DESCRIPTION

TISSUE OCCLUDING AGENT

TECHNICAL FIELD

[0001]

The present invention relates to a tissue occluding agent comprising a self-assembling peptide hydrogel.

BACKGROUND ART

[0002]

Tissue occlusion to prevent leakage of body fluids (blood, tissue fluids and the like) caused by tissue damage have major significance in clinical situations, including surgery. Effectively inhibiting body fluid leakage from damage sites is associated with improved life support during surgery and improved post-surgical quality of life (QOL).

[0003]

Hemostasis is considered clinically important for the following reasons.

1. Blood loss is a major cause of death, with causes of blood loss including serious trauma, aneurysm, esophageal and gastric ulcers, and esophageal varix rupture. Probability of death is high when hemorrhage cannot be immediately arrested.

2. Hemorrhage during surgery is a major concern, since hemorrhage can lead to systemic infection or organ dysfunction. Hemorrhage not only interferes with the object of surgery, but removal of hemorrhaged blood is also a delaying factor in surgery.

3. Hemorrhage is also a problem with minimally invasive surgery (laparoscopic surgery and the like), and switching to incisive surgery may be necessary when hemorrhage cannot be sufficiently prevented.

[0004]

The following methods exist for hemostasis.

1. Methods of directly compressing the blood vessels at the site of hemorrhage (astriction). The drawback to this method is that time and effort are required to maintain pressure, while the patient is also at risk of hematoma.

2. Other methods of arresting hemorrhage by physical means, such as methods of clamping or clipping near the site of hemorrhage, or methods of placing a plug or sponge on the site of hemorrhage. The drawback to these hemorrhage arresting methods is difficulty of management when the hemorrhaging is from numerous

microvessels.

3. Methods of clotting the blood by heat and cauterizing the hemorrhaging blood vessel (electrocautery). The drawback of such methods is that the surrounding tissue is subjected to thermal injury and the patient undergoes increased invasion, while the medical instruments used require expert skill (the method cannot be used outside of a medical institution).

[0005]

The following agents exist for hemostasis.

1. Alginic acid
2. Gelatin sponges
3. Collagen fibers
4. Fibrin paste

Collagen fibers and fibrin paste are often clinically used as effective hemorrhage arresting materials, but their drawbacks include the fact that (1) gelatin and collagen fibers are animal collagen and fibrin paste is an animal-derived product obtained using a blood preparation and bovine thrombin, and therefore the risk of infection exists, and (2) they are non-transparent and therefore interfere at the site of surgery.

[0006]

Heparinemia may sometimes be induced, wherein the blood clotting function of the patient is artificially reduced during surgery. Heparin is used to suppress blood clotting during surgery when using an artificial heart-lung machine. An artificial heart-lung machine is foreign to the body, and when blood is circulated through the artificial heart-lung machine the blood immediately coagulates and clogs the circuit, so that administration of heparin into the blood is essential before extracorporeal circulation.

[0007]

Collagen fibers and fibrin paste utilize the blood clotting system of the body for hemostasis, and therefore have a lower hemostatic effect for heparinemia. A lower hemostatic effect tends to lead to greater hemorrhage and thus increased need for blood transfusion, while a longer time is also required for complete hemostasis when extracorporeal circulation is terminated. Thus, a hemorrhage arresting material has been desired that does not have lower performance with heparinemia and that does not utilize blood clotting.

[0008]

Blood vessel suture is necessary not only for cardiac and vascular surgery, but

also for general intraperitoneal surgery. Since a small amount of blood leakage occurs from blood vessel sutures following operation, a hemorrhage arresting material with a persistent suppressing effect is desired.

[0009]

Biliary or pancreatic fistula is a symptom wherein leakage of bile or pancreatic fluid due to biliary system surgery or pancreatitis or pancreatic surgery adversely affects other organs. Currently, no substance is known that effectively inhibits leakage of bile or pancreatic fluid and is clinically applicable, and therefore a method for safely and effectively preventing biliary and pancreatic fistula is desired.

[0010]

Also, leakage of air in the lungs is known as a symptom of spontaneous pneumothorax involving rupture of the alveolar cyst, or traumatic pneumothorax occurring with rib fracture or catheter paracentesis. Depending on the symptoms it may be necessary to wait for natural healing, and a method of simply providing an upper layer on the affected area and adhering it to the lung tissue to occlude the cyst hole is considered a simple and highly safe method for treatment of pneumothorax.

[0011]

Techniques for endoscopic excision of lesions continue to be developed with advances in endoscope technology. In particular, surgical methods are being established for endoscopic excision of lesions of polyps or early-stage cancer in the gastrointestinal tract, including the esophagus, stomach and intestines (superficial cancer believed to be without lymph node metastasis). In endoscopic demucosation, hypertonic saline or the like is usually injected into the submucous layer including the lesion site to swell the lesion site, and the excision site is held while excising the tissue containing the lesion site by electrocautery, for example.

In this technique, a solution such as hypertonic saline is injected into the submucous layer to separate the lesion site from the proper muscle layer, but low-viscosity solutions such as saline cannot maintain lesion site swelling during surgery, and therefore an infusion solution that allows swelling of affected areas to be maintained during the course of surgery is desired.

Methods of suppressing hemorrhage from lesion excision sites by injection of a vasoconstrictor such as thrombin through a catheter are also employed, but no effective measures for complete hemostasis have been established, and therefore a method for rapidly stopping post-excision hemorrhage is also desired.

[0012]

Advances in catheter treatments have led to establishment of surgical methods

for killing tumors or myomas by occlusion of the arteries flowing into lesion sites, that control the blood flow to the tumors and myomas. Specifically, these include hepatic artery occlusion, uterine artery occlusion and cerebral artery occlusion.

In such techniques, collagen extracted from a heterogeneous animal, or a liquid such as ethylene-vinyl alcohol, is infused for occlusion of the artery, but this raises concerns regarding risk of infection and toxicity. The development of an infusion solution with no risk of infection and low toxicity is therefore desired.

An infusion solution that may contain an added anticancer agent or contrast agent is also desired.

[0013]

Self-assembling peptides have a property whereby the peptide molecules form regularly arranged self-assemblies according to their amino acid sequence. In recent years, these have attracted much attention as novel materials because of their physical, chemical and biological properties.

Self-assembling peptides have an alternating structure of electrically charged hydrophilic amino acids and electrically neutral hydrophobic amino acids, and alternating distribution of positive charge and negative charge, whereby they adopt a β-structure at physiological pH and salt concentration.

[0014]

Hydrophilic amino acids that can be used include acidic amino acids such as aspartic acid and glutamic acid, and basic amino acids such as arginine, lysine, histidine and ornithine. As hydrophobic amino acids there may be used alanine, valine, leucine, isoleucine, methionine, phenylalanine, tyrosine, tryptophan, serine, threonine or glycine.

[0015]

The self-assembly of such peptides occurs under the following conditions.

(1) The peptide molecules adopt a β-structure in aqueous solution, wherein the charged hydrophilic amino acids and electrically neutral hydrophobic amino acids are maldistributed on the two sides of the peptide molecules.

(2) The β-structure results in a complementary electrical distribution between adjacent molecules.

(3) The β-structure leads to sufficient hydrophobic bonding between adjacent molecules.

(4) The electrical charge of the amino acid side chains is screened by monovalent inorganic salts.

(5) The molecules are electrostatically neutral near the isoelectric point of the peptide.

[0016]

It is believed that self-assembly occurs by the following mechanism when these conditions are all satisfied.

(1) The alternating distribution of positive charge and negative charge in the peptide molecules causes attraction between the molecules.

(2) Hydrophobic bonds are formed between the neutral amino acid side chains of adjacent molecules.

(3) The positive/negative electrical distribution results in complementary alignment between adjacent molecules, and associative force between the molecules is strengthened.

(4) The molecular aggregates gradually extend, forming nanofibers.

[0017]

The nanofibers are superfine fibers with thicknesses of about 10 nm-20 nm, and it has been reported that they aggregate to form meshwork and exhibit a macroscopically gel-like form.

[0018]

The gel network structure strongly resembles a natural extracellular matrix (ECM) in terms of its fiber size and pore size, and its use as a scaffold for cell culture is being studied.

[0019]

Since the peptide hydrogel is biodegradable and its decomposition product does not adversely affect tissue, while it is also highly bioabsorbable, it is suitable for cellular engraftment and growth.

[0020]

Because self-assembling peptides are chemical synthetic products obtained by solid phase synthesis and do not carry the risk of animal-derived infectious disease, they are even more promising as substitutes for collagen and the like, given concerns in recent years regarding animal viruses and other unknown infectious agents, such as mad cow disease.

[0021]

The application of self-assembling peptides for hemostasis is indicated in Patent document 1, but the video showing hemostasis at a hepatic incision site, provided in an article cited in the examples thereof, shows persistent blood leakage from the end of the incision site, and the reported complete hemostasis was not achieved. It is conjectured that the reason for incomplete hemostasis was insufficient adhesion between the self-assembling peptide gel and the tissue. Thus, further improvement is

necessary to take advantage of the hemostatic effect of self-assembling peptides to a level allowing their clinical application.

[Patent document 1] International Patent Publication No. WO2006-116524

DISCLOSURE OF THE INVENTION

PROBLEMS TO BE SOLVED BY THE INVENTION

[0022]

The objective of the present invention is to provide a self-assembling peptide tissue occluding agent that can effectively occlude sites of tissue damage in large mammals including humans and that does not carry the risk of infection by viruses and the like, as well as a method for use of the same.

MEANS FOR SOLVING THE PROBLEMS

[0023]

The present inventors have completed this invention upon finding that a tissue occluding effect equivalent to or greater than that of existing tissue occluding agents is exhibited when a self-assembling peptide hydrogel utilized as a scaffold for cell culture is applied for tissue occlusion. It has been a noted issue that simply applying aqueous peptide solutions to body fluid leakage sites does not provide a sufficient tissue occluding effect. As a result of diligent research, however, it was found that a sufficient tissue occluding effect and biological safety can be achieved by removing excess body fluid from body fluid leakage sites.

[0024]

Specifically, the invention relates to a tissue occluding agent containing a peptide, wherein the peptide is an amphiphilic peptide having 8-200 amino acid residues with the hydrophilic amino acids and hydrophobic amino acids alternately bonded, and is a self-assembling peptide exhibiting a β-structure in aqueous solution in the presence of physiological pH and/or a cation.

[0025]

In this tissue occluding agent, the peptide is preferably a self-assembling peptide having a repeating sequence which is a sequence comprising arginine, alanine, aspartic acid and alanine, a sequence comprising isoleucine, glutamic acid, isoleucine and lysine, or a sequence comprising lysine, leucine, aspartic acid and leucine, and more preferably it is a self-assembling peptide comprising the amino acid sequence listed as SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3.

[0026]

The tissue occluding agent also preferably comprises small molecular drugs to prevent hemolysis, inflammation and infection.

[0027]

The small molecular drugs are preferably selected from the group consisting of glucose, saccharose, purified saccharose, lactose, maltose, trehalose, dextran, iodine, lysozyme chloride, dimethylisopropylazulene, tretinoin tocoferil, povidone iodine, alprostadil alfadex, anise alcohol, isoamyl salicylate, α,α-dimethylphenylethyl alcohol, bacdanol, helional, sulfazin silver, bucladesine sodium, alprostadil alfadex, gentamycin sulfate, tetracycline hydrochloride, sodium fusidate, mupirocin calcium hydrate and isoamyl benzoate.

[0028]

The invention also relates to agents comprising the aforementioned tissue occluding agent, which are hemostatic agents for hemorrhage of blood that have reduced coagulating power due to addition of anticoagulants, hemostatic agents for hemorrhage wound surfaces on parenchymal organs, hemostatic agents for arterial hemorrhage and phleborrhagia, inhibitors of bile leakage from the gall bladder or bile duct, inhibitors of hemorrhage or air leakage from the lungs, hemostatic agents or transcatheter application during endoscopic demucosation, infusions for mucosal tissue for swelling of excision sites, inhibitors of hemorrhage and body fluid leakage from excision sites in methods of excising mucosal tissue resections that have been swelled by infusion of liquids into the mucosal tissue, or arteriovenous occluding agents in arteriovenous occlusion or varix sclerotherapy agents used in varix sclerotherapy. Anticancer agents and/or contrast agents may also be added to the arteriovenous occluding agent or varix sclerotherapy agent.

EFFECT OF THE INVENTION

[0029]

The self-assembling peptide as the major component in the tissue occluding agent of the invention can also serve as a scaffold for migrating cells in addition to its role as a occluding agent, thus allowing it to have a higher curative effect after surgery instead of simple occlusion. In addition, the tissue occluding agent of the invention has improved adhesion to tissue when excess body fluid has been removed from a body fluid leakage site (for example, when it has been applied to a hemorrhage arrest site where hemorrhage has been stopped), and thus exhibits an adequate tissue occluding effect with biological safety.

[0030]

The self-assembling peptide as the major component of the tissue occluding agent of the invention can be produced by synthesis, and therefore does not carry the risk of viral or other infection compared to conventional tissue-derived biomaterials, and is itself bioabsorbable, eliminating concerns of inflammation.

BRIEF DESCRIPTION OF THE DRAWINGS

[0031]

Fig. 1 shows a comparison between the hemostatic effects of a 1% aqueous peptide solution and 3% aqueous peptide solution in a abdominal aorta injection needle perforation model. (a) Hemorrhage confirmed following aortal puncture, perivascular hemorrhage (arrow tip; 1% aqueous peptide solution-treated group), (b) coating aqueous peptide solution (1% aqueous peptide solution-treated group), (c) blood vessel indiscernible due to blood of resumed hemorrhage following declamp (arrow tip; 1% aqueous peptide solution-treated group), (d) hemorrhage confirmed following aortal puncture, perivascular hemorrhage (arrow tip; 3% aqueous peptide solution-treated group), (e) coating aqueous peptide solution (3% aqueous peptide solution-treated group), (f) after rinsing with physiological saline, completely hemorrhage-arrested blood vessel can be seen at center of photograph (arrow tip; 3% aqueous peptide solution-treated group).

Fig. 2 shows a comparison between the hemostatic effects of a 1% aqueous peptide solution and 3% aqueous peptide solution in a hepatic partial excision model. (a) Hemorrhage confirmed following hepatic excision (1% aqueous peptide solution-treated group), (b) coating aqueous peptide solution (1% aqueous peptide solution-treated group), (c) no hemorrhage observed from cut surface after rinsing with physiological saline (arrow tip; 1% aqueous peptide solution-treated group), (d) hemorrhage confirmed following hepatic excision, (3% aqueous peptide solution-treated group), (e) coating aqueous peptide solution (3% aqueous peptide solution-treated group), (f) hemorrhage observed from cut surface after rinsing with physiological saline (arrow tip; 3% aqueous peptide solution-treated group).

Fig. 3 shows a histopathological comparison of the cut surfaces of a liver with arrested hemorrhage using an aqueous peptide solution and liver from a control group (physiological saline). (a) Intravascular histology (aqueous peptide solution-treated group), (b) intravascular histology (physiological saline-treated group), (c) intravascular histology (aqueous peptide solution-treated group), (d) intravascular histology (aqueous peptide solution-treated group), (e) intravascular histology (aqueous peptide solution-treated group), (f) intravascular histology (aqueous peptide solution-treated

group).

Fig. 4 shows the hemostatic effect of a 3% aqueous peptide solution in a abdominal aorta injection needle perforation model of rabbits with suppressed blood clotting function. (a) Condition before aortal puncture, (b) perivascular hemorrhage observed after aortal puncture (arrow tip), (c) coating aqueous peptide solution, (d) perivascular hemorrhage of (b) not seen after declamp (arrow tip).

Fig. 5 shows the hemostatic effect of a saccharose-containing 3% aqueous peptide solution in a abdominal aorta injection needle perforation model. (a) Condition before aortal puncture, (b) perivascular hemorrhage observed after aortal puncture (arrow tip), (c) coating aqueous peptide solution, (d) perivascular hemorrhage of (b) not seen after declamp (arrow tip).

Fig. 6 shows a comparison of the hemostatic effects and lung leakage occlusion effects of saccharose-containing 3% aqueous peptide solution and physiological saline in a lung leakage model. (a) Hemorrhage confirmed in lung (arrow tip; saccharose-containing 3% aqueous peptide solution-treated group), (b) coating aqueous peptide solution (arrow tip; saccharose-containing 3% aqueous peptide solution-treated group), (c) wound site immersed in physiological saline to confirm air leaks, but no air leakage or hemorrhage observed (arrow tip; saccharose-containing 3% aqueous peptide solution-treated group), (d) hemorrhage confirmed in lung (arrow tip; physiological saline-treated group), (e) coating physiological saline (arrow tip; physiological saline-treated group), (f) no distinct hemorrhage since wound site was immersed in physiological saline to confirm air leaks, but continuous hemorrhage was observed (arrow tip; physiological saline-treated group).

Fig. 7 bile duct wall occlusion effect of a 3% aqueous peptide solution in bile duct wall perforation model. (a) Condition before bile duct puncture, (b) bile leakage confirmed following bile duct puncture (arrow tip), (c) coating aqueous peptide solution (arrow tip), (d) bile leakage of (b) not observed around bile duct after rinsing with physiological saline (arrow tip).

Fig. 8 shows mucosal swelling formation and hemostatic effect of a 3% aqueous peptide solution in an intravesical tumor. (a) Before infusion of aqueous peptide solution, (b) swelling of affected area confirmed after infusion of aqueous peptide solution, (c) no hemorrhage at excision site during tumor excision by electrocautery, (d) no hemorrhage at excision site after tumor excision by electrocautery.

Fig. 9 shows the hemostatic effect of a 2% aqueous peptide solution in a gastric mucosa incision model. (a) hemorrhage confirmed after hepatic incision (arrow tip),

(b) coating 2% aqueous peptide solution (arrow tip), (c) no hemorrhage from incision site after rinsing with physiological saline (arrow tip).

Fig. 10 shows the hemostatic effect of a 1% aqueous peptide solution in a hepatic transection model. (a) Hemorrhage confirmed after hepatic incision (arrow tip), (b) coating 1% IEIK9 aqueous peptide solution (arrow tip), (c) hemorrhage observed from incision site after rinsing with physiological saline (arrow tip), (d) hemorrhage confirmed after hepatic incision (arrow tip), (e) coating 1% IEIK13 aqueous peptide solution (arrow tip), (f) no hemorrhage observed from incision site after rinsing with physiological saline (arrow tip), (g) hemorrhage confirmed after hepatic incision (arrow tip), (h) coating 1% KLD aqueous peptide solution (arrow tip), (i) no hemorrhage observed from incision site after rinsing with physiological saline (arrow tip).

Fig. 11 shows self-assembling of a 1.5% aqueous peptide solution by bile. (a) 1.5% PuraMatrix aqueous peptide solution before self-assembling, (b) 1.5% PuraMatrix aqueous peptide solution after coating bile, (c) bile removed, self-assembly confirmed, (d) physical impact applied to disrupt self-assembled gel, (e) 1% IEIK9 aqueous peptide solution before self-assembly, (f) 1% IEIK9 aqueous peptide solution after coating bile, (g) virtually no self-assembly seen after removal of bile, (h) physical impact applied to disrupt self-assembled gel, (i) 1% IEIK13 aqueous peptide solution before self-assembly, (j) 1% IEIK13 aqueous peptide solution after coating bile, (k) bile removed, self-assembly confirmed, (l) physical impact applied to disrupt self-assembled gel, (m) 1% KLD aqueous peptide solution before self-assembly, (n) 1% KLD aqueous peptide solution after coating bile, (o) bile removed, self-assembly confirmed, (p) physical impact applied to disrupt self-assembled gel.

Fig. 12 shows the portal vein embolization effect with a 3% aqueous peptide solution. (a), (b) Embolization of portal vein confirmed (arrow tip).

Fig. 13 shows self-assembly of an iopamidol-containing aqueous peptide solution.

(a) Iopamidol-containing 3% aqueous peptide solution before self-assembly, (b) iopamidol-containing 3% aqueous peptide solution after coating cell culturing medium, (c) cell culturing medium removed, self-assembly confirmed, (d) iopamidol-containing 0.0468% aqueous peptide solution before self-assembly, (e) iopamidol-containing 0.0468% aqueous peptide solution after coating cell culturing medium, (f) cell culturing medium removed, self-assembly confirmed.

Fig. 14 shows self-assembly of an iopamidol-containing 3% aqueous peptide solution after catheter passage (arrow tip).

BEST MODE FOR CARRYING OUT THE INVENTION

[0032]

The tissue occluding agent of the invention will now be explained in detail.

[0033]

The main component of the tissue occluding agent of the invention is a self-assembling peptide which is an amphiphilic peptide having 8-200 amino acid residues with the hydrophilic amino acids and hydrophobic amino acids alternately bonded, and it exhibits a β-structure in aqueous solution in the presence of physiological pH and/or a cation.

According to the invention, physiological pH is pH 6-8, preferably pH 6.5-7.5 and more preferably pH 7.3-7.5. A "cation" according to the invention is, for example, 5 mM-5 M sodium ion or potassium ion.

[0034]

Self-assembling peptides used for the invention can be represented by the following 4 general formulas, for example.

$((XY)_l - (ZY)_m)_n$ (I)

$((YX)_l - (YZ)_m)_n$ (II)

$((ZY)_l - (XY)_m)_n$ (III)

$((YZ)_l - (YX)_m)_n$ (IV)

(In formulas (I)-(IV), X represents an acidic amino acid, Y represents a hydrophobic amino acid and Z represents a basic amino acid, and l, m and n are all integers ($n \times (l+m) < 200$)).

The N-terminals may be acetylated, and the C-terminals may be amidated.

[0035]

Hydrophilic amino acids that can be used include acidic amino acids such as aspartic acid and glutamic acid, and basic amino acids such as arginine, lysine, histidine and ornithine. As hydrophobic amino acids there may be used alanine, valine, leucine, isoleucine, methionine, phenylalanine, tyrosine, tryptophan, serine, threonine or glycine.

[0036]

Preferred among these self-assembling peptides are self-assembling peptides having the repeating sequence of arginine, alanine, aspartic acid and alanine (RADA), and such peptide sequences are represented by $Ac-(RADA)_p-CONH_2$ ($p = 2-50$). There are also preferred self-assembling peptides having the repeating sequence of isoleucine, glutamic acid, isoleucine and lysine (IEIK), and such peptide sequences are represented by $Ac-(IEIK)_pI-CONH_2$ ($p = 2-50$). There are additionally preferred

self-assembling peptides having the repeating sequence of lysine, leucine, aspartic acid and leucine (KLDL), and such peptide sequences are represented by $Ac-(KLDL)_p-CONH_2$ (p = 2-50). These self-assembling peptides may be composed of 8-200 amino acid residues, with 8-32 residue self-assembling peptides being preferred and self-assembling peptides having 12-16 residues being more preferred.

[0037]

As specific examples of self-assembling peptides according to the invention there may be mentioned peptide RAD16-I having the sequence ($Ac-(RADA)_4-CONH_2$) (SEQ ID NO: 1), peptide IEIK13 having the sequence ($Ac-(IEIK)_3I-CONH_2$) (SEQ ID NO: 2) and peptide KLD having the sequence ($Ac-(KLDL)_3-CONH_2$) (SEQ ID NO: 3), and a 1% aqueous solution of RAD16-I is available as the product PuraMatrix™ by 3D-Matrix Co., Ltd. PuraMatrix™ contains 1% peptide having the sequence ($Ac-(RADA)_4-CONH_2$) (SEQ ID NO: 1), with hydrogen ion and chloride ion.

[0038]

PuraMatrix™, IEIK13 and KLD are oligopeptides of 12-16 amino acid residues and having a length of about 5 nm, and although their solutions are liquid at acidic pH, the peptides undergo self-organization upon change to neutral pH, forming nanofibers with diameters of about 10 nm, causing gelling of the peptide solutions.

[0039]

PuraMatrix™ is an amphiphilic peptide having an amino acid sequence with alternate repeats of positively charged arginine and negatively charged aspartic acid as hydrophilic amino acids, and alanine as a hydrophobic amino acid, IEIK13 is an amphiphilic peptide having an amino acid sequence with alternate repeats of positively charged lysine and negatively charged glutamic acid as hydrophilic amino acids and isoleucine as a hydrophobic amino acid, and KLD is an amphiphilic peptide having an amino acid sequence with alternate repeats of positively charged lysine and negatively charged aspartic acid as hydrophilic amino acids and leucine as a hydrophobic amino acid; the self-assembly of the peptides is due to hydrogen bonding and hydrophobic bonding between the peptide molecules by the amino acids composing the peptides.

[0040]

In the self-assembling peptides used for the invention, the nanofiber diameter is 10-20 nm and the pore size is 5-200 nm, as averages. These numerical value ranges are approximately the same as collagen, which is a natural extracellular matrix.

[0041]

Physiological pH and salt concentration are conditions for self-assembly of the self-assembling peptides of the invention. The presence of a monovalent alkali

metal ion is especially important. That is, sodium ion and potassium ion present in large amounts in the body help promote gelling. Once gelling has occurred, the gel does not decompose even under common protein denaturing conditions such as high temperature or with denaturing agents such as acids, alkalis, proteases, urea, guanidine hydrochloride or the like.

[0042]

These self-assembling peptides, such as PuraMatrix™, are peptide sequences lacking a distinct physiologically active motif, and therefore intrinsic cell function is not impaired. Physiologically active motifs control numerous intracellular phenomena such as transcription, and the presence of physiologically active motifs can lead to phosphorylation of intracytoplasmic or cell surface proteins by enzymes that recognize the motifs. When a physiologically active motif is present in a peptide tissue occluding agent, transcription of proteins with various functions can be activated or suppressed. The self-assembling peptides, such as PuraMatrix™, lack such physiologically active motifs and therefore do not carry this risk.

[0043]

Since the self-assembling peptide used for the invention is produced by chemical synthesis, it does not contain unidentified components derived from the extracellular matrix of another animal. This property therefore eliminates concerns of infection, including BSE, making the peptide highly safe even for medical use.

[0044]

Furthermore, a self-assembling peptide composed of natural amino acids also has satisfactory biocompatibility and biodegradability, and it has been reported that infusion of PuraMatrix™ into murine cardiac muscle, for example, results in infiltration of cells into the PuraMatrix™ and formation of normal tissue. The decomposition time differs depending on the conditions such as the location of infusion, but the fibers decompose and are excreted by about 2 to 8 weeks after infusion.

[0045]

The tissue occluding agent of the invention may further contain added smallmolecular drugs. There are no particular restrictions on such smallmolecular drugs, and there may be mentioned glucose, saccharose, purified saccharose, lactose, maltose, trehalose, dextran, iodine, lysozyme chloride, dimethylisopropylazulene, tretinoin tocoferil, povidone iodine, alprostadil alfadex, anise alcohol, isoamyl salicylate, α,α-dimethylphenylethyl alcohol, bacdanol, helional, sulfazin silver, bucladesine sodium, alprostadil alfadex, gentamycin sulfate, tetracycline hydrochloride, sodium fusidate, mupirocin calcium hydrate and isoamyl benzoate.

[0046]

A sugar may be added to the tissue occluding agent of the invention to improve the osmotic pressure of the solution from hypotonicity to isotonicity without reducing the tissue occluding effect, thereby allowing the biological safety to be increased.

[0047]

The tissue occluding agent of the invention may be in the form of a powder, a solution, a gel, or the like. Since the self-assembling peptide gelates in response to changes in solution pH and salt concentration, it can be distributed as a liquid drug that gelates upon contact with the body during application.

[0048]

Modes of clinical use include cylinder-equipped syringes or pipettes that are prefilled with chemical solution containing components such as self-assembling peptides (prefilled syringes), or methods of supplying a chemical solution to a syringe or pipette chip by means that supplies the components through the opening of the syringe or pipette chip (an aspirator or valve), and applying it to the affected area through the discharge section. A construction with two or more syringes or pipettes is sometimes used.

[0049]

The components may be used as a coating on an instrument such as a stent or catheter, to suppress body fluid leakage.

[0050]

Also, the components may be anchored on a support such as gauze or a bandage, or a lining, that is commonly used in the field. The components may also be soaked into a sponge for use.

[0051]

In addition, an atomizing sprayer filled with a powder or solution of the components may be prepared. When such a spray is used for spraying onto an affected area, the pH and salt concentration increase upon contact with the body causing gelling, and therefore this form can be applied for a greater variety of sites and conditions than a gel form.

[0052]

The tissue occluding agent of the invention will now be explained in greater detail through the following examples, but the invention is not limited thereto so long as its gist and range of application is maintained.

[Example 1]

[0053]

Hemostatic effects of 1% aqueous peptide solution and 3% aqueous peptide solution in rabbit abdominal aorta/portal vein stem injection needle perforation model.

A rabbit abdominal aorta and portal vein stem injection needle perforation model was prepared under vascular clamp, and the hemostatic effects of 1% aqueous peptide solution and 3% aqueous peptide solution were evaluated.

[0054]

<Materials>

· Aqueous peptide solutions

1. 1% Aqueous peptide solution (peptide sequence: Ac-$(RADA)_4$-NH_2, CPC Scientific, Inc; concentration: wt/vol)

2. 3% Aqueous peptide solution (peptide sequence: Ac-$(RADA)_4$-NH_2, CPC Scientific, Inc; concentration: wt/vol)

· Animals

Japanese white rabbits (3.0-4.0 kg, Japan White, conventional, purchased from Funabashi Farm Co., Ltd.). The animals were bred with breeding pellets (JA Higashinihon Kumiai Shiryou) supplied in a breeding room controlled at a room temperature of 25°C, a humidity of 65% and an illumination time of 12 hours (7:00-19:00), and water freely supplied with a water bottle. Fasting was from the morning of the test day, with water freely supplied.

[0055]

<Method>

· The rabbits were subcutaneously administered (3 mg/kg) Celactar 2% injection (2.0 g content as xylazine in 100 mL, product of Bayer Ltd.), and then ketamine (50 mg content as ketamine in 1 mL, product of Fuji Chemical Industries, Ltd.) was intravenously administered (10 mg/kg) for anesthesia.

· The rabbits were laparotomized by median section. Approximately 10 cm of the abdominal aorta and portal vein stem was exposed, each blood vessel was ablated from the surrounding tissue, and the abdominal aorta was punctured with a 23G injection needle (Terumo Corp.) while the portal vein stem was punctured with a 26G injection needle (Terumo Corp.).

· Upon confirming hemorrhage, blood flow at the peripheral end and central end was clampped with a hemostatic clamp, and after removing the hemorrhaged blood with physiological saline and gauze, it was immediately treated with the aqueous peptide solution.

· After 1-2 minutes of treatment, blood flow was reoleased and the presence of hemorrhage from the puncture site was visually examined.

[0056]

<Results>

Fig. 1 shows the hemostatic effect of the aqueous peptide solution on hemorrhage from the blood vessel in this example. As seen in Table 1, two of two rabbits of the abdominal aorta perforation model demonstrated complete hemostasishemostasis with 3% aqueous peptide solution. With 1% aqueous peptide solution, however, both rabbits had projectile hemorrhage following release of the clamp, and therefore hemostasis was not complete.hemostasis In the portal vein stem perforation model, , two of two cases also demonstrated complete hemostasis with 3% aqueous peptide solution, but had persistent phleborrhagia with 1% aqueous peptide solution.

[0057]

[Table 1]

No.	Aqueous peptide solution	Puncture site	Procedure time	Result	Observations
1	3%	Abdominal aorta	1:00	G	Complete hemostasis
2	3%	Abdominal aorta	1:00	G	Complete hemostasis
3	1%	Abdominal aorta	2:00	P	Projectile hemorrhage following release of blockage
4	1%	Abdominal aorta	2:00	P	Projectile hemorrhage following release of blockage
5	3%	Portal vein stem	2:00	G	Complete hemostasis
6	3%	Portal vein stem	1:00	G	Complete hemostasis
7	1%	Portal vein stem	2:00	P	Continuous hemorrhage following release of blockage

[Example 2]

[0058]

Hemostatic effects of 1% aqueous peptide solution and 3% aqueous peptide solution in rabbit partial liver resection model.

A rabbit partial liver resection model was prepared and the hemostatic effects of 1% aqueous peptide solution and 3% aqueous peptide solution were evaluated.

[0059]

<Materials>

· Aqueous peptide solutions

1. 1% Aqueous peptide solution (peptide sequence: Ac-$(RADA)_4$-NH_2, CPC Scientific, Inc.)

2. 3% Aqueous peptide solution (peptide sequence: Ac-$(RADA)_4$-NH_2, CPC Scientific, Inc.)

[0060]

· Animals

Japanese white rabbits (3.0-4.0 kg, Japan White, conventional, purchased from Funabashi Farm Co., Ltd.). The animals were bred with breeding pellets (JA Higashinihon Kumiai Shiryou) supplied in a breeding room controlled to a room temperature of 25°C, a humidity of 65% and an illumination time of 12 hours (7:00-19:00), and water freely supplied with a water bottle. Fasting was from the morning of the test day, with water freely supplied.

[0061]

<Method>

· The rabbits were subcutaneously administered (3 mg/kg) Celactar 2% injection (2.0 g content as xylazine in 100 mL, product of Bayer Ltd.), and then ketamine (50 mg content as ketamine in 1 mL, product of Fuji Chemical Industries, Ltd.) was intravenously administered (10 mg/kg) for anesthesia.

· The rabbits were laparotomized by median section, and the hepatic left or right lobe was sharply excised to a size of approximately 5 cm width × 3 cm length from the edge using a scalpel, to prepare a liver section.

· After confirming projectile hemorrhage from the resected surface, the liver parenchyma including the arteries, portal vein and veins was manually astricted to immediately block blood flow, and the hemorrhaged blood was removed with physiological saline and gauze, after which the prepared resected liver surface was treated with aqueous peptide solution.

· Blood flow was released 1-2 minutes after treatment, the aqueous peptide solution that was gelled was removed by physiological saline, and the presence of any hemorrhage from the resected liver surface s was visually confirmed.

· The resected liver surface in which a hemostatic effect was seen were fixed with 20% formalin and histopathologically evaluated by HEstaining.

[0062]

<Results>

Fig. 2 shows the hemostatic effect of the aqueous peptide solutions on hemorrhage from the resected liver surface in this example. As seen in Table 2, two of two rabbits showed a complete hemostasis with 1% aqueous peptide solution, but a

complete hemostasis was observed in only 1 of 4 rabbits with 3% aqueous peptide solution. In histological observation, blood vessel occlusion was confirmed in the vascular surface due to tight contact of the gelled aqueous peptide solution to the resected liver surface (Fig. 3).

[0063]

[Table 2]

No.	Concentration	Procedure time	Result	Observations
1	3%	2:00	P	Continuous hemorrhage from aorta
2	3%	2:00	G	Complete hemostasis
3	3%	1:00	F	Blood oozing
4	3%	2:00	P	Continuous hemorrhage from aorta
5	1%	2:00	G	Complete hemostasis
6	1%	2:00	G	Complete hemostasis

[Example 3]

[0064]

Hemostatic effect of aqueous peptide solution in abdominal aorta injection needle perforation model of rabbits with suppressed blood clotting function.

The hemostatic effect of an aqueous peptide solution in rabbits administered an anticoagulant (heparin) was evaluated in an abdominal aorta injection needle perforation model.

[0065]

<Materials>

· Aqueous peptide solution

3% Aqueous peptide solution (peptide sequence: Ac-$(RADA)_4$-NH_2, CPC Scientific, Inc.)

· Animals

Japanese white rabbits (3.0-4.0 kg, Japan White, conventional, purchased from Funabashi Farm Co., Ltd.). The animals were bred with breeding pellets (JA Higashinihon Kumiai Shiryou) supplied in a breeding room controlled to a room temperature of 25°C, a humidity of 65% and an illumination time of 12 hours (7:00-19:00), and water freely supplied with a water bottle. Fasting was from the morning of the test day, with water freely supplied.

[0066]

<Method>

· The rabbits were subcutaneously administered (3 mg/kg) Celactar 2% injection (2.0 g content as xylazine in 100 mL, product of Bayer Ltd.), and then ketamine (50 mg

content as ketamine in 1 mL, product of Fuji Chemical Industries, Ltd.) was intravenously administered (10 mg/kg) for anesthesia.

· 1,000 units of heparin (Novo-Heparin for injection, 5000 units, Mochida Pharmaceutical Co., Ltd.) was administered through the inferior vena cava to artificially lower blood clotting function.

· The rabbits were laparotomized by median section. Approximately 10 cm of the abdominal aorta was exposed, the blood vessel was ablated from the surrounding tissue, and the vessel was punctured with a 26G, 25G or 23G injection needle (Terumo Corp.).

· Upon confirming hemorrhage, blood flow at the peripheral end and center end was clamped with a hemostatic clamp, and after removing the hemorrhaged blood with physiological saline and gauze, it was immediately treated with the aqueous peptide solution.

· After 1-2 minutes of treatment, blood flow was releaseed and the presence of hemorrhage was visually examined.

[0067]

<Results>

Fig. 4 shows the hemostatic effect of the aqueous peptide solution on hemorrhage from the abdominal aorta of rabbits with reduced blood clotting function in this example. As seen in Table 3, complete hemostasis was observed with 3% aqueous peptide solution in the 26G, 25G and 23G injection needle perforation models.

[0068]

[Table 3]

No.	Needle size (G)	Procedure time	Result	Observations
1	23G	1:00	G	Complete hemostasis
2	25G	1:00	G	Complete hemostasis
3	25G	1:00	G	Complete hemostasis
4	26G	2:00	G	Complete hemostasis

[Example 4]

[0069]

Hemostatic effect of sugar-containing aqueous peptide solutions in rabbit abdominal aorta/portal vein stem injection needle perforation model.

The hemostatic effects of sugar-containing aqueous peptide solutions were evaluated using an abdominal aorta/portal vein stem injection needle perforation model.

[0070]

<Materials>

· Aqueous peptide solutions

1. Saccharose-containing aqueous peptide solutions (peptide sequence: Ac-$(RADA)_4$-NH_2, CPC Scientific, Inc; concentrations: 2% and 3%, saccharose: Wako Pure Chemical Industries, Ltd., 10% concentration)

2. Glucose-containing 2% aqueous peptide solution (peptide sequence: Ac-$(RADA)_4$-NH_2, CPC Scientific, Inc; glucose: Wako Pure Chemical Industries, Ltd., 5% concentration)

3. Trehalose-containing 2% aqueous peptide solution (peptide sequence: Ac-$(RADA)_4$-NH_2, CPC Scientific, Inc; trehalose: Wako Pure Chemical Industries, Ltd., 5% concentration)

4. 3% Aqueous peptide solution (peptide sequence: Ac-$(RADA)_4$-NH_2, CPC Scientific, Inc.)

5. 2% Aqueous peptide solution (peptide sequence: Ac-$(RADA)_4$-NH_2, CPC Scientific, Inc.)

· Animals

Japanese white rabbits (3.0-4.0 kg, Japan White, conventional, purchased from Funabashi Farm Co., Ltd.). The animals were bred with breeding pellets (JA Higashinihon Kumiai Shiryou) supplied in a breeding room controlled to a room temperature of 25°C, a humidity of 65% and an illumination time of 12 hours (7:00-19:00), and water freely supplied with a water bottle. Fasting was from the morning of the test day, with water freely supplied.

[0071]

<Method>

· The rabbits were subcutaneously administered (3 mg/kg) Celactar 2% injection (2.0 g content as xylazine in 100 mL, product of Bayer Ltd.), and then ketamine (50 mg content as ketamine in 1 mL, product of Fuji Chemical Industries, Ltd.) was intravenously administered (10 mg/kg) for anesthesia.

· The rabbits were laparotomized by median section. Approximately 10 cm of the abdominal aorta and portal vein stem was exposed, each blood vessel was ablated from the surrounding tissue, and the abdominal aorta was punctured with a 26G, 25G or 23G injection needle (Terumo Corp.) while the portal vein stem was punctured with a 26G injection needle (Terumo Corp.).

· Upon confirming hemorrhage, blood flow at the peripheral end and center end was clamped with a hemostatic clamp, and after removing the hemorrhaged blood with physiological saline and gauze, it was immediately treated with the aqueous peptide solution.

· After 1-2 minutes of treatment, blood flow was released and the presence of hemorrhage was visually examined.

[0072]

<Results>

Fig. 5 shows the hemostatic effect of a sugar-containing aqueous peptide solution on hemorrhage from a blood vessel in this example. As seen in Table 4, the hemostatic effect of the saccharose-containing 3% aqueous peptide solution was equivalent to that of 3% aqueous peptide solution in the abdominal aorta 23G, 25G and 26G injection needle perforation models.

[0073]

As seen in Table 5, the hemostatic effects of 2% aqueous peptide solution, saccharose-containing 2% aqueous peptide solution, glucose-containing 2% aqueous peptide solution and trehalose-containing 2% aqueous peptide solution were equivalent in the portal vein stem 26G injection needle perforation model.

[0074]

[Table 4]

No.	Aqueous peptide solution	Puncture site	Needle size (G)	Procedure time	Result	Observation
1	3%	Abdominal aorta	23	1:00	G	Complete hemostasis
2	3%	Abdominal aorta	23	1:00	G	Complete hemostasis
3	3%	Abdominal aorta	25	1:00	G	Complete hemostasis
4	3%	Abdominal aorta	25	1:00	G	Complete hemostasis
5	3%	Abdominal aorta	25	1:00	G	Complete hemostasis
6	3%	Abdominal aorta	26	2:00	G	Complete hemostasis
7	Saccharose-containing 3%	Abdominal aorta	23	2:00	G	Complete hemostasis
8	Saccharose-containing 3%	Abdominal aorta	23	1:00	G	Complete hemostasis
9	Saccharose-containing 3%	Abdominal aorta	23	2:00	G	Complete hemostasis
10	Saccharose-containing	Abdominal aorta	23	2:00	P	Projectile hemorrhage

	3%					
11	Saccharose-containing 3%	Abdominal aorta	25	2:00	G	Complete hemostasis
12	Saccharose-containing 3%	Abdominal aorta	26	1:00	G	Complete hemostasis
13	Saccharose-containing 3%	Abdominal aorta	26	1:00	G	Complete hemostasis
14	Saccharose-containing 3%	Abdominal aorta	26	2:00	G	Complete hemostasis
15	Saccharose-containing 3%	Abdominal aorta	26	2:00	G	Complete hemostasis
16	Saccharose-containing 3%	Abdominal aorta	26	2:00	G	Complete hemostasis

[0075]

[Table 5]

No.	Aqueous peptide solution	Puncture site	Needle size (G)	Procedure time	Result	Observation
1	2%	Portal vein stem	26G	2:00	G	Complete hemostasis
2	2%	Portal vein stem	26G	2:00	G	Complete hemostasis
3	Glucose-containing 2%	Portal vein stem	26G	2:00	G	Complete hemostasis
4	Glucose-containing 2%	Portal vein stem	26G	2:00	G	Complete hemostasis
5	Glucose-containing 2%	Portal vein stem	26G	2:00	P	Continuous hemorrhage
6	Saccharose-containing 2%	Portal vein stem	26G	2:00	G	Complete hemostasis
7	Trehalose-containing 2%	Portal vein stem	26G	2:00	G	Complete hemostasis

[Example 5]

[0076]

Lung leakage blocking effect of saccharose-containing aqueous peptide solution in rabbit lung leakage model

A rabbit lung leakage model was prepared, and the lung leakage blocking effects of a saccharose-containing 3% aqueous peptide solution and physiological saline were compared.

[0077]

<Materials>

· Aqueous peptide solutions

1. Saccharose-containing 3% aqueous peptide solution (peptide sequence: Ac-$(RADA)_4$-NH_2, CPC Scientific, Inc; saccharose: Wako Pure Chemical Industries, Ltd., concentration: 10%)

2. Physiological saline (product of Otsuka Pharmaceutical Factory, Inc.)

· Animals

Japanese white rabbits (3.0-4.0 kg, Japan White, conventional, purchased from Funabashi Farm Co., Ltd.). The animals were bred with breeding pellets (JA Higashinihon Kumiai Shiryou) supplied in a breeding room controlled to a room temperature of 25°C, a humidity of 65% and an illumination time of 12 hours (7:00-19:00), and water freely supplied with a water bottle. Fasting was from the morning of the test day, with water freely supplied.

[0078]

<Method>

· The rabbits were subcutaneously administered (3 mg/kg) Celactar 2% injection (2.0 g content as xylazine in 100 mL, product of Bayer Ltd.), and then ketamine (50 mg content as ketamine in 1 mL, product of Fuji Chemical Industries, Ltd.) was intravenously administered (10 mg/kg) for anesthesia.

· The rabbits were thoracotomized under respiratory assistance with an artificial respirator. The lungs were exposed and bluntly wounded with forceps to create lung leakage with hemorrhage.

· After removing the hemorrhaged blood with physiological saline and gauze, the hemorrhage surface was treated with saccharose-containing 3% aqueous peptide solution and physiological saline.

· Approximately 30 seconds after treatment, the presence of hemorrhage and air leakage from the lung leak in the physiological saline was visually confirmed.

[0079]

<Results>

Fig. 6 shows the lung leakage blocking effect of the saccharose-containing aqueous peptide solution in this example. As seen in Table 6, complete hemostasis and lung leak blockage were confirmed with saccharose-containing 3% aqueous peptide

solution, but persistent hemorrhage and air leakage from the lung leak was observed with physiological saline.

[0080]

[Table 6]

Solution	Procedure time	Result	Remarks
Saccharose-containing 3%	0:30	G	Complete hemostasis. No air leakage from lung leak.
Physiological saline	-	P	Continuous hemorrhage and air leakage from lung leak.

[Example 6]

[0081]

Bile duct wall occlusion effect of aqueous peptide solution in rabbit bile duct injection needle perforation model.

A rabbit injection needle bile duct perforation model was prepared, and the bile duct wall occlusion effect of an aqueous peptide solution was evaluated.

[0082]

<Materials>

· Aqueous peptide solution

3% Aqueous peptide solution (peptide sequence: Ac-$(RADA)_4$-NH_2, CPC Scientific, Inc.)

· Animals

Japanese white rabbits (3.0-4.0 kg, Japan White, conventional, purchased from Funabashi Farm Co., Ltd.). The animals were bred with breeding pellets (JA Higashinihon Kumiai Shiryou) supplied in a breeding room controlled to a room temperature of 25°C, a humidity of 65% and an illumination time of 12 hours (7:00-19:00), and water freely supplied with a water bottle. Fasting was from the morning of the test day, with water freely supplied.

[0083]

<Method>

· The rabbits were subcutaneously administered (3 mg/kg) Celactar 2% injection (2.0 g content as xylazine in 100 mL, product of Bayer Ltd.), and then ketamine (50 mg content as ketamine in 1 mL, product of Fuji Chemical Industries, Ltd.) was intravenously administered (10 mg/kg) for anesthesia.

· The rabbits were laparotomized by median section. Approximately 10 cm of the bile duct was exposed, and after ablation from the surrounding tissue, the bile duct was

punctured with a 26G injection needle.

· After confirming discharge of bile and continuous flow of bile, the bile flow was blocked with a hemostatic clamp.

· The leaked bile was removed with physiological saline and gauze, and then treated with 3% aqueous peptide solution.

· After 2 minutes of treatment, bile flow blockage was released and the presence of bile flow from the puncture site was visually examined.

[0084]

<Results>

Fig. 7 shows the bile duct wall occlusion effect of the aqueous peptide solution in this example. As seen in Table 7, a complete bile duct wall occlusion effect was observed with 3% aqueous peptide solution.

[0085]

[Table 7]

No.	Needle size (G)	Procedure time	Result	Observations
1	26G	2:00	G	Aqueous peptide solution gelled with bile, bile duct puncture site completely blocked

[Example 7]

[0086]

Sustained elevation of excision site and hemostatic effect of aqueous peptide solution with canine intravesical tumorectomy.

Aqueous peptide solutions were submucosally injected into the bladder during canine intravesical tumorectomy, and the sustained elevation and hemostatic effects of the aqueous peptide solutions at the excision site were evaluated.

[0087]

<Materials>

· Aqueous peptide solution

3% Aqueous peptide solution (peptide sequence: Ac-$(RADA)_4$-NH_2, CPC Scientific, Inc.)

· Animals

Dogs (male)

[0088]

<Method>

Canine intravesical tumorectomy was carried out under general anesthesia

management, by the following procedure.

· After hypogastric incision with an electrocautery scalpel (Erbe, Inc.), the electrocautery scalpel was used for incision of the bladder to expose the intravesical tumor (pedunculated tumor with a base site diameter of approximately 0.5 cm that iselevated from the vesical mucosa).

· 3% Aqueous peptide solution was submucosally injected around the tumor base in 4 injections of 0.5 mL each, and elevation of the tumor on the mucosa was confirmed.

· After elevating the tumor, the tumor was excised with an electronic scalpel.

· The time from aqueous peptide solution injection to complete tumor excision was approximately 2 minutes.

[0089]

<Results>

As shown in Fig. 8, the tumors elevated by injection of aqueous peptide solution into the vesical mucosa, and the elevated state was maintained even during excision. No hemorrhage was observed either during or after tumor excision.

[Example 8]

[0090]

Hemostatic effect of 2% aqueous peptide solution in rabbit gastric mucosa incision model

A rabbit gastric mucosa incision hemorrhage model was prepared, and the hemostatic effect of a 2% aqueous peptide solution was evaluated.

[0091]

<Materials>

· Aqueous peptide solution

1.2% Aqueous peptide solution (peptide sequence: Ac-$(RADA)_4$-NH_2, CPC Scientific, Inc.)

[0092]

· Animals

Japanese white rabbits (3.0-4.0 kg, Japan White, conventional, purchased from Funabashi Farm Co., Ltd.). The animals were bred with breeding pellets (JA Higashinihon Kumiai Shiryou) supplied in a breeding room controlled to a room temperature of 25°C, a humidity of 65% and an illumination time of 12 hours (7:00-19:00), and water freely supplied with a water bottle. Fasting was from the morning of the test day, with water freely supplied.

[0093]

<Method>

· The rabbits were subcutaneously administered (3 mg/kg) Celactar 2% injection (2.0 g content as xylazine in 100 mL, product of Bayer Ltd.), and then ketamine (50 mg content as ketamine in 1 mL, product of Fuji Chemical Industries, Ltd.) was intravenously administered (10 mg/kg) for anesthesia.

· The rabbits were laparotomized by median section and the stomachs were incised, after which the gastric mucosa was exposed and sharply incised with a scalpel to about 1 cm to induce hemorrhage.

· After confirming blood oozing from the incision, the blood was removed as much as possible with gauze and the gastric mucosa incision was treated with aqueous peptide solution.

· The gelled aqueous peptide solution was removed 1 minute after treatment using physiological saline, and the presence of any hemorrhage from the gastric mucosa incision was visually confirmed.

[0094]

<Results>

Fig. 9 shows the hemostatic effect of the aqueous peptide solution on hemorrhage from the gastric mucosa incision in this example. No hemorrhage was observed after applying the aqueous peptide solution.

[Example 9]

[0095]

Hemostatic effect of 1% aqueous peptide solution in rabbit hepatic transection model

A rabbit hepatic transection hemorrhage model was prepared, and the hemostatic effect of a 1% aqueous peptide solution was evaluated.

[0096]

<Materials>

· Aqueous peptide solutions

1. 1% Aqueous peptide solution (peptide sequence: IEIK9 (SEQ ID NO: 4), CPC Scientific, Inc)

2. 1% Aqueous peptide solution (peptide sequence: IEIK13, CPC Scientific, Inc)

3. 1% Aqueous peptide solution (peptide sequence: KLD, CPC Scientific, Inc)

[0097]

· Animals

Japanese white rabbits (3.0-4.0 kg, Japan White, conventional, purchased from Funabashi Farm Co., Ltd.). The animals were bred with breeding pellets (JA Higashinihon Kumiai Shiryou) supplied in a breeding room controlled to a room

temperature of 25°C, a humidity of 65% and an illumination time of 12 hours (7:00-19:00), and water freely supplied with a water bottle. Fasting was from the morning of the test day, with water freely supplied.

[0098]

<Method>

· The rabbits were subcutaneously administered (3 mg/kg) Celactar 2% injection (2.0 g content as xylazine in 100 mL, product of Bayer Ltd.), and then ketamine (50 mg content as ketamine in 1 mL, product of Fuji Chemical Industries, Ltd.) was intravenously administered (10 mg/kg) for anesthesia.

· The rabbits were laparotomized by median section and the hepatic left lobes were exposed and sharply transected with a scalpel to about 1 cm to induce hemorrhage.

· After confirming exudative hemorrhage from the incision, the blood was removed as much as possible with gauze and the hepatic incision was treated with aqueous peptide solution.

· The gelled aqueous peptide solution was removed 1 minute after treatment using physiological saline, and the presence of any hemorrhage from the hepatic incision was visually confirmed.

[0099]

<Results>

Fig. 10 shows the hemostatic effect of the aqueous peptide solution on hemorrhage from the hepatic incision in this example. Absolutely no gelling and no hemostatic effect was observed when the IEIK9 aqueous peptide solution was used as the upper layer on the hemorrhage wound surface, but the IEIK13 aqueous peptide solution and KLD aqueous peptide solution gelled after applying, and no hemorrhage was observed.

[Example 10]

[0100]

Self-assembly of aqueous peptide solution with rabbit bile

Self-assembly of aqueous peptide solutions with rabbit bile was evaluated with different aqueous peptide solutions.

[0101]

<Materials>

· Aqueous peptide solutions

1. 1.5% Aqueous peptide solution (peptide sequence: Ac-$(RADA)_4$-NH_2, CPC Scientific, Inc.)

2. 1% Aqueous peptide solution (peptide sequence: IEIK9, CPC Scientific, Inc)

3. 1% Aqueous peptide solution (peptide sequence: IEIK13, CPC Scientific, Inc)

4. 1% Aqueous peptide solution (peptide sequence: KLD, CPC Scientific, Inc)

[0102]

· Animals

Japanese white rabbits (3.0-4.0 kg, Japan White, conventional, purchased from Funabashi Farm Co., Ltd.). The animals were bred with breeding pellets (JA Higashinihon Kumiai Shiryou) supplied in a breeding room controlled to a room temperature of 25°C, a humidity of 65% and an illumination time of 12 hours (7:00-19:00), and water freely supplied with a water bottle. Fasting was from the morning of the test day, with water freely supplied.

[0103]

<Method>

· The rabbits were subcutaneously administered (3 mg/kg) Celactar 2% injection (2.0 g content as xylazine in 100 mL, product of Bayer Ltd.), and then ketamine (50 mg content as ketamine in 1 mL, product of Fuji Chemical Industries, Ltd.) was intravenously administered (10 mg/kg) for anesthesia.

· The rabbits were laparotomized by median section, and bile was sampled from the gallbladder using a 23G injection needle (Terumo Corp.).

· Droplets of each aqueous peptide solution before self-assembly were prepared (diameter: approximately 5-8 mm), and the sampled bile was gently poured thereover so as to cover the aqueous peptide solution.

· After approximately 30 seconds the bile was removed and self-assembly was confirmed, and the self-assembled gel was physically disrupted with a 23G injection needle.

[0104]

<Results>

Fig. 11 shows an example of self-assembly of the aqueous peptide solutions of this example by bile. Bile-induced self-assembly was confirmed in all of the aqueous peptide solutions except for IEIK9.

[Example 11]

[0105]

Confirmation of vascular embolization effect of 3% aqueous peptide solution in rat portal vein embolization

A 3% aqueous peptide solution was injected through the rat portal vein, and the vascular embolization effect was evaluated.

[0106]

<Materials>

· 3% Aqueous peptide solution (peptide sequence: Ac-$(RADA)_4$-NH_2, CPC Scientific, Inc.)

[0107]

· Animals

SD rats (250 g, male, purchased from Japan SLC, Inc.) were raised in a breeding room controlled to temperature/humidity: 22 ±3°C/50 ±20%, ventilation frequency: 10-15 time/hr, illumination time: artificial illumination for 12 hours (8:00-20:00), and were given free access to solid feed CRF-1 (Oriental Yeast Co., Ltd.) using a metal feeder, with tap water made freely available using an automatic water supplier.

[0108]

<Method>

· The rats were subjected to inhalation anesthesia with diethyl ether (Kishida Chemical Co., Ltd.).

· The rats were laparotomized by median section and the portal veins were exposed.

· A 4 mL portion of aqueous peptide solution was injected through the portal vein stem with a 26G injection needle (Terumo Corp.), and hemorrhage from the puncture site was immediately arrested with aqueous peptide solution.

· The livers were extracted 5 minutes after injection and immediately fixed with 10% formalin (Wako Pure Chemical Industries, Ltd.).

· The tissue was stained with haematoxylin eosin (HE) 1 week after fixing.

[0109]

<Results>

As shown in Fig. 12, portal vein embolization with aqueous peptide solution was confirmed.

[Example 12]

[0110]

Confirmation of self-assembly of aqueous peptide solution dissolving iopamidol

Self-assembly of an aqueous peptide solution dissolving iopamidol was evaluated.

<Materials>

[0111]

· Aqueous peptide solution

3% Aqueous peptide solution (peptide sequence: Ac-$(RADA)_4$-NH_2, CPC

Scientific, Inc.)

· Cell culturing medium (Dulbecco's Modified Eagle Medium, Gibco)

· Iopamidol (Wako Pure Chemical Industries, Ltd.)

[0112]

<Method>

· 306.2 mg of iopamidol was dissolved in 1 ml of 3% aqueous peptide solution.

· The iopamidol-containing 3% aqueous peptide solution was diluted with MilliQ water to prepare a 0.0468% aqueous peptide solution. A 300 μl portion of cell culturing medium was added in 6 portions, 50 μl at a time, to 100 μl of the 3% iopamidol-containing aqueous peptide solution and 0.0468% iopamidol-containing aqueous peptide solution, surrounding and contacting the 3% iopamidol-containing aqueous peptide solution and 0.0468% iopamidol-containing aqueous peptide solution. At 15 minutes after addition of the culture medium, the surrounding culture medium was removed and gellation was visually confirmed.

[0113]

<Results>

As shown in Fig. 13, self-assembly was confirmed with the iopamidol-containing aqueous peptide solutions.

[Example 13]

[0114]

Confirmation of self-assembly of iopamidol-containing 3% aqueous peptide solution after microcatheter passage

Self-assembly of a 3% aqueous peptide solution dissolving iopamidol was evaluated.

<Materials>

[0115]

· Aqueous peptide solution

3% Aqueous peptide solution (peptide sequence: Ac-$(RADA)_4$-NH_2, CPC Scientific, Inc.)

· Cell culturing medium (Dulbecco's Modified Eagle Medium, Gibco)

· Iopamidol (Wako Pure Chemical Industries, Ltd.)

· Microcatheter (2.4 Fr, 150/20, *Boston Scientific*)

[0116]

<Method>

· 306.2 mg of iopamidol was dissolved in 1 ml of 3% aqueous peptide solution.

· The iopamidol-containing 3% aqueous peptide solution was discharged into the cell

culturing medium through a catheter.

[0117]

<Results>

As shown in Fig. 14, self-assembly was confirmed with the iopamidol-containing 3% aqueous peptide solution.

CLAIMS

1. A tissue occluding agent comprising a peptide, wherein the peptide is an amphiphilic peptide having 8-200 amino acid residues with the hydrophilic amino acids and hydrophobic amino acids alternately bonded, and is a self-assembling peptide exhibiting a β-structure in aqueous solution at physiological pH and/or in the presence of a cation.

2. The tissue occluding agent according to claim 1, wherein the peptide has a repeating sequence which is a sequence consisting of arginine, alanine, aspartic acid and alanine, a sequence consisting of isoleucine, glutamic acid, isoleucine and lysine, or a sequence consisting of lysine, leucine, aspartic acid and leucine.

3. The tissue occluding agent according to claim 1 or 2, wherein the peptide consists of the amino acid sequence of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3.

4. The tissue occluding agent according to any one of claims 1 to 3, further comprising a small molecule drug.

5. The tissue occluding agent according to claim 4, wherein the smallmolecule drug is selected from the group consisting of glucose, saccharose, purified saccharose, lactose, maltose, trehalose, dextran, iodine, lysozyme chloride, dimethylisopropylazulene, tretinoin tocoferil, povidone iodine, alprostadil alfadex, anise alcohol, isoamyl salicylate, α,α-dimethylphenylethyl alcohol, bacdanol, helional, sulfazin silver, bucladesine sodium, alprostadil alfadex, gentamycin sulfate, tetracycline hydrochloride, sodium fusidate, mupirocin calcium hydrate and isoamyl benzoate.

6. The hemostatic agent for hemorrhage of blood in a condition of reduced clotting function induced by addition of an anticoagulant, comprising the tissue occluding agent according to any one of claims 1 to 5.

7. The hemostatic agent for a hemorrhaging wound surface of a parenchymal organ, comprising the tissue occluding agent according to any one of claims 1 to 5.

8. The hemostatic agent for arterial hemorrhage and phleborrhagia, comprising the tissue occluding agent according to any one of claims 1 to 5.

9. An agent for preventing bile leakage from the gall bladder or bile duct, comprising the tissue occluding agent according to any one of claims 1 to 5.

10. An agent for preventing hemorrhage or air leakage from the lungs, comprising the tissue occluding agent according to any one of claims 1 to 5.

11. A mucosal tissue infusion for elevating an excision site in endoscopic demucosation, the infusion comprising the tissue occluding agent according to any one

of claims 1 to 5.

12. An agent for preventing hemorrhage and body fluid leakage from an excision site in a method of excising a mucosal tissue section that has been elevated by infusion of a liquid into the mucosal tissue, the agent comprising the tissue occluding agent according to any one of claims 1 to 5.

13. A hemostatic agent applicable for transcatheter application for hemostasis from an excision site in endoscopic demucosation, the agent comprising the tissue occluding agent according to any one of claims 1 to 5.

14. An arteriovenous occluding agent for arteriovenous occlusion or a varix sclerotherapy agent for varix sclerotherapy, comprising the tissue occluding agent according to any one of claims 1 to 5.

15. An arteriovenous occluding agent for arteriovenous occlusion or a varix sclerotherapy agent for varix sclerotherapy according to claim 14, further comprising an anticancer agent and/or a contrast agent.

SEQUENCE LISTING

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