M, Li RH, Friess W (November 2003). Collagen sponges for bone regeneration with rhBMP-2. *Adv. Drug Deliv. Rev.* 55 (12): 1613–29.). Recombinant human BMP2 is available for orthopaedic use in USA (e.g. INFUSE® Bone Graft, Medtronic Inc, USA).

Summary

The present disclosure concerns a heparan sulphate preparation, heparan sulphate isolated or obtained from bone marrow stroma. In one embodiment the heparan sulphate is HS5.

Heparan sulphate from bone marrow stroma has been found to enhance the lineage-commitment of hematopoietic stem cells and enhance BMP-2 induced osteogenesis.

In an aspect, there is provided heparan sulphate HS5 substantially purified from the core protein, wherein the heparan sulphate has the following heparan lyase disaccharide digestion profile:

Disaccharide	% Composition
ΔHexUA – GlcN	22.11 ±10%
ΔHexUA – GlcN,6S	38.79 ±10%
ΔHexUA – GlcNS	4.20 ±10%
ΔHexUA – GlcNAc,6s	4.53 ±10%
Other	30.37 ±10%

In the present disclosure, heparan sulphate from bone marrow stroma is provided. Heparan sulphate from bone marrow stroma may be provided in isolated form or in substantially purified form. This may comprise providing a composition in which the heparan sulphate component is at least 80% heparan sulphate from bone marrow stroma, more preferably one of at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100%.

Accordingly, isolated heparan sulphate HS5 is provided, and heparan sulphate isolated from bone marrow stroma is provided.

Heparan sulphates according to the present disclosure may be isolated or obtained from human or mammalian bone marrow stroma.

Heparan sulphates according to the present disclosure may have the following heparan lyase disaccharide digestion profile:

Disaccharide	% Composition
ΔHexUA – GlcN	22.11 ±10%
ΔHexUA – GlcN,6S	38.79 ±10%
ΔHexUA – GlcNS	4.20 ±10%
ΔHexUA – GlcNAc,6s	4.53 ±10%
Other	30.37 ±10%10%

In some preferred embodiments the percentage composition of these disaccharides may be within one of ± one of 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% of the values shown for each disaccharide. The digestion profile is preferably obtained by complete digestion

of the heparan sulphate with heparan lyases, more preferably with heparitinases I, and/or II and/or III, and most preferably with heparitinases I, and II or heparitinases I, II and III.

In one aspect, a method of increasing the number of committed clonogenic cells in a population of hematopoietic stem cells (HSCs) is provided, the method comprising culturing HSCs *in vitro* in the presence of isolated heparan sulphate HS5 or heparan sulphate isolated from bone marrow stroma.

The method may comprise increasing the number of myeloid lineage-committed progenitor cells. The method may comprise increasing the number of colony forming cells (CFC). The method may comprise increasing the number of burst forming unit-erythroid (BFU-E). The method may comprise increasing the number of colony forming unit-granulocyte macrophage (CFU-GM). The initial HSC population in the culture may comprise at least 50% CD34+ cells, more preferably one of 60%, 70%, 75%, 80%, 85%, 90% or 95% CD34+ cells. The HSC population may be obtained from cord blood. The concentration of heparan sulphate in or added to the culture media may be 500 ng/ml or less, or one of 250 ng/ml or less, 100 ng/ml or less, 90 ng/ml or less, 80 ng/ml or less,, 70 ng/ml or less, 60 ng/ml or less, 50 ng/ml or less, 40 ng/ml or less, 30 ng/ml or less, 20 ng/ml or less, 10 ng/ml or less, or 5 ng/ml or less.

In one embodiment, myeloid lineage-committed progenitor cells obtained by *in vitro* culture of hematopoietic stem cells (HSCs) in the presence of heparan sulphate from bone marrow stroma are provided.

In the present disclosure, a method of promoting or enhancing osteogenesis is provided, the method comprising administering heparan sulphate from bone marrow stroma to osteoprogenitor cells. The method may be a method of promoting or enhancing BMP-2 induced osteogenesis. The method may comprise contacting the osteoprogenitor cells with heparan sulphate from bone marrow stroma *in vitro* or *in vivo*. The osteoprogenitor cells may also be contacted with BMP2 protein, e.g. simultaneously with said heparan sulphate. The osteoprogenitor cells may be mesenchymal stem cells, osteoblast cells, myoblast cells or other bone stem cells or bone precursor cells.

In another aspect, a method of promoting osteogenesis, the method comprising administering heparan sulphate as defined herein to osteoprogenitor cells *in vitro* is provided.

In another embodiment, bone tissue obtained by *in vitro* culture of osteoprogenitor cells in the presence of heparan sulphate from bone marrow stroma is

provided. The bone tissue may be obtained through culture of the osteoprogenitor cells in the presence of BMP2 protein.

In another embodiment, a method of culturing osteoprogenitor cells is provided, the method comprising culturing osteoprogenitor cells in contact with heparan sulphate from bone marrow stroma.

In another aspect, there is provided a method of culturing osteoprogenitor cells comprising culturing osteoprogenitor cells in contact with heparan sulphate as defined above.

In another embodiment, culture media comprising heparan sulphate from bone marrow stroma is provided.

In another aspect, there is provided culture media comprising heparan sulphate as defined above.

In another embodiment, the use of heparan sulphate from bone marrow stroma in cell culture *in vitro* is provided.

In another aspect, there is provided the use of heparan sulphate as defined above in cell culture *in vitro*.

In another embodiment, a kit is provided comprising a predetermined amount of heparan sulphate from bone marrow stroma and a predetermined amount of BMP2 protein.

In another aspect, there is provided a kit comprising a predetermined amount of heparan sulphate as defined above and a predetermined amount of BMP2 protein.

In another embodiment, a pharmaceutical composition or medicament is provided comprising heparan sulphate from bone marrow stroma.

In another aspect, there is provided a pharmaceutical composition or medicament comprising heparan sulphate as defined above.

In another embodiment, a composition or pharmaceutical composition is provided comprising heparan sulphate from bone marrow stroma and BMP2 protein.

In another aspect, there is provided a composition or pharmaceutical composition comprising heparan sulphate as defined above and BMP2 protein.

In another embodiment, heparan sulphate from bone marrow stroma or a composition or pharmaceutical composition comprising said heparan sulphate is provided for use in a method of medical treatment. The use of heparan sulphate from bone marrow stroma for the manufacture of a pharmaceutical composition or medicament for the treatment of disease or repair/replacement/regeneration of cells or tissue in a patient is also provided.

In another aspect, there is provided heparan sulphate as defined above or a composition or pharmaceutical composition as defiend above for use in medical treatment.

In one embodiment the method of medical treatment may comprise administration of the heparan sulphate or composition or pharmaceutical composition to a patient receiving HSC transplantation. The heparan sulphate or composition or pharmaceutical composition may be administered to the patient in combination with transplanted HSCs.

In another embodiment the method of medical treatment comprises a method of wound healing *in vivo*. The method of medical treatment may comprise repair and/or regeneration of bone and/or treatment of bone fracture.

In another aspect, there is provided the use of heparan sulphate in the manufacture of a medicament for medical treatment, wherein the medical treatment comprises wound healing.

In another aspect, there is provided the use of heparan sulphate as defined above or a composition or pharmaceutical composition as defined above in the manufacture of a medicament for use in medical treatment, wherein the medical treatment comprises repair and/or regeneration of bone and/or treatment of bone fracture.

A method of transplanting hematopoietic stem cells (HSCs) to a patient in need thereof is provided, the method comprising transplanting a therapeutically effective amount of HSCs to the patient and administering a therapeutically effective amount of heparan sulphate from bone marrow stroma to the site of transplantation of the HSCs in the patient or to tissue that surrounds, is near to, or adjacent to the site of transplantation. The site of transplantation may be bone marrow. The HSCs and heparan sulphate may be administered in combination with the HSCs, or separately.

In another embodiment, products containing therapeutically effective amounts of:

(i) heparan sulphate from bone marrow stroma; and

(ii) hematopoietic stem cells (HSCs),

for simultaneous, separate or sequential use in a method of medical treatment are provided.

In another aspect, there is provided products containing therapeutically effective amounts of:

(i) heparan sulphate as defined above; and

(ii) hematopoietic stem cells (HSCs),

for simultaneous, separate or sequential use in medical treatment.

In another embodiment, a biocompatible implant or prosthesis comprising a biomaterial and heparan sulphate from bone marrow stroma is provided. The implant or

prosthesis may be coated or impregnated with said heparan sulphate. The implant or prosthesis may be further coated or impregnated with BMP2 protein and/or with osteoprogenitor cells.

In another aspect, there is provided a biocompatible implant or prosthesis comprising a biomaterial and heparan sulphate as defined above.

In another embodiment, a method of forming a biocompatible implant or prosthesis is provided, the method comprising the step of coating or impregnating a biomaterial with heparan sulphate from bone marrow stroma. The method may comprise coating or impregnating the biomaterial with one or both of BMP2 protein and osteoprogenitor cells.

In an aspect, there is provided a method of forming a biocompatible implant or prosthesis, the method comprising the step of coating or impregnating a biomaterial with heparan sulphate as defined above.

In the present disclosure, a method of treating a bone fracture in a patient is provided, the method comprising administration of a therapeutically effective amount of heparan sulphate from bone marrow stroma to the patient. The method may comprise administering said heparan sulphate to tissue surrounding the fracture. Administration of said heparan sulphate may comprise injection of the heparan sulphate

to the tissue surrounding the fracture. The heparan sulphate may be formulated as a pharmaceutical composition or medicament comprising the heparan sulphate and a pharmaceutically acceptable carrier, adjuvant or diluent. The method may further comprise administering BMP2 protein to the patient. Said heparan sulphate and BMP2 protein may be formulated as a pharmaceutical composition comprising the heparan sulphate and BMP2 protein and a pharmaceutically acceptable carrier, adjuvant or diluent.

In the present disclosure, a method of treating a bone fracture in a patient is provided, the method comprising surgically implanting a biocompatible implant or prosthesis, which implant or prosthesis comprises a biomaterial and a therapeutically effective amount of heparan sulphate from bone marrow stroma into tissue of the patient at or surrounding the site of fracture. The implant or prosthesis may be coated or impregnated with said heparan sulphate. The implant or prosthesis may be further coated or impregnated with one or both of BMP2 protein and osteoprogenitor cells.

In another embodiment, products containing therapeutically effective amounts of:

(i) heparan sulphate from bone marrow stroma;

and one or both of

(ii) BMP2 protein;

(iii) osteoprogenitor cells,

for simultaneous, separate or sequential use in a method of medical treatment are provided.

In another aspect, there is provided products containing therapeutically effective amounts of:

(i) heparan sulphate as defined above;

and one or both of

(ii) BMP2 protein;

(iii) osteoprogenitor cells,

for simultaneous, separate or sequential use in medical treatment.

In the present disclosure, a method for the repair, replacement or regeneration of cells or tissue in a human or animal patient in need of such treatment is provided, the method comprising:

(i) culturing stem cells *in vitro* in contact with heparan sulphate from bone marrow stroma for a period of time sufficient for some or all of said cells to differentiate

(ii) or become lineage-committed;

(iii) collecting said differentiated or lineage-committed cells;

(iv) implanting said differentiated or lineage-committed cells into the body of the patient at a site of injury or disease to repair, replace or regenerate cells or tissue in the patient.

Claims:

1. Heparan sulphate HS5 substantially purified from the core protein, wherein the heparan sulphate has the following heparan lyase disaccharide digestion profile:

Disaccharide	% Composition
ΔHexUA – GlcN	22.11 ±10%
ΔHexUA – GlcN,6S	38.79 ±10%
ΔHexUA – GlcNS	4.20 ±10%
ΔHexUA – GlcNAc,6s	4.53 ±10%
Other	30.37 ±10%

2. A method of increasing the number of committed clonogenic cells in a population of hematopoietic stem cells (HSCs), the method comprising culturing HSCs *in vitro* in the presence of isolated heparan sulphate HS5 or heparan sulphate isolated from bone marrow stroma.

3. The method of claim 2 wherein the method comprises increasing the number of myeloid lineage-committed progenitor cells.

4. The method of claim 2 or 3 wherein the method comprises increasing the number of colony forming cells (CFC).

5. The method of any one of claims 2 to 4 wherein the method comprises increasing the number of burst forming unit-erythroid (BFU-E).

6. The method of any one of claims 2 to 5 wherein the method comprises increasing the number of colony forming unit-granulocyte macrophage (CFU-GM).

7. The method of any one of claims 2 to 6 wherein the initial HSC population in the culture comprises at least 50% CD34+ cells.

8. The method of any one of claims 2 to 7 wherein the HSC population is obtained from cord blood.

9. The method of any one of claims 2 to 8 wherein the concentration of heparan sulphate in the culture media is 500 ng/ml or less.

10. A method of promoting osteogenesis, the method comprising administering heparan sulphate according to claim 1 to osteoprogenitor cells *in vitro*.

11. The method of claim 10 wherein the osteoprogenitor cells are contacted with BMP2 protein simultaneously with said heparan sulphate.

12. The method of claim 10 or 11 wherein the osteoprogenitor cells are mesenchymal stem cells, osteoblast cells or myoblast cells.

13. A method of culturing osteoprogenitor cells comprising culturing osteoprogenitor cells in contact with heparan sulphate according to claim 1.

14. Culture media comprising heparan sulphate according to claim 1.

15. Use of heparan sulphate according to claim 1 in cell culture *in vitro*.

16. A kit comprising a predetermined amount of heparan sulphate according to claim 1 and a predetermined amount of BMP2 protein.

17. A pharmaceutical composition or medicament comprising heparan sulphate according to claim 1.

18. A composition or pharmaceutical composition comprising heparan sulphate according to claim 1 and BMP2 protein.

19. Heparan sulphate according to claim 1 or a composition or pharmaceutical composition according to claims 17 or 18 for use in medical treatment.

20. Heparan sulphate or a composition or pharmaceutical composition for use medical treatment according to claim 19 wherein the medical treatment comprises the heparan sulphate or composition or pharmaceutical composition to be administered to a patient receiving HSC transplantation.

21. Heparan sulphate or a composition or pharmaceutical composition for use in medical treatment according to claim 19 wherein the heparan sulphate or composition or pharmaceutical composition is to be administered in combination with transplanted HSCs.

22. Products containing therapeutically effective amounts of:

(i) heparan sulphate according to claim 1; and

(ii) hematopoietic stem cells (HSCs),

for simultaneous, separate or sequential use in medical treatment.

23. Use of heparan sulphate according to claim 1, in the manufacture of a medicament for medical treatment, wherein the medical treatment comprises wound healing.

24. Use of heparan sulphate according to claim 1 or a composition or pharmaceutical composition according to any one of claims 17 or 18 in the manufacture of a medicament for use in medical treatment, wherein the medical treatment comprises repair and/or regeneration of bone and/or treatment of bone fracture.

25. A biocompatible implant or prosthesis comprising a biomaterial and heparan sulphate according to claim 1.

26. The implant or prosthesis of claim 25 wherein the implant or prosthesis is coated with heparan sulphate according to claim 1.

27. The implant or prosthesis of claim 26 wherein the implant or prosthesis is impregnated with heparan sulphate according to any one of claims 1 to 4.

28. The implant or prosthesis of claim 26 to 27 wherein the implant or prosthesis is further coated or impregnated with BMP2 protein and/or with osteoprogenitor cells.

29. A method of forming a biocompatible implant or prosthesis, the method comprising the step of coating or impregnating a biomaterial with heparan sulphate according to claim 1.

30. The method of claim 29 wherein the method further comprises coating or impregnating the biomaterial with one or both of BMP2 protein and osteoprogenitor cells.

31. Products containing therapeutically effective amounts of:

(i) heparan sulphate according to claim 1;

and one or both of

(ii) BMP2 protein;

(iii) osteoprogenitor cells,

for simultaneous, separate or sequential use in medical treatment.