The FDA-approved purified human thrombin is packaged as a solution, but this can only be stored at room temperature for up to 24 hours; long-term storage requires freezing (Lew and Weaver, Biologics: Targets & Therapy 2008:2(4) 593-599). A further disadvantage of using thrombin is that it takes time for the enzyme to convert fibrinogen to fibrin, so there is a delay before blood coagulation is accelerated.

Conventional FTAs also use very high amounts of fibrinogen. Other haemostats rely on the patient's own fibrinogen for promotion of clot formation. A haemostatic matrix, termed "FLOSEAL", consists of a mixture of bovine-derived gelatin matrix, human derived thrombin, and calcium chloride. The thrombin is provided in freeze-dried form, and must be dissolved in calcium chloride solution, then mixed with the gelatin matrix, prior to use. The product must be used within eight hours of preparation. Again the requirement to prepare the mixture shortly before use may be particularly disadvantageous, for example, if the product is required in an emergency.

WO 2008/065388 describes formation of a biogel using an agent that is able to polymerise fibrinogen in the absence of thrombin. The agent comprises several fibrinogen-binding peptides conjugated to a soluble human serum albumin (HSA) carrier using the cross-linking agent succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC). US 2012/0114682 describes use of conjugates of fibrin knob peptides to form fibrin polymers, and their use in wound repair. This document also describes the production of a conjugate comprising the fibrinogen-binding peptide GPRP (SEQ ID NO: 1) attached to polyethylene glycol (PEG). The "knob-PEG" conjugate was made by reacting a maleimide-activated PEG with a C-terminal cysteine of the synthesised knob peptide.

Conjugation methods are often complex, requiring multiple steps, some of which may need to be completed at different locations, and often result in relatively low yield of the desired product. A further disadvantage of conjugated products is that they may be sensitive to sterilising radiation, due to the carrier and/or linker materials used in their synthesis.

There is a need, therefore, to provide haemostats that can rapidly polymerise fibrinogen, can readily be produced without use of immunogenic reagents, are resistant to sterilising radiation, and can be provided in ready-to-use form.

Summary

According to one aspect, the present disclosure refers to a peptide dendrimer that comprises a branched core, and a plurality of fibrinogen-binding peptides separately covalently attached to the branched core, wherein the branched core comprises:

herein to refer to any organic compound with a first functional group that is an amine ($-NH_2$), a second functional group that is a carboxylic acid (-COOH), a third functional group, and a fourth functional group. The third and fourth functional group may be any functional group that is capable of reaction with a carboxy-terminal end of a fibrinogen-binding peptide, or with a functional group of a linker attached to the carboxy-terminal end of a fibrinogen-binding peptide.

Multifunctional amino acids may comprise a central carbon atom (α- or 2-) bearing an amino group, a carboxyl group, and a side chain bearing a further functional group (thereby providing a tri-functional amino acid), or a further two functional groups (thereby providing a tetra-functional amino acid.

The, or each, multi-functional amino acid residue may be a residue of a proteinogenic or non-proteinogenic multi-functional amino acid, or a residue of a natural or unnatural multi-functional amino acid.

Proteinogenic tri-functional amino acids possess a central carbon atom (α- or 2-) bearing an amino group, a carboxyl group, a side chain and an α-hydrogen levo conformation. Examples of suitable tri-functional proteinogenic amino acids include L-lysine, L-arginine, L-aspartic acid, L-glutamic acid, L-asparagine, L-glutamine, and L-cysteine.

Examples of suitable tri-functional non-proteinogenic amino acid residues include D-lysine, beta-Lysine, L-ornithine, D-ornithine, and D-arginine residues.

Thus, examples of suitable tri-functional amino acid residues for use in a peptide dendrimer of the disclosure include lysine, ornithine, arginine, aspartic acid, glutamic acid, asparagine, glutamine, and cysteine residues, such as L-lysine, D-lysine, beta-Lysine, L-ornithine, D-ornithine, L-arginine, D-arginine, L-aspartic acid, D-aspartic acid, L-glutamic acid, D-glutamic acid, L-asparagine, D-asparagine, L-glutamine, D-glutamine, L-cysteine, and D-cysteine residues.

Examples of suitable multi-functional unnatural amino acids suitable for use in a peptide dendrimer of the disclosure include Citrulline, 2,4-diaminoisobutyric acid, 2,2'-diaminopimelic acid, 2,3-diaminopropionic acid, and *cis*-4-amino-L-proline. Multi-functional unnatural amino acids are available from Sigma-Aldrich.

In some examples, the branched core may comprise a homopolymeric multi-functional amino acid sequence, for example a poly-lysine, poly-arginine, or poly-ornithine sequence, such as a branched core comprising from two to ten, or from two to six, consecutive lysine,

arginine, or ornithine residues. In other examples, the branched core may comprise different multi-functional amino acid residues, for example one or more lysine residues, one or more arginine residues, and/or one or more ornithine residues.

In other examples, the branched core may comprise a plurality of multi-functional amino acid residues, and one or more other amino acid residues.

Where the branched core comprises a plurality of multi-functional amino acid residues, adjacent multi-functional amino acid residues may be linked together by amino acid side chain links, by peptide bonds, or some adjacent multi-functional amino acid residues may be linked together by side chain links and others by peptide bonds.

In further examples, the branched core may comprise two or more multi-functional amino acid residues, and at least one fibrinogen-binding peptide is separately attached to each of two or more of the multi-functional amino acid residues, and two or more fibrinogen-binding peptides are separately attached to at least one of the multi-functional amino acid residues of the branched core.

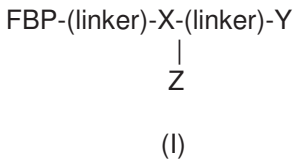
According to other examples, two fibrinogen-binding peptides are separately attached to a terminal multi-functional amino acid residue of the branched core.

Examples of structures of peptide dendrimers of the disclosure include peptide dendrimers in which:

- the branched core comprises a first tri-functional amino acid residue to which two fibrinogen-binding peptides are attached, and a second tri-functional amino acid residue to which one fibrinogen-binding peptide is attached;
- the branched core comprises a first tri-functional amino acid residue to which two fibrinogen-binding peptides are attached, and a second tri-functional amino acid residue to which two fibrinogen-binding peptides are attached;
- the branched core comprises a first tri-functional amino acid residue to which two fibrinogen-binding peptides are attached, a second tri-functional amino acid residue to which one fibrinogen-binding peptide is attached, and a third tri-functional amino acid residue to which one fibrinogen-binding peptide is attached; or
- the branched core comprises a first tri-functional amino acid residue to which two fibrinogen-binding peptides are attached, a second tri-functional amino acid residue to which one fibrinogen-binding peptide is attached, a third tri-functional amino acid

residue to which one fibrinogen-binding peptide is attached, and a fourth tri-functional amino acid residue to which one fibrinogen-binding peptide is attached.

A peptide dendrimer of the disclosure may comprise the following general formula (I):



(I)

where:

FBP is a fibrinogen-binding peptide;

–(linker)- is an optional linker, preferably a non-peptide linker;

X is a tri-functional amino acid residue, preferably lysine, ornithine, or arginine;

Y is –FBP, or -NH_2;

Z is –(linker)-FBP when Y is -FBP, or -[-X_n-(linker)-FBP]$_a$-(linker)-FBP when Y is -NH_2;

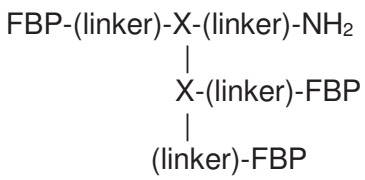
where:

X_n is a tri-functional amino acid residue, preferably lysine, L-ornithine, or arginine; and

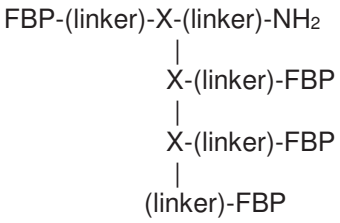
a is 1-10, preferably 1-3.

For example, when Y is NH_2, Z is -[-X_n-(linker)-FBP]$_a$-(linker)-FBP, the structure of the dendrimer is as follows:

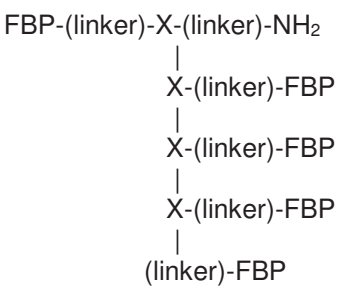
where a is 1:



or, where a is 2:



or, where a is 3:



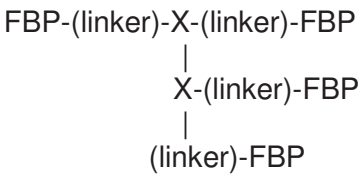
Alternatively, Z is -[-X_n-(linker)-FBP]$_a$-(linker)-FBP when Y is -FBP;

where:

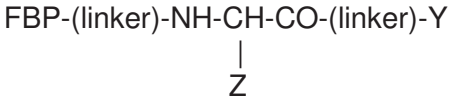
X_n is a tri-functional amino acid residue, preferably lysine, L-ornithine, or arginine; and

a is 1-10, preferably 1-3.

For example, when Y is -FBP, Z is -[-X_n-(linker)-FBP]$_a$-(linker)-FBP and a is 1, the structure of the dendrimer is as follows:



A peptide dendrimer of the disclosure may comprise the following general formula (II):



(II)

where:

FBP is a fibrinogen-binding peptide;

–(linker)- is an optional linker, preferably comprising $–NH(CH_2)_5CO–$;

Y is –FBP, or $-NH_2$;

Z is:

-R-(linker)-FBP, when Y is -FBP, or

-R-COCHNH-(linker)-FBP
|
R-(linker)-FBP, when Y is $-NH_2$; or

-R-COCHNH-(linker)-FBP
|
R-COCHNH-(linker)-FBP
|
R-(linker)-FBP, when Y is $-NH_2$; or

-R-COCHNH-(linker)-FBP
|
R-COCHNH-(linker)-FBP
|
R-COCHNH-(linker)-FBP
|
R-(linker)-FBP, when Y is $-NH_2$;

where R is $-(CH_2)_4NH-$, $-(CH_2)_3NH-$, or $-(CH_2)_3NHCNHNH-$.

Consequently, in one example, Z may be:

$-[-R-COCHNH-(linker)-FBP]_a$
|
R-(linker)-FBP, when Y is $-NH_2$;

where R is $-(CH_2)_4NH-$, $-(CH_2)_3NH-$, or $-(CH_2)_3NHCNHNH-$;

where a is 1-3.

Alternatively, a may be 4-10, or it may be 1-10.

In another example, Z is:

$-[-R-COCHNH-(linker)-FBP]_a$
|
R-(linker)-FBP, when Y is –FBP;

where R is $-(CH_2)_4NH-$, $-(CH_2)_3NH-$, or $-(CH_2)_3NHCNHNH-$;

where a is 1-10, preferably 1-3.

For example, Z is:

-R-COCHNH-(linker)-FBP
|
R-(linker)-FBP, when Y is –FBP and a is 1.

A peptide dendrimer of the disclosure may comprise the following general formula (III):

FBP–(linker)-NH-CH-CO-(linker)-Y
|
Z

(III)

where:

FBP is a fibrinogen-binding peptide;

–(linker)- is an optional linker, preferably comprising $–NH(CH_2)_5CO–$;

Y is –FBP, or $-NH_2$;

Z is:

$-(CH_2)_4NH$-(linker)-FBP, when Y is -FBP; or

$-(CH_2)_4NHCOCHNH$-(linker)-FBP
|
$(CH_2)_4NH$-(linker)-FBP, when Y is $-NH_2$; or

$-(CH_2)_4NHCOCHNH$-(linker)-FBP
|
$(CH_2)_4NHCOCHNH$-(linker)-FBP
|
$(CH_2)_4NH$-(linker)-FBP, when Y is $-NH_2$; or

$-(CH_2)_4NHCOCHNH$-(linker)-FBP
|
$(CH_2)_4NHCOCHNH$-(linker)-FBP
|
$(CH_2)_4NHCOCHNH$-(linker)-FBP
|
$(CH_2)_4NH$-(linker)-FBP, when Y is $-NH_2$.

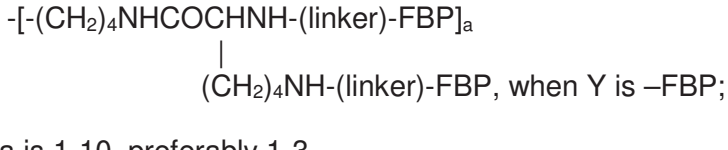
Consequently, in one example, Z may be:

$-[-(CH_2)_4NHCOCHNH$-(linker)-FBP$]_a$
|
$(CH_2)_4NH$-(linker)-FBP, when Y is $-NH_2$;

where a is 1-3.

Alternatively a is 4-10, or it may be 1-10.

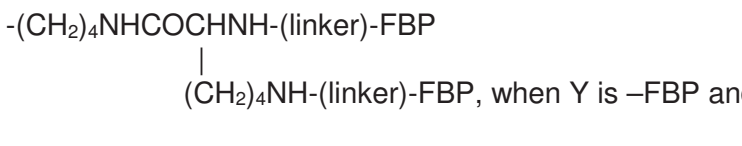
In another example, Z is:



-[-$(CH_2)_4$NHCOCHNH-(linker)-FBP]$_a$
|
$(CH_2)_4$NH-(linker)-FBP, when Y is –FBP;

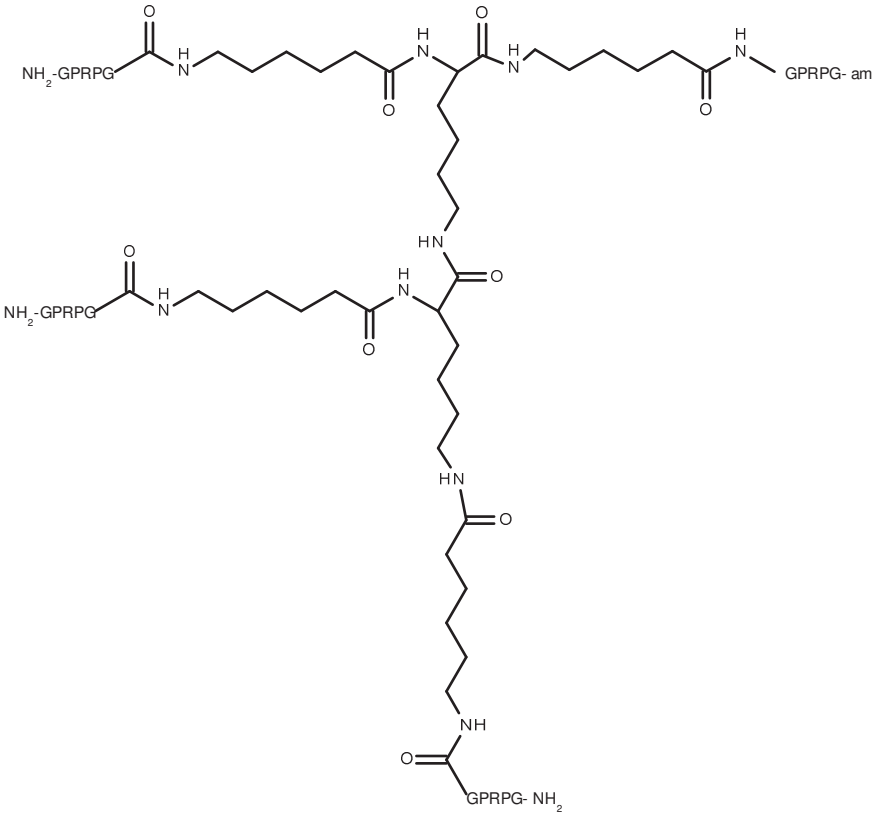
where a is 1-10, preferably 1-3.

For example, Z is:



-$(CH_2)_4$NHCOCHNH-(linker)-FBP
|
$(CH_2)_4$NH-(linker)-FBP, when Y is –FBP and a is 1.

In one example, the peptide dendrimer does not comprise the following structure:



Any suitable fibrinogen-binding peptide (FBP) may be used. For example, the peptide may be capable of binding to a region of fibrinogen that is naturally bound to fibrin or by the platelet membrane glycoproteins GPIIb-IIIa. Fibrin binding to fibrinogen is discussed in Mosesson *et al.* 2001, *Ann. N.Y. Acad. Sci.*, **936**, 11-30. Binding of GPIIb-IIIa to fibrinogen is discussed in Bennett, 2001, *Annals of NY Acad. Sci.*, **936**, 340-354.

The term “peptide” as used herein also incorporates peptide analogues. Several peptide analogues are known to the skilled person. Any suitable analogue may be used provided fibrinogen is able to bind the fibrinogen binding peptide.

Examples of suitable fibrinogen binding peptides and how they may be identified are provided in WO 2005/035002, WO 2007/015107 and WO 2008/065388.

Preferably the fibrinogen-binding peptides are each 3-60, preferably 3-30, more preferably 3-10, amino acid residues in length.

Preferably each fibrinogen binding peptide binds to fibrinogen with a dissociation constant (K_D) of between 10^{-9} to 10^{-6} M, for example around 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 200, 250, 300, 350, 400, or more nM. A K_D of around 100nM is preferred. The dissociation constant can be measured at equilibrium. For example, radio-labelled fibrinogen of known concentration can be incubated with microspheres to which the fibrinogen binding moiety has been cross-linked. Typically 5μM peptide is cross-linked to 1gm microspheres, or 15-40 μmoles of peptide is cross-linked to 1gm of microspheres. The peptide-linked microspheres are diluted to 0.5 mg/ml, and incubated in isotonic buffer at pH 7.4 (for example 0.01M Hepes buffer containing 0.15M NaCl) with radio labelled fibrinogen at concentrations of between 0.05 and 0.5mg/ml for up to 1hr at 20^{O}C. The fibrinogen bound to the fibrinogen binding moiety on the microspheres can be separated from the free fibrinogen by centrifugation and the amount of free and bound fibrinogen measured. The dissociation constant can then be calculated by Scatchard analysis by plotting concentration of bound fibrinogen against the ratio of the concentrations of bound: free fibrinogen, where the slope of the curve represents K_D.

According to some examples, the fibrinogen-binding peptides of peptide dendrimers of the disclosure bind preferentially to hole ‘a’ of fibrinogen over hole ‘b’ of fibrinogen. Examples of sequences of suitable fibrinogen-binding peptides that bind preferentially to hole ‘a’ over hole ‘b’ of fibrinogen include: GPR-; GPRP- (SEQ ID NO: 1); GPRV- (SEQ ID NO: 2); GPRPFPA- (SEQ ID NO: 3); GPRVVAA- (SEQ ID NO: 4); GPRPVVER- (SEQ ID NO: 5); GPRPAA- (SEQ ID NO: 6); GPRPPEC- (SEQ ID NO: 7); GPRPPER- (SEQ ID NO: 8); GPSPAA- (SEQ ID NO: 9).

According to other examples, the fibrinogen-binding peptides of peptide dendrimers of the disclosure bind preferentially to hole ‘b’ of fibrinogen over hole ‘a’ of fibrinogen. Examples of sequences of fibrinogen-binding peptides that bind preferentially to hole ‘b’

over hole ‘a’ of fibrinogen include: GHR-, GHRP- (SEQ ID NO: 10), GHRPY- (SEQ ID NO: 11), GHRPL- (SEQ ID NO: 12), GHRPYamide- (SEQ ID NO: 13).

Each fibrinogen-binding peptide of a peptide dendrimer of the disclosure may, independently, be attached at its carboxy-terminal end (optionally via a linker), or at its amino-terminal end (optionally via a linker) to the branched core of the dendrimer. If the fibrinogen-binding peptide is attached at its amino-terminal end, the carboxy-terminal end of the peptide may comprise an amide group. The presence of an amide group, rather than a carboxyl group (or a negatively charged carboxylate ion), at the exposed carboxy-terminal end of the peptide may help to optimise binding of the fibrinogen-binding peptide to fibrinogen.

In some examples, each fibrinogen-binding peptide is attached (optionally via a linker) at its carboxy-terminal end to the branched core of the dendrimer. In other examples, at least one fibrinogen-binding peptide is attached (optionally via a linker) at its amino-terminal end to the branched core of the dendrimer. For example, at least one fibrinogen-binding peptide that binds preferentially to hole ‘a’ over hole ‘b’ of fibrinogen, such as a peptide comprising sequence APFPRPG (SEQ ID NO: 14), may be attached (optionally via a linker) at its amino-terminal end to the branched core of the dendrimer.

Advantageously, a peptide dendrimer of the disclosure comprises fibrinogen-binding peptides of different sequence (referred to herein as a ‘chimeric’ peptide dendrimer). For example, in some examples a peptide dendrimer of the disclosure comprises fibrinogen-binding peptides that have different selectivity of binding to hole ‘a’ over hole ‘b’ of fibrinogen.

According to an example of the disclosure there is provided an agent that comprises a plurality of carriers, wherein each carrier has a plurality of fibrinogen-binding peptides attached to the carrier, and wherein the fibrinogen-binding peptides attached to the carriers comprise fibrinogen-binding peptides of different sequence.

In some examples of the disclosure, the plurality of carriers comprise a first plurality of carriers, and a second plurality of carriers, wherein the fibrinogen-binding peptides attached to the first plurality of carriers are of different sequence to the fibrinogen-binding peptides attached to the second plurality of carriers.

In other examples of the disclosure, each carrier has fibrinogen-binding peptides of different sequence attached thereto.

The carrier may be a soluble or insoluble carrier, but is preferably not a platelet. The carrier may be suitable for topical administration to a tissue site of a subject, for example a bleeding wound site, or a mucosal site. Soluble carriers may be suitable for intravenous rather than topical administration. The carrier may comprise a soluble or insoluble protein, a therapeutic drug, a polymer (for example a biocompatible polymer, such as polyethylene glycol), or a combination of any of these. Examples of protein carriers are an enzyme or a protein which is not an enzyme, such as human serum albumin.

An insoluble carrier may be a microparticle (including a solid, hollow, or porous microparticle, preferably a substantially spherical microparticle). The microparticle may be formed of any suitable substance, for example cross-linked protein. A suitable protein is albumin (serum-derived or recombinant, human or non-human in sequence) or gelatin. Microparticles suitable for use as insoluble carriers in the present disclosure may be formed by spray drying human serum albumin (HSA) using well known spray-drying technology, for example as in WO 92/18164. Alternatives to use of microparticles as carriers include liposomes, synthetic polymer particles (such as polylactic acid, polyglycolic acid and poly(lactic/glycolic) acid), or cell membrane fragments.

At least a majority of the carriers may have a maximum dimension that is less than 6μm. This may be preferred if the agents of the disclosure are for intravenous administration.

Alternatively, at least a majority of the carriers may have a maximum dimension that is greater than 6μm. This may be preferred if the agents of the disclosure are for topical administration.

In theory there is no upper limit to the number of fibrinogen-binding peptides per carrier molecule. The optimum number is likely to depend on many factors, such as the nature of the carrier, and the number of reactive groups on each carrier for attaching the fibrinogen-binding peptides. However, it is preferred that on average there are up to 100 fibrinogen-binding peptides per carrier molecule. Preferably, on average there are at least three, preferably at least four or five fibrinogen-binding peptides per carrier molecule. A preferred range is 10-20 fibrinogen-binding peptides per carrier molecule.

The carrier may comprise groups which permit attachment of the fibrinogen-binding peptides to the carrier. For example, the carrier may comprise thiol moieties or amine moieties on its surface. If the carrier is proteinaceous, the thiol or amine moieties may be provided by side chains of amino acids, for example cysteine or lysine. Non-peptide groups may be added to the carrier. This is particularly advantageous if the carrier is formed from

protein, such as HSA. For example, thiol groups may be added to the carrier using reagents such as 2-iminothiolane (2-IT) which is able to react with primary amine groups on the carrier.

The fibrinogen-binding peptides of different sequence may comprise a first fibrinogen-binding peptide that binds preferentially to hole ‘a’ over hole ‘b’ of fibrinogen, and a second fibrinogen-binding peptide that binds with higher selectivity to hole ‘a’ over hole ‘b’ of fibrinogen than the first fibrinogen-binding peptide. Such peptide dendrimers have been found to polymerise fibrinogen rapidly over a relatively wide range of peptide dendrimer concentration.

For example, the first fibrinogen-binding peptide may comprise an amino acid sequence GPRP- (SEQ ID NO: 1) at its amino-terminal end, and/or the second fibrinogen-binding peptide may comprise an amino acid sequence -APFPRPG (SEQ ID NO: 14) at its carboxy-terminal end, where the amino acid residues of the sequences are denoted in amino- to carboxy- order, and “-“ denotes the end of the sequence that is attached to the branched core of the peptide dendrimer, or to the carrier. A fibrinogen-binding peptide with the sequence –APFPRPG (SEQ ID NO: 14) at its carboxy-terminal end binds with higher selectivity to hole ‘a’ over hole ‘b’ of fibrinogen than a fibrinogen-binding peptide with the sequence GPRP- (SEQ ID NO: 1) at its amino-terminal end.

In other examples, the fibrinogen-binding peptides of different sequence may comprise a first fibrinogen-binding peptide that binds preferentially to hole ‘a’ over hole ‘b’ of fibrinogen, and a second fibrinogen-binding peptide that binds preferentially to hole ‘b’ over hole ‘a’ of fibrinogen. Such peptide dendrimers have been found to polymerise with fibrinogen to form relatively dense hydrogels compared to equivalent peptide dendrimers containing only fibrinogen-binding peptides that bind preferentially to hole ‘a’ over hole ‘b’ of fibrinogen. It is believed that the increased density of the hydrogels formed is due to binding of fibrinogen-binding peptides of the dendrimers to hole ‘a’ and hole ‘b’ of fibrinogen, thereby strengthening the network of polymerised fibrinogen.

For example, the first fibrinogen-binding peptide may comprise an amino acid sequence GPRP- (SEQ ID NO: 1) at its amino-terminal end and/or the second fibrinogen-binding peptide may comprise an amino acid sequence GHRP- (SEQ ID NO: 10), or an amino acid sequence GHRPY- (SEQ ID NO: 11), at its amino terminal end. Fibrinogen-binding peptides with the sequence GPRP- (SEQ ID NO: 1) at the amino-terminal end bind with some selectivity to hole ‘a’ of fibrinogen. Fibrinogen-binding peptides with the sequence

GHRP- (SEQ ID NO: 10), or GHRPY- (SEQ ID NO: 11), at the amino-terminal end bind preferentially to hole ‘b’ of fibrinogen.

One or more, or each, fibrinogen-binding peptide may be covalently attached to the branched core of a peptide dendrimer of the disclosure by a non-peptide linker. The linker may be any suitable linker that does not interfere with binding of fibrinogen to fibrinogen-binding peptides of the peptide dendrimer. The linker may comprise a flexible, straight-chain linker, suitably a straight-chain alkyl group. Such linkers allow the fibrinogen-binding peptides of the peptide dendrimer to extend away from each other. For example, the linker may comprise a $–NH(CH_2)_nCO–$ group, where n is any number, suitably 1-10, for example 5. A linker comprising a $-NH(CH_2)_5CO-$ group may be formed by use of ε-amino acid 6-aminohexanoic acid (εAhx).

In theory, there is no limit to the total number of fibrinogen-binding peptides that may be present in a peptide dendrimer of the disclosure. However, in practice, for any particular structure, the number of fibrinogen-binding peptides can be varied and tested to determine the optimum number for the desired fibrinogen polymerisation properties, for example, for the speed fibrinogen polymerisation or for the density of the hydrogel produced by polymerisation with fibrinogen. Peptide dendrimers may comprise a total of up to twenty fibrinogen-binding peptides per dendrimer, for example up to ten fibrinogen-binding peptides per dendrimer, or up to five fibrinogen-binding peptides per dendrimer.

The Applicant has found that, surprisingly, mixtures of a peptide dendrimer of the disclosure with a peptide conjugate, comprising two or more fibrinogen-binding peptides, are able to polymerise fibrinogen more rapidly than either the peptide dendrimer, or the peptide conjugate, alone.

According to one aspect, the present disclosure refers to a composition comprising a peptide dendrimer of the disclosure, and a peptide conjugate comprising two or more fibrinogen-binding peptides.

The peptide conjugate may comprise fibrinogen-binding peptides of the same sequence, or of different sequence. For example, the peptide conjugate may comprise only fibrinogen-binding peptides that bind preferentially to hole ‘a’ over hole ‘b’ of fibrinogen, or only fibrinogen-binding peptides that bind preferentially to hole ‘b’ over hole ‘a’ of fibrinogen, or one or more fibrinogen-binding peptides that bind preferentially to hole ‘a’ over hole ‘b’ of

fibrinogen and one or more fibrinogen-binding peptides that bind preferentially to hole ‘b’ over hole ‘a’ of fibrinogen.

The peptide conjugate may comprise a carrier to which the fibrinogen-binding peptides are attached. A suitable carrier may comprise one or more amino acid residues, for example a single amino acid residue, such as a lysine amino acid residue. An advantage of conjugates comprising carriers that comprise one or more amino acid residues is that they can readily be made using solid-phase peptide synthesis methods.

Each fibrinogen-binding peptide of the peptide conjugate may, independently, be attached at its carboxy-terminal end (optionally via a linker), or at its amino-terminal end (optionally via a linker), to the carrier. If the fibrinogen-binding peptide is attached at its amino-terminal end, the carboxy-terminal end of the peptide may comprise an amide group.

In some examples, the peptide conjugate may be a peptide dendrimer of the disclosure.

The fibrinogen-binding peptides of the peptide dendrimer of a composition of the disclosure may bind preferentially to hole ‘a’ of fibrinogen over hole ‘b’ of fibrinogen, and the fibrinogen-binding peptides of the peptide conjugate may bind preferentially to hole ‘b’ of fibrinogen over hole ‘a’ of fibrinogen.

Such compositions have been found to have synergistic effects in that they are able to polymerise fibrinogen more rapidly than either the peptide dendrimer or the peptide conjugate alone. The mechanism of this synergistic effect is not fully understood, but without being bound by theory, it is believed that it may occur because the composition provides more ‘A’ and ‘B’ fibrinogen polymerisation sites.

Alternatively, the fibrinogen-binding peptides of the peptide dendrimer of a composition of the disclosure may bind preferentially to hole ‘b’ of fibrinogen over hole ‘a’ of fibrinogen, and the fibrinogen-binding peptides of the peptide conjugate bind preferentially to hole ‘a’ of fibrinogen over hole ‘b’ of fibrinogen.

According to the disclosure there is also provided a pharmaceutical composition, which comprises a peptide dendrimer of the disclosure, an agent of the disclosure, or a composition of the disclosure, and a pharmaceutically acceptable carrier, excipient, or diluent.

In one aspect, the present disclosure refers to a pharmaceutical composition, which comprises a peptide dendrimer of the disclosure, or a composition of the disclosure, and a pharmaceutically acceptable carrier, excipient, or diluent.

Suitable pharmaceutically acceptable carriers, excipients, and diluents are well-known to the skilled person. Pharmaceutically acceptable carriers, excipients, and diluents include

those suitable for topical administration with a peptide dendrimer, an agent, or a composition, of the disclosure to a wound site. Examples of suitable pharmaceutically acceptable carriers include carriers, preferably in flowable form, such as gelatin, fibrin, chitosan, fibronectin, collagen, starch, hyaluronic acid. Suitable pharmaceutically acceptable diluents or excipients include buffers, such as Tris-HCl, acetate, or phosphate buffers, additives such as detergents or solubilizing agents (for example, Tween 80, Polysorbate 80), anti-oxidants (for example, ascorbic acid, sodium metabisulfite), preservatives (for example, meta-cresol, parabens (methyl, propyl, or butyl), chlorobutanol, phenylmercuric salts (for example, acetate, borate, nitrate), sorbic acid, benzyl alcohol), and bulking substances (for example, lactose, mannitol), tonicity agents (for example, sugars, sodium chloride), polymeric compounds, such as polylactic acid, polyglycolic acid.

A particular advantage of peptide dendrimers, agents, and compositions, of the disclosure is that they can readily be sterilised, for example by exposure to irradiation, suitably gamma irradiation, without significant loss of the ability of the peptide dendrimer, or composition, to polymerise with fibrinogen.

According to the disclosure, there is provided a method of sterilising a peptide dendrimer of the disclosure, an agent of the disclosure, or a composition of the disclosure, which comprises exposing the peptide dendrimer, agent, or composition to gamma irradiation, preferably up to 30 kGy. The peptide dendrimer, agent, or composition may be in dry, wet, or solvent form.

In one aspect, the present disclosure refers to a method of sterilising a peptide dendrimer of the disclosure, or a composition of the disclosure, which comprises exposing the peptide dendrimer, or composition to gamma irradiation, preferably up to 30 kGy

According to the disclosure there is also provided a peptide dendrimer of the disclosure, an agent of the disclosure, or a composition of the disclosure, which is sterile.

Peptide dendrimers, agents, or compositions, of the disclosure may advantageously be provided as a sterile, ready-to-use formulation, in particular, as a sterile, ready-to-use haemostatic or wound treatment formulation.

In some examples, a peptide dendrimer of the disclosure may be formulated into a hydrated flowable gelatin paste and packaged into a syringe that can be irradiated to provide a sterile, ready-to-use, flowable product.

According to the disclosure, there is also provided a method of polymerising fibrinogen, which comprises contacting fibrinogen with a peptide dendrimer of the disclosure, with an agent of the disclosure, or with a composition of the disclosure.

The relative concentration of the dendrimer and the fibrinogen used for polymerisation will depend on the nature of the dendrimer, for example how many fibrinogen-binding peptides are present, and the sequence of the fibrinogen-binding peptides. The Applicant has observed rapid polymerisation times using peptide dendrimers of the disclosure at concentrations ranging from 0.005mg/ml to 2mg/ml with physiological levels of fibrinogen (3mg/ml).

For some peptide dendrimers of the disclosure, as the concentration of the dendrimer is increased, the speed of fibrinogen polymerisation (i.e. the "clotting time") is reduced. Without being bound by theory, this is believed to be due to saturation of the 'a' and/or 'b' holes of the fibrinogen molecules by the fibrinogen-binding peptides of the dendrimer. At these higher dendrimer concentrations, there is an excess of fibrinogen-binding peptides competing for free fibrinogen binding holes (i.e. for empty 'a' and/or 'b' holes), and this competition is believed to reduce the rate at which polymerisation takes place.

There is also provided according to the disclosure a kit for formation of a hydrogel, which comprises a peptide dendrimer of the disclosure, an agent of the disclosure, or a composition of the disclosure, and, separately, fibrinogen.

In one aspect, the present disclosure refers to a kit for formation of a hydrogel, which comprises a peptide dendrimer of the disclosure, or a composition of the disclosure, and, separately, fibrinogen.

There is further provided according to the disclosure a hydrogel comprising a copolymer of a peptide dendrimer of the disclosure, of an agent of the disclosure, or of a composition of the disclosure, and fibrinogen.

In one aspect, the present disclosure refers to a hydrogel comprising a copolymer of a peptide dendrimer disclosure, or of a composition of the disclosure, and fibrinogen.

Peptide dendrimers, agents, and compositions of the disclosure may be used as haemostatic agents, for example to treat bleeding, or to treat a wound.

According to the disclosure there is provided a method of treating bleeding, or of treating a wound, which comprises administering a peptide dendrimer of the disclosure, an agent of the disclosure, or a composition of the disclosure, to a site of bleeding or to a wound.

According to the disclosure, there is also provided the use of a peptide dendrimer as disclosed herein, an agent as disclosed herein or a composition as disclosed herein, in the manufacture of a medicament for the treatment of bleeding or for treating a wound.

In one aspect, the present disclosure refers to use of a peptide dendrimer of the disclosure, or a composition of the disclosure, in the manufacture of a medicament for the treatment of bleeding or for treating a wound.

The peptide dendrimer, agent, or composition, may polymerise endogenous (i.e. host) fibrinogen present at the site of bleeding or the wound. In some examples, exogenous fibrinogen may be administered as well as the peptide dendrimer, the agent, or the composition, of the disclosure to the site of bleeding or to the wound.

The term "fibrinogen" is used herein to include natural fibrinogen, recombinant fibrinogen, or a derivative of fibrinogen that can be converted by thrombin to form fibrin (for example, natural or recombinant fibrin monomer, or a derivative of fibrin monomer that may or may

not be capable of spontaneous assembly). The fibrinogen should be able to bind at least two fibrinogen binding peptides. The fibrinogen may be obtained from any source, and from any species (including bovine fibrinogen), but is preferably human fibrinogen. Human fibrinogen may be obtained from autologous or donor blood. Autologous fibrinogen, or recombinant fibrinogen, is preferred because this reduces the risk of infection when administered to a subject.

A suitable amount of the peptide dendrimer for administration to a human subject will depend, for example, on the type of dendrimer, for example how many fibrinogen-binding peptides are present per dendrimer molecule, and on the type and size of wound or bleeding site. However, a typical amount of the dendrimer is 0.1ml to 50ml, for example 0.1ml to 5ml, or 1 to 50ml, of a preparation (for example, an aqueous preparation) containing the dendrimer at a concentration of 0.005 to 25mg/ml.

A suitable amount of exogenous fibrinogen for administration to a human subject is from 0.1mg to 200mg, for example 3mg to 200mg.

Peptide dendrimers, agents, or compositions of the disclosure may be provided as a fluid for administration directly to a wound, or applied to a sponge or fabric (for example, impregnated or coated), optionally with fibrinogen, prior to application. Alternatively, peptide dendrimers, agents, or compositions of the disclosure may be mixed with a flowable paste for administration with a syringe.

According to the disclosure there is also provided a peptide dendrimer of the disclosure, an agent of the disclosure, or a composition of the disclosure, for use as a medicament.

In one aspect, the present disclosure refers to a peptide dendrimer of the disclosure, or a composition of the disclosure, for use as a medicament.

There is further provided according to the disclosure a peptide dendrimer of the disclosure, an agent of the disclosure, or a composition of the disclosure, for use in the treatment of bleeding or for use in treating a wound.

In one aspect, the present disclosure refers to a peptide dendrimer of the disclosure, or a composition of the disclosure, for use in the treatment of bleeding or for use in treating a wound.

There is also provided according to the disclosure use of a peptide dendrimer of the disclosure, an agent of the disclosure, or a composition of the disclosure, in the manufacture of a medicament for use in the treatment of bleeding or for use in treating a wound.

Peptide dendrimers, agents, and compositions, of the disclosure have several important advantages. In particular, in certain examples, the peptide dendrimers, agents, and compositions, can readily be manufactured using conventional solid-phase peptide synthesis procedures. At optimum concentrations, peptide dendrimers, agents, and

compositions, of the disclosure can polymerise fibrinogen, in the absence of thrombin, in less than a second. Peptide dendrimers and agents of the disclosure can also polymerise fibrinogen in human plasma in less than a second.

The structure of a peptide dendrimer or agent of the disclosure can be selected so as to optimise its properties for the intended use of the dendrimer. For example, a peptide dendrimer comprising five fibrinogen-binding peptides of the same sequence that bind preferentially to the 'a' hole of fibrinogen is able to polymerise fibrinogen almost instantaneously. In contrast, a 'chimeric' peptide dendrimer with one or more fibrinogen-binding peptides that bind preferentially to the 'a' hole of fibrinogen, and one or more different fibrinogen-binding peptides that bind preferentially to the 'b' hole of fibrinogen, may polymerise fibrinogen more slowly, but forms hydrogels of greater density and size.

Peptide dendrimers, agents, and compositions, of the disclosure can be sterilised without loss of fibrinogen polymerisation activity. This is an important advantage because it allows the peptide dendrimers, agents, and compositions to be provided in sterile, ready-to-use, formulations, for example as ready-to-use haemostatic or wound treatment formulations.

Description of drawings

Embodiments of the invention are now described by way of example only, with reference to the accompanying drawings in which:

Figure 1 shows the ability of a peptide dendrimer of a preferred embodiment to polymerise fibrinogen at varying concentrations;

Figure 2 shows the ability of several different peptide dendrimers to polymerise fibrinogen at varying concentrations. The numbering refers to the identity of the peptide dendrimer;

Figure 3 shows the ability of several different peptide dendrimers to polymerise fibrinogen at varying concentrations. The numbering refers to the identity of the peptide dendrimer;

Figure 4 shows the ability of several different peptide dendrimers to polymerise fibrinogen at varying concentrations. The numbering refers to the identity of the peptide dendrimer;

Figure 5 shows a photograph of hydrogels formed by polymerisation of fibrinogen using different peptide dendrimers of the invention;

Figure 6 shows the ability of different combinations of peptide dendrimers of the invention with peptide conjugates to polymerise fibrinogen at varying concentrations; and

Claims

1. A peptide dendrimer that comprises a branched core, and a plurality of fibrinogen-binding peptides separately covalently attached to the branched core, wherein the branched core comprises:

from two to ten multi-functional amino acid residues, wherein each fibrinogen-binding peptide is separately covalently attached to a multi-functional amino acid residue of the branched core;

a plurality of multi-functional amino acid residues, wherein one or more fibrinogen-binding peptides are separately covalently attached to each of at least two adjacent multi-functional amino acid residues of the branched core;

a plurality of multi-functional amino acid residues, wherein two or more fibrinogen-binding peptides are separately covalently attached to at least one of the multi-functional amino acid residues of the branched core;

a plurality of multi-functional amino acid residues, wherein two or more multi-functional amino acid residues are covalently linked through a side chain of an adjacent multi-functional amino acid residue; or

a single multi-functional amino acid residue, and a fibrinogen-binding peptide is separately covalently attached to each functional group of the multi-functional amino acid residue;

wherein the multi-functional amino acid residues comprise tri- or tetra-functional amino acid residues, or tri- and tetra-functional amino acid residues, or the single multi-functional amino acid residue is a tri- or tetra-functional amino acid residue.

2. A peptide dendrimer according to claim 1, wherein the branched core comprises a plurality of consecutive multi-functional amino acid residues and preferably up to ten multi-functional amino acid residues.

3. A peptide dendrimer according to claim 2, wherein the multi-functional amino acid residue, or the plurality of multi-functional amino acid residues, comprise a lysine, ornithine, arginine, aspartic acid, glutamic acid, asparagine, glutamine, or cysteine residue.

4. A peptide dendrimer according to any preceding claim, of the following general formula (I):

FBP-(linker)-X-(linker)-Y
|
Z

(I)

where:

FBP is a fibrinogen-binding peptide;

–(linker)- is an optional linker, preferably a non-peptide linker;

X is a tri-functional amino acid residue, preferably a lysine, ornithine, arginine, aspartic acid, glutamic acid, asparagine, glutamine, or cysteine residue;

Y is –FBP, or -NH_2;

Z is –(linker)-FBP when Y is -FBP, or -[-X_n-(linker)-FBP]$_a$-(linker)-FBP when Y is -NH_2, or -[-X_n-(linker)-FBP]$_a$-(linker)-FBP when Y is -FBP;

where:

X_n is a tri-functional amino acid residue, preferably a lysine, ornithine, arginine, aspartic acid, glutamic acid, asparagine, glutamine, or cysteine residue; and

a is 1-10, preferably 1-3.

5. A peptide dendrimer according to any preceding claim, of the following general formula (II):

FBP-(linker)-NH-CH-CO-(linker)-Y
|
Z

(II)

where:

FBP is a fibrinogen-binding peptide;

–(linker)- is an optional linker, preferably comprising –NH$(CH_2)_5$CO–;

Y is –FBP, or -NH_2;

Z is:

-R-(linker)-FBP, when Y is -FBP, or

-R-COCHNH-(linker)-FBP
|
R-(linker)-FBP, when Y is -NH_2; or

-R-COCHNH-(linker)-FBP
|
R-COCHNH-(linker)-FBP
|
R-(linker)-FBP, when Y is -NH_2; or

-R-COCHNH-(linker)-FBP
|
R-COCHNH-(linker)-FBP
|
R-COCHNH-(linker)-FBP
|
R-(linker)-FBP, when Y is -NH_2; or

-[-R-COCHNH-(linker)-FBP]$_a$
|
R-(linker)-FBP, when Y is –FBP and a is 1-10, preferably 1-3

where R is -$(CH_2)_4NH$-, -$(CH_2)_3NH$-, or -$(CH_2)_3NHCNHNH$.

6. A peptide dendrimer according to any preceding claim, of the following general formula (III):

FBP–(linker)-NH-CH-CO-(linker)-Y
|
Z

(III)

where:

FBP is a fibrinogen-binding peptide;

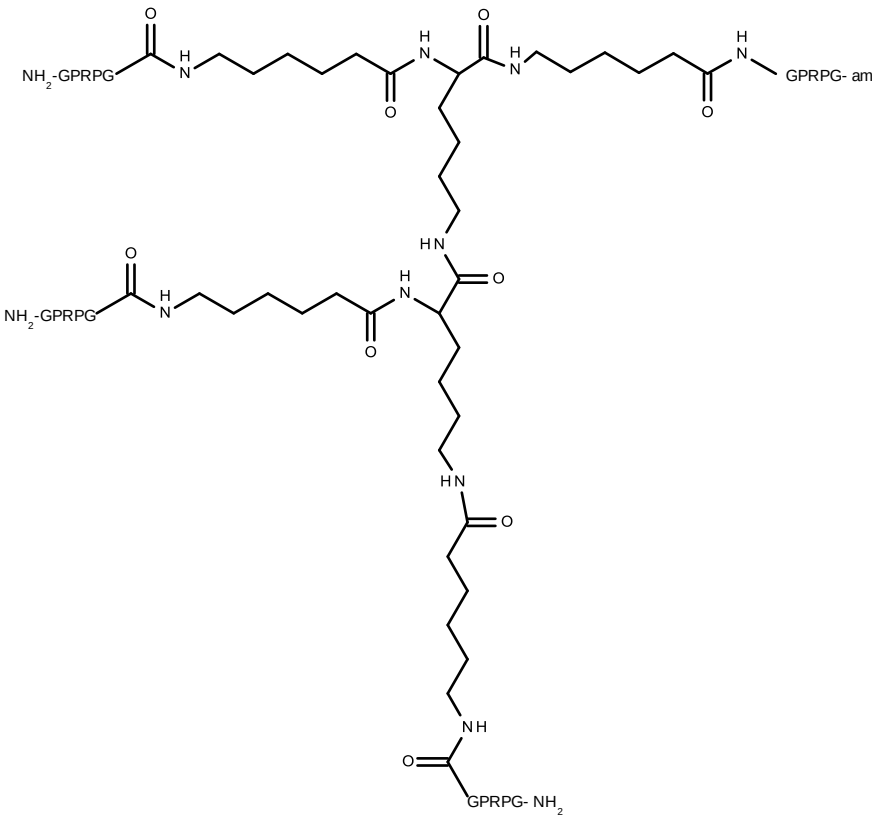
–(linker)- is an optional linker, preferably comprising –$NH(CH_2)_5CO$–;

Y is –FBP, or -NH_2;

Z is:

-$(CH_2)_4NH$-(linker)-FBP, when Y is -FBP; or

$-(CH_2)_4NHCOCHNH\text{-(linker)-FBP}$
$\quad\quad\quad



12. A peptide dendrimer according to any preceding claim comprising fibrinogen-binding peptides of different sequence.

13. A peptide dendrimer according to claim 12, wherein the fibrinogen-binding peptides of different sequence have different selectivity of binding to hole ‘a’ over hole ‘b’ of fibrinogen.

14. A peptide dendrimer according to claim 12 or 13, wherein the fibrinogen-binding peptides of different sequence comprise a first fibrinogen-binding peptide that binds preferentially to hole ‘a’ of fibrinogen over hole ‘b’ of fibrinogen, and a second fibrinogen-binding peptide that binds with higher selectivity of binding to hole ‘a’ of fibrinogen over hole ‘b’ of fibrinogen than the first fibrinogen-binding peptide.

15. A peptide dendrimer according to claim 14, wherein the first fibrinogen-binding peptide comprises an amino acid sequence GPRP- (SEQ ID NO: 1) at its amino-terminal end.

16. A peptide dendrimer according to claim 14 or 15, wherein the second fibrinogen-binding peptide comprises an amino acid sequence -APFPRPG (SEQ ID NO: 14) at its carboxy-terminal end.

17. A peptide dendrimer according to claim 12 or 13, wherein the fibrinogen-binding peptides of different sequence comprise a first fibrinogen-binding peptide that binds preferentially to hole ‘a’ of fibrinogen over hole ‘b’ of fibrinogen, and a second fibrinogen-binding peptide that binds preferentially to hole ‘b’ of fibrinogen over hole ‘a’ of fibrinogen.

18. A peptide dendrimer according to claim 17, wherein the first fibrinogen-binding peptide comprises an amino acid sequence GPRP- (SEQ ID NO: 1) at its amino-terminal end.

19. A peptide dendrimer according to claim 17 or 18, wherein the second fibrinogen-binding peptide comprises an amino acid sequence GHRP- (SEQ ID NO: 10), preferably an amino acid sequence GHRPY- (SEQ ID NO: 11), at its amino terminal end.

20. A composition comprising a peptide dendrimer according to any preceding claim, and a peptide conjugate comprising two or more fibrinogen-binding peptides.

21. A composition according to claim 20, wherein the peptide conjugate is a peptide dendrimer according to any of claims 1 to 19.

22. A composition according to claim 20 or 21, wherein the fibrinogen-binding peptides of the peptide dendrimer bind preferentially to hole ‘a’ of fibrinogen over hole ‘b’ of fibrinogen, and the fibrinogen-binding peptides of the peptide conjugate bind preferentially to hole ‘b’ of fibrinogen over hole ‘a’ of fibrinogen.

23. A composition according to claim 20 or 21, wherein the fibrinogen-binding peptides of the peptide dendrimer bind preferentially to hole ‘b’ of fibrinogen over hole ‘a’ of fibrinogen, and the fibrinogen-binding peptides of the peptide conjugate bind preferentially to hole ‘a’ of fibrinogen over hole ‘b’ of fibrinogen.

24. A pharmaceutical composition, which comprises a peptide dendrimer according to any of claims 1 to 19, or a composition according to any of claims 20 to 23, and a pharmaceutically acceptable carrier, excipient, or diluent.

25. A pharmaceutical composition according to claim 24, which is a ready-to-use haemostatic formulation in which the pharmaceutically acceptable carrier, excipient, or diluent comprises hydrated gelatin.

26. A peptide dendrimer according to any of claims 1 to 19, or a composition according to any of claims 20 to 25, which is sterile.

27. A method of sterilising a peptide dendrimer according to any of claims 1 to 19, or a composition according to any of claims 20 to 25, which comprises exposing the peptide dendrimer or composition to gamma irradiation, preferably up to 30 kGy.

28. A kit for formation of a hydrogel, which comprises a peptide dendrimer according to any of claims 1 to 19, or a composition according to any of claims 20 to 25, and, separately, fibrinogen.

29. A hydrogel comprising a copolymer of a peptide dendrimer according to any of claims 1 to 19, or of a composition according to any of claims 20 to 25, and fibrinogen.

30. Use of a peptide dendrimer according to any of claims 1 to 19, or a composition according to any of claims 20 to 25, in the manufacture of a medicament for the treatment of bleeding or for treating a wound.

31. A use according to claim 30, wherein the medicament is to be administered to the site of bleeding or to the wound.

32. A peptide dendrimer according to any of claims 1 to 19, or a composition according to any of claims 20 to 25, for use as a medicament.

33. A peptide dendrimer according to any of claims 1 to 19, or a composition according to any of claims 20 to 25, for use in the treatment of bleeding or for use in treating a wound.