vital to understanding the rules of HS-protein interaction and design of HS mimics that can target proteins in human diseases. The minimal length and structural features of heparin/HS motifs have been identified in only a few cases (27-30). The first well studied example for the minimal length of the HS motif required for ligand binding and activity was the heparin-derived pentasaccharide were sufficient to interact with antitrombin III to inhibit clotting factors thrombin (IIa) and factor Xa (28-30). However, heparin-derived tetrasaccharide were sufficient to interact with fibroblast growth factor–2 (FGF-2), but oligosaccharides of degree of polymerization (dp) 10-12 are required for optimizing the proliferative activity of FGF-2. However, the structural sequence required for antithrombin III binding, where the most characteristic feature is the unusual 3-*O* sulphate group on a 6-*O* sulphate *N*-sulphoglucosamine residue (31). Furthermore, the structural characterization of a HS pattern that could bind FGF-2 has illustrated the importance of continuous stretches of the disulphide disaccharide *N*-sulphoglucosamine-iduronate 2-*O* sulphate for highest binding ability (32). These examples clearly demonstrated that the minimal oligosaccharide length units and specific structural features of HS pattern required for either binding or biological activity are not strictly related.

In this context, the characterization of the minimal HS unit and specific sulphate group of HS that binds BMP2 is of importance in an attempt to clarify the molecular mechanism that underline the requirement of HS in the regulation of BMP2 activity. However, HS is extremely heterogeneous in sequence and size and the source is limited. Heparin is similar in structure to the sulphated regions of HS. Therefore, in this study we examine the relationship between minimum length and specific sulphate group of heparin-derived oligosaccharides, which is required for binding to BMP2 and their capability to enhance BMP2 induced osteoblast differentiation on C2C12 myoblasts.

Previously, we identified and isolated an heparan sulphate that binds BMP2 with high affinity and showed that this HS has activity in the repair/regeneration of bone. Our work is reported in WO2010/030244 A1 and US9498494.

Summary

In one aspect of the present disclosure provides a composition comprising an isolated heparin or heparan sulphate oligosaccharide for use in therapy, wherein the isolated heparin or heparan sulphate oligosaccharide having a chain length of at least 10 saccharides and no more than 50 saccharides and a BMP2 protein, wherein the oligosaccharide is capable of binding BMP2. In one aspect, the present disclosure refers to a composition comprising an isolated heparin or heparan sulphate oligosaccharide having a chain length 36 to 50 saccharides and a BMP2 protein, wherein the oligosaccharide is capable of binding BMP2, for use in therapy. In one aspect, the present disclosure refers to a use of the isolated ~~heparin or~~ heparan sulphate as disclosed herein in the manufacture of a medicament for in wound healing *in vivo*, wherein said medicament is to be administered simultaneously or sequentially with BMP2 protein. In another aspect, the present disclosure refers to a mixture for use in therapy, wherein the mixture comprises or consists of a plurality of BMP2 binding ~~heparin or~~ heparan sulphate oligosaccharides each having a chain length of ~~at least 10 saccharides and no more than~~ 36 to 50 saccharides and BMP2 protein. In another aspect, the present disclosure refers to a use of mixture comprising or consisting of a plurality of BMP2 binding ~~heparin or~~ heparan sulphate oligosaccharides each having a chain length of ~~at least 10 saccharides and no more than~~ 36 to 50 saccharides, in the manufacture of a medicament for wound healing *in vivo*,

wherein said medicament is to be administered simultaneously or sequentially with BMP2 protein. In another aspect, the present disclosure refers to a composition comprising a fragment, optionally isolated, of a BMP2 binding heparan sulphate, wherein the fragment comprises or consists of an oligosaccharide having a chain length of ~~at least 10~~36 to 50 saccharides, wherein the oligosaccharide is capable of binding BMP2, and BMP2 protein, for use in therapy. In another aspect, the present disclosure refers to a use of a fragment, optionally isolated, of a BMP2 binding heparan sulphate, wherein the fragment comprises or consists of an oligosaccharide having a chain length of ~~at least 10~~36 to 50 saccharides, wherein the oligosaccharide is capable of binding BMP2 in the manufacture of a medicament for wound healing *in vivo*, wherein said medicament is to be administered simultaneously or sequentially with BMP2 protein. In still another aspect, the present disclosure refers to a biocompatible implant or prosthesis comprising a biomaterial, BMP2 protein, and either: a. an isolated ~~heparin or~~ heparan sulphate oligosaccharide capable of binding BMP2 and having a chain length of ~~at least 10 saccharides and no more than~~36 to 50 saccharides, b. a mixture comprising or consisting of a plurality of BMP2 binding ~~heparin or~~ heparan sulphate oligosaccharides each having a chain length of ~~at least 10 saccharides and no more than~~36 to 50 saccharides, or c. a fragment, optionally isolated, of a BMP2 binding heparan sulphate, wherein the fragment comprises or consists of an oligosaccharide having a chain length of ~~at least 10~~36 to 50 saccharides, wherein the oligosaccharide is capable of binding BMP2. In yet another aspect, the present disclosure refers to a method of forming a biocompatible implant or prosthesis, the method comprising the step of coating or impregnating a biomaterial with BMP-2 protein and either: a. an isolated ~~heparin or~~ heparan sulphate oligosaccharide capable of binding BMP2 and having a chain length of ~~at least 10 saccharides and no more than~~36 to 50 saccharides, b. a mixture comprising or consisting of a plurality of BMP2 binding ~~heparin or~~ heparan sulphate oligosaccharides each having a chain length of ~~at least 10 saccharides and no more than~~ 36 to 50 saccharides, or c. a fragment, optionally isolated, of a BMP2 binding heparan sulphate, wherein the fragment comprises or consists of an oligosaccharide having a chain length of ~~at least 10~~ 36 to 50 saccharides, wherein the oligosaccharide is capable of binding BMP2. In yet another aspect, the present disclosure refers to a use of an isolated ~~heparin or~~ heparan sulphate oligosaccharide capable of binding BMP2 and having a chain length of ~~at least 10 saccharides and no more than~~ 36 to 50 saccharides, a mixture comprising or consisting of a plurality of BMP2 binding ~~heparin or~~ heparan sulphate oligosaccharides each having a chain length of ~~at least 10 saccharides and no more than~~ 36 to 50 saccharides, or a BMP-2 binding fragment, optionally isolated, of a BMP2 binding heparan sulphate, wherein the fragment comprises or consists of an oligosaccharide having a chain length of ~~at least 10~~36 to 50 saccharides, in the manufacture of a biocompatible implant or prosthesis for treating a bone fracture in a patient.

Claims:

1. A composition comprising an isolated ~~heparin or~~ heparan sulphate oligosaccharide having a chain length of ~~at least 10~~ 36 ~~saccharides and no more than~~ to 50 saccharides and a BMP2 protein, wherein the oligosaccharide is capable of binding BMP2, for use in therapy.

2. Use of an isolated ~~heparin or~~ heparan sulphate having a chain length of ~~at least~~ 36 ~~10 saccharides and no more than~~to 50 saccharides, wherein the oligosaccharide is capable of binding BMP2, in the manufacture of a medicament for in wound healing *in vivo,* wherein said medicament is to be administered simultaneously or sequentially with BMP2 protein.

3. The use according to claim 2, wherein the medicament is for
   - repairing and/or regenerating of connective tissue,
   - repairing and/or regenerating of bone,
   - repairing and/or regenerating of bone in a mammal or a human, or
   - repairing and/or regenerating of a broken bone.

~~4. The composition for use, or use, according to any one of claims 1 to 3, wherein the isolated heparin or heparan sulphate has a chain length of at least 12 saccharides.~~

~~5. The composition for use, or use, according to any one of claims 1 to 3, wherein the isolated heparin or heparan sulphate has a chain length of 10 or 12 saccharides.~~

~~6. The composition for use, or use, according to any one of claims 1 to 3, wherein the isolated heparin or heparan sulphate has a chain length of 10 to 50 saccharides, optionally one of 12 to 50 saccharides, 14 to 50 saccharides, 16 to 50 saccharides, 18 to 50 saccharides, 20 to 50 saccharides, 22 to 50 saccharides, 24 to 50 saccharides, 26 to 50 saccharides, 28 to 50 saccharides, 30 to 50 saccharides, 32 to 50 saccharides, 34 to 50 saccharides, 36 to 50 saccharides, 38 to 50 saccharides, 40 to 50 saccharides, 42 to 50 saccharides, 44 to 50 saccharides, 46 to 50 saccharides, or 48 to 50 saccharides.~~

~~7. The composition for use, or use, according to any one of claims 1 to 3, wherein the isolated heparin or heparan sulphate has a chain length of 10 to 36 saccharides, optionally one of 12 to 36 saccharides, 14 to 36 saccharides, 16 to 36 saccharides, 18 to 36 saccharides, 20 to 36 saccharides, 22 to 36 saccharides, 24 to 36 saccharides, 26 to 36 saccharides, 28 to 36 saccharides, 30 to 36 saccharides, 32 to 36 saccharides, or 34 to 36 saccharides.~~

~~8~~4. The composition for use, or use, according to any one of claims 1 to 3, wherein the isolated ~~heparin or~~ heparan sulphate has a chain length of ~~36 to 50 saccharides, optionally~~ one of 38 to 50 saccharides, 40 to 50 saccharides, 42 to 50 saccharides, 44 to 50 saccharides, 46 to 50 saccharides, or 48 to 50 saccharides.

~~9. The composition for use, or use, according to any one of claims 1 to 3, wherein the isolated heparin or heparan sulphate has a chain length of 18 to 40 saccharides, optionally one of 20 to 40 saccharides, 22 to~~

~~40 saccharides, 24 to 40 saccharides, 26 to 40 saccharides, 28 to 40 saccharides, 30 to 40 saccharides, 32 to 40 saccharides, 34 to 40 saccharides, 36 to 40 saccharides, or 38 to 40 saccharides.~~

~~10~~5. The composition for use, or use, according to any one of claims 1 to ~~4~~9, wherein the isolated ~~heparin or~~ heparan sulphate enhances BMP2-mediated ALP activity, e.g. as measured in an in vitro assay of BMP2-mediated ALP activity.

~~11~~6. The composition for use, or use, according to any one of claims 1 to 5~~10~~, wherein the isolated ~~heparin or~~ heparan sulphate enhances BMP2-mediated Smad 1/5/9 phosphorylation, e.g. as measured in an in vitro assay of BMP2-mediated Smad 1/5/9 phosphorylation.

~~12~~7. The composition for use, or use, according to any one of claims 1 to 6~~11~~, wherein the isolated ~~heparin or~~ heparan sulphate is N-sulphated.

~~13~~8. The composition for use, or use, according to any one of claims 1 to 7~~12~~, wherein the isolated ~~heparin or~~ heparan sulphate is 6-O sulphated.

~~14~~9. The composition for use, or use, according to any one of claims 1 to 8~~13~~, wherein the isolated ~~heparin or~~ heparan sulphate is 2-O de-sulphated.

~~15~~10. A mixture comprising or consisting of a plurality of BMP2 binding ~~heparin or~~ heparan sulphate oligosaccharides each having a chain length of ~~at least 10~~ 36 ~~saccharides and no more than~~ to 50 saccharides, and BMP2 protein, for use in therapy.

11~~16~~. Use of mixture comprising or consisting of a plurality of BMP2 binding ~~heparin or~~ heparan sulphate oligosaccharides each having a chain length of ~~at least 10~~ 36 ~~saccharides and no more than~~to 50 saccharides, in the manufacture of a medicament for wound healing *in vivo,* wherein said medicament is to be administered simultaneously or sequentially with BMP2 protein.

12~~17~~. The use according to claim 11~~16~~, wherein the medicament is for

- repairing and/or regenerating of connective tissue,
- repairing and/or regenerating of bone,
- repairing and/or regenerating of bone in a mammal or a human, or
- repairing and/or regenerating of a broken bone.

13~~18~~. The mixture for use or use according to any one of claims 10~~15~~ to 12~~17~~, wherein the mixture is provided in isolated or substantially pure form.

~~19. The mixture for use or use according to any one of claims 15 to 17, wherein the oligosaccharides each have a chain length of at least 12 saccharides.~~

~~20. The mixture for use or use according to any one of claims 15 to 17, wherein the oligosaccharides each have a chain length of 10 or 12 saccharides.~~

~~21. The mixture for use or use of any one of claims 15 to 20, wherein the oligosaccharides each have a chain length of 10 to 50 saccharides, optionally one of 12 to 50 saccharides, 14 to 50 saccharides, 16 to 50 saccharides, 18 to 50 saccharides, 20 to 50 saccharides, 22 to 50 saccharides, 24 to 50 saccharides, 26 to 50 saccharides, 28 to 50 saccharides, 30 to 50 saccharides, 32 to 50 saccharides, 34 to 50 saccharides, 36 to 50 saccharides, 38 to 50 saccharides, 40 to 50 saccharides, 42 to 50 saccharides, 44 to 50 saccharides, 46 to 50 saccharides, or 48 to 50 saccharides.~~

~~22. The mixture for use or use of any one of claims 15 to 20, wherein the oligosaccharides each have a chain length of 10 to 36 saccharides, optionally one of 12 to 36 saccharides, 14 to 36 saccharides, 16 to 36 saccharides, 18 to 36 saccharides, 20 to 36 saccharides, 22 to 36 saccharides, 24 to 36 saccharides, 26 to 36 saccharides, 28 to 36 saccharides, 30 to 36 saccharides, 32 to 36 saccharides, or 34 to 36 saccharides.~~

14~~23~~. The mixture for use or use of any one of claims 10~~15~~ to 13~~20~~, wherein the oligosaccharides each have a chain length of ~~36 to 50 saccharides, optionally~~ one of 38 to 50 saccharides, 40 to 50 saccharides, 42 to 50 saccharides, 44 to 50 saccharides, 46 to 50 saccharides, or 48 to 50 saccharides.

~~24. The mixture for use or use of any one of claims 15 to 20, wherein the oligosaccharides each have a chain length of 18 to 40 saccharides, optionally one of 20 to 40 saccharides, 22 to 40 saccharides, 24 to 40 saccharides, 26 to 40 saccharides, 28 to 40 saccharides, 30 to 40 saccharides, 32 to 40 saccharides, 34 to 40 saccharides, 36 to 40 saccharides, or 38 to 40 saccharides.~~

15~~25~~. The mixture for use or use of any one of claims 10~~15~~ to 14~~24~~, wherein the BMP2 binding ~~heparin or~~ heparan sulphate oligosaccharides enhance BMP2-mediated ALP activity, e.g. as measured in an in vitro assay of BMP2-mediated ALP activity.

16~~26~~. The mixture for use or use of any one of claims 10~~15~~ to 15~~25~~, wherein the BMP2 binding ~~heparin or~~ heparan sulphate oligosaccharides enhance BMP2-mediated Smad 1/5/9 phosphorylation, e.g. as measured in an in vitro assay of BMP2-mediated Smad 1/5/9 phosphorylation.

17~~27~~. The mixture for use or use of any one of claims 10~~15~~ to 16~~26~~, wherein the BMP2 binding ~~heparin or~~ heparan sulphate oligosaccharides are N-sulphated.

18~~28~~. The mixture for use or use of any one of claims 10~~15~~ to 17~~27~~, wherein the BMP2 binding ~~heparin or~~ heparan sulphate oligosaccharides are 6-O sulphated.

19~~29~~. The mixture for use or use of any one of claims 10~~15~~ to 18~~28~~, wherein the BMP2 binding ~~heparin or~~ heparan sulphate oligosaccharides are 2-O de-sulphated.

2030. A composition comprising a fragment, optionally isolated, of a BMP2 binding heparan sulphate, wherein the fragment comprises or consists of an oligosaccharide having a chain length of ~~at least 10~~36 to 50 ~~-~~saccharides, wherein the oligosaccharide is capable of binding BMP2, and BMP2 protein, for use in therapy.

2131. Use of a fragment, optionally isolated, of a BMP2 binding heparan sulphate, wherein the fragment comprises or consists of an oligosaccharide having a chain length of ~~at least 10~~ 36 to 50 saccharides, wherein the oligosaccharide is capable of binding BMP2 in the manufacture of a medicament for wound healing *in vivo,* wherein said medicament is to be administered simultaneously or sequentially with BMP2 protein.

2232. The use according to claim 2131, wherein the medicament is for

- repairing and/or regenerating of connective tissue,
- repairing and/or regenerating of bone,
- repairing and/or regenerating of bone in a mammal or a human, or
- repairing and/or regenerating of a broken bone.

2333. The composition for use or use according to any one of claims 2030 to 2232, wherein the BMP2 binding heparan sulphate is HS3.

~~34. The composition for use or use according to any one of claims 30 to 33, wherein the oligosaccharide has a chain length of no more than 50 saccharides.~~

~~35. The composition for use or use according to any one of claims 30 to 34, wherein the oligosaccharide has a chain length of at least 12 saccharides.~~

~~36. The composition for use or use according to any one of claims 30 to 35, wherein the oligosaccharide has a chain length of 10 or 12 saccharides.~~

~~37. The composition for use or use according to any one of claims 30 to 36, wherein the oligosaccharide has a chain length of 10 to 50 saccharides, optionally one of 12 to 50 saccharides, 14 to 50 saccharides, 16 to 50 saccharides, 18 to 50 saccharides, 20 to 50 saccharides, 22 to 50 saccharides, 24 to 50 saccharides, 26 to 50 saccharides, 28 to 50 saccharides, 30 to 50 saccharides, 32 to 50 saccharides, 34 to 50 saccharides, 36 to 50 saccharides, 38 to 50 saccharides, 40 to 50 saccharides, 42 to 50 saccharides, 44 to 50 saccharides, 46 to 50 saccharides, or 48 to 50 saccharides.~~

~~38. The composition for use or use according to any one of claims 30 to 36, wherein the oligosaccharide has a chain length of 10 to 36 saccharides, optionally one of 12 to 36 saccharides, 14 to 36 saccharides, 16 to 36 saccharides, 18 to 36 saccharides, 20 to 36 saccharides, 22 to 36 saccharides, 24 to 36 saccharides, 26 to 36 saccharides, 28 to 36 saccharides, 30 to 36 saccharides, 32 to 36 saccharides, or 34 to 36 saccharides.~~

2439. The composition for use or use according to any one of claims 2030 to 2336, wherein the oligosaccharide has a chain length of ~~36 to 50 saccharides, optionally~~ one of 38 to 50 saccharides, 40 to 50 saccharides, 42 to 50 saccharides, 44 to 50 saccharides, 46 to 50 saccharides, or 48 to 50 saccharides.

~~40. The composition for use or use according to any one of claims 30 to 36, wherein the oligosaccharide has a chain length of 18 to 40 saccharides, optionally one of 20 to 40 saccharides, 22 to 40 saccharides, 24 to 40 saccharides, 26 to 40 saccharides, 28 to 40 saccharides, 30 to 40 saccharides, 32 to 40 saccharides, 34 to 40 saccharides, 36 to 40 saccharides, or 38 to 40 saccharides.~~

2541. The composition for use or use according to any one of claims 2030 to 2440, wherein the fragment enhances BMP2-mediated ALP activity, e.g. as measured in an in vitro assay of BMP2-mediated ALP activity.

2642. The composition for use or use according to any one of claims 2030 to 2541, wherein the fragment enhances BMP2-mediated Smad 1/5/9 phosphorylation, e.g. as measured in an in vitro assay of BMP2-mediated Smad 1/5/9 phosphorylation.

2743. The composition for use or use according to any one of claims 2030 to 2642, wherein the heparin or heparan sulphate is N-sulphated.

2844. The composition for use or use according to any one of claims 2030 to 2743, wherein the heparin or heparan sulphate is 6-O sulphated.

2945. The composition for use or use according to any one of claims 2030 to 2844, wherein the heparin or heparan sulphate is 2-O de-sulphated.

3046. A biocompatible implant or prosthesis comprising a biomaterial, BMP2 protein, and either:

a. an isolated ~~heparin or~~ heparan sulphate oligosaccharide capable of binding BMP2 and having a chain length of ~~at least 10 saccharides and no more than~~36 to 50 saccharides,
b. a mixture comprising or consisting of a plurality of BMP2 binding ~~heparin or~~ heparan sulphate oligosaccharides each having a chain length of ~~at least 10 saccharides and no more than~~36 to 50 saccharides, or
c. a fragment, optionally isolated, of a BMP2 binding heparan sulphate, wherein the fragment comprises or consists of an oligosaccharide having a chain length of ~~at least 10~~36 to 50 saccharides, wherein the oligosaccharide is capable of binding BMP2.

3147. A method of forming a biocompatible implant or prosthesis, the method comprising the step of coating or impregnating a biomaterial with BMP-2 protein and
either:

a. an isolated ~~heparin or~~ heparan sulphate oligosaccharide capable of binding BMP2 and having a chain length of ~~at least 10 saccharides and no more than~~ 36 to 50 saccharides,

b. a mixture comprising or consisting of a plurality of BMP2 binding ~~heparin or~~ heparan sulphate oligosaccharides each having a chain length of ~~at least 10 saccharides and no more than~~ 36 to 50 saccharides, or

c. a fragment, optionally isolated, of a BMP2 binding heparan sulphate, wherein the fragment comprises or consists of an oligosaccharide having a chain length of ~~at least 10~~36 to 50 saccharides, wherein the oligosaccharide is capable of binding BMP2.

3248. Use of an isolated ~~heparin or~~ heparan sulphate oligosaccharide capable of binding BMP2 and having a chain length of ~~at least 10 saccharides and no more than~~ 36 to 50 saccharides, a mixture comprising or consisting of a plurality of BMP2 binding ~~heparin or~~ heparan sulphate oligosaccharides each having a chain length of ~~at least 10 saccharides and no more than~~36 to 50 saccharides, or a BMP-2 binding fragment, optionally isolated, of a BMP2 binding heparan sulphate, wherein the fragment comprises or consists of an oligosaccharide having a chain length of ~~at least 10~~36 to 50 saccharides, in the manufacture of a biocompatible implant or prosthesis for treating a bone fracture in a patient.