human intestine (typically around 15 μm). This means that even co-culture systems cannot be integrated with whole digestive fluids due to the likelihood of cell death.

[0007] It has been known to use porcine mucus in such *in vitro* systems. However, although porcine mucus reflects human intestinal mucus, Caco-2 cells do not survive prolonged co-incubation. To overcome this problem, a biosimilar mucus has been developed. Biosimilar mucus, which is compatible with Caco-2 monolayers, mimics the rheological properties of porcine intestinal mucus and has been used to assess drug absorption *in vitro*. Boegh, Marie, et al. (European Journal of Pharmaceutics and Biopharmaceutics 87.2 (2014): 227-235) describe the design and characterization of biosimilar mucus compatible with Caco-2 cell monolayers cultured *in vitro* to establish a more representative *in vitro* model for the intestinal mucosa. The biosimilar mucus mixture is composed of degraded commercial gastric mucin, BSA, cholesterol, phosphatidylcholine, linoleic acid, and polyacrylic acid (Carbopol 974P NF at 0.3 to 0.9% w/v). Polyacrylic acid is a synthetic polymer, which is added to the composition to provide the desired viscoelastic properties and a microstructure comparable to freshly isolated porcine intestinal mucus (PIM). The biosimilar mucus was optimized with regard to the lipid content in order to obtain cellular compatibility with well-differentiated Caco-2 cell monolayers. However, significant differences in peptide permeability were found between the isolated PIM and the biosimilar mucus. In addition, Carbopol is known to interact with mucin, and so will alter mucus properties and/or function. Commercially available porcine gastric mucin (Sigma Aldrich™) has been denatured during isolation, and consequently its ability to form mucin: mucin interactions is lost, which results in its inability to form a gel. Furthermore, gastric mucin is comprised of MUC5AC and MUC6 gene products, whereas small intestinal mucin is MUC2.

[0008] There are a number of problems with other approaches to modelling intestinal tissue comprising a mucus layer. Human mucus is difficult to obtain, and furthermore the isolation process can alter the mucins. In addition, synthetic gastro-intestinal secretions are used to mimic the native intestinal environment, but such synthetic secretions have been found to cause cell death when applied to cell cultures in the absence of a protective mucus layer as would be found *in vivo*. Native porcine mucus collected from animals has also been found to kill cells, and therefore is not suitable for use in a tissue model.

[0009] The present invention aims to overcome or ameliorate some or all of these problems associated with the prior art.

SUMMARY

[0010] The present disclosure describes, in one aspect, a purified, biocompatible mucus, wherein the mucus:

i) substantially lacks nucleic acids, protein and/or lipids, and/or molecules below 100kDa, preferably below 70kDa; and ii) comprises mucin, and wherein the purified, biocompatible mucus is a gel, and wherein said gel has a phase angle within the linear viscoelastic region of 3 to 20 degrees. Suitably, the purified, biocompatible mucus contains mucin which has a density of 1.3 to 1.6g/mL. Suitably, the purified, biocompatible mucus does not comprise a synthetic polymer. Suitably, the purified, biocompatible mucus contains mucin, and wherein the mucin is not substantially denatured, hydrolysed

or degraded. Suitably, the mucin present in the mucus comprises sufficient intermolecular interactions to enable the purified biocompatible mucus to form a gel. In an example, the mucus is a gel.

[0011] The present disclosure also describes, in another aspect, a tissue model, comprising:

a substrate having a cell support portion;

a cell population provided on the substrate;

a purified, biocompatible mucus;

optionally, a fluid provided on the mucus layer and/or below the substrate layer, wherein the purified, biocompatible mucus: i) substantially lacks nucleic acids, protein or lipids or molecules below 100kDa, preferably 70kDa; and ii) comprises mucin, wherein the purified, biocompatible mucus is a gel and wherein said gel has a phase angle within the linear viscoelastic region of 3 to 20 degrees.

[0012] In another aspect, there is provided a method of purifying mucus, wherein the method comprises removal of substantially all nucleic acids, protein and/or lipids, and/or molecules below 100kDa, preferably below 70kDa from the mucus, wherein the step of removal comprises dialysis and/or equilibrium density gradient centrifugation and wherein said method comprises i) providing a sample comprising mucus; ii) solubilising any mucin in the mucus; iii) separating from the mucus any nucleic acids, proteins, lipids by equilibrium density centrifugation; and iv) dialysing the mucus to remove nucleic acids, protein or lipids or molecules under 100kDa and wherein said method comprises the exposure of the sample to one or more enzyme inhibitors to obtain a mucus according as disclosed herein and/or wherein said purified mucus is for use in a tissue model as disclosed herein. Suitably, the method does not substantially remove mucin from the mucus. Suitably, the method does not comprise denaturation, degradation or hydrolysis of mucin. Optionally, the method may comprise a further separation step, for example centrifugation. Suitably, the method provides a purified biocompatible mucus as disclosed herein.

[0013] One example, the method may comprise i) providing a sample comprising mucus; ii) solubilising any mucin in the mucus; iii) separating from the mucus any nucleic acids, proteins, and/or lipids; and optionally iv) removing any contaminants, undesirable components or reagents from the mucus; to provide a purified, biocompatible mucus. Step iii) may comprise equilibrium density gradient centrifugation. Step iv) may comprise removing any cytotoxic components, reagents, and/or enzymes, and may comprise dialysis of the mucus. Suitably, the method comprises separating from the mucus a fraction having a density gradient of 1.3 to 1.6g/mL. Suitably, the mucin present in the mucus has a density of 1.3 to 1.6g/mL.

[0014] Another example, the method may comprise i) providing a sample comprising mucus; ii) dialysing the mucus to separate from the mucus any molecules below 100kDa, preferably below 70kDa; and optionally iii) separating the mucus.

[0015] In one example, there is described a method for determining or predicting absorption of a test compound through a tissue model, wherein the tissue model comprises i) a substrate comprising a cell support portion; ii) a cell population in contact with the cell support portion of the substrate; and iii) purified biocompatible mucus, in contact with the cell; and wherein the method comprises contacting the purified biocompatible mucus with the test compound, and detecting movement of the test compound in the tissue model;, thereby determining the ability of the test compound to traverse the purified biocompatible mucus and cellular layers of the tissue model.

[0016] In another example, the tissue model may comprise purified biocompatible mucus or a composition comprising the purified biocompatible mucus as described herein. The tissue model may be as described herein. The tissue

model used in a method of detecting an effect of a test compound may be a stomach tissue model, small intestine tissue model, large intestine tissue model, gastro-intestinal tissue model, female reproductive tissue model, nasal tissue model, optic tissue model and/or airway tissue model.

[0017] The step of detecting movement of the test compound in the tissue model may comprise observing, monitoring or measuring any one or more of, but not limited to, the distance travelled by the test compound into the tissue model, the absorption of the test compound into the tissue model, the path travelled in the tissue model, the rate of travel or perfusion into the tissue model, cellular uptake in the tissue model, and the location of the test compound in the tissue model. The detecting may also comprise determining the concentration of the test compound at or below the substrate. The step of detecting the movement of the test compound may comprise detecting the test compound in the purified biocompatible mucus layer, the cell population, and/or in contact with a surface of the substrate. The presence or absence of the test compound at the luminal surface and/or at the basolateral surface of the tissue mode may be detected. The presence of the test compound at the basolateral surface indicates the ability of the compound to be absorbed through the tissue explant.

[0018] The method may comprise the step of applying food to the purified biocompatible mucus or fluid layer of the model. The method may comprise the step of applying native intestinal media to the purified biocompatible mucus or fluid layer of the model.

[0019] In one example, there is described a kit comprising, in a suitable container, purified biocompatible mucus or a composition comprising the purified biocompatible mucus as described herein, and/or a tissue model as disclosed herein, and optionally a substrate, a buffer, reagents, instructions for use, and other standard ingredients well known in the art.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] Embodiments of the invention are further described hereinafter with reference to the accompanying drawings, in which:

[0021] Figure 1 is a graph showing Caco-2 cell viability in the presence of simulated digestive fluids with and without native mucus in a 24 well plate transwell over a period of 4 hours. The Cytotoxic effects of porcine small intestinal surface mucus on a Caco-2 cell monolayer, with either luminal PBS or simulated small intestinal fluids are shown with a Cell titre Blue cell viability assay. Native mucus kills cells, but confers some protection of the remaining live cells by GI fluids. Under agitation, the ability of native mucus to protect cells is diminished.

[0022] Figure 2 is a representation of the problems with viscosity of commercial mucins. Human mucus is difficult to obtain. The commercial isolation process alters the mucin glycoproteins and disrupts the ability to form mucin:mucin interactions. Purification methods including boiling, or exposure to proteolytic or glycolytic enzymes can alter mucins. The purification method described herein in the third aspect does not affect mucin:mucin interactions, and allows mucin to retain the ability to form a gel at higher concentrations.

CLAIMS

1. A tissue model, comprising in discrete layers:

i. a substrate having a cell support portion;

ii. a cell population provided on the substrate;

iii. a purified, biocompatible mucus;

iv. optionally, a physiological fluid provided on the mucus layer and/or below the substrate layer,

wherein the purified, biocompatible mucus: i) substantially lacks nucleic acids, protein or lipids or molecules below 100kDa, preferably 70kDa; and ii) comprises mucin, wherein the purified, biocompatible mucus is a gel and wherein said gel has a phase angle within the linear viscoelastic region of 3 to 20 degrees.

2. The tissue model according to claim 1 wherein said gel has a shear strain in the linear viscoelastic region greater than 5%

3. A tissue model according to claim 1 or claim 2 wherein the purified, biocompatible mucus does not contain a synthetic polymer and/or is not substantially denatured, hydrolysed or degraded.

4. A tissue model according to any one of claims 1 to 3, wherein the purified, biocompatible mucus is provided in combination with one or more of a lipid, salt, protein, nucleic acid (DNA or RNA), carbohydrate, glycoprotein, cell, and water.

5. A tissue model according to any one of claims 1 to 4, wherein the cells of the cell population are, or are derived from, ileum, jejunum, stomach, duodenum, oesophagus, buccal, lingual or colon of the gastrointestinal tract, or from the nose, lung, bronchi, bronchioles, or mouth; or from the cervix or vagina, or from the eye; or any combination thereof.

6. A tissue model according to any one of claims 1 to 5, wherein the fluid is digestive fluid, saliva, vaginal secretion, tears, or airways secretions, a synthetic fluid, or a microbial cell population; or any combination thereof.

7. A tissue model according to any one of claims 1 to 6 wherein the tissue model is a stomach tissue model, a small intestine tissue model, a large intestine tissue model or a gastro-intestinal tissue model

8. A combination of two or more tissue models according to any one of claims 1 to 7, wherein the two or more tissue models are placed in series, and preferably wherein the two or more tissue models are each selected from the group consisting of an airway model, a stomach tissue model, a small intestine tissue model, and a large intestine tissue model.

9. A purified, biocompatible mucus, wherein the mucus: i) substantially lacks nucleic acids, protein or lipids or molecules below 100kDa, preferably 70kDa; and ii) comprises mucin, and wherein

the purified, biocompatible mucus is a gel, and wherein said gel has a phase angle within the linear viscoelastic region of 3 to 20 degrees.

10. The purified, biocompatible mucus according to claim 9 wherein said gel has a shear strain in the linear viscoelastic region greater than 5%.

11. A purified, biocompatible mucus according to claim 9 or claim 10 wherein the purified, biocompatible mucus does not contain a synthetic polymer.

12. A purified, biocompatible mucus according to any one of claims 9 to 11 wherein the mucin is not substantially denatured, hydrolysed or degraded.

13. A purified, biocompatible mucus according to any one of claims 9 to 12, and one or more of lipid, salts, protein, nucleic acid (DNA or RNA), carbohydrates, glycoproteins, cells, and water.

14. A method for determining or predicting absorption of a test compound through a tissue model as defined in any one of claims 1 to 8; wherein the method comprises contacting the purified biocompatible mucus with the test compound, and detecting movement of the test compound in the tissue model.

15. A method according to claim 14, wherein the method comprises detecting and optionally determining the concentration of the test compound in the purified biocompatible mucus layer, the cell population, in contact with a surface of the substrate, below the substrate, at the luminal surface and/or at the basolateral surface.

16. A method according to claim 14 or 15 wherein the test compound is detected by a binding assay, mass spectrometry, a colorimetric assay, liquid chromatography, nucleic acid sequencing, SDS PAGE, Western blotting, or in situ hybridisation.

17. A method according to claim 16 wherein the test compound is a small molecule, an organic molecule, antibody, peptide, protein, hormone, antagonist, nucleic acid (e.g. antisense, siRNA, shRNA, RNAi, expressible coding sequences), saccharide, fatty acid, steroid, purine, pyrimidine; derivatives or structural analogs thereof; bacteria, fungi, or extracts thereof; plant or animal extract; or any combination of two or more thereof.

18. A method according to any one of claims 14 to 17 wherein the test compound is a pharmaceutical agent.

19. A method according to any one of claims 14 to 18 for use in any one or more of drug absorption testing, drug formulation development, profiling GI damage/side effects, nutrient uptake analysis, drug-microbiome interaction studies, profiling intestinal damage, and pre/pro biotic testing.

20. A method according to any one of claims 14 to 19, wherein the method comprises the step of applying a food and/or intestinal media to the tissue model.

21. A method according to any one of claims 14 to 20 wherein the test compound is measured at one or more different time intervals.

22. A kit comprising, in a suitable container, a purified mucus as defined in any one of claims 9 to 13 or a tissue model as defined in any one of claims 1 to 8, and optionally a substrate, a buffer, a reagent, and instructions for use.

23. A method of purifying mucus, wherein the method comprises removal of substantially all nucleic acids, protein and/or lipids, and/or molecules below 100kDa, preferably below 70kDa, from the mucus, wherein the step of removal comprises dialysis and/or equilibrium density gradient centrifugation and wherein said method comprises i) providing a sample comprising mucus; ii) solubilising any mucin in the mucus; iii) separating from the mucus any nucleic acids, proteins, lipids by equilibrium density centrifugation; and iv) dialysing the mucus to remove nucleic acids, protein or lipids or molecules under 100kDa and wherein said method comprises the exposure of the sample to one or more enzyme inhibitors to obtain a mucus according to claims 9-13 and/or wherein said purified mucus is for use in a tissue model according to claims 1-8.

24. The method according to claim 23 wherein said enzyme inhibitors are one or more protease inhibitors.

25. The method according to claims 23-24 wherein said sample is exposed to one or more enzyme inhibitors prior to solubilisation.

26. A method according to any one of claims 23 to 25, wherein the separation step comprises equilibrium density gradient centrifugation, and optionally selecting a fraction having a density of 1.3-1.6g/mL.

27. A method according to any one of claims 23 to 26 wherein the method uses a caesium salt density gradient.

28. A method according to any one of claims 23 to 27 further comprising providing a tissue sample comprising mucus; and optionally extracting the mucus from the tissue sample.

29. A method according to any one of claims 20 to 25, further comprising sterilising one or more components of the purified mucus.

30. A method according to any one of claims 20 to 26, further comprising combining the purified mucin with a buffer, a lipid, salt, protein, nucleic acid (DNA or RNA), glycoprotein, cell, and/or water.

31. A method according to any one of claims 20 to 27, further comprising placing the purified mucus under conditions to allow gelation of the mucin.

32. A method according to any one of claims 23 to 31, further comprising lyophilising, storing, and/or combining the purified mucus with a buffer.

33. A method according to any one of claims 23 to 32, further comprising applying the purified mucus to a cell population provided on a substrate to form a tissue model, and optionally applying a fluid to the purified mucin.