sequences are generally at least 5-6 disaccharides in length (24-26). The precise structures of HS that are involved in these interactions have remained elusive. Information on minimal binding sequences on HS is 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 an isolated heparin or heparan sulphate oligosaccharide is provided, the isolated heparin or heparan sulphate oligosaccharide having a chain length of at least 6 saccharides and no more than 50 saccharides. In one aspect, the present disclosure refers to an isolated heparin or heparan sulphate oligosaccharide having a chain length of at least 10 saccharides and no more than 50 saccharides, wherein the oligosaccharide is capable of binding BMP2. In another aspect, the present disclosure refers to mixture of isolated heparin or heparan sulphate oligosaccharides according to the present invention. In another aspect, the present disclosure refers to 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 50 saccharides. In another aspect, the present disclosure refers to 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 saccharides, wherein the oligosaccharide is capable of binding BMP2. In still another aspect, the present disclosure refers to a mixture comprising or consisting of a

plurality of fragments according to the present invention. In another aspect, the present disclosure refers to isolated heparin or heparan sulphate of the present invention, mixture of the present invention or fragment of the present invention for use in therapy. In another aspect, the present disclosure refers to the use of the isolated heparin or heparan sulphate of the present invention, mixture of the present invention or fragment of the present invention in the manufacture of a medicament for wound healing *in vivo*, the repair and/or regeneration of connective tissue, the repair and/or regeneration of bone, the repair and/or regeneration of bone in a mammal or a human, or the repair and/or regeneration of a broken bone. In another aspect, the present disclosure refers to the use of the isolated heparin or heparan sulphate of the present invention, mixture of the present invention or fragment of the present invention in the manufacture of a biocompatible implant or prosthesis for treating a bone fracture in a patient. In another aspect, the present disclosure refers to products containing therapeutically effective amounts of the isolated heparin or heparan sulphate of the present invention, mixture of the present invention or fragment of the present invention; and BMP2 protein; for simultaneous, separate or sequential use in therapy.

Other aspects and embodiments are described in the appended claims.

In some examples the oligosaccharide has a chain length of one of: at least 8, at least 10, or at least 12 saccharides.

In some examples the oligosaccharide comprises or consists of a chain length of 8, 10 or 12 saccharides.

In some examples the oligosaccharide has a chain length of no more than one of:49, 48, 47, 46, 45, 44, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 32, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11 or 10 saccharides.

In some examples the oligosaccharide has a chain length of one of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 or 50 saccharides.

In some embodiments the oligosaccharide is N-sulphated. This may comprise N-sulphation of N-acetyl-D-glucosamine (GlcNAc) residues in the heparin or heparan sulphate oligosaccharide chain. Preferably at least 80% of N-acetyl-D-glucosamine (GlcNAc) residues in the isolated heparin or heparan sulphate are N-sulphated. In some examples this may be one of at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100%.

In some embodiments the oligosaccharide is 6-O sulphated (O-sulphation at C6 of N-sulphoglucosamine (GlcNS) residues). Preferably at least 80% of N-sulphoglucosamine (GlcNS) residues in the heparin or heparan sulphate oligosaccharide chain are 6-O-sulphated. In some examples this may be one of at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100%.

Although the oligosaccharide may be 2-O sulphated (O-sulphation at C2 of IdoA and GlcA) to a varying degree, in some embodiments the isolated heparin or heparan sulphate is 2-O de-sulphated.

In some examples the oligosaccharide binds BMP2 protein, optionally with a K_D of less than one of: 100μM, 50μM, 10μM, 1 μM, 100nM, 50nM, 10nM or 1nM.

Preparations or compositions comprising the isolated heparin or heparan sulphate are provided.

In one aspect of the present invention a pharmaceutical composition or medicament is provided comprising the isolated heparin or heparan sulphate in accordance with the aspects described above. The pharmaceutical composition or medicament may further comprise a pharmaceutically acceptable carrier, adjuvant or diluent.

In another example a composition comprising the isolated heparin or heparan sulphate according to any one of the aspects above and BMP2 protein is provided.

In one example the isolated heparin or heparan sulphate is provided for use in a method of treatment.

In a related example the use of the isolated heparin or heparan sulphate in the manufacture of a medicament for use in a method of medical treatment is provided.

In another example a method of treatment is provided, the method comprising the step of administering the isolated heparin or heparan sulphate to a subject in need of treatment thereof.

In examples concerning a method of medical treatment the method of treatment may comprise a method of wound healing *in vivo*, the repair and/or regeneration of connective tissue, the repair and/or regeneration of bone and/or the repair and/or regeneration of bone in a mammal or a human. In some preferred examples the method of treatment comprises the repair and/or regeneration of a broken bone. In some examples the method of treatment may comprise simultaneous or sequential administration of BMP2 protein.

In some examples a method of treating a bone fracture in a patient is provided, the method comprising administration of a therapeutically effective amount of the isolated heparin or heparan sulphate to the patient. In some examples the method comprises administering the isolated heparin or heparan sulphate to the tissue surrounding the fracture. In some examples the method comprises injection of the isolated heparin or heparan sulphate to the tissue surrounding the fracture. In such methods the isolated heparin or heparan sulphate may be formulated as a pharmaceutical composition or medicament comprising the isolated heparin or heparan sulphate and a pharmaceutically acceptable carrier, adjuvant or diluent.

In some examples the method may further comprise administering BMP2 protein to the patient. In such methods the isolated heparin or heparan sulphate and BMP2 protein may be formulated as a pharmaceutical composition comprising the isolated heparin or heparan sulphate and BMP2 protein and a pharmaceutically acceptable carrier, adjuvant or diluent.

In a further aspect of the present invention a biocompatible implant or prosthesis comprising a biomaterial and the isolated heparin or heparan sulphate is provided. In some embodiments the implant or prosthesis is

coated with the isolated heparin or heparan sulphate. In some embodiments the implant or prosthesis is impregnated with the isolated heparin or heparan sulphate.

In another aspect of the present invention a method of forming a biocompatible implant or prosthesis is provided, the method comprising the step of coating or impregnating a biomaterial with the isolated heparin or heparan sulphate. In some embodiments the method further comprises coating or impregnating the biomaterial with BMP2 protein.

In some examples 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 the isolated heparin or heparan sulphate, into tissue of the patient at or surrounding the site of fracture.

In some examples the implant or prosthesis is coated with the isolated heparin or heparan sulphate. In some examples the implant or prosthesis is impregnated with the isolated heparin or heparan sulphate. In some examples the implant or prosthesis is further impregnated with BMP2 protein.

In yet a further aspect of the present invention a kit of parts is provided, the kit comprising a predetermined amount of the isolated heparin or heparan sulphate and a predetermined amount of BMP2. The kit may comprise a first container containing the predetermined amount of the isolated heparin or heparan sulphate and a second container containing the predetermined amount of BMP2. The kit may be provided for use in a method of medical treatment. The method of medical treatment may comprise a method of wound healing *in vivo*, the repair and/or regeneration of connective tissue, the repair and/or regeneration of bone and/or the repair and/or regeneration of bone in a mammal or a human. The kit may be provided together with instructions for the administration of the isolated heparin or heparan sulphate and BMP2 protein separately, sequentially or simultaneously in order to provide the medical treatment.

In a further example products are provided, the products containing therapeutically effective amounts of:

(i) the isolated heparin or heparan sulphate; and

(ii) BMP2 protein;

for simultaneous, separate or sequential use in a method of medical treatment. The method of medical treatment may comprise a method of wound healing *in vivo*, the repair and/or regeneration of connective tissue, the repair and/or regeneration of bone and/or the repair and/or regeneration of bone in a mammal or a human. The products may optionally be formulated as a combined preparation for co-administration.

In a further example there is provided a method of designing a heparin or heparan sulphate, optionally a heparin or heparan sulphate for use in a method of treatment as described herein, the method comprising determining one or more of the chain length, sulphation pattern and saccharide (or disaccharide) content or sequence of a heparin or heparan sulphate that has BMP2 binding activity.

In another example there is provided a method of manufacturing, producing or preparing a heparin or heparan sulphate, optionally a heparin or heparan sulphate for use in a method of treatment as described herein, the method comprising one or more of the following steps:

(i) determining one or more of the chain length, sulphation pattern and saccharide (or disaccharide) content or sequence of a heparin or heparan sulphate that has BMP2 binding activity;

(ii) synthesising one or a plurality of heparin or heparan sulphate oligosaccharides having a chain length, and/or sulphation pattern and/or saccharide (or disaccharide) content or sequence correlated with BMP2 binding activity;

(iii) formulating one or a plurality of heparin or heparan sulphate oligosaccharides having a chain length, and/or sulphation pattern and/or saccharide (or disaccharide) content or sequence correlated with BMP2 binding activity as a pharmaceutical composition or medicament.

Detailed Description of the Invention

In some embodiments heparin or heparan sulphate oligosaccharides may be obtained by size fractionation of heparin or heparan sulphate preparations. As such the heparin and heparan sulphate oligosaccharides may be fragments of larger heparin or heparan sulphate molecules. Suitable sources of heparin and heparan sulphate preparations for size fractionation include commercially available heparin and heparan