of repeating disaccharide units of glucoronic acid (GlcA) and glucosamine (GlcN), and plays essential roles in controlling cell phenotype and tissue development [9,10]. Certain HS species, together with the likes of neuropillin-1, are required to form a complex with VEGF ligand and receptor to stabilise and enhance the $VEGF_{165}$-VEGFR2 interaction [4]. Specifically, HS binds to the 55-residue COOH-terminal amino acid sequence of $VEGF_{165}$, and regulates $VEGF_{165}$ -mediated endothelial proliferation, tube formation, as well as vascular hyperpermeability[5,6]. In combination with polymer-based biomaterials, heparin has shown favourable ability in maintaining a sustained release of VEGF and producing localised vascularisation [11,12]. However, heparin is not suitable to stimulating angiogenesis in clinical applications. First, this highly charged HS species has notable anti-coagulant effects that cause serious adverse events such as haemorrhage (bleeding), thrombocytopenia, and hyperkalemia [13,14]. Overdoses of heparin have also been reported and are fatal [15]. Second, instead of selectively binding pro-angiogenic factors, heparin and mixtures of HS variants ubiquitously bind a variety of other soluble growth factors; given their inherent lack of specificity. Some of these nonspecific bindings may bring forward unpredictable effects that may antagonise the fundamental process needed for controlled angiogenesis.

Summary ~~of the Invention~~

The present ~~invention~~ disclosure provides the heparan sulphate HS7, which may be provided in isolated or substantially purified form.

In some ~~embodiments~~ examples HS7 is capable of binding SEQ ID NO:1, optionally with a K_D of less than 100μM, or one of less than 50μM, 40μm, 30μM, 20μM, or 10μM.

HS7 may be obtained by a method comprising:

(i) providing a solid support having polypeptide molecules adhered to the support, wherein the polypeptide comprises a heparin-binding domain having the amino acid sequence of SEQ ID NO:1;

(ii) contacting the polypeptide molecules with a mixture comprising glycosaminoglycans such that polypeptide-glycosaminoglycan complexes are allowed to form;

(iii) partitioning polypeptide-glycosaminoglycan complexes from the remainder of the mixture;

(iv) dissociating glycosaminoglycans from the polypeptide-glycosaminoglycan complexes;

(v) collecting the dissociated glycosaminoglycans.

In some examples ~~embodiments~~ the mixture comprising glycosaminoglycans is a heparan sulphate preparation obtained from porcine mucosa.

In ~~another aspect of~~ the present invention a composition comprising HS7 is provided. In some examples ~~embodiments~~ a pharmaceutical composition or medicament comprising HS7 is provided.

In one aspect of the present disclosure, a composition, pharmaceutical composition or medicament comprising HS7 is provided, wherein HS7 is heparan sulphate having the disaccharide composition:

Disaccharide	Normalised weight percentage
ΔUA,2S-GlcNS,6S	11.08±1.0
ΔUA,2S-GlcNS	4.46±0.4
ΔUA-GlcNS,6S	15.84±1.0
ΔUA,2S-GlcNAc,6S	4.76±0.6
ΔUA-GlcNS	20.27±3.0
ΔUA,2S-GlcNAc	1.01±0.4
ΔUA-GlcNAc,6S	10.63±1.0
ΔUA-GlcNAc	31.95±1.6

following digestion with heparin lyases I, II and III and then subjecting the resulting disaccharide fragments to capillary electrophoresis analysis, and
wherein the HS7 is capable of binding SEQ ID NO:1.

In some examples ~~embodiments~~ the pharmaceutical composition or medicament further comprises VEGF protein. In ~~some~~ another aspect~~s~~ of the present ~~invention~~ disclosure the pharmaceutical composition or medicament is provided for use in a method of medical treatment.

In ~~a further aspect of~~ the present disclosure ~~invention~~ HS7 is provided for use in a method of medical treatment. In another aspect of the present disclosure HS7 is provided for use in a method of medical treatment, wherein HS7 is heparan sulphate having the disaccharide composition:

Disaccharide	Normalised weight percentage
ΔUA,2S-GlcNS,6S	11.08±1.0
ΔUA,2S-GlcNS	4.46±0.4
ΔUA-GlcNS,6S	15.84±1.0
ΔUA,2S-GlcNAc,6S	4.76±0.6
ΔUA-GlcNS	20.27±3.0
ΔUA,2S-GlcNAc	1.01±0.4
ΔUA-GlcNAc,6S	10.63±1.0
ΔUA-GlcNAc	31.95±1.6

following digestion with heparin lyases I, II and III and then subjecting the resulting disaccharide fragments to capillary electrophoresis analysis, and wherein the HS7 is capable of binding SEQ ID NO:1. In some ~~embodiments~~ examples the method of treatment involves the stimulation or promotion of the growth of blood vessels.

In ~~another aspect of~~ the present disclosure ~~invention~~ the use of HS7 in the manufacture of a medicament for the treatment of a disease, condition or injury to tissue is provided, wherein the method involves the stimulation or promotion of the growth of blood vessels. In another aspect of the present disclosure the use of HS7 in the manufacture of a medicament for the treatment of vascular disease, cardiovascular disease, heart disease, ischemia, ischemic disease, stroke, ischemic vascular disease, myocardial infarction, or a disease or condition characterised by decreased blood flow to tissues and/or organs due to blocked or partially blocked arteries or injury to tissue, wherein the treatment involves the stimulation or promotion of the growth of blood vessels is provided, wherein HS7 is heparan sulphate having the disaccharide composition:

Disaccharide	Normalised weight percentage
ΔUA,2S-GlcNS,6S	11.08±1.0
ΔUA,2S-GlcNS	4.46±0.4
ΔUA-GlcNS,6S	15.84±1.0
ΔUA,2S-GlcNAc,6S	4.76±0.6

ΔUA-GlcNS	20.27±3.0
ΔUA,2S-GlcNAc	1.01±0.4
ΔUA-GlcNAc,6S	10.63±1.0
ΔUA-GlcNAc	31.95±1.6

following digestion with heparin lyases I, II and III and then subjecting the resulting disaccharide fragments to capillary electrophoresis analysis, and wherein the HS7 is capable of binding SEQ ID NO:1.

In ~~a further aspect of~~ the present disclosure ~~invention~~ a method of treating a disease, condition or injury to tissue in a patient is provided, the method comprising administration of a therapeutically effective amount of HS7 to the patient. In some ~~embodiments~~ examples the method involves the stimulation or promotion of the growth of blood vessels in the patient. In some examples ~~embodiments~~ the method further comprises administering VEGF protein to the patient.

In some examples ~~embodiments~~ methods of treatment comprise administering HS7 to tissue at or surrounding a wound or location on/in the patient's body at which blood vessel growth is required.

In ~~a further aspect of~~ the present disclosure ~~invention~~ a method of treating a disease, condition or injury to tissue in a patient is provided, the method comprising surgically implanting a biocompatible implant or prosthesis, which implant or prosthesis comprises a biomaterial

and HS7, into tissue of the patient at or surrounding the site of the disease, condition or injury.

In ~~another aspect of~~ the present disclosure ~~invention~~ a biocompatible implant or prosthesis comprising a biomaterial and HS7 is provided. In another aspect of the present disclosure a biocompatible implant or prosthesis comprising a biomaterial and isolated or purified HS7 is provided, wherein HS7 is heparan sulphate having the disaccharide composition:

Disaccharide	Normalised weight percentage
ΔUA,2S-GlcNS,6S	11.08±1.0
ΔUA,2S-GlcNS	4.46±0.4
ΔUA-GlcNS,6S	15.84±1.0
ΔUA,2S-GlcNAc,6S	4.76±0.6
ΔUA-GlcNS	20.27±3.0
ΔUA,2S-GlcNAc	1.01±0.4
ΔUA-GlcNAc,6S	10.63±1.0
ΔUA-GlcNAc	31.95±1.6

following digestion with heparin lyases I, II and III and then subjecting the resulting disaccharide fragments to capillary electrophoresis analysis, and
wherein the HS7 is capable of binding SEQ ID NO:1.

In ~~a further aspect of~~ the present disclosure ~~invention~~ a method of forming a biocompatible implant or prosthesis is provided, the method comprising the step of coating or impregnating a biomaterial with HS7. In another aspect of the present disclosure a method of forming a biocompatible implant or prosthesis is provided, the method comprising the step of coating or impregnating a biomaterial with isolated or purified HS7, wherein HS7 is heparan sulphate having the disaccharide composition:

Disaccharide	Normalised weight percentage
ΔUA,2S-GlcNS,6S	11.08±1.0
ΔUA,2S-GlcNS	4.46±0.4
ΔUA-GlcNS,6S	15.84±1.0
ΔUA,2S-GlcNAc,6S	4.76±0.6
ΔUA-GlcNS	20.27±3.0
ΔUA,2S-GlcNAc	1.01±0.4
ΔUA-GlcNAc,6S	10.63±1.0
ΔUA-GlcNAc	31.95±1.6

following digestion with heparin lyases I, II and III and then subjecting the resulting disaccharide fragments to capillary electrophoresis analysis, and
wherein the HS7 is capable of binding SEQ ID NO:1.

In ~~another aspect of~~ the present disclosure ~~invention~~ a method of promoting growth of blood vessels is provided, the method comprising administering HS7 to vascular cells or to vascular tissue. In some ~~embodiments~~ examples the vascular cells or vascular tissue are contacted with HS7 *in vitro*. In another aspect of the present disclosure a method of promoting growth of blood vessels *in vitro* is provided, the method comprising administering HS7 to vascular cells or to vascular tissue, wherein the vascular cells or vascular tissue are contacted with HS7 *in vitro* In other examples ~~embodiments~~ the vascular cells or vascular tissue are contacted with HS7 *in vivo*.

In another aspect of the present disclosure a biological scaffold comprising HS7 is provided, wherein HS7 is heparan sulphate having the disaccharide composition:

Disaccharide	Normalised weight percentage
ΔUA,2S-GlcNS,6S	11.08±1.0
ΔUA,2S-GlcNS	4.46±0.4
ΔUA-GlcNS,6S	15.84±1.0
ΔUA,2S-GlcNAc,6S	4.76±0.6
ΔUA-GlcNS	20.27±3.0
ΔUA,2S-GlcNAc	1.01±0.4
ΔUA-GlcNAc,6S	10.63±1.0
ΔUA-GlcNAc	31.95±1.6

following digestion with heparin lyases I, II and III and then subjecting the resulting disaccharide fragments to capillary electrophoresis analysis, and
wherein the HS7 is capable of binding SEQ ID NO:1.

Description of Preferred Embodiments

The present invention concerns the novel heparan sulphate HS7, which has been isolated by the inventors. Isolated HS7 is heparin-free and provides high activating affinity for VEGF**.**

Applications of HS7 include:

1. Providing agents capable of being used in medical treatment as an alternative to exogenously applied VEGF, whilst optionally being capable of sequestering endogenous VEGF at sites of ischemia.

2. As an adjuvant that, when bound to VEGF, reduces the amounts of exogenous VEGF required to promote angiogenesis, wherein the HS7 optionally:
   (a) protects and/or potentiates the effects of VEGF,
   (b) acts as a reservoir for VEGF,
   (c) contributes to the release of bioavailable VEGF at infarct sites, and/or
   (d) provides for a longer term sustained effect of VEGF.

HS7

Claims:

1. Isolated or substantially purified heparan sulphate HS7, wherein following digestion with heparin lyases I, II and III and then subjecting the resulting disaccharide fragments to capillary electrophoresis analysis the heparan sulphate HS7 has a disaccharide composition comprising:

Disaccharide	Normalised weight percentage
ΔUA,2S-GlcNS,6S	11.08±3.0
ΔUA,2S-GlcNS	4.46±2.0
ΔUA-GlcNS,6S	15.84±3.0
ΔUA,2S-GlcNAc,6S	4.76±2.0
ΔUA-GlcNS	20.27±3.0
ΔUA,2S-GlcNAc	1.01±0.5
ΔUA-GlcNAc,6S	10.63±3.0
ΔUA-GlcNAc	31.95±3.0

2. Isolated or substantially purified heparan sulphate HS7 wherein following digestion with heparin lyases I, II and III and then subjecting the resulting disaccharide fragments to capillary electrophoresis analysis the heparan sulphate HS7 has a disaccharide composition comprising:

Disaccharide	Normalised weight percentage
ΔUA,2S-GlcNS,6S	11.08±1.0
ΔUA,2S-GlcNS	4.46±0.4
ΔUA-GlcNS,6S	15.84±1.0
ΔUA,2S-GlcNAc,6S	4.76±0.6
ΔUA-GlcNS	20.27±3.0
ΔUA,2S-GlcNAc	1.01±0.4
ΔUA-GlcNAc,6S	10.63±1.0
ΔUA-GlcNAc	31.95±1.6

3. HS7 according to claim 1 or 2 wherein the HS7 is capable of binding SEQ ID NO:1 or 2.

4. HS7 according to any one of claims 1 to 3 obtained by a method comprising:

(i) providing a solid support having polypeptide molecules adhered to the support, wherein the polypeptide comprises a heparin-binding domain having the amino acid sequence of SEQ ID NO:1;

(ii) contacting the polypeptide molecules with a mixture comprising glycosaminoglycans such that polypeptide-glycosaminoglycan complexes are allowed to form;

(iii) partitioning polypeptide-glycosaminoglycan complexes from the remainder of the mixture;

(iv) dissociating glycosaminoglycans from the polypeptide-glycosaminoglycan complexes;

(v) collecting the dissociated glycosaminoglycans.

5. HS7 according to claim 4 wherein the mixture comprising glycosaminoglycans is a heparan sulphate preparation obtained from porcine mucosa.

16. A composition, pharmaceutical composition or medicament comprising HS7, wherein HS7 is heparan sulphate having the disaccharide composition:

Disaccharide	Normalised weight percentage
ΔUA,2S-GlcNS,6S	11.08±1.0
ΔUA,2S-GlcNS	4.46±0.4
ΔUA-GlcNS,6S	15.84±1.0
ΔUA,2S-GlcNAc,6S	4.76±0.6
ΔUA-GlcNS	20.27±3.0
ΔUA,2S-GlcNAc	1.01±0.4
ΔUA-GlcNAc,6S	10.63±1.0
ΔUA-GlcNAc	31.95±1.6

following digestion with heparin lyases I, II and III and then subjecting the resulting disaccharide fragments to capillary electrophoresis analysis, and wherein the HS7 is capable of binding SEQ ID NO:1 according to any one of claims 1 to 5.

27. The pharmaceutical composition or medicament of claim 61 wherein the pharmaceutical composition or medicament further comprises Vascular Endothelial Growth Factor (VEGF) protein.

~~8~~3. The pharmaceutical composition or medicament of claim ~~6~~1 or ~~7~~2 for use in a method of medical treatment.

~~4~~9. HS7 ~~according to any one of claims 1 to 5~~ for use in a method of medical treatment, wherein HS7 is heparan sulphate having the disaccharide composition:

Disaccharide	Normalised weight percentage
ΔUA,2S-GlcNS,6S	11.08±1.0
ΔUA,2S-GlcNS	4.46±0.4
ΔUA-GlcNS,6S	15.84±1.0
ΔUA,2S-GlcNAc,6S	4.76±0.6
ΔUA-GlcNS	20.27±3.0
ΔUA,2S-GlcNAc	1.01±0.4
ΔUA-GlcNAc,6S	10.63±1.0
ΔUA-GlcNAc	31.95±1.6

following digestion with heparin lyases I, II and III and then subjecting the resulting disaccharide fragments to capillary electrophoresis analysis, and wherein the HS7 is capable of binding SEQ ID NO:1.

~~5~~10. HS7 ~~according to any one of claims 1 to 5 for use in a method of medical treatment~~for use according to claim 4, wherein the method involves the stimulation or promotion of the growth of blood vessels.

~~6~~11. Use of HS7 ~~according to any one of claims 1 to 5~~ in the manufacture of a medicament for the treatment of vascular disease, cardiovascular disease, heart disease, ischemia, ischemic disease, stroke, ischemic vascular disease, myocardial infarction, or a disease or condition characterised by decreased blood flow to tissues and/or organs due to blocked or partially blocked arteries ~~a disease, condition~~ or injury to tissue, wherein the ~~method~~ treatment involves the stimulation or promotion of the growth of blood vessels, wherein HS7 is heparan sulphate having the disaccharide composition:

Disaccharide	Normalised weight percentage
ΔUA,2S-GlcNS,6S	11.08±1.0
ΔUA,2S-GlcNS	4.46±0.4
ΔUA-GlcNS,6S	15.84±1.0

ΔUA,2S-GlcNAc,6S	4.76±0.6
ΔUA-GlcNS	20.27±3.0
ΔUA,2S-GlcNAc	1.01±0.4
ΔUA-GlcNAc,6S	10.63±1.0
ΔUA-GlcNAc	31.95±1.6

following digestion with heparin lyases I, II and III and then subjecting the resulting disaccharide fragments to capillary electrophoresis analysis, and wherein the HS7 is capable of binding SEQ ID NO:1.

712. The use according to claim 611 wherein the ~~method comprises administering HS7~~medicament is to be administered to tissue at or surrounding a wound or location on the patient's body at which blood vessel growth is required.

813. The use of claim 611 or 712 wherein ~~the method further comprises administering~~ a VEGF protein is to be further administered to the patient.

914. A biocompatible implant or prosthesis comprising a biomaterial and isolated or ~~substantially~~ purified HS7, wherein HS7 is heparan sulphate having the disaccharide composition:

Disaccharide	Normalised weight percentage
ΔUA,2S-GlcNS,6S	11.08±1.0
ΔUA,2S-GlcNS	4.46±0.4
ΔUA-GlcNS,6S	15.84±1.0
ΔUA,2S-GlcNAc,6S	4.76±0.6
ΔUA-GlcNS	20.27±3.0
ΔUA,2S-GlcNAc	1.01±0.4
ΔUA-GlcNAc,6S	10.63±1.0
ΔUA-GlcNAc	31.95±1.6

following digestion with heparin lyases I, II and III and then subjecting the resulting disaccharide fragments to capillary electrophoresis analysis, and wherein the HS7 is capable of binding SEQ ID NO:1 ~~according to any one of claims 1 to 5~~.

10~~15~~. The implant or prosthesis of claim 9~~13~~ wherein the implant or prosthesis is coated with said HS7.

11~~16~~. The implant or prosthesis of claim 9~~13~~ wherein the implant or prosthesis is impregnated with said HS7.

12~~17~~. A method of forming a biocompatible implant or prosthesis, the method comprising the step of coating or impregnating a biomaterial with isolated or ~~substantially~~ purified HS7, wherein HS7 is heparan sulphate having the disaccharide composition:

Disaccharide	Normalised weight percentage
ΔUA,2S-GlcNS,6S	11.08±1.0
ΔUA,2S-GlcNS	4.46±0.4
ΔUA-GlcNS,6S	15.84±1.0
ΔUA,2S-GlcNAc,6S	4.76±0.6
ΔUA-GlcNS	20.27±3.0
ΔUA,2S-GlcNAc	1.01±0.4
ΔUA-GlcNAc,6S	10.63±1.0
ΔUA-GlcNAc	31.95±1.6

following digestion with heparin lyases I, II and III and then subjecting the resulting disaccharide fragments to capillary electrophoresis analysis, and wherein the HS7 is capable of binding SEQ ID NO:1 ~~according to any one of claims 1 to 5~~.

13~~18~~. A method of promoting growth of blood vessels in vitro, the method comprising administering HS7 to vascular cells or to vascular tissue, wherein the vascular cells or vascular tissue are contacted with HS7 *in vitro.*

14. A biological scaffold comprising HS7, wherein HS7 is heparan sulphate having the disaccharide composition:

Disaccharide	Normalised weight percentage
ΔUA,2S-GlcNS,6S	11.08±1.0
ΔUA,2S-GlcNS	4.46±0.4
ΔUA-GlcNS,6S	15.84±1.0

ΔUA,2S-GlcNAc,6S	4.76±0.6
ΔUA-GlcNS	20.27±3.0
ΔUA,2S-GlcNAc	1.01±0.4
ΔUA-GlcNAc,6S	10.63±1.0
ΔUA-GlcNAc	31.95±1.6

following digestion with heparin lyases I, II and III and then subjecting the resulting disaccharide fragments to capillary electrophoresis analysis, and wherein the HS7 is capable of binding SEQ ID NO:1.

15. The biological scaffold of claim 14, wherein the biological scaffold is an extended-release drug delivery device, tissue valve, tissue valve leaflet, drug-eluting stent, vascular graft, wound healing or skin graft, or orthopaedic prosthesis.

16. The biological scaffold of claim 14 or 15, for use in a method of medical treatment.

17. The composition, pharmaceutical composition or medicament of claims 1-3, use according to claims 4-8, implant or prosthesis of claims 9-11, method of claims 12 or 13, or biological scaffold of claims 14-16, wherein the HS7 is obtained by a method comprising:

(i) providing a solid support having polypeptide molecules adhered to the support, wherein the polypeptide comprises a heparin-binding domain having the amino acid sequence of SEQ ID NO:1;

(ii) contacting the polypeptide molecules with a mixture comprising glycosaminoglycans such that polypeptide-glycosaminoglycan complexes are allowed to form;

(iii) partitioning polypeptide-glycosaminoglycan complexes from the remainder of the mixture;

(iv) dissociating glycosaminoglycans from the polypeptide-glycosaminoglycan complexes;

(v) collecting the dissociated glycosaminoglycans.

18. The composition, pharmaceutical composition or medicament, use, implant or prosthesis or method according to claim 17 wherein the mixture comprising glycosaminoglycans is a heparan sulphate preparation obtained from porcine mucosa.