SUMMARY

[0012a] Disclosed herein is the use of mammalian amniotic exosomes derived from allogeneic mammalian amnion epithelial cells derived from a donor mammal of the same species in the manufacture of a medicament for the treatment of a fibrotic disease, condition or disorder of the lung, liver, heart, kidney or pancreas, or a neurodegenerative disease or condition, in a mammalian subject, wherein the medicament is to be systemically or locally administered to the subject.

[0012b] Also disclosed herein is the use of mammalian amniotic exosomes derived from allogeneic mammalian amnion epithelial cells derived from a donor mammal of the same species in the manufacture of a medicament for the treatment of exercise induced pulmonary hemorrhage in a mammalian subject, wherein the medicament is to be systemically or locally administered to the subject.

[0013] In accordance with the present disclosure a vesicular vehicle for cellular communication is identified as being released from mammalian amnion epithelial cells (AECs). The vesicles, referred to herein as "amniotic exosomes", are nanometer-sized extracellular vesicles (50-100nm) derived from late endosomes and released from cell surfaces.

[0014] Taught herein is an improved form of mammalian amnion epithelial cell-based therapy. The improvement comprises the use of the nano-sized amniotic exosomes which are released by the epithelial cells and exert reparative effects by activating endogenous repair mechanisms. Amniotic exosomes are shown herein to act directly on immune cells to *inter alia* reduce T-cell proliferation, increase macrophage phagocytosis, activate stem cells and inhibit collagen production in activated fibroblasts. It is proposed herein that the amniotic exosomes release a profile of exosomal cargo in the form of proteins (e.g. cytokines) and genetic molecules (e.g. miRNA, mRNAs and non-coding RNAs).

[0015] The biogenesis of exosomes involves the formation of intraluminal vesicles by the inward budding of the late endosome's limiting membrane. Late endosomes then fuse with the plasma membrane to release the exosomes. Once secreted, exosomes can either be taken up by target cells located in close proximity to the parent cell or travel to distal sites through the circulation. Mechanistically, exosomes operate as complex vectors that contain parental cell material. They can contain proteins and genetic material, which are then transferred to their target cells.

[0016] The present disclosure is predicated, therefore, on the development of an enhanced approach to cell-based therapy. The present disclosure teaches the use of the amniotic exosomes which are released from mammalian amnion epithelial cells and which have immunomodulatory, pro-regenerative and reparative effects. The amniotic exosomes exert an effect on immune cells to reduce T-cell proliferation, increase macrophage phagocytosis and activate endogenous stem cells

through the release of beneficial proteomic and genetic molecules such as miRNA, mRNA and non-coding RNAs. The amniotic exosomes are proposed herein to facilitate tissue repair, regeneration and reparation including wound healing, promote cellular maintenance, induce neuronal protection including ameliorating the effects of neurodegeneration and injury and promoting repair and neuroregeneration. The amniotic exosomes, also suppress collagen production in activated fibroblasts. The exosomes are further proposed to promote repair and regeneration following disease or adverse event in the systemic vasculature such as ischemic reperfusion injury or organ damage including ameliorating kidney, liver, pancreas, heart and lung damage as well as the treatment of fibrotic conditions in those organs (e.g. liver or lung fibrosis). The exosomes are also useful in promoting myelination and hence are proposed to be useful in the treatment of demyelination diseases or disorders such as multiple sclerosis..

[0017] The amniotic exosomes have beneficial effects not only in humans but also non-human mammals. Hence, the present disclosure extends to human and veterinary applications. AECs from human subjects are referred to herein as "hAECs".

[0018] An example of a veterinary application is the treatment of racing animals including horses, racing dogs and camels for exercise induced pulmonary haemorrhage (EIPH).

[0019] The amniotic exosomes can be produced in large quantity by culturing mammalian amnion epithelial cells in a bioreactor and isolating the amniotic exosomes from the conditioned culture medium. The epithelial cells can be maintained as an immortalized cell line. The amniotic exosomes can be isolated when required or stored in a lyophilized state.

[0020] An innovative feature of the present disclosure is that it is not necessary to identify a compatible donor of the mammalian amnion epithelial cells in order to use the amniotic exosomes. The exosomes do not induce an adverse immunological reaction. Rather, donors are selected on the basis of gestational stage and/or other characteristics such as health of a neonate or term babies. In an embodiment, however, the amniotic exosomes are derived from hAECs from patients at the terminal end period of a pregnancy.

The amnion epithelial cells produce amniotic exosomes which are at least as good at promoting tissue or neuronal repair, regeneration and/or reparation for different physiological conditions as are amnion epithelial cells. However, there is none of the disadvantages of a cell-based therapy. Hence, an aspect of the present disclosure is donor selection in order to identify amnion epithelial cells which produce amniotic exosomes useful in treating a desired condition. This can lead to the generation of a bank of amnion epithelial cells. A particular batch of cells can then be selected based on the disease or condition to be treated.

[0021] Pharmaceutical compositions comprising the amniotic exosomes, therapeutic kits comprising amniotic exosomes and/or reagents for screening for a suitable donor or amnion epithelial cell line and bioreactor kits are also encompassed by the teachings of the present disclosure.

[0022] Taught herein is an enhanced or modified form cell-based therapy. Hence, enabled herein is an improved cell-based therapeutic protocol for treating a mammalian including a human subject by the use of amnion epithelial cells the improvement comprising isolating amniotic exosomes from an immortalized amnion epithelial cell line and systemically or locally administering to the subject in need of tissue or neuronal repair, regeneration and/or reparation including promotion of remyelination.

muscle actin within 24 hours.

[0031] Figure 8A-C are diagrammatic representations showing that amniotic exosomes contain miRNAs that target cytokine-cytokine receptor signaling pathways. Yellow boxes indicate a target by one or more miRNAs.

[0032] Figure 9A-B are diagrammatic representations showing that amniotic exosomes contain miRNAs that target Wnt signaling pathways. Yellow boxes indicate a target by one or more miRNAs.

[0033] Figure 10 is a diagrammatic representation showing that amniotic exosomes contain miRNAs that target PI3K-Akt signaling pathways. Yellow boxes indicate a target by one or more miRNAs.

[0034] Figure 11 is a diagrammatic representation showing that amniotic exosomes contain miRNAs target TGFβ signaling pathways. Yellow boxes indicate a target by one or more miRNAs.

[0035] Figure 12A is a graphical representation and **Figures 12B, C and D** are photographic representations showing the lung regenerative effects of amniotic exosomes comprising tissue airspace ratio (%) between healthy control hAECs, term exosomes and preterm exosomes. A "term" exosome is an exosome isolated from hAEC at the end of a pregnancy. The "preterm" exosome is isolated prior to pregnancy term.

[0036] Figure 13A through C are photographic representations showing that amniotic exosomes trigger regeneration in the lungs as do hAECs. The dark stain is evidence of elastin-positive tips.

[0037] Figure 14 is a graphical representation showing that amniotic exosomes, but not fibroblast exosomes, trigger an endogenous stem cell response in the lungs. This response is significantly greater than the response induced by hAECs.

[0038] Figures 15A and B show that amniotic exosomes were anti-fibrotic in the liver as evidenced using the Sirius red stain (A) and α-smooth muscle activin (α-SMA)

myofibroblasts (α-smooth muscle actin positive) in the lungs. This was consistent with the reduction in collagen deposition in the lungs. The results are shown in Figure 6.

[0117] Amniotic exosomes directly reverse activation of primary human lung fibroblasts *in vitro.* When cultured in the presence of 5ng/mL transforming growth factor β, amniotic exosomes decreased protein levels of α-smooth muscle actin within 24 hours. The results are shown in Figure 7.

[0118] Amniotic exosomes contain miRNAs that target the cytokine-cytokine receptor signaling pathways as shown in Figure 8A-C, where yellow boxes indicate a target by one or more miRNAs.

[0119] Amniotic exosomes contain miRNAs that target the Wnt signaling pathways as shown in Figure 9A-B, where yellow boxes indicate a target by one or more miRNAs.

[0120] Amniotic exosomes contain miRNAs that target the PI3K-Akt signaling pathways as shown in Figure 10 where yellow boxes indicate a target by one or more miRNAs.

[0121] Amniotic exosomes contain miRNAs that target the TGFβ signaling pathways as shown in Figure 11 where yellow boxes indicate a target by one or more miRNAs.

[0122] It is clear that amniotic exosomes are as, if not more, effective than AECs such as hAECs and have a great capacity to induce cellular and molecular repair mechanisms in a diverse range of physiological and neural processes.

[0123] Those skilled in the art will appreciate that the disclosure described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the disclosure contemplates all such variations and modifications. The disclosure also enables all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of the steps or features or compositions or compounds.

CLAIMS:

1. Use of mammalian amniotic exosomes derived from allogeneic mammalian amnion epithelial cells derived from a donor mammal of the same species in the manufacture of a medicament for the treatment of a fibrotic disease, condition or disorder of the lung, liver, heart, kidney or pancreas, or a neurodegenerative disease or condition, in a mammalian subject, wherein the medicament is to be systemically or locally administered to the subject.

2. The use of Claim 1 wherein the mammalian subject to be treated is a human.

3. The use of Claim 1 wherein the neurodegenerative disease or condition is a demyelination disease.

4. The use of Claim 3 wherein the demyleination disease is multiple sclerosis, optic neuritis, Devic's disease, transverse myelitis, acute disseminated encephalomyelitis, adrenoleukodystrophy or adrenomyeloneuropathy.

5. The use of Claim 1 wherein neurodegenerative disease or condition is selected from the group consisting of motor neuron disease, a stroke, spinal cord injury, traumatic brain injury, Alzheimer’s disease, Parkinson’s disease, Huntington’s diseases and multiple sclerosis.

6. The use of Claim 2 wherein the human subject is to be treated for a disease or condition selected from the group consisting of bronchopulmonary dysplasia, cystic fibrosis, lung fibrosis, liver fibrosis, chronic lung infection, asthma, allergic rhinitis and chronic obstructive pulmonary disease (COPD).

7. The use of Claim 1 wherein the amniotic exosomes reverse lung infection and fibrosis and reverse activation of primary lung fibroblasts.

8. The use of Claim 1 wherein the amniotic exosomes contain miRNAs which target cytokine-cytokine receptor, Wnt, PI3K-Akt and TGFβ signaling pathways.

9. Use of mammalian amniotic exosomes derived from allogeneic mammalian amnion epithelial cells derived from a donor mammal of the same species in the manufacture of a medicament for the treatment of exercise induced pulmonary hemorrhage in a mammalian subject, wherein the medicament is to be systemically or locally administered to the subject.

10. The use of Claim 9 wherein the mammalian subject to be treated is a non-human mammal.

11. The use of Claim 10 wherein the non-human mammalian subject to be treated is a racing animal selected from the group consisting of a horse, dog and camel.

12. The use of Claim 9 wherein the amniotic exosomes reverse lung infection and fibrosis and reverse activation of primary lung fibroblasts.

13. The use of Claim 9 wherein the amniotic exosomes contain miRNAs which target cytokine-cytokine receptor, Wnt, PI3K-Akt and TGFβ signaling pathways.

14. The use of any one of Claims 1 to 13 wherein the amniotic exosomes are derived from a bank of immortalized mammalian amnion epithelial cell lines.

15. The use of any one of Claims 1 to 13 wherein the amniotic exosomes are selected from a bank of lyophilized amniotic exosomes derived from immortalized mammalian amniotic epithelial cell lines.