable for conjugation with the quantum dots described herein may be any therapeutically-active protein or peptide, up to about 200 kilodaltons (kDa) in size, e.g., it may be about or up to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 30, 50, 60, 70, 80, 90, 10, 110, 120, 130, 140, 150, 160, 170, 180, 190, or 200 kDa in size. In order to attach the protein or peptide to the QD surface via EDC/NHS, they also require a primary amine group available. Non-limiting examples of proteins and peptides of this size and comprising a primary amine suitable for conjugation with quantum dots for oral administration, as described herein, may be, for example, insulin (e.g., 51 amino acids; about 6 kDa) and analogues or derivatives thereof, growth hormone (e.g., 191 amino acids; about 22 kDa), glucagon-like peptide-1 (GLP-1) agonists such as liraglutide (30 amino acids; about 4 kDa) and exenatide (39 amino acids; about 4 kDa), glucagon-like peptide-2 (GLP-2) agonists such as apraglutide (33 amino acids; about 4 kDa), platelet derived growth factor (PDGF) beta receptor modulators such as BOT191 (Fibroferon) (about 9 kDa), or integrin alpha-4/beta-7 antagonists such as PN-10943, vasopressin, interleukins (less than 30 kDa in size), enkephalins, endorphins and the like, Cas 9 (about 160 kDa), peanut allergen such as Ara h 1, Ara h 3 or a combination thereof

(about 60 kDa to about 120 kDa). These therapeutically effective proteins and peptides above may be used in the prevention and/or treatment of type I or type II diabetes (insulin and GLP-1 agonists); obesity (liraglutide); growth hormone deficiency, particularly in subjects in old age (growth hormone); diabetic nephropathy, liver fibrosis, NASH or renal fibrosis (BOT191); celiac disease and other gastrointestinal diseases (apraglutide); or inflammatory bowel disease or ulcerative colitis (PN-10943). The above peptides and proteins are only examples of current therapeutics that are not currently orally bioavailable but would be advantageous to a subject to be able to self-administer orally.

Examples

Materials & methods

Ethics

[0267] The study was approved by the Animal Welfare Committee of the Sydney Local Health District and was performed in accordance with the Australian Code of Practice for the care and use of animals for scientific research (AWC approval: 2018/010, 2019/044, 2022/2024 and 2022/025). All information provided accords with the ARRIVE guidelines.

Materials

[0268] Materials were obtained as follows: Digital Heating Mantle (cat no: DMS631, Adelab Scientific, AUS), silver diethyldithiocarbamate (cat no: D93503, Merck, AUS), 1-dodecanethiol (cat no: 471364 Merck, AUS), cyclohexane (cat no: 227048, Merck, AUS), ethanol (cat no: 02851, Merck, AUS), acetone (cat no: 179124, Merck, AUS), N2 gas (cat no: 032, BOC, AUS), Ar gas (cat no: 062, BOC, AUS), 3-mercaptopropionic acid [3-MPA] (cat no: M5801, Merek, AUS), citric acid (cat no: C0759, Merck, AUS), glycerine (cat no: G2289, Merck, AUS), cytidine (cat no: C6727, Merck, AUS), chitosan (cat no: C3646, Merck, AUS), glucose (cat no: G8270, Merck, AUS), glacial acetic acid (cat no: A6283, Merck, AUS), phosphate buffered saline (cat no: P4417, Merck, AUS), N-(3-dimethylaminopropyl)-N′-ethyl carbodiimide hydrochloride [EDC] (cat no: E6383 Merek, AUS), N-Hydroxysulfosuccinimide sodium salt [NHS] (cat no: 56485, Merek, AUS), SnakeSkin dialysis tubing 10,000 MWCO (cat no: 88243, ThermoFisher Scientific, AUS), bovine serum albumin (cat no: A7906, Merck, AUS), recombinant

human serum albumin (cat no: 205-0005, Sartorus, UK), formaldehyde solution 37% (cat no: F1635, Merck AUS), sodium bicarbonate (cat no: S6014, Merck, AUS), sodium carbonate (anhydrous) (cat no: PHR1948, Merck, AUS), scintillation fluid (Ultima Gold 2x, cat no: 6013329, PerkinElmer, AUS), 30% H_2O_2 (cat no: 18312, Merck, AUS), Solvable solution (cat no: 6NE9100, PerkinElmer, AUS), Liquid scintillation vials glass (cat no: 986541, Merck, AUS), (14C)-insulin human (cat no: ARC 3146, Bio Scientific, AUS), insulin human recombinant (cat no: 91077C, SAFC, AUS), recombinant human GAD65 (cat no: 17802-H09B, Sino Biological, China) Accu-Chek Performa strips (cat no: p-4015630981946, Amcal, AUS), Accu-Chek Performa blood glucose meter kit (cat no: p-4015630982219, Amcal, AUS), Mouse IAA/Insulin Autoantibodies Elisa (cat no: MOFI01452, assay Genie, AUS), RBC Lysis buffer (cat no: 00-4333-57, ThermoFisher, AUS), (14C)-citric acid (cat no: NEC160050UC, Perkin Elmer, AUS), Cas9 Protein (cat no: CAS9PROT, Merck, AUS), miRNA mimic 2.0 std (cat no: 4464066, Thermo Fisher Scientific, AUS), miRNA mimic 2.0 neg (cat no: 4464058, Thermo Fisher Scientific, AUS), miRNA mimic 2.0 mir-1 (cat no: 4464058, Thermo Fisher Scientific, AUS), sil sel pre-dsg siRNA std (cat no: 4390771, Thermo Fisher Scientific, AUS), sil sel gapdh siRNA (cat no: 4390849, Thermo Fisher Scientific, AUS), sil gapdh siRNA (cat no: AM4631, Thermo Fisher Scientific, AUS), sil sel neg control (cat no: 4390844, Thermo Fisher Scientific, AUS), CD98 siRNA (cat no: sc-35034, Santa Cruz Biotechnology Inc., USA), anti-CD4-BV650 (cat no: 563747, BD Biosciences, AUS), anti-CD3e-BV421 (cat no: 562600, BD Biosciences, AUS), anti-CD25-PE (cat no: 12-0251-82, ThermoFisher, AUS), anti-Foxp3-APC (cat no: 17-5773-82, ThermoFisher, AUS), anti-CD16/32 (cat no: 14-0161-85, ThermoFisher, AUS), anti-CD45R/B220-APC (cat no: 103212, BioLegend, AUS), anti-CD8a-PerCP/Cy5.5 (cat no: 100734, BioLegend, AUS), anti-TIM4-BUV395 (cat no: 742779, BD Biosciences, AUS), anti-CD161-BV421 (cat no: 108731, BioLegend, AUS), anti-stephtavin-BV421 (cat no: xxx, xxx, AUS), anti-CD64-PE (cat no: 139303, BioLegend, AUS), anti-CD45-APC (cat no: 157606, BioLegend, AUS), anti-MHC II (I-Ad)-FITC (cat no: 115005, BioLegend, AUS), anti-CD11c-BV605 (cat no: 117333, BioLegend, AUS), anti-CD11b-PerCP-Cy5.5 (cat no: 101227, BioLegend, AUS), anti-MHC I-AF700 (cat no: NB100-65938AF700, Novus Biologicals, AUS), anti-Lyve-1-PE-Cy7 (cat no: 25-0443-80, ThermoFisher Scientific, AUS), MAB monoclonal antibody (MHCII-A-Ig7) (cat no: xxx, Merck, AUS), N,N-Dimethylformamide [DMF] (cat no: 227056, Merck, AUS), diethylenetriamine [DET] (cat no: 51851, Merck, AUS), Dichloromethane (cat no: 270997, Merck, AUS), hexane anhydrous (cat no: 296090,

Merck, AUS), ethyl acetate anhydrous (cat no: 270989, Merck, AUS), Benzene (cat no: 270709, Merck, AUS), β-Benzyl L-Aspartic Acid N-carboxyanhydride [BLA-NCA] (cat no: santsc-503431, Bio-Strategy, AUS), n-Butylamine (cat no: 471305, Merck, AUS), dichloromethane (cat no: 34856, Merck, AUS), Acetic anhydride (cat no: A6404, Merck, AUS), diethyl ether (cat no: 676845, Merck, AUS). sgRNA gapdh primer (accession no. NM_008084) F: 5' TGCACCACCAACTGCTTAGC (SEQ ID No:1), R: 5' GGCATGGACTGTGGTCATGAG (SEQ ID No:2); b-actin primer (accession no. NM_007393) F: 5' CTCTAGACTTCGAGCAGGAGATGG (SEQ ID No:3), R: 5'ATGCCACAGGATTCCATACCCAAG (SEQ ID No:4); cd98 primer F: 5' GAACCAGAAGGATGAAAT (SEQ ID No:5), R: 5' AATGATGTGAATGCTCTT (SEQ ID No:6).

Analytical methods

High voltage transmission electron microscopy

[0269] Visualization of QD, QD-insulin, QD-insulin-Pasp(DET), Ag_2S QD-insulin-Pasp(DET)-CS/GS and Ag_2S QD-insulin-Pasp(DET)-CS/GS-FSA were taken, with either Ag_2S or CQDs cores from non-biological samples was performed using high voltage (120 kV) transmission electron microscopy (HV-TEM). HV-TEM was performed on a JEOL 1400 (JEOL, AUS), at the transmission electron microscope facilities at the Australian Centre for Microscopy and Microanalysis, the University of Sydney, AUS. Non-biological samples were prepared by evaporative deposition on copper-based TEM grids.

Scanning electron microscopy

[0270] Samples were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, osmicated, dehydrated in graded ethanol, and hexamethyl disilazane, mounted on stubs, sputter coated with platinum, and examined using a JEOL 6380 Scanning Electron Microscope (JEOL, Tokyo, Japan). Images at 10,000 magnification were collected by a blinded observer and used to measure fenestration diameter and LSEC porosity using ImageJ [National Institutes of Health (NIH), Bethesda, MD].

ZetaSizer characterization

[0271] Hydrodynamic diameter, PDI and ζ potential were measured using a Zetasizer Nano ZS (Malvern Bioanalytical, AUS), at Sydney Analytical, the University of Sydney, AUS. Measurements were performed using 1 µM QD, QD-insulin, QD-insulin-Pasp(DET), Ag_2S QD-insulin-Pasp(DET)-CS/GS and Ag_2S QD-insulin-Pasp(DET)-CS/GS-FSA in MQ water. Hydrodynamic diameter size and PDI measurements were performed using backscatter (173o) data collection with 3 repeats of 12-15 measurements per sample. All samples were analyzed in disposable folded capillary cells. Z potential was measured with 5 repeats of 10-12 measurements per sample (maximal setting was 100 measurements). All data was collected with triplicate data points.

Fourier transformed infrared microscopy (FTIR)

[0272] FTIR was performed on a LUMOS FTIR microscope (Bruker, USA), at the vibrational spectrometry facilities at Sydney Analytical, the University of Sydney, AUS. Samples were collected from dried QD, QD-insulin, QD-insulin-Pasp(DET), Ag_2S QD-insulin-Pasp(DET)-CS/GS, Ag_2S QD-insulin-Pasp(DET)-CS/GS-FSA, Ag_2S QD-insulin-Pasp(DET)-CS/GS-BSA, Ag_2S QD-insulin-Pasp(DET)-CS/GS-OxBSA, Ag_2S QD-insulin-Pasp(DET)-CS/GS-glucBSA, Ag_2S QD-insulin-Pasp(DET)-CS/GS-galBSA and Ag_2S QD- Pasp(DET)-CS/GS-FSA were taken, with either Ag_2S or CQDs cores. A minimum of 10 measurements per material performed. Data show the average spectrum across 3500-700 nm in ATR mode, following atmosphere correction and normalization performed using OPUS 7.0 software (Bruker, USA). An average spectrum was produced from 10-20 individual measurements per material.

Mice

[0273] 3-4-week-old non-obese-diabetic (NOD) mice were obtained from the Animal Resource Centre in Perth, Western Australia. Animals were housed at the ANZAC Research Institute animal house on a 12-hour light/dark cycle and provided with ad libitum access to food, water and enrichment. Mice were housed under these conditions until 12 months of age.

Non-human primates

[0274] Eight-year-old male Papio hamadryas from the Australian National Baboon Colony (Sydney) were used in this study. Studies were approved by the SLHD AWC: 2024-023 and meet all ethical guidelines required and specified.

[0275] Single ascending dosing study of EA-2 with insulin tolerance testing (ITT) to investigate incidence of adverse hypoglycaemia. To investigate the safety and tolerability of single ascending dosages of EA-2 in male and female NHPs. N=4 (female) N=6 (male), dosages investigated were 36 & 360 µg kg–1 for males 75 & 750 µg kg–1 for females.

[0276] Baseline blood samples were collected by a licensed veterinarian following anaesthesia via intramuscular ketamine (8 mg kg–1). Baseline was collected one week prior to the lowest dose treatment. Baseline bloods were collected at day 0, low dose was given on day 7 via a 2g piece of sugar free chocolate, high dose was given on day 14 via a 2g piece of sugar free chocolate and post-treatment bloods were collected on day 21. Blood samples were prepared and analysed for biochemistry, lipid, urinary and haematology parameters using SLHD labs meeting accreditation for human samples.

[0277] On the day on EA-2 dosing, baboons were fasted overnight (16 h) before being given dosages of nano-insulin between 7-9 am. Nano-insulin was given at t = 0 with blood sampled at t = 15, 30, 45, 60 and 90 mins. The low and high dosages of nano-insulin were given one week apart. The dosages of EA-2 examined were 36 & 360 µg kg–1 for males 75 & 750 µg kg–1 for females. The same animals received a low and a high dose one week apart. Nano-insulin was given via reformulation with sugar-free chocolate squares (2 g).

[0278] 28- day multi dosing study of EA-2 with immunophenotyping to investigate effect on regulatory CD4+ T cells and tolerance dendric cell populations in peripheral blood monoclonal cells. To investigate the safety and tolerability of multiple ascending dosages of nano-insulin in male and female NHPs. Secondary outcomes were to investigate the effect 28 treatment on regulatory CD4+ T cells and tolerance dendric cell populations in peripheral blood monoclonal cells. N=5 per sex, dosages investigated were 360 µg kg–1 for males 750 µg kg–1 for females. Baseline blood samples were collected by a licensed veterinarian following anaesthesia via intramuscular ketamine (8 mg kg–1). Baseline was collected one week prior to dosing with nano-insulin. Nano-insulin (360 µg kg–1 for males 750 µg kg–1 for females) was given once daily from day

1 to day 28. On Day 29 blood samples were collected, and animal received the daily nano-insulin in the afternoon. EA-2 treatment was washed out to day 56 with bloods collected on day 57. Blood samples were prepared and analysed for biochemistry, lipid, urinary and haematology parameters using SLHD labs meeting accreditation for human samples.

Blood glucose testing

[0279] Blood glucose was measured used a handheld Glucometer using Accu-check proforma strips. Blood glucose was recorded weekly.

Blood and tissue collection

[0280] NOD mice tissue collection was performed at weeks 8 or 12. Mice were euthanized by a single intraperitoneal injection of 100 mg/kg ketamine and 10 mg/kg xylazine following all experiments. 200-250 mg of tissue samples was collected from the liver, spleen, kidney, brain, heart, lungs, stomach, pancreas and small intestine (duodenum, ileum, colon) along with 500 µL of plasma blood collected via cardiac puncture. Tissue samples were snap frozen with liquid N_2 or placed in 2.5% glutaraldehyde/ 4% paraformaldehyde.

Biodistribution analysis sample preparation and radiolabelled activity analysis

[0281] Tissue samples were weighed and mixed in a reaction vial with 1 mL Solvable solution and incubated at 60°C for 4 hours to dissolve the tissue. 0.2 mL 30% H_2O_2 was added to samples to reduce the dark colour saturation. Samples were mixed with 10 mL scintillation fluid. Radioactivity was measured using a scintillation counter (Tri-carb 2100 TR, PerkinElmer, AUS). All samples were mixed with 10 mL scintillation fluid (5 measurements per sample). Baseline measurements were collected from control mice that were not treated with radioactivity QDs. Data were collected as disintegrations per minute and used for analysis. All radioactivity data presented in this manuscript used n=3 mice per group, for *in vitro* hepatocyte endocytosis the experiments were performed in triplicate.

Splenocyte isolation

[0282] The spleen was excised and placed in RPMI with 2% FCS 4°C. The spleen was manually burst and passed through a 35 µm cell filter. Cells were centrifuged at

1500 rpm at 4°C for 5 minutes and resuspended in 1 mL RBC lysis buffer and incubated at room temperature for 3 minutes. 9 mL FACS (2 mM EDTA, 1 g/L BSA, in PBS) buffer was then added and cells were centrifuged at 1500 rpm at 4°C for 5 minutes and resuspended in 10 mL FACS buffer.

Non-hepatocyte cell isolations

[0283] The isolation of non-hepatocyte cells (liver sinusoidal endothelial cells, Kupffer cells, dendritic cells and macrophages) has previous been reported in our methods paper and our previous studies (Hunt et al., **2020**, *ACS Nano 14*, 2, 1492–1507). In brief, mice were anesthetized with a mixture of 10 mg/kg xylazine and 100 mg/kg ketamine with liver cannulated via the portal vein and perfused with Krebs buffer solution (0.142 M NaCl, 6.71 mM KCl, 9.63 mM HEPES and 4.6 mM $CaCl_2$) and collagenase at 37 °C. The liver was removed and dissociated by forceps in Krebs buffer solution (without $CaCl_2$) at 4 °C. Cells filtered and collected in Krebs buffer solution (without $CaCl_2$ but with 10 g/L BSA). All centrifugation steps, including the Percoll gradients were performed at 4 °C. Hepatocytes were isolated by 3× 10 minute centrifugations at 50 g. Dead cells were removed by collecting the fraction between a two-step Percoll gradient (40%, 50%). Non-hepatocyte cells were collected from the hepatocyte supernatant and isolated using another two-step Percoll gradient (25%, 45%) and centrifugation at 1350 g at 4 °C. Cells were suspended in FACS buffer followed by cell counting and prepared for flow cytometry.

Flow cytometry

[0284] Following cell isolations splenocytes and non-hepatocytes cell number were determined by a TC20 Automated Cell Counter (Bio-Rad Laboratories, AUS) with 2 x 10^6 cells per sample were transferred to FACS tubes. From a single sample 1 x 10^6 cell were transferred to compensation FACS tubes and a negative control. FACS tubes were centrifuged (1500 RPM, 4 °C, 5 minutes) with the supernatant discarded. Blocking was performed with Fc Block (CD16/32) 1:200 for 10 minutes at 4 °C in the dark. Splenocytes were stained with a T cell panel (CD3e-BV421 [1:50], CD4-BV650 [1:150], Cd8-PerCP/Cy5.5 [1:100] and CD25-PE [1:100]) for 20 minutes at 4 °C in the dark. Non-hepatocyte liver cells were stained with a cell type and MHC panel (MHCI-AF700 [1:25], TIM4-BUV395 [1:50], CD161-BV421 [1:50], biotinylated I-A^{g7} -BV421 [1:50], CD64-PE [1:50], Lyve1-PE/Cy7 [1:50], CD45-APC [1:100], CD11c-BV605 [1:100],

MHCII-FITC [1:150], CD11b-PerCP/Cy5.5 [1:150]) for 20 minutes at 4 °C in the dark. Non-hepatocyte liver cells were then centrifuged (1500 RPM, 4 °C, 5 minutes), washed in FACS buffer, centrifuged and resuspended in 600 µL FACS buffer. Splenocytes were then prepared for FoxP3 staining using a fixation and permeabilization buffer set as per kit instructions. Cells were permeabilization and fixed for 60 minutes at 4 °C in the dark using the fix/ permeabilization buffer, then centrifuged at 400 g for 5 minutes at room temperature. Cells were stained with FoxP3-APC (1:100) for 30 minutes at room temperature in the dark. Cells were centrifuged (400 g, 5 minutes, room temperature) and resuspended in 300 µL FACS buffer.

[0285] To discriminate between live and dead cell events, samples were further stained with the cell viability dyes DAPI (180 µM). Flow cytometry was performed on a BD LSR Fortessa X-20 flow cytometer (BD Biosciences, AUS) with data analysed on FlowJo (v20, FlowJo LLC, ON, USA).

Assays

[0286] Auto Insulin antibody (IAA) concentrations were determined used a Mouse IAA/Insulin Autoantibodies Elisa as per kit instruction. Isolated blood plasma was diluted 1:10 in sample dilution buffer and incubated for 90 minutes at 37 °C in well plates. Wells were washed with wash buffer, incubated with Biotin-labelled antibody working solution for 60 minutes at 37 °C, washed and incubated with HRP-Streptavidin Conjugate for 30 minutes, washed and developed with TMB Substrate. Samples were analysed in triplicate and performed with Elisa standards and controls.

Statistics

[0287] All statistical analysis was performed using one-way-ANOVA with post hoc Bonferroni test (n=5-20 experiments); post hoc methods were applied for comparison between multiple groups (GraphPad Prism 8.4.0, GraphPad Software, Inc., USA). Power calculations were performed as previously described. All data presented as mean ± SD (n=5-20).

Example 1 – Synthesis of Ag_2S and carbon quantum dots

Ag_2S quantum dot (QD) formation

[0288] Ag_2S QD synthesis has previously been reported (Hunt et al. **2020**, *ACS Nano 14*, 2, 1492–1507). Briefly, 256 mg silver diethyldithiocarbamate was mixed with 12 mL 1-dodecanethiol under vigorous magnetic stirring. An N_2 vacuum was created followed by an argon vacuum to remove oxygen from the mixture. The solution was heated to 200 °C at a rate of 12 °C/min and held at this temperature for 1 hour. Following synthesis, EtOH was added (88 mL) to the solution followed by centrifugation at 4000 rpm for 30 minutes in glass vials. Ag_2S QDs were resuspended in minimal cyclohexane (5 mL), washed twice with EtOH with each wash resulted in precipitation of Ag_2S QDs and isolated with centrifugation (4000 rpm for 30 minutes). Aqueous phase transfer was performed with QDs suspended in a 1:1 (v/v) mixture of cyclohexane and acetone under magnetic stirring. 1 mL of 3-mercaptopropionic acid was added per 250 mg of Ag_2S QDs and mixed at room temperature for 1 hour. Following this QDs were mixed with EtOH and centrifuged 3000 rpm for 5 minutes. The pellet was redispersed in minimal MQ (5 mL), washed with EtOH twice and dispersed in MQ. Samples were then filtered using Whatman Grade 4 filter paper. Ag_2S QDs were dried with a Speedvac for storage at 4 °C in the dark.

Carbon QD (CQD) formation

[0289] CQD synthesis was performed by adding 600 mg cytidine or methionine with 7 mL glycerine in a three-neck flask with a magnetic stirrer. An argon vacuum (20 minutes) removed oxygen from the mixture. The solution was then heated to 200 °C at a rate of 12 °C/minute. In a separate glass vial, 600 mg citric acid was dissolved in 1.0 mL glycerine by gentile mixing and heating to 100 °C. The citric acid solution was then hot injected into the three-neck flask and mixed at 200 °C for 30 minutes. The solution was then cooled to room temperature, mixed 1:1 with MQ and filtered with 0.45 µm filter followed by a 0.22 µm filter. The filtered materials were centrifuged at 4000 rpm for 30 minutes in 10 kDa spin columns. The filtered solution was collected and transferred to 3.5 kDa spin columns and centrifuged at 4000 rpm for 30 minutes. The collected sample between 3.5-10 kDa was collected, resuspended and washed twice with MQ by repeat centrifugation. CQDs were dried with a Speedvac for storage at 4 °C in the dark.

Example 2 – Ag_2S or carbon QD conjugation to insulin and/or GAD, siRNA, Cas9, or peanut allergen

Ag_2S QD conjugation to insulin

[0290] For 1 mL of 2.6 mg/mL Ag_2S QD-INS (72 IU/mL). Combine 188.5 µL Ag_2S QD stock (1.0 mg/mL) with 1.862 mg EDC, 2 mg NHS in 275 µL MQ and mix for 1 hr at 4 °C. Prepare 100x Carbonate-Bicarbonate Buffer (1.05 g Sodium bicarbonate (Sigma), 9.27 g Sodium carbonate (anhydrous) (Sigma), 10 µL: MQ). Add 75 µL 100x Carbonate-Bicarbonate Buffer to QD-EDC-NHS mixture and mix for 2 minutes. Prepare 4.36 mg insulin human recombinant in 375 µL MQ with 3 µL 1M HCl until dissolved. Add all dissolved insulin solution (378 µL) to QD-EDC-NHS mixture with or without 250 ng GAD65 (glutamate decarboxylase or glutamic acid decarboxylase 65 kDa) and mix for 4 hours. The solution was then dialysed with snakeskin tubing (10 kDa) in 1000 mL of MQ for 2, 4 and 16 hours at 4 °C. to provide Ag_2S QD-INS. The solution was stored at 4 °C in the dark until use.

CQD conjugation to insulin

[0291] For 1 mL of 2.6 mg/mL carbon QD-INS (72 IU/mL). Combine 8 µL CQD stock (1.0 mg/mL) with 1.862 mg EDC, 2 mg NHS in 275 µL MQ and mix for 1 hr at 4 °C. Prepare 100x Carbonate-Bicarbonate Buffer (1.05 g Sodium bicarbonate (Sigma), 9.27 g Sodium carbonate (anhydrous) (Sigma), 10 µL: MQ). Add 75 µL 100x Carbonate-Bicarbonate Buffer to QD-EDC-NHS mixture and mix for 2 minutes. Prepare 4.36 mg insulin human recombinant in 375 µL MQ with 3 µL 1M HCl until dissolved. Add all dissolved insulin solution (378 µL) to QD-EDC-NHS mixture with or without 250 ng GAD65 and mix for 4 hours. The solution was then dialysed with snakeskin tubing (10 kDa) in 1000 mL of MQ for 2, 4 and 16 hours at 4 °C to provide carbon QD-INS. The solution was stored at 4 °C in the dark until use.

Ag_2S QD conjugation to siRNA

[0292] Combine 5 nmol Ag_2S QDs with 9.59 ng EDC and 10.86 ng NHS in RNAse-free water, gently agitating at 4°C for 1 hour. Adjust the solution to pH 10 by adding carbonate-bicarbonate buffer (0.79 mg sodium bicarbonate and 6.95 mg sodium carbonate per 1 mL RNAse-free water) and adding 1uL buffer per 54.17uL of solution. Next, add 5 nmol siRNA (in RNAse-free water), mixing gently at 4°C for 4 hours. Dialyse using a 10 kDa 15 mL spin column, centrifuging three times at 4000 rpm for 5 minutes each, adding 2mL RNAse-free water prior to centrifugation. Store the remaining 300uL solution at 4°C in the dark until use.

Ag_2S QD conjugation to Cas9

[0293] Combine 130 µL Ag_2S QD (1 mg/mL, 520 pmol) with 99.684 µL EDC (10 mg/mL) and 113 µL NHS (10 mg/mL). Mix gently at 4°C for 1 hour. Adjust the solution to pH 10 by adding 7.36 µL carbonate-bicarbonate buffer (0.79 mg sodium bicarbonate and 6.95 mg sodium carbonate per 1 mL RNAse-free water; add 1 µL buffer per 54.17 µL solution). Add 17.42 µL Cas9 protein (5 mg/mL stock, 100 µg total) and gently mix at 4°C for 4 hours. Dialyse using a 10 kDa 15mL spin column, centrifuging three times at 4000 rpm for 5 minutes each, adding 2 mL RNAse-free water prior to each centrifugation. Store the remaining ~300 µL solution at 4°C in the dark until use. Add ~700uL to the AgQD-Cas9 stock to make a 1mL solution.

Ag_2S QD conjugation to peanut allergen

[0294] For 1 mL of 130 ug/mL Ag_2S QD-Arah1/Arah3. Combine 9.43 µL Ag_2S QD stock (1.0 mg/mL) with 93.1 ug EDC, 100 ug NHS in 275 µL MQ and mix for 1 hr at 4 °C. Prepare 100x Carbonate-Bicarbonate Buffer (1.05 g Sodium bicarbonate (Sigma), 9.27 g Sodium carbonate (anhydrous) (Sigma), 10 µL: MQ). Add 3.75 µL 100x Carbonate-Bicarbonate Buffer to QD-EDC-NHS mixture and mix for 2 minutes. Prepare 100 ug Arah1 and 100 ug Arah3 in 375 µL MQ and add solution (378 µL) to QD-EDC-NHS mixture and mix for 4 hours. The solution was then dialysed with snakeskin tubing (10 kDa) in 1000 mL of MQ for 2, 4 and 16 hours at 4 °C. to provide Ag2S QD-arah1/arah3. The solution was stored at 4 °C in the dark until use.

Radiolabelling

[0295] Carbon-14: (^{14}C)-insulin was conjugated to QDs via EDC/NHS coupling by adding (^{14}C)-insulin with insulin as described above. Samples were prepared at 1,000,000 DPM/mL. (^{14}C)-Cas9 was conjugated to QDs via EDC/NHS coupling by adding (^{14}C)-Cas9 with Cas9 as described above. Samples were prepared at 1,000,000 DPM/mL.

Example 3 – Synthesis of coated QDs

Pasp(DET) formation

[0296] Pasp(DET) formation has been described previously (Miyata et al., **2008,** *JACS,* 130, 16287-16294). Firstly, the formation of poly(β-benzyl L-aspartate) (PBLA)

was performed by n-butylamine initiated ring-opening polymerization of BLA-NCA followed by acetylation. N-Butylamine (50.0 µL, 0.506 mmol) in dichloromethane (5.0 mL) was added to the BLA-NCA (10.0 g, 40.1 mmol), dissolved in the mixture of DMF (10.0 mL) and dichloromethane (100 mL) under an argon atmosphere, and stirred at 35 °C for 72 hours. Acetic anhydride (167 µL, 1.77 mmol) was added to the reaction mixture, and stirred at 35 °C for 1 hour to acetylate the N-terminus. The solution was poured into diethyl ether to precipitate PBLA. The recovered PBLA was dissolved in dichloromethane (1.25 L). Removal of dichloromethane was performed by evaporation, samples were dried by lyophilization to obtain PBLA. The introduction of amino groups into the PBLA side chain was performed by the aminolysis reaction of PBLA with DET. DET (1.31 mL, 121 mmol) was added to the PBLA (50.0 mg, 24.9 µmol) in DMF (1.0 mL) under an argon atmosphere and stirred at 40 °C for 24 hours. The reaction mixture was slowly poured into 20% v/v acetic acid (10.4 mL) and dialyzed against 0.01 M HCl solution and distilled water. The final solution was lyophilized to obtain Pasp(DET) and diluted to 200 mg/mL, pH 5, and stored at 4 °C in the dark until use.

Chitosan/glucose copolymer (CS/GS)

[0297] Chitosan/glucose copolymer (CS/GS) was produced by combining 5 mg/mL chitosan, with 10% glacial acetic acid in MQ. Under gentle mixing the solution was heated to 50 °C for 1 hour until chitosan dissolved. 2.5 mg/mL glucose was added to the solution and mixed for 1 hour at 50 °C until all powder was dissolved. The solution is allowed to cool to room temperature and then dialysed with snakeskin tubing (10 kDa) in MQ for 2, 4 and 16 hours at room temperature. The solution was diluted to 10 mg/mL, pH 5, and stored at 4 °C in the dark until use.

[0298] To add additional carboxylic acid functional groups to CS/GS to produce CS/GS-COOH the inventors performed an iodine haloform reaction. Excess NaOH (1 mM final volume) and I_2 (1 mM final volume) solution was added to 10 mg/mL CS/GS and mixed for 24 hours at room temperature. Following iodine attachment, the solution was dialysed in 1 mM NaOH with 3.5 kDa filter to remove excess I_2 followed by removal of I groups with the addition of 10 mM NaOH. Repeat dialysis in 10 mM NaOH was performed followed by dialysis in MQ water to remove NaOH. HCl 1 mM was added to shift the pH to 5 and the solution was stored at 4 °C in the dark until use.

Formaldehyde treated bovine and human serum albumin

[0299] Formaldehyde treated serum albumin (FSA) was produced as previously described (Mego & McQueen, **1967,** *J Cell Physiol,* 70, 115– 120). Briefly, BSA or human serum albumin (HAS) (20 mg/ml) was added to 20% formaldehyde with 0.4 M Carbonate-Bicarbonate Buffer, pH 10. The solution was slowly dissolved over 72 hours at room temperature with gentle stirring. FSA was then dialysed against MQ for 2, 4 and 16 hours. The solution was diluted to 10 mg/mL, pH 7, and stored at 4 °C in the dark until use.

Oxidised bovine serum albumin

[0300] Oxidised bovine serum albumin (OxBSA) was produced as previously described (S. Guedes et al., **2009**, *Rapid Commun Mass Spectrom.*, (15):2307-15). Briefly, BSA (20 mg/mL) was added to 0.5 mM hydrogen peroxide (H_2O_2) with 0.01M $NaCO_3$ buffer, pH 10. The solution was mixed at 37 °C with gentle stirring for 24 hours. OxBSA was then dialysed against MQ for 2, 4 and 16 hours. The solution was diluted to 1 mg/mL, pH 7, and stored at 4 °C in the dark until use.

Glycolated bovine serum albumin (glucose)

[0301] Glycolated bovine serum albumin (glucose) (glucBSA) was produced as previously described (Al Ledesma-Osuna, G. Ramons-Clamont, L. Vázquez-Moreno, **2008**, *Acta Biochim Pol.* 55(3):491-7. Epub). Briefly, BSA (20 mg/mL) was added to 1 mM glucose. The solution was mixed at 23 °C with gentle stirring for 24 hours. (glucBSA) was then dialysed against MQ for 2, 4 and 16 hours. The solution was diluted to 1 mg/mL, pH 7, and stored at 4 °C in the dark until use.

Glycolated bovine serum albumin (galactose)

[0302] Glycolated bovine serum albumin (galactose) (galBSA) was produced as previously described (Al Ledesma-Osuna, G. Ramons-Clamont, L. Vázquez-Moreno, **2008**, *Acta Biochim Pol.* 55(3):491-7. Epub). Briefly, BSA (20 mg/mL) was added to 1 mM galactose with 0.01M $NaCO_3$ Buffer, pH 10. The solution was mixed at 50 °C with gentle stirring for 24 hours. (galBSA) was then dialysed against MQ for 2, 4 and 16 hours. The solution was diluted to 1 mg/mL, pH 7, and stored at 4 °C in the dark until use.

(a) Formation of Nano-BITS conjugates (Ag_2S and carbon QD)

QD coating with Pasp(DET) and CS/GS

[0303] Combine 94.24 ul QD stock (1.0 mg/ml) to 653 ul MQ water with 5.0 ul Pasp(DET) polymer (200 mg/ml) and mix for 4 hours at 4 °C. QD stock may be either Ag_2S QD stock or CQD stock solution. Following this add 20 ul CS/GS polymer stock (10 mg/ml) and mix for another 4 hours at 4 °C. Add 25 ul 100x Carbonate-Bicarbonate Buffer and mix for 3 hours at 4 °C, (pH 7).

QD-Pasp(DET)-CS/GS conjugation to FSA

[0304] For FSA attachment, add 3.8 mg EDC and 4 mg NHS and mix for 1 hr at 4 °C. Add 50 ul 100x Carbonate-Bicarbonate Buffer and mix for 2 mins. Add 70 ul FSA stock (10 mg/ml) to solution and mix for 4 hrs. The solution was then dialysed with snakeskin tubing (10 kDa) in 100 mL of MQ for 2, 4 and 16 hours at 4 °C. The solution was stored at 4 °C in the dark until use.

(b) Formation of Nano-insulin conjugates (Ag_2S and carbon QD)

QD-insulin coating with Pasp(DET)

[0305] For 1 mL of 1.3 mg/mL QD- insulin-Pasp(DET) combine the 1 mL QD-insulin with 5.0 µL Pasp(DET) polymer (200 mg/mL). QD-insulin may be either Ag_2S QD-insulin or carbon QD-insulin. Combine slowly and add 2 µL 1 M NaOH to adjust pH to 9 for solubility. Mix for 4 hours at 4 °C, filter with Whatman filter paper 4. Stored at 4 °C in the dark until use. QD-insulin-Pasp(DET) will precipitate in MQ at pH 5, resuspendable with vortexing or centrifuge at 3500 RPM for 15 minutes at 4 °C, remove supernatant and resuspend at desired concentration or freeze dry to form a powder (approx. 1.3 mg final volume).

QD-insulin-Pasp(DET) coating with CS/GS or CS/GS-COOH

[0306] For 1 mL of 1.3 mg/mL QD- insulin-Pasp(DET)-CS/GS combine the 1 mL QD-insulin-Pasp(DET) with 20 µL CS/GS polymer stock (10 mg/mL) add MQ up to 1 mL. Mix for 4 hours at 4 °C, filter with Whatman filter paper 4. Stored at 4 °C in the dark until use. QD-insulin-Pasp(DET)-CS/GS will precipitate in MQ at pH 5, resuspendable with vortexing or centrifuge at 3500 RPM for 15 minutes at 4 °C, remove supernatant and resuspend at desired concentration or freeze dry to form a powder (approx. 1.3 mg final volume).

Ag_2S QD-insulin-Pasp(DET)-CS/GS conjugation to BSA, OxBSA, GlucBSA, galBSA or FSA

[0307] For 1 mL of 1.3 mg/mL Ag_2S QD-INS–Pasp(DET)-CS/GS, 1 mL Ag_2S QD-insulin-Pasp(DET)-CS/GS was combined with 3.8 mg EDC, 4 mg NHS in and mixed for 1 hour at 4 °C. 75 uL 100x Carbonate-Bicarbonate Buffer was added to the Ag_2S QD-INS- insulin-Pasp(DET)-CS/GS-EDC-NHS mixture and mixed for 2 minutes. 70 µL BSA, OxBSA, GlucBSA, galBSA orFSA stock (10 mg/mL) was added to the solution and mixed for 4 hours. The solution was then dialysed with snakeskin tubing (10 kDa) in 100 mL of MQ for 2, 4 and 16 hours at 4 °C. The solution (nano-insulin) was stored at 4 °C in the dark until use.

[0308] Table 1 shows a summary of the synthetic steps for an exemplary QD conjugate of the present invention. It will be appreciated that combining Pasp(DET) and CS/GS due to both being positively charged ligands is difficult as attachment does not occur naturally. Therefore, the method requires sensitive pH adjustment.

Table 1. Summary of the method steps and optimal pH.

Step	**pH**	**Cause of pH change**	**Importance**
QDs alone	7	None	Insignificant
QDs with EDC and NHS (1 hour closed solution, 4 °C)	5	EDC and NHS added	Optimal for EDC attachment to QDs
QD/EDC/NHS with $NaCO_3$ buffer and insulin (4 hour closed solution, 4 °C)	9	$NaCO_3$ buffer	Attachment of insulin to QDs via the formation of an amide bridge mediated by EDC/NHS
Dialysis of QD-Insulin (24 hour open solution, 4 °C)	7	Removal of $NaCO_3$ buffer and EDC, NHS	Insignificant, removing unreacted materials and any other negatively charged materials
Addition of Pasp(DET) (4 hour closed solution, 4 °C)	7	No change	The triamines of the Pasp(DET) attach to negatively charged species of QD-INS, including the QD itself, and negatively charged amino acids of insulin. Particularly B chain 20-30 aa. This encapsulates QD-INS in Pasp(DET)

Addition of CS/GS (1 hour closed solution, 4 °C)	5	CS/GS solution	Pasp(DET) in a lysosome promotes disruption by the primary amines (from DET) interacting with the lysosomal membrane when the pH is lowered to 5.5. The use of a solution of about pH 5 facilitates Pasp(DET) interaction with CS/GS. The diethyltriamine groups of Pasp(DET) and the acetal groups of CS/GS (from the use of 75% deacetylated chitosan) perform reductive aminisation and lead to an imine or hemialminate group. Removal of all acetal groups (eg CS/GS-COOH) prevents the bilayer from forming.
CS/GS – glucose degeneration to form gluconic acid tail. (3 hour closed solution, 4 °C)	7	$NaCO_3$ buffer	Oxidation of glucose on CS/GS is promoted with $NaCO_3$ acting as a reduction agent.
Q-I-P-C with EDC and NHS (1 hour closed solution, 4°C)	5	EDC and NHS added	Optimal for EDC attachment to CS/GS-COOH group.
Q-I-P-C/EDC/NHS with $NaCO_3$ buffer and FSA (4 hour closed solution, 4 °C)	9	$NaCO_3$ buffer	Attachment of FSA to complex via the formation of an amide bridge mediated by EDC/NHS between the COOH group on gluconic tail of CS/GS.
Dialysis of Q-I-P-C-F (24 hour open solution, 4 °C)	7	Removal of $NaCO_3$ buffer and EDC, NHS	Insignificant, removing unreacted materials.

(c) Formation of Nano-siRNA conjugates

Ag_2S QD-siRNA coating with Pasp(DET)

[0309] Add 700uL of RNAse-free water to Ag_2S QD-siRNA solution. Add11.5ul/mL of Pasp(DET) 87x stock (100 µg/mL excess) to the Ag_2S QD-siRNA conjugate, mixing at 4°C for 4 hours. Store at 4°C in the dark until use.

Ag_2S QD-siRNA-Pasp(DET) coating with CS/GS or CS/GS-COOH

[0310] Add 9uL/mL 111x CS/GS polymer solution (100 µg/mL excess) to the Ag_2S QD-siRNA-Pasp(DET) complex and mix at 4°C for 4 hours. Store at 4°C in the dark until use.

Ag_2S QD-siRNA-Pasp(DET)-CS/GS conjugation to FSA

[0311] Mix the Ag_2S QD-siRNA-Pasp(DET)-CS/GS complex with 3.84 mg EDC and 4 mg NHS and mixing for 1 hour at 4 °C. Adjust the solution to pH 10 by adding 18.47uL of carbonate-bicarbonate buffer (0.79 mg sodium bicarbonate and 6.95 mg sodium carbonate per 1 mL RNAse-free water). Add 46.3uL of 2.16mg/mL FSA stock (100 µg/mL excess), mixing at 4°C for 4 hours. Dialyse using a 10 kDa 15mL spin column, centrifuging three times at 4000 rpm for 5 minutes each, adding 2mL RNAse-free water prior to centrifugation. Store the final Ag_2S QD-siRNA-Pasp(DET)-CS/GS-FSA (~0.5 mL, pH ~7) at 4°C in the dark until use.

(d) Formation of Nano-Cas9 conjugates

Ag_2S QD-Cas9 coating with Pasp(DET)

[0312] Add 38.76 µL Pasp(DET) (2.58 mg/mL stock) to Ag_2S QD-Cas9 solution to achieve a final concentration of 100 µg/mL and mix at 4°C for 4 hours. Store at 4°C in the dark until use.

Ag_2S QD-Cas9-Pasp(DET) coating with CS/GS or CS/GS-COOH

[0313] Add 5.33 µL CS/GS polymer solution (18.75 mg/mL stock, final concentration 100 µg/mL) to the Ag_2S QD-Cas9-Pasp(DET) complex and mix at 4°C for 4 hours. Store at 4°C in the dark until use.

Ag_2S QD-Cas9-Pasp(DET)-CS/GS conjugation to FSA

[0314] Combine the Ag_2S QD-Cas9-Pasp(DET)-CS/GS complex with 99.684 µL EDC (10 mg/mL) and 113 µL NHS (10 mg/mL). Mix gently at 4°C for 1 hour. Add 46.3 µL FSA stock (2.16 mg/mL, final concentration 100 µg/mL) and mix at 4°C for 4 hours. Dialyse using a 10 kDa 15mL spin column, centrifuging three times at 4000 rpm for 5 minutes each, adding 2 mL RNAse-free water prior to each centrifugation. Store the final QD-Cas9-Pasp(DET)-CS/GS-FSA conjugate (~0.4 mL final volume, pH ~7) at 4°C in the dark until use.

(e) Formation of Nano-peanut allergen conjugates

Ag_2S QD-Arah1/Arah3 coating with Pasp(DET)

[0315] For 1 mL of 65 ug/mL QD-Arah1/Arah3-Pasp(DET) combine 1 mL QD-Arah1/Arah3 with 5.0 µL Pasp(DET) polymer (10 mg/mL). Combine slowly and add 2 µL 1 M NaOH to adjust pH to 9 for solubility. Mix for 4 hours at 4 °C, filter with Whatman filter paper 4. Stored at 4 °C in the dark until use. QD-Arah1/Arah3-Pasp(DET) will precipitate in MQ at pH 5, resuspendable with vortexing or centrifuge at 3500 RPM for 15 minutes at 4 °C, remove supernatant and resuspend at desired concentration or freeze dry to form a powder (approx. 65 ug final volume).

Ag_2S QD- Arah1/Arah3 -Pasp(DET) coating with CS/GS or CS/GS-COOH

[0316] For 1 mL of 65 ug/mL QD-Arah1/Arah3-Pasp(DET)-CS/GS combine the 1 mL QD-Arah1/Arah3-Pasp(DET) with 1 µL CS/GS polymer stock (10 mg/mL). Mix for 4 hours at 4 °C, filter with Whatman filter paper 4. Stored at 4 °C in the dark until use. QD-Arah1/Arah3-Pasp(DET)-CS/GS will precipitate in MQ at pH 5, resuspendable with vortexing or centrifuge at 3500 RPM for 15 minutes at 4 °C, remove supernatant and resuspend at desired concentration or freeze dry to form a powder (approx. 1.3 mg final volume).

Ag_2S QD- Arah1/Arah3-Pasp(DET)-CS/GS conjugation to FSA

[0317] For 1 mL of 65 ug/mL Ag_2S QD- Arah1/Arah3–Pasp(DET)-CS/GS, 1 mL Ag_2S QD-Arah1/Arah3-Pasp(DET)-CS/GS-FSA was combined with 0.19 mg EDC, 0.2 mg NHS in and mixed for 1 hour at 4 °C. 3.75 uL 100x Carbonate-Bicarbonate Buffer was added to the Ag_2S QD- Arah1/Arah3- insulin-Pasp(DET)-CS/GS-EDC-NHS mixture and mixed for 2 minutes. 3.5 µL BSA, OxBSA, GlucBSA, galBSA or FSA stock (10 mg/mL) was added to the solution and mixed for 4 hours. The solution was then dialysed with snakeskin tubing (10 kDa) in 100 mL of MQ for 2, 4 and 16 hours at 4 °C. The solution (nano-peanut) was stored at 4 °C in the dark until use.

[0318] **Figure 1** shows characterisation parameters for Ag_2S nano-BITS, Ag_2S nano-insulin and CQD nano-insulin. Nano-insulin stock was prepared at 1 mg/mL. Nano-insulin was negatively charged (**B**) with a diameter of 26.7 nm (Ag_2S) or 30.3 nm (CQD) (**D**), hydrodynamic diameter of 251 nm (Ag_2S) or 185 nm (CQD) (**A**) with a high PDI (**C**).

Analysis was performed on a Malvern Zetasizer Nano ZS (ATA Scientific, AUS) or ImageJ (NIH, USA). Data shows mean ± SD (n=3 zeta sizer, n=10 TEM).

[0319] **Figure 2** shows the FTIR spectra of Ag_2S QD nano-BITS and Ag_2S QD nano-insulin. These formulations were successful in *in vitro* and *in vivo* studies and used the targeting protein FSA (formaldehyde treated serum albumin). Ag_2S QD nano-BITS and Ag_2S QD nano-insulin stock was prepared at 1 mg/mL. Data was collected from a dried sample of 0.1 mg/mL at pH 7. Both Ag_2S QD nano-BITS and Ag_2S QD nano-insulin demonstrates amine I and amine II fingerprint spectra with a high prevalence of NH functional groups.

[0320] **Figure 3** shows FTIR of different targeting proteins and modifications to CS/GS for the Ag_2S QD nano-insulin and Ag_2S QD nano-BITS formulations. Ag_2S QD nano-insulin with attached BSA, oxidised BSA or glycated (glucose or galactose) BSA with stocks was prepared at 1 mg/mL. Ag_2S QD nano-insulin and Ag_2S QD nano-BITS were also formulated with COOH enriched CS/GS with or without FSA attachment with stocks prepared at 1 mg/mL for analysis. Data as collected from a dried sample of 0.1 mg/mL at pH 7.

[0321] **Figures 4 – 6** show SEMs of dried Ag_2S nano-insulin (**Fig 4**), TEMs of Ag_2S nano-insulin (**Fig 5**), and TEMS of CQD nano-insulin (**Fig 6**). The data confirm the sizes of the QD conjugates in **Figure 1**.

Example 4 - Treatment of mice

[0322] Therapeutic was administered by drinking water spiked with Ag_2S QD-insulin-Pasp(DET)-CS/GS-FSA (Ag_2S QD nano-insulin), Ag_2S QD-Pasp(DET)-CS/GS-FSA (Ag_2S QD nano-BITS), Ag_2S QD-insulin-GAD65-Pasp(DET)-CS/GS-FSA, Ag_2S QD-insulin-GAD-65-Pasp(DET)-CS/GS, Ag_2S QD-insulin-CS/GS-FSA, carbon QD-insulin-Pasp(DET)-CS/GS-FSA and QDs. Dosages were determined based on the daily water intake, weight of the mice and insulin concentrations. Dosages used were: Ag_2S QD-insulin-Pasp(DET)-CS/GS-FSA (0.4, 1, 2, 5, 10 and 20 IU/kg/day), Ag_2S QD-insulin-CS/GS-FSA, (2 IU/kg/day), Ag_2S QD (2 IU/kg). All other dosages were 20 IU/kg/day insulin with 1.5 ng/kg/day GAD65.

[0323] NOD mice were treated with increasing dosages of Ag_2S QD nano-insulin (0.4, 1, 2, 5, 10 and 20 IU/kg), oral insulin (2 IU/kg/day) (current state of the art therapy in

clinical trials), nano-insulin without Pasp(DET), QDs alone and untreated. Mice were treated continuously from either: 1) 3 weeks to 12 weeks, 2) 3 weeks to 52 weeks, 3) intermittently weeks 3-4, 7-8 and 11-12 and; 4) 8 weeks to 52 weeks.

[0324] NOD mice were treated with Ag_2S QD nano-insulin (1, 5 or 20 IU/kg/day) or oral insulin (1 IU/kg/day) from 4 weeks of age (n=8-12 per group). Blood glucose was recorded weekly with a measurement above 11.1 mmol/L considered to be hyperglycaemic. Anti-CD3 treatments were given to untreated mice following repeat blood glucose reading >11.1 mmol/L, mice were treated with 5x 5 µg/kg daily injections. Mice were monitored daily post hyperglycaemia with back-to-back hyperglycaemic scores categorising a mouse as diabetic. The mice were monitored until 40 weeks of age for the onset of hyperglycaemia and diabetes.

[0325] **Figure 7** shows a survival curve for the development of diabetes relative to the week of diabetes development. The data demonstrated Ag_2S QD nano-insulin has a dose dependent effect of the EC_{50} of diabetes development and delays the onset of diabetes in female NOD mice compared to untreated and oral insulin treated mice ($P = 0.0001$) and is shown in **Figure 8**.

[0326] **Figure 9** shows a survival curve for the development of diabetes relative to the week of diabetes development in NOD mice. The data demonstrates that nano-insulin with a Ag_2S QD core had a greater effect than a carbon QD core with both treatments delaying the onset of diabetes in NOD mice compared to controls ($P = 0.0001$).

[0327] **Figure 10** shows the effects of Ag_2S QD nano-insulin on auto insulin antibodies (IAA) in female NOD mice. For this experiment, female NOD mice were treated with nano-insulin (2 IU/kg/day) or oral insulin (2 IU/kg/day) from weeks 3-8 and 3-12 (n=3-7 per group). IAA was measured from blood serum samples used a commercially available Elisa. IAA was reduced in mice treated with Ag_2S QD nano-insulin (2 IU/kg/day) but not with Ag_2S QD nano-insulin treatment without Pasp(DET) or CS/GS (2 IU/kg/day) or oral insulin (2 IU/kg/day) at 8 weeks. At 12 weeks Ag_2S QD nano-insulin (2 IU/kg/day) approached significance ($p=0.0645$) compared to untreated 12-week NOD mice. The data shows mean ± SD, ** $P < 0.01$ and demonstrated nano-insulin was ineffective without the Pasp(DET) polymer or CS/GS polymer.

[0328] **Figure 11** shows the effects of Ag_2S QD nano-insulin treatments on splenocytes T cell subsets in female NOD mice. Female NOD mice were treated with Ag_2S QD nano-insulin (2 or 5 IU/kg/day) or oral insulin (2 IU/kg/day) from week 4 to week 12 (n=2-6). Splenocytes were isolated at week 12 and analysed by flow cytometry for CD3, CD4 (**A**), CD25 (**B**), Foxp3 (**C**) and CD8 (**D**). Ag_2S QD nano-insulin (2 IU/kg/day) demonstrated increased CD4+ regulatory T cells (Foxp3+CD25+) compared to diabetic untreated mice. No changes in CD4, CD25 or CD8 T cells were observed between groups.

[0329] **Figure 12** shows the effects of Ag_2S QD nano-insulin treatment on liver non parenchymal and immune cells in female NOD mice. Female NOD mice were treated with Ag_2S QD nano-insulin (2 or 5 IU/kg/day) from week 3 to week 12 (n=2-6). Untreated mice liver cells were collected at weeks 8 or 12. Liver cells were analysed by flow cytometry for Tim4, Lyve1, CD11b, CD11c, CD45, CD64, CD161, MHCI, MHCII and DAPI. Untreated mice between weeks 8 and 12 demonstrated elevated autoimmunity but increased dendritic cells (DCs), Kupffer cells (KCs) along with increased MHCII expression on DCs (**A-B**), macrophages (**C-D**), KCs (**E-F**), monocyte-derived macrophages (MoMFs) (**G-H**) and liver sinusoidal endothelial cells (LSECs) (**I-K**). Treatment with Ag_2S QD nano-insulin (2 or 5 IU/kg/day) decreased MHCII expression on macrophages, MoMFs and LSECs similarly to 8-week control mice. 2 IU/kg/day treatment only was observed to decreased DCs and increase MoMFs, comparatively 5 IU/kg/day increased DCs and reduced MoMFs. 5 IU/kg/day was also observed to decreased KC MHCII expression.

[0330] **Figure 13** shows the biodistribution of Ag_2S QD nano-(^{14}C)-insulin over 24 hours. Male C57BL/6J mice (n=5) were treated with an oral gavage of 100,000 DPM nano-(^{14}C)-insulin. Pharmacokinetic (PK) data from blood (**A**) and faeces (**B**) were collected between 0-24 hours. Mice were euthanized at 24 hours and all major organs were collected and examined for ^{14}C-insulin distribution (**C-F**). Circulating blood (^{14}C)-insulin concentrations peaked 1 hour following gavage. Peak distribution in faecal matter was observed at 4 hours post gavage. 24 hours following gavage, 3% ^{14}C-insulin remained in the liver with trace amounts observable in other organs.

[0331] **Figure 14** shows the effects of chronic Ag_2S QD nano-insulin treatment on splenocytes T cell subsets. Female NOD mice were treated with nano-insulin (20 IU/kg/day) from week 4 to week 40 (n=4). Anti-CD3 treatments (n=5) were given to

untreated mice following repeat blood glucose reading >11.1 mmol/L, mice were treated with 5x 5 µg/kg daily injections. Control mice were untreated for 40 weeks. Only mice that were non-diabetic at the completion of 40w were analysed. Splenocytes were isolated and analysed by flow cytometry for CD3 (**A**), CD4 (**B**), CD25, Foxp3 (**C**) and CD8 (**D**). Nano-insulin (20 IU/kg/day) demonstrated increased CD4+ regulatory T cells (Foxp3+CD25+) compared to control and anti-CD3 treated mice. No changes in CD4, CD25 or CD8 T cells were reported between nano-insulin and controls. Anti-CD3 treated mice demonstrated reduced Cd8 positive cells compared to controls and nano-insulin treated mice.

[0332] **Figure 15** shows the effects of Ag_2S QD nano-insulin on insulitis in female NOD mice. Female NOD mice were treated with nano-insulin (2 IU/kg) from weeks 4-18 (**A**) or with either nano-insulin (20 IU/kg) from weeks 4-40 or anti-CD3 (5x 5 µg/kg/day) (**B**). Insulitis was measured based on the histological evaluation (**C**) with a scores equal to 0 = normal islets, 1 = peri-insulitis (minor immune cell infiltration), 2 insulitis (<50% immune cell infiltration), 3 = severe insulitis (>50% immune cell infiltration). Insulitis was reduced in mice treated with nano-insulin 18 and 40 weeks.

[0333] **Figure 16** is a survival curve showing the development of hyperglycaemia in female NOD mice treated with Ag_2S QD-insulin (50 ug/kg/day), Ag_2S QD nano-BITS (50 ug/kg/day), Ag_2S QD nano-insulin-GAD-FSA and Ag_2S QD nano-insulin-GAD without the targeting protein. NOD mice were treated with Ag_2S QD nano-insulin-GAD with targeting protein FSA (20 IU/kg/day, 315 ng/kg/day), Ag_2S QD nano-insulin-GAD without the targeting protein FSA (20 IU/kg/day, 315 ng/kg/day) and Ag_2S QD-insulin and Ag_2S QD nano-BITS from 8 weeks of age (n=5-8 per group). Blood glucose was recorded weekly with a measurement above 11.1 mmol/L considered to be hyperglycaemic. Nano-insulin and Anti-CD3 treatments groups were provided from those in Figure 7.

[0334] Mice were monitored daily post hyperglycaemia with back-to-back hyperglycaemic scores categorising a mouse as diabetic. Mice were monitored until 40 weeks of age for the onset of hyperglycaemia and diabetes. The data demonstrated that Ag_2S QD nano-insulin with GAD provides no additional benefit in NOD mice and showed that the removal of FSA as a targeting protein drastically reduced the effectiveness of Ag_2S QD nano-insulin-GAD. The data also showed Ag_2S QD nano-BITS has similar effectiveness to anti-CD3 therapy to delay the onset of diabetes in

NOD mice. The Ag_2S QD nano-BITS did not contain any antigen. Control treatment was provided with oral Ag_2S QDs alone.

[0335] **Figure 17** shows changes in dendritic cell MHC-II A-I^{g7} expression following 24 hour *in vivo* and *in vitro* treatments. In **Figure 17A**, Female NOD mice were given a gavage of either Ag_2S QD nano-BITS or Ag_2S QD nano-insulin (50 IU/kg) and were euthanized 24 hours later with isolated liver non-parenchymal cells analysed for changes in MHC-II A-I^{g7} expression. MHC-II A-I^{g7} is an insulin specific MHC-II complex associated with immune tolerance. Liver non-parenchymal cells included, liver sinusoidal endothelial cells, macrophages, Kupffer cells and dendritic cells. Increased I-Ag7 expression was only observed with nano-insulin treatment.

[0336] In **Figure 17B**, liver non-parenchymal cells were isolated from female NOD mice. The cells were treated with the individual Ag_2S QD nano-insulin constituents (Ag_2S QDs, CS/GS, Past(DET), FSA, insulin), the combined materials (Ag_2S QD-DET, Ag_2S QD-CS/GS, Ag_2S QD-DET-CS/GS) or Ag_2S QD nano-insulin (Ag_2S QD-INS-DET-CS/GS-FSA). The concentration of materials were based on the exposure to 10 ng/mL nano-insulin (Ag_2S QD 0.2 ng/mL; INS 10 ng/mL; DET 1 ng/mL; CS/GS 1 ng/mL; FSA 1 ng/mL). Increased I-A^{g7} expression was only observed with nano-insulin treatment.

[0337] In **Figure17C**, cells were treated with Ag_2S QD nano-insulin with different targeting proteins (none, BSA, glucose glycated BSA, galactose glycated BSA, oxidized BSA and FSA). The concentration of targeting protein was fixed per material (1 ng/mL) and the cells were treated for 24 hours. Increased MHC-II A-I^{g7} expression was only observed with nano-insulin with the FSA targeting protein.

[0338] In **Figure 17D**, cells were treated with Ag_2S QD nano-insulin with modified CS/GS. Additional COOH groups were applied to CS/GS by iodine haloform reactions. Similarly, *in vitro* cells were treated for 24 hours and the expression of MHCII A-I^{g7} was examined. The increased expression observed with nano-insulin treatment was abolished by modification of the CS/GS polymer.

[0339] **Figure 18** shows changes in Kupffer cell MHC-II A-I^{g7} expression following 24 hour *in vivo* and *in vitro* treatment. In **Figure 18A**, female NOD mice were given a gavage of either Ag_2S QD nano-BITS or Ag_2S QD nano-insulin (50 IU/kg) and were euthanized 24 hours later with isolated liver non-parenchymal cells analysed for

changes in MHC-II A-I^{g7} expression. Liver non-parenchymal cells included, liver sinusoidal endothelial cells, macrophages, Kupffer cells and dendritic cells. No changes in I-A^{g7} expression were observed in Kupffer cells following *in vivo* treatment.

[0340] In **Figure 18B**, liver non-parenchymal cells were isolated from female NOD mice. The cells were treated with the individual Ag_2S QD nano-insulin constituents (Ag_2S QDs, CS/GS, Past(DET), FSA, insulin), the combined materials (Ag_2S QD-DET, Ag_2S QD-CS/GS, Ag_2S QD-DET-CS/GS) or Ag_2S QD nano-insulin (Ag_2S QD-INS-DET-CS/GS-FSA). The concentration of materials were based on the exposure to 10 ng/mL nano-insulin (Ag_2S QD 0.2 ng/mL; INS 10 ng/mL; DET 1 ng/mL; CS/GS 1 ng/mL; FSA 1 ng/mL No changes in I-A^{g7} expression were observed in Kupffer cells following *in vitro* treatment.

[0341] In **Figure 18C**, cells were treated with Ag_2S QD nano-insulin with different targeting proteins (none, BSA, glucose glycated BSA, galactose glycated BSA, oxidized BSA and FSA). The concentration of targeting protein was fixed per material (1 ng/mL) and the cells were treated for 24 hours. No changes in I-A^{g7} expression were observed in Kupffer cells following *in vitro* treatment.

[0342] In **Figure 18D**, the cells were treated with Ag_2S QD nano-insulin with modified CS/GS. Additional COOH groups were applied to CS/GS by iodine haloform reactions. Similarly to previous panels, *in vitro* cells were treated for 24 hours and the expression of MHCII A-I^{g7} was examined. No changes in I-A^{g7} expression were observed in Kupffer cells following *in vitro* treatment.

[0343] **Figure 19** shows changes in liver sinusoidal endothelial cells (LSECs) MHC-II A-I^{g7} expression following 24 hour *in vivo* and *in vitro* treatment. In **Figure 19A**, female NOD mice were given a gavage of either Ag_2S QD nano-BITS or Ag_2S QD nano-insulin (50 IU/kg), mice were euthanized 24 hours later with isolated liver non-parenchymal cells analysed for changes in MHC-II A-I^{g7} expression. Liver non-parenchymal cells included, liver sinusoidal endothelial cells, macrophages, Kupffer cells and dendritic cells. Increased I-A^{g7} expression was observed with nano-BITS and nano-insulin treatment.

[0344] In **Figure 19B**, liver non-parenchymal cells were isolated from female NOD mice and the cells were treated with individual Ag_2S QD nano-insulin constituents (Ag_2S

QDs, CS/GS, Past(DET), FSA, insulin), combined materials (Ag_2S QD-DET, Ag_2S QD-CS/GS, Ag_2S QD-DET-CS/GS) or Ag_2S QD nano-insulin (Ag_2S QD-INS-DET-CS/GS-FSA). The concentration of materials were based on the exposure to 10 ng/mL nano-insulin (Ag_2S QD 0.2 ng/mL; INS 10 ng/mL; DET 1 ng/mL; CS/GS 1 ng/mL; FSA 1 ng/mL). Increased I-A^{g7} expression was only observed with nano-insulin treatment.

[0345] In **Figure 19C**, the cells were treated with Ag_2S QD nano-insulin with different targeting proteins (none, BSA, glucose glycated BSA, galactose glycated BSA, oxidized BSA and FSA). The concentration of targeting protein was fixed per material (1 ng/mL) Cells were treated for 24 hours. Increased MHC-II A-I^{g7} expression was only observed with Ag_2S QD nano-insulin with the FSA targeting protein.

[0346] In **Figure 19D**, the cells were treated with Ag_2S QD nano-BITS or Ag_2S QD nano-insulin with modified CS/GS. Additional COOH groups were applied to CS/GS by iodine haloform reactions. *In vitro* cells were treated for 24 hours and the expression of MHCII A-I^{g7} was examined. The increased expression observed with Ag_2S QD nano-BITS or Ag_2S QD nano-insulin treatment was abolished by modification of the CS/GS polymer.

[0347] **Figure 20** shows changes in macrophage MHC-II A-I^{g7} expression following 24 hour *in vivo* and *in vitro* treatment. In **Figure 20A**, female NOD mice were given a gavage of either Ag_2S QD nano-BITS or Ag_2S QD nano-insulin (50 IU/kg), mice were euthanized 24 hours later with isolated liver non-parenchymal cells analysed for changes in MHC-II A-I^{g7} expression. Liver non-parenchymal cells included, liver sinusoidal endothelial cells, macrophages, Kupffer cells and dendritic cells. No changes in I-A^{g7} expression were observed in macrophages following *in vivo* treatment.

[0348] In **Figure 20B**, liver non-parenchymal cells were isolated from female NOD mice and the cells were treated with individual Ag_2S QD nano-insulin constituents (QDs, CS/GS, Past(DET), FSA, insulin), combined materials (Ag_2S QD-DET, Ag_2S QD-CS/GS, Ag_2S QD-DET-CS/GS) or Ag_2S QD nano-insulin (Ag_2S QD-INS-DET-CS/GS-FSA). The concentration of materials were based on the exposure to 10 ng/mL nano-insulin (Ag_2S QD 0.2 ng/mL; INS 10 ng/mL; DET 1 ng/mL; CS/GS 1 ng/mL; FSA 1 ng/mL. No changes in I-A^{g7} expression were observed in macrophages following *in vivo* treatment.

[0349] In **Figure 20C**, cells were treated with Ag_2S QD nano-insulin with different targeting proteins (none, BSA, glucose glycated BSA, galactose glycated BSA, oxidized BSA and FSA). The concentration of targeting protein was fixed per material (1 ng/mL) and the cells were treated for 24 hours. No changes in I-A^{g7} expression were observed in macrophages following *in vitro* treatment.

[0350] In **Figure 20D**, cells were treated with Ag_2S QD nano-insulin with modified CS/GS. Additional COOH groups were applied to CS/GS by iodine haloform reactions. *In vitro* cells were treated for 24 hours and the expression of MHCII A-I^{g7} was examined. No changes in I-A^{g7} expression were observed in macrophages following *in vitro* treatment.

[0351] **Figure 21** shows the effects of Ag_2S QD nano-siRNA of GAPDH mRNA following 4 hour *in vitro* and 24 hour *in vivo* treatments. Hepatocytes (**Figure 21A**) and liver sinusoidal endothelial cells (LSECs) (**Figure 21B**) were isolated from male C57BL/6J mice (n=5). The cells were treated for 4 hours with escalating dosages of nano-siRNA(GAPDH). No changes in GAPDH mRNA were observed for hepatocytes and LSECs demonstrated a dose dependent reduction in mRNA expression.

[0352] In **Figure 21C**, *in vivo* experimentation was performed in male C57BL/6J mice. The mice were given a single gavage of 15 nM Ag_2S QD nano-siRNA(GAPDH) and the controls were untreated. The mice were euthanized 24 hours later with hepatocytes and LSECs isolated for analysis. LSECs demonstrated a 50% reduction in GAPDH mRNA while hepatocytes showed no change in expression.

[0353] In **Figure 22**, Male C57BL/6J mice(n=3) were treated with an oral gavage of 100,000 DPM Ag_2S QD nano-(^{14}C)-Cas9. Mice were euthanised at 24 hrs and all major organs were collected and examined for ^{14}C-Cas9 distribution. 24 hrs following gavage, 2% ^{14}C-Cas9 remained in the liver with trace amounts observable in other organs. The biodistribution of Ag_2S QD nano-CRISPRCas9 matches the biodistribution of Ag_2S QD nano-insulin (Figure **13-C**) (ie the delivery of a small peptide (insulin 5.8 kda) and a large protein (Cas9 160 kDa) occurs to the same location). This demonstrates that the compositions described herein can transport a broad range of sized proteins *in vivo* following oral administration.

[0354] **Figure 23** demonstrates that the attachment of the Ag_2S QD to Cas9 changes the uptake *in vitro* of Cas9 in different cell cultures. Ag_2S QD attachment inhibits non-specific uptake of Cas9 into cells. This demonstrates a unique, unexpected benefit that Ag_2S QD attachment to Cas9 controls endocytosis.

[0355] **Figure 24** shows relative CD98 gene expression changes to beta-actin in the liver sinusoid endothelial cells (LSECs), hepatocytes (Hep), splenocytes (Spleen) and payers patches (PPs) in healthy Bl6 mice 24 hours post-treatment with 0.02 mg/kg Ag_2S QD nano-CD98-siRNA. Ag_2S QD Nano-siRNA has minimal but some actions in the gut in addition to the liver. This indicates that gut actions may also be promoted with this therapy.

Example 5 – further experiments with Ag_2S QD-insulin

[0356] Non-human primates (NHPs) were treated with Ag_2S QD containing 9 mg insulin antigen for 28 days. As shown in **Figure 25**, Ag_2S QD-insulin showed no changes between baseline (day 0) bloods (haematological analysis) and following 28 days treatment. After treatment there was a 28-day washout period. Key outcome for the data was that an inverse vaccine technology that promoted immunomodulation had no effect on haematological markers (haemoglobin, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration mean, mean cell volume, pact cell volume, platelets, RCC, WCC, neutrophils, lymphocytes, monocytes, eosinophils and basophils).

[0357] As shown in **Figure 26**, NHPs treated for 28 days with Ag_2S QD-insulin showed increased CD62L on Tregs compared to washout but no change in Treg cell numbers in peripheral blood. NHP also showed increased cDC1 cells but no change sin cDC2 or pDCs in peripheral blood. Key outcome was that 28 days treatment led to increased population of regulatory T cells with CD62L expression, also known as L-selectin. This distinct subset are more potent suppressors of effector T cells and are better at migrating to secondary lymphoid organs. This may be used as a marker of Ag_2S QD activity during treatment. **Figure 26** also shows that treatment increased conventional dendric cells (cDC1) but not peripheral DCs or cDC2 cells, indicating a role in Th1 responses but not Th2.

[0358] As shown in **Figure 27**, non-obese diabetic (NOD) mice treated for 28 days with Ag_2S QD-insulin showed increased CD62L (combined central memory (CM) like Foxp3+ Tregs compared to control matching NHP data). Reduced proliferation and effector Treg populations were observed with Ag_2S QD-insulin treatment. The data shows the same pathway (where populations of T regulatory cells were upregulated) in NOD mice as in NHPs with the same duration of treatment.

[0359] As shown in **Figure 28**, these NOD mice also showed increased naïve CD4 and naïve CD8 cells, and reduced proliferating and memory CD4+, indicating that treatment reduced transduction to effector T cells. The results suggest that CD62L Tregs were preventing the conversion of naïve to effector T cells in the NOD mouse animal model of disease.

[0360] The NHP studies also investigated the effects of Ag_2S QD-insulin treatment in an insulin tolerance test (ITT) as Ag_2S QD containing 9 mg of insulin may have induced a reduction in blood sugar. As shown in **Figure 29**, there was no observed changes in blood sugar in either males or female NHPs. The same NHPs were also checked for responses to an oral insulin, which induced a 20% reduction in blood sugar. These results show Ag_2S QD-insulin does not reduce blood glucose despite containing the same insulin that, in oral form, reduced blood glucose.

Example 6 – Ag_2S QD-peanut allergen

[0361] Ag_2S QD-peanut allergen was produced (see **Figure 30** for the corresponding AFTR-FTIR analyses).

[0362] Ag_2S QD-peanut allergen was given to the CH3 mouse model of peanut allergy for 14 days. 3 dosages were given (low, moderate and high dose at 0.01, 0.1 and 1 ug/dose, respectively) every day for 14 days prior to a peanut allergen challenge. As shown in **Figure 31,** both moderate dose and omalizumab (positive control and conventional treatment) were effective in suppressing IgE levels across sexes (A-C females, E-G males), indicating successful allergenic modulation. The most effective results were observed in mice treated with 3 moderate dosages.

[0363] As shown in **Figure 32**, Ag_2S QD-peanut allergen treatment induced a Th1 response with a dose-specific pattern. IgG2a elevation across all treatment doses and omalizumab suggests a Th1-skewed immune modulation, likely beneficial in countering

Th2 dominance in allergic contexts. The highest IgG2a levels were observed in low-dose groups. The medium and high-dose groups demonstrated a more controlled IgG2a increase, aligning more closely with the response seen in omalizumab-treated animals, supporting a moderated Th1 engagement at higher doses. These results indicate that the mechanism of both Ag_2S QD-insulin and Ag_2S QD-peanut allergen may be via modulation of the Th1 response.

[0364] As shown in **Figure 33**, Ag_2S QD-peanut allergen reduced IL-10 across low, medium and high dosages, similarly to omalizumab. These results indicate an effective allergy treatment.

[0365] Overall, these data show that QD compositions described herein are immunomodulating without immune suppression with a mechanism of action likely related to regulatory T cells and the Th1 response. This effect was observed in both NOD mice (autoimmune type 1 diabetes model) and NHPs.

[0366] The inventors also showed that QD compositions described herein are a platform technology and provided proof of concept for a type 4 hypersensitivity in Type 1 Diabetes and in a type 1 hypersensitivity in peanut allergy.

CLAIMS

1. A quantum dot polymer composition comprising:

- a quantum dot, and

- a polymer shell,

wherein the polymer shell comprises a first polymer layer comprising an endosomal disruptive polymer, and a second polymer layer comprising a biopolymer, wherein the biopolymer is suitable for oral administration.

2. The composition according to claim 1, wherein the endosomal disruptive polymer is selected from the group consisting of: polyethylene imine; poly(arginine); poly(lysine); poly(histidine); poly-[2-{(2-aminoethyl)amino}-ethyl-aspartamide] (pAsp(DET)); a block co-polymer of poly(ethylene glycol) (PEG) and poly(arginine); a block co-polymer of PEG and poly(lysine); a block co-polymer of PEG and poly{N—[N-(2-aminoethyl)-2-aminoethyl]aspartamide} (PEG-pAsp(DET)); Poly-l-lysine (PLL); DET functionalised polyphenol (-)-epi-gallocatechin gallate (EGCG); Poly(amidoamine) dendrimers; amine functionalised dextran; Poly(histidine-arginine) 6-modified chitosan; PEG-PCL-PEI; a polymer comprising a backbone including poly-aspartic acid, glutamic acid, or a combination thereof, functionalised with diamine, triamine, lysine, histidine, arginine, or a combination thereof; and combinations thereof.

3. The composition according to claim 1 or 2, wherein the biopolymer is selected from the group consisting of: Poly(N-isopropylacrylamide) (PNIPAM); Polyacrylamide (PAM); Poly(acrylic acid); Polymethacrylate and other acrylic polymers; hyaluronic acid; heparin; chondroitin sulfate; chitosan; polyglutamate; poly-lysine; poly-histidine; poly (glutamic acid); poly-aspartic acid; N-acetylgalactosamine; glucose; sucrose; maltose; fructose; galactose; gelatin; and combinations thereof.

4. The composition according to any one of claims 1-3, wherein the biopolymer is conjugated to the endosomal disruptive polymer.

5. The composition according to any one of claims 1-4, wherein the endosomal disruptive polymer is conjugated to the quantum dot.

6. The composition according to any one of claims 1-5, wherein the quantum dot is formed from a material which comprises or consists of: carbon, silver, gold, platinum, aluminium, palladium, copper, cobalt, indium, zinc, nickel, silicon, and combinations thereof.

7. The composition according to any one of claims 1-6, wherein:

- the quantum dot is selected from an Ag_2S quantum dot and a carbon quantum dot,

- the endosomal disruptive polymer is pAsp(DET), and

- the biopolymer is selected from: chitosan, galactose, glucose or a combination thereof.

8. The composition according to any one of claims 1-7, further comprising a target molecule.

9. The composition according to claim 8, wherein the target molecule is cross-linked serum albumin.

10. The composition according to claim 8 or 9, wherein the target molecule is conjugated to the biopolymer.

11. The composition according to any one of claims 1-10, wherein the quantum dot is conjugated to a therapeutic agent.

12. The composition according to claim 11, wherein the therapeutic agent is selected from the group consisting of: protein, peptide, DNA, RNA, microRNA, siRNA, CRISPR-Cas9, and combinations thereof.

13. The composition according to claim 12, wherein the therapeutic agent is selected from insulin, Arah1/Arah3, siRNA, and Cas9.

14. The composition according to any one of claims 11-13, wherein the endosomal disruptive polymer is conjugated to the therapeutic agent.

15. The composition according to any one of claims 11-14, wherein the therapeutic agent is conjugated to a surface of the quantum dot or a surface ligand of the quantum dot.

16. The composition according to any one of claims 1-15, wherein the quantum dot has an average diameter of between about 1 nm and about 10 nm.

17. The composition according to claim 16, wherein the quantum dot has an average diameter of less than 10 nm.

18. The composition according to any one of claims 1-17, wherein the composition has an average diameter of between about 20 to 30 nm.

19. A method of preparing the composition of any one of claims 1 – 18, the method comprising;

- contacting the quantum dot with the endosomal disruptive polymer to form a quantum dot – endosomal disruptive polymer composition,

- contacting the quantum dot – endosomal disruptive polymer composition with the biopolymer to form the composition.

20. The method according to claim 19, further comprising contacting the composition with a target molecule.

21. The method according to claim 19 or 20, further comprising contacting the composition with a therapeutic agent.

22. A method of preventing or treating an autoimmune disease or condition in a subject in need thereof, the method comprising administering to the subject a therapeutic amount of a composition according to any one of claims 1-18.

23. The method of claim 22, wherein the autoimmune disease or condition is a Type I-IV hypersensitivity.

24. The method of claim 23, wherein the Type I-IV hypersensitivity is selected from type I or II diabetes and peanut allergy.

25. A method of treating insufficient endogenous peptide production in a subject in need thereof, the method comprising administering to the subject a therapeutic amount of a composition according to any one of claims 1-18.

26. A method of treating a subject suffering from a condition, wherein the condition is treatable with administration of a therapeutic exogenous peptide or protein, the method comprising administering to the subject a therapeutic amount of a composition according to any one of claims 1-18.

27. A method of delivering a therapeutic agent to an organ or tissue of a subject, the method comprising administering a composition according to any one of claims 1-18 to the subject, wherein the organ or tissue is selected from the liver, pancreas, intestine, small bowel or kidneys.

28. The method of any one of claims 22-27, wherein the composition is administered to the subject orally.

29. A method of evaluating a subject's response to treatment of an auto-immune disease or condition with a composition of any one of claims 1-18, the method comprising:

- providing a subject who has received or is receiving a composition of any one of claims 1-18;
- measuring or determining the level of regulatory T cells with CD26L expression in the subject;
- determining that the subject is responding to treatment when the level of regulatory T cells with CD26L expression is higher than the level in a reference data set in the form of one or more subjects who have the auto-immune disease or condition and have not received the treatment; or
- determining the subject is not responding to treatment when the level of regulatory T cells with CD26L expression is the same or lower than the level in the reference data set.