

SYNERGISTIC PERMEATION ENHANCERS FOR GASTROINTESTINAL SYNTHETIC EPITHELIAL LININGS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This patent application claims priority benefit of U.S. Provisional Patent Application No. 63/541,237, filed September 28, 2023, of U.S. Provisional Patent Application No. 63/623,109, filed January 19, 2024, and of U.S. Provisional Patent Application No. 63/673,614, filed July 19, 2024. The contents of those applications are hereby incorporated by reference herein in their entirety.

FIELD OF THE INVENTION

[0002] The invention relates to synthetic linings for the gastrointestinal (GI) tract, which can modulate absorption of drugs, nutrients, and other substances in the GI tract, and synergistic combinations of permeation enhancers to aid in such absorption.

BACKGROUND OF THE INVENTION

[0003] The gastrointestinal (GI) tract in humans, vertebrates, and other animals serves as a highly versatile organ system with multiple functions. A primary function of the GI tract is absorption of substances, such as nutrients from food. Due to the convenience of oral administration of drugs versus other methods such as injection or inhalation, absorption of substances in the GI tract is also widely utilized for administration of pharmaceuticals. Multiple diverse modes are available for oral administration of drugs, including both immediate release and sustained release formulations.

[0004] Many pharmaceuticals cannot be administered orally, however, due to the function of the epithelial lining as a barrier against certain types of substances. Various physical and chemical properties of certain pharmaceuticals prevent passage across the epithelium lining the GI tract. Pharmaceuticals, especially biologics, may be too large for transport across the epithelium. Other properties which affect transport include charge and hydrophobicity.

[0005] The current disclosure provides gastrointestinal synthetic epithelial linings (GSELs) in combination with synergistic combinations of permeation enhancers which are particularly effective at promoting absorption of pharmaceuticals in the gastrointestinal tract. The synergistic combinations of permeation enhancers disclosed herein for use with the gastrointestinal synthetic epithelial linings provide high levels of drug absorption as compared to individual permeation enhancers.

SUMMARY OF THE INVENTION

[0006] The present disclosure relates to synthetic linings formed *in situ* in the gastrointestinal tract, such as in the small intestine, used in combination with an agent and synergistic combinations of permeation enhancers, where the permeation enhancers synergistically increase the absorption of the agent across the intestinal tissue. The synthetic lining itself is referred to as a Gastrointestinal Synthetic Epithelial Lining (GSEL).

[0007] Disclosed herein is an orally administrable composition for forming a polymer *in situ* in a subject and enhancing permeation of an agent, comprising: a polymer precursor; an oxygen source; and a combination of permeation enhancers comprising at least one of an ammonium salt, a carbonate salt, or a bicarbonate salt, and at least one of a bile salt or bile acid. In some embodiments the composition further comprises one or more agents. In some embodiments, the one or more agents comprise one or more active pharmaceutical ingredients. In some embodiments, the ammonium salt, carbonate salt, or bicarbonate salt is an ammonium salt. In some embodiments, the ammonium salt, carbonate salt, or bicarbonate salt is a carbonate salt. In some embodiments, the ammonium salt, carbonate salt, or bicarbonate salt is a bicarbonate salt. In some embodiments, the bile salt or bile acid is a bile salt. In some embodiments, the bile salt or bile acid is a bile acid. In some embodiments, the ammonium salt is ammonium carbonate (NHCO), ammonium sulfate (NH₂SO₄), ammonium citrate (NHCA), ammonium phosphate (NHPO), diammonium phosphate (DAP), monoammonium phosphate (MAP), ammonium bicarbonate (NHHCO), or ammonium chloride (NHCl). In some embodiments, the ammonium salt is ammonium carbonate. In some embodiments, the ammonium salt is ammonium bicarbonate. In some embodiments, the carbonate salt is sodium carbonate or potassium carbonate. In some embodiments, the bicarbonate salt is sodium bicarbonate or potassium bicarbonate. In some embodiments, the bile salt or bile acid is a bile acid, and the bile acid is cholic acid (CHA), glycocholic acid (GCA), deoxycholic acid (DCA), glychenodeoxycholic acid (GCDCA), glycodeoxycholic acid (GDCA), taurodeoxycholic acid (TDCA), taurocholic acid (TCA), bovine bile (OX), chenodeoxycholic acid (CDCA), taurochenodeoxycholic acid (TCDCA), lithocholic acid (LCA), glycolithocholate (GLC), glycohyocholate (GHC), tauroolithocholate (TLC), ursodeoxycholic acid (UDCA), tauroursodeoxycholic acid (TUDCA), glyoursodeoxycholic acid (GUDCA), 12-monoketocholeic acid (12-MKC), 7-monoketocholeic acid (7-MKC), 7,12-diketocholeic acid (7,12-DKC), 3,7,12-triketocholeic acid (3,7,12-TKC), 12-monoketodeoxycholeic acid (12-MKDC), or 3-[(3-cholamidopropyl)dimethylammonio]-1-

propanesulfonate (CHAPS). In some embodiments, the bile salt or bile acid is a bile acid, and the bile acid is glycocholic acid. In some embodiments, the ammonium salt, carbonate salt, or bicarbonate salt is an ammonium salt and the ammonium salt is ammonium carbonate; and wherein the bile salt or bile acid is a bile acid, and the bile acid is glycocholic acid. In some embodiments, the ammonium salt, carbonate salt, or bicarbonate salt is an ammonium salt and the ammonium salt is ammonium bicarbonate; and wherein the bile salt or bile acid is a bile acid, and the bile acid is glycocholic acid. In some embodiments, the bile salt or bile acid is a bile salt, and the bile salt is a salt of cholic acid, a salt of glycocholic acid, a salt of deoxycholic acid, a salt of glychochenodeoxycholic acid, a salt of glycodeoxycholic acid, a salt of taurodeoxycholic acid, a salt of taurocholic acid, a salt of bovine bile, a salt of chenodeoxycholic acid, a salt of taurochenodeoxycholic acid, a salt of lithocholic acid, a salt of glycolithocholate, a salt of glycohyocholate, a salt of tauroolithocholate, a salt of ursodeoxycholic acid, a salt of tauroursodeoxycholic acid, a salt of glycoursodeoxycholic acid, a salt of 12-monoketocholeic acid, a salt of 7-monoketocholeic acid, a salt of 7,12-diketocholic acid, a salt of 3,7,12-triketocholic acid, a salt of 12-monoketodeoxycholic acid, or a salt of 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate. In some embodiments, the bile salt or bile acid is a bile salt, and the bile salt is a salt of glycocholic acid. In some embodiments, the ammonium salt, carbonate salt, or bicarbonate salt is an ammonium salt and the ammonium salt is ammonium carbonate; and wherein the bile salt or bile acid is a bile salt, and the bile salt is a salt of glycocholic acid. In some embodiments, the ammonium salt, carbonate salt, or bicarbonate salt is an ammonium salt and the ammonium salt is ammonium bicarbonate; and wherein the bile salt or bile acid is a bile salt, and the bile salt is a salt of glycocholic acid. In some embodiments, the bile salt or bile acid is a bile salt, and the bile salt is sodium cholate, sodium glycocholate, sodium deoxycholate, sodium glychochenodeoxycholate, sodium glycodeoxycholate, sodium taurodeoxycholate, sodium taurocholate, a sodium salt of bovine bile, sodium chenodeoxycholate, sodium taurochenodeoxycholate, sodium lithocholate, sodium glycolithocholate, sodium glycohyocholate, sodium tauroolithocholate, sodium ursodeoxycholate, sodium tauroursodeoxycholate, sodium glycoursodeoxycholate, sodium 12-monoketocholeate, sodium 7-monoketocholeate, sodium 7,12-diketocholeate, sodium 3,7,12-triketocholeate, sodium 12-monoketodeoxycholate, or sodium 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate. In some embodiments, the bile salt or bile acid is a bile salt, and the bile salt is sodium glycocholate. In some embodiments, the ammonium salt, carbonate salt, or bicarbonate salt is an ammonium salt and the ammonium salt is ammonium carbonate; and

wherein the bile salt or bile acid is a bile salt, and the bile salt is sodium glycocholate. In some embodiments, the ammonium salt, carbonate salt, or bicarbonate salt is an ammonium salt and the ammonium salt is ammonium bicarbonate; and wherein the bile salt or bile acid is a bile salt, and the bile salt is sodium glycocholate.

[0008] In some embodiments, the composition further comprises a buffering agent.

[0009] In some embodiments, the polymer precursor comprises one or both of a monomer and an oligomeric precursor to a polymer. In some embodiments, the polymer precursor is selected from Table 1 or Table 2, or combinations thereof. In some embodiments, the monomer is dopamine, levodopa, norepinephrine, methyldopa, levodopa methyl ester, levodopa ethyl ester, or combinations thereof. In some embodiments, the monomer is dopamine. In some embodiments, the oligomeric precursor to a polymer is an oligomer of dopamine, an oligomer of levodopa, an oligomer of norepinephrine, an oligomer of methyldopa, an oligomer of levodopa methyl ester, an oligomer of levodopa ethyl ester, or an oligomer of any combination of dopamine, levodopa, norepinephrine, methyldopa, levodopa methyl ester, or levodopa ethyl ester. In some embodiments, the oligomeric precursor to a polymer is an oligomer of dopamine. In some embodiments, the monomer is dopamine and the oligomeric precursor to a polymer is an oligomer of dopamine. In some embodiments, the oxygen source is a substrate for an endogenous catalyst. In some embodiments, the oxygen source is urea hydrogen peroxide or hydrogen peroxide.

[0010] In some embodiments, the composition is in an oral dosage form. In some embodiments, the oral dosage form is a solution, a gel, a tablet, a powder, or a capsule. In some embodiments, the oral dosage form comprises one or more solutions, gels, tablets, powders, or capsules. In some embodiments, the oral dosage form comprises an enteric dosage form.

[0011] In some embodiments, the agent is a macromolecule having a molecular weight between about 1 kD and about 160 kD. In some embodiments, the macromolecule is a polypeptide or polynucleotide. In some embodiments, the macromolecule is a polypeptide. In some embodiments, the polypeptide has a molecular weight of between about 1 kD and about 10 kD. In some embodiments, the polypeptide comprises between about 8 and about 80 amino acids. In some embodiments, the polypeptide comprises insulin, a GLP-1 receptor agonist, liraglutide, desmopressin, octreotide, an analgesic peptide, difelikefalin, H-20, an antibiotic, cyclosporin, vancomycin, lactase, beta galactosidase, exenatide, teriparatide, nafarelin, buserelin, captopril, daptomycin, an antibody, caplacizumab, ozoralizumab, brolocizumab, ranibizumab, bevacizumab, trastuzumab, rituximab, adalimumab, an enzyme,

lipase, a protease, phenylalanine hydroxylase, carbamoylphosphate synthetase I, glucose oxidase, or L-asparaginase. In some embodiments, the polypeptide comprises semaglutide. In some embodiments, the polypeptide comprises tirzepatide. In some embodiments, the macromolecule is a polynucleotide. In some embodiments, the polynucleotide has a molecular weight of between about 5 kD and about 1500 kD. In some embodiments, the polynucleotide is single-stranded. In some embodiments, the polynucleotide is double-stranded. In some embodiments, the polynucleotide is single-stranded and comprises between about 10 and about 5000 bases. In some embodiments, the polynucleotide is single-stranded and comprises between about 10 and about 1000 bases. In some embodiments, the polynucleotide is single-stranded and comprises between about 10 and about 30 bases. In some embodiments, the polynucleotide is double-stranded and comprises between about 5 and about 2500 base pairs. In some embodiments, the polynucleotide is double-stranded and comprises between about 5 and about 500 base pairs. In some embodiments, the polynucleotide is double-stranded and comprises between about 5 and about 15 base pairs. In some embodiments, the polynucleotide comprises an antisense oligonucleotide, mipomersen, patisiran, exondys, an siRNA, or an inflammatory bowel disease (IBD) targeting siRNA. In some embodiments, the agent is a small molecule having a molecular weight of 1 kD or less.

[0012] Disclosed herein is a method of forming a polymer coating in the gastrointestinal tract of a subject, the method comprising orally administering to a subject a composition of any one of the compositions disclosed herein. In some embodiments, the polymer coating is formed in the small intestine of the subject. In some embodiments, the polymer coating is formed in the duodenum of the subject.

[0013] Disclosed herein is a method of forming a polymer coating in the gastrointestinal tract of a subject, the method comprising orally administering to a subject: a polymer precursor; an oxygen source; and a permeation enhancer combination comprising at least one of an ammonium salt, a carbonate salt, or a bicarbonate salt, and at least one of a bile salt or bile acid; wherein the polymer precursor and the oxygen source contact a catalyst endogenous to the subject and the catalyst polymerizes the polymer precursor. In some embodiments, the polymer coating is formed in the small intestine of the subject. In some embodiments, the polymer coating is formed in the duodenum of the subject. In some embodiments, the method further comprises administering one or more agents to the subject. In some embodiments, the polymer precursor, the oxygen source, and the permeation enhancer combination are administered as a single composition. In some embodiments, the polymer precursor, the oxygen source, the permeation enhancer combination, and the one or more agents are

administered as a single composition. In some embodiments, the one or more agents are administered in a separate composition from the administration of the polymer precursor, the oxygen source, and the permeation enhancer combination. In some embodiments, the agent is an active pharmaceutical ingredient.

[0014] Disclosed herein is an orally administrable composition for forming a polymer *in situ* in a subject and enhancing permeation of an agent, comprising: a polymer precursor; an oxygen source; a combination of permeation enhancers comprising a first permeation enhancer and a second permeation enhancer; wherein the combination of the first permeation enhancer and the second permeation enhancer increase the permeation of the agent in comparison to the sum of the permeation of the agent by each enhancer individually. In some embodiments, the first permeation enhancer is an ammonium salt. In some embodiments, the first permeation enhancer is a carbonate salt. In some embodiments, the first permeation enhancer is a bicarbonate salt. In some embodiments, the second permeation enhancer is a bile salt. In some embodiments, the second permeation enhancer is a bile acid. In some embodiments, the composition further comprises an agent. In some embodiments, the agent is an active pharmaceutical ingredient. In some embodiments, the agent comprises semaglutide. In some embodiments, the agent comprises tirzepatide. In some embodiments, the composition further comprises one or more additional agents.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] FIG. 1A shows a schematic of the GSEL formulation mechanism and the GSEL delivery mechanism.

[0016] FIG. 1B shows a schematic of the GSEL formulation mechanism and the GSEL delivery mechanism.

[0017] FIG. 1C shows a schematic of the 96 well plate screen.

[0018] FIG. 1D shows the percent mean permeability of active pharmaceutical ingredient (API) semaglutide. Each data point represents the average permeability of a separate formulation. The short horizontal bar represents the mean. Data is normalized to the permeability of GSEL_40 and sorted by the mean permeability. The x-axis is labeled with the excipient abbreviation and concentration in mg/ml (e.g., CHA_80 indicates 80 mg/ml of CHA; CHA indicates Cholic Acid Sodium Salt). All formulations contained GSEL_40 (9.8 mg/mL dopamine HCl, 25 mg/mL PDA nanoparticles, 0.67 mg/mL hydrogen peroxide, 2.2 mg/mL Tris HCl, 4.3 mg/mL Tris base). Table E1 in Example 1 provides a key to the abbreviations used for the excipients.

[0019] FIG. 1E shows the percent mean permeability of active pharmaceutical ingredient (API) tirzepatide. P_m = (amount that crossed tissue) divided by (amount initially placed in the upper chamber), multiplied by 100. CV represents the coefficient of variation and is defined as: (standard deviation) divided by (mean), multiplied by 100. Each data point represents the average permeability of a separate formulation. The short horizontal bar represents the mean. Data is sorted by the mean permeability. The x-axis is labeled with the excipient abbreviation and concentration in mg/ml (e.g., CHA_80 indicates 80 mg/ml of CHA; CHA indicates Cholic Acid Sodium Salt). All formulations contained GSEL_40 (9.8 mg/mL dopamine HCl, 25 mg/mL PDA nanoparticles, 0.67 mg/mL hydrogen peroxide, 2.2 mg/mL Tris HCl, 4.3 mg/mL Tris base; values can vary by plus or minus 5%; and at pH 8.5). Table E1 in Example 1 provides a key to the abbreviations used for the excipients, and Table E2 reports the % P_m .

[0020] FIG. 1F shows the summary of values determined for each GSEL-single excipient combination. Active pharmaceutical ingredient (API) transported is semaglutide. Fold change GSEL (FC GSEL) measures the fold change of permeability of each formulation with excipient, and normalized by the permeability of GSEL only. Data is sorted by the mean permeability. The numerical value indicates excipient concentration (e.g., GSEL-GCA_40-40 indicates 40 mg/ml GSEL and 40 mg/ml sodium glycocholate). P_m = (amount that crossed tissue) divided by (amount initially placed in the upper chamber), multiplied by 100. CV represents the coefficient of variation and is defined as: (standard deviation) divided by (mean), multiplied by 100.

[0021] FIG. 1G shows the summary of values determined for each GSEL-single excipient combination. Active pharmaceutical ingredient (API) transported is tirzepatide. Fold change GSEL (FC GSEL) measures the fold change of permeability of each formulation with excipient, and normalized by the permeability of GSEL only. Data is sorted by the mean permeability. The value indicates excipient concentration. P_m = (amount that crossed tissue) divided by (amount initially placed in the upper chamber), multiplied by 100. CV represents the coefficient of variation and is defined as: (standard deviation) divided by (mean), multiplied by 100.

[0022] FIG. 1H shows the average fold change of gastrointestinal synthetic epithelial lining (GSEL) permeability, clustered by classes of excipients. Active pharmaceutical ingredient (API) transported is semaglutide. Fold change GSEL (FC GSEL) measures the fold change of permeability of each formulation with excipient, and normalized by the permeability of GSEL only. Each data point represents the average permeability of a separate

formulation. The short horizontal bar represents the mean. Data is sorted by the mean permeability of the classes. The value indicates excipient concentration (mg/ml).

[0023] FIG. 1I shows the average fold change of gastrointestinal synthetic epithelial lining (GSEL) permeability, clustered by classes of excipients. Active pharmaceutical ingredient (API) transported is tirzepatide. Fold change GSEL (FC GSEL) measures the fold change of permeability of each formulation with excipient, and normalized by the permeability of GSEL only. Each data point represents the average permeability of a separate formulation. The short horizontal bar represents the mean. Data is sorted by the mean permeability of the classes. The value indicates excipient concentration (mg/ml).

[0024] FIG. 1J shows the effect of excipient concentration on the percent mean permeability of active pharmaceutical ingredient (API) tirzepatide. Each data point represents the average permeability of a separate formulation. The short horizontal bar represents the mean. Data is normalized to the permeability of GSEL_40 and sorted by the mean permeability. The x-axis is labeled with the excipient abbreviation and concentration in mg/ml (e.g., CHA_80 indicates 80 mg/ml of CHA; CHA indicates Cholic Acid Sodium Salt). All formulations contained GSEL_40 (9.8 mg/mL dopamine HCl, 25 mg/mL PDA nanoparticles, 0.67 mg/mL hydrogen peroxide, 2.2 mg/mL Tris HCl, 4.3 mg/mL Tris base).

[0025] FIG. 1K shows the effect of excipient concentration on the percent mean permeability of active pharmaceutical ingredient (API) tirzepatide. Each data point represents the average permeability of a separate formulation. The short horizontal bar represents the mean. Data is normalized to the permeability of GSEL_40 and sorted by the mean permeability. The x-axis is labeled with the excipient abbreviation and concentration in mg/ml (e.g., CHA_80 indicates 80 mg/ml of CHA; CHA indicates Cholic Acid Sodium Salt). All formulations contained GSEL_40 (9.8 mg/mL dopamine HCl, 25 mg/mL PDA nanoparticles, 0.67 mg/mL hydrogen peroxide, 2.2 mg/mL Tris HCl, 4.3 mg/mL Tris base).

[0026] FIG. 2A shows the average fold change of gastrointestinal synthetic epithelial lining (GSEL) permeability for each combination of excipient. Active pharmaceutical ingredient (API) transported is tirzepatide. Percent Pm = (amount that crossed tissue) divided by (amount initially placed in the upper chamber), multiplied by 100. CV represents the coefficient of variation and is defined as: (standard deviation) divided by (mean), multiplied by 100. Each data point represents the average permeability of a separate formulation. The short horizontal bar represents the mean. Data is sorted by the mean permeability of the classes. The numerical value (e.g., 20, 40, 80) on the x-axis indicates excipient concentration (mg/ml).

[0027] FIG. 2B shows the average fold change of gastrointestinal synthetic epithelial lining (GSEL) permeability for each combination of excipient. Active pharmaceutical ingredient (API) transported is tirzepatide. Percent Pm = (amount that crossed tissue) divided by (amount initially placed in the upper chamber), multiplied by 100. CV represents the coefficient of variation and is defined as: (standard deviation) divided by (mean), multiplied by 100. Each data point represents the average permeability of a separate formulation. The short horizontal bar represents the mean. Data is sorted by the mean permeability of the classes. The numerical value (e.g., 20, 40, 80) on the x-axis indicates excipient concentration (mg/ml).

[0028] FIG. 2C shows the average fold change of gastrointestinal synthetic epithelial lining (GSEL) permeability for each excipient. Active pharmaceutical ingredient (API) transported is tirzepatide. Percent Pm = (amount that crossed tissue) divided by (amount initially placed in the upper chamber), multiplied by 100. CV represents the coefficient of variation and is defined as: (standard deviation) divided by (mean), multiplied by 100. Each data point represents the average permeability of a separate formulation. The short horizontal bar represents the mean. Data is sorted by the mean permeability of the classes. The numerical value (e.g., 20, 40, 80) on the x-axis indicates excipient concentration (mg/ml).

[0029] FIG. 2D shows the average fold change of gastrointestinal synthetic epithelial lining (GSEL) permeability for single excipients and combinations of excipients. Active pharmaceutical ingredient (API) transported is tirzepatide. Percent Pm = (amount that crossed tissue) divided by (amount initially placed in the upper chamber), multiplied by 100. CV represents the coefficient of variation and is defined as: (standard deviation) divided by (mean), multiplied by 100. Each data point represents the average permeability of a separate formulation. The short horizontal bar represents the mean. Data is sorted by the mean permeability of the classes. The numerical value (e.g., 20, 40, 80) on the x-axis indicates excipient concentration (mg/ml).

[0030] FIG. 3A shows the calculated degree of permeation (Pm) of active pharmaceutical ingredient (API) transported (semaglutide) by GSELs with combinations of a bile salt (CHA = Cholic Acid Sodium Salt; GCA = sodium glycocholate) with ammonium carbonate (NHCO) at various concentrations, as well as GSELs with only ammonium carbonate, ammonium carbonate without GSEL, and GSEL without permeation enhancer. Each data point represents the average permeability of a separate formulation. The short horizontal bar represents the mean. Data is sorted by the mean permeability. The numerical values (e.g., 20, 40, 80) on the x-axis indicate excipient concentrations (mg/ml).

[0031] FIG. 3B shows the calculated degree of permeation (P_m) of active pharmaceutical ingredient (API) transported (semaglutide) by GSELS with combinations of a bile salt (CHA = Cholic Acid Sodium Salt; GCA = sodium glycocholate) with ammonium carbonate (NHCO) at various concentrations, as well as GSELS with only ammonium carbonate, ammonium carbonate without GSEL, and GSEL without permeation enhancer. Each data point represents the average permeability of a separate formulation. The short horizontal bar represents the mean. Data is sorted by the mean permeability. The numerical values (e.g., 20, 40, 80) on the x-axis indicate excipient concentrations (mg/ml).

[0032] FIG. 3C shows the screening results of 50 combinations of bile salts with other excipients demonstrated a synergistic effect on semaglutide permeability, $n=4-8$.

[0033] FIG. 3D shows the results of a permeability test where GSEL is combined with bile salt CHA and different ammonium salts on Franz cells, where GSEL is composed of 6.25 mg PDA nanoparticles, 2.45 mg DA-HCl, with a 14.6:1 DA-HCl:H₂O₂ ratio and a 4 $\mu\text{L}/\text{mm}^2$ dispensing volume and the ammonium salts were tested at 40 mg/mL (mass 10 mg), GSEL concentration, $n=7-24$, Semaglutide was quantified using ELISA.

[0034] FIG. 3E shows the results of a permeability test where GSEL is combined with bile salt CHA and SNAC or other benzene ring-based excipients, where GSEL is composed of 6.25 mg PDA nanoparticles, 2.45 mg DA-HCl, with a 14.6:1 DA-HCl:H₂O₂ ratio and a 4 $\mu\text{L}/\text{mm}^2$ dispensing volume and the benzene ring-based excipients were tested at 40 mg/mL (mass 10 mg), $n=7-24$, Semaglutide was quantified using ELISA.

[0035] FIG. 4 shows the percent mean permeability (P_m) of active pharmaceutical ingredient (API) (semaglutide). Data is ordered by total mass of CHA (cholic acid sodium salt) and/or NHCO (ammonium carbonate). The numerical values (e.g., 20, 40, 80) on the x-axis indicate excipient concentrations (mg/ml).

[0036] FIG. 5 shows the percent mean permeability (P_m) of tirzepatide transported across porcine duodenal tissue 20 hours after delivery of GSEL as a liquid suspension. The numerical values (e.g., 20, 40, 80) on the x-axis indicate excipient concentrations (mg/ml).

[0037] FIG. 6A shows the FD4 permeation on Franz cells over time, GSEL and GSEL_CHA-20 had a significant statistical difference at 22 hours, $**P=0.029$, t-test Mann-Whitney test, Confidence level=95%, $n=7$.

[0038] FIG. 6B shows percent FITC-Dextran-40K (FD40) permeated at 20 hours after GSEL delivery of GSEL. The numerical values on the x-axis indicate excipient concentrations (mg/ml). The table summarizes the percent FITC-Dextran-40K (FD40) permeated at 6 hours and 20 hours after GSEL delivery.

[0039] FIG. 7A shows amount FITC-Dextran-4K (FD4) permeated (μg) over time after delivery of GSEL with or without various ratios of permeation enhancers (PE, where the permeation enhancer is a 1:1 combination of sodium glycocholate (GCA) and ammonium carbonate (NHCO), at 40 mg/ml each, i.e. GCA-NHCO_40-40). The values indicate excipient concentrations (mg/ml).

[0040] FIG. 7B shows amount FITC-Dextran-4K (FD4) permeated (μg) over time after delivery of various GSEL formulations. The values indicate excipient concentrations (mg/ml).

[0041] FIG. 7C shows a table summarizing the amount FITC-Dextran-4K (FD4) (μg) permeated over time after delivery of various GSEL formulations. The values indicate excipient concentrations (mg/ml).

[0042] FIG. 8 shows the percent mean permeability (P_m) of active pharmaceutical ingredient (API) (semaglutide). Porcine colon tissue is used as the barrier.

[0043] FIG. 9 shows the percent mean permeability of semaglutide in a Franz cell experiment comparing bile salts alone and in combination with other excipients as permeation enhancers.

[0044] FIG. 10A shows the percent mean permeability of semaglutide over twenty hours in a Franz cell test comparing a combination of sodium glycocholate (GCA) and ammonium carbonate (NHCO) versus a combination of sodium glycocholate (GCA) and sodium carbonate (NaCO) as a permeation enhancer.

[0045] FIG. 10B shows the amount (μg) of semaglutide over seven hours in a Franz cell test comparing a combination of sodium glycocholate (GCA) and ammonium carbonate (NHCO) versus a combination of sodium glycocholate (GCA) and sodium carbonate (NaCO) as a permeation enhancer. FIG. 10B is an expanded view of the initial hours of the graph shown in FIG. 10C.

[0046] FIG. 10C shows the amount (μg) of semaglutide permeated over twenty hours in a Franz cell test comparing a combination of sodium glycocholate (GCA) and ammonium carbonate (NHCO) versus a combination of sodium glycocholate (GCA) and sodium carbonate (NaCO) as a permeation enhancer. FIG. 10B is an expanded view of the initial hours of the graph shown in FIG. 10C.

[0047] FIG. 10D shows the percent mean permeability calculated for semaglutide permeated over twenty hours in a Franz cell test comparing a combination of sodium glycocholate (GCA) and ammonium carbonate (NHCO) versus a combination of sodium glycocholate (GCA) and sodium carbonate (NaCO) as permeation enhancers.

[0048] FIG. 11A shows the percent mean permeability of semaglutide in a comparison of various ammonium carbonate (NHCO) salts in combination with Cholic Acid Sodium Salt (CHA) as a permeation enhancer.

[0049] FIG. 11B shows the values of determined percent mean permeability of semaglutide in a comparison of various ammonium carbonate (NHCO) salts in combination with Cholic Acid Sodium Salt (CHA) as a permeation enhancer after 20 hours.

[0050] FIG. 12A shows the percent mean permeability of semaglutide in a comparison of bile salts taurocholic acid (TCA) and taurochenodeoxycholic acid (TCDCA) as a permeation enhancer.

[0051] FIG. 12B shows the values of determined percent mean permeability of semaglutide in a comparison of bile salts as a permeation enhancer after 20 hours.

[0052] FIG. 13A shows the percent mean permeability of semaglutide across duodenal tissue in a comparison of GCA and NHCO, or GCA, NHCO and GSEL as a permeation enhancer after 20 hours.

[0053] FIG. 13B shows the values of determined percent mean permeability of semaglutide across duodenal tissue in a comparison of GCA and NHCO, or GCA, NHCO and GSEL as permeation enhancers after 20 hours.

[0054] FIG. 14 shows that percent coverage of surface by GSEL does not increase significantly after 30 minutes of incubation. GSEL is composed of 10 mg PDA nanoparticles, 3.92 mg DA-HCl, with a 14.6:1 DA-HCl:H₂O₂ weight ratio and a 1.966 $\mu\text{L}/\text{mm}^2$ dispensing volume, n=4.

[0055] FIG. 15 shows a schematic for Franz cell experiments that test co-administration versus co-formulation.

[0056] FIG. 16 shows the percent dye (FITC-Dextran-4K) entrapped FITC-Dextran-4K on duodenum tissue with and without GSEL and/or CHA (as permeation enhancer), where the formulation is administered as a liquid (e.g., suspension) compared to solid formulation. ***P<0.0001 using one-way ANOVA. Confidence interval: 95% , n=4-8.

[0057] FIG. 17 shows the percent dye (FITC-Dextran-4K) permeated on duodenum tissue when administered as a liquid (e.g., suspension) compared to a solid formulation. * indicates p<0.05.

[0058] FIG. 18A shows a schematic of the colocalization assay where entrapment of FITC-dextran dyes is evaluated by measuring dye in the supernatant after the GSEL has formed on the surface of the tissue.

[0059] FIG. 18B shows the percent dye (FITC-Dextran 4K, FITC-Dextran 10K, or FITC-Dextran 40K) entrapped on porcine duodenal tissue in the presence of GSEL, or GSEL and permeation enhancer (CHA). Values were compared using 2-way ANOVA followed by Dunnett's multiple comparisons test. ***** indicates $p < 0.0001$. The table summarizes the percent of FITC-Dextran 4K, FITC-Dextran 10K, or FITC-Dextran 40K entrapped on the tissue in the presence of GSEL, or GSEL and permeation enhancer (CHA). Formulation was delivered as a liquid (e.g., suspension).

[0060] FIG. 19A shows a schematic depiction of permeability test on Franz cells, where receptor chamber fluid is collected to measure FITC-dextran dye that permeated through the tissue.

[0061] FIG. 19B shows the percent dye (FITC-Dextran 4K, FITC-Dextran 10K, or FITC-Dextran 40K) permeated through porcine duodenal in the presence of GSEL, or GSEL and permeation enhancer (CHA). Values were compared using 2-way ANOVA followed by Sidak's multiple comparisons test. ***** indicates $p < 0.0001$; *** indicates $p < 0.0002$. Formulation was delivered as a liquid (e.g., suspension). The table summarizes the percent of FITC-Dextran 4K, FITC-Dextran 10K, or FITC-Dextran 40K permeated through the tissue in the presence of GSEL, or GSEL and permeation enhancer (CHA). Formulation was delivered as a liquid (e.g., suspension).

[0062] FIG. 20A shows the results of a screen with bile salts with GSEL platform on Franz cells, where GSEL is composed of 6.25 mg PDA nanoparticles, 2.45 mg DA-HCl, with a 14.6:1 DA-HCl:H₂O₂ ratio and a 4 $\mu\text{L}/\text{mm}^2$ dispensing volume and CHA was tested at 40 mg/mL (mass 10 mg), $n=4-8$, Semaglutide was quantified using ELISA. Formulation was delivered as a liquid (e.g., suspension).

[0063] FIG. 20B shows the results of a permeability test where GSEL was combined with the bile salt CHA at increasing concentrations, and GSEL was composed of 6.25 mg PDA nanoparticles, 2.45 mg DA-HCl, with a 14.6:1 DA-HCl:H₂O₂ ratio and a 4 $\mu\text{L}/\text{mm}^2$ dispensing volume and CHA was tested in the range 40-160 mg/mL (mass 10-40 mg), $n=7-24$, Semaglutide was quantified using ELISA. Formulation was delivered as a liquid (e.g., suspension).

[0064] FIG. 21 shows the set up of a dynamic colocalization test conducted on porcine jejunal tissue (6 inches in length) at an angle of 17.92° for FD4 or semaglutide with and without GSEL and at different GSEL concentrations. The suspensions are dispensed at 3mL/min and the flow-through is collected to analyze how much FD4 or semaglutide does not colocalize.

[0065] FIG. 22A shows the results of a Franz cell permeability test where GSEL is combined with bile salt CHA and different ammonium salts on Franz cells, where GSEL is composed of 6.25 mg PDA nanoparticles, 2.45 mg DA-HCl, with a 14.6:1 DA-HCl:H₂O₂ ratio and a 4 $\mu\text{L}/\text{mm}^2$ dispensing volume and the ammonium salts were tested at 40 mg/mL (mass 10 mg), GSEL concentration, n=7-24, Semaglutide was quantified using ELISA.

[0066] FIG. 22B shows the results of a Franz cell permeability test where GSEL is combined with bile salt CHA and SNAC or other benzene ring-based excipients, where GSEL is composed of 6.25 mg PDA nanoparticles, 2.45 mg DA-HCl, with a 14.6:1 DA-HCl:H₂O₂ ratio and a 4 $\mu\text{L}/\text{mm}^2$ dispensing volume and the benzene ring-based excipients were tested at 40 mg/mL (mass 10 mg), n=7-24, Semaglutide was quantified using ELISA.

[0067] FIG. 22C shows the results of permeability tests on Franz cells where GSEL:permeation enhancer (PE):semaglutide (SEMA) (PE=GCA-NHCO) is compared against controls; semaglutide only, semaglutide with SNAC at 14:300, SEMA with GCA-NHCO, SEMA with GSEL, The GSEL composition is composed of 6.25 mg PDA nanoparticles, 2.45 mg DA-HCl, with 14.6:1 DA-HCl:H₂O₂ ratio and 4 $\mu\text{L}/\text{mm}^2$ dispensing volume, GCA and NHCO are tested at 40 mg/mL (mass 10 mg). ****P<0.0001, using one-way ANOVA, Confidence interval: 95%, n=8.

[0068] FIG. 22D shows the results of permeability tests on Franz cells where 1xGSEL includes 6.25 mg PDA nanoparticles and 2.45 mg DA-HCl, with 14.6:1 DA-HCl:H₂O₂ ratio, 2 $\mu\text{L}/\text{mm}^2$ dispensing volume and PE mass (GCA+NHCO) is 2, 10, 20, or 40 mg, **P=0.0016, ****P<0.0001, using one-way ANOVA, Confidence interval: 95%, n=22.

[0069] FIG. 22E shows the results of permeability tests on Franz cells where PE mass is 20 mg (10 mg GCA+ 10mg NHCO) and PDA nanoparticles and DA-HCl masses change (1x= PDA nanoparticles 6.25 mg and DA-HCl=2.45 mg, 2x= PDA nanoparticles 12.5 mg, DA-HCl= 4.9 mg, 4x= PDA nanoparticles 25 mg, DA-HCl= 9.8mg, 14.6:1 DA-HCl:H₂O₂ ratio, 2 $\mu\text{L}/\text{mm}^2$ dispensing volume), P=0.6906, *P=0.0498, using one-way ANOVA, Confidence interval: 95%, n=8-12.

[0070] FIG. 22F shows dynamic colocalization tests conducted on porcine jejunal tissue for FD4 with and without GSEL and at different GSEL concentrations where permeation enhancer (PE): 40 mg/mL GCA+ 40 mg/mL NHCO; and the permeation enhancer: semaglutide ratio = 20:0.5; PDA 25 mg/mL and DA-HCl=9.8 mg/mL for 1xGSEL; PDA nanoparticles 50 mg/mL and DA-HCl= 19.6 mg/mL for 2xGSEL, 14.6:1 DA-HCl:H₂O₂ ratio, 3 mL through 6 inches tissue over 1 minute at 17.92°, ****P<0.0001 using one-way ANOVA, Confidence interval: 95%, n=3.

[0071] FIG. 22G shows static colocalization tests on porcine duodenum tissue for FD4, PE 20 mg (10 mg GCA+ 10mg NHCO), PDA nanoparticles 6.25 mg and DA-HCl=2.45 mg for 1xGSEL, PDA nanoparticles 12.5 mg and DA-HCl= 4.9 mg for 2xGSEL, 14.6:1 DA-HCl:H₂O₂ ratio, 2 μ L/mm² dispensing volume, ***P=0.0004, ****P<0.0001, using one-way ANOVA, Confidence interval: 95%, n=4.

[0072] FIG. 22H shows the results of a dynamic colocalization test on porcine jejunal tissue, for semaglutide with and without GSEL and at different permeation enhancer (PE) concentrations where 1xPE (40 mg/mL GCA+ 40 mg/mL NHCO), or 0.1xPE (4 mg/mL GCA+ 4 mg/mL NHCO), PDA nanoparticles 50 mg/mL and DA-HCl=19.6 mg/mL, 14.6:1 DA-HCl:H₂O₂ ratio, 3 mL through 6 inches of tissue over 1 minute at 17.92°, n=2.

[0073] FIG. 22I shows the porcine jejunal tissue after the dynamic colocalization test using FD4.

[0074] FIG. 22J shows the permeability of semaglutide (mass, ug) on Franz cells for different permeation enhancer: semaglutide ratios (20:0.5, 20:1, 20: 2, 20:4, 20:8) for 2xGSEL (PDA nanoparticles 12.5 mg, DA-HCl= 4.9 mg, 14.6:1 DA-HCl:H₂O₂ ratio, 2 μ L/mm² dispensing volume), and 1x permeation enhancer (PE) (GCA 10mg, NHCO 10mg (total PE 20mg)), and semaglutide is increased **P=0.0044, ****P<0.0001 using one-way ANOVA, confidence interval: 95%, n=8-9. The permeation enhancer:semaglutide ratio was changed by modulating the amount of semaglutide inside.

[0075] FIG. 22K shows the results of a static colocalization test on porcine duodenum tissue for semaglutide, where the total mass of permeation enhancer used is 20 mg (10 mg GCA+ 10mg NHCO), permeation enhancer : semaglutide ratio is 20:2, PDA nanoparticles 6.25 mg and DA-HCl=2.45 mg for 1xGSEL, PDA nanoparticles 12.5 mg and DA-HCl= 4.9 mg for 2xGSEL, 14.6:1 DA-HCl:H₂O₂ ratio, 2 μ L/mm² dispensing volume, *P=0.0155, using one-way ANOVA, Confidence interval: 95%, n=4-10.

[0076] FIG. 22L shows the results of the dynamic colocalization test on porcine jejunal tissue for semaglutide, PE: 40 mg/mL GCA+ 40 mg/mL NHCO), PE:SEMA= 20:0.5, PDA nanoparticles 25 mg/mL and DA-HCl=9.8 mg/mL for 1xGSEL, PDA nanoparticles 50 mg/mL and DA= 19.6 mg/mL for 2xGSEL, 14.6:1 DA-HCl:H₂O₂ ratio, 4x GSEL= 100 mg/ml PDA, DA-HCl= 39.2 mg/ml 14.6:1 DA-HCl:H₂O₂ ratio; 3 mL through 6 inches of tissue over 1 minute at 17.92°, **P=0.0286, using unpaired two-tailed t-test, no significant difference using one-way ANOVA, Confidence interval: 95%, n=4.

[0077] FIG. 22M shows the results of a Franz cell permeability of semaglutide (%) on Franz cells for different permeation enhancer: semaglutide ratios for 2xGSEL, for different

permeation enhancer: semaglutide ratios (20:0.5, 20:1, 20:2, 20:4, 20:8) where 2xGSEL is composed of PDA nanoparticles 12.5 mg, DA-HCl= 4.9 mg, 14.6:1 DA- HCl:H₂O₂ ratio, and 1xPE (GCA 10 mg, NHCO 10 mg (total PE 20 mg)), 2 μ L/mm² dispensing volume, no significant change for any of the groups, using one-way ANOVA, Confidence interval: 95%, n=8.

[0078] FIG. 23A shows an example of endoscopic delivery of the suspension *in vivo*.

[0079] FIG. 23B shows the pharmacokinetic curve for intravenous administration of 1 mg of semaglutide per pig. N=2.

[0080] FIG. 23C shows the pharmacokinetic curves for the control groups (e.g., 1x permeation enhancer alone) and experimental groups that received formulation by endoscopic placement to the duodenum (2x GSEL combined with 1x permeation enhancer). Graph shows the bioavailabilities achieved for the Control (1xPE) and 2xGSEL_1xPE formulations for 168 h studies. *P=0.0316, using non-parametric t-test, Confidence interval: 95%, n=4-5.

[0081] FIG. 23D shows the AUC values for up to 24 hours for control, (semaglutide alone), Control (1x permeation enhancer alone), 1xGSEL combined with 1x permeation enhancer (1xGSEL_1xPE) and 2x GSEL combined with 1x permeation enhancer (2xGSEL_1xPE). 1xPE corresponds to 6.4 mg/kg GCA and 6.4 mg/kg NHCO, 1xGSEL includes 4.55 mg/kg PDA nanoparticles and 1.8 mg/kg DA-HCl, 2xGSEL includes 9.1 mg/kg PDA nanoparticles and 3.6 mg/kg DA-HCl.

[0082] FIG. 23E shows the AUC values for up to 48 hours for control, (semaglutide alone), Control (1x permeation enhancer alone), 1xGSEL combined with 1x permeation enhancer (1xGSEL_1xPE) and 2x GSEL combined with 1x permeation enhancer (2xGSEL_1xPE). 1xPE corresponds to 6.4 mg/kg GCA and 6.4 mg/kg NHCO, 1xGSEL includes 4.55 mg/kg PDA nanoparticles and 1.8 mg/kg DA-HCl, 2xGSEL includes 9.1 mg/kg PDA nanoparticles and 3.6 mg/kg DA-HCl.

[0083] FIG. 23F shows the AUC values for up to 72 hours for control, (semaglutide alone), Control (1x permeation enhancer alone), 1xGSEL combined with 1x permeation enhancer (1xGSEL_1xPE) and 2x GSEL combined with 1x permeation enhancer (2xGSEL_1xPE). 1xPE corresponds to 6.4 mg/kg GCA and 6.4 mg/kg NHCO, 1xGSEL includes 4.55 mg/kg PDA nanoparticles and 1.8 mg/kg DA-HCl, 2xGSEL includes 9.1 mg/kg PDA nanoparticles and 3.6 mg/kg DA-HCl.

[0084] FIG. 23G shows the AUC values for up to 168 hours for control, (semaglutide alone), Control (1x permeation enhancer alone), 1xGSEL combined with 1x permeation enhancer (1xGSEL_1xPE) and 2x GSEL combined with 1x permeation enhancer

(2xGSEL_1xPE). 1xPE corresponds to 6.4 mg/kg GCA and 6.4 mg/kg NHCO, 1xGSEL includes 4.55 mg/kg PDA nanoparticles and 1.8 mg/kg DA-HCl, 2xGSEL includes 9.1 mg/kg PDA nanoparticles and 3.6 mg/kg DA-HCl.

[0085] FIG. 24 shows AUC values up to 48h for different formulations with varying GSEL concentrations.

[0086] FIG. 25 shows the pharmacokinetic curves for Control (1xPE), Control (2xPE), and formulation groups with different GSEL concentrations and GSEL:PE ratios.

[0087] FIG. 26A shows the results from an experiment where pigs were dosed with Rybelsus® (1 mg/kg semaglutide) by endoscopically placing Rybelsus® tablets (1 mg/kg total semaglutide) into the stomach. Plasma semaglutide results are shown for the two pigs dosed.

[0088] FIG. 26B shows the Rybelsus® results overlayed over the Control (semaglutide + permeation enhancer) and two GSEL:PE formulations (1.5 mg/kg semaglutide). The control curve is the lowest curve on the figure.

[0089] FIG. 26C shows the bioavailabilities for each group demonstrates a ~43-70% improvement in bioavailability of the GSEL:PE formulation groups compared to the Rybelsus® group.

DETAILED DESCRIPTION OF THE INVENTION

[0090] The present disclosure relates to synthetic linings formed *in situ* in the gastrointestinal tract, such as in the small intestine. Such a lining is called a Gastrointestinal Synthetic Epithelial Lining (GSEL). A GSEL is a temporary polymeric lining that rests against (i.e., contacts) the internal surface of the gastrointestinal tract. For example, dopamine can be polymerized in the small intestine to form a GSEL coating in the interior of the duodenum, jejunum, or ileum. The present disclosure describes the use of GSELs in combination with an agent and synergistic combinations of permeation enhancers, where the permeation enhancers synergistically increase the absorption of the agent across the intestinal tissue.

[0091] GSELs can be used to localize an active pharmaceutical ingredient (API) in a specific location, resulting in prolonged contact with the gastrointestinal mucosa. Due to the prolonged contact with the mucosa, the API can be delivered over an extended period, which can be advantageous for APIs that are absorbed slowly, or which are best administered over a lengthy time period. A GSEL can permit a higher percentage of an API to be absorbed as compared to a standard dosage form, and this higher percentage of absorption with a GSEL

can allow attainment of similar total API absorption with administration of a lower dosage of API. A GSEL can also reduce the frequency of administration of an API by provided for extended release of API, as compared to multiple administrations of a conventional dose formulation over a period of time.

[0092] Despite the prolonged contact afforded by a GSEL, some APIs may not permeate through the gastrointestinal barrier in sufficient amounts to be therapeutically effective, or may not permeate at all. The present disclosure describes synergistic permeation enhancers for use in GSELs which enhance the permeation of an active pharmaceutical ingredient (API) across the gastrointestinal mucosa, as compared to GSELs having only a single permeation enhancer, GSELs with non-synergistic combinations of permeation enhancers, or GSELs lacking a permeation enhancer. GSELs with such synergistic combinations of permeation enhancers can provide for efficient and effective administration of APIs via enteral administration.

[0093] In order to form a GSEL, a GSEL composition is administered to a subject orally. The composition can alternatively be administered by feeding tube, for example, in subjects with impaired swallowing ability; or, if necessary, the composition can be administered using an endoscope to deliver the composition to the small intestine, stomach, or esophagus. The GSEL composition for administration comprises a polymer precursor (e.g., a monomer, oligomer, or other prepolymer, such as dopamine, dopamine HCl, dopamine oligomers, or dopamine prepolymers). This polymer precursor is polymerized to form the GSEL. The GSEL composition for administration also comprises an oxygen source (such as hydrogen peroxide or urea hydrogen peroxide), which will provide oxygen at the appropriate location in the gastrointestinal tract to cause polymerization of the polymer precursor. The GSEL composition for administration also comprises a synergistic combination of permeation enhancers as described herein (such as sodium glycocholate and ammonium carbonate), to increase the permeation of the active pharmaceutical ingredient across the gastrointestinal barrier. The composition relies on one or more catalysts endogenous to the gastrointestinal tract to convert the oxygen precursor to oxygen. Liberation of oxygen in turn leads to polymerization of the polymer precursor. The small intestine contains endogenous catalysts with peroxidase activity such as catalase, which enables liberation of oxygen, resulting in formation and deposition of the GSEL in the specific anatomical location where the endogenous catalyst is located. The GSEL composition for administration can also include an active pharmaceutical ingredient (API). Formation of the GSEL with its associated API thus localizes the API and the synergistic combination of permeation enhancers to the

vicinity of the polymer. In this manner, a depot of the API and the synergistic combination of permeation enhancers can be retained at a specific anatomical location over an extended period of time for absorption at that location. For example, when the GSEL containing API and the synergistic combination of permeation enhancers forms in the small intestine, the GSEL keeps the API in proximity to the intestinal epithelium for an extended period. In contrast, when an API is administered as a tablet which dissolves in the intestinal tract, without a GSEL, the API may transit the small intestine much more rapidly, leading to lower absorption of API, or requiring much more API to be administered to obtain equivalent absorption.

[0094] Alternatively, the API can be administered in a separate composition from the GSEL precursor composition. If the API is administered in a separate composition, it can be administered before administration of the GSEL precursor composition, concurrently with administration of the GSEL precursor composition, or subsequent to administration of the GSEL precursor composition.

[0095] The GSEL with the combination of synergistic permeation enhancers aids the API in crossing the gastrointestinal barrier, leading to increased permeation of the API across the gastrointestinal barrier in the presence of the synergistic permeation enhancers compared to permeation of the API using a GSEL without permeation enhancer, or the API with a single permeation enhancer or non-synergistic combinations of permeation enhancers. Even if the API is administered separately from the GSEL and the synergistic permeation enhancers, enhanced permeation of the API can occur when the API traverses the portion of the small intestine coated by the GSEL with the synergistic permeation enhancers.

[0096] The components of the GSEL composition for administration are discussed in more detail herein, as follows: 1) polymer precursors, 2) oxygen sources, and 3) synergistic combinations of permeation enhancers. GSELs and GSEL compositions for administration may also contain 4) one or more active pharmaceutical ingredients (APIs). A GSEL or GSEL composition for administration may not contain any API, in which case the one or more APIs can be administered separately. Alternatively, a GSEL or GSEL composition for administration may contain one or more APIs, and one or more APIs can optionally also be administered separately from the GSEL. Also discussed are endogenous catalysts which allow for formation of the GSEL *in situ*.

Definitions

[0097] An “agent” is any substance intended for therapeutic, diagnostic, or nutritional use in a patient, individual, or subject. Agents include, but are not limited to, drugs, nutrients, vitamins, and minerals. An “active pharmaceutical ingredient” (API) is an agent which is the component in a pharmaceutical dosage that produces the desired biological effect on a patient, individual, or subject to whom the dosage is administered.

[0098] An “excipient” is any substance added to a formulation of an agent that is not the agent itself. Excipients include, but are not limited to, binders, coatings, diluents, disintegrants, emulsifiers, flavorings, glidants, lubricants, and preservatives.

[0099] A “patient,” “individual,” or “subject” refers to a human, or a non-human animal. Non-human animals include mammals, including domestic animals such as a dog or cat; or an animal used in commerce, such as a cow, pig, horse, sheep, or goat. In a preferred embodiment, a patient, individual, or subject is a human.

[0100] “Treating” a disease or disorder with the compositions and methods disclosed herein is defined as administering one or more of the compositions disclosed herein to a patient in need thereof, with or without additional agents, in order to reduce or eliminate either the disease or disorder, or one or more symptoms of the disease or disorder, or to suppress symptoms of the disease or disorder, or to retard the progression of the disease or disorder or of one or more symptoms of the disease or disorder, or to reduce the severity of the disease or disorder or of one or more symptoms of the disease or disorder. Treatment can commence after emergence of symptoms of a disease or disorder, or can commence before emergence of symptoms of a disease or disorder. Treatment can also be continued after symptoms have been resolved.

[0101] “Therapeutic use” of the compositions disclosed herein is defined as using one or more of the compositions disclosed herein to treat a disease or disorder, as defined above. A “therapeutically effective amount” of a therapeutic agent, such as a drug, is an amount of the agent, which, when administered to a patient, individual, or subject, is sufficient to achieve the desired biological result, such as an amount sufficient to reduce or eliminate either a disease or disorder or one or more symptoms of a disease or disorder, or to retard the progression of a disease or disorder or of one or more symptoms of a disease or disorder, or to reduce the severity of a disease or disorder or of one or more symptoms of a disease or disorder. A therapeutically effective amount can be administered to a patient, individual, or subject as a single dose, or can be divided and administered as multiple doses.

[0102] “Permeation” of an agent or of an active pharmaceutical ingredient (API) across a barrier refers to the amount of the agent or API able to cross the barrier.

[0103] A “permeation enhancer” refers to a substance which is able to increase the permeation of an agent or an active pharmaceutical ingredient across a barrier.

[0104] The term “polymer” refers to a compound comprising eleven or more covalently connected repeating units.

[0105] The term “oligomer” refers to a compound comprising between two to ten covalently connected repeating units.

[0106] The unit “kD” refers to kilodaltons.

[0107] As used herein, the singular forms “a”, “an”, and “the” include plural references unless indicated otherwise or the context clearly dictates otherwise.

[0108] When numerical values are expressed herein using the term “about” or the term “approximately,” it is understood that both the value specified, as well as values reasonably close to the value specified, are included. For example, the description “about 50° C” or “approximately 50° C” includes both the disclosure of 50° C itself, as well as values close to 50° C. Thus, the phrases “about X” or “approximately X” include a description of the value X itself. If a range is indicated, such as “approximately 50° C to 60° C” or “about 50° C to 60° C,” it is understood that both the values specified by the endpoints are included, and that values close to each endpoint or both endpoints are included for each endpoint or both endpoints; that is, “approximately 50° C to 60° C” (or “about 50° C to 60° C”) is equivalent to reciting both “50° C to 60° C” and “approximately 50° C to approximately 60° C” (or “about 50° C to 60° C”).

[0109] With respect to numerical ranges disclosed in the present description, any disclosed upper limit for a component may be combined with any disclosed lower limit for that component to provide a range (provided that the upper limit is greater than the lower limit with which it is to be combined). Each of these combinations of disclosed upper and lower limits are explicitly envisaged herein. For example, if ranges for the amount of a particular component are given as 10% to 30%, 10% to 12%, and 15% to 20%, the ranges 10% to 20% and 15% to 30% are also envisaged, whereas the combination of a 15% lower limit and a 12% upper limit is not possible and hence is not envisaged.

[0110] Unless otherwise specified, percentages of ingredients in compositions are expressed as weight percent, or weight/weight percent. It is understood that reference to relative weight percentages in a composition assumes that the combined total weight percentages of all components in the composition add up to 100. It is further understood that

relative weight percentages of one or more components may be adjusted upwards or downwards such that the weight percent of the components in the composition combine to a total of 100, provided that the weight percent of any particular component does not fall outside the limits of the range specified for that component.

[0111] Some embodiments described herein are recited as “comprising” or “comprises” with respect to their various elements. In alternative embodiments, those elements can be recited with the transitional phrase “consisting essentially of” or “consists essentially of” as applied to those elements. In further alternative embodiments, those elements can be recited with the transitional phrase “consisting of” or “consists of” as applied to those elements. Thus, for example, if a composition or method is disclosed herein as comprising A and B, the alternative embodiment for that composition or method of “consisting essentially of A and B” and the alternative embodiment for that composition or method of “consisting of A and B” are also considered to have been disclosed herein. Likewise, embodiments recited as “consisting essentially of” or “consisting of” with respect to their various elements can also be recited as “comprising” as applied to those elements. Finally, embodiments recited as “consisting essentially of” with respect to their various elements can also be recited as “consisting of” as applied to those elements, and embodiments recited as “consisting of” with respect to their various elements can also be recited as “consisting essentially of” as applied to those elements.

[0112] When a composition or system is described as “consisting essentially of” the listed elements, the composition or system contains the elements expressly listed, and may contain other elements which do not materially affect the condition being treated (for compositions for treating conditions), or the properties of the described system (for compositions comprising a system). However, the composition or system either does not contain any other elements which do materially affect the condition being treated other than those elements expressly listed (for compositions for treating systems) or does not contain any other elements which do materially affect the properties of the system (for compositions comprising a system); or, if the composition or system does contain extra elements other than those listed which may materially affect the condition being treated or the properties of the system, the composition or system does not contain a sufficient concentration or amount of those extra elements to materially affect the condition being treated or the properties of the system. When a method is described as “consisting essentially of” the listed steps, the method contains the steps listed, and may contain other steps that do not materially affect the condition being treated by the method or the properties of the system produced by the

method, but the method does not contain any other steps which materially affect the condition being treated or the system produced other than those steps expressly listed.

[0113] This disclosure provides several embodiments. It is contemplated that any features from any embodiment can be combined with any features from any other embodiment where possible. In this fashion, hybrid configurations of the disclosed features are within the scope of the present disclosure.

Gastrointestinal Synthetic Epithelial Linings (GSELs) for Administration of Agents

[0114] The gastrointestinal synthetic epithelial lining (GSEL) precursor compositions for formation of the GSEL comprise 1) a polymer precursor; 2) an oxygen source; and 3) a synergistic combination of permeation enhancers. In one embodiment, each component of the composition can be administered as a separate dosage form, to combine in the gastrointestinal tract of a subject. In one embodiment, the polymer precursor and oxygen source can be administered together in one dosage form, and the synergistic combination of permeation enhancers can be administered in a separate dosage form. In one embodiment, the polymer precursor, oxygen source, synergistic combination of permeation enhancers can be administered together in one dosage form.

[0115] The GSEL precursor composition can also comprise 4) an agent, such as an active pharmaceutical ingredient (API); or the GSEL precursor composition can comprise multiple agents such as multiple APIs. As with the polymer precursor, oxygen source, and synergistic combination of permeation enhancers, the agent can be administered together with the other components of the GSEL precursor composition. Alternatively, an API (or multiple APIs) can be administered in a separate composition, either before administration of the other components of the GSEL precursor composition, concurrently with administration of the other components of the GSEL precursor composition, or subsequent to administration of the other components of the GSEL precursor composition.

Polymer Precursors for Gastrointestinal Synthetic Epithelial Lining Precursor Compositions

[0116] In one aspect, the disclosure provides a method of forming a polymer *in situ* in a subject, the method comprising administering to a subject a composition comprising a polymer precursor, an oxygen source, and a combination of synergistic permeation enhancers, wherein the polymer precursor and the oxygen source contact a catalyst endogenous to the subject and the catalyst polymerizes the polymer precursor.

[0117] In some embodiments, polymerization of the polymer precursor is catalyzed when the oxygen source and polymer precursor contact the endogenous catalyst. In some embodiments, polymerization of the polymer precursor is catalyzed when the oxygen source and polymer precursor contact a peroxidase in the small intestine. In some embodiments, polymerization of the polymer precursor is catalyzed when the oxygen source and polymer precursor contact a peroxidase in the duodenum.

[0118] In some embodiments, the polymer precursor is a catechol-based polymer precursor. In some embodiments, the polymer precursor comprises a 1,2-dihydroxybenzene moiety. In some embodiments, the polymer precursor comprises an optionally substituted 1,2-dihydroxybenzene moiety. In some embodiments, the polymer precursor comprises a 1,2-dihydroxyphenyl moiety. In some embodiments, the polymer precursor comprises an optionally substituted 1,2-dihydroxyphenyl moiety.

[0119] In some embodiments, the polymer precursor is a 2-(3,4-dihydroxyphenyl)ethylamine-based polymer precursor. (2-(3,4-dihydroxyphenyl)ethylamine is commonly known as dopamine.) In certain embodiments, the polymer precursor comprises a 3,4-dihydroxyphenethylamine moiety. In certain embodiments, the polymer precursor comprises an optionally substituted 3,4-dihydroxyphenethylamine moiety. In certain embodiments, the polymer precursor comprises 2-(3,4-dihydroxyphenyl)ethylamine. In certain embodiments, the polymer precursor comprises an optionally substituted 2-(3,4-dihydroxyphenyl)ethylamine moiety.

[0120] In some embodiments, the polymer precursor is selected from the group consisting of dopamine, levodopa, norepinephrine, methyl dopa, levodopa methyl ester, levodopa ethyl ester, derivatives thereof, and combinations thereof. In certain embodiments, the polymer precursor is selected from the group consisting of dopamine, levodopa, norepinephrine, methyl dopa, levodopa methyl ester, and levodopa ethyl ester.

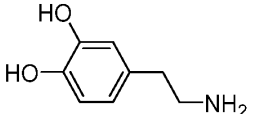
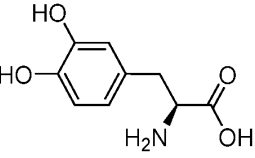
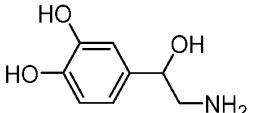
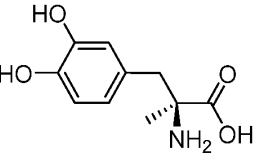
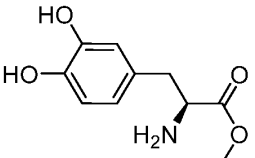
[0121] In certain embodiments, the polymer precursor is a derivative of dopamine. In some embodiments, the polymer precursor comprises dopamine, levodopa, norepinephrine, methyl dopa, levodopa methyl ester, or levodopa ethyl ester.

[0122] In some embodiments, the derivative of the polymer precursor comprises a macromolecule. In some embodiments, the polymer precursor comprises dopamine, levodopa, norepinephrine, methyl dopa, levodopa methyl ester, or levodopa ethyl ester and at least one macromolecule. In certain embodiments the macromolecule is alginate, hyaluronic acid, polyacrylic acid, polyethylene glycol, chondroitin sulfate, chitosan, or a combination thereof. In some embodiments the macromolecule is alginate, hyaluronic acid, polyacrylic

acid, polyethylene glycol, chondroitin sulfate, or chitosan. In certain embodiments the polymer precursor comprises (A) dopamine, levodopa, norepinephrine, methyl dopa, levodopa methyl ester, or levodopa ethyl ester moieties bound to (B) alginate, hyaluronic acid, polyacrylic acid, polyethylene glycol, chondroitin sulfate, or chitosan. In certain embodiments the polymer precursor comprises (A) more than one of dopamine, levodopa, norepinephrine, methyl dopa, levodopa methyl ester, and levodopa ethyl ester moieties bound to (B) alginate, hyaluronic acid, polyacrylic acid, polyethylene glycol, chondroitin sulfate, or chitosan. In certain embodiments the polymer precursor comprises (A) more than one of dopamine, levodopa, norepinephrine, methyl dopa, levodopa methyl ester, and levodopa ethyl ester moieties bound to (B) more than one of alginate, hyaluronic acid, polyacrylic acid, polyethylene glycol, chondroitin sulfate, and chitosan.

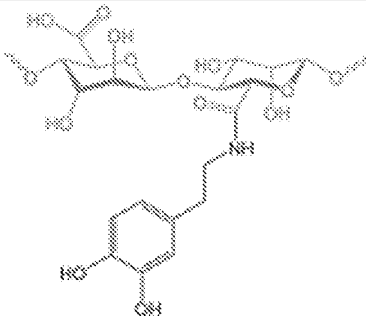
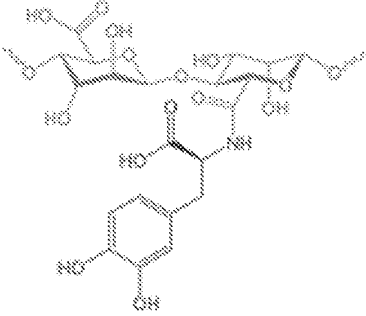
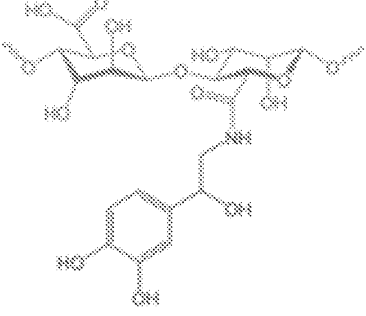
[0123] In certain embodiments, the polymer precursor is selected from the structures listed in Table 1 or 2.

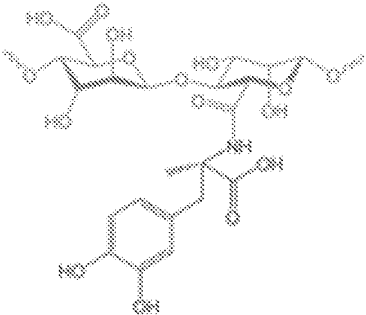
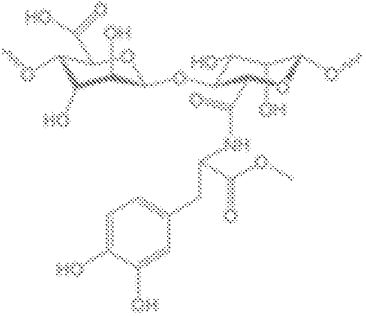
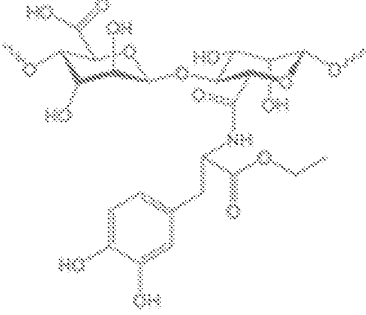
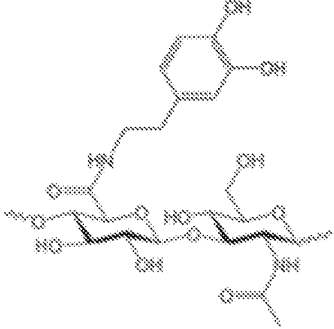
Table 1: Polymer precursors

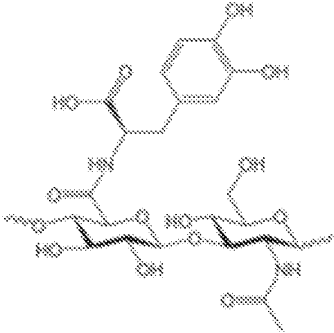
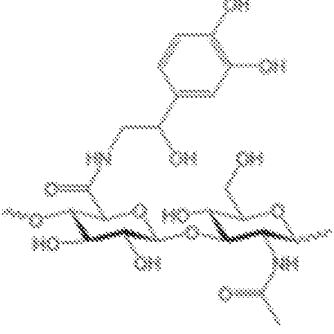
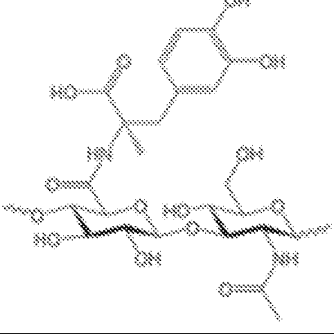
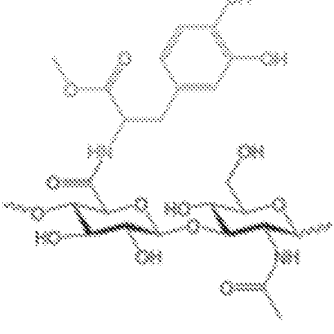
Polymer precursor	Structure
M1	
M2	
M3	
M4	
M5	

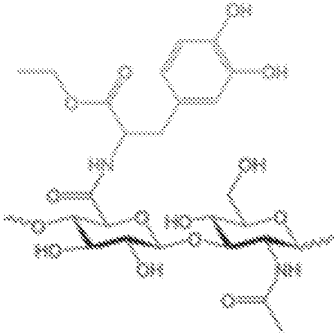
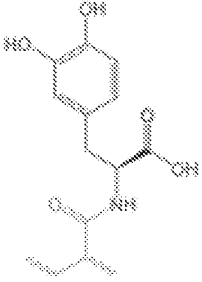
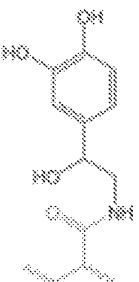
Polymer precursor	Structure
M6	

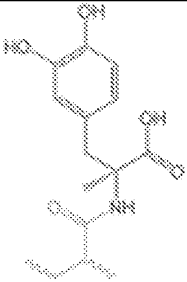
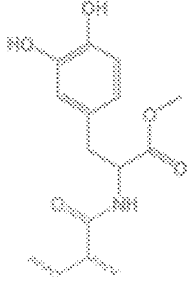
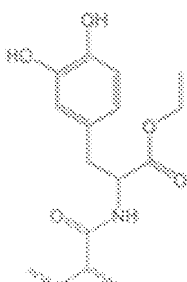
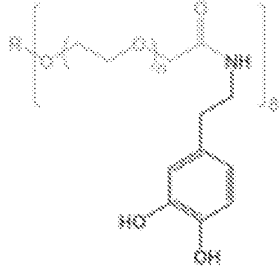
Table 2: Macromolecules for Use as Polymer Precursors

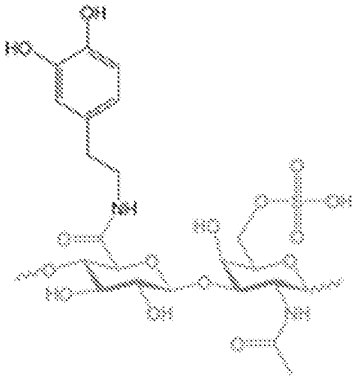
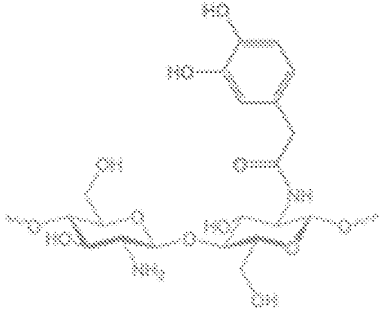
Macromolecule	Components	Structure
C1	M1 and alginate	
C2	M2 and alginate	
C3	M3 and alginate	

Macromolecule	Components	Structure
C4	M4 and alginate	
C5	M5 and alginate	
C6	M6 and alginate	
C7	M1 and hyaluronic acid	

Macromolecule	Components	Structure
C8	M2 and hyaluronic acid	
C9	M3 and hyaluronic acid	
C10	M4 and hyaluronic acid	
C11	M5 and hyaluronic acid	

Macromolecule	Components	Structure
C12	M6 and hyaluronic acid	
C13	M1 and polyacrylic acid	
C14	M2 and polyacrylic acid	
C15	M3 and polyacrylic acid	

Macromolecule	Components	Structure
C16	M4 and polyacrylic acid	
C17	M5 and polyacrylic acid	
C18	M6 and polyacrylic acid	
C19	M1 and polyethylene with tripentaerythritol core	 R=tripentaerythritol core structure

Macromolecule	Components	Structure
C20	M1 and chondroitin sulfate	
C21	M1 and chitosan	

[0124] In some embodiments, the polymer precursor comprises a single type of polymer precursor. In certain embodiments, the polymer precursor comprises a combination of polymer precursors. In certain embodiments, the polymer precursor comprises a combination of polymer precursors listed in Tables 1 and 2. In some embodiments, the combination of polymer precursors consists of two different polymer precursors. In some embodiments, the combination of polymer precursors consists of three different polymer precursors. In some embodiments, the combination of polymer precursors consists of four different polymer precursors. In some embodiments, the composition comprises an amount of polymer precursor to (polymer precursor, oxygen source, and permeation enhancer) of 40% to 90%. In some embodiments, the polymer precursor is dopamine (which may be provided in the form of dopamine HCl or another pharmaceutically acceptable salt of dopamine). In some embodiments, the composition comprises about 0.001 to about 1000 mg/mL of dopamine. In some embodiments, the composition comprises about 0.001 to about 500 mg/mL of dopamine. In some embodiments, the composition comprises about 0.01 to about 100 mg/mL of dopamine. In some embodiments, the composition comprises about 1 to about 100 mg/mL of dopamine. In some embodiments, the composition comprises about 1 to about 50 mg/mL

of dopamine. In some embodiments, the composition comprises about 10 to about 40 mg/mL of dopamine. In some embodiments, the composition comprises about 1 to about 20 mg/mL of dopamine. In some embodiments, the composition comprises about 60 mg/mL of dopamine. In some embodiments, the composition comprises about 50 mg/mL of dopamine. In some embodiments, the composition comprises about 40 mg/mL of dopamine. In some embodiments, the composition comprises about 30 mg/mL of dopamine. In some embodiments, the composition comprises about 20 mg/mL of dopamine. In some embodiments, the composition comprises about 10 mg/mL of dopamine. In some embodiments, the composition comprises about 9.8 mg/mL of dopamine. In some embodiments, the composition comprises about 18 mg/ml to about 20 mg/ml of dopamine. In some embodiments, the composition comprises about 18.5 mg/ml of dopamine. In some embodiments, the composition comprises about 19 mg/ml of dopamine. In some embodiments, the composition comprises about 19.5 mg/ml of dopamine. In some embodiments, the composition comprises about 19.6 mg/ml of dopamine. In some embodiments, the composition comprises about 20 mg/ml of dopamine. The amount of dopamine that the composition comprises can also be expressed as a weight-based dosage, such as mg/kg, wherein the amount of dopamine administered is determined by the body weight of the subject receiving the composition. In some embodiments, the composition comprises about 1 mg/kg to about 9 mg/kg of dopamine. In some embodiments, the composition comprises about 3 mg/kg to about 10 mg/kg of dopamine. In some embodiments, the composition comprises about 2 mg/kg to about 9 mg/kg of dopamine. In some embodiments, the composition comprises about 3 mg/kg to about 9 mg/kg of dopamine. In some embodiments, the composition comprises about 4 mg/kg to about 9 mg/kg of dopamine. In some embodiments, the composition comprises about 5 mg/kg to about 9 mg/kg of dopamine. In some embodiments, the composition comprises about 6 mg/kg to about 9 mg/kg of dopamine. In some embodiments, the composition comprises about 7 mg/kg to about 9 mg/kg of dopamine. In some embodiments, the composition comprises about 4 mg/kg to about 6 mg/kg of dopamine. In some embodiments, the composition comprises about 8 mg/kg to about 9 mg/kg of dopamine. In some embodiments, the composition comprises about 2 mg/kg to about 3 mg/kg of dopamine. In some embodiments, the composition comprises about 3 mg/kg to about 4 mg/kg of dopamine. In some embodiments, the composition comprises about 4 mg/kg to about 7 mg/kg of dopamine. In some embodiments, the composition comprises about 5 mg/kg to about 6 mg/kg of dopamine. In some embodiments, the composition comprises about 5 mg/kg to about 7 mg/kg of dopamine.

In some embodiments, the composition comprises about 7 mg/kg to about 8 mg/kg of dopamine. In some embodiments, the composition comprises about 7 mg/kg to about 9 mg/kg of dopamine. In some embodiments, the composition comprises about 8 mg/kg to about 9 mg/kg of dopamine. In some embodiments, the composition comprises about 1.7 mg/kg of dopamine. In some embodiments, the composition comprises about 1.75 mg/kg of dopamine. In some embodiments, the composition comprises about 1.8 mg/kg of dopamine. In some embodiments, the composition comprises about 1.85 mg/kg of dopamine. In some embodiments, the composition comprises about 3.4 mg/kg of dopamine. In some embodiments, the composition comprises about 3.45 mg/kg of dopamine. In some embodiments, the composition comprises about 3.5 mg/kg of dopamine. In some embodiments, the composition comprises about 3.55 mg/kg of dopamine. In some embodiments, the composition comprises about 3.6 mg/kg of dopamine. In some embodiments, the composition comprises about 3.65 mg/kg of dopamine. In some embodiments, the composition comprises about 5.2 mg/kg of dopamine. In some embodiments, the composition comprises about 5.25 mg/kg of dopamine. In some embodiments, the composition comprises about 5.3 mg/kg of dopamine. In some embodiments, the composition comprises about 5.35 mg/kg of dopamine. In some embodiments, the composition comprises about 5.4 mg/kg of dopamine. In some embodiments, the composition comprises about 5.45 mg/kg of dopamine. In some embodiments, the composition comprises about 7.0 mg/kg of dopamine. In some embodiments, the composition comprises about 7.05 mg/kg of dopamine. In some embodiments, the composition comprises about 7.1 mg/kg of dopamine. In some embodiments, the composition comprises about 7.15 mg/kg of dopamine. In some embodiments, the composition comprises about 7.2 mg/kg of dopamine. In some embodiments, the composition comprises about 7.25 mg/kg of dopamine. In some embodiments, the composition comprises about 8.8 mg/kg of dopamine. In some embodiments, the composition comprises about 8.9 mg/kg of dopamine. In some embodiments, the composition comprises about 9 mg/kg of dopamine. In any of these embodiments, a pharmaceutically acceptable salt of dopamine can be used, such as dopamine hydrochloride. In any of these embodiments, polydopamine, a mixture of dopamine and polydopamine, or a pharmaceutically acceptable salt thereof can be used in place of dopamine or a pharmaceutically acceptable salt of dopamine. In some embodiments, polydopamine are polydopamine nanoparticles. For example, in some embodiments, the composition comprises about 0.001 to about 1000 mg/mL of polydopamine or polydopamine

nanoparticles. In some embodiments, the composition comprises about 0.001 to about 500 mg/mL of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 0.01 to about 100 mg/mL of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 1 to about 100 mg/mL of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 1 to about 50 mg/mL of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 10 to about 40 mg/mL of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 1 to about 20 mg/mL of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 60 mg/mL of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 50 mg/mL of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 40 mg/mL of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 30 mg/mL of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 25 mg/mL of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 20 mg/mL of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 10 mg/mL of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 9.8 mg/mL of polydopamine or polydopamine nanoparticles. The amount of polydopamine or polydopamine nanoparticles that the composition comprises can also be expressed as a weight-based dosage, such as mg/kg, wherein the amount of polydopamine or polydopamine nanoparticles administered is determined by the body weight of the subject receiving the composition. In some embodiments, the composition comprises about 3 mg/kg to about 23 mg/kg of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 4 mg/kg to about 23 mg/kg of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 5 mg/kg to about 23 mg/kg of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 6 mg/kg to about 23 mg/kg of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 7 mg/kg to about 23 mg/kg of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 8 mg/kg to about 23 mg/kg of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 9 mg/kg to about 23 mg/kg of polydopamine or polydopamine nanoparticles. In some embodiments, the

[illegible]

nanoparticles. In some embodiments, the composition comprises about 13 mg/kg to about 14 mg/kg of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 12 mg/kg to about 15 mg/kg of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 15 mg/kg to about 19 mg/kg of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 13 mg/kg to about 19 mg/kg of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 17 mg/kg to about 19 mg/kg of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 18 mg/kg to about 19 mg/kg of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 18 mg/kg to about 20 mg/kg of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 21 mg/kg to about 22 mg/kg of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 20 mg/kg to about 22 mg/kg of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 22.25 mg/kg to about 23 mg/kg of polydopamine or polydopamine nanoparticles.

[0125] In some embodiments, the composition comprises about 4.45 mg/kg of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 4.50 mg/kg of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 4.25 mg/kg of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 4.35 mg/kg of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 4.45 mg/kg of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 4.55 mg/kg of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 4.56 mg/kg of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 4.57 mg/kg of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 4.58 mg/kg of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 4.59 mg/kg of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 4.6 mg/kg of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 4.61 mg/kg of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 8.9 mg/kg of polydopamine or polydopamine nanoparticles. In some embodiments, the

[illegible]

polydopamine nanoparticles. In some embodiments, the composition comprises about 18.5 mg/kg of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 22 mg/kg of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 22.5 mg/kg of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 22.75 mg/kg of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 23 mg/kg of polydopamine or polydopamine nanoparticles. In some embodiments, the composition comprises about 0.001 to about 1000 mg/mL of a mixture of dopamine and polydopamine. In some embodiments, the composition comprises about 0.001 to about 500 mg/mL of a mixture of dopamine and polydopamine. In some embodiments, the composition comprises about 0.01 to about 100 mg/mL of a mixture of dopamine and polydopamine. In some embodiments, the composition comprises about 1 to about 100 mg/mL of a mixture of dopamine and polydopamine. In some embodiments, the composition comprises about 1 to about 50 mg/mL of a mixture of dopamine and polydopamine. In some embodiments, the composition comprises about 10 to about 40 mg/mL of a mixture of dopamine and polydopamine. In some embodiments, the composition comprises about 1 to about 20 mg/mL of a mixture of dopamine and polydopamine. In some embodiments, the composition comprises about 60 mg/mL of a mixture of dopamine and polydopamine. In some embodiments, the composition comprises about 50 mg/mL of a mixture of dopamine and polydopamine. In some embodiments, the composition comprises about 40 mg/mL of a mixture of dopamine and polydopamine. In some embodiments, the composition comprises about 30 mg/mL of a mixture of dopamine and polydopamine. In some embodiments, the composition comprises about 20 mg/mL of a mixture of dopamine and polydopamine. In some embodiments, the composition comprises about 10 mg/mL of a mixture of dopamine and polydopamine. In some embodiments, the composition comprises about 9.8 mg/mL of a mixture of dopamine and polydopamine. In some embodiments, the composition comprises about 0.001 to about 1000 mg/mL of a mixture of dopamine and polydopamine nanoparticles. In some embodiments, the composition comprises about 0.001 to about 500 mg/mL of a mixture of dopamine and polydopamine nanoparticles. In some embodiments, the composition comprises about 0.01 to about 100 mg/mL of a mixture of dopamine and polydopamine nanoparticles. In some embodiments, the composition comprises about 1 to about 100 mg/mL of a mixture of dopamine and polydopamine nanoparticles. In some embodiments, the composition comprises about 1 to about 50 mg/mL of a mixture of dopamine and polydopamine nanoparticles. In some embodiments, the

composition comprises about 10 to about 40 mg/mL of a mixture of dopamine and polydopamine nanoparticles. In some embodiments, the composition comprises about 1 to about 20 mg/mL of a mixture of dopamine and polydopamine nanoparticles. In some embodiments, the composition comprises about 60 mg/mL of a mixture of dopamine and polydopamine nanoparticles. In some embodiments, the composition comprises about 50 mg/mL of a mixture of dopamine and polydopamine nanoparticles. In some embodiments, the composition comprises about 40 mg/mL of a mixture of dopamine and polydopamine nanoparticles. In some embodiments, the composition comprises about 30 mg/mL of a mixture of dopamine and polydopamine nanoparticles. In some embodiments, the composition comprises about 20 mg/mL of a mixture of dopamine and polydopamine nanoparticles. In some embodiments, the composition comprises about 10 mg/mL of a mixture of dopamine and polydopamine nanoparticles. In some embodiments, the composition comprises about 9.8 mg/mL of a mixture of dopamine and polydopamine nanoparticles. In some embodiments, the composition comprises dopamine and polydopamine.

[0126] When a mixture of dopamine and polydopamine is used, the amount of dopamine to polydopamine by weight (i.e., dopamine:polydopamine) can range from about 0.05:1 dopamine:polydopamine to about 1:0.05 dopamine: polydopamine on a weight/weight basis, such as about 0.1:1, about 0.2:1, about 0.25:1, about 0.3:1, about 0.4:1, about 0.5:1, about 0.6:1, about 0.7:1, about 0.75:1, about 0.8:1, about 0.9:1, about 1:1, about 1:0.9, about 1:0.8, about 1:0.75, about 1:0.7, about 1:0.6, about 1:0.5, about 1:0.4, about 1:0.3, about 1:0.25, about 1:0.2, or about 1:0.1 dopamine: polydopamine. In some embodiments, the amount of dopamine to polydopamine by weight can range from about 0.2:1 to about 0.6:1. In some embodiments, the amount of dopamine to polydopamine by weight can range from about 0.3:1 to about 0.5:1. In some embodiments, the amount of dopamine to polydopamine by weight can range from about 0.2:1, about 0.22:1, about 0.23:1, about 0.24:1, about 0.25:1, about 0.26:1, about 0.27:1, about 0.28:1, about 0.29:1, about 0.3:1, about 0.31:1, about 0.32:1, about 0.33:1, about 0.34:1, about 0.35:1, about 0.36:1, about 0.37:1, about 0.38:1, about 0.39:1, about 0.4:1, about 0.41:1, about 0.42:1, about 0.43:1, about 0.44:1, about 0.45:1, about 0.46:1, about 0.47:1, about 0.48:1, about 0.49:1, or about 0.5:1. In some embodiments, the amount of dopamine to polydopamine by weight is about 0.35:1. In some embodiments, the amount of dopamine to polydopamine by weight is about 0.36:1. In some embodiments, the amount of dopamine to polydopamine by weight is about 0.37:1. In some embodiments, the amount of dopamine to polydopamine by weight is about 0.38:1. In some

embodiments, the amount of dopamine to polydopamine by weight is about 0.39:1. In some embodiments, the amount of dopamine to polydopamine by weight is about 0.4:1. In some embodiments, the amount of dopamine to polydopamine by weight is about 0.41:1. In some embodiments, the amount of dopamine to polydopamine by weight is about 0.42:1. In some embodiments, the amount of dopamine to polydopamine by weight is about 0.43:1. In some embodiments, the amount of dopamine to polydopamine by weight is about 0.44:1. In some embodiments, the amount of dopamine to polydopamine by weight is about 0.45:1. In some embodiments, the composition comprises dopamine and polydopamine nanoparticles. When a mixture of dopamine and polydopamine nanoparticles is used, the amount of dopamine to polydopamine nanoparticles by weight (i.e., dopamine: polydopamine nanoparticles) can range from about 0.05:1 dopamine: polydopamine nanoparticles to about 1:0.05 dopamine: polydopamine nanoparticles on a weight/weight basis, such as about 0.1:1, about 0.2:1, about 0.25:1, about 0.3:1, about 0.4:1, about 0.5:1, about 0.6:1, about 0.7:1, about 0.75:1, about 0.8:1, about 0.9:1, about 1:1, about 1:0.9, about 1:0.8, about 1:0.75, about 1:0.7, about 1:0.6, about 1:0.5, about 1:0.4, about 1:0.3, about 1:0.25, about 1:0.2, or about 1:0.1 dopamine: polydopamine nanoparticles. In some embodiments, the amount of dopamine to polydopamine nanoparticles by weight can range from about 0.2:1 to about 0.6:1. In some embodiments, the amount of dopamine to polydopamine nanoparticles by weight can range from about 0.3:1 to about 0.5:1. In some embodiments, the amount of dopamine to polydopamine nanoparticles by weight can range from about 0.2:1, about 0.22:1, about 0.23:1, about 0.24:1, about 0.25:1, about 0.26:1, about 0.27:1, about 0.28:1, about 0.29:1, about 0.3:1, about 0.31:1, about 0.32:1, about 0.33:1, about 0.34:1, about 0.35:1, about 0.36:1, about 0.37:1, about 0.38:1, about 0.39:1, about 0.4:1, about 0.41:1, about 0.42:1, about 0.43:1, about 0.44:1, about 0.45:1, about 0.46:1, about 0.47:1, about 0.48:1, about 0.49:1, or about 0.5:1. In some embodiments, the amount of dopamine to polydopamine nanoparticles by weight is about 0.35:1. In some embodiments, the amount of dopamine to polydopamine nanoparticles by weight is about 0.36:1. In some embodiments, the amount of dopamine to polydopamine nanoparticles by weight is about 0.37:1. In some embodiments, the amount of dopamine to polydopamine nanoparticles by weight is about 0.38:1. In some embodiments, the amount of dopamine to polydopamine nanoparticles by weight is about 0.39:1. In some embodiments, the amount of dopamine to polydopamine nanoparticles by weight is about 0.4:1. In some embodiments, the amount of dopamine to polydopamine nanoparticles by weight is about 0.41:1. In some embodiments, the amount of dopamine to polydopamine nanoparticles by weight is about 0.42:1. In some embodiments, the amount of

dopamine to polydopamine nanoparticles by weight is about 0.43:1. In some embodiments, the amount of dopamine to polydopamine nanoparticles by weight is about 0.44:1. In some embodiments, the amount of dopamine to polydopamine nanoparticles by weight is about 0.45:1.

[0127] In some embodiments, a particular composition falling within these ranges is: 6.25 mg polydopamine nanoparticles, 2.45 mg dopamine-HCl, 0.17 mg hydrogen peroxide.

[0128] In some embodiments, a particular composition falling within these ranges is: 12.5 mg polydopamine nanoparticles, 4.9 mg dopamine-HCl, 0.34 mg hydrogen peroxide.

[0129] In some embodiments, a particular composition falling within these ranges is: 25 mg polydopamine nanoparticles, 9.8 mg dopamine-HCl, 0.68 mg hydrogen peroxide.

[0130] In some embodiments, a particular composition falling within these ranges is 4.567 mg/kg polydopamine nanoparticles (PDA), 1.789 mg/kg dopamine hydrochloride, 0.122 mg/kg hydrogen peroxide, in Tris buffer, pH 8.5. The mg/kg reflects weight-based dosages, wherein the weight is determined by the body weight of the subject receiving the composition,

[0131] In some embodiments, a particular composition falling within these ranges is: 9.133 mg/kg polydopamine nanoparticles, 3.596 mg/kg dopamine hydrochloride, 0.244 mg/kg hydrogen peroxide, in Tris buffer, pH 8.5. The mg/kg reflects weight-based dosages, wherein the weight is determined by the body weight of the subject receiving the composition,

[0132] In some embodiments, a particular composition falling within these ranges is: 13.7 mg/kg polydopamine nanoparticles, 5.394 mg/kg dopamine hydrochloride, 0.366 mg/kg hydrogen peroxide, in Tris buffer, pH 8.5. The mg/kg reflects weight-based dosages, wherein the weight is determined by the body weight of the subject receiving the composition,

[0133] Where chemically possible and pharmaceutically desirable, alternative pharmaceutically acceptable salts or the free acids or free bases of any of the foregoing compounds can be used.

[0134] International Patent Application Nos. WO 2021/119350 and WO 2021/119354, which are incorporated herein by reference in their entirety, describe methods of forming polymers *in situ* in a subject.

Oxygen Sources for Gastrointestinal Synthetic Epithelial Lining Precursor Compositions

[0135] In one aspect, the disclosure provides for a composition comprising a polymer precursor, an oxygen source, and a permeation enhancer. In some embodiments, the oxygen source is hydrogen peroxide or urea hydrogen peroxide. In some embodiments, the oxygen

source is hydrogen peroxide. In some embodiments, the oxygen source is urea hydrogen peroxide. When the oxygen source contacts a catalyst endogenous to the subject, such as a catalase or a peroxidase, oxygen is liberated from the oxygen source, resulting in polymerization of the polymer precursor.

[0136] In some embodiments, the endogenous catalyst is selected from catalases or peroxidases. In some embodiments, the endogenous catalyst is a peroxidase. In certain embodiments, the peroxidase is eosinophil peroxidase, lactoperoxidase, or myeloperoxidase.

[0137] In some embodiments, the endogenous catalyst is a catalase. In some embodiments, the catalase is a bacterial catalase. In some embodiments, the catalase is a human catalase.

[0138] In some embodiments, the endogenous catalyst is located in the gastrointestinal (GI) tract of the subject. In some embodiments, the endogenous catalyst is located in the small intestine of the subject. In some embodiments, the endogenous catalyst is located in the duodenum of the subject.

[0139] In some embodiments, the endogenous catalyst is located in the upper GI of the subject. In some embodiments, the endogenous catalyst is located in the stomach of the subject.

[0140] In some embodiments, the composition comprises an amount of oxygen source to (polymer precursor, oxygen source, and permeation enhancer) of 1% to 15%.

[0141] In some embodiments, the composition comprises about 0.01 to about 100 mM of the oxygen source. In some embodiments, the composition comprises about 0.1 to about 50 mM of the oxygen source. In some embodiments, the composition comprises about 1 to about 30 mM of the oxygen source. In some embodiments, the composition comprises about 20 mM of the oxygen source. In some embodiments, the composition comprises a concentration of oxygen source compatible with ingestion by the subject. In some embodiments, the composition comprises an amount of oxygen source to dopamine (oxygen source: dopamine) of about 10:1, about 20:1, about 15:1, about 14:1, or about 13:1. In some embodiments, the composition comprises an amount of oxygen source to dopamine of 14.6:1. In some embodiments, the oxygen source is hydrogen peroxide. In some embodiments, the composition comprises an amount of hydrogen peroxide to dopamine (hydrogen peroxide: dopamine) of about 10:1, about 20:1, about 15:1, about 14:1, or about 13:1. In some embodiments, the composition comprises an amount of hydrogen peroxide to dopamine of 14.6:1.

Synergistic Combinations of Permeation Enhancers for Gastrointestinal Synthetic Epithelial Linings (GSELs)

[0142] The current disclosure provides combinations of permeation enhancers for Gastrointestinal Synthetic Epithelial Linings (GSELs), which have been found to provide synergistic permeation enhancing effects for active pharmaceutical ingredients (APIs). GSELs localize an API in a specific location, resulting in prolonged contact with the gastrointestinal mucosa. However, some APIs may not permeate through the gastrointestinal barrier in sufficient amounts to be therapeutically effective, or may not permeate at all, despite the prolonged contact afforded by a GSEL. Permeation enhancers can be used in conjunction with a GSEL to increase the penetration of an API across the gastrointestinal mucosa. Certain combinations of permeation enhancers have been found to work in synergistic combination. In particular, combinations of a) at least one of an ammonium salt, a carbonate salt, or a bicarbonate salt, and b) at least one of a bile salt or bile acid, have been found to have synergistic effects on permeation of agents such as active pharmaceutical ingredients.

[0143] The first component of the synergistic permeation enhancer combination comprises one or more of an ammonium salt, a carbonate salt, or a bicarbonate salt. The first component can comprise one or more ammonium salts, one or more carbonate salts, one or more bicarbonate salts, or any number of ammonium salts, any number of carbonate salts, and/or any number of bicarbonate salts in combination.

[0144] In some embodiments, the composition comprises ammonium salt, which can be determined by weight of the subject. the composition comprises about 1 mg/kg to about 100 mg/kg of ammonium salt. In some embodiments, the composition comprises about 1 mg/kg to about 50 mg/kg of ammonium salt. In some embodiments, the composition comprises about 1 mg/kg to about 25 mg/kg of ammonium salt. In some embodiments, the composition comprises about 5 mg/kg to about 100 mg/kg of ammonium salt. In some embodiments, the composition comprises about 5 mg/kg to about 50 mg/kg of ammonium salt. In some embodiments, the composition comprises about 5 mg/kg to about 25 mg/kg of ammonium salt. In some embodiments, the composition comprises about 10 mg/kg to about 30 mg/kg of ammonium salt. In some embodiments, the composition comprises about 10 mg/kg to about 70 mg/kg of ammonium salt. In some embodiments, the composition comprises about 10 mg/kg to about 100 mg/kg of ammonium salt. In some embodiments, the composition comprises about 25 mg/kg to about 30 mg/kg of ammonium salt. In some embodiments, the composition comprises about 25 mg/kg to about 50 mg/kg of ammonium salt. In some

embodiments, the composition comprises about 25 mg/kg to about 75 mg/kg of ammonium salt. In some embodiments, the composition comprises about 25 mg/kg to about 100 mg/kg of ammonium salt. In some embodiments, the composition comprises about 30 mg/kg to about 60 mg/kg of ammonium salt. In some embodiments, the composition comprises about 30 mg/kg to about 80 mg/kg of ammonium salt. In some embodiments, the composition comprises about 30 mg/kg to about 100 mg/kg of ammonium salt. In some embodiments, the composition comprises about 50 mg/kg to about 100 mg/kg of ammonium salt. In some embodiments, the composition comprises about 50 mg/kg to about 80 mg/kg of ammonium salt. In some embodiments, the composition comprises about 50 mg/kg to about 100 mg/kg of ammonium salt. In some embodiments, the composition comprises about 60 mg/kg to about 70 mg/kg of ammonium salt. In some embodiments, the composition comprises about 70 mg/kg to about 80 mg/kg of ammonium salt. In some embodiments, the composition comprises about 80 mg/kg to about 90 mg/kg of ammonium salt. In some embodiments, the composition comprises about 70 mg/kg to about 80 mg/kg of ammonium salt. In some embodiments, the composition comprises about 75 mg/kg to about 95 mg/kg of ammonium salt. In some embodiments, the composition comprises about 80 mg/kg to about 95 mg/kg of ammonium salt.

[0145] Ammonium salts for use in the synergistic combinations include, but are not limited to, ammonium carbonate (NHCO), ammonium sulfate (NH₂SO₄), ammonium citrate (NHCA), ammonium phosphate (NHPO), diammonium phosphate (DAP), monoammonium phosphate (MAP), ammonium bicarbonate (NH₂HCO₃), or ammonium chloride (NHCl). Ammonium lactate, ammonium acetate, ammonium sulfate, ammonium sulfite, triammonium citrate, ammonium propionate, and ammonium sulfamate can also be used. One particularly useful ammonium salt is ammonium carbonate. Another particularly useful ammonium salt is ammonium bicarbonate. Abbreviations used herein, such as NHCO to indicate ammonium carbonate, are not intended to designate chemical formulas, as the chemical formula for ammonium carbonate is (NH₄)₂CO₃.

[0146] In some embodiments, the composition comprises about 10 mg/ml to about 50 mg/ml NHCO. In some embodiments, the composition comprises about 15 mg/ml to about 40 mg/ml NHCO. In some embodiments, the composition comprises about 20 mg/ml to about 45 mg/ml NHCO. In some embodiments, the composition comprises about 30 mg/ml to about 42 mg/ml NHCO. In some embodiments, the composition comprises about 40 mg/ml to about 45 mg/ml NHCO. In some embodiments, the composition comprises about 20 mg/ml to about 35 mg/ml NHCO. In some embodiments, the composition comprises about 35 mg/ml to about 43 mg/ml

NHCO. In some embodiments, the composition comprises about 39 mg/ml to about 45 mg/ml NHCO. In some embodiments, the composition comprises about 39 mg/ml to about 41 mg/ml NHCO. In some embodiments, the composition comprises about 40 mg/ml NHCO.

[0147] In some embodiments, the composition comprises, by weight-based dose, from about 6 mg/kg NHCO to about 8 mg/kg NHCO. In some embodiments, the composition comprises, by weight-based dose, from about 6 mg/kg NHCO to about 7 mg/kg NHCO. In some embodiments, the composition comprises, by weight-based dose, about 6 mg/kg NHCO. In some embodiments, the composition comprises, by weight-based dose, about 6.4 mg/kg NHCO. In some embodiments, the composition comprises, by weight-based dose, about 7 mg/kg NHCO. In some embodiments, the composition comprises, by weight-based dose, from about 10 mg/kg NHCO to about 13 mg/kg NHCO. In some embodiments, the composition comprises, by weight-based dose, from about 11 mg/kg NHCO to about 13 mg/kg NHCO. In some embodiments, the composition comprises, by weight-based dose, from about 12 mg/kg NHCO to about 13 mg/kg NHCO. In some embodiments, the composition comprises, by weight-based dose, 12.8 mg/kg NHCO.

[0148] Carbonate salts for use in the synergistic combinations include, but are not limited to, sodium carbonate (NaCO) or potassium carbonate (KCO). As previously noted, these are abbreviations, not chemical formulas.

[0149] Bicarbonate salts for use in the invention include, but are not limited to, sodium bicarbonate (NaHCO) or potassium bicarbonate (KHCO). As previously noted, these are abbreviations, not chemical formulas.

[0150] The second component of the synergistic permeation enhancer combination comprises one or more of a bile salt or a bile acid. The second component can comprise one or more bile salts, one or more bile acids, or any number of bile salts and/or bile acids in combination. Liquid GSELs are formulated in aqueous solution, and depending on the pH of the formulation, addition of a single bile acid, or a single bile salt, can result in a mixture of the bile acid and its bile salt (i.e., the conjugate base of the bile acid). For example, the pKa of cholic acid is given as 4.98 (see URL pubchem.ncbi.nlm.nih.gov/compound/Cholic-acid, referencing Serjeant EP, Dempsey B; Ionisation constants of organic acids in aqueous solution. IUPAC Chem Data Ser No.23. NY,NY: Pergamon pp.989 (1979)). If cholic acid is added to a formulation, and the pH raised to approximately 8, most of the material will be in the form of the cholate anion. Conversely, if a cholate salt is added to a formulation, and the pH lowered to approximately 2, most of the material will be in the form of cholic acid. Accordingly, when a bile salt or bile acid is recited, it is understood that the form of the

material may change depending on the pH—that is, a bile salt may convert partially or mostly to a bile acid, and/or a bile acid may convert partially or mostly to a bile salt, depending on the pH of the formulation containing the bile salt or bile acid. A typical pH value for GSEL formulations is about 8.5, plus or minus 1 pH unit.

[0151] In some embodiments, the composition comprises bile salt, which can be determined by weight of the subject. the composition comprises about 1 mg/kg to about 100 mg/kg of bile salt. In some embodiments, the composition comprises about 1 mg/kg to about 50 mg/kg of bile salt. In some embodiments, the composition comprises about 1 mg/kg to about 25 mg/kg of bile salt. In some embodiments, the composition comprises about 5 mg/kg to about 100 mg/kg of bile salt. In some embodiments, the composition comprises about 5 mg/kg to about 50 mg/kg of bile salt. In some embodiments, the composition comprises about 5 mg/kg to about 25 mg/kg of bile salt. In some embodiments, the composition comprises about 10 mg/kg to about 30 mg/kg of bile salt. In some embodiments, the composition comprises about 10 mg/kg to about 70 mg/kg of bile salt. In some embodiments, the composition comprises about 10 mg/kg to about 100 mg/kg of bile salt. In some embodiments, the composition comprises about 25 mg/kg to about 30 mg/kg of bile salt. In some embodiments, the composition comprises about 25 mg/kg to about 50 mg/kg of bile salt. In some embodiments, the composition comprises about 25 mg/kg to about 75 mg/kg of bile salt. In some embodiments, the composition comprises about 25 mg/kg to about 100 mg/kg of bile salt. In some embodiments, the composition comprises about 30 mg/kg to about 60 mg/kg of bile salt. In some embodiments, the composition comprises about 30 mg/kg to about 80 mg/kg of bile salt. In some embodiments, the composition comprises about 30 mg/kg to about 100 mg/kg of bile salt. In some embodiments, the composition comprises about 50 mg/kg to about 100 mg/kg of bile salt. In some embodiments, the composition comprises about 50 mg/kg to about 80 mg/kg of bile salt. In some embodiments, the composition comprises about 50 mg/kg to about 100 mg/kg of bile salt. In some embodiments, the composition comprises about 60 mg/kg to about 70 mg/kg of bile salt. In some embodiments, the composition comprises about 70 mg/kg to about 80 mg/kg of bile salt. In some embodiments, the composition comprises about 80 mg/kg to about 90 mg/kg of bile salt. In some embodiments, the composition comprises about 70 mg/kg to about 80 mg/kg of bile salt. In some embodiments, the composition comprises about 75 mg/kg to about 95 mg/kg of bile salt. In some embodiments, the composition comprises about 80 mg/kg to about 95 mg/kg of bile salt.

[0152] Bile acids for use in the synergistic combinations include, but are not limited to, cholic acid (CHA), glycocholic acid (GCA), deoxycholic acid (DCA), glychochenodeoxycholic acid (GCDCA), glycodeoxycholic acid (GDCA), taurodeoxycholic acid (TDCA), taurocholic acid (TCA), bovine bile (OX), chenodeoxycholic acid (CDCA), taurochenodeoxycholic acid (TCDCA), lithocholic acid (LCA), glycolithocholate (GLC), glycohyocholate (GHC), tauroolithocholate (TLC), ursodeoxycholic acid (UDCA), tauroursodeoxycholic acid (TUDCA), glycoursodeoxycholic acid (GUDCA), 12-monoketocholeic acid (12-MKC), 7-monoketocholeic acid (7-MKC), 7,12-diketocholeic acid (7,12-DKC), 3,7,12-triketocholeic acid (3,7,12-TKC), 12-monoketodeoxycholic acid (12-MKDC), or 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS). A particularly useful bile acid is glycocholic acid.

[0153] Bile salts for use in the synergistic combinations include, but are not limited to, a salt of cholic acid, a salt of glycocholic acid, a salt of deoxycholic acid, a salt of glychochenodeoxycholic acid, a salt of glycodeoxycholic acid, a salt of taurodeoxycholic acid, a salt of taurocholic acid, a salt of bovine bile, a salt of chenodeoxycholic acid, a salt of taurochenodeoxycholic acid, a salt of lithocholic acid, a salt of glycolithocholate, a salt of glycohyocholate, a salt of tauroolithocholate, a salt of ursodeoxycholic acid, a salt of tauroursodeoxycholic acid, a salt of glycoursodeoxycholic acid, a salt of 12-monoketocholeic acid, a salt of 7-monoketocholeic acid, a salt of 7,12-diketocholeic acid, a salt of 3,7,12-triketocholeic acid, a salt of 12-monoketodeoxycholic acid, or a salt of 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate. A particularly useful bile salt is a salt of glycocholic acid.

[0154] The bile salts can be sodium salts, including, but not limited to, sodium cholate, sodium glycocholate, sodium deoxycholate, sodium glychochenodeoxycholate, sodium glycodeoxycholate, sodium taurodeoxycholate, sodium taurocholate, a sodium salt of bovine bile, sodium chenodeoxycholate, sodium taurochenodeoxycholate, sodium lithocholate, sodium glycolithocholate, sodium glycohyocholate, sodium tauroolithocholate, sodium ursodeoxycholate, sodium tauroursodeoxycholate, sodium glycoursodeoxycholate, sodium 12-monoketocholate, sodium 7-monoketocholate, sodium 7,12-diketocholate, sodium 3,7,12-triketocholate, sodium 12-monoketodeoxycholate, or sodium 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate. A particularly useful sodium salt of a bile acid is sodium glycocholate (GCA). Specific synergistic combinations of permeation enhancers include:
glycocholic acid and ammonium carbonate;

a salt of glycocholic acid and ammonium carbonate and sodium glycocholate and ammonium carbonate.

[0155] In some embodiments, the composition comprises about 10 mg/ml to about 50 mg/ml GCA. In some embodiments, the composition comprises about 15 mg/ml to about 40 mg/ml GCA. In some embodiments, the composition comprises about 20 mg/ml to about 45 mg/ml GCA. In some embodiments, the composition comprises about 30 mg/ml to about 42 mg/ml GCA. In some embodiments, the composition comprises about 40 mg/ml to about 45 mg/ml GCA. In some embodiments, the composition comprises about 20 mg/ml to about 35 mg/ml GCA. In some embodiments, the composition comprises about 35 mg/ml to about 43 mg/ml GCA. In some embodiments, the composition comprises about 39 mg/ml to about 45 mg/ml GCA. In some embodiments, the composition comprises about 39 mg/ml to about 41 mg/ml GCA. In some embodiments, the composition comprises about 40 mg/ml GCA.

[0156] In some embodiments, the composition comprises, by weight-based dose, from about 6 mg/kg GCA to about 8 mg/kg GCA. In some embodiments, the composition comprises, by weight-based dose, from about 6 mg/kg GCA to about 7 mg/kg GCA. In some embodiments, the composition comprises, by weight-based dose, about 6 mg/kg GCA. In some embodiments, the composition comprises, by weight-based dose, about 6.4 mg/kg GCA. In some embodiments, the composition comprises, by weight-based dose, about 7 mg/kg GCA. In some embodiments, the composition comprises, by weight-based dose, from about 10 mg/kg GCA to about 13 mg/kg GCA. In some embodiments, the composition comprises, by weight-based dose, from about 11 mg/kg GCA to about 13 mg/kg GCA. In some embodiments, the composition comprises, by weight-based dose, from about 12 mg/kg GCA to about 13 mg/kg GCA. In some embodiments, the composition comprises, by weight-based dose, 12.8 mg/kg GCA.

[0157] A specific synergistic combination of permeation enhancers (expressed as a weight-based dosage) includes: 6.4 mg/kg glycocholic acid (GCA) and 6.4 mg/kg ammonium carbonate (NHCO).

[0158] A specific synergistic combination of permeation enhancers (expressed as a weight-based dosage) includes: 12.8 mg/kg glycocholic acid (GCA) and 12.8 mg/kg ammonium carbonate (NHCO).

[0159] Where chemically possible and pharmaceutically desirable, alternative pharmaceutically acceptable salts or the free acids or free bases of any of the foregoing compounds can be used.

Exemplary Formulations comprising permeation enhancers

[0160] Disclosed herein are also formulations for GSEL precursors.

[0161] In some embodiments, the GSEL precursor formulation comprises about 1-200 mg/ml of dopamine, about 1-200 mg/ml of polydopamine, about 0-8 mg/mL of oxygen source, 1-100 mg/mL of bile salt, and 1-100 mg/mL of ammonium salt. In some embodiments, the ratio of dopamine to oxygen source is from 20:1 (wt/wt) to 10:1 (wt/wt).

[0162] A particular formulation falling within the ranges disclosed herein ranges is: 28 mg/kg polydopamine nanoparticles, 11 mg/kg dopamine hydrochloride, 1.6 mg/kg hydrogen peroxide, and one or more permeation enhancers. In some embodiments, the amount of one or more permeation enhancers is determined by a weight by weight (wt/wt) ratio of dopamine: hydrogen peroxide. In some embodiments, the ratio of dopamine: hydrogen peroxide is 10:1. In some embodiments, the ratio of dopamine: hydrogen peroxide is 20:1. In some embodiments, the ratio of dopamine: hydrogen peroxide is 15:1. In some embodiments, the ratio of dopamine: hydrogen peroxide is 15:1. In some embodiments, the ratio of dopamine: hydrogen peroxide is 14:1. In some embodiments, the ratio of dopamine: hydrogen peroxide is 14.6:1.

[0163] A particular formulation falling within these ranges is: 4.567 mg/kg polydopamine nanoparticles, 1.789 mg/kg dopamine hydrochloride, 0.122 mg/kg hydrogen peroxide, 6.4 mg/kg GCA and 6.4 mg/kg NHCO.

[0164] A particular formulation falling within these ranges is: 9.133 mg/kg polydopamine nanoparticles, 3.596 mg/kg dopamine hydrochloride, 0.244 mg/kg hydrogen peroxide, 6.4 mg/kg GCA and 6.4 mg/kg NHCO.

[0165] A particular formulation falling within these ranges is: 13.7 mg/kg polydopamine nanoparticles, 5.394 mg/kg dopamine hydrochloride, 0.366 mg/kg hydrogen peroxide, 6.4 mg/kg GCA and 6.4 mg/kg NHCO.

[0166] A particular formulation falling within these ranges is: 4.567 mg/kg polydopamine nanoparticles, PDA, 1.789 mg/kg dopamine hydrochloride, 0.122 mg/kg hydrogen peroxide, 12.8 mg/kg GCA and 12.8 mg/kg NHCO.

[0167] A particular formulation falling within these ranges is: 9.133 mg/kg polydopamine nanoparticles, 3.596 mg/kg dopamine hydrochloride, 0.244 mg/kg hydrogen peroxide, 12.8 mg/kg GCA and 12.8 mg/kg NHCO.

[0168] A particular formulation falling within these ranges is: 13.7 mg/kg polydopamine nanoparticles, 5.394 mg/kg dopamine hydrochloride, 0.366 mg/kg hydrogen peroxide, 12.8 mg/kg GCA and 12.8 mg/kg NHCO.

[0169] Where chemically possible and pharmaceutically desirable, alternative pharmaceutically acceptable salts or the free acids or free bases of any of the foregoing compounds can be used.

[0170] In some embodiments, any of the formulations disclosed herein is a powder formulation. In some embodiments, any of the formulations disclosed herein is a liquid formulation.

EXAMPLES

[0171] The disclosure is further illustrated by the following non-limiting examples.

Example 1

Initial Evaluation of Permeation Enhancers by 96 well plate screening

[0172] Methods:

[0173] Polydopamine nanoparticle synthesis:

[0174] Polydopamine (PDA) nanoparticles were synthesized based on a previously established method. According to the protocol 716.3 mL of ethanol, 1611.7 mL of deionized distilled water, and 72 mL of 28-30% w/v ammonium hydroxide were added under stirring at room temperature. After 30 minutes, 44.7 g of dopamine hydrochloride (dopamine-HCl) were added under stirring and the reaction was incubated for 18h at room temperature. The formed nanoparticles were pelleted by ultracentrifugation at 18,000 g for 30 minutes at room temperature. After centrifugation, the nanoparticles were washed by discarding 15% of the supernatant and replacing it with an equal volume of deionized water, the pellets were redispersed, and then the suspension was centrifuged again at 18,000 g for 15 min at room temperature (1st wash cycle). For the 2nd and 3rd wash cycle, 60-70% of the supernatant were removed respectively. PDA nanoparticles were then collected and freeze-dried at the following conditions: -80°C, vacuum <300 mTorr, yielding ~10 g of PDA nanoparticles.

[0175] Ex vivo tissue preparation:

[0176] Porcine duodenal or jejunal tissue was resected and rinsed with antibiotic-antimycotic solution diluted 1:20 in 1x PBS buffer pH 7.4. The tissue was dissected into pieces of different sizes depending on the test performed.

[0177] 96 well plate screening:

[0178] Fresh porcine jejunum tissue was resected and washed in a series of saline solutions supplemented with 5% antibiotic-antimycotic solution. The tissue was then mounted onto an augmented 96-well plate (the plate that will receive permeated drug) filled

with buffer (FIG. 1A, FIG. 1B). Tissues from multiple pigs were used to calculate an average permeability and standard deviations from the mean. Another 96-well plate was sandwiched on top and secured by magnets. To prepare the donor solutions, a master plate was generated by aliquoting from 3 plates: (1) The excipient and API plate included the excipients and drug at 2.5x the final concentration. The tris buffer and hydrogen peroxide were at 1.25x the final concentration. (2) The polydopamine (PDA) plate had PDA nanoparticles at 2.5x the final concentration, and tris buffer and hydrogen peroxide at 1.25x the final concentration. (3) The dopamine plate had dopamine at 5x the final concentration. All formulations contained GSEL_40 (9.8 mg/mL dopamine HCl, 25 mg/mL PDA nanoparticles, 0.67 mg/mL hydrogen peroxide, 2.2 mg/mL Tris HCl, 4.3 mg/mL Tris base. The master plate was aliquoted from each plate at 0.5:0.5:1 volume ratio. Then the donor solution in the master plate was pipetted into the donor 96-well plate. The entire system was then covered and placed at room temperature for 20-24 hours. Samples from the receiving 96-well plate were then analyzed using LC-MS. Experimental samples and control samples tested on the same tissue were compared to determine percent mean permeability and/or fold change. Permeability was calculated by comparing permeate to how much was added to the donor chamber. Fold change was calculated by comparing experimental samples to control samples. More specifically, a master plate was prepared using concentrated solutions of each component of GSEL. A concentrated permeation enhancer solution was made with 1 or 2 excipients in 50 mM Tris buffer, pH 8.5. The dilutions were made such that the final concentration of permeation enhancers in the master plate was 40 mg/mL or 80 mg/mL. The concentrated PDA nanoparticles, hydrogen peroxide and semaglutide solution was made such that the final concentration in the master plate was 25 mg/mL of PDA nanoparticles, 5 mg/mL of semaglutide, and 19.6 mM (0.67 mg/mL) of hydrogen peroxide. The concentrated dopamine solution was made with dopamine HCl and deionized water such that the final dopamine concentration in the master plate was 9.8 mg/mL. The master plate was made by adding the concentrated excipients solution, followed by a concentrated PDA nanoparticles /hydrogen peroxide/drug solution, and then a concentrated dopamine solution. The volume over surface area was 50 μ L/40.15 mm². A total of 61 single excipients were prepared and screened with 4 replicates per formulation. From the binary screen, 12 excipients were selected for further testing as combinations. The combinations were studied at different permeation enhancers to GSEL mass ratios (1:1 and 1:2). The combination screen was carried out with the same experimental conditions as discussed above.

[0179] The receiver compartment was filled with 0.5% w/v ovalbumin in 1X PBS. Two plates contained jejunum tissue from one animal and the other two plates contained tissue from another animal. From the master plate, 50 μ l was pipetted into each tissue plate. To simulate the stomach dilution that GSEL would undergo in the jejunum, 20 μ L of donor fluid was replaced with Tris buffer at 15, 30 and 60 minutes after stamping. A total of 4 tissue plates were stamped, then incubated at 37°C for 20 hours. After 20 hours the plates were inverted and 80 μ L of receiver fluid collected into deep well plates. Replicate wells from tissue plates from the same animal were pooled. The deep well plates were analyzed via LC-MS to determine semaglutide concentration. Permeability was calculated using the following equation.

$$\text{Permeability}(\%) = 100 * 3 * \frac{[\text{Receiver}]}{[\text{Donor}]}$$

[0180] Excipients tested and their corresponding names are listed in **Table E1**.

Table E1: Excipient table

Excipient	Name
C08	Sodium Octanoate
C10	Decanoic Acid
C12	Dodecanoic acid
C14	Tetradecanoic acid
CACL	Calcium Chloride
CCO:75n	Calcium Carbonate Nanoparticles, Cubic, 75-105nm
CDCA	Chenodeoxycholic acid sodium salt
CHA	Cholic Acid Sodium Salt
CHAPS	CHAPS hydrate
CHLORG	Chlorogenic acid
CINA	Cinnamic Acid Sodium Salt
CITR	Sodium Citrate
DADMAC	Diallyldimethylammonium chloride
DCA	Deoxycholic acid sodium salt
DENDAC2.5	PAMAM Dendrimer Carboxylate Surface Groups G2.5
DENDOH4	PAMAM Dendrimer Hydroxyl Surface Groups G4
DGLC	D-(+)-Glucose
DOC	Docusate Sodium
EDTA	Ethylenediaminetetraacetic Acid Tetrasodium Salt
EPE:2	Poly(ethylene glycol)-block-Poly(propylene glycol)-block-Poly(ethylene glycol) 2.8K
GAPS	Gibberellic Acid Potassium Salt
GCA	Sodium Glycocholate
GDCA	Glycodeoxycholic acid sodium salt
GCDCA	Glycochenodeoxycholic Acid Sodium Salt
GERAN	Geranic Acid

Excipient	Name
GSEL	Gastrointestinal synthetic epithelial lining
HAP:5u	Hydroxyapatite, Spherical, 5um
HCO3	Sodium bicarbonate
HPBCD	(2-Hydroxypropyl)-beta cyclodextrin
HPGCD	(2-Hydroxypropyl)-gamma-cyclodextrin 4.5 DS
IAA	Indole 3-acetic acid Sodium Salt
KCL	Potassium Chloride
LABS	Caprylcaproyl Macrogol Glycerides (Labrasol)
LFCS	Oleoyl polyoxyl-6 glycerides
NHCO	Ammonium Carbonate
NHP	Ammonium phosphate dibasic
OX	Bile (BOVINE) (Sigma-Aldrich catalog # b3883)
PEG:1	Poly(ethylene glycol) 1K
PEG:6	Poly(ethylene glycol) 6K
PEI:B25	Poly(ethylenimine) Hydrochloride, Branched 25KDa
PEI:L4	Poly(ethylenimine) Hydrochloride, Linear 4k
PEP:2	Poly(propylene glyco)-block-poly(ethylene glycol)-block-poly(propylene glycol) Mn ~ 2000
PHYT	Phytic Acid Sodium Salt Hydrate
POXZ:5	Poly(2-ethyl-2-oxazoline) 5K
PVA:30	Poly(vinyl alcohol) 30-70k
PVP:10	Poly(vinylpyrrolidone) 10K
PVSA	Polyvinyl Sulfonic Acid
SBECD	Sulfobutylated beta-cyclodextrin sodium salt
SDS	Sodium dodecyl sulfate
SHB	Sodium 4-hydroxybenzoate
SNAC	Salcaprozate Sodium
SPMD	Spermidine Trihydrochloride
STC	Sodium taurocholate
TDCA	Taurodeoxycholic acid sodium salt
VB9	Folic Acid
ZnO:R10n	Zinc Oxide Nanoparticles Round 10 nm
MOGRO	Mogroside V
HDE	Hydroxyectoine
HAP 2.5U	Hydroxyapatite, Spherical, 2.5 um
MAIS	Linoleic Acid Monoglyceride
TRHP	Diethylene glycol monoethyl ether
QUILZ	Quillaja Saponin
MAND	Mandelic acid
SUCC	Sodium succinate
MALI	DL-Malic acid
NANB	Sodium 4-(nicotinamido)butanoate
HAP:R20N	Hydroxyapatite, Rod-Shaped, 20nm

Excipient	Name
FERUL	trans-Ferulic acid
GRRHZ	Glycyrrhizin
CITRA	Citric acid

[0181] Results: Active pharmaceutical ingredient (API) permeation was significantly enhanced when the GSEL was combined with select single excipients (FIG. 1A, FIG. 1B, FIG. 1C, FIG. 1D, FIG. 1E, FIG. 1F, Table E1). Excipients belonging to specific classes such as bile salts, chelators and permeation enhancers were identified to significantly increase API permeability compared to GSEL alone (FIG. 1G).

[0182] The effect of excipient concentration on API permeability was also evaluated (FIG. 1H, 1I). Concentration influenced API permeability for some excipients, but did not impact mean permeability for others. Increasing excipient concentrations above 25 mg/mL resulted in diminishing improvements in permeability.

[0183] Excipients were also tested in combination with each other, and with varying ratios of formulations (FIGs. 2A-D, Table E3, Table E4). Top combinations of excipients that enhanced permeability included at least one bile salt, cholic acid or a salt thereof, or glycocholic acid or a salt thereof. Bile salt combinations of note included 80 mg/mL poly(vinylsulfonic acid) sodium salt, 40 mg/mL ammonium carbonate, 80 mg/mL salcaprozate sodium, 20 mg/mL folic acid, 80 mg/mL phytic acid, and 40 mg/mL sodium dodecyl sulfate (FIG. 3A, FIG. 3B, FIG. 3C, FIG. 3D, FIG. 3E, FIG. 4, Table E5).

Table E2: Mean permeability of tirzepatide from 96 well plate screen

Formulation ID	Pm (%)
GSEL-CHA_40-80	14.617225
GSEL-STC_40-80	14.3403775
GSEL-GCDCA_40-80	12.6334625
GSEL-GDCA_40-80	11.37472575
GSEL-OX_40-80	11.2640775
GSEL-TDCA_40-80	10.5118075
GSEL-GCA_40-80	9.494401
GSEL-DCA_40-80	9.4135005
GSEL-SDS_40-80	6.18036225
GSEL-CDCA_40-80	4.8189925
GSEL-C10_40-80	3.363229
GSEL-GRRHZ_40-80	3.152514
GSEL-HPBCD_40-80	2.56573275
GSEL-CHLORG_40-80	1.39729125
GSEL-HAP:5U_40-80	1.308657
GSEL-NHBICA_40-80	1.24080575

GSEL-NHCO_40-80	1.03374675
GSEL-SNAC_40-80	0.94380975
GSEL-IAA_40-80	0.87040475
GSEL-PVSA_40-80	0.75541025
GSEL-SBECN_40-80	0.73141475
GSEL-PEG:1_40-80	0.68227725
GSEL-ZNO:R10N_40-80	0.5287995
GSEL-C08_40-80	0.5231195
GSEL-PVP:10_40-80	0.45121925
GSEL-K188_40-80	0.40641325
GSEL-PEP:2_40-80	0.39455775
GSEL-SHB_40-80	0.38235425
GSEL-GAPS_40-80	0.38149425
GSEL-LFCS_40-80	0.3741175
GSEL-DADMAC_40-80	0.370069
GSEL-HPGCD_40-80	0.35656225
GSEL-CCO:75N_40-80	0.32975825
GSEL-GERAN_40-80	0.31747375
GSEL-DENDAC2.5_40-80	0.3140605
GSEL-NHP_40-80	0.26399475
GSEL-DGLC_40-80	0.25976275
GSEL-DENDOH4_40-80	0.25540425
GSEL-PHYT_40-80	0.23173875
GSEL_40	0.229966
GSEL-PEG:6_40-80	0.227398
GSEL-PEI:B25_40-80	0.161895
GSEL-KCL_40-80	0.158575
GSEL-LABS_40-80	0.075777333
GSEL-NAC_40-80	0.0685045
GSEL-EPE:2_40-80	0.041631
GSEL-SPMD_40-80	0
GSEL-LCARN_40-80	0

Table E3: Mean permeability of semaglutide from 96 well plate screen

Formulation ID	Pm	Formulation ID	Pm
GSEL-GCA-NHCO_40-120-80	88.28	GSEL-C10_40-40	1.10
GSEL-GCA-NHCO_40-80-80	84.60	GSEL-HCO3_40-40	1.00
GSEL-GCA-NHCO_40-40-80	63.10	GSEL-PVSA_40-80	0.94
GSEL-GCA-NHCO_40-120-40	34.94	GSEL-SBECN_40-40	0.92
GSEL-GCA-PVSA_40-80-80	26.43	GSEL-C10-HPBCD_40-40-80	0.89
GSEL-GCA-NHCO_40-40-40	24.82	GSEL-PHYT-SDS_80-40-40	0.86
GSEL-CHA-PVSA_40-80-80	23.81	GSEL-C08_40-80	0.82

Formulation ID	Pm	Formulation ID	Pm
GSEL-CHA-NHCO_80-40-40	21.41	GSEL-CINA_40-40	0.81
GSEL-GCA-NHCO_40-30-90	18.49	GSEL-HAP:5u_40-20	0.80
GSEL-GCA-NHCO_40-80-40	18.18	GSEL-FERUL_40-40	0.78
GSEL-GCA-NHCO_40-120-20	16.77	GSEL-VB9_40-20	0.72
GSEL-GCA-NHCO_80-40-40	16.29	GSEL-PVSA_40-40	0.68
GSEL-GCA-NHCO_40-60-60	16.08	GSEL-CHLORG_40-40	0.66
GSEL-GCA-NHCO_40-80-20	15.75	GSEL-PHYT-SNAC_40-80-80	0.61
GSEL-GCA-SNAC_40-80-80	15.59	GSEL-QUILZ_40-40	0.61
GSEL-GCA-NHCO_40-90-30	15.41	GSEL-GCA-C10_40-10-30	0.59
GSEL-CHA-SNAC_40-80-80	15.20	GSEL-IAA_40-40	0.58
GSEL-GCA-C10_40-120-80	14.93	GSEL-CITR_40-40	0.55
GSEL-CHA-VB9_40-80-20	14.15	GSEL-PHYT-HPBCD_40-80-80	0.53
GSEL-GCA-VB9_40-80-20	14.10	GSEL-SNAC_40-40	0.53
GSEL-NHCO_40-120	13.71	GSEL-HAP:2.5u_40-40	0.49
GSEL-GCA-C10_40-80-80	13.50	GSEL-GCA-PHYT_40-80-80	0.47
GSEL-CHA-PHYT_40-80-80	13.29	GSEL-C08_40-40	0.45
GSEL-CHA-SDS_80-40-40	12.05	GSEL-PEP:2_40-40	0.42
GSEL-GCA-C10_40-120-40	11.15	GSEL-CINA_40-20	0.41
GSEL-CHA-C10_40-80-80	10.88	GSEL-SHB_40-40	0.40
GSEL-CHA-C10_80-40-40	10.19	GSEL-DGLC_40-40	0.40
GSEL-CHA_40-80	9.64	GSEL-HCO3_40-20	0.38
GSEL-GCA-C10_40-90-30	9.57	GSEL-HPGCD_40-40	0.36
GCA_80	9.56	GSEL-SUCC_40-40	0.34
GSEL-GCA-C10_40-80-40	9.15	GSEL-GCA-C10_40-20-20	0.34
GSEL-GCA-C10_40-120-20	9.12	GSEL-KCL_40-80	0.32
GSEL-GCA-SDS_80-40-40	9.07	GSEL-KCL_40-40	0.32
GSEL-C10_40-120	9.05	GSEL-PHYT-C10_40-80-80	0.26
GSEL-GCA-NHCO_40-60-20	9.01	GSEL-PVP:10_40-40	0.25
GSEL-GCA-C10_80-40-40	8.56	GSEL-NANB_40-40	0.24
GSEL-GCA-C10_40-40-80	8.33	GSEL-DENDAC2.5_40-40	0.23

Formulation ID	Pm	Formulation ID	Pm
GSEL-GCA-C10_40-80-20	6.83	GSEL-PVA:30_40-40	0.22
GSEL-GCA_40-80	6.49	GSEL-HAP:R20n_40-40	0.21
GSEL-GCA-NHCO_40-20-60	5.78	GSEL-GSEL_40-40	0.20
GSEL-CHA-HPBCD_40-80-80	5.29	GSEL-LFCS_40-40	0.20
GSEL-NHCO_40-80	5.09	GSEL-GAPS_40-40	0.17
GSEL-GCA-C10_40-40-40	5.07	GSEL-PEG:6_40-40	0.16
GSEL-GCA-C10_40-60-20	5.02	GSEL-PHYT-PVSA_40-80-80	0.16
GSEL-GCDCA_40-40	4.95	GSEL-CACL_40-40	0.15
GSEL-GCA-HPBCD_40-80-80	4.84	GSEL-CHLORG_40_40	0.15
GSEL-GCA_40-120	4.84	GSEL-EPE:2_40-40	0.14
GSEL-GCA-NHCO_40-40-20	4.49	GSEL-DADMAC_40-40	0.13
GSEL-CHA_40-40	4.44	GSEL-CCO:75n_40-40	0.13
GSEL-GCA_40-40	4.23	GSEL-PHYT-VB9_40-80-20	0.12
GSEL-GCA-C10_40-60-60	4.08	GSEL-MAIS_40-40	0.12
GSEL-GCA-C10_40-30-90	4.02	GSEL-PHYT-C10_80-40-40	0.11
GSEL-C10-PVSA_40-80-80	3.97	GSEL-C10_40-20	0.11
GSEL-GCA-NHCO_40-30-10	3.79	GSEL-HDE_40-40	0.09
GSEL-C10-SNAC_40-80-80	3.71	GSEL-NHCO_40-20	0.09
GSEL-GRRHZ_40-40	3.29	GSEL-LABS_40-40	0.09
GSEL-GCA-C10_40-40-20	3.11	GSEL-PEG:1_40-40	0.08
GSEL-C10-VB9_40-80-20	3.01	GSEL-ZNO:R10n_40-40	0.07
GSEL-C10-PVSA_40-40-80	2.84	GSEL-TRHP_40-40	0.05
GSEL-GCA-NHCO_40-10-30	2.78	GSEL-PEI:L4_40-40	0.00
GSEL-C10-SDS_80-40-40	2.58	GSEL-PEI:B25_40-40	0.00
GSEL-OX_40-40	2.45	GSEL-SPMD_40-40	0.00
GSEL-C10-HPBCD_40-80-80	2.36	GSEL-PEG:6_40_40	0.00
GSEL-GCA-C10_40-30-10	2.26	GSEL-DENDOH4_40-40	0.00
GSEL-C10-SDS_40-40-40	2.20	GSEL-POXZ:5_40-40	0.00
GSEL-C10_40-80	2.12	GSEL-DOC_40-40	0.00
GSEL-PHYT_40-80	2.11	GSEL-GERAN_40-40	0.00

Formulation ID	Pm	Formulation ID	Pm
GSEL-HPBCD_40-80	2.09	GSEL_40	0.00
GSEL-CHAPS_40-40	2.00	GSEL-MAND_40-40	0.00
GSEL-C10-SNAC_40-40-80	1.96	GSEL-MALI_40-40	0.00
GSEL-CHLORG_40-80	1.67		
GSEL-PHYT_40-40	1.65		
GSEL-MOGRO_40-40	1.53		
GSEL-HPBCD_40-40	1.49		
GSEL-GCA-C10_40-20-60	1.46		
GSEL-VB9_40-40	1.34		
GSEL-HAP:5u_40-40	1.34		
GSEL-NHCO_40-40	1.34		
GSEL-SDS_40-40	1.33		
GSEL-GCA-NHCO_40-20-20	1.32		
GSEL-EDTA_40-40	1.29		
GSEL-C10-VB9_40-40-20	1.28		
GSEL-SNAC_40-80	1.21		
GSEL-IAA_40-80	1.19		
GSEL-SDS_40-80	1.18		

Table E4: Mean permeability of Tirzepatide from 96 well plate screen

Formulation ID	Pm	Formulation ID	Pm
GSEL-OX-SNAC_40-80-80	7.120595	GSEL-C10-PHYT_40-80-40	0.312469
GSEL-OX-PHYT_40-80-40	6.806515	GSEL-C10-HPBCD_40-80-80	0.296566
GSEL-C08-OX_40-40-80	5.808677	GSEL-SHB_40-40	0.286986
GSEL-OX-VB9_40-80-20	5.733973	GSEL-HPBCD_40-40	0.282702
GSEL-OX-SNAC_80-40-80	5.728415	GSEL-C10-SNAC_40-80-80	0.272402
GSEL-OX_40-40	5.618745	GSEL-PHYT-VB9_40-40-20	0.270446
GSEL-OX-PVSA_40-80-80	4.966895	GSEL-DENDOH4_40-40	0.264641
GSEL-C10-OX_80-40-80	4.392149	GSEL-C10-SNAC_40-40-80	0.261132
GSEL-C10-OX_40-40-80	4.202414	GSEL-SDS_40-40	0.24696
GSEL-CHAPS_40-40	4.177833	GSEL-VB9_40-20	0.230579
GSEL-NHCO-OX_40-40-80	4.040422	GSEL-C10-PVSA_40-40-80	0.220385

Formulation ID	Pm	Formulation ID	Pm
GSEL-C10-OX_40-80-80	3.406085	GSEL-SPMD_40-40	0.203597
GSEL-CHA_40-40	3.255406	GSEL-C10-VB9_40-40-20	0.176163
GSEL-GCDCA_40-40	2.337877	GSEL-PHYT-PVSA_40-40-80	0.173233
GSEL-HPBCD-OX_40-80-80	2.313999	GSEL-DADMAC_40-40	0.155933
GSEL-OX_40-80	2.209829	GSEL-PVP:10_40-40	0.146657
GSEL-GCA_40-40	2.007605	GSEL-PHYT-SNAC_40-40-80	0.143743
GSEL-NHCO_40-40	1.699936	GSEL-SNAC_40-40	0.137929
GSEL-POXZ:5_40-40	1.630138	GSEL-CACL_40-40	0.129819
GSEL-PHYT_40-40	1.5604	GSEL-PEG:6_40-40	0.12388
GSEL-HPBCD-PHYT_40-80-40	1.526263	GSEL-CINA_40-40	0.110599
GSEL-VB9_40-40	1.405658	GSEL-DGLC_40-40	0.109028
GSEL-PVSA_40-40	1.116028	GSEL-HPBCD_40-80	0.094392
GSEL-C10_40-40	0.998059	GSEL-PEI:B25_40-40	0.092415
GSEL-C10_40-80	0.959741	GSEL-HAP:5u_40-40	0.091132
GSEL-CITR_40-40	0.908472	GSEL-EPE:2_40-40	0.076187
GSEL-C10-PVSA_40-80-80	0.834144	GSEL-DENDAC2.5_40-40	0.056057
GSEL-PEG:1_40-40	0.807619	GSEL-ZNO:R10n_40-40	0.053071
GSEL-CHLORG_40-40	0.767041	GSEL_40	0.020832
GSEL-SBECD_40-40	0.576162	GSEL-PEI:L4_40-40	0
GSEL-IAA_40-40	0.537574	GSEL-PEP:2_40-40	0
GSEL-EDTA_40-40	0.5367	GSEL-CCO:75n_40-40	0
GSEL-C10-HPBCD_40-40-80	0.512985	GSEL-GAPS_40-40	0
GSEL-KCL_40-40	0.470564	GSEL-LABS_40-40	0
GSEL-HPGCD_40-40	0.463945	GSEL-LFCS_40-40	0
GSEL-C10-VB9_40-80-20	0.447494	GSEL-DOC_40-40	0
GSEL-C08_40-40	0.438409	GSEL-GERAN_40-40	0
GSEL-C10-C08_40-80-40	0.393746	OX_80	0
GSEL-HCO3_40-40	0.388316	GSEL-PVSA_40-80	0
GSEL-PVA:30_40-40	0.371791	GSEL-SNAC_40-80	0
GSEL-C10-C08_40-40-40	0.320874	GSEL-C08-PHYT_40-40-40	0

Formulation ID	Pm	Formulation ID	Pm
GSEL-C10-PHYT_40-40-40	0.320217		

Table E5: Permeability data of tirzepatide delivered by GSELS including ammonium carbonate with GCA or ammonium carbonate with CHA

Formulation	Pm (%)
GSEL-GCA-NHCO_40-120-80	88.28
GSEL-GCA-NHCO_40-80-80	84.60
GSEL-GCA-NHCO_40-40-80	63.10
GSEL-GCA-NHCO_40-120-40	34.94
GSEL-GCA-NHCO_40-40-40	24.82
GSEL-CHA-NHCO_40-80-40	21.41
GSEL-GCA-NHCO_40-30-90	18.49
GSEL-GCA-NHCO_40-80-40	17.42
GSEL-GCA-NHCO_40-120-20	16.77
GSEL-GCA-NHCO_40-60-60	16.08
GSEL-GCA-NHCO_40-80-20	15.75
GSEL-GCA-NHCO_40-90-30	15.41
GSEL-NHCO_40-120	13.71
GSEL-GCA-NHCO_40-60-20	9.01
GSEL-GCA-NHCO_40-20-60	5.78
GSEL-NHCO_40-80	5.38
GSEL-GCA-NHCO_40-40-20	4.49
GSEL-GCA-NHCO_40-30-10	3.79
GSEL-GCA-NHCO_40-10-30	2.78
GSEL-GCA-NHCO_40-20-20	1.32
GSEL-NHCO_40-40	1.26
NHCO_40	0.47
GSEL-NHCO_40-20	0.09
GSEL_40	0.00

Example 2A*Evaluation of Permeation Enhancers – Franz cell experiments*

[0184] Franz cells were used for testing different permeation enhancers on ex vivo tissue from the small intestine, such as porcine duodenal tissue (or colon tissue where indicated). Water pumps were attached to the Franz cell rack to maintain each Franz cell at 37°C. The racks were loaded with Franz cells, and each cell was filled with 5 mL of phosphate buffered saline. Tissue was cut into ~3.2x3.2 cm squares and any large clumps of mucus or excrement was removed before mounting onto the Franz cells. Next, gastrointestinal synthetic epithelial lining (GSEL) solutions was prepared by first weighing out dopamine HCl, polydopamine, and a permeation enhancer into a vial. The chosen active pharmaceutical ingredient (API), Tris buffer (50 mM, pH=8.5), and hydrogen peroxide (1M) were then added to the vial, which was then vortexed until all solids were dispersed (brief sonication was used if necessary). 250 µL of the final mixture was then added to the donor compartment of each Franz cell. Samples from the receiving compartment was periodically collected from 0 to 24 hours and the concentration of API is measured. At each sampling point, 200 µL of phosphate buffered saline is added to replace the removed volume (200 µL).

[0185] The same experiment was carried out, except without using a permeation enhancer in the GSEL solution. The amount of API detected in the receiving compartment for GSELs with permeation enhancer was compared to the amount of API detected in the receiving compartment for GSELs without permeation enhancer at each time point to determine the increase in permeation provided by the permeation enhancer.

[0186] Sample preparation consisted of protein precipitation in methanol. Samples were analyzed via LC-MS. For some tests an ELISA was used for quantification of semaglutide. Permeability (Pm %) was calculated as follows:

$Pm(\%) =$

$$\left(\frac{\text{Concentration of dye or semaglutide in receiver chamber} \left(\frac{\mu\text{g}}{\text{mL}} \right) * \text{Volume in receiver chamber (mL)}}{\text{Initial dye or semaglutide added in donor chamber (ug)}} \right) *$$

100

Table E6: Percent mean permeability of semaglutide delivered by GSELS including bile salt and ammonium carbonate combinations (Franz cell data)

Formulation	Pm (%)
GSEL-CHA-NHCO_40-80-40	29.57
GSEL-CHA-SNAC_80-80-80	27.00
GSEL-CHA-NHCO_40-40-40	24.00
GSEL-CHA_40-160	23.14
GSEL-GCDCA_40-80	17.50
GSEL-CHA_40-80	15.77
GSEL-GCDCA_40-40	14.00
GSEL-CHA_40-40	8.95
GSEL-GCA_40-40	8.55
GSEL-C12_40-40	8.33
GSEL-OX_40-40	7.00
GSEL-SPAN_40-40	6.43
GSEL-C10_40-40	3.40
GSEL-NHCO_40-40	3.38
GSEL-PhyticA_40-40	1.29
GSEL-DCA_40-40	1.14
GSEL_40	0.86

[0187] Results: Excipients identified in the 96 well screen were further evaluated for efficacy was also tested in Franz cells. API (such as semaglutide and tirzepatide) delivery by liquid and powder formulations of GSEL was measured (FIG. 5, FIG. 13A, FIG. 13B, and Table E6). Permeability was significantly enhanced for some of the combinations of excipients when the API was delivered by liquid formulation compared to a powder formulation (FIG. 9, FIG. 10).

[0188] Screening results of 50 single excipients demonstrated that bile salts were the most promising class for further investigation in combination with other excipients (n=4-15). From the executed screenings on semaglutide it became evident that bile salts such as sodium glycocholate (GCA) and CHA in combination with ammonium carbonate (NHCO) enhanced

permeability to 24.8-35.8% (FIG. 3B). Further permeability tests on Franz cells demonstrated the same synergistic effect when bile salts were combined with other ammonium salts (FIG. 3D). Based on the dual excipient screen, another combination that demonstrated a synergistic effect was CHA or GCA with SNAC (FIG. 3C). This result was also supported by supplementary tests on Franz cells where additional benzene ring-based molecules were tested (FIG. 3E).

[0189] FITC-Dextran-4K (FD4) and FITC-Dextran-40K (FD40) are molecules with an approximate molecular weight of 4 kilodaltons and 40 kilodaltons, respectively. Excipients were tested for transporting FD4, demonstrating that including excipient increased transport (FIG. 6A). Excipient combinations were also tested by assessing FD40 permeability across the GSEL (FIG. 11A, FIG. 11B). Permeation of the API using formulations that included GSEL, GCA and NHCO were comparable to GCA and NHCO alone at 6 and 20 hours, suggesting that the 40 mg/mL GSEL did not inhibit the efficacy of GCA and NHCO as general excipients (FIG. 6B).

[0190] For FD4, the total amount of FD4 that permeated increased as the ratio of excipient (GCA (40 mg/mL) and NHCO (40 mg/mL) increased relative to GSEL, but the amount of FD4 permeated over time remained similar irrelevant of GSEL formulation (FIG. 7A, FIG. 7B, FIG. 7C). Permeation was observed to occur at a linear rate (FIG. 7A, FIG. 7B). The permeation enhancers (PE) used were a 1:1 combination of sodium glycocholate (GCA) and ammonium carbonate (NHCO), at 40 mg/ml each, i.e. GCA-NHCO_40-40.

[0191] To confirm that the excipient-GSEL combination was effective across multiple tissue types, the assay was also performed using colon tissue. The total amount of semaglutide permeated was significantly enhanced by the addition of NHCO and GCA to the GSEL (FIG. 8, FIG. 9, FIG. 10A, FIG. 10B, FIG. 10C, FIG. 10D, FIG. 11A, FIG. 11B). Rate of API permeation was low during the initial six hours after GSEL delivery, but then increased linearly after six hours (FIG. 10B, FIG. 10C).

[0192] Other excipient combinations were also confirmed by Franz cell experiments (FIG. 12A, FIG. 12B, FIG. 13A, FIG. 13B). Permeation of semaglutide over duodenal tissue using formulations that included GSEL, GCA and NHCO was observed to be greater compared to GCA and NHCO alone at 20 hours, suggesting that the 40 mg/mL GSEL did not inhibit the efficacy of GCA and NHCO as general excipients (FIG. 13A, FIG. 13B). The data also suggests that certain combinations of excipients enhanced the permeation of the API through the GSEL.

Example 2B*Evaluation of Permeation Enhancer Formulations*

[0193] Methods:

[0194] *Ex vivo* GSEL coverage test:

[0195] Porcine duodenum samples were cut into ~16.5 mm diameter circular pieces and added to a 24-well plate with luminal side facing up. GSEL composition consisted of 25 mg/mL PDA nanoparticles, 9.8 mg/mL dopamine-HCl, 0.67 mg/mL hydrogen peroxide (H₂O₂). 400 µL of GSEL suspension were immediately added to the tissue in the 24-well plate. Tissue samples were removed in replicates of 3 from the solution at timepoints 0, 5, 10, 15, 20, 25, 30 and 120 min post solution addition. At each time point, tissue samples were removed from the well and washed 3 times in PBS. The washed samples were then imaged and analyzed to assess percent of GSEL coverage on the tissue.

[0196] *Ex vivo* static colocalization tests:

[0197] Porcine duodenum samples (2.54 x 2.54 cm²) were placed on an aluminum foil tray where the tissue was laid flat. The luminal surface of the duodenum was exposed to conduct testing on the mucosal layer of the tissue, while the serosal side of the tissue was laid flat on the foil tray. The tissue was capped with a cylinder with 9 mm orifice diameter, where the GSEL suspension was added for static colocalization tests. GSEL composition for tests with FD dyes was: 6.25 mg PDA, 2.45 mg DA-HCl, with a 14.6:1 DA-HCl:H₂O₂ weight ratio, suspension in Tris buffer 50mM pH 8.5. FD dyes fluorescein isothiocyanate-dextran 4kDa, 10kDa or 40kDa were co-formulated at 0.078-0.156 mg, with 2 µL/mm² (125 µL/63.61 mm²) or 4 µL/mm² (250 µL/63.61 mm²), dispensing volume. PE system included CHA 5-20 mg or GCA 10 mg +NHCO 10 mg. The groups were incubated for 30 min. Samples were collected from the donor chamber and FD dyes quantification was conducted by measuring fluorescence at excitation wavelength 490 nm and emission wavelength 520 nm.

[0198] For tests with semaglutide, different GSEL or PE formulation concentrations were tested. GSEL compositions are follows: 1xGSEL: 6.25 mg PDA, 2.45 mg dopamine-HCl, 0.17mg H₂O₂, 2xGSEL: 12.5 mg PDA, 4.9 mg, 0.34 mg H₂O₂, 4xGSEL: 25 mg PDA, 9.8 mg, 0.68 mg H₂O₂. PE system was composed of GCA/NHCO; 0.1xPE: 1 mg GCA, 1 mg NHCO mg, 1xPE: 10 mg GCA, 10 mg NHCO. Semaglutide was co-formulated at different amounts between 0.25-0.5 mg and all formulations were suspended in Tris buffer 50 mM, pH 8.5. The volume over surface area was 125 µL/63.61 mm². 1 M hydrogen peroxide stock was prepared fresh before each experiment. GSEL suspension test groups were prepared by

weighing out PDA, dopamine hydrochloride, and the PEs in glass scintillation vials. Semaglutide stock solutions were prepared in Tris buffer 50 mM, pH 8.5. The semaglutide solution and hydrogen peroxide were added in the vials and mixed by vortexing and sonication for 1 min. Once the suspensions were dispensed the groups were incubated for 0.5-1h. Samples were collected from the apical surface of the tissue, centrifuged at 16,000xg for 8 minutes at 4°C and the supernatants were collected, diluted in Tris buffer (50 mM, pH 8.5) and then mixed with boric acid (80mg/mL, pH ~8.0) at a volume ratio 1:2 (sample: boric acid) to inhibit any further dopamine polymerization and precipitation. Next the samples were stored into micronic HDPE vessels and kept at -20 °C until analysis. Samples were thawed, then diluted in methanol prior to analysis by HPLC.

[0199] Dye or semaglutide entrapped (%) was calculated as follows:

Dye or Semaglutide entrapped(%)

$$= \left(1 - \frac{\text{Dye or semaglutide in donor chamber(mg)}}{\text{Initial dye or semaglutide added in donor chamber (mg)}} \right) * 100$$

[0200] Co-administration versus co-formulation test:

[0201] In co-formulation groups, all GSEL components and cholic acid (CHA) were mixed and suspended simultaneously with FD 4kDa, in co-administration groups GSEL components and CHA were mixed and suspended first, FD 4kDa was added 10 min later. Co-formulation vs co-administration groups were tested as liquids or solids' form. For liquids in coformulation test all components (6.25 mg PDA, 2.45 mg DA-HCl, with a 14.6:1 DA-HCl:H₂O₂ weight ratio, 10 mg CHA, 1 mg FD4) were suspended in Tris buffer with 4 μL/mm² (250 μL/63.61 mm²) dispensing volume, whereas in coadministration GSE+CHA were suspended and added first in Tris buffer and FD4 was added 10 min later dissolved in Tris buffer as well. For powders in coformulation test all components (6.25 mg PDA, 2.45 mg DA-HCl, with a 5.33:1 DA-HCl: urea hydrogen peroxide weight ratio, 10 mg CHA, 1 mg FD4, 1.04 mg Tris base, 0.56 mg Tris acid) were added simultaneously as a blend in the donor chamber and 10 min later water was added with 4 μL/mm² (250 μL/63.61 mm²). In coadministration GSEL components and CHA were added first and FD4 was added 10 min later dissolved in water with 4 μL/mm² (250 μL/63.61 mm²) dispensing volume. All groups were incubated for 24 hours, when samples were collected from the receiver chamber and FD4 quantification was conducted by measuring fluorescence at excitation wavelength 490 nm and emission wavelength 520 nm.

[0202] Results: FITC-dextran 4 kDa (FD4), 10 kDa (FD10) and 40 kDa (FD40) were used as model macromolecules to validate the ability of GSEL to entrap macromolecules, and assess GSEL permeability through ex vivo tissue when integrated with permeation enhancers. To determine the rate of GSEL formation, the GSEL components were dispensed on tissue and left to react for different durations. These experiments showed that GSEL incubation past 30 minutes did not seem to significantly change coverage (FIG. 14).

[0203] To test colocalization of FD4 on duodenal tissue, liquid (e.g., suspension) formulations were prepared by adding all components into an aliquot of Tris buffer simultaneously, then directly administered to the tissue (FIG. 15). For solid (e.g., powder) formulations, all powders and Tris salts were added as solids on the tissue surface and water was added 10 minutes later (FIG. 15, FIG. 16, FIG. 17). Afterwards, the supernatants were measured for FD4 content.

[0204] Co-formulation in suspension tests showed an increase in permeability of 38.3% compared to co-administration, albeit not statistically significant, while for solids the increase (69.4%) was more evident for the co-formulation delivery method as opposed to the co-administration method with 69.4% improvement, suggesting that FD4 being present in the matrix during GSEL formation is critical for co-localization (FIG. 17).

[0205] GSEL alone was sufficient to entrap dye (FIG. 16, FIG. 18). Further colocalization tests using FD4, FD10, and FD40 showed that co-delivery with GSEL suspensions led to $31.0 \pm 6.7\%$, $29.3 \pm 1.7\%$, and $25.8 \pm 3.0\%$ entrapment (FIG. 18), respectively, compared to the $<1\%$ entrapped amount of the control groups (FIG. 18). The addition of a permeation enhancer, sodium cholate (CHA) led to increased entrapment for FD10 and FD40 ($39.8 \pm 0.5\%$ and 38.5 ± 5.3 , respectively), suggesting potential synergistic effects of certain PEs with GSEL formulation (FIG. 18).

[0206] GSEL powders were also tested with FD4 and illustrated an improved entrapment effect ($62.3 \pm 9.0\%$ colocalization) when compared to delivery as a suspension (FIG. 16).

[0207] Substrates of various sizes were found to permeate the porcine duodenal tissue at a higher percentage when applied with GSEL that comprised a permeation enhancer, versus GSEL with no permeation enhancer (FIG. 19, FIG. 20A, FIG. 20B). More specifically, the addition of CHA led to 2.25, 2.74, and 3.58-fold increase for FD4, FD10, and FD40 (respectively) after 24 hours (FIG. 19).

Example 2C

Evaluation of Permeation Enhancers – Dynamic assays

[0208] Methods:

[0209] *Ex vivo* dynamic colocalization test

[0210] Porcine duodenum tissue was dissected into 6-inch segments and washed using an Antibiotic-Antimycotic solution that was diluted 1:20 in 1x PBS, pH 7.4. Tissue was placed on an angled surface (17.92°). GSEL suspension (3 mL) with different GSEL concentrations and/or permeation enhancers were dispensed through the tissue over 1 min. GSEL composition was as follows: PDA 25 mg/mL and DA-HCl=9.8 mg/mL for 1xGSEL, PDA 50 mg/mL and DA= 19.6 mg/mL for 2xGSEL, 14.6:1 DA-HCl:H₂O₂ ratio, in Tris buffer 50mM, pH 8.5. PE composition was: 40 mg/mL GCA+ 40 mg/mL NHCO, FD4 dye or semaglutide concentration was at or 1 mg/mL or 2 mg/mL, respectively. The flow-through was collected in tubes and dye was quantified as mentioned previously for static colocalization tests. For semaglutide, entrapment was also assessed with the same protocol followed for static colocalization tests.

[0211] The gastrointestinal tract is a dynamic environment. Low entrapment could still permit increased permeability in static conditions, however in a dynamic environment it could translate to low residence time and poorer absorption. On the other hand, high levels of entrapment of semaglutide within the GSEL platform might impair permeability through the tissue because the semaglutide diffusion out of the network is too slow. Thus, it was investigated how changing the ratios among GSEL, permeation enhancer (PE) and semaglutide affected colocalization and permeability. The dynamic assay comprised assaying GSEL delivery on tissue mounted at an angle (FIG. 21).

[0212] GCA and NHCO were identified to be highly synergistic with semaglutide and GSEL (FIG. 22A, FIG. 22B), and formulation was modified to improve co-localization and permeation. The formulation (1xGSEL_1xPE) was identified to comprise: 1xGSEL= 6.25 mg polydopamine nanoparticles (PDA), 2.45 mg dopamine hydrochloride (DA-HCl), 1xPE= 10 mg GCA and 10 mg NHCO.

[0213] Using this formulation, a permeability test was executed to test semaglutide co-formulated with GSEL and GCA-NHCO against controls: semaglutide only, semaglutide+SNAC (14:300 by mass based on the Rybelsus[®] tablet), semaglutide+GSEL, and semaglutide+GCA+NHCO (FIG. 22C). In this static Franz cell test, it was evident that GCA-NHCO is a permeation enhancer system that significantly improves permeability to

19.9-fold or 15.5-fold without or with GSEL, respectively (FIG. 22C). In contrast, SNAC did not promote permeation of semaglutide as much (FIG. 22C). Notably, as the Franz tests were static tests, it was hypothesized that GSEL could provide a localized depot to increase residence time at the deposition spot and lead to higher absorption.

[0214] Changing the ratio of permeation enhancer to GSEL from 1x to 0.5x or 2x did not improve permeability in a statistically significant manner, indicating that decreasing permeation enhancer concentration by half would not impact the enhancement of permeability (FIG. 22D).

[0215] Changing GSEL content revealed that 1x GSEL and 2x GSEL did not seem to have an effect, while 4x GSEL significantly decreased permeability, indicating that increasing the GSEL:PE ratio could have a negative effect on semaglutide release and tissue absorption (FIG. 22E). Under static conditions, increasing GSEL content relative to PE concentration increased semaglutide entrapment (FIG. 22K). However, under dynamic conditions, the GSEL content did not impact entrapment in a statistically significant manner (FIG. 22L). FD4 was used as an exemplary molecule of 4KDa in size to further verify that increasing the concentration of GSEL relative to permeation enhancer led to greater entrapment in both static and dynamic assays (FIG. 22F, FIG. 22G).

[0216] In *ex vivo* experiments, when permeation enhancer content decreased 10-fold (0.1xPE to GSEL), there was higher colocalization (>60%, FIG. 2H, 2I), in a preliminary dynamic test but permeability decreased to 0.5 % (FIG. 2J).

[0217] Different ratios of permeation enhancer: semaglutide were examined where increasing amount of semaglutide was co-formulated with 2xGSEL and 1x permeation enhancer. All formulations exhibited similar percent permeability (FIG. 2M).

Example 3

Gastrointestinal Synthetic Epithelial Lining Formulation with Permeation Enhancer for Administration to Subject: Evaluation in Pig Model

[0218] Methods:

[0219] *In vivo* pharmacokinetic studies

[0220] All animal work was executed in accordance to animal protocol G2022-142 approved by IACUC. Studies were performed at Cummings School of Veterinary Medicine at Tufts University (Division of Animal Resources). Yorkshire pigs, weighing 40-80 kg each over 7 months and approximately 4-9 months of age were used. Following routine preoperative fast, pigs were anesthetized, intubated, and maintained on isoflurane anesthesia

during the procedure. An indwelling marginal ear vein catheter was be inserted for blood collection, which occurred over 1-7 days. Dry GSEL powders containing permeation enhancers and semaglutide were solubilized with Tris buffer immediately before administration. An endoscope guided catheter was placed into the proximal small intestines, where the GSEL suspensions were injected. Blood was taken periodically from the ear vein catheter and the blood was immediately centrifuged down in serum collection tubes. Samples were analyzed via ELISA and LC-MS. Pigs were woken up after 6 hours and monitored until full recovery.

[0221] More specifically, following a preoperative fast, the pigs were anesthetized, intubated, and maintained on isoflurane anesthesia during the procedure. An endoscope guided catheter was placed into the duodenum to allow for direct delivery of formulations. Experimental or control groups were mixed and delivered into the duodenum of fasted Yorkshire pigs via catheter with 1-3 mL/min administration rate. For 1xGSEL pigs the delivered formulation consisted of 4.567 mg/kg PDA, 1.789 mg/kg dopamine hydrochloride, 0.122 mg/kg hydrogen peroxide, in Tris buffer, pH 8.5. For 2xGSEL pigs the delivered formulation consisted of 9.133 mg/kg PDA, 3.596 mg/kg dopamine hydrochloride, 0.244 mg/kg hydrogen peroxide, in Tris buffer, pH 8.5. For 3xGSEL pigs the delivered formulation consisted of 13.7 mg/kg PDA, 5.394 mg/kg dopamine hydrochloride, 0.366 mg/kg hydrogen peroxide, in Tris buffer, pH 8.5. For 1xPE pigs the formulation consisted of 6.4 mg/kg GCA and 6.4 mg/kg NHCO. For 2xPE pigs the formulation consisted of 12.8 mg/kg GCA and 12.8 mg/kg NHCO. Semaglutide dose in all cases was 1.5 mg/kg semaglutide. Suspensions dosing was 0.183 mL/kg. 1 pig received a semaglutide solution without the addition of permeation enhancers at 1.5 mg/kg semaglutide via endoscopic placement into the duodenum. 2 pigs (IV group) received an intravenous injection of 1 mg semaglutide in PBS (1 mg/mL) to enable calculations of absolute bioavailability (F%). Plasma samples were collected at timepoints 0, 5min, 15min, 30min, 45 min, 1h, then hourly up to 12h and 24h, 48h, 72h, 144h, 168h and analyzed with LC-MS/MS (see below). Pharmacokinetic (PK) curves were plotted and the area under the curves (AUC) were calculated using Prism. Bioavailability (F%) was calculated using the following formula:

$$F\% = \left(\left(\frac{AUC_{ORAL}}{AUC_{IV}} \right) * \left(\frac{Dose_{IV}}{Dose_{ORAL}} \right) \right) * 100$$

[0222] For semaglutide analysis from porcine plasma the following procedure was followed:

[0223] Blood samples were drawn and collected in K₃EDTA tubes and immediately centrifuged at 10,000 rpm for 5 minutes to isolate the plasma layer. Plasma samples were stored in micronic storage vessels at -80 °C until analysis. Sample preparation consisted of protein precipitation in methanol. An LC-MS/MS (liquid chromatography tandem mass spectrometry) method was developed and validated to measure the concentration of semaglutide in plasma.

[0224] For the following experiment, a ratio of formulation components as presented in **Table E7** was used (e.g., 2x GSEL, 6.4 mg/kg GCA, 6.4 mg/kg NHCO, with semaglutide at a final ratio of 20:2.35 permeation enhancer to semaglutide; or 3xGSEL with 2xPE (12.73 mg/kg GCA and 12.73 mg/kg NHCO)).

Table E7: Formulation components

Table E7: Formulation components							
Ex vivo, Mass (mg), dispensing volume 125 μ L/63.61 mm ² or 250 μ L/63.61 mm ² , in 50mM Tris, pH 8.5							
GSEL Components	1x GSEL	2x GSEL	4x GSEL				
PDA	6.25	12.50	25.00				
Dopamine HCl	2.45	4.90	9.80				
Tris acid	0.56 or 0.28	0.56 or 0.28	0.56 or 0.28				
Tris base	1.08 or 0.54	1.08 or 0.54	1.08 or 0.54				
Hydrogen peroxide	0.17	0.34	0.68				
PE Components				0.1x PE	0.5x PE	1x PE	2x PE
NHCO				1	5	10	20
GCA				1	5	10	20
PE:Semaglutide							
		20:0.5	20:1	20:2	20:4	20:8	
Semaglutide (mg)		0.25					
			0.5				
				1			
					2		
							4
In vivo, Mass per pig weight (mg/kg)							
GSEL Components	1x GSEL	2x GSEL	3x GSEL				
PDA	4.567	9.133	13.700				
Dopamine HCl	1.798	3.596	5.394				
Tris acid	0.395	0.395	0.395				
Tris base	0.762	0.762	0.762				
Hydrogen peroxide	0.122	0.244	0.366				
PE Components				1x PE	2x PE		
NHCO				6.4	12.8		
GCA				6.4	12.8		
Semaglutide				1.5			

[0225] Results: GSEL formulations comprising permeation enhancers were identified which result in semaglutide plasma concentrations significantly greater in the pigs receiving the GSEL formulation compared to the pigs receiving the non-GSEL formulation or the pigs receiving semaglutide alone. The GSEL suspensions led to increased plasma semaglutide levels and overall semaglutide exposure over 24 or 168 h (**FIG. 23C and Table E8**).

[0226] PK curves are presented for intravenous administration of semaglutide alone, endoscopic placement of semaglutide (control), versus endoscopic placement of semaglutide delivered with GSEL with permeation enhancers at various formulations, versus endoscopic placement of semaglutide delivered with the permeation enhancers alone as a control (FIG. 23A, FIG. 23B, FIG. 23C). Based on the PK values, the area under the curve (AUC) was calculated for each group and the results were plotted for different timepoints at 24, 48, 72 and 168 hours (FIG. 23D, FIG. 23E, FIG. 23F, FIG. 23G). In all cases, the delivery of semaglutide with GSEL and permeation enhancers demonstrated at least a 3-fold increase in semaglutide uptake, as compared to the control (semaglutide administered with permeation enhancers), while delivery of semaglutide without any PEs showed zero absorption for 1.5 mg/kg dose for n=1. This result is likely because of the depot effect that the GSEL provides, increasing the retention time of the macromolecule on the duodenal tissue resulting in enhanced absorption.

[0227] The absolute bioavailabilities (e.g., F = fraction absorbed) are shown in **Table E8**, with semaglutide delivered with GSEL/permeation enhancers achieving bioavailabilities ranging from 0.5-5.0 %. The AUC, C_{max}, t_{max} for each individual pig test in all groups are reported in **Table E9 and E10**. All groups were endoscopically delivered to the duodenum except for the IV and the Rybelsus[®] groups, which were endoscopically delivered to the stomach.

[0228] The GSEL/permeation enhancer formulation was observed to be more effective in comparison to the GSEL alone dose, as shown from the AUC up to 48 hours post administration (**Table E8**). A 3-fold increase in relative bioavailability compared to the control and an absolute bioavailability of up to 2.4±1.6%, ranging from 0.5 to 4.5% (n=4) was observed, while further tuning of the formulation resulted in improved bioavailability of 5.0% (n=1).

[0229] Upon recalculation and/or addition of new data, it was observed from the area under the curve (AUC) calculation, GSEL:PE was found to exhibit 6-fold improvement compared to the control group where only the PEs was formulated with semaglutide. To evaluate absolute bioavailability, 2 pigs received semaglutide as an intravenous injection,

demonstrating an elimination half-life of 63.4 ± 1.5 h based on the PK curves of the 2 pigs (FIG. 23B). Semaglutide delivered intraduodenally without any PEs showed zero absorption at 1.5 mg/kg dose (n=1, Table E8). Notably, the GSEL:PE suspension system led to an average bioavailability of $2.4 \pm 1.6\%$ (n=4, ranging from 0.5 to 4.5%) when compared to the $0.4 \pm 0.3\%$ of the 1xPE control group (n=5), ranging from 0.1% to 0.8% (Fig. 23C). This result is attributed to the depot and colocalization effect GSEL:PE provides, which increased the retention time of semaglutide on the duodenal tissue resulting in enhanced absorption.

[0230] Next, a set of preliminary tests were performed to establish correlations between drug absorption and formulation parameters. For these tests, the effects of different GSEL concentrations and GSEL:PE ratios were evaluated (**Tables E8 and E9**). The 2xGSEL_1xPE was more effective in comparison to the 1xGSEL_1xPE (n=3) or 3xGSEL_1xPE (n=1) dose as shown from the AUC for up to 48h, (**Table E8 and FIG. 24**), suggesting there is an optimum GSEL:PE ratio for a fixed concentration of PE. Comparisons were only done with 48 hours of data (AUC_{48h}) due to constraints limiting blood draws from pigs in the 1xGSEL_1xPE group. Additional experiments changed the GSEL:PE ratio by altering the PE concentration or the GSEL concentration (**Tables E8, E9, E10**). These tests showed that increasing the PE concentration with or without GSEL does not improve bioavailability, as also shown from the PK curves' trend (FIG. 25). These results corroborate results from ex vivo permeability tests and suggest that a more complex interaction takes place between the PEs, GSEL, and semaglutide (FIGs. 22C, 22D, 22E, 22J, 22K, 22L).

[0231] To compare to the current marketed oral product, Rybelsus[®] tablets were endoscopically delivered to the stomach of two pigs (Table E8 and FIGs. 26A-C). These pigs resulted in bioavailability values of 1.7% and 1.2% (average $1.4\% \pm 0.3$), similar to reported results for SNAC-based semaglutide formulations in large animals. The GSEL:PE formulations of 2xGSEL_1xPE and 3xGSEL_2xPE reached its highest bioavailability of 5.0% and respective average bioavailabilities of $2.4\% \pm 1.7\%$ and $1.9\% \pm 2.1$, representing a ~70% and ~40% improvement over Rybelsus[®] (FIGs. 26A-C and Table E9). These data demonstrate that GSEL:PEs can enable novel SNAC-less formulations to further improve oral bioavailability of peptide drugs. Interestingly, it was observed that the two Rybelsus[®] dosed pigs experienced consistent adverse effects (nausea, abdominal discomfort, lethargy) post dosing, while these effects were rarely seen in the GSEL:PE treated pigs. It was hypothesized that the increased gastric emptying time in pigs could contribute towards increased nausea, but also note that this increased time could also help increase bioavailability since Rybelsus[®] has been shown to absorb in the stomach.²⁴ The Rybelsus[®]

dosed pigs also received a smaller semaglutide dose (1 mg/kg) compared to the GSEL:PE treated pigs (1.5 mg/kg) to mitigate adverse effects, so the repeated observation of adverse effects in the Rybelsus® treated pigs stood out. Regardless, further experimentation is needed to draw clear conclusions about the tolerability of SNAC versus PEs used in this work and about how location of absorption (stomach vs small intestines) may affect adverse effects.

[0232] A set of tests were performed in pigs to establish correlations between drug absorption and formulation parameters. For these tests, the effects of different GSEL concentrations and GSEL:PE ratios were evaluated (**Table E8**). The 2xGSEL_1xPE was more effective in comparison to the 1xGSEL_1xPE (n=3) or 3xGSEL_1xPE (n=1) dose as shown from the AUC for up to 48h, (**FIG. 24, Table E8**), suggesting that there is an optimum ratio for GSEL:PE for a fixed concentration of PE. Increasing the PE concentration with or without GSEL did not improve bioavailability, as also shown from the PK curves' trend (FIG. 25).

[0233] Oral macromolecule delivery and tolerable use of permeation enhancers remain a significant challenge due to insufficient co-localization of large molecular weight macromolecules with often small molecular weight PEs and low residency times of all components. These challenges severely reduce dose efficiency, which in turn constrain viable dosage forms for commercialization. The formulations disclosed herein addresses these challenges by co-formulating macromolecules with GSEL and permeation enhancers and providing a localized, targeted drug depot in the duodenum. Using the high-throughput screening shown in FIG. 1C, synergistic combinations of GSEL and different permeation enhancer systems were identified for semaglutide. Using these synergistic combinations, the Yorkshire pig studies showed that combining semaglutide with GSEL and synergistic, SNAC-less PEs led a 2.4% absolute bioavailability which corresponds to a 6-fold increase in relative bioavailability compared to the control and a 1.7-fold increase in relative bioavailability compared to Rybelsus®. These studies demonstrate GSEL's ability to co-localize macromolecule drugs to the duodenum and provide a targeted depot to enhance oral bioavailability.

Table E8: Bioavailability results for all groups tested in vivo.

	¹ SEMA dose	AUC/ dose (24 h)	AUC/ dose (48 h)	AUC/ dose (72 h)	AUC/ dose (168 h)	F (24 h)	F (48 h)	F (72 h)	F (168 h)	F (std)	n
IV	0.02	332984			977349						2

Control (SEMA only)	1.5	0	0	0	0	0.0%	0.0%	0.0%	0.0%	0	1
Rybelsus® (stomach delivery)	1.0	4742			14000					1.4% (0.3)	2
Control (1xPE)	1.5	1781	2592	2933	3694	0.5%	0.5%	0.4%	0.8%	0.4% (0.3)	5
Control (2xPE)	1.5	1787	1049	1250	5694	0.2%	0.3%	0.2%	0.2%	0.8% (0.8)	2
1xGSEL_1xPE	1.5	1702				0.5%				0.5% ³	3
2xGSEL_1xPE	1.5	9269	14540	17334	23131	2.8%	2.8%	2.6%	2.4%	2.4% (1.7)	4
3xGSEL_1xPE	1.5	4942	6187	6996	7640	1.5%	1.2%	1.1%	0.8%	0.8%	1
2xGSEL_2xPE	1.5	1461	2250	2784	3556	0.5%	0.5%	0.4%	0.4%	0.4% (0.0)	2
3xGSEL_2xPE	1.5	6368	27705	34737	18507	5.1%	5.3%	5.3%	5.0%	1.9% (2.1)	4

¹Dose= mg/kg, ²AUC= ng*h/mL, ³1xGSEL_1xPE studies were run only up to 24h

Table E9: Pharmacokinetic parameters for individual pigs.

Group	Pig	Weight (kg)	Dose (mg/kg)	t _{max} (h)	C _{max} (ng/mL)	AUC 24h (ng*h/mL)	AUC 168h (ng*h/mL)	F 168h
IV	396	47	0.02	0.08	596	6709	19679	-
IV	398	49	0.02	0.08	550	7156	21016	-
Control, SEMA only	808	54	1.5	-	0	0	0	0
Control, 1xPE	808	42	1.5	5	126	2327	4882	0.33%
	809	51	1.5	9	400	7037	11568	0.79%
	399	43	1.5	3	123	1083	2252	0.15%
	397	50	1.5	7	169	2642	7107	0.48%
	398	69	1.5	0.08	102	269	1895	0.13%
1xGSEL, 1xPE	810*	52	1.5	6	120	1467	-	-
	807*	47	1.5	10	182	3042	-	-
	806	43	1.5	5	126	3149	7058	0.48 %
2xGSEL, 1xPE	806	55	1.5	9	1269	13154	26593	1.81%
	394	50	1.5	6	149	2165	7680	0.52%
	395	50	1.5	1	920	15197	39191	2.67%
	396	62	1.5	6	1618	25096	65319	4.46%
3xGSEL, 1xPE	396	75	1.5	2	802	7413	11460	0.78%
Control, 2xPE	399	62.5	1.5	2	75	1054	2525	0.17%
	125	49.5	1.5	1	503	6468	19581	1.34%
2xGSEL, 2xPE	394	67.5	1.5	2	130	1694	4882	0.33%
	395	71.5	1.5	0.08	338	2689	5785	0.39%
3xGSEL, 2xPE	397	65	1.5	1	1942	25319	73096	4.99%
	124	47.2	1.5	3	233	5022	11885	0.81%
	987	65	1.5	11	197	3557	11505	0.78%
	988	70.5	1.5	8	235	4308	14556	0.99%

Rybelsus® (stomach)	124	64.5	1.1	10	296	5197	16414	1.68%
	125	67.3	1.0	9	490	4287	11587	1.19%

*no timepoints after 24h

Table E10: Additional pharmacokinetic parameters for individual pigs.

Group	Pig	Clearance (L/h/kg)	Mean Residence Time (h)	Volume of Distribution (steady state) (L/kg)
IV	396	0.00102	52.15	0.0530
IV	398	0.00095	51.70	0.0492
Control, SEMA only	808	-	-	-
Control, 1xPE	808	93.11	23.58	-
	809	16.41	18.17	-
	399	444.05	28.36	-
	397	43.97	52.18	-
	398	608.89	128.71	-
1xGSEL, 1xPE	810*	-	-	-
	807*	-	-	-
	806	44.28	2.98	-
2xGSEL, 1xPE	806	3.12	32.15	-
	394	37.56	35.90	-
	395	1.43	47.70	-
	396	0.51	47.30	-
3xGSEL, 1xPE	396	16.78	22.23	-
Control, 2xPE	399	349.45	45.82	-
	125	5.72	52.77	-
2xGSEL, 2xPE	394	93.11	49.02	-
	395	66.48	31.67	-
3xGSEL, 2xPE	397	0.41	49.30	-
	124	15.58	44.51	-
	987	16.72	60.73	-
	988	10.41	54.61	-
Rybelsus® (stomach)	124	3.99	54.67	-
	125	7.25	49.68	-

*no timepoints retrieved after 24h

Example 4

*Gastrointestinal Synthetic Epithelial Lining Formulation with Permeation Enhancer for
Administration to Subject: Evaluation in Dog Model*

[0234] A total of 12 (6 male, 6 female) beagles weighing 9-12 kg and approximately 6-14 months of age are used. Dogs will be trained to swallow capsules/tablets and test articles will be administered orally following an overnight fast. Following dosing, 15 mL of saline will be administered as a post-dose flush using a syringe. Blood samples will be collected via

cephalic venipuncture over 48 hours (0, 0.33, 0.66, 1, 2, 4, 6, 8, 24, 48) and then daily over 7 days. Samples will be collected into serum separation tubes. Samples will be analyzed via ELISA and LC-MS. The table below illustrates one potential formulation for GSELs with permeation enhancers for the dog studies.

Component
Semaglutide
Excipient for permeation enhancement
Dopamine
Polydopamine
Urea H ₂ O ₂
Tris acid
Tris base
Aerosil (other excipients)

[0235] The disclosures of all publications, patents, patent applications and published patent applications referred to herein by an identifying citation are hereby incorporated herein by reference in their entirety.

[0236] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it is apparent to those skilled in the art that certain changes and modifications will be practiced. Therefore, the description and examples should not be construed as limiting the scope of the invention.

CLAIMS

What is claimed is:

1. An orally administrable composition for forming a polymer *in situ* in a subject and enhancing permeation of an agent, comprising:
 - a polymer precursor;
 - an oxygen source; and
 - a combination of permeation enhancers comprising at least one of an ammonium salt, a carbonate salt, or a bicarbonate salt, and at least one of a bile salt or bile acid.
2. The composition of claim 1, further comprising one or more agents.
3. The composition of claim 2, wherein the one or more agents comprise one or more active pharmaceutical ingredients.
4. The composition of any one of claims 1-3, wherein the ammonium salt, carbonate salt, or bicarbonate salt is an ammonium salt.
5. The composition of any one of claims 1-3, wherein the ammonium salt, carbonate salt, or bicarbonate salt is a carbonate salt.
6. The composition of any one of claims 1-3, wherein the ammonium salt, carbonate salt, or bicarbonate salt is a bicarbonate salt.
7. The composition of any one of claims 1-6, wherein the bile salt or bile acid is a bile salt.
8. The composition of any one of claims 1-6, wherein the bile salt or bile acid is a bile acid.
9. The composition of any one of claims 1-4, 7, or 8, wherein the ammonium salt is ammonium carbonate (NHCO), ammonium sulfate (NH₂SO₄), ammonium citrate (NHCA), ammonium phosphate (NH₂PO₄), diammonium phosphate (DAP), monoammonium phosphate (MAP), ammonium bicarbonate (NH₄HCO₃), or ammonium chloride (NH₄Cl).

10. The composition of any one of claims 1-5, 7, or 8, wherein the ammonium salt is ammonium carbonate.
11. The composition of any one of claims 1-4 or 6-8, wherein the ammonium salt is ammonium bicarbonate.
12. The composition of any one of claims 1-3, 5, 7, or 8, wherein the carbonate salt is sodium carbonate or potassium carbonate.
13. The composition of any one of claims 1-3 or 6-8, wherein the bicarbonate salt is sodium bicarbonate or potassium bicarbonate.
14. The composition of any one of claims 1-6 or 8-13, wherein the bile salt or bile acid is a bile acid, and the bile acid is cholic acid (CHA), glycocholic acid (GCA), deoxycholic acid (DCA), glychenodeoxycholic acid (GCDCA), glycodeoxycholic acid (GDCA), taurodeoxycholic acid (TDCA), taurocholic acid (TCA), bovine bile (OX), chenodeoxycholic acid (CDCA), taurochenodeoxycholic acid (TCDCA), lithocholic acid (LCA), glycolithocholate (GLC), glycohyocholate (GHC), tauroolithocholate (TLC), ursodeoxycholic acid (UDCA), tauroursodeoxycholic acid (TUDCA), glyoursodeoxycholic acid (GUDCA), 12-monoketocholic acid (12-MKC), 7-monoketocholic acid (7-MKC), 7,12-diketocholic acid (7,12-DKC), 3,7,12-triketocholic acid (3,7,12-TKC), 12-monoketodeoxycholic acid (12-MKDC), or 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS).
15. The composition of any one of claims 1-6 or 8-13, wherein the bile salt or bile acid is a bile acid, and the bile acid is glycocholic acid.
16. The composition of any one of claims 1-3, wherein the ammonium salt, carbonate salt, or bicarbonate salt is an ammonium salt and the ammonium salt is ammonium carbonate; and wherein the bile salt or bile acid is a bile acid, and the bile acid is glycocholic acid.
17. The composition of any one of claims 1-3, wherein the ammonium salt, carbonate salt, or bicarbonate salt is an ammonium salt and the ammonium salt is ammonium bicarbonate; and wherein the bile salt or bile acid is a bile acid, and the bile acid is glycocholic acid.

18. The composition of any one of claims 1-7 or 9-13, wherein the bile salt or bile acid is a bile salt, and the bile salt is a salt of cholic acid, a salt of glycocholic acid, a salt of deoxycholic acid, a salt of glychochenodeoxycholic acid, a salt of glycodeoxycholic acid, a salt of taurodeoxycholic acid, a salt of taurocholic acid, a salt of bovine bile, a salt of chenodeoxycholic acid, a salt of taurochenodeoxycholic acid, a salt of lithocholic acid, a salt of glycolithocholate, a salt of glycohyocholate, a salt of tauroolithocholate, a salt of ursodeoxycholic acid, a salt of tauroursodeoxycholic acid, a salt of glyoursodeoxycholic acid, a salt of 12-monoketocholeic acid, a salt of 7-monoketocholeic acid, a salt of 7,12-diketocholeic acid, a salt of 3,7,12-triketocholeic acid, a salt of 12-monoketodeoxycholic acid, or a salt of 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate.
19. The composition of any one of claims 1-7 or 9-13, wherein the bile salt or bile acid is a bile salt, and the bile salt is a salt of glycocholic acid.
20. The composition of any one of claims 1-3, wherein the ammonium salt, carbonate salt, or bicarbonate salt is an ammonium salt and the ammonium salt is ammonium carbonate; and wherein the bile salt or bile acid is a bile salt, and the bile salt is a salt of glycocholic acid.
21. The composition of any one of claims 1-3, wherein the ammonium salt, carbonate salt, or bicarbonate salt is an ammonium salt and the ammonium salt is ammonium bicarbonate; and wherein the bile salt or bile acid is a bile salt, and the bile salt is a salt of glycocholic acid.
22. The composition of any one of claims 1-7 or 9-13, wherein the bile salt or bile acid is a bile salt, and the bile salt is sodium cholate, sodium glycocholate, sodium deoxycholate, sodium glychochenodeoxycholate, sodium glycodeoxycholate, sodium taurodeoxycholate, sodium taurocholate, a sodium salt of bovine bile, sodium chenodeoxycholate, sodium taurochenodeoxycholate, sodium lithocholate, sodium glycolithocholate, sodium glycohyocholate, sodium tauroolithocholate, sodium ursodeoxycholate, sodium tauroursodeoxycholate, sodium glyoursodeoxycholate, sodium 12-monoketocholeate, sodium 7-monoketocholeate, sodium 7,12-diketocholeate, sodium 3,7,12-triketocholeate, sodium 12-monoketodeoxycholate, or sodium 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate.

23. The composition of any one of claims 1-7 or 9-13, wherein the bile salt or bile acid is a bile salt, and the bile salt is sodium glycocholate.
24. The composition of any one of claims 1-3, wherein the ammonium salt, carbonate salt, or bicarbonate salt is an ammonium salt and the ammonium salt is ammonium carbonate; and wherein the bile salt or bile acid is a bile salt, and the bile salt is sodium glycocholate.
25. The composition of any one of claims 1-3, wherein the ammonium salt, carbonate salt, or bicarbonate salt is an ammonium salt and the ammonium salt is ammonium bicarbonate; and wherein the bile salt or bile acid is a bile salt, and the bile salt is sodium glycocholate.
26. The composition of any one of claims 1-25, further comprising a buffering agent.
27. The composition of any one of claims 1-26, wherein the polymer precursor comprises one or both of a monomer and an oligomeric precursor to a polymer.
28. The composition of any one of claims 1-27, wherein the polymer precursor is selected from Table 1 or Table 2, or combinations thereof.
29. The composition of claim 27 or 28, wherein the monomer is dopamine, levodopa, norepinephrine, methyl dopa, levodopa methyl ester, levodopa ethyl ester, or combinations thereof.
30. The composition of claim 27 or 28, wherein the monomer is dopamine.
31. The composition of any one of claims 27-30, wherein the oligomeric precursor to a polymer is an oligomer of dopamine, an oligomer of levodopa, an oligomer of norepinephrine, an oligomer of methyl dopa, an oligomer of levodopa methyl ester, an oligomer of levodopa ethyl ester, or an oligomer of any combination of dopamine, levodopa, norepinephrine, methyl dopa, levodopa methyl ester, or levodopa ethyl ester.
32. The composition of any one of claims 27-30, wherein the oligomeric precursor to a polymer is an oligomer of dopamine.

33. The composition of claim 27, wherein the monomer is dopamine and the oligomeric precursor to a polymer is an oligomer of dopamine
34. The composition of any one of claims 1-33, wherein the oxygen source is a substrate for an endogenous catalyst.
35. The composition of any one of claims 1-34, wherein the oxygen source is urea hydrogen peroxide or hydrogen peroxide.
36. The composition of any one of claims 1-35, wherein the composition is in an oral dosage form.
37. The composition of claim 36, wherein the oral dosage form is a solution, a gel, a tablet, a powder, or a capsule.
38. The composition of claim 36, wherein the oral dosage form comprises one or more solutions, gels, tablets, powders, or capsules.
39. The composition of claim 36, wherein the oral dosage form comprises an enteric dosage form.
40. The composition of any one of claims 2-39 wherein the agent is a macromolecule having a molecular weight between about 1 kD and about 160 kD.
41. The composition of claim 40, wherein the macromolecule is a polypeptide or polynucleotide.
42. The composition of claim 40, wherein the macromolecule is a polypeptide.
43. The composition of claim 42, wherein the polypeptide has a molecular weight of between about 1 kD and about 10 kD.
44. The composition of claim 42, wherein the polypeptide comprises between about 8 and about 80 amino acids.

45. The composition of claim 42, wherein the polypeptide comprises insulin, a GLP-1 receptor agonist, liraglutide, desmopressin, octreotide, an analgesic peptide, difelikefalin, H-20, an antibiotic, cyclosporin, vancomycin, lactase, beta galactosidase, exenatide, teriparatide, nafarelin, buserelin, captopril, daptomycin, an antibody, caplacizumab, ozoralizumab, brolucizumab, ranibizumab, bevacizumab, trastuzumab, rituximab, adalimumab, an enzyme, lipase, a protease, phenylalanine hydroxylase, carbamoylphosphate synthetase I, glucose oxidase, or L-asparaginase.
46. The composition of claim 42, wherein the polypeptide comprises semaglutide.
47. The composition of claim 42, wherein the polypeptide comprises tirzepatide.
48. The composition of claim 40, wherein the macromolecule is a polynucleotide.
49. The composition of claim 48, wherein the polynucleotide has a molecular weight of between about 5 kD and about 1500 kD.
50. The composition of claim 48 or claim 49, wherein the polynucleotide is single-stranded.
51. The composition of claim 48 or claim 49, wherein the polynucleotide is double-stranded.
52. The composition of claim 48, wherein the polynucleotide is single-stranded and comprises between about 10 and about 5000 bases.
53. The composition of claim 48, wherein the polynucleotide is single-stranded and comprises between about 10 and about 1000 bases.
54. The composition of claim 48, wherein the polynucleotide is single-stranded and comprises between about 10 and about 30 bases.
55. The composition of claim 48, wherein the polynucleotide is double-stranded and comprises between about 5 and about 2500 base pairs.

56. The composition of claim 48, wherein the polynucleotide is double-stranded and comprises between about 5 and about 500 base pairs.
57. The composition of claim 48, wherein the polynucleotide is double-stranded and comprises between about 5 and about 15 base pairs.
58. The composition of claim 48, wherein the polynucleotide comprises an antisense oligonucleotide, mipomersen, patisiran, exondys, an siRNA, or an inflammatory bowel disease (IBD) targeting siRNA.
59. The composition of any one of claims 2-39, wherein the agent is a small molecule having a molecular weight of 1 kD or less.
60. A method of forming a polymer coating in the gastrointestinal tract of a subject, the method comprising orally administering to a subject a composition of any one of claims 1-59.
61. The method of claim 60, wherein the polymer coating is formed in the small intestine of the subject.
62. The method of claim 61, wherein the polymer coating is formed in the duodenum of the subject.
63. A method of forming a polymer coating in the gastrointestinal tract of a subject, the method comprising orally administering to a subject:
- a polymer precursor;
 - an oxygen source; and
 - a permeation enhancer combination comprising at least one of an ammonium salt, a carbonate salt, or a bicarbonate salt, and at least one of a bile salt or bile acid;
- wherein the polymer precursor and the oxygen source contact a catalyst endogenous to the subject and the catalyst polymerizes the polymer precursor.
64. The method of claim 63, wherein the polymer coating is formed in the small intestine of the subject.

65. The method of claim 64, wherein the polymer coating is formed in the duodenum of the subject.

66. The method of any one of claims 63-65, further comprising administering one or more agents to the subject.

67. The method of any one of claims 63-66, wherein the polymer precursor, the oxygen source, and the permeation enhancer combination are administered as a single composition.

68. The method of claim 66, wherein the polymer precursor, the oxygen source, the permeation enhancer combination, and the one or more agents are administered as a single composition.

69. The method of claim 66, wherein the one or more agents are administered in a separate composition from the administration of the polymer precursor, the oxygen source, and the permeation enhancer combination.

70. The method of any one of claims 66 to 69, wherein the agent is an active pharmaceutical ingredient.

71. An orally administrable composition for forming a polymer *in situ* in a subject and enhancing permeation of an agent, comprising:

a polymer precursor;

an oxygen source;

a combination of permeation enhancers comprising a first permeation enhancer and a second permeation enhancer;

wherein the combination of the first permeation enhancer and the second permeation enhancer increase the permeation of the agent in comparison to the sum of the permeation of the agent by each enhancer individually.

72. The composition of claim 71, wherein the first permeation enhancer is an ammonium salt.

73. The composition of claim 71, wherein the first permeation enhancer is a carbonate salt.

74. The composition of claim 71, wherein the first permeation enhancer is a bicarbonate salt.
75. The composition of any one of claims 71 to 74, wherein the second permeation enhancer is a bile salt.
76. The composition of any one of claims 71 to 74, wherein the second permeation enhancer is a bile acid.
77. The composition of any one of claims 71 to 76, further comprising an agent.
78. The composition of claim 77, wherein the agent is an active pharmaceutical ingredient.
79. The composition of any one of claims 71 to 77, wherein the agent comprises semaglutide.
80. The composition of any one of claims 71 to 77, wherein the agent comprises tirzepatide.
81. The composition of any one of claims 71 to 80, further comprising one or more additional agents.