AMPHIPHILIC LINEAR PEPTIDE/PEPTOID AND HYDROGEL COMPRISING THE SAME

FIELD OF THE INVENTION

The present invention provides an amphiphilic linear peptide and/or peptoid as well as a hydrogel that includes the amphiphilic linear peptide/peptoid. The amphiphilic linear peptide/peptoid is capable of self-assembling into three-dimensional macromolecular nanofibrous networks, which entrap water, and forming a hydrogel. These peptides/peptoids include short amphiphilic sequences with a hydrophobic portion of aliphatic amino acids and at least one acidic, neutral, or basic polar amino acid. The amphiphilic linear peptide/peptoid is build up of non repetitive aliphatic amino acids, which may be in the L- or D-form. A plurality of such peptides/peptoids assembles to supramolecular helical fibers and forms peptide hydrogels after assembly. A corresponding hydrogel is formed in aqueous solutions at physiological pH and is thus useful for inter alia cell culture, tissue engineering, tissue regeneration, wound healing and release of bioactive moieties (including cells, nucleic acids, antimicrobials, micro-/nanoparticles, cosmetic agents and small molecule therapeutics), as well as for providing mechanical support for damaged or missing tissues. Such hydrogels can also be formed *in situ*, wherein the gelation process occurs within the body following the injection of a peptide solution. Such hydrogels, which are rigid, biocompatible and entrap up to 99.9% of water, are also well suited for applications utilizing electronic devices.

BACKGROUND OF THE INVENTION

Supramolecular structures are held together by intermolecular bondings that are responsible for the organization of polymolecular systems. The non-covalent, intermolecular forces which are required for the assembly of the defined supramolecular structures are mainly electrostatic interactions, hydrogen bondings, van der Waals forces, etc. Supramolecular chemistry or biology gathers a vast body of two or three dimensional complex structures and entities formed by association of chemical or biological species. These associations are governed by the principles of molecular complementarity or molecular recognition and self-assembly. The knowledge of the rules of intermolecular association can be used to design polymolecular assemblies in form of membranes, films, layers, micelles, tubules, gels for a variety of biomedical or technological applications (J.-M. Lehn, Science, 295, 2400-2403, 2002).

Peptides have been used for the fabrication of supramolecular structures through molecular self-assembly (S. Zhang, Nature Biotechnology, 21, 1171-1178, 2003). Peptides are for instance able to assemble into nanotubes (US 7,179,784) or into supramolecular hydrogels consisting of three dimensional scaffolds with a large amount of around 98-99% immobilized water or aqueous solution. The peptide-based biomaterials are powerful tools for potential applications in biotechnology, medicine and even technical applications. Depending on the individual properties these peptide-based hydrogels are thought to serve in the development of new materials for tissue engineering, regenerative medicine, as drug and vaccine delivery vehicles or as peptide chips for pharmaceutical research and diagnosis (E. Place et al., Nature Materials, 8, 457-470, 2009). There is also a strong interest to use peptide-based self-assembled biomaterial such as gels for the development of molecular electronic devices (A. R. Hirst et al. Angew. Chem. Int. Ed., 47, 8002-8018, 2008)

A variety of "smart peptide hydrogels" have been generated that react on external manipulations such as temperature, pH, mechanical influences or other stimuli with a dynamic behavior of swelling, shrinking or decomposing. Nevertheless, these biomaterials are still not "advanced" enough to mimic the biological variability of natural tissues as for example the extracellular matrix (ECM) or cartilage tissue or others. The challenge for a meaningful use of peptide hydrogels is to mimic the replacing natural tissues not only as "space filler" or mechanical scaffold, but to understand and cope with the biochemical signals and physiological requirements that keep the containing cells in the right place and under "*in vivo*" conditions (R. Fairman and K. Akerfeldt, Current Opinion in Structural Biology, 15, 453-463, 2005).

Much effort has been undertaken to understand and control the relationship between peptide sequence and structure for a rational design of suitable hydrogels. In general hydrogels contain macroscopic structures such as fibers that entangle and form meshes. Most of the peptide-based hydrogels utilize as their building blocks β-pleated sheets which assemble to fibers. Later it was shown that it is possible to design hydrogelating self-assembling fibers purely from α-helices. Besides β-sheet structure-based materials (S. Zhang et al., PNAS, 90, 3334-3338, 1993: A. Aggeli et al., Nature, 386, 259-262, 1997, etc.) a variety of α-helical hydrogels has been developed (W. A. Petka et al., Science, 281, 389-392, 1998; C. Wang et al., Nature, 397, 417-420, 1999; C. Gribbon et al., Biochemistry, 47, 10365-10371, 2008; E. Banwell et al., Nature Materials, 8, 596-600, 2009, etc.).

Nevertheless, the currently known peptide hydrogels are in most of the cases associated with low rigidity, sometimes unfavourable physiological properties and/or complexity and the

requirement of substantial processing thereof which leads to high production costs. There is therefore a widely recognized need for peptide hydrogels that are easily formed, non-toxic and have a sufficiently high rigidity for standard applications. The hydrogels should also be suitable for the delivery of bioactive moieties (such as nucleic acids, small molecule therapeutics, cosmetic and anti-microbial agents) and/or for use as biomimetic scaffolds that support the *in vivo* and *in vitro* growth of cells and facilitate the regeneration of native tissue. Stimuli-responsive gelation of the peptides would also be desirable, as they could then be applied as a minimally invasive injectable therapy or implanted as a biological construct to replace damaged/missing tissue.

SUMMARY OF THE INVENTION

It is therefore desirable to provide a biocompatible compound that is capable of forming a hydrogel that meets at least some of the above requirements to a higher extent than currently available hydrogels and that is not restricted by the above mentioned limitations.

The objects of the present invention are solved by an amphiphilic peptide and/or peptoid capable of self-assembling into three-dimensional macromolecular nanofibrous networks, which entrap water and form a hydrogel, the amphiphilic peptide and/or peptoid comprising an amphiphilic sequence consisting of:

a hydrophobic sequence stretch of n aliphatic amino acids, wherein *n* is an integer from 2 to 15, and

a hydrophilic sequence stretch linked to said hydrophobic sequence stretch and having a polar moiety which is acidic, neutral or basic, said polar moiety comprising *m* adjacent hydrophilic amino acids, wherein *m* is an integer from 1 to 5.

In one embodiment, the amphiphilic peptide and/or peptoid has a C-terminus and an N-terminus, wherein both said C-terminus and said N-terminus do not carry any protecting groups attached to them.

In one embodiment, the amphiphilic peptide and/or peptoid has a C-terminus and an N-terminus, wherein the N-terminus is protected by an N-terminal protecting group.

In one embodiment, the amphiphilic peptide and/or peptoid has a C-terminus and an N-terminus, wherein the C-terminus is protected by a C-terminal protecting group.

In one embodiment, said N-terminal protecting group has the general formula –C(O)–R, wherein R is selected from the group consisting of H, unsubstituted or substituted alkyls, and unsubstituted or substituted aryls.

Preferred alkyls in accordance with the present invention are methyl, ethyl, propyl, isopropyl, butyl and isobutyl.

In one embodiment, said N-terminal protecting group is an acetyl group.

In one embodiment, said N-terminal protecting group is a peptidomimetic molecule, including natural and synthetic amino acid derivatives, wherein the N-terminus of said peptidomimetic molecule may be modified with a functional group selected from the group consisting of carboxylic acid, amide, alcohol, aldehyde, amine, imine, nitrile, an urea analog, thiol, phosphate, carbonate, sulfate, nitrate, maleimide, vinyl sulfone, azide, alkyne, alkene, carbohydrate, imide, peroxide, ester, thioester, aryl, ketone, sulphite, nitrite, phosphonate and silane.

In one embodiment, said C-terminal protecting group is an amide group.

In one embodiment, the C-terminus of said amphiphilic peptide and/or peptoid has the formula -CONHR or -CONRR', with R and R' being selected from the group consisting of H, unsubstituted or substituted alkyls, and unsubstituted or substituted aryls.

In one embodiment, said C-terminal protecting group is an ester group.

In one embodiment, the C-terminus of said amphiphilic peptide and/or peptoid has the formula $–CO_2R$, with R being selected from the group consisting of H, unsubstituted or substituted alkyls, and unsubstituted or substituted aryls.

In one embodiment, said C-terminal protecting group is a peptidomimetic molecule, including natural and synthetic amino acid derivatives, wherein the C-terminus of said peptidomimetic molecule may be modified with a functional group selected from the group consisting of carboxylic acid, amide, alcohol, aldehyde, amine, imine, nitrile, an urea analog, thiol, phosphate, carbonate, sulfate, nitrate, maleimide, vinyl sulfone, azide, alkyne, alkene, carbohydrate, imide, peroxide, ester, thioester, aryl, ketone, sulphite, nitrite, phosphonate and silane.

In one embodiment, the amphiphilic peptide and/or peptoid has an N-terminal protecting group, which is an acetyl group, and a C-terminal protecting group, which is an amide group.

In one embodiment, n is an integer from 2 to 6, preferably 2 to 5.

In one embodiment, m is an integer from 1 to 2, preferably 1.

In one embodiment, the amphiphilic peptide and/or peptoid consists of o amphiphilic sequences, as defined above, which amphiphilic sequences are linked to each other, o being an integer from 1 to 50.

In one embodiment, for a given amphiphilic peptide and/or peptoid, said aliphatic amino acids and said hydrophilic amino acids are either D-amino acids or L-amino acids.

In one embodiment, each of the hydrophilic amino acids has a polar group which is independently selected from a hydroxyl, an ether, a carboxyl, an imido, an amido, an ester, an amino, a guanidino, a thio, a thioether, a seleno, and a telluro group.

In one embodiment, said polar moiety of said hydrophilic sequence stretch comprises m adjacent hydrophilic amino acids, m being as defined above, said hydrophilic amino acids being selected from the group comprising aspartic acid, asparagine, glutamic acid, glutamine, 5-N-

ethyl-glutamine (theanine), citrulline, thio-citrulline, cysteine, homocysteine, methionine, ethionine, selenomethionine, telluromethionine, threonine, allo-threonine, serine, homoserine, arginine, homoarginine, ornithine (Orn), 2,4-diaminobutyric acid (Dab or Dbu), 2,3-diaminopropionic acid (Dap or Dpr), lysine and N(6)-carboxy-methyllysine, histidine, and wherein said hydrophobic sequence stretch comprises n aliphatic amino acids, n being as above, said aliphatic amino acids being selected from the group comprising isoleucine, nor-leucine, leucine, valine, alanine, glycine, homoallylglycine and homopropargylglycine.

In one embodiment, m is 1 or 2.

In one embodiment, m is 2 and said polar moiety comprises two identical amino acids, or m is 1 and said polar moiety comprises any one of aspartic acid, asparagine, glutamic acid, glutamine, serine, threonine, cysteine, methionine, lysine, ornithine (Orn), 2,4-diaminobutyric acid (Dab or Dbu), 2,3-diaminopropionic acid (Dap or Dpr) and histidine.

In one embodiment, said polar moiety is adjacent to the hydrophobic sequence stretch of n aliphatic amino acids.

In one embodiment, said polar moiety has a sequence selected from Asp, Asn, Glu, Gln, Ser, Thr, Cys, Met, Lys, Orn, Dab, Dap, His, Asn-Asn, Asp-Asp, Glu-Glu, Gln-Gln, Asn-Gln, Gln-Asn, Asp-Gln, Gln-Asp, Asn-Glu, Glu-Asn, Asp-Glu, Glu-Asp, Gln-Glu, Glu-Gln, Asp-Asn, Asn-Asp Thr-Thr, Ser-Ser, Thr-Ser, Ser-Thr, Asp-Ser, Ser-Asp, Ser-Asn, Asn-Ser, Gln-Ser, Ser-Gln, Glu-Ser, Ser-Glu, Asp-Thr, Thr-Asp, Thr-Asn, Asn-Thr, Gln-Thr, Thr-Gln, Glu-Thr, Thr-Glu, Cys-Asp, Cys-Lys, Cys-Ser, Cys-Thr, Cys-Orn, Cys-Dab, Cys-Dap, Lys-Lys, Lys-Ser, Lys-Thr, Lys-Orn, Lys-Dab, Lys-Dap, Ser-Lys, Ser-Orn, Ser-Dab, Ser-Dap, Orn-Lys, Orn-Orn, Orn-Ser, Orn-Thr, Orn-Dab, Orn-Dap, Dab-Lys, Dab-Ser, Dab-Thr, Dab-Orn, Dab-Dab, Dab-Dap, Dap-Lys, Dap-Ser, Dap-Thr, Dap-Orn, Dap-Dab, Dap-Dap.

In one embodiment, said polar moiety comprises the C-terminus of the amphiphilic peptide and/or peptoid, or said polar moiety comprises the N-terminus of the amphiphilic peptide and/or peptoid.

In one embodiment, said polar moiety comprises the C-terminus of the amphiphilic peptide and/or peptoid.

In one embodiment, said polar moiety consists of at least one amino acid positioned at the C-terminus of the amphiphilic peptide and/or peptoid.

In one embodiment, said hydrophobic sequence stretch comprises and/or forms the N-terminus of the amphiphilic peptide and/or peptoid.

In one embodiment, all or a portion of the aliphatic amino acids of the hydrophobic sequence stretch are arranged in an order of decreasing amino acid size in the direction from N- to C-terminus of the amphiphilic peptide and/or peptoid, wherein the size of the aliphatic amino acids is defined as I = L > V > A > G.

In one embodiment, said aliphatic amino acids arranged in an order of decreasing amino acid size have a sequence which is a repetitive or non-repetitive sequence.

In one embodiment, said aliphatic amino acids arranged in order of decreasing amino acid size have a sequence with a length of 2 to 7, preferably 2 to 6, more preferably 2 to 5 amino acids.

In one embodiment, said aliphatic amino acids arranged in an order of decreasing amino acid size have a sequence selected from LIVAG, ILVAG, LIVAA, LAVAG, IVAG, LIVA, LIVG, IVA and IV, wherein, optionally, there is an A preceding such sequence at the N-terminus.

In one embodiment, all or a portion of the aliphatic amino acids of the hydrophobic sequence stretch are arranged in an order of identical amino acid size in the amphiphilic peptide and/or peptoid.

In one embodiment, said aliphatic amino acids arranged in order of identical amino acid size have a sequence with a length of 2 to 4 amino acids.

In one embodiment, said aliphatic amino acids arranged in an order of identical size have a sequence selected from LLLL, LLL, LL, IIII, III, II, VVVV, VVV, VV, AAAA, AAA, AA, GGGG, GGG, and GG.

In one embodiment, the amphiphilic sequence undergoes a conformational change during self-assembly, preferably a conformational change from a random coil conformation to a helical intermediate structure to a final beta conformation.

In one embodiment, the conformational change is dependent on the concentration of the amphiphilic peptide and/or peptoid, dependent on the ionic environment (e.g. the salt concentration), pH dependent and/or temperature dependent. In one embodiment, the conformational change is facilitated or triggered by changes in pH, ionic environment and/or temperature.

In one embodiment, the amphiphilic linear sequence comprises a single hydrophilic and at least two aliphatic amino acids.

In one embodiment, the amphiphilic sequence is one of SEQ ID NO: 1 to 86.

In one embodiment, the amphiphilic peptide and/or peptoid is stable in aqueous solution at physiological conditions at ambient temperature for a period of time in the range from 1 day to at least 6 months, preferably to at least 8 months more preferably to at least 12 months.

In one embodiment, the amphiphilic peptide and/or peptoid is stable in aqueous solution at physiological conditions, at a temperature up to 90 °C, for at least 1 hour.

In one embodiment, the amphiphilic peptide and/or peptoid has the general formula:

$$Z_p\text{-}(X)_n\text{-}(Y)_m\text{-}Z'_q,$$

wherein

Z is an N-terminal protecting group,

X is, at each occurrence, independently selected from an aliphatic amino acid,

Y is, at each occurrence, independently selected from a hydrophilic amino acid,

Z' is a C-terminal protecting group,

n is an integer selected from 2 to 6, preferably 2 to 5,

m is selected from 1 and 2, wherein, preferably, m is 1,
and p and q are independently selected from 0 and 1, wherein, preferably, p is 1.

Preferably, said aliphatic amino acid is selected from the group comprising isoleucine, norleucine, leucine, valine, alanine, glycine, homoallylglycine and homopropargylglycine. Preferably, said hydrophilic amino acid is selected from the group comprising aspartic acid, asparagine, glutamic acid, glutamine, 5-N-ethyl-glutamine (theanine), citrulline, thio-citrulline, cysteine, homocysteine, methionine, ethionine, selenomethionine, telluromethionine, threonine, allo-threonine, serine, homoserine, arginine, homoarginine, ornithine (Orn), 2,4-diaminobutyric acid (Dab or Dbu), 2,3-diaminopropionic acid (Dap or Dpr), lysine and N(6)-carboxy-methyllysine, histidine.

In one embodiment, the C-terminal amino acid of the amphiphilic peptide and/or peptoid is a neutral or basic hydrophilic (polar) amino acid. In one embodiment, in the above general formula, m is 1, and Y is selected from neutral or basic hydrophilic amino acids. Preferred neutral hydrophilic amino acids include serine and threonine. Preferred basic hydrophilic amino acids include lysine (K), ornithine (Orn), 2,4-diaminobutyric acid (Dab or Dbu) and 2,3-diaminopropionic acid (Dap or Dpr).

In one embodiment, the C-terminal amino acid of the amphiphilic peptide and/or peptoid is selected from the group consisting of a lysine (K), an ornithine (Orn), a 2,4-diaminobutyric acid (Dab or Dbu) and a 2,3-diaminopropionic acid (Dap or Dpr). In one embodiment, in the above general formula, m is 1, and Y is selected from the group consisting of a lysine (K), an ornithine (Orn), a 2,4-diaminobutyric acid (Dab or Dbu) and a 2,3-diaminopropionic acid (Dap or Dpr).

In one embodiment, the amphiphilic peptide and/or peptoid has a sequence selected from the group comprising LIVAGK (SEQ ID NO: 19 or 39), LIVAG(Orn) (SEQ ID NO: 43 or 44), LIVAG(Dab) (SEQ ID NO: 45 or 46), LIVAG(Dap) (SEQ ID NO: 47 or 48), ILVAGK (SEQ ID NO: 49 or 50), ILVAG(Orn) (SEQ ID NO: 51 or 52), ILVAG(Dab) (SEQ ID NO: 53 or 54), ILVAG(Dap) (SEQ ID NO: 55 or 56), AIVAGK (SEQ ID NO: 57 or 58), AIVAG(Orn) (SEQ ID NO: 59 or 60), AIVAG(Dab) (SEQ ID NO: 61 or 62), AIVAG(Dap) (SEQ ID NO: 63 or 64), IIIK (SEQ ID NO: 27 or 28), III(Orn) (SEQ ID NO: 65 or 66), III(Dab) (SEQ ID

NO: 67 or 68), III(Dap) (SEQ ID NO: 69 or 70), IVK (SEQ ID NO: 71 or 72), IV(Orn) (SEQ ID NO: 73 or 74), IV(Dab) (SEQ ID NO: 75 or 76), IV(Dap) (SEQ ID NO: 77 or 78), LVK (SEQ ID NO: 79 or 80), LV(Orn) (SEQ ID NO: 81 or 82), LV(Dab) (SEQ ID NO: 83 or 84) and LV(Dap) (SEQ ID NO: 85 or 86). In one embodiment, the amphiphilic peptide and/or peptoid consists of L-amino acids (L-form).

In one embodiment, the amphiphilic peptide and/or peptoid has an amidated C-terminus.

In one embodiment, the amphiphilic peptide and/or peptoid has an acetylated N-terminus.

In one embodiment, the N-terminal amino acid of the amphiphilic peptide and/or peptoid is an isoleucine (I).

The objects are also solved by a hydrogel comprising at least one amphiphilic peptide and/or peptoid as defined above. The hydrogel can comprise more than one amphiphilic peptide and/or peptoid, such as two, three, four or more amphiphilic peptides and/or peptoids, which can differ in their amino acid sequence, N- and/or C-terminal protecting group.

In one embodiment, the hydrogel is stable in aqueous solution at ambient temperature for a period of at least 7 days, preferably at least 2 to 4 weeks, more preferably at least 1 to 6 months.

In one embodiment, the hydrogel is characterized by a storage modulus G' to loss modulus G'' ratio that is greater than 2.

In one embodiment, the hydrogel is characterized by a storage modulus G' from 100 Pa to 80,000 Pa at a frequency in the range of from 0.02 Hz to 16 Hz.

In one embodiment, the hydrogel has a higher mechanical strength than collagen or its hydrolyzed form (gelatine).

In one embodiment, the hydrogel further comprises a non-peptidic polymer. Preferably, the at least one non-peptidic polymer is present at a concentration of 50% (w/w) or less, preferably

40% (w/w) or less, with respect to the total weight of the hydrogel. Such non-peptidic polymer may be used to modify the mechanical properties of the hydrogel (e.g. increasing its elasticity) and/or for the coupling of bioactive agents/moieties. Composite hydrogels comprising an additional non-peptidic polymer are described in detail in PCT/SG2012/000421, which is hereby incorporated herein by reference in its entirety. Alternatively or additionally, the hydrogel may comprise ultrasmall self-assembling natural peptides (dimers to hexamers) that form organogels when dissolving them in organic solvents, oils and/or mixtures of oils. Such organogels are described in detail in SG201201239-9, which is also incorporated herein by reference in its entirety.

In one embodiment, the hydrogel comprises fibers of the at least one amphiphilic peptide and/or peptoid as defined above, said fibers defining a network that is capable of entrapping at least one of a microorganism, a cell, a virus particle, a peptide, a peptoid, a protein, a nucleic acid, an oligosaccharide, a polysaccharide, a vitamin, an inorganic molecule, a nano- or microparticle, a synthetic polymer, a small organic molecule, a cosmetic agent or a pharmaceutically active compound.

In one embodiment, the hydrogel comprises at least one of a microorganism, a cell, a virus particle, a peptide, a peptoid, a protein, a nucleic acid, an oligosaccharide, a polysaccharide, a vitamin, an inorganic molecule, a nano- or microparticle, a synthetic polymer, a small organic molecule, a cosmetic agent or a pharmaceutically active compound entrapped by the network of fibers of the amphiphilic peptide and/or peptoid.

In one embodiment, the fibers of the amphiphilic peptide and/or peptoid are coupled to the at least one of a microorganism, a cell, a virus particle, a peptide, a peptoid, a protein, a nucleic acid, an oligosaccharide, a polysaccharide, a vitamin, an inorganic molecule, a nano- or microparticle, a synthetic polymer, a small organic molecule, a cosmetic agent or a pharmaceutically active compound.

In one embodiment, the at least one of a microorganism, a cell, a virus particle, a peptide, a peptoid, a protein, a nucleic acid, an oligosaccharide, a polysaccharide, a vitamin, an inorganic molecule, a nano- or microparticle, a synthetic polymer, a small organic molecule, a

cosmetic agent or a pharmaceutically active compound is coupled to said non-peptidic polymer.

In one embodiment, said pharmaceutically active compound is selected from the group comprising haemostatic agents, antibiotics, anti-microbial agents, anti-fungal agents, anti-inflammatory agents, analgesics, anti-coagulants, antibodies, antigens, growth factors and cytokines.

In one embodiment, said hydrogel is provided in an injectable form and gels *in situ*.

In one embodiment, the hydrogel is comprised in at least one of a fuel cell, a solar cell, an electronic cell, a biosensing device, a medical device, an implant, a wound dressing, a pharmaceutical composition and a cosmetic composition.

In one embodiment, the pharmaceutical composition or the cosmetic composition are provided in the form of a topical gel or cream, a spray, a powder, or a sheet, patch or membrane.

In one embodiment, the pharmaceutical composition or the cosmetic composition is provided in the form of an injectable solution, which, preferably, gels *in situ* following injection into the body.

The objects are also solved by a hydrogel as defined above for use in at least one of the following: release of a pharmaceutically active compound, medical tool kit, a fuel cell, a solar cell, an electronic cell, tissue regeneration, tissue replacement, wound healing, skin care, stem cell therapy and gene therapy.

The objects are also solved by a method of preparing a hydrogel, the method comprising dissolving at least one amphiphilic peptide and/or peptoid as defined above in an aqueous solution.

In one embodiment, the dissolved at least one amphiphilic peptide and/or peptoid in aqueous solution is further exposed to temperature, wherein the temperature is in the range from 20 °C to 90 °C, preferably from 20 °C to 70 °C.

In one embodiment, the dissolved at least one amphiphilic peptide and/or peptoid in aqueous solution is exposed to a temperature in the range from 35 °C to 40 °C (body temperature).

In one embodiment, the at least one amphiphilic peptide and/or peptoid is dissolved at a concentration from 0.01 µg/ml to 100 mg/ml, preferably at a concentration from 1 mg/ml to 50 mg/ml, more preferably at a concentration from about 1 mg/ml to about 20 mg/ml.

In one embodiment, the method further comprises at least one of the steps of:

- adding at least one bioactive agent;
- adding at least one non-peptidic polymer;
- adding at least one gelation enhancer;
- adding at least one buffer, preferably at least one physiologically acceptable buffer.

In one embodiment, said gelation enhancer is a salt or a solution of a salt.

In one embodiment, said gelation enhancer is a cross-linking agent.

In one embodiment, said adding at least one non-peptidic polymer further comprises mixing or cross-linking the at least one non-peptidic polymer with the at least one amphiphilic peptide and/or peptoid.

The objects are also solved by a surgical implant or stent, comprising a peptide and/or peptoid scaffold, wherein the peptide and/or peptoid scaffold is formed by a hydrogel as defined above.

The objects are also solved by a pharmaceutical and/or cosmetic composition and/or a biomedical device and/or electronic device comprising at least one amphiphilic peptide and/or peptoid as defined above or a hydrogel as defined above.

In one embodiment, the pharmaceutical and/or cosmetic composition and/or the biomedical device, and/or the electronic devices further comprise(s) a pharmaceutically active compound.

In one embodiment, the pharmaceutical and/or cosmetic composition further comprise(s) a pharmaceutically acceptable carrier.

In one embodiment, the pharmaceutical and/or cosmetic composition is applied topically.

In one embodiment, the pharmaceutical and/or cosmetic composition is injectable.

The objects are also solved by a kit of parts, the kit comprising a first container with at least one amphiphilic peptide and/or peptoid as defined above and a second container with an aqueous solution.

In one embodiment, the aqueous solution of the second container further comprises a pharmaceutically active compound.

In one embodiment, the first container with at least one amphiphilic peptide and/or peptoid further comprises a pharmaceutically active compound.

In one embodiment, the kit of parts further comprises a third container with a gelation enhancer.

In one embodiment, said gelation enhancer is a salt or a solution of a salt.

In one embodiment, at least one of said first, second or third container is provided as a spray bottle or a syringe. In one embodiment, all of said first, second and third container are provided as spray bottles.

The objects are also solved by a kit of parts, the kit comprising a first container with an aqueous solution of at least one amphiphilic peptide and/or peptoid as defined above and a second container with a gelation enhancer.

In one embodiment, the first container further comprises a pharmaceutically active compound.

In one embodiment, said gelation enhancer is a salt or a solution of a salt.

In one embodiment, at least one of said first and said second container is provided as a spray bottle or a syringe. In one embodiment, both said first and said second container are provided as spray bottles or syringes.

In one embodiment, said first and said second container are provided in the form of separate compartments of a spray bottle or syringe.

The objects are also solved by a method of tissue regeneration or tissue replacement comprising the steps:

a) providing a hydrogel as defined above;
b) exposing said hydrogel to cells which are to form regenerated tissue;
c) allowing said cells to grow on or in said hydrogel.

In one embodiment, the method is performed *in vitro* or *in vivo* or *ex vivo*. The term "*ex vivo*" refers, for example, to a scenario, where said cells are taken from the patient and cultured on a scaffold formed by a hydrogel as defined above, which scaffold is then implanted back into the patient.

In one embodiment, the method is performed *in vivo*, wherein, in step a), said hydrogel is provided at a place in the body of a patient where tissue regeneration or tissue replacement is intended.

In one embodiment, said tissue is selected from the group comprising skin tissue, nucleus pulposus in the intervertebral disc, cartilage tissue, synovial fluid and submucosal connective tissue in the bladder neck.

In one embodiment, said step a) is performed by injecting said hydrogel or a solution of at least one amphiphilic peptide and/or peptoid as defined above (i.e. a precursor of the hy-

drogel) at a place in the body of a patient where tissue regeneration or tissue replacement is intended.

In one embodiment, said step a) further comprises the co-injection of a gelation enhancer, preferably of a solution of a salt.

In one embodiment, said step a) further comprises the co-injection of cells.

In one embodiment, the method is performed *ex vivo*, wherein, in step a) or b), cells from a patient or from a donor are mixed with said hydrogel, and the resulting mixture is provided at a place in the body of a patient where tissue regeneration or tissue replacement is intended.

In one embodiment, said hydrogel comprises one or more bioactive therapeutics that stimulate regenerative processes and/or modulate the immune response.

The objects are also solved by a method of wound treatment comprising the steps:

a) providing a hydrogel as defined above;
b) exposing said hydrogel to said wound.

In one embodiment, said wound is a burn wound.

In one embodiment, said hydrogel induces autolytic debridement.

In one embodiment, said hydrogel comprises at least one amphiphilic peptide and/or peptoid, wherein the C-terminal amino acid of the amphiphilic peptide and/or peptoid is selected from the group consisting of a lysine (K), an ornithine (Orn), a 2,4-diaminobutyric acid (Dab or Dbu) and a 2,3-diaminopropionic acid (Dap or Dpr).

In one embodiment, said hydrogel functions as a haemostatic agent.

In one embodiment, said hydrogel functions as an anti-inflammatory agent, which acts by scavenging extracellular nucleic acids that stimulate the innate immune system.

In one embodiment, in step a), said hydrogel is mixed with cells that repopulate the wound and expedite wound healing.

In one embodiment, said hydrogel comprises one or more bioactive therapeutics that diffuse into the wound and facilitate the healing response by modulating the inflammatory response and/or stimulating regenerative processes.

In a first aspect the present invention provides an amphiphilic peptide and/or peptoid capable of self-assembling into three-dimensional macromolecular nanofibrous networks, which entrap water and form a hydrogel. The amphiphilic peptide and/or peptoid includes a hydrophobic and a hydrophilic sequence. This hydrophobic sequence has a length of *n* L- or D- amino acids. *n* is an integer, which may typically range from 2 to about 15. The hydrophilic sequence has a polar and/or charged moiety comprising *m* L- or D- amino acids. *m* is an integer from 1 to 5. Each of the *m* aliphatic amino acids carries an independently selected polar group. The amphiphilic linear sequence has a net charge at physiological pH and, preferably, an N-terminus carrying a protecting group. The protecting group can be an acetyl group. The amphiphilic peptide and/or peptoid may comprise *o* linked amphiphilic peptide and/or peptoid sequences of *n* hydrophobic and m hydrophilic L- and D- amino acids, wherein *o* is an integer from 1 to about 50. The amphiphilic peptide and/or peptoid may consist of *o* linked amphiphilic peptide and/or peptoid sequences of *n* hydrophobic and m hydrophilic L- and D- amino acids. The value of *n* may be an integer from 2 to about 15. The value of *m* may be 1 to 5. The charged and/or polar group of each of the *m* hydrophilic L- and D- amino acids may be independently selected from a hydroxyl, an ether, a carboxyl, an amido, an ester, an amino, a guanidino, a thio, a thioether, a seleno, and a telluro group. The charged or polar moiety of the hydrophilic sequence may comprise *m* L-or D-amino acids selected from the group consisting of aspartic acid, asparagine, glutamic acid, glutamine, 5-N-ethyl-glutamine (theanine), citrulline, thio-citrulline, cysteine, homocysteine, methionine, ethionine, selenomethionine, telluromethionine, threonine, allo-threonine, serine, homoserine, arginine, homoarginine, ornithine (Orn), 2,4-diaminobutyric acid (Dab or Dbu), 2,3-diaminopropionic acid (Dap or Dpr), lysine and N(6)-carboxymethyllysine. The charged and/or polar moiety of the hydrophilic sequence may comprise two identical amino acids. The two identical amino acids may be adjacent to the non-polar hydrophobic moiety. The charged and/or polar moi-

ety may consist of two amino acids with a sequence selected from Asn-Asn, Asp-Asp, Glu-Glu, Gln-Gln, Asn-Gln, Gln-Asn, Asp-Gln, Gln-Asp, Asn-Glu, Glu-Asn, Asp-Glu, Glu-Asp, Gln-Glu, Glu-Gln, Asp-Asn, Asn-Asp, Thr-Thr, Ser-Ser, Thr-Ser, Ser-Thr, Asp-Ser, Ser-Asp, Ser-Asn, Asn-Ser, Gln-Ser, Ser-Gln, Glu-Ser, Ser-Glu, Asp-Thr, Thr-Asp, Thr-Asn, Asn-Thr, Gln-Thr, Thr-Gln, Glu-Thr, Thr-Glu, Cys-Asp, Cys-Lys, Cys-Ser, Cys-Thr, Cys-Orn, Cys-Dab, Cys-Dap, Lys-Lys, Lys-Ser, Lys-Thr, Lys-Orn, Lys-Dab, Lys-Dap, Ser-Lys, Ser-Orn, Ser-Dab, Ser-Dap, Orn-Lys, Orn-Orn, Orn-Ser, Orn-Thr, Orn-Dab, Orn-Dap, Dab-Lys, Dab-Ser, Dab-Thr, Dab-Orn, Dab-Dab, Dab-Dap, Dap-Lys, Dap-Ser, Dap-Thr, Dap-Orn, Dap-Dab, Dap-Dap. The charged and/or polar moiety may comprise the C-terminus of the amphiphilic peptide and/or peptoid. The charged and/or polar moiety may comprise (i) the C-terminus, the C-terminus carrying an unprotected C-terminal carboxyl group or (ii) the N-terminus, the N-terminus carrying an unprotected N-terminal amino group. The charged and/or polar moiety may comprise the C-terminus of the amphiphilic peptide and/or peptoid, the C-terminus carrying an unprotected C-terminal carboxyl group and wherein the N-terminus carries a protecting group preferably the acetyl group. The charged and/or polar moiety may comprise the C-terminus of the amphiphilic peptide and/or peptoid, the C-terminus carrying a protected C-terminal carboxyl group, preferably protected with an amido or ester group, and the N-terminus carrying a protecting group, preferably the acetyl group. The protecting group may be an amido protecting group. The charged and/or polar moiety may consist of at least one amino acid positioned at the C-terminus of the amphiphilic peptide and/or peptoid. The hydrophobic sequence may comprise at least two aliphatic amino acids that is defined by a main chain comprising 1 to about 20 carbon atoms. A portion of the amino acids of the non-polar moiety may be arranged in a general sequence of decreasing size in the direction from N- to C-terminus of the amphiphilic peptide and/or peptoid, and the size of adjacent amino acids of the non-polar moiety may be identical or smaller in the direction of the general sequence of decreasing size. The general sequence of decreasing size may be preferably a non-repetitive sequence. The direction of the general sequence of decreasing size in which adjacent amino acids may be of identical or smaller size may be the direction toward the charged and/or polar moiety of the sequence. The portion of the amino acids arranged in a general sequence of decreasing size may have a length of 2-7, preferably 2-6, more preferably 2, 3, 4, 5 or 6 amino acids. The portion of the amino acids arranged in a general sequence of decreasing size may also have a length of *n-m-1* amino acids and wherein the portion of the amino acids arranged in the general sequence of decreasing size may be

positioned between the remaining non-polar amino acid of the non-polar moiety of *n-m* amino acids and the polar moiety. The remaining non-polar amino acid of the non-polar moiety of *n-m* amino acids may define the N-terminus or the C-terminus of the amphiphilic peptide and/or peptoid. The remaining non-polar amino acid of the non-polar moiety of *n-m* amino acids may be one of alanine, valine and glycine. The amphiphilic linear sequence may undergo a conformational change from a random coil conformation to a helical conformation during self-assembly. The conformational change may be concentration, pH, temperature and salt concentration dependent. The non-polar moiety of the amphiphilic linear sequence may comprise at least one L-or D-amino acid selected from the group consisting of glycine, homoallylglycine, homopropargylglycine, alanine, valine, leucine, norleucine and isoleucine. The amphiphilic linear sequence may comprise a single polar and/or charge and a single non-polar moiety. The amphiphilic linear sequence may have a positive or a negative net charge. The net charge may be from about -1 to about -4 or from about +5 to about +1. The net charge may be from about -1 to about -2. The net charge may be -2. The net charge may be +1 or +2 or +5. The amphiphilic peptide and/or peptoid may be stable in aqueous solution at physiological conditions at ambient temperature for a period of time in the range from 1 day to at least 6 months, preferably at least 8 months, more preferably at least 12 months. The amphiphilic peptide and/or peptoid may be stable in aqueous solution at physiological conditions at a temperature to 90 °C for at least 1 hour. The C-terminal amino acid of the amphiphilic peptide and/or peptoid may be selected from the group consisting of a lysine (K), an ornithine (Orn), a 2,4-diaminobutyric acid (Dab or Dbu) and a 2,3-diaminopropionic acid (Dap or Dpr). In some embodiments, the N-terminal amino acid of the amphiphilic peptide and/or peptoid is an isoleucine (I). The hydrogel may comprise more than one amphiphilic peptide and/or peptoid, such as two, three, four or more amphiphilic peptides and/or peptoids, which can differ in their amino acid sequence, N- and/or C-terminal protecting group. The hydrogel may further comprise a non-peptidic polymer.

In a second aspect the invention provides a hydrogel. The hydrogel includes an amphiphilic peptide and/or peptoid according to the first aspect. The hydrogel may be stable in aqueous solution at ambient temperature for a period of at least 7 days. The hydrogel may be stable in aqueous solution at ambient temperature for a period of at least 2 to 4 weeks. The hydrogel may be stable in aqueous solution at ambient temperature for a period of at least 1 to 6 months. The hydrogel mechanical property may be characterized by a loss modulus G'' to

storage modulus G' ratio that is less than1. The hydrogel may be characterized by magnitude of storage modulus G' greater than loss modulus G" by minimum factor of 1.5. The hydrogel may be characterized by a storage modulus G' of from 100 Pa to 80,000 Pa at a frequency in the range of from 0.02 Hz to 16 Hz. The hydrogel may be characterized by higher storage modulus G' with increase in the concentration of peptide. The hydrogel may have a higher mechanical strength than collagen or hydrolyzed form (gelatine). The hydrogel may comprise fibers of an amphiphilic peptide and/or peptoid described herein. The fibers may define a network that is capable of entrapping at least one of a microorganism, a cell, a virus particle, a peptide, a peptoid, a protein, a nucleic acid, an oligosaccharide, a polysaccharide, a vitamin, an inorganic molecule, a nano- or microparticle, a synthetic polymer, a small organic molecule, a cosmetic agent or a pharmaceutically active compound. The hydrogel may comprise at least one of a microorganism, a cell, a virus particle, a peptide, a peptoid, a protein, a nucleic acid, an oligosaccharide, a polysaccharide, a vitamin, an inorganic molecule, a nano- or microparticle, a synthetic polymer, a small organic molecule, a cosmetic agent or a pharmaceutically active compound entrapped by the network of fibers of the amphiphilic polymer. The fibers of the amphiphilic polymer may be coupled to the at least one of a microorganism, a cell, a virus particle, a peptide, a peptoid, a protein, a nucleic acid, an oligosaccharide, a polysaccharide, a vitamin, an inorganic molecule, a nano- or microparticle, a synthetic polymer, a small organic molecule, a cosmetic agent or a pharmaceutically active compound entrapped by the network of fibers of the amphiphilic polymer. The at least one of a microorganism, a cell, a virus particle, a peptide, a peptoid, a protein, a nucleic acid, an oligosaccharide, a polysaccharide, a vitamin, an inorganic molecule, a nano- or microparticle, a synthetic polymer, a small organic molecule, a cosmetic agent or a pharmaceutically active compound may also be coupled to the non-peptidic polymer. The hydrogel may be comprised in at least one of a fuel cell, a solar cell, a electronic cell, a biosensing device, a medical device, an implant**,** a wound dressing, a pharmaceutical composition, drug and vaccine delivery system, tissue culture medium, biosensor devices and a cosmetic composition. The pharmaceutical composition or the cosmetic composition may be provided in the form of a topical gel or cream, a spray, a powder, or a sheet, patch or membrane. The pharmaceutical composition or the cosmetic composition may also be provided in the form of an injectable solution, which, preferably, gels *in situ* following injection into the body. The hydrogel may be for at least one of (controlled or sustained) release of a pharmaceutically active compound, medical tool kit, a fuel cell, a solar cell, an electronic cell, tissue regeneration, tissue replacement, wound heal-

ing, skin care, stem cell therapy and gene therapy. In some embodiments the hydrogel may be used for tissue regeneration, drug release or gene therapy.

In a third aspect the invention provides a method of preparing a hydrogel. The method includes providing an amphiphilic peptide and/or peptoid according to the first aspect. The method further includes dissolving and/or dispersing the amphiphilic peptide and/or peptoid in an aqueous solution. The dissolved/dispersed amphiphilic peptide and/or peptoid in aqueous solution may be further exposed to a temperature. The temperature may be selected in the range from about 20 °C to about 90, preferably from 20°C to 70°C. The amphiphilic peptide and/or peptoid may be dissolved at a concentration from about 0.01 µg/ml to about 100 mg/ml. The amphiphilic peptide and/or peptoid may be dissolved at a concentration from about 1 mg/ml to about 50 mg/ml. The amphiphilic peptide and/or peptoid may be dissolved and/or dispersed at a concentration from about 1 mg/ml to about 30 mg/ml. The method may further comprise at least one of the steps of adding at least one bioactive agent; adding at least one non-peptidic polymer; adding at least one gelation enhancer; adding at least one buffer, preferably at least one physiologically acceptable buffer. In some embodiments, the gelation enhancer may be a salt or a solution of a salt, in other embodiments, the gelation enhancer may be a cross-linking agent. In some embodiments, said adding at least one non-peptidic polymer further comprises mixing or cross-linking the at least one non-peptidic polymer with the amphiphilic peptide and/or peptoid.

In a fourth aspect the invention provides a surgical implant or stent. The surgical implant or stent includes a peptide and/or peptoid scaffold. The peptide and/or peptoid scaffold is defined by a hydrogel according to the second aspect.

In a fifth aspect the invention provides a pharmaceutical and/or cosmetic composition. The pharmaceutical and/or cosmetic composition includes the amphiphilic peptide and/or peptoid according to the first aspect or a hydrogel according to the second aspect. The pharmaceutical and/or cosmetic composition may comprise a pharmaceutically active compound. The pharmaceutical and/or cosmetic composition may comprise a pharmaceutically acceptable carrier.

In a sixth aspect the invention provides a kit of parts. The kit includes a first container and a second container. The first container includes a peptide and/or peptoid according to the first

aspect. The second container includes an aqueous solution. The aqueous solution of the second container may further comprise a pharmaceutically active compound. The fist container with an amphiphilic peptide and/or peptoid may further comprise a pharmaceutically active compound. Alternatively, the kit includes a first container with an aqueous solution of an amphiphilic peptide and/or peptoid according to the first aspect and a second container with a gelation enhancer. In some embodiments the container(s) may be provided as spray bottles or syringes or compartments of syringes.

In a seventh aspect the invention provides for a method of tissue regeneration or tissue replacement.

In an eighth aspect the invention provide for a method of wound treatment.

Other aspects and features of the present invention will become apparent to those skilled in the art upon review of the following description of specific embodiments of the invention in conjunction with the accompanying figures.

BRIEF DESCRIPTION OF THE DRAWINGS

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

Figures 1A to **1J** represent a sorted list of some exemplary peptides of the invention capable of forming hydrogels. These peptides are embodiments in which the entire peptide consists of a single linear amphiphilic sequence. Peptides which are forming hydrogels are named with a short code, but their individual sequence is disclosed. The peptides of these examples consist of a sequence of natural amino acids containing 3 to 7 amino acids. The N-terminus is acetylated which removes the charge that would otherwise restrain the amphiphilic character of the peptides.

Figure 2 depicts gelation pictures for peptide based hydrogels at lowest concentrations.

Figure 3 depicts gelation pictures for Ac-AS-6 (Ac-AIVAGS) (L) at concentrations of 5 mg/ml, 10 mg/ml, 15 mg/ml.

Figure 4 depicts a hypothesis of self-assembly from peptide monomers to supramolecular network of condensed fibers. (A) Assembly is believed to initiate with antiparallel pairing of two peptide monomers by changing to α-helical conformations. Subsequently, peptide pairs assemble to fibers and nanostructures. Condensation of peptide fibers to fiber aggregates results in hydrogel formation.

Figure 5 depicts environmental scanning electron microscopy (ESEM) images of hydrogels of Ac-LD6 (Ac-LIVAGD) (L) (10 mg/ml), where Fig. 5A, Fig. 5B and Fig. 5C are images obtained at magnification of 260X, 1000X, 2000X, 2400X, 4000X at a temperature of 4 °C with HV at 10KV. The images indicate the formation of fibrous structures.

Figure 6 shows field emission scanning electron microscopy (FESEM) images of hydrogels of Ac-LD6 (Ac-LIVAGD) (L) (15 mg/ml), where figures 6A-D are images obtained at magnifications of 6000X, 45000X, 45000X and 40000X with HV at 10 KV.

Figure 7 depicts field emission scanning electron microscopy (FESEM) images of Ac-AD6 (Ac-AIVAGD) (D) hydrogels (20 mg/ml) at a magnification of 50 X (fig. 7A) and 20000 X (fig. 7 B) at 12 KV.

Figure 8 shows field emission scanning electron microscopy (FESEM) images of hydrogels of Ac-AD6 (Ac-AIVAGD) (D) (20 mg/ml) obtained at 120 X (fig. 8A), and 450 X (fig. 8B).

Figure 9A a)-f) shows the morphology and structure evaluation of the peptide scaffolds as determined by field emission scanning electron microscopy **(a-f) (a)** A honeycomb porous structure is observed following lyophilization of 20 mg/mL Ac-AD_6 (Ac-AIVAGD) (D) hydrogel. The pores are bounded by membranes of condensed fibers as shown in close-up views of 15 mg/mL **(b)** and 20 mg/mL **(c)** Ac-ID_3 (Ac-IVD) (L) hydrogels. Further magnification of 20 mg/mL Ac-AD_6 (L) hydrogel revealed single fibers **(d, e).** At lower concentrations, 0.1 mg/mL Ac-LD_6 (Ac-LIVAGD) (L), nanostructures are observed **(f)**.

Figure 9B shows an image obtained at a magnification of 1000x, HV of 12 KV, **Fig. 9C** obtained at a magnification of 2500x, HV of 12 KV, **Fig. 9D** obtained at a magnification of 4000x, HV of 10 KV, **Fig. 9E** obtained at a magnification of 35000x, HV of 10 KV, **Fig. 9F** at a magnification of 80000x, HV of 5 KV, **Fig. 9G** obtained at a magnification of 120000x, HV of 10 KV, and **Fig. 9H** at a magnification of 200000x, HV of 10 KV.

Figure 10 shows **(a)** Far-UV CD spectra demonstrate that with increasing concentration there is the transition of Ac-LD_6 (Ac-LIVAGD) peptide conformation from random coil (below threshold concentration) to α-helical (222 and 208 nm peaks) and further β-type (negative band at 218 nm) structures. Heating the samples to facilitate gelation increased the β type aggregation. **(b)** Below threshold concentration, the random coil conformations of 0.2 mg/mL Ac-LD_6 were reversibly affected by step-wise temperature increases (solid lines) from 25˚C to 90˚C and cooling (dotted lines). **(c, d)** Above the threshold concentration in 1 mg/mL Ac-LD_6 gel, stepwise temperature increases **(c)** stabilized the β -type structures irreversibly, such that subsequent cooling **(d)** did not alter the CD spectra. **(e)** Far-UV CD spectra of $AcID_3$ (Ac-IVD) at different concentrations. All curves were done at 25 ˚C.

Figure 11 shows Rheology.**(a, b)** The high mechanical strengths of different peptide hydrogels at 20 mg/mL concentration was determined by measuring storage moduli (G') as a function of angular frequency under 0.1% strain, at 25˚C and 50˚C respectively. The gels demonstrate good thermal stability compared to gelatin, which liquidified at 50˚C (hence excluded in 4B). **(c)** Mechanical strength is a function of concentration, as determined from oscillatory frequency sweep studies using Ac-LD_6 (Ac-LIVAGD) (L) under 0.1% strain at 25˚C. **(d)** Increasing salt concentration (NaCl) decreases G', reducing the rigidity of 10 mg/mL Ac-LD_6 (L) hydrogels, demonstrating the tuneability and reversibility of gelation.

Figure 12 shows a further example of a rheology measurement for peptide based hydrogels. **Figure 12A** and **Figure 12B** depict oscillatory amplitude sweep studies at temperatures of 25°C and 50°C for Ac-AD6 (Ac-AIVAGD) (L) and Ac-AD6 (D) at a concentration of 20 mg/ml with a constant frequency of [1rad-s] and a gap of 0.8 mm. The graphs indicate the plot of moduli [Pa] versus strain (%) at temperatures of 25°C and 50°C. The linear viscoelastic range was observed at 0.07% to 0.2 strain % at temperatures of 25°C and 50°C. **Fig. 12C** and **Fig. 12D** depict oscillatory frequency sweep Studies at temperatures of 25°C and 50°C

for Ac-AD6(L) and Ac-AD6(D) at a concentration of 20 mg/ml with varying frequency ranges from 0.1 to 100 [Rad/s] with a constant strain [%] of 0.1% linear viscoelastic range and a gap of 0.8 mm.

Figure 13 shows a further example of a rheology measurement for peptide based hydrogels. Depicted is a frequency sweep study of a uv cross-linked peptide at a temperature of 25 °C with 0.1 % strain.

Figure 14 depicts rheology measurements for gelatin-1890 (type A, porcine skin). This figure shows moduli data obtained at 25 °C when applying different frequencies.

Figure 15 illustrates the biocompatibility of peptide-based hydrogels of the invention using further cell lines. Fig. 15A shows a microscopy image of human primary renal tubule cells (HPRTC) after 72 hours after seeding on a hydrogel of Ac-LD_6 (Ac-LIVAGD) (L) in DMEM medium, grown at optimum conditions. Fig. 15B shows microscopy images of human primary renal tubule cells (HPRTC) after 72 hrs after seeding on tissue culture plastic, grown at optimum conditions. Fig. 15C shows microscopy images of human umbilical vein endothelial cells (HUVEC) after 72 hrs after seeding on gels of Ac-LD_6 (L) in DMEM medium, grown at optimum conditions. Fig. 15D shows microscopy images of human umbilical vein endothelial cells (HUVEC) after 72 hrs after seeding on tissue culture plastic, grown at optimum conditions.

Figure 16 is a further illustration on the viability of cells in presence of a hydrogel of the invention. Human fibroblast cells were cultured in the presence (Fig. 16A) and absence (Fig. 16B) of Ac-LD_6 (Ac-LIVAGD) (L) (5 mg/ml). Fluorescein isothiocyanate (FITC) stained cells (left panels), Texas red stained cells (center panels) and cells stained with both FITC and Texas red (right panels) are shown.

Figure 17 shows the production of composite polymer-peptide hydrogels by incorporating (A) linear and (B) branched polymers with ultrashort peptides during self-assembly. The resulting hydrogels have better mechanical properties (due to cross-linking and increased elasticity) and (C) offer opportunities to incorporate bioactive functionalities to modulate the immune and physiological response.

Figure 18 shows that ultrashort peptide hydrogels facilitate autolytic debridement of necrotic eschar tissue in burn wounds and enhance wound contracture. (A) Ac-LK_6 (Ac-LIVAGK-NH_2) and Ac-IK_6 (Ac-ILVAGK-NH_2) hydrogel candidates demonstrated earlier onset and completion of autolytic debridement, compared to Mepitel®, as observed by gross examination. (B) Gross histological analysis of skin samples (day 7) confirmed the completion of autolytic debridement for wounds dressed with peptide hydrogels. The loss of the necrotic epidermal layer, as marked by red arrows, for burnt area treated with $AcIK_6$ and $AcLK_6$ hydrogels, is contrasted with remnants of epidermal tissue adhered to the dermis for the wounds dressed with Mepitel®.(C) Ultrashort peptide hydrogels $AcIK_6$ and $AcLK_6$ promote hemostasis and wound closure, resulting in almost complete wound healing by day 14. (D) Quantitative evaluation of wound healing using digital planimetry revealed that $AcIK_6$ and $AcLK_6$ hydrogels stimulated recovery of the burnt area (mixed model analysis, $p<0.05$).

Figure 19 shows the histological evaluation of burn wound healing at day 7 and 14. (A) The tissue damage penetrated mid/deep dermis tissue, for all injuries, as examined at day 7. Necrotic epidermal tissue is observed for the Mepitel®-dressed samples, while complete debridement of the eschar epidermal tissues was observed for Ac-IK_6 (Ac-ILVAGK-NH_2) and Ac-LK_6 (Ac-LIVAGK-NH_2) hydrogel-dressed injuries. (B) At the boundary of the injured and healthy tissue, infiltration of the healthy basal cells from the epidermal-dermis junction and the adjacent undamaged hair shafts, into the damaged tissue is observed for the Ac-IK_6 and Ac-LK_6 hydrogel-dressed injuries, at day 7. (C) Recovery of the damaged tissue at day 14, as compared to day 7. In particular, almost complete epidermal regeneration was attained for Ac-IK_6 and Ac-LK_6 hydrogel treated wounds. (D) At the boundary of the injury site, significantly higher extent of cell replication has occurred amongst basal cells located in the hair follicles, for wounds dressed with Ac-IK_6 and Ac-LK_6 hydrogels.

Figure 20 shows various formulations of the hydrogels according to the present invention. (A) Hydrogel patch. (B) Membrane that can be re-constituted into a hydrogel. (C) Spray incorporating secondary components (such as bioactive therapeutics). A subclass of peptides (having a lysine or a similar amino acid as polar head group) that is stimuli-responsive can be further exploited to produce products that will only gel in situ when the two spray streams

meet. (D) Topical hydrogels and organogels. (E) Rehydration of membranes into hydrogels, which can be accelerated by sonication.

Figure 21 shows examples of a subclass of peptides that allow stimuli-responsive gelation, e.g. gelation in the presence of salts at physiological concentrations. (A) The minimum gelation concentration decreases in the presence of salts. Interchanging the amino acids Ile and Leu at the N-terminus further facilitates gelation. (B) The mechanical strength of this subclass increases in the presence of higher salt concentration such as saline and phosphate-buffered saline, as compared to hydrogels solvated in pure water. (C) The aliphatic chain length of the lysine residue has no significant effect on gelation, as observed by comparing minimum gelation concentrations of Ac-LK_6 (Ac-LIVAGK-NH_2), Ac-$L(Orn)_6$ (Ac-LIVAG(Orn)-NH_2) and Ac-$L(Dab)_6$ (Ac-LIVAG(Dab)-NH_2).

Figure 22 demonstrates the ability of peptides with a lysine polar head to bind nucleic acids. (A) The peptide Ac-LK_6 (Ac-LIVAGK-NH_2) was extremely efficient in binding and trapping the DNA, possibly through electrostatic interactions. There was minimal release of the DNA, as observed over the course of several days. Ac-LK_6 also effectively protected the DNA against nuclease degradation (B). When hydrogels encapsulating plasmid DNA were incubated with DNAse, digested DNA fragments were not observed, compared to the naked DNA control. The composite hydrogel fragments did not migrate out of the well during electrophoresis, indicating that the peptide strongly interacts with oligonucleotides, increasing the mass and thereby hindering migration through the agarose gel.

Figure 23 shows that peptide hydrogels according to the present invention enhance hemostasis.

Figure 24 illustrates injectable therapy for degenerative disc disease. The mechanical properties of ex vivo extracted porcine nucleus pulposus (A) was determined to be slightly lower than that of our tuneable peptide hydrogels (B). Using a rabbit model of degenerative disc disease (C), 2 different treatments using peptide hydrogels encapsulating MRI contrast agent or cells were evaluated. (D) The hydrogel persists and can still be detected in the nucleus pulposis space using MRI after 2 months, and upon dissection of the intervertebral discs, more matrix-like material was detected in the treated discs compared to untreated discs.

Figure 25 shows the use of peptide hydrogels as dermal and adipose fillers. (B) Peptide hydrogels were subcutaneous implanted in C57BL/6 mice. After 2 months the animals were sacrificed and the implant site was collected for histological analysis. In all the animals, the inflammatory reaction to the implants was minimal to mild, as evident from (C)the mild foreign body type histiocytic reaction around the hydrogel implant (amorphous eosinophilic material beneath the skeletal muscle layer). (D) When human adipose stem cells were encapsulated in Ac-LK_6 (Ac-LIVAGK-NH_2) peptide hydrogels and subsequently implanted subcutaneously into SCID mice, fat pads formed in vivo after 6 weeks. (E) A thick layer of adipose tissue, populated by mature adipocytes (F) was noted at the implant site. This can potentially be applied in fat grafting wherein currently only 30-50% retention of the transplanted fat tissue can be achieved.

DETAILED DESCRIPTION OF THE INVENTION

An exemplary embodiment of the invention provides a novel class of hydrogel-forming peptides/peptoids derived from inter alia natural amino acids. These peptides/peptoids are small amphiphilic peptides with a hydrophobic portion of aliphatic amino acids and one or two polar amino acids. The peptides/peptoids (typically 3-7-mers) are typically in the L- or D-form and can self assemble into supramolecular fibers which are organized into mesh-like structures. The hydrogels are generally characterized by a remarkable rigidity and are biocompatible and non-toxic. Depending on the peptide/peptoid sequence these hydrogels can show stimuli-responsive, thermoresponsive and/or thixotropic character. By selecting the peptide assembling conditions, the thickness and length of the fibers as well as the mechanical properties of the resulting hydrogel scaffold can be controlled. The rigid hydrogels can be used for cultivation of a variety of primary human cells, providing peptide scaffolds that can be useful in the repair and replacement of various tissues. Also disclosed is the procedure of preparing these hydrogels. Disclosed is further the use of respective hydrogels in applications such as cell culture, tissue engineering, plastic surgery, drug and vaccine delivery, oral applications, cosmetics, packaging and the like as well as for technical applications, as for example for use in electronic devices which may include solar or fuel cells.

An exemplary of the present invention provides an amphiphilic peptide and/or peptoid capable of forming a hydrogel, i.e. a polymer network in which water is the dispersion medium.

The amphiphilic peptide and/or peptoid includes one or more linear amphiphilic sequences, each having a polar and a non-polar portion. For sake of simplicity explanations are in the following to a large extent focused on amphiphilic peptides and/or peptoids that consist of a single respective linear sequence. In these explanations a respective peptide and/or peptoid is denominated a "linear peptide and/or peptoid". Respective explanations apply to any linear sequence, which may also be included in an amphiphilic peptide and/or peptoid with a plurality of these linear sequences. Each of these linear sequences is individually selected. In some embodiments an amphiphilic peptide and/or peptoid disclosed herein includes several linear amphiphilic sequences, each of them differing from any other of the linear amphiphilic sequences. In some embodiments an amphiphilic peptide and/or peptoid disclosed herein includes several identical linear amphiphilic sequences. In one embodiment an amphiphilic peptide and/or peptoid disclosed herein includes a plurality of linear amphiphilic sequences, each linear amphiphilic sequence being identical to each other linear amphiphilic sequence.

A peptide and/or peptoid according to an exemplary embodiment of the invention includes *o* amphiphilic linear sequences. The symbol *o* represents an integer selected in the range from 1 to about 25, such as from 1 to about 20, from 1 to about 18, from 1 to about 15, from 1 to about 12, from 1 to about 10, from 1 to about 8, from 1 to about 6, from 1 to about 5 from 1 to about 4 or from 1 to about 3. In some embodiments these amphiphilic linear sequences are linked in a consecutive manner, thereby defining a linear portion of the peptide and/or peptoid. In some embodiments the peptide and/or peptoid has a backbone with one or more branches. In such an embodiment these amphiphilic linear sequences may be included on different branches.

As mentioned above, each of the *o* amphiphilic linear sequences is independently selected. A respective amphiphilic linear sequence has a length of *n* aliphatic amino acids. The symbol *n* represents an integer selected in the range from 3 to about 18, such as from 3 to about 15, from 3 to about 14, from 3 to about 13, from 3 to about 12, from 3 to about 11, from 3 to about 10, from 3 to about 9, from 3 to about 8 or from 3 to about 7, such as 3, 4, 5, 6, 7, 8, 9 or 10 aliphatic amino acids.

In some embodiments an amphiphilic linear sequence of a peptide and/or peptoid described herein is chiral, rendering the entire amphiphilic peptide and/or peptoid chiral. A corresponding linear peptide and/or peptoid, i.e. an embodiment that consists of a single respective linear sequence, is accordingly a chiral peptide or peptoid. A respective amphiphilic linear sequence may include any linear non-aromatic amino acid. The term "amino acid" as used

herein refers to an alpha-amino carboxylic acid, i.e. a carboxylic acid with an amino group in the α-position. The respective amino group may be an $-NH_2$ group or an $-NHR^1$ group. The moiety R^1 may be any aliphatic group, whether alkyl, alkenyl or alkynyl, with a main chain that includes 1 to 5, to 10, to 15 or to 20 carbon atoms. Examples of alkenyl radicals are straight-chain or branched hydrocarbon radicals which contain one or more double bonds. Alkenyl radicals generally contain about two to about twenty carbon atoms and one or more, for instance two, double bonds, such as about two to about ten carbon atoms, and one double bond. Alkynyl radicals normally contain about two to about twenty carbon atoms and one or more, for example two, triple bonds, preferably such as two to ten carbon atoms, and one triple bond. Examples of alkynyl radicals are straight-chain or branched hydrocarbon radicals which contain one or more triple bonds. Examples of alkyl groups are methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, the n isomers of these radicals, isopropyl, isobutyl, isopentyl, *sec*-butyl, *tert*-butyl, neopentyl, 3,3 dimethylbutyl.

A peptoid is an oligo(N-alkyl) glycine that, similar to the side chain connected to the α carbon atom (see below) of a peptide, at the amide nitrogen carries a moiety that is in the present invention an aliphatic moiety. Accordingly, in embodiments where an $-NHR^1$ group (supra) is included in the amino acid and the α carbon atom is included in a $-CH_2-$ group, the reaction product of coupling a plurality of such amino acids may be called a peptoid. A peptoid can also be taken to differ from a peptide in that it carries its side chain at the amide nitrogen rather than at the α carbon atom. Peptoids are typically resistant to proteases and other modifying enzymes and can have a much higher cell permeability than peptides (see e.g. Kwon, Y.-U., and Kodadek, T., J. Am. Chem. Soc. (2007) 129, 1508-1509).

The term “amino acid” includes compounds in which the carboxylic acid group is shielded by a protecting group in the form of an ester (including an ortho ester), a silyl ester, an amide, a hydrazide, an oxazole, an 1,3-oxazoline or a 5-oxo-1,3,-oxazolidine. The term “amino acid” also includes compounds in which an amino group of the form $-NH_2$ or $-NHR^1$ (supra) is shielded by a protecting group. Suitable amino protecting groups include, but are not limited to, a carbamate, an amide, a sulfonamide, an imine, an imide, histidine, a N-2,5,-dimethylpyrrole, an N-1,1,4,4-tetramethyldisilylazacyclopentane adduct, an N-1,1,3,3-tetramethyl-1,3-disilisoindoline, an N-diphenylsilyldiethylene, an 1,3,5-dioxazine, a N-[2-(trimethylsilyl)ethoxy]methylamine, a N-(5,5-dimethyl-3-oxo-1-cyclohexenyl)amine, a N-4,4,4-trifluoro-3-oxo-1-butenylamine, a N-9-borabicyclononane and a nitroamine. A protecting group may also be present that shields both the amino and the carboxylic group such as

e.g. in the form of a 2,2-dimethyl-4-alkyl-2-sila-5-oxo-1,3-oxazolidine. The alpha carbon atom of the amino acid typically further carries a hydrogen atom. The so called “side chain” attached to the alpha carbon atom, which is in fact the continuing main chain of the carboxylic acid, is an aliphatic moiety that may be linear or branched. The term “side chain” refers to the presence of the amino acid in a peptide (supra), where a backbone is formed by coupling a plurality of amino acids. An aliphatic moiety bonded to the α carbon atom of an amino acid included in such a peptide then defines a side chain relative to the backbone. As explained above, the same applies to an aliphatic moiety bonded to the amino group of the amino acid, which likewise defines a side chain relative to the backbone of a peptoid.

The term "aliphatic" means, unless otherwise stated, a straight or branched hydrocarbon chain, which may be saturated or mono- or poly-unsaturated and include heteroatoms. The term "heteroatom" as used herein means an atom of any element other than carbon or hydrogen. An unsaturated aliphatic group contains one or more double and/or triple bonds (alkenyl or alkynyl moieties). The branches of the hydrocarbon chain may include linear chains as well as non-aromatic cyclic elements. The hydrocarbon chain, which may, unless otherwise stated, be of any length, and contain any number of branches. Typically, the hydrocarbon (main) chain includes 1 to 5, to 10, to 15 or to 20 carbon atoms. Examples of alkenyl radicals are straight-chain or branched hydrocarbon radicals which contain one or more double bonds. Alkenyl radicals generally contain about two to about twenty carbon atoms and one or more, for instance two, double bonds, such as about two to about ten carbon atoms, and one double bond. Alkynyl radicals normally contain about two to about twenty carbon atoms and one or more, for example two, triple bonds, preferably such as two to ten carbon atoms, and one triple bond. Examples of alkynyl radicals are straight-chain or branched hydrocarbon radicals which contain one or more triple bonds. Examples of alkyl groups are methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl, octyl, nonyl, decyl, the n isomers of these radicals, isopropyl, isobutyl, isopentyl, sec-butyl, tert-butyl, neopentyl, 3,3 dimethylbutyl. Both the main chain as well as the branches may furthermore contain heteroatoms as for instance N, O, S, Se or Si or carbon atoms may be replaced by these heteroatoms.

An aliphatic moiety may be substituted or unsubstituted with one or more functional groups. Substituents may be any functional group, as for example, but not limited to, amino, amido, azido, carbonyl, carboxyl, keto, cyano, isocyano, dithiane, halogen, hydroxyl, nitro, organometal, organoboron, seleno, silyl, silano, sulfonyl, thio, thiocyano, trifluoromethyl sulfonyl, p-toluenesulfonyl, bromobenzenesulfonyl, nitrobenzenesulfonyl, and methanesulfonyl.

As should be apparent from the above, the side chain of an amino acid in a peptide/peptoid described herein may be of a length of 0 to about 5, to about 10, to about 15 or to about 20 carbon atoms. It may be branched and include unsaturated carbon-carbon bonds. In some embodiments one or more natural amino acids are included in the peptide or peptoid. Such a natural amino acid may be one of the 20 building blocks of naturally occurring proteins.

In a peptide or peptoid, including a peptide/peptoid disclosed herein individual amino acids are covalently coupled via amide bonds between a carboxylic group of a first and an amino group of a second amino acid. A peptide and/or peptoid disclosed herein is non-repetitive, such that two amino acids coupled to each other are always different from one another.

The term amphiphilic refers to a compound that is soluble in both polar and non-polar fluids. It also encompasses multiphase compounds. The amphiphilic properties of the peptide and/or peptoid are due to the presence of both polar and non-polar moieties within the same peptide and/or peptoid. In this regard the peptide and/or peptoid may be of surfactant nature. Accordingly, the polar properties of a peptide and/or peptoid according to an embodiment of the invention are based on a polar moiety. Two such moiety are a -COOH side group, in particular in the form of a charged COO^- group and an amino group. A further such moiety is a C-terminal -COOH group if it is present in free, unprotected form. Generally, a surfactant molecule includes a polar, typically hydrophilic, head group attached to a non-polar, typically hydrocarbon, moiety. Non-polar moieties of a peptide or peptoid include a hydrocarbon chain that does not carry a functional group.

An amphiphilic linear sequence included in a peptide and/or peptoid of an embodiment of the invention thus includes a polar moiety and a non-polar moiety. The polar moiety includes an aliphatic amino acid that carries a polar group such as a hydroxyl group, a thiol group, a seleno group, an amino group, an amide group, an ether group, a thioether group or a seleno ether group. Accordingly, the polar moiety may include an amino acid that carries a functional polar group with a proton such as hydroxyl, thiol, selenol, amine or amide. The polar moiety may also include the C-terminus or the N-terminus of the peptide and/or peptoid. The C-terminus or the N-terminus may in such a case be present in the form of the free carboxyl or amino group, respectively, i.e. free of a protecting group.

Generally the polar moiety of a linear amphiphilic sequence of an amphiphilic peptide and/or peptoid of an embodiment of the invention is defined by a single amino acid, by two consecutive amino acids or by three consecutive amino acids that is/are coupled to the non-polar moiety of the peptide/peptoid. Accordingly, in some embodiments the polar moiety of the

peptide/peptoid consists of two amino acids that are covalently coupled via an amide bond, both amino acids carrying a polar peptide/peptoid side chain. One of these two amino acids may be a terminal amino aid of the peptide/peptoid, defining its N- or C-terminus. In some embodiments the amphiphilic peptide/peptoid has a single amino acid with a polar side chain with the residual portion of the peptide/peptoid defining the non-polar moiety. In some embodiments the amphiphilic peptide/peptoid has two amino acids with a polar side chain while the residual portion of the peptide/peptoid defines the non-polar moiety. As three illustrative examples of a respective polar side chain may serve 4-methyl-4-thio-pentyl, 6-ethoxycarbonyl-4,5-dimethyl-hexyl and 6-hydroxy-4-(1-hydroxyethyl)-hexyl groups. As used herein, the numbering of corresponding peptide/peptoid side chains is started with "1" at the carbon atom that is covalently bonded to the α-carbon atom of the amino acid or to the amino group of the amino acid, respectively. Amino acids included in the polar moiety may be or include, but are not limited to, aspartic acid, asparagine, glutamic acid, 4-fluoro-glutamic acid, 2-aminoadipic acid, γ-carboxy-glutamic acid, 4-tert-butyl aspartic acid, glutamine, 5-N-ethyl-glutamine (theanine), citrulline, thio-citrulline, cysteine, homocysteine, methionine, ethionine, selenomethionine, telluromethionine, threonine, allo-threonine, serine, homoserine, arginine, homoarginine, ornithine, lysine, 5-hydroxy-lysine and N(6)-carboxymethyllysine. Any such amino acid may be present in the L-or D-form.

The amphiphilic linear sequence of the amphiphilic peptide/peptoid of an embodiment of the invention can be defined as having *n* amino acids. Where a single amino acid with a polar side chain is included in the amphiphilic linear sequence, the non-polar moiety may then be taken to have *n-1* amino acids. In this case the polar moiety consists of exactly one amino acid, such amino acid being selected from any amino acids of the foregoing paragraph. Where two consecutive amino acids with a polar side chain are included in the amphiphilic linear sequence of the peptide/peptoid, the non-polar moiety may then be taken to have *n-2* amino acids. In this case the polar moiety consists of exactly two amino acids. Where three consecutive amino acids with a polar side chain are included in the amphiphilic linear sequence, the non-polar moiety may then be taken to have *n-3* amino acids. In this case the polar moiety consists of exactly three amino acids. In embodiments where the polar moiety consists of two amino acids, the polar moiety may have a sequence selected from Asn-Asn, Asp-Asp, Glu-Glu, Gln-Gln, Asn-Gln, Gln-Asn, Asp-Gln, Gln-Asp, Asn-Glu, Glu-Asn, Asp-Glu, Glu-Asp, Gln-Glu, Glu-Gln, Asp-Asn, Asn-Asp, Thr-Thr, Ser-Ser, Thr-Ser, Ser-Thr, Asp-Ser, Ser-Asp, Ser-Asn, Asn-Ser, Gln-Ser, Ser-Gln, Glu-Ser, Ser-Glu, Asp-Thr, Thr-Asp, Thr-

Asn, Asn-Thr, Gln-Thr, Thr-Gln, Glu-Thr, Thr-Glu, Cys-Asp, Cys-Lys, Cys-Ser, Cys-Thr, Cys-Orn, Cys-Dab, Cys-Dap, Lys-Lys, Lys-Ser, Lys-Thr, Lys-Orn, Lys-Dab, Lys-Dap, Ser-Lys, Ser-Orn, Ser-Dab, Ser-Dap, Orn-Lys, Orn-Orn, Orn-Ser, Orn-Thr, Orn-Dab, Orn-Dap, Dab-Lys, Dab-Ser, Dab-Thr, Dab-Orn, Dab-Dab, Dab-Dap, Dap-Lys, Dap-Ser, Dap-Thr, Dap-Orn, Dap-Dab, Dap-Dap. In embodiments where the polar moiety consists of three amino acids, the polar moiety may have a sequence selected from Asn-Asn-Asn, Asn-Asn-Asp, Asn-Asp-Asn, Asp-Asn-Asn, Asp-Asp-Asn, Asp-Asn-Asp, Asp-Asp-Asp, Asn-Asn-Glu, Asn-Asn-Gln, Asn-Glu-Asn, Asn-Gln-Asn, Glu-Glu-Glu, Gln-Gln-Gln, Asn-Gln-Gln, Asn-Glu-Gln, Asp-Asn-Glu, Gln-Asn-Asn, Gln-Asn-Asn, Glu-Asp-Gln, Asp-Gln-Asp, Asn-Glu-Asp, Glu-Asn-Gln, Asp-Glu-Gln, Asn-Glu-Gln, Glu-Asp-Asn, and Gln-Asp-Asn, Thr-Thr-Thr, Ser-Ser-Ser, Asn-Thr-Thr, Asn-Ser-Ser Asn-Ser-Thr, Asn-Thr-Ser Asp-Asn-Ser, Ser-Asn-Asn, Thr-Asn-Asn, Ser-Asp-Thr, to name a few.

The amphiphilic linear sequence of the peptide/peptoid has a net charge at physiological pH. The term" physiological pH" is known to those in the art to refer to the pH value of blood, which has typically a pH value of about 7.4. In embodiments where the amphiphilic linear sequence is arranged at the C- or N-terminus of the peptide/peptoid, the respective terminus may provide the corresponding net charge. In embodiments where the amphiphilic linear sequence is not arranged at the C- or N-terminus of the peptide/peptoid, the polar moiety of the amphiphilic linear sequence includes one or more amino acids that have a side chain with a functional group that is charged at physiological pH. Illustrative examples of a respective functional group include an amino, a nitro-, a guanidino, a esteryl, a sulfonyl or a carboxyl group. In some embodiments the net charge of the amphiphilic linear sequence is, as a positive or as a negative charge, equal to or smaller than the number of amino acids included in the polar moiety thereof. In some embodiments the net charge of the amphiphilic linear sequence is one of -3, -2 or -1. In some embodiments the net charge of the amphiphilic linear sequence is +1, +2 or +3.

The respective polar side chain of an amino acid of the polar moiety, coupled to the α-carbon atom of the amino acid (supra) and/or to the amino group thereof, may typically be defined by a main chain that includes 1 to about 20, including 1 to about 15, 1 to about 10 or 1 to about 5 carbon atoms. For sake of clarity it is recited that the term "side chain" is used relative to the backbone of the peptide and/or peptoid. This peptide and/or peptoid side chain may be branched and thus be defined by a main chain and branches. Both the main chain and

branches, if present, of the peptide and/or peptoid side chain may include one or more double or triple bonds (supra). Examples of side chains include, but are not limited to, methyl, ethyl, propyl, isopropyl, propenyl, propinyl, butyl, butenyl, *sec*-butyl, *tert*-butyl, isobutyl, pentyl, neopentyl, isopentyl, pentenyl, hexyl, 3,3 dimethylbutyl, heptyl, octyl, nonyl or decyl groups. The functional polar group is bonded to this the peptide and/or peptoid side chain.

In some embodiments the polar moiety of the amphiphilic linear sequence includes two identical amino acids. Where these amino acids are naturally occurring amino acids, they may for example define one of the sequences Lys-Lys, Gln-Gln, Glu-Glu, Asp-Asp, Asn-Asn, Met-Met, Thr-Thr, Arg-Arg or Ser-Ser. The term "naturally occurring" in this context refers to the 20 amino acids into which the genetic code is directly being translated by any organism. Such two identical polar amino acids may for example be adjacent to the non-polar moiety.

In some embodiments the amphiphilic linear sequence of the peptide/peptoid has a hydrophobic tail of aliphatic amino acids and at least one polar, including a charged, amino acid head group.

The non-polar moiety includes an amino acid, generally at least two amino acids, with a hydrocarbon chain that does not carry a functional group. The respective side chain, coupled to the α-carbon atom of the amino acid (supra), may have a main chain that includes 0 to about 20 or 1 to about 20, including 0 to about 15, 1 to about 15, 0 to about 10, 1 to about 10, 1 to about 5 or 0 to about 5 carbon atoms. The non-polar moiety may thus include an amino acid without side chain, i.e. glycine. The peptide and/or peptoid side chain may be branched (supra) and include one or more double or triple bonds (supra). Examples of peptide and/or peptoid side chains include, but are not limited to, methyl, ethyl, propyl, isopropyl, propenyl, propinyl, butyl, butenyl, *sec*-butyl, *tert*-butyl, isobutyl, pentyl, neopentyl, isopentyl, pentenyl, hexyl, 3,3 dimethylbutyl, heptyl, octyl, nonyl or decyl groups. As a few illustrative examples, the non-polar moiety may include an amino acid of alanine, valine, leucine, isoleucine, norleucine, norvaline, 2-(methylamino)-isobutyric acid, 2-amino-5-hexynoic acid. Such an amino acid may be present in any desired configuration. Bonded to the non-polar moiety may also be the C-terminus or the N-terminus of the peptide/peptoid. Typically the C-terminus or the N-terminus is in such a case shielded by a protecting group (supra).

In some embodiments the non-polar moiety includes a sequence of amino acids that is arranged in decreasing or increasing size. Hence, a portion of the amino acids of the non-polar moiety may be arranged in a general sequence of decreasing or increasing size. Relative to the direction from N- to C-terminus or from C- to N-terminus this general sequence can thus

be taken to be of decreasing size. By the term “general sequence” of decreasing or increasing size is meant that embodiments are included in which adjacent amino acids are of about the same size as long as there is a general decrease or increase in size. Within a general sequence of decreasing size the size of adjacent amino acids of the non-polar moiety is accordingly identical or smaller in the direction of the general sequence of decreasing size. In some embodiments the general sequence of decreasing or increasing size is a non-repetitive sequence.

As an illustrative example, where a respective portion of amino acids is a sequence of five amino acids, the first amino acid may have a 3,4-dimethyl-hexyl side chain. The second amino acid may have a neopentyl side chain. The third amino acid may have a pentyl side chain. The fourth amino acid may have a butyl side chain. The fifth amino acid may be glycine, i.e. have no side chain. Although a neopently and a pentyl side chain are of the same size, the general sequence of such a non-polar peptide portion is decreasing in size. As a further illustrative example of a general sequence of decreasing size in a non-polar moiety the respective non-polar portion may be a sequence of three amino acids. The first amino acid may have an n-nonyl side chain. The second amino acid may have a 3-ethyl-2-methyl-pentyl side chain. The third amino acid may have a tert-butyl side chain. As yet a further illustrative example of a general sequence of decreasing size in a non-polar moiety, the non-polar moiety may be a sequence of nine amino acids. The first amino acid may have a 4-propyl-nonyl side chain. The second amino acid may have an n-dodecyl side chain. The third amino acid may have a 6,6-diethyl-3-octenyl side chain. An n-dodecyl side chain and a 6,6-diethyl-3-octenyl side chain both have 12 carbon atoms and thus again have a comparable size, Nevertheless, the 6,6-diethyl-3-octenyl group includes an unsaturated carbon-carbon bond and is thus of slightly smaller size than the dodecyl group. The fourth amino acid may have a 2-methyl-nonyl side chain. The fifth amino acid may have a 3-propyl-hexyl side chain. The sixth amino acid may have an n-hexyl side chain. The seventh amino acid may have a 2-butynyl side chain. The 8th amino acid may have an isopropyl side chain. The ninth amino acid may have a methyl side chain.

Where a portion of the amino acids of the non-polar moiety arranged in a general sequence of decreasing (or increasing) size only contains naturally occurring amino acids (whether in the D- or the L-form), it may for example have a length of five amino acids, such as the sequence leucine-isoleucine-valine-alanine-glycine or isoleucine-leucine-valine-alanine-glycine, A general sequence of decreasing size of only natural amino acids may also have a length of four amino acids. Illustrative examples include the sequences isoleucine-leucine-valine-

alanine, leucine-isoleucine-valine-alanine, isoleucine-valine-alanine-glycine, leucine-valine-alanine-glycine, leucine-isoleucine-alanine-glycine, leucine-isoleucine-valine-glycine, isoleucine-leucine-alanine-glycine or isoleucine-leucine-valine-glycine. A general sequence of decreasing size of only natural amino acids may also have a length of three amino acids. Illustrative examples include the sequences isoleucine-valine-alanine, leucine-valine-alanine, isoleucine-valine-glycine, leucine-valine-glycine, leucine-alanine-glycine, isoleucine-alanine-glycine or isoleucine-leucine-alanine. A general sequence of decreasing size of only natural amino acids may also have a length of two amino acids. Illustrative examples include the sequences isoleucine-valine, leucine-valine, isoleucine-alanine, leucine-alanine, leucine-glycine, isoleucine-glycine, valine-alanine, valine-glycine or alanine-glycine.

In some embodiments the direction of decreasing size of the above defined general sequence of decreasing size is the direction toward the polar moiety of the amphiphilic linear sequence. Accordingly, in such embodiments the size of adjacent amino acids within this portion of the non-polar moiety is accordingly identical or smaller in the direction of the polar moiety. Hence, as a general trend in such an embodiment, the closer to the polar moiety of the amphiphilic linear sequence, the smaller is the overall size of a peptide and/or peptoid side chain throughout the respective general sequence of decreasing size. In the above illustrative example of a general sequence of three amino acids with a n-nonyl, a 3-ethyl-2-methyl-pentyl and a tert-butyl side chain, the next amino acid may be polar in that it carries a peptide/peptoid side chain with a polar functional group. As an illustrative example, adjacent to the tert-butyl side chain within the peptide/peptoid there may be a 3-carboxy-n-butyl side chain.

In some embodiments the entire non-polar moiety of the amphiphilic linear peptide and/or peptoid or the amphiphilic linear sequence, respectively, consists of the general sequence of decreasing (or increasing) size. In such an embodiment the general sequence of decreasing (or increasing) size may have a length of *n-m* amino acids (cf. above). In some embodiments the general sequence of decreasing or increasing size is flanked by further non-polar side chains of the peptide/peptoid. In one embodiment the general sequence of decreasing (or increasing) size has a length of *n-m-1* amino acids. In this embodiment there is one further amino acid included in the peptide/peptoid, providing a non-polar peptide/peptoid side chain. This amino acid may be positioned between the general sequence of decreasing (or increasing) size and the polar amino acid, the polar amino acid may be positioned between this additional non-polar amino acid and the general sequence of decreasing (or increasing) size or the general sequence of decreasing (or increasing) size may be positioned between the polar

amino acid and this additional non-polar amino acid. Typically the general sequence of decreasing (or increasing) size is positioned between the polar amino acid and this additional non-polar amino acid. The additional non-polar amino acid may for example define the N-terminus of the peptide/peptoid, which may be shielded by a protecting group such as an amide, e.g. a propionic acyl or an acetyl group. Together with the general sequence of decreasing (or increasing) size as defined above it may define the non-polar portion of the peptide/peptoid. The polar amino acid may define the C-terminus of the peptide/peptoid. In this example the general sequence of decreasing (or increasing) size is thus flanked by the polar amino acid on one side and by the additional non-polar amino acid on the other side. In one embodiment where embodiment the general sequence of decreasing (or increasing) size has a length of *n-m-1* amino acids, the remaining non-polar amino acid of the non-polar moiety of n-m amino acids is one of alanine and glycine.

As explained above, the polar moiety of the amphiphilic linear sequence may in some embodiments be defined by two or three consecutive amino acids. The polar moiety includes *m* aliphatic amino acids. Each of the *m* aliphatic amino acids is independently selected and carries an independently selected polar group. The symbol *m* represents an integer selected from 1, 2 and 3. The at least essentially non-polar moiety (supra) accordingly has a number *of n-m*, i.e. *n-1, n-2* or *n-3*amino acids. In some embodiments *n* is equal to or larger than *m + 2*. In such an embodiment *m* may thus represent a number of *n-2* or smaller.

In an embodiment where the entire non-polar moiety of the amphiphilic linear peptide and/or peptoid consists of the general sequence of decreasing (or increasing) size (supra), this non-polar moiety may thus have a length of *n-2* or *n-3* amino acids. In an embodiment where the amphiphilic linear peptide and/or peptoid has a further non-polar side chain in addition to the non-polar moiety of decreasing (or increasing) size, this additional non-polar side chain may be included in an amino acid that is directly bonded to an amino acid of the general sequence of decreasing (or increasing) size. The non-polar moiety may thus be defined by the non-polar moiety of decreasing (or increasing) size and the respective further amino acid with a non-polar side chain. In one such an embodiment where *m* = 1, the non-polar moiety may thus have a length of *n-2* amino acids, of which the non-polar moiety of decreasing (or increasing) size has a length of *n-3* amino acids. The general sequence of decreasing (or increasing) size may be positioned between the two polar amino acids and this additional non-polar amino acid, or the additional non-polar amino acid may be positioned between the general sequence of decreasing (or increasing) size and the two polar amino acids. Typically the

general sequence of decreasing (or increasing) size is positioned between the two polar amino acids and this additional non-polar amino acid. As mentioned above, one of the two polar amino acids may define the C-terminus of the peptide/peptoid. In this example the general sequence of decreasing (or increasing) size may thus be flanked by the two consecutive polar amino acids on one side and by the additional non-polar amino acid on the other side. Again, in some embodiments where $m = 1$ the two consecutive polar amino acids may also be positioned between the general sequence of decreasing (or increasing) size and the additional non-polar amino acid, in which case the non-polar moiety has a first portion with a length of *n-3* amino acids and a further portion of one amino acid.

Electrostatic forces, hydrogen bonding and van der Waals forces between amphiphilic linear sequences as defined above, including amphiphilic linear peptides and/or peptoids, result in these amphiphilic linear sequences to be coupled to each other. Without being bound by theory, thereby a cross-linking effect occurs that allows the formation of a hydrogel. In this regard the inventors have observed the formation of fibers based on helical structures.

The fibers formed of amphiphilic linear sequences of amphiphilic peptides and/or peptoids of an embodiment of the invention typically show high mechanical strength, which renders them particularly useful in tissue regeneration applications, for instance the replacement of damaged tissue. Amphiphilic peptides and/or peptoids of an embodiment of the invention have been observed to generally assemble into a fiber structure that resembles collagen fibers. Collagen, a component of soft tissue in the animal and human body, is a fibrous protein that provides most of the tensile strength of tissue. The mechanical strength of fibers of amphiphilic peptides and/or peptoids of an embodiment of the invention has been found to typically be much higher than that of collagen and of gelatine, the hydrolysed form of collagen. An amphiphilic peptide and/or peptoid of an embodiment of the invention may thus be included in a hydrogel that is used as permanent or temporary prosthetic replacement for damaged or diseased tissue.

The amphiphilic linear sequence of the peptide/peptoid, which may represent the entire amphiphilic peptide/peptoid (supra) has been found to show remarkable stability at physiological conditions, even at elevated temperatures. It is in some embodiments stable in aqueous solution at physiological conditions at ambient temperature for a period of time in the range from 1 day to 1 month or more. It may in some embodiments be stable in aqueous solution at physiological conditions at 90 °C for at least 1 hour, at least 2 hours, at least 3 hours, at least 4 hours or at least 5 hours.

An amphiphilic linear sequence of an amphiphilic peptide and/or peptoid of an embodiment of the invention, including an amphiphilic linear peptide and/or peptoid, is capable of providing a self assembling α-helical fiber in aqueous solution under physiological conditions. The peptides/peptoids (typically 3-7-mers) in the L- or D-form can self assemble into supramolecular helical fibers which are organized into mesh-like structures mimicking biological substances such as collagen. It has previously been observed in X-ray crystallography that peptides of a length of 3 to 6 amino acids with repetitive alanine containing sequences and an acetylated C-terminus take a helical conformation (Hatakeyama, Y, et al, Angew. Chem. Int. Ed. (2009) 8695-8698). Using peptides with an amphiphilic sequence of an embodiment of the invention, AcLD6 (L), the formation of aggregates has for example been observed already at 0.1 mg/ml. As the concentration of peptide is increased to 1 mg/ml, the peptide monomers were found to align to form fibrous structures. With a formation of fibers occurring under physiological conditions at concentrations below 2 mM a peptide/peptoid of an embodiment of the invention is well suited as an injectable hydrogel material that can form a hydrogel under physiological conditions. An embodiment of the invention thus also relates to an amphiphilic linear peptide and/or peptoid as defined above for tissue engineering as well as to a tissue engineering method that involves applying, including injecting a respective amphiphilic linear peptide and/or peptoid.

A hydrogel according to an embodiment of the present invention is typically characterized by a remarkable rigidity and are generally biocompatible and non-toxic. Depending on the selected peptide/peptoid sequence these hydrogels can show thermoresponsive or thixotropic character. Reliant on the peptide/peptoid assembling conditions the fibers differ in thickness and length. Generally rigid hydrogels are obtained that are well suited for cultivation of a variety of primary human cells, providing peptide/peptoid scaffolds that can be useful in the repair and replacement of various tissues. Disclosed is also a process of preparing these hydrogels. The exemplary usage of these hydrogels in applications such as cell culture, tissue engineering, orthopaedic surgery, aesthetic/plastic surgery, drug and vaccine delivery, oral applications, cosmetics, packaging and the like is described, as well as for technical applications, as for example for use in electronic devices which might include solar or fuel cells.

As an amphiphilic linear sequence of the peptide/peptoid, a hydrogel of an embodiment of the invention shows high stability at physiological conditions, even at elevated temperatures. In some embodiments such a hydrogel is stable in aqueous solution at ambient temperature

for a period of at least 7 days, at least 14 days, at least a month or more, such as at least 1 to about 6 months.

In some embodiments a hydrogel disclosed herein is coupled to a molecule or a particle, including a quantum dot, with characteristic spectral or fluorometric properties, such as a marker, including a fluorescent dye and a MRI contrast agent. A respective molecule may for instance allow monitoring the fate, position and/or the integrity of the hydrogel.

In some embodiments a hydrogel disclosed herein is coupled to a molecule with binding affinity for a selected target molecule, such as a microorganism, a virus particle, a peptide, a peptoid, a protein, a nucleic acid, a peptide, an oligosaccharide, a polysaccharide, an inorganic molecule, a synthetic polymer, a small organic molecule, a drug or a cell.

The term "nucleic acid" or nucleid acid molecule" as used herein refers to any nucleic acid in any possible configuration, such as single stranded, double stranded or a combination thereof. Nucleic acids include for instance DNA molecules (e.g., cDNA or genomic DNA), RNA molecules (e.g., mRNA), analogues of the DNA or RNA generated using nucleotide analogues or using nucleic acid chemistry, locked nucleic acid molecules (LNA), and protein nucleic acids molecules (PNA). DNA or RNA may be of genomic or synthetic origin and may be single or double stranded. In the present method of an embodiment of the invention typically, but not necessarily, an RNA or a DNA molecule will be used. Such nucleic acid can be e.g. mRNA, cRNA, synthetic RNA, genomic DNA, cDNA synthetic DNA, a copolymer of DNA and RNA, oligonucleotides, etc. A respective nucleic acid may furthermore contain non-natural nucleotide analogues and/or be linked to an affinity tag or a label. In some embodiments the nucleic acid molecule may be isolated, enriched, or purified. The nucleic acid molecule may for instance be isolated from a natural source by cDNA cloning or by subtractive hybridization. The natural source may be mammalian, such as human, blood, semen, or tissue. The nucleic acid may also be synthesized, e.g. by the triester method or by using an automated DNA synthesizer.

Many nucleotide analogues are known and can be used in nucleic acids and oligonucleotides used in the methods of exemplary embodiments of the invention. A nucleotide analogue is a nucleotide containing a modification at for instance the base, sugar, or phosphate moieties. Modifications at the base moiety include natural and synthetic modifications of A, C, G, and T/U, different purine or pyrimidine bases, such as uracil-5-yl, hypoxanthin-9-yl, and 2-

aminoadenin-9-yl, as well as non-purine or non-pyrimidine nucleotide bases. Other nucleotide analogues serve as universal bases. Universal bases include 3-nitropyrrole and 5-nitroindole. Universal bases are able to form a base pair with any other base. Base modifications often can be combined with for example a sugar modification, such as for instance 2'-O-methoxyethyl, e.g. to achieve unique properties such as increased duplex stability.

A peptide may be of synthetic origin or isolated from a natural source by methods well-known in the art. The natural source may be mammalian, such as human, blood, semen, or tissue. A peptide, including a polypeptide may for instance be synthesized using an automated polypeptide synthesizer. Illustrative examples of polypeptides are an antibody, a fragment thereof and a proteinaceous binding molecule with antibody-like functions. Examples of (recombinant) antibody fragments are Fab fragments, Fv fragments, single-chain Fv fragments (scFv), diabodies, triabodies (Iliades, P., et al., FEBS Lett (1997) 409, 437-441), decabodies (Stone, E., et al., Journal of Immunological Methods (2007) 318, 88–94) and other domain antibodies (Holt, L.J., et al., Trends Biotechnol. (2003), 21, 11, 484-490). An example of a proteinaceous binding molecule with antibody-like functions is a mutein based on a polypeptide of the lipocalin family (WO 03/029462, Beste et al., Proc. Natl. Acad. Sci. U.S.A. (1999) 96, 1898-1903). Lipocalins, such as the bilin binding protein, the human neutrophil gelatinase-associated lipocalin, human Apolipoprotein D or glycodelin, posses natural ligand-binding sites that can be modified so that they bind to selected small protein regions known as haptens. Examples of other proteinaceous binding molecules are the so-called glubodies (see e.g. internation patent application WO 96/23879), proteins based on the ankyrin scaffold (Mosavi, L.K., et al., Protein Science (2004) 13, 6, 1435-1448) or crystalline scaffold (e.g. internation patent application WO 01/04144) the proteins described in Skerra, J. Mol. Recognit. (2000) 13, 167-187, AdNectins, tetranectins and avimers. Avimers contain so called A-domains that occur as strings of multiple domains in several cell surface receptors (Silverman, J., et al., Nature Biotechnology (2005) 23, 1556-1561). Adnectins, derived from a domain of human fibronectin, contain three loops that can be engineered for immunoglobulin-like binding to targets (Gill, D.S. & Damle, N.K., Current Opinion in Biotechnology (2006) 17, 653-658). Tetranectins, derived from the respective human homotrimeric protein, likewise contain loop regions in a C-type lectin domain that can be engineered for desired binding (ibid.). Where desired, a modifying agent may be used that further increases the affinity of the respective moiety for any or a certain form, class etc. of target matter.

An example of a nucleic acid molecule with antibody-like functions is an aptamer. An aptamer folds into a defined three-dimensional motif and shows high affinity for a given target structure. Using standard techniques of the art such as solid-phase synthesis an aptamer with affinity to a certain target can accordingly be formed and immobilized on a hollow particle of an embodiment of the invention.

As a further illustrative example, a linking moiety such as an affinity tag may be used to immobilise the respective molecule. Such a linking moiety may be a molecule, e.g. a hydrocarbon-based (including polymeric) molecule that includes nitrogen-, phosphorus-, sulphur-, carben-, halogen- or pseudohalogen groups, or a portion thereof. As an illustrative example, the peptide/peptoid included in the hydrogel may include functional groups, for instance on a side chain of the peptide/peptoid, that allow for the covalent attachment of a biomolecule, for example a molecule such as a protein, a nucleic acid molecule, a polysaccharide or any combination thereof. A respective functional group may be provided in shielded form, protected by a protecting group that can be released under desired conditions. Examples of a respective functional group include, but are not limited to, an amino group, an aldehyde group, a thiol group, a carboxy group, an ester, an anhydride, a sulphonate, a sulphonate ester, an imido ester, a silyl halide, an epoxide, an aziridine, a phosphoramidite and a diazoalkane.

Examples of an affinity tag include, but are not limited to, biotin, dinitrophenol or digoxigenin, oligohistidine, polyhistidine, an immunoglobulin domain, maltose-binding protein, glutathione-S-transferase (GST), calmodulin binding peptide (CBP), FLAG'-peptide, the T7 epitope (Ala-Ser-Met-Thr-Gly-Gly-Gln-Gln-Met-Gly), maltose binding protein (MBP), the HSV epitope of the sequence Gln-Pro-Glu-Leu-Ala-Pro-Glu-Asp-Pro-Glu-Asp of herpes simplex virus glycoprotein D, the hemagglutinin (HA) epitope of the sequence Tyr-Pro-Tyr-Asp-Val-Pro-Asp-Tyr-Ala, the "myc" epitope of the transcription factor c-myc of the sequence Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu, or an oligonucleotide tag. Such an oligonucleotide tag may for instance be used to hybridise to an immobilised oligonucleotide with a complementary sequence. A further example of a linking moiety is an antibody, a fragment thereof or a proteinaceous binding molecule with antibody-like functions (see also above).

A further example of linking moiety is a cucurbituril or a moiety capable of forming a complex with a cucurbituril. A cucurbituril is a macrocyclic compound that includes glycoluril units, typically self-assembled from an acid catalyzed condensation reaction of glycoluril and formaldehyde. A cucurbit[n]uril, (CB[n]), that includes n glycoluril units, typically has two

portals with polar ureido carbonyl groups. Via these ureido carbonyl groups cucurbiturils can bind ions and molecules of interest. As an illustrative example cucurbit[7]uril (CB[7]) can form a strong complex with ferrocenemethylammonium or adamantylammonium ions. Either the cucurbit[7]uril or e.g. ferrocenemethylammonium may be attached to a biomolecule, while the remaining binding partner (e.g. ferrocenemethylammonium or cucurbit[7]uril respectively) can be bound to a selected surface. Contacting the biomolecule with the surface will then lead to an immobilisation of the biomolecule. Functionalised CB[7] units bound to a gold surface via alkanethiolates have for instance been shown to cause an immobilisation of a protein carrying a ferrocenemethylammonium unit (Hwang, I., et al., J. Am. Chem. Soc. (2007) 129, 4170-4171).

Further examples of a linking moiety include, but are not limited to an oligosaccharide, an oligopeptide, biotin, dinitrophenol, digoxigenin and a metal chelator (cf. also below). As an illustrative example, a respective metal chelator, such as ethylenediamine, ethylenediaminetetraacetic acid (EDTA), ethylene glycol tetraacetic acid (EGTA), diethylenetriaminepentaacetic acid (DTPA), N,N-bis(carboxymethyl)glycine (also called nitrilotriacetic acid, NTA), 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA), 2,3-dimercapto-1-propanol (dimercaprol), porphine or heme may be used in cases where the target molecule is a metal ion. As an example, EDTA forms a complex with most monovalent, divalent, trivalent and tetravalent metal ions, such as e.g. silver (Ag^{+}), calcium (Ca^{2+}), manganese (Mn^{2+}), copper (Cu^{2+}), iron (Fe^{2+}), cobalt (Co^{3+}) and zirconium (Zr^{4+}), while BAPTA is specific for Ca^{2+}. In some embodiments a respective metal chelator in a complex with a respective metal ion or metal ions defines the linking moiety. Such a complex is for example a receptor molecule for a peptide of a defined sequence, which may also be included in a protein. As an illustrative example, a standard method used in the art is the formation of a complex between an oligohistidine tag and copper (Cu^{2+}), nickel (Ni^{2+}), cobalt (Co^{2+}), or zink (Zn^{2+}) ions, which are presented by means of the chelator nitrilotriacetic acid (NTA).

Avidin or streptavidin may for instance be employed to immobilise a biotinylated nucleic acid, or a biotin containing monolayer of gold may be employed (Shumaker-Parry, J.S., et al., Anal. Chem. (2004) 76, 918). As yet another illustrative example, the biomolecule may be locally deposited, e.g. by scanning electrochemical microscopy, for instance via pyrrole-oligonucleotide patterns (e.g. Fortin, E., et al., Electroanalysis (2005) 17, 495). In other embodiments, in particular where the biomolecule is a nucleic acid, the biomolecule may be directly synthesised on the surface of the immobilisation unit, for example using photoactiva-

tion and deactivation. As an illustrative example, the synthesis of nucleic acids or oligonucleotides on selected surface areas (so called “solid phase” synthesis) may be carried out using electrochemical reactions using electrodes. An electrochemical deblocking step as described by Egeland & Southern (Nucleic Acids Research (2005) 33, 14, e125) may for instance be employed for this purpose. A suitable electrochemical synthesis has also been disclosed in US patent application US 2006/0275927. In some embodiments light-directed synthesis of a biomolecule, in particular of a nucleic acid molecule, including UV-linking or light dependent 5’-deprotection, may be carried out.

The molecule that has a binding affinity for a selected target molecule may be immobilised on the nanocrystals by any means. As an illustrative example, an oligo- or polypeptide, including a respective moiety, may be covalently linked to the surface of nanocrystals via a thio-ether-bond, for example by using ω functionalized thiols. Any suitable molecule that is capable of linking a nanocrystal of an embodiment of the invention to a molecule having a selected binding affinity may be used to immobilise the same on a nanocrystal. For instance a (bifunctional) linking agent such as ethyl-3-dimethylaminocarbodiimide, N-(3-aminopropyl) 3-mercapto-benzamide, 3-aminopropyl-trimethoxysilane, 3-mercaptopropyl-trimethoxysilane, 3-(trimethoxysilyl) propyl-maleimide, or 3-(trimethoxysilyl) propyl-hydrazide may be used. Prior to reaction with the linking agent, the surface of the nanocrystals can be modified, for example by treatment with glacial mercaptoacetic acid, in order to generate free mercaptoacetic groups which can then employed for covalently coupling with an analyte binding partner via linking agents.

Embodiments of the present invention also include a hydrogel, which can be taken to be a water-swollen water-insoluble polymeric material. The hydrogel includes, including contains and consists of, a peptide and/or peptoid as defined above. Since a hydrogel maintains a three-dimensional structure, a hydrogel of an embodiment of the invention may be used for a variety of applications. Since the hydrogel has a high water content and includes amino acids, it is typically of excellent biocompatibility.

A hydrogel according to an embodiment of the invention is typically formed by self-assembly. The inventors have observed that the peptides/peptoids assemble into fibers that form mesh-like structures. Without being bound by theory hydrophobic interaction between non-polar portions of peptides/peptoids of an embodiment of the invention are contemplated to assist such self-assembly process.

The method of forming the hydrogel includes dissolving the peptide/peptoid in aqueous solution. Agitation, including mixing such as stirring, and/or sonication may be employed to facilitate dissolving the peptide/peptoid. In some embodiments the aqueous solution with the peptide/peptoid therein is exposed to a temperature below ambient temperature, such as a temperature selected from about 2 °C to about 15 °C. In some embodiments the aqueous solution with the peptide/peptoid therein is exposed to an elevated temperature, i.e. a temperature above ambient temperature. Typically the aqueous solution is allowed to attain the temperature to which it is exposed. The aqueous solution may for example be exposed to a temperature from about 25 °C to about 85 °C or higher, such as from about 25 °C to about 75 °C, from about 25 °C to about 70 °C, from about 30 °C to about 70 °C, from about 35 °C to about 70 °C, from about 25 °C to about 60 °C, from about 30 °C to about 60 °C, from about 25 °C to about 50 °C, from about 30 °C to about 50 °C or from about 40 °C to about 65 °C, such as e.g. a temperature of about 40 °C, about 45 °C, about 50 °C, about 55 °C, about 60 °C or about 65 °C. The aqueous solution with the peptide/peptoid therein may be maintained at this temperature for a period of about 5 min to about 10 hours or more, such as about 10 min to about 6 hours, about 10 min to about 4 hours, about 10 min to about 2.5 hours, about 5 min to about 2.5 hours, about 10 min to about 1.5 hours or about 10 min to about 1 hour, such as about 15 min, about 20 min, about 25 min, about 30 min, about 35 min or about 40 min.

A hydrogel according to an embodiment of the invention may be included in a fuel cell, where it may for example provide a substrate between the anode and the cathode. a liquid electrolyte may be encompassed by the hydrogel. Likewise, a hydrogel according to an embodiment of the invention may provide a substrate between two electrodes in an electrophoresis apparatus. The hydrogel may also be conducting. The hydrogel may also serve in enhancing the efficiency of charge-separated states and/or slowing down charge recombination. The hydrogel may thus be applied in any form photovoltaics, including a solar cell.

In some embodiments a hydrogel disclosed herein is a biocompatible, including a pharmaceutically acceptable hydrogel. The term "biocompatible" (which also can be referred to as "tissue compatible"), as used herein, is a hydrogel that produces little if any adverse biological response when used in vivo. The term thus generally refers to the inability of a hydrogel to promote a measurably adverse biological response in a cell, including in the body of an animal, including a human. A biocompatible hydrogel can have one or more of the following properties: non-toxic, non-mutagenic, non-allergenic, non-carcinogenic, and/or non-irritating. A biocompatible hydrogel, in the least, can be innocuous and tolerated by the respective cell

and/or body. A biocompatible hydrogel, by itself, may also improve one or more functions in the body.

Depending on the amino acids that are included in the peptide/peptoid that is included in a hydrogel, a respective hydrogel may be biodegradable. A biodegradable hydrogel gradually disintegrates or is absorbed in vivo over a period of time, e.g., within months or years. Disintegration may for instance occur via hydrolysis, may be catalysed by an enzyme and may be assisted by conditions to which the hydrogel is exposed in a human or animal body, including a tissue, a blood vessel or a cell thereof. Where a peptide is made up entirely of natural amino acids, a respective peptide can usually be degraded by enzymes of the human/animal body.

A hydrogel according to an embodiment of the invention may also serve as a depot for a pharmaceutically active compound such as a drug and/or micro- and nanoparticles. A hydrogel according to an embodiment of the invention may be designed to mimic the natural extracellular matrix of an organism such as the human or animal body. A fiber formed from the peptide/peptoid of an embodiment of the invention, including a respective hydrogel, may serve as a biological scaffold. A hydrogel of an embodiment of the invention may be included in an implant, in a contact lens or may be used in tissue engineering. In one embodiment, the peptides consist typically of 3-7 amino acids and are able to self-assemble into complex fibrous scaffolds which are seen as hydrogels, when dissolved in water or aqueous solution. These hydrogels can retain water up to 99.9% and possess sufficiently high mechanical strength. Thus, these hydrogels can act as artificial substitutes for a variety of natural tissues without the risk of immunogenicity. The hydrogels in accordance with the present invention may be used for cultivating suitable primary cells and thus establish an injectable cell-matrix compound in order to implant or re-implant the newly formed cell-matrix in vivo. Therefore, the hydrogels in accordance with the present invention are particularly useful for tissue regeneration or tissue engineering applications. As used herein, a reference to an "implant" or "implantation" refers to uses and applications of/for surgical or arthroscopic implantation or injection of a hydrogel containing device (or of a peptide solution that gels in situ) into a human or animal, e.g. mammalian, body or limb. Arthroscopic techniques are taken herein as a subset of surgical techniques, and any reference to surgery, surgical, etc., includes arthroscopic techniques, methods and devices. A surgical implant that includes a hydrogel according to an embodiment of the invention may include a peptide and/or peptoid scaffold. This the peptide and/or peptoid scaffold may be defined by the respective hydrogel. A hydrogel of an embodiment of the invention may also be included in a wound cover such as a gauze or

sheet or membrane or cream or spray, serving in maintaining the wound in a moist state to promote healing.

Depending on the amino acid sequence used in the peptide/peptoid the hydrogel may be temperature-sensitive. It may for instance have a lower critical solution temperature or a temperature range corresponding to such lower critical solution temperature, beyond which the gel collapses as hydrogen bonds by water molecules are released as water molecules are released from the gel.

The disclosed subject matter also provides improved chiral amphiphilic natural-based peptides and/or peptoids that assemble to peptide/peptoid hydrogels with very favorable material properties. The advantage of these peptide/peptoid hydrogels is that they are accepted by a variety of different primary human cells, thus providing peptide scaffolds that can be useful in the repair and replacement of various tissues. Depending on the chirality of the peptide monomer the character of the hydrogels can be designed to be more stable and less prone to degradation though still biocompatible.

A hydrogel and/ or a peptide/peptoid described herein can be administered to an organism, including a human patient per se, or in pharmaceutical compositions where it may include or be mixed with pharmaceutically active ingredients or suitable carriers or excipient(s). Techniques for formulation and administration of respective hydrogels or peptides/peptoids resemble or are identical to those of low molecular weight compounds well established in the art. Exemplary routes include, but are not limited to, oral, transdermal, and parenteral delivery. A hydrogel or a peptide/peptoid may be used to fill a capsule or tube, or may be provided in compressed form as a pellet. The peptide/peptoid or the hydrogel may also be used in injectable or sprayable form, for instance as a suspension of a respective peptide/peptoid.

A hydrogel of an embodiment of the invention may for instance be applied onto the skin or onto a wound. Further suitable routes of administration may, for example, include depot, oral, rectal, transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intravenous, intramedullary injections, as well as intrathecal, direct intraventricular, intraperitoneal, intranasal, or intraocular injections. It is noted in this regard that for administering microparticles a surgical procedure is not required. Where the microparticles include a biodegradable polymer there is no need for device removal after release of the anticancer agent. Nevertheless the microparticles may be included in or on a scaffold, a coating, a patch, composite material, a gel or a plaster.

In some embodiments one may administer a hydrogel and/or a peptide/peptoid in a local rather than systemic manner, for example, via injection.

Pharmaceutical compositions that include a hydrogel and/or a peptide/peptoid of an embodiment of the present invention may be manufactured in a manner that is itself known, e. g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with an embodiment of the present invention thus may be formulated in conventional manner using one or more physiologically acceptable carriers including excipients and auxiliaries that facilitate processing of the hydrogel and/or peptide/peptoid into preparations that can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the peptide/peptoid of an embodiment of the invention may be formulated in aqueous solutions, for instance in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the hydrogel and/or peptide/peptoid can be formulated readily by combining them with pharmaceutically acceptable carriers well known in the art. Such carriers enable the hydrogel and/or peptide/peptoid, as well as a pharmaceutically active compound, to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated. Pharmaceutical preparations for oral use can be obtained by adding a solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatine, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or

dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations that can be used orally include push-fit capsules made of gelatine, as well as soft, sealed capsules made of gelatine and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the peptides/peptoids may be suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for such administration. For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

The hydrogel and/or peptide/peptoid may be formulated for parenteral administration by injection, e.g., by intramuscular injections or bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e. g., in ampules or in multi-dose containers, with an added preservative. The respective compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

The hydrogel and/or peptide/peptoid may be formulated for other drug delivery systems like implants, or transdermal patches or stents.

The ultrashort peptide hydrogels (peptide alone or polymer-peptide composite) can be applied as versatile formulations that provide sustained and controlled release of bioactive components.

The bioactive moieties of interest include (but are not limited to):

- antibiotics (small molecules, ions, nano- and microparticles),
- anti-fungals,
- anti-inflammatories,
- analgesics,
- antibodies,
- antigens,
- adjuvants,

- cosmetic agents, such as serum proteins, anti-ageing, whitening agents, lubricants, sunscreen agents,
- vitamins and small molecule compounds (such as vitamin E, retinoic acid, alpha-hydroxyacids)
- nano- and microparticles such as silver (for antibacterial properties), zinc oxide (anti-bacterial) and titanium oxide (sun-screen), as well as polymeric nano- and microparticles encapsulating above-mentioned bioactive moieties,
- cells, including platelets, hematopoietic stem cells, adult mesenchymal stem cells, cord blood cells, adipose stem cells, induced pluripotent stem cells, keratinocytes, cartilage and fibroblasts.

Bioactive moieties and cells can be encapsulated into the hydrogels by mixing during the gelation process. Alternatively, bioactive moieties can be conjugated to the peptides or non-peptidic polymers to be incorporated in composite polymer-peptide hydrogels (Figure 17).

Use for wound healing, cosmetics and skin care applications

Barrier function

As the peptides according to the present invention self-assemble into nanofibrous porous scaffolds, they form a barrier against the external environment and can protect against dehydration and infection and also arrest bleeding. As hydrogels are soft biomaterials, trauma to the regenerated tissue is minimized. This is particularly important if the wound requires frequent dressing changes. A major disadvantage of traditional gauze dressings is the entanglement of recovering tissue with the gauze fibers. Consequently, during dressing changes, the recovering tissue is often re-injured causing significant pain and discomfort. An additional advantage of the hydrogels according to the present invention is their transparency – wound recovery can be monitored without completely cleaning or undressing the wound. Furthermore, due to the propensity of the hydrogels to absorb or release water, wound exudates are effectively managed.

Hemostatic capabilities

In addition to forming a physical barrier to seal leaking blood vessels, the subclass of peptides containing lysine and similar amino acids as the polar head group can potentially facilitate hemostasis. Analogues and derivatives of lysine, such as ε-aminocaproic acid and tran-

sexamic acid, are antifibronolytics that prevent excessive blood loss by competitively inhibiting the activation of plasminogen to plasmin. The mechanism of interaction involves offering alternative enzymatic binding sites to lysine residues in fibrin, thereby preventing the breakdown of blood clots. In other words, these hydrogelating hemostatic peptides can also potentially be used to seal open and surgical wounds.

Wound healing

The application of ultrashort peptide hydrogels according to the present invention expedites the recovery of partial thickness burn wounds. They combine the advantages of commercial hydrogel dressings with those of nanofibrous scaffolds in providing topographical cues for tissue regeneration. Their optical transparency facilitates wound observation without having to remove the primary dressing. Yet frequent changes as necessitated by the extent of injury will also not inflict trauma on the recovering tissues since the soft biomaterial can be simply wiped away with gauze. When used as a wound dressing, the hydrogel according to the present invention (1) forms a barrier to prevent bacteria and fungal spores from entering the wound, (2) forms a physical barrier to prevent cells from leaking out of damaged blood vessels (hemostatic function), (3) limits dehydration of tissues, (4) offers protection against mechanical stress and abrasion and (5) provides mechanical cues for cellular and tissue regeneration (biomimetic scaffold). A subclass of peptides/peptoids with lysine and similar polar head groups with primary amines, can bind nucleic acids released by dead and damaged cells. These nucleic acids include ssRNA, dsRNA and unmethylated DNA, which bind nucleic acid-sensing toll-like receptors, and subsequently activate the hosts' innate immune system. By acting as molecular scavengers (Lee et al, PNAS 2011), this subclass of peptides/peptoids can potentially block the immune stimulatory effects of extracellular nucleic acids which induce pathological inflammatory responses. As such, the peptide hydrogels can potentially significantly reduce tissue trauma due to inflammatory response post-burn injury.

Maintenance of hydration and induction of autolytic debridement

Due to the high water content of the hydrogels according to the present invention, the underlying tissues are kept hydrated, which is particularly attractive for cosmetic applications and for the treatment of wounds. In particular, a key advantage of using hydrogels as wound dressings is their propensity to induce autolytic debridement of the necrotic eschar tissue. Autolytic debridement is preferred over physical methods (such as scraping, surgical excision

and cleaning with gauze), which damages portions of the tissue that have started to recover. By accelerating autolytic removal of the necrotic tissue, the ultrashort peptide hydrogels according to the present invention create room for cells to infiltrate the damage region and commence tissue regeneration.

Skin substitute

The ultrashort peptide hydrogels according to the present invention can potentially be applied as a synthetic skin substitute. Their nanofibrous macromolecular architecture resembles extracellular matrix, and can thus be applied as scaffolds in place of (donor and cadaveric) skin grafts. Commercially available skin substitutes are typically of animal (bovine and porcine) and bacterial origin and could present with immunogenic problems and societal (religious) objections. The skin substitute formulation could further be enhanced by the incorporation of regenerative factors and stem cells.

Versatile formulation

Self-assembling ultrashort peptides hydrogels can be applied as topical dressings (hydrogels and organogels), sprays, hydrogel patches and membranes that can be re-hydrated into hydrogels (Figure 20). The semi-viscous peptide solution (mixed with cells or small molecule therapeutics) can be applied as a topical gel or cream, a spray, hydrogel sheet, patch or membrane. In particular, sprays, topical gels and creams can physically mix with the adjacent tissue thereby allowing for uniform dispersion of encapsulated therapeutic agents. As the applied peptide solution is warmed (to body temperature), gelation is enhanced. For a subclass of peptides containing amidated lysine (exemplified by Ac-LIVAGK-NH_2) and amidated lysine-substitutes as the polar head group, stimuli-responsive gelation is observed in the presence of increased salt concentration, such as in normal and phosphate buffered saline (Figure 21). Stimuli-responsive gelation behavior facilitates the development of sprays, as the gelation can be stimulated by a change in salt concentration. For example, sprays can be formulated, wherein the fluid peptide solution is initially separated from the gelation-trigger agent. Upon dispersion, the solutions mix and gelation occurs. This also simplifies the delivery device, as the peptide solution is significantly less viscous at lower salt concentrations. The gelation process is endothermic and does not release any heat.

The nanofibrous network of the hydrogels can be used to maintain the active ingredients in suspension, preventing aggregation or precipitation over time, thereby increasing the shelf-

life. The high water content can also be used to maintain tissue hydration, while allowing gaseous permeability. As the hydrogels (at low concentrations) are clear and transparent, they will not significantly affect physical appearance.

The peptide hydrogels are biocompatible *in vitro* and *in vivo*, and could potentially encourage the regeneration of native cells. In a clinical setting, gelation *in situ* (within the body cavity) can generate nanofibrous scaffolds to replace damaged or missing tissue. The biomimetic nanotopography can potentially provide cues for cell migration, regeneration and enhance the growth of co-delivered cells. *In situ* gelation can also be applied to form hemostatic plugs and thus prevent excessive blood loss during surgical procedures.

The peptides are also resistant to degradation by enzymes and would thus be more durable. The material can also be sterilized by UV exposure or autoclaving, without compromising the molecular structure of the dry peptide powder. Due to their small size of the ultrashort peptides (3-7 amino acids), they can potentially diffuse through intact skin and penetrate the underlying tissue to form a scaffold for tissue regeneration.

Considering the long shelf-life stability of sealed peptides stored at room temperature, "just-add-water" formulations are also possible. The hydrogels can be re-constituted by adding a fixed volume of clean water to lyophilized peptide powder at the point of application, and subsequently administered as a gel, spray or patch. This formulation design will greatly reduce transportation costs, thereby facilitating adoption as part of basic first aid kits particularly in war zones and third world countries. This development can potentially revolutionize emergency medicine, providing a convenient, easy-to-use first-line treatment for partial thickness burn injuries, and filling a niche that is sorely neglected by commercial skin substitutes and topical hydrogel dressings currently available on the market.

<u>Use as injectable therapy to treat degenerative disc disease or urinary incontinence and use as a dermal and/or adipose filler</u>

Self-assembly and stimuli-responsive nature

Self-assembly behavior of the peptides in aqueous conditions allows for the development of an injectable therapy in which the peptide solution is injected and subsequently gels *in situ* into a hydrogel. The semi-viscous peptide solution (mixed with cells or small molecule therapeutics) can be injected into small cavities in the body, and is hence minimally invasive. The fluid may also physically mix with the adjacent tissue, thereby allowing for uniform dispersion of the cells or therapeutic agents. Current treatment options for degenerative disc disease

are highly invasive and require surgical intervention in the form of spinal fusions or disc replacements with a metal or ceramic implant. Thus, an injectable therapy is significantly less invasive and offers an alternative treatment for patients who are not eligible for surgery.

Stimuli-responsive gelation behavior facilitates the development of an injectable therapy, as the gelation can be stimulated by a change in temperature or salt concentration. This allows time for the clinician to position the needle and to inject the solution before the gel sets. Injected as a cold or room temperature solution, some peptides gel *in situ* as the aqueous mixture warms to body temperature within the body. Alternatively, co-injection with a high salt content solution (such as via a co-axial syringe, or T-junction needle) also stimulates the gelation of salt-sensitive peptides. The gelation process is endothermic and does not release any heat.

Rigid and tuneable mechanical properties

The peptide hydrogels have high mechanical strength, which can provide interim mechanical support for the damaged and recovering tissue. When applied to treat degenerative disc disease, the injectable therapy will gel in situ and increase the height of the intervertebral disc, thereby increasing the cushioning effect for the vertebral bones and relieving the compression on the spinal nerve.

The mechanical properties of the hydrogel can be further modulated to match that of native tissue by varying the peptide sequence, composition, concentration, counter-ion and salt concentration of the solution. The peptide hydrogels have demonstrated good biocompatibility, stability and resistance to enzymatic degradation, and would thus persist in the tissue for several months. As such, the therapeutic effect is expected to last for months, reducing the need for repeated treatments.

Biocompatibility and adipose tissue regeneration

The peptide hydrogels are biocompatible and can support the proliferation of native cells or stem cells injected. This would facilitate regeneration of the tissue. Cell attachment, proliferation and differentiation could potentially be enhanced by conjugating or encapsulating small molecule, short peptide motifs, cytokines, growth factors and oligonucleotides (DNA, mRNA, shRNA and siRNA).

EXAMPLES

Experiments have been performed to illustrate the technical aspects of exemplary embodiments of the present invention. The following examples are described in the Experimental Methods and Results. The skilled artisan will readily recognize that the examples are intended to be illustrative and are not intended to limit the scope of the present invention.

EXPERIMENTAL METHODS AND RESULTS

Peptides

The peptide sequences were designed to represent an amphiphilic peptide structure containing a hydrophilic head group and a hydrophobic tail. The rationale for the peptides design was to create a peptide monomer of decreasing size resembling a cone shaped structure. The hydrophobic tail differs by using different aliphatic amino acids. It is consisting of the following aliphatic amino acids such as glycine, alanine, valine, leucine and isoleucine and the hydrophilic head group is consisting of one or two polar or charged amino acids. The sequence order of the hydrophobic tail differed by using different aliphatic amino acids. The peptides were commercially synthesized from GL Biochem, Shanghai, China. In order to verify the reproducibility of the peptide hydrogel-forming behavior peptides were also synthesized from other companies (Biomatik Corp., Anaspec. Inc, American Peptide Company, USA). The peptides have a purity of equal or higher than 95% verified by High-performance liquid chromatography (HPLC) and mass spectrometry. The peptide stock solutions were dissolved in water at 5 to 10 mg/ml. Most of the peptides are acetylated at the N-terminus.

Peptide-based hydrogel preparation

All peptides (GL Biochem, Shanghai, China, ≥ 98% purity) were freshly prepared in order to avoid premature peptide aggregation. The peptides were dissolved in water and left at room temperature to form hydrogels. Depending on the peptide concentration, the self-assembly process occurred immediately, within hours or even within days (experimental time frame for gelation). For higher peptide concentrations peptides were dissolved in milliQ water by vortexing. If a forced and accelerated hydrogel preparation was needed, the peptide solution was subjected to sonication in a water bath (Barnstead Labline 9319 UltrasonicLC60H). No significant structural differences were observed between hydrogels produced via self-assembly

and those whose assembly was facilitated by sonication. Few peptides formed hydrogels more easily at elevated temperatures, i.e. at 50°C.

To study the effect of concentration variation, both $AcLD_6$ (L) and AcID3 (L) hydrogels were prepared with varying concentration as specified above. To study the effect of monovalent and divalent cations, $AcLD_6$ (L) hydrogels were prepared by dissolving peptide in 10, 50, 100 and 150 mM NaCl and $CaCl_2$ solutions. FESEM and rheology studies were further performed to characterize the morphology and strength of these hydrogels.

Preparation of gelatin and collagen gels: Gelatin (Type A, G1890; Sigma Aldrich) hydrogels was prepared by first dissolving gelatin in milli Q water by heating followed by cooling till the gelation was observed. Collagen (Type I from bovine, Advanced Biomatrix, USA) was diluted with PBS buffer to a concentration of 1.5 mg/ml and titrated to pH 7.4 using 0.1M NaOH. Gelation was achieved by incubating the solution at 37˚C for 1 hour.

Circular dichroism (CD) spectroscopy

Secondary peptide structures were analyzed by measuring ellipticity spectra using the Aviv Circular Dichroism Spectrometer, model 410. CD samples were prepared by diluting stock peptides solutions (5-10 mg/ml) in water. The diluted peptide solutions were filled in to a cuvette with 1 mm path length and spectra were acquired. As a blank reference water was used and the reference was subtracted from the raw data before molar ellipticity was calculated. The calculation was based on the formula: $[\theta]_\lambda = \theta_{obs}$ x 1/(10 Lcn), where $[\theta]_\lambda$ is the molar ellipticity at λ in deg cm^2 d/mol, is the observed ellipticity atλ in mdeg, L is the path length in cm, c is the concentration of the peptide in M, and n is the number of amino acids in the peptide. Secondary structure analysis was done using CDNN software.

Environmental Scanning Electron Microscopy (ESEM)

Samples were placed onto a sample holder of FEI Quanta 200 Environmental Scanning Electron Microscopy. The surface of interest was then examined using accelerating voltage of 10kV at a temperature of 4 ˚C.

Field Emission Scanning Electron Microscopy (FESEM)

Samples were frozen at -20°C and subsequently to -80°C. Frozen samples were further freeze dried. Freeze dried samples were fixed onto a sample holder using conductive tape and sputtered with platinum from both the top and the sides in a JEOL JFC-1600 High Resolution

Sputter Coater. The coating current used was 30 mA and the process lasted for 60 sec. The surface of interest was then examined with a JEOL JSM-7400F Field Emission Scanning Electron Microscopy system using an accelerating voltage of 5-10 kV.

Rheological measurements

To determine the viscoelastic properties of the peptide-based hydrogels, hydrogels were subjected to dynamic time, strain and frequency sweep experiments using the ARES-G2 rheometer (TA Instruments, Piscataway, NJ) with the 25.0 mm diameter titanium parallel plate geometry and a 0.8 mm gap distance. Oscillatory frequency study was performed to compare the strength of peptide based hydrogel with varying concentration of peptides, or for peptide in presence of monovalent or divalent ions. Oscillatory frequency sweep studies were performed at 0.1-100 rad/s frequency and 0.1% strain at 25°C and 50°C.

[A] Ac-LD_6 [L]:

Peptide sequence: Ac-LIVAGD-COOH

Molecular weight: 629.56

(1) Temperature sweep study for Ac-LD_6 (L):

(a) The peptide mixture was then placed on rheometer lower plate. Following parameters were optimized:

Gap between two plates: 1 mm

Strain: 10 %

Frequency: 6.28 rad/sec

Temperature scan: 4°C to 60°C

Sample volume: 500 µl

(2) Frequency sweep study for Ac-LD_6 (L):

Optimized parameter required to perform frequency sweep study

Gap between two plates: 0.8 mm

Strain: 0.1 %

Temperature: 25 and 50°C

Sample volume: 1ml

Frequency scan: 0.1 rad/sec to 100 rad/sec

Concentration of Ac-LD-6 (L) in hydrogel: 10 mg/ml

(3) Effect of concentration variation of Ac-LD_6 (L) on gel strength:

Optimized parameters that are required to perform frequency sweep studies for measuring gel strength are as follows:

Gap between two plates: 0.8 mm

Strain: 0.1 %

Temperature: 25 and 50°C

Sample volume: 1ml

Frequency scan: 0.1 rad/sec to 100 rad/sec

Concentrations of Ac-LD_6 (L) in hydrogels: 5 mg/ml, 10 mg/ml, 15 mg/ml and ,20 mg/ml and 30mg/ml in water.

(4) Effect of sodium chloride (NaCl) on the gel strength of Ac-LD_6 (L):

Effect of sodium chloride on Ac-LD_6 (L) based hydrogels, were studied by performing a frequency sweep study on hydrogels prepared by dispersing 10 mg of Ac-LD-6 (L) in varying concentration of NaCl solution for example 10mM, 50mM, 100mM and 150mM of NaCl solution using optimized procedure to form hydrogels. Optimized parameter required to perform frequency sweep study to measure gel strength in presence of NaCl are as follows:

Gap between two plates: 0.5 mm and 0.8 mm

Strain: 10 % and 0.1% respectively

Temperature: 25°C and 50 °C

Sample volume: 1ml

Frequency scan: 0.1 rad/sec to 100 rad/sec

Concentrations of NaCl solutions used to prepare 10 mg/ml of Ac-LD-6 (L) Hydrogels: 10mM, 50 mM, 100 mM, 150 mM NaCl solution.

Cell growth experiments

In order to find out whether the peptide hydrogels can serve as a scaffold for tissue engineering, its biocompatibility was investigated. Different primary human cells were seeded on top of the hydrogel after its gelation in tissue culture medium (DMEM without serum) in 6-well, 24-well or 96-well culture plates, see the culture conditions below. During the next 2-4 days

no change of medium was necessary, but eventually fresh media was added to the wells. The cells were analyzed for viability.

Primary human renal proximal tubule cells (HPTCs) and primary human umbilical vein endothelial cells (HUVECs) were obtained from ScienCell Research Laboratories (Carlsbad, CA, USA). HPTCs were cultivated in basal epithelial cell medium supplemented with 2% fetal bovine serum (FBS) and 1% epithelial cell growth supplement (all components obtained from ScienCell Research Laboratories). The culture medium for HUVECs was endothelial cell medium containing 5% FBS and 1% endothelial cell growth supplement (ScienCell Research Laboratories). All cell culture media used were supplemented with 1% penicillin/streptomycin solution (ScienCell Research Laboratories), and all cells were cultivated at 37°C in a 5% CO_2 atmosphere. The seeding density of the cells was about 5 x 10^4 cells /cm^2. However since HUVECs are bigger than HPTCs the cell number would be slightly lower than one for HPTC cells (~ 4.5 x 10^4 cells /cm^2). Both cell types had a confluency of about 80% in the wells after seeding.

Treatment of wounds

Two ultrashort peptide candidates Ac-ILVAGK-NH_2 (AcIK6) and Ac-LIVAGK-NH_2(AcLK6) were formulated into hydrogel patches of 25mm diameter and 1.5mm thick. Gelation occurred within minutes, producing stable hydrogels amendable to handling. The two peptide candidates chosen from this study both contained lysine as a polar head group, which could potentially facilitate hemostasis of open wounds and reduce inflammation caused by extracellular nucleic acids released by dead and damaged cells. Analogues and derivatives of lysine, such as ε-aminocaproic acid and transexamic acid, are anti-fibronolytics that prevent excessive blood loss by competitively inhibiting the activation of plasminogen to plasmin. The mechanism of interaction involves offering alternative enzymatic binding sites to lysine residues in fibrin, thereby preventing the breakdown of blood clots (Figure 23). The primary amine group present on the lysine can also act as a molecular scavenger to bind extracellular nucleic acids (such as ssRNA, dsRNA and unmethylated DNA) and thus block their immune stimulatory effects (Figure 22). The hydrogel patches were evaluated in a rat partial thickness burn model. The standard-of-care treatment Mepitel®, a flexible polyamide net coated with silicone to discourage adhesion of the regenerating tissue, was used as a control.

Both hydrogels stimulated autolytic debridement. The onset of debridement of hydrogel-treated wounds was observed by day 8 for all the animals, compared to day 10 for control samples (Figure 18A). Complete debridement of the eschar was approximately delayed by 2 days comparing hydrogel-treated and control samples. Histological analysis of skin samples taken at day 7 corroborated the completion of autolytic debridement for wounds dressed with peptide hydrogels. The loss of the necrotic epidermal layer, as marked by red arrows in Figure 18B, for burnt area treated with Ac-IK_6 and Ac-LK_6 hydrogels, is contrasted with remnants of epidermal tissue adhered to the dermis for the wounds dressed with Mepitel®. By accelerating autolytic removal of the necrotic tissue, the ultrashort peptide hydrogels create room for cells to infiltrate the damage region and commence tissue regeneration.

Wound closure was in part facilitated by the rapid onset of autolytic debridement. As such, it is not surprising that the burn injuries treated with hydrogels contracted at a higher rate compared to Mepitel® (Figure 18C). Quantifying the wound area using digital planimetry (Figure 18D), 56.96% and 55.16% wound contracture is observed for Ac-IK_6 and Ac-LK_6 hydrogels respectively by day 10. In contrast, only 46.67% of the wound area had healed for Mepitel®-dressed wounds. By day 14, 85.09% and 92.95% coverage of the burn is achieved for the hydrogels, which is dramatically higher than the 62.46% for Mepitel® dressings (mixed-model analysis, $p<0.05$).

Histological analysis of the injury suggests that the technique used consistently generated deep partial thickness burns. The structural integrity of the tissue was significantly altered, giving rise to more disorganized extracellular matrix and loss of hair-shaft follicles in the dermis, and detachment of the epidermis in some specimens. There was also extensive lymphocyte infiltration at the interface of injured and healthy tissue. The damage extended more than 50% through the dermis even after 7 days. As illustrated in Figure 19A, necrotic epidermal tissue is observed for the Mepitel®-dressed samples, while complete debridement of the eschar epidermal tissues was observed for Ac-IK_6 and Ac-LK_6 hydrogel-dressed injuries. While extracellular matrix re-modelling had not yet occurred in the dermis, there are some signs of recovery, as demonstrated by the proliferation of hair shaft follicles in the surrounding healthy tissue. At the boundary of the injured and healthy tissue (Figure 19B), infiltration of the healthy basal cells from the epidermal-dermis junction and the adjacent undamaged hair shafts, into the damaged tissue is observed for the Ac-IK_6 and Ac-LK_6 hydrogel-dressed

injuries, under higher magnification. The basal cells are the stem cells responsible for skin tissue regeneration following injury. By day 14 (Figure 19C), the regeneration of the epidermis is almost complete for Ac-IK_6 and Ac-LK_6 hydrogel treated wounds. Basal cells at the epidermis-dermis interface have also begun to penetrate the dermis to form precursors to hair shaft follicles. This corroborates well with observations gleaned from gross examinations. In comparison, the epidermal tissue in Mepitel®-dressed wounds was thinner and more fragile. Hydrogel-treated wounds also demonstrate a greater degree of matrix remodelling in the dermis. At the boundary of the injury site (Figure 19D), a significantly higher extent of cell replication has occurred amongst basal cells located in the hair follicles, for wounds dressed with Ac-IK_6 and Ac-LK_6 hydrogels. In contrast, hair shaft follicle regeneration in Mepitel®-dressed wounds is minimal.

Injectable therapy for degenerative disc disease

The peptide Ac-LK_6 (Ac-LIVAGK-NH_2) was used to formulate an injectable therapy to treat degenerative disc disease in a rabbit model (Figure 24). Two treatments were evaluated, the peptide hydrogel (encapsulating an MRI contrast agent) and hydrogel encapsulating rabbit nucleus pulposus cells (labeled with iron oxide nanoparticles). All the animals responded well to treatment, and did not experience any adverse immunological or physiological reactions. After 2 months, the animals were sacrificed and their vertebral column was collected for ex vivo MRI imaging. There was minimal leakage of the MRI contrast agent from the nucleus pulposus space, indicating that the hydrogel was still present even after 2 months. In dissection of the disc, an increase in nucleus pulposus mass was observed for several samples treated with cells.

Dermal filler and scaffold for adipose tissue regeneration

The compatibility of various peptides evaluated in vivo using C57BL6 mice. 30uL of hydrogel was implanted subcutaneously and the immune response was evaluated after 1 and 2 months (Figure 25). Interestingly, hydrogel was observed as amorphous eosinophilic (pink), polarisable foreign body material beneath skeletal muscle layer. Minimal to mild inflammatory response noted in the form of multi-nucleated giant cell histiocytes, but attributed to the implantation surgery. Hydrogel persists even after 2 months, demonstrating good *in vivo* stability.

Ac-LIVAGK-NH_2 hydrogels encapsulating human adipose derived stem cells were implanted in SCID mice. 6 weeks post-implantation, the mice were sacrificed and fat pads were observed at the implantation site, below muscles and the native adipose tissue present in the subcutaneous space. As this was not observed for sites implanted with human mesenchymal stem cells, this result suggests that the peptide hydrogels can potentially support the proliferation of adipose precursor cells and reduce cellular necrosis and migration following adipose cell transplantation.

The listing or discussion of a previously published document in this specification should not necessarily be taken as an acknowledgement that the document is part of the state of the art or is common general knowledge. All documents listed are hereby incorporated herein by reference in their entirety for all purposes.

Exemplary embodiments of the invention illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms “comprising”, “including,” containing”, etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by exemplary embodiments and optional features, modification and variation of the inventions embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

Other embodiments are within the following claims. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

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Claims

1. An amphiphilic peptide and/or peptoid capable of self-assembling into three-dimensional macromolecular nanofibrous networks, which entrap water and form a hydrogel, the amphiphilic peptide and/or peptoid comprising an amphiphilic sequence consisting of:

a hydrophobic sequence stretch of n aliphatic amino acids, wherein *n* is an integer from 2 to 15, and

a hydrophilic sequence stretch linked to said hydrophobic sequence stretch and having a polar moiety which is acidic, neutral or basic, said polar moiety comprising *m* adjacent hydrophilic amino acids, wherein *m* is an integer from 1 to 5.

2. The amphiphilic peptide and/or peptoid of claim 1, wherein the amphiphilic peptide and/or peptoid has a C-terminus and an N-terminus, wherein the N-terminus is protected by an N-terminal protecting group.

3. The amphiphilic peptide and/or peptoid of claim 1 or 2, wherein the amphiphilic peptide and/or peptoid has a C-terminus and an N-terminus, wherein the C-terminus is protected by a C-terminal protecting group.

4. The amphiphilic peptide and/or peptoid of claim 2, wherein said N-terminal protecting group has the general formula –C(O)–R, wherein R is selected from the group consisting of H, unsubstituted or substituted alkyls, and unsubstituted or substituted aryls.

5. The amphiphilic peptide and/or peptoid of claim 4, wherein said N-terminal protecting group is an acetyl group.

6. The amphiphilic peptide and/or peptoid of claim 2, wherein said N-terminal protecting group is a peptidomimetic molecule, including natural and synthetic amino acid derivatives, wherein the N-terminus of said peptidomimetic molecule may be modified with a functional group selected from the group consisting of carboxylic acid, amide, alcohol, aldehyde, amine, imine, nitrile, an urea analog, thiol, phosphate, carbonate, sulfate, nitrate, maleimide, vinyl

sulfone, azide, alkyne, alkene, carbohydrate, imide, peroxide, ester, thioester, aryl, ketone, sulphite, nitrite, phosphonate and silane.

7. The amphiphilic peptide and/or peptoid of claim 3, wherein said C-terminal protecting group is an amide group.

8. The amphiphilic peptide and/or peptoid of claim 7, wherein the C-terminus of said amphiphilic peptide and/or peptoid has the formula -CONHR or -CONRR', with R and R' being selected from the group consisting of H, unsubstituted or substituted alkyls, and unsubstituted or substituted aryls.

9. The amphiphilic peptide and/or peptoid of claim 3, wherein said C-terminal protecting group is an ester group.

10. The amphiphilic peptide and/or peptoid of claim 9, wherein the C-terminus of said amphiphilic peptide and/or peptoid has the formula $–CO_2R$, with R being selected from the group consisting of H, unsubstituted or substituted alkyls, and unsubstituted or substituted aryls.

11. The amphiphilic peptide and/or peptoid of claim 3, wherein said C-terminal protecting group is a peptidomimetic molecule, including natural and synthetic amino acid derivatives, wherein the C-terminus of said peptidomimetic molecule may be modified with a functional group selected from the group consisting of carboxylic acid, amide, alcohol, aldehyde, amine, imine, nitrile, an urea analog, thiol, phosphate, carbonate, sulfate, nitrate, maleimide, vinyl sulfone, azide, alkyne, alkene, carbohydrate, imide, peroxide, ester, thioester, aryl, ketone, sulphite, nitrite, phosphonate and silane.

12. The amphiphilic peptide and/or peptoid of any one of the foregoing claims, wherein n is an integer from 2 to 6, preferably 2 to 5.

13. The amphiphilic peptide and/or peptoid of any one of the foregoing claims, wherein m is an integer from 1 to 2.

14. The amphiphilic peptide and/or peptoid of any one of the foregoing claims, wherein the amphiphilic peptide and/or peptoid consists of o amphiphilic sequences, as defined in any one of the foregoing claims, which amphiphilic sequences are linked to each other, o being an integer from 1 to 50.

15. The amphiphilic peptide and/or peptoid of any one of the foregoing claims, wherein, for a given amphiphilic peptide and/or peptoid, said aliphatic amino acids and said hydrophilic amino acids are either D-amino acids or L-amino acids.

16. The amphiphilic peptide and/or peptoid of any one of the foregoing claims, wherein each of the hydrophilic amino acids has a polar group which is independently selected from a hydroxyl, an ether, a carboxyl, an imido, an amido, an ester, an amino, a guanidino, a thio, a thioether, a seleno, and a telluro group.

17. The amphiphilic peptide and/or peptoid of any one of the foregoing claims, wherein said polar moiety of said hydrophilic sequence stretch comprises m adjacent hydrophilic amino acids, m being defined as in any one of claims 1 to 13, said hydrophilic amino acids being selected from the group comprising aspartic acid, asparagine, glutamic acid, glutamine, 5-N-ethyl-glutamine (theanine), citrulline, thio-citrulline, cysteine, homocysteine, methionine, ethionine, selenomethionine, telluromethionine, threonine, allo-threonine, serine, homoserine, arginine, homoarginine, ornithine (Orn), 2,4-diaminobutyric acid (Dab), 2,3-diaminopropionic acid (Dap), lysine and N(6)-carboxy-methyllysine, histidine, and wherein said hydrophobic sequence stretch comprises n aliphatic amino acids, n being as defined in any one of claims 1 to 12, said aliphatic amino acids being selected from the group comprising isoleucine, norleucine, leucine, valine, alanine, glycine, homoallylglycine and homopropargylglycine.

18. The amphiphilic peptide and/or peptoid of claim 17, wherein m is 1 or 2.

19. The amphiphilic peptide and/or peptoid of claim 18, wherein m is 2 and wherein said polar moiety comprises two identical amino acids, or wherein m is 1 and wherein said polar moiety comprises any one of aspartic acid, asparagine, glutamic acid, glutamine, serine,

threonine, cysteine, methionine, lysine, ornithine (Orn), 2,4-diaminobutyric acid (Dab), 2,3-diaminopropionic acid (Dap) and histidine.

20. The amphiphilic peptide and/or peptoid of claim 19, wherein said polar moiety is adjacent to the hydrophobic sequence stretch of n aliphatic amino acids.

21. The amphiphilic peptide and/or peptoid of any one of claims 18 to 20, wherein said polar moiety has a sequence selected from Asp, Asn, Glu, Gln, Ser, Thr, Cys, Met, Lys, Orn, Dab, Dap, His, Asn-Asn, Asp-Asp, Glu-Glu, Gln-Gln, Asn-Gln, Gln-Asn, Asp-Gln, Gln-Asp, Asn-Glu, Glu-Asn, Asp-Glu, Glu-Asp, Gln-Glu, Glu-Gln, Asp-Asn, Asn-Asp Thr-Thr, Ser-Ser, Thr-Ser, Ser-Thr, Asp-Ser, Ser-Asp, Ser-Asn, Asn-Ser, Gln-Ser, Ser-Gln, Glu-Ser, Ser-Glu, Asp-Thr, Thr-Asp, Thr-Asn, Asn-Thr, Gln-Thr, Thr-Gln, Glu-Thr, Thr-Glu, Cys-Asp, Cys-Lys, Cys-Ser, Cys-Thr, Cys-Orn, Cys-Dab, Cys-Dap, Lys-Lys, Lys-Ser, Lys-Thr, Lys-Orn, Lys-Dab, Lys-Dap, Ser-Lys, Ser-Orn, Ser-Dab, Ser-Dap, Orn-Lys, Orn-Orn, Orn-Ser, Orn-Thr, Orn-Dab, Orn-Dap, Dab-Lys, Dab-Ser, Dab-Thr, Dab-Orn, Dab-Dab, Dab-Dap, Dap-Lys, Dap-Ser, Dap-Thr, Dap-Orn, Dap-Dab, Dap-Dap.

22. The amphiphilic peptide and/or peptoid of any one of the foregoing claims, wherein said polar moiety comprises the C-terminus of the amphiphilic peptide and/or peptoid, or wherein said polar moiety comprises the N-terminus of the amphiphilic peptide and/or peptoid.

23. The amphiphilic peptide and/or peptoid of claim 22, wherein said polar moiety comprises the C-terminus of the amphiphilic peptide and/or peptoid.

24. The amphiphilic peptide and/or peptoid of any one of claims 1 to 23, wherein said polar moiety consists of at least one amino acid positioned at the C-terminus of the amphiphilic peptide and/or peptoid.

25. The amphiphilic peptide and/or peptoid of any one of claims 1 to 24, wherein said hydrophobic sequence stretch comprises and/or forms the N-terminus of the amphiphilic peptide and/or peptoid.

26. The amphiphilic peptide and/or peptoid of any one of claims 1 to 25, wherein all or a portion of the aliphatic amino acids of the hydrophobic sequence stretch are arranged in an order of decreasing amino acid size in the direction from N- to C-terminus of the amphiphilic peptide and/or peptoid, wherein the size of the aliphatic amino acids is defined as I = L > V > A > G.

27. The amphiphilic peptide and/or peptoid of claim 26, wherein said aliphatic amino acids arranged in an order of decreasing amino acid size have a sequence which is a repetitive or non-repetitive sequence.

28. The amphiphilic peptide and/or peptoid of any one of claims 26 and 27, wherein said aliphatic amino acids arranged in order of decreasing amino acid size have a sequence with a length of 2 to 7, preferably 2 to 6, more preferably 2 to 5 amino acids.

29. The amphiphilic peptide and/or peptoid of any one of claims 26 to 28, wherein said aliphatic amino acids arranged in an order of decreasing amino acid size have a sequence selected from LIVAG, ILVAG, LIVAA, LAVAG, IVAG, LIVA, LIVG, IVA and IV, wherein, optionally, there is an A preceding such sequence at the N-terminus.

30. The amphiphilic peptide and/or peptoid of any one of claims 1 to 25, wherein all or a portion of the aliphatic amino acids of the hydrophobic sequence stretch are arranged in an order of identical amino acid size in the amphiphilic peptide and/or peptoid.

31. The amphiphilic peptide and/or peptoid of claim 30, wherein said aliphatic amino acids arranged in order of identical amino acid size have a sequence with a length of 2 to 4 amino acids.

32. The amphiphilic peptide and/or peptoid of any one of claims 30 and 31, wherein said aliphatic amino acids arranged in an order of identical size have a sequence selected from LLLL, LLL, LL, IIII, III, II, VVVV, VVV, VV, AAAA, AAA, AA, GGGG, GGG, and GG.

33. The amphiphilic peptide and/or peptoid of any one of claims 1 to 32, wherein the amphiphilic sequence undergoes a conformational change during self-assembly, preferably a

conformational change from a random coil conformation to a helical intermediate structure to a final beta conformation.

34. The amphiphilic peptide and/or peptoid of claim 33, wherein the conformational change is dependent on the concentration of the amphiphilic peptide and/or peptoid, dependent on the ionic environment, pH dependent and/or temperature dependent.

35. The amphiphilic peptide and/or peptoid of any one of claims 1 to 34, wherein the amphiphilic linear sequence comprises a single hydrophilic and at least two aliphatic amino acids.

36. The amphiphilic peptide and/or peptoid of any one of the foregoing claims, wherein the amphiphilic sequence is one of SEQ ID NO: 1 to 86.

37. The amphiphilic peptide and/or peptoid of any one of claims 1 to 36, being stable in aqueous solution at physiological conditions at ambient temperature for a period of time in the range from 1 day to at least 6 months, preferably to at least 8 months more preferably to at least 12 months.

38. The amphiphilic peptide and/or peptoid of any one of claims 1 to 37, being stable in aqueous solution at physiological conditions, at a temperature up to 90 °C, for at least 1 hour.

39. The amphiphilic peptide and/or peptoid of any one of the foregoing claims having the general formula:

$$Z_p\text{-}(X)_n\text{-}(Y)_m\text{-}Z'_q,$$

wherein

Z is an N-terminal protecting group,

X is, at each occurrence, independently selected from an aliphatic amino acid,

Y is, at each occurrence, independently selected from a hydrophilic amino acid,

Z' is a C-terminal protecting group,

n is an integer selected from 2 to 6, preferably 2 to 5,

m is selected from 1 and 2, wherein, preferably, m is 1,

and p and q are independently selected from 0 and 1, wherein, preferably, p is 1.

40. The amphiphilic peptide and/or peptoid of any one of the foregoing claims, wherein the C-terminal amino acid of the amphiphilic peptide and/or peptoid is selected from the group consisting of a lysine (K), an ornithine (Orn), a 2,4-diaminobutyric acid (Dab) and a 2,4-diaminopropionic acid (Dap).

41. The amphiphilic peptide and/or peptoid of claim 40, wherein the amphiphilic peptide and/or peptoid has a sequence selected from the group comprising LIVAGK (SEQ ID NO: 19 or 39), LIVAG(Orn) (SEQ ID NO: 43 or 44), LIVAG(Dab) (SEQ ID NO: 45 or 46), LIVAG(Dap) (SEQ ID NO: 47 or 48), ILVAGK (SEQ ID NO: 49 or 50), ILVAG(Orn) (SEQ ID NO: 51 or 52), ILVAG(Dab) (SEQ ID NO: 53 or 54), ILVAG(Dap) (SEQ ID NO: 55 or 56), AIVAGK (SEQ ID NO: 57 or 58), AIVAG(Orn) (SEQ ID NO: 59 or 60), AIVAG(Dab) (SEQ ID NO: 61 or 62), AIVAG(Dap) (SEQ ID NO: 63 or 64), IIIK (SEQ ID NO: 27 or 28), III(Orn) (SEQ ID NO: 65 or 66), III(Dab) (SEQ ID NO: 67 or 68), III(Dap) (SEQ ID NO: 69 or 70), IVK (SEQ ID NO: 71 or 72), IV(Orn) (SEQ ID NO: 73 or 74), IV(Dab) (SEQ ID NO: 75 or 76), IV(Dap) (SEQ ID NO: 77 or 78), LVK (SEQ ID NO: 79 or 80), LV(Orn) (SEQ ID NO: 81 or 82), LV(Dab) (SEQ ID NO: 83 or 84) and LV(Dap) (SEQ ID NO: 85 or 86).

42. The amphiphilic peptide and/or peptoid of any one of claims 40 and 41, wherein the amphiphilic peptide and/or peptoid has an amidated C-terminus.

43. The amphiphilic peptide and/or peptoid of any one of claims 40 to 42, wherein the amphiphilic peptide and/or peptoid has an acetylated N-terminus.

44. The amphiphilic peptide and/or peptoid of any one of claims 40 to 43, wherein the N-terminal amino acid of the amphiphilic peptide and/or peptoid is an isoleucine (I).

45. A hydrogel comprising at least one amphiphilic peptide and/or peptoid of any one of claims 1 to 44, preferably at least one amphiphilic peptide and/or peptoid of any one of claims 40 to 44.

46. The hydrogel of claim 45, wherein the hydrogel is stable in aqueous solution at ambient temperature for a period of at least 7 days, preferably at least 2 to 4 weeks, more preferably at least 1 to 6 months.

47. The hydrogel of any one of claims 45 and 46, wherein the hydrogel is characterized by a storage modulus G' to loss modulus G'' ratio that is greater than 2.

48. The hydrogel of any one of claims 45 to 47, wherein the hydrogel is characterized by a storage modulus G' from 100 Pa to 80,000 Pa at a frequency in the range of from 0.02 Hz to 16 Hz.

49. The hydrogel of any one of claims 45 to 48, wherein the hydrogel has a higher mechanical strength than collagen or its hydrolyzed form (gelatine).

50. The hydrogel of any one of claims 45 to 49, further comprising a non-peptidic polymer.

51. The hydrogel of any one of claims 45 to 50, comprising fibers of the at least one amphiphilic peptide and/or peptoid of any one of claims 1 to 44, said fibers defining a network that is capable of entrapping at least one of a microorganism, a cell, a virus particle, a peptide, a peptoid, a protein, a nucleic acid, an oligosaccharide, a polysaccharide, a vitamin, an inorganic molecule, a nano- or microparticle, a synthetic polymer, a small organic molecule, a cosmetic agent or a pharmaceutically active compound.

52. The hydrogel of claim 51, wherein the hydrogel comprises at least one of a microorganism, a cell, a virus particle, a peptide, a peptoid, a protein, a nucleic acid, an oligosaccharide, a polysaccharide, a vitamin, an inorganic molecule, a nano- or microparticle, a synthetic polymer, a small organic molecule, a cosmetic agent or a pharmaceutically active compound entrapped by the network of fibers of the amphiphilic peptide and/or peptoid.

53. The hydrogel of claim 52, wherein the fibers of the amphiphilic peptide and/or peptoid are coupled to the at least one of a microorganism, a cell, a virus particle, a peptide, a peptoid, a protein, a nucleic acid, an oligosaccharide, a polysaccharide, a vitamin, an inor-

ganic molecule, a nano- or microparticle, a synthetic polymer, a small organic molecule, a cosmetic agent or a pharmaceutically active compound.

54. The hydrogel of claim 52, wherein the at least one of a microorganism, a cell, a virus particle, a peptide, a peptoid, a protein, a nucleic acid, an oligosaccharide, a polysaccharide, a vitamin, an inorganic molecule, a nano- or microparticle, a synthetic polymer, a small organic molecule, a cosmetic agent or a pharmaceutically active compound is coupled to said non-peptidic polymer.

55. The hydrogel of any one of claims 51 to 54, wherein said pharmaceutically active compound is selected from the group comprising haemostatic agents, antibiotics, anti-microbial agents, anti-fungal agents, anti-inflammatory agents, analgesics, anti-coagulants, antibodies, antigens, growth factors and cytokines.

56. The hydrogel of any one of claims 45 to 55, wherein said hydrogel is provided in an injectable form and gels *in situ*.

57. The hydrogel of any one of claims 45 to 56, wherein the hydrogel is comprised in at least one of a fuel cell, a solar cell, an electronic cell, a biosensing device, a medical device, an implant, a wound dressing, a pharmaceutical composition and a cosmetic composition.

58. The hydrogel of claim 57, wherein the pharmaceutical composition or the cosmetic composition are provided in the form of a topical gel or cream, a spray, a powder, or a sheet, patch or membrane.

59. The hydrogel of claim 57, wherein the pharmaceutical composition or the cosmetic composition is provided in the form of an injectable solution.

60. The hydrogel of any one of claims 45 to 56 for use in at least one of the following: release of a pharmaceutically active compound, medical tool kit, a fuel cell, a solar cell, an electronic cell, tissue regeneration, tissue replacement, wound healing, skin care, stem cell therapy and gene therapy.

61. A method of preparing a hydrogel, the method comprising dissolving at least one amphiphilic peptide and/or peptoid of any one of claims 1 to 44 in an aqueous solution.

62. The method of claim 61, wherein the dissolved at least one amphiphilic peptide and/or peptoid in aqueous solution is further exposed to temperature, wherein the temperature is in the range from 20 °C to 90 °C, preferably from 20 °C to 70 °C.

63. The method of claim 62, wherein the dissolved at least one amphiphilic peptide and/or peptoid in aqueous solution is exposed to a temperature in the range from 35 °C to 40 °C.

64. The method of any one of claims 61 to 63, wherein the at least one amphiphilic peptide and/or peptoid is dissolved at a concentration from 0.01 µg/ml to 100 mg/ml, preferably at a concentration from 1 mg/ml to 50 mg/ml, more preferably at a concentration from about 1 mg/ml to about 20 mg/ml.

65. The method of any one of claims 61 to 64, further comprising at least one of the steps of:

- adding at least one bioactive agent;
- adding at least one non-peptidic polymer;
- adding at least one gelation enhancer;
- adding at least one buffer, preferably at least one physiologically acceptable buffer.

66. The method of claim 65, wherein said gelation enhancer is a salt or a solution of a salt.

67. The method of claim 65, wherein said gelation enhancer is a cross-linking agent.

68. The method of claim 65, wherein said adding at least one non-peptidic polymer further comprises mixing or cross-linking the at least one non-peptidic polymer with the at least one amphiphilic peptide and/or peptoid.

69. A surgical implant or stent, comprising a peptide and/or peptoid scaffold, wherein the peptide and/or peptoid scaffold is formed by a hydrogel of any one of claims 45 to 56.

70. A pharmaceutical and/or cosmetic composition and/or a biomedical device and/or electronic device comprising at least one amphiphilic peptide and/or peptoid of any one of claims 1 to 44, preferably at least one amphiphilic peptide and/or peptoid of any one of claims 40 to 44, or a hydrogel of any one of claims 45 to 56.

71. The pharmaceutical and/or cosmetic composition and/or the biomedical device, and/or the electronic devices of claim 70, further comprising a pharmaceutically active compound.

72. The pharmaceutical and/or cosmetic composition of claims 70 or 71, further comprising a pharmaceutically acceptable carrier.

73. The pharmaceutical and/or cosmetic composition of any one of claims 70 to 72, which is injectable.

74. A kit of parts, the kit comprising a first container with at least one amphiphilic peptide and/or peptoid of any one of claims 1 to 44, preferably of at least one amphiphilic peptide and/or peptoid of any one of claims 40 to 44, and a second container with an aqueous solution.

75. The kit of parts of claim 74, wherein the aqueous solution of the second container further comprises a pharmaceutically active compound.

76. The kit of parts of claims 74 or 75, wherein the first container with at least one amphiphilic peptide and/or peptoid further comprises a pharmaceutically active compound.

77. The kit of parts of any one of claims 74 to 76, further comprising a third container with a gelation enhancer.

78. The kit of parts of claim 77, wherein said gelation enhancer is a salt or a solution of a salt.

79. The kit of parts of any one of claims 74 to 78, wherein at least one of said first, second or third container is provided as a spray bottle or a syringe.

80. A kit of parts, the kit comprising a first container with an aqueous solution of at least one amphiphilic peptide and/or peptoid of any one of claims 1 to 44, preferably of at least one amphiphilic peptide and/or peptoid of any one of claims 40 to 44, and a second container with a gelation enhancer.

81. The kit of parts of claim 80, wherein the first container further comprises a pharmaceutically active compound.

82. The kit of parts of claim 80 or 81, wherein said gelation enhancer is a salt or a solution of a salt.

83. The kit of parts of any one of claims 80 to 82, wherein at least one of said first and said second container is provided as a spray bottle or a syringe.

84. A method of tissue regeneration or tissue replacement comprising the steps:

a) providing a hydrogel as defined in any one of claims 45 to 56;
b) exposing said hydrogel to cells which are to form regenerated tissue;
c) allowing said cells to grow on or in said hydrogel.

85. The method of claim 84, which is performed *in vitro* or *in vivo* or *ex vivo*.

86. The method of claim 85, which is performed *in vivo*, wherein, in step a), said hydrogel is provided at a place in the body of a patient where tissue regeneration or tissue replacement is intended.

87. The method of claim 86, wherein said tissue is selected from the group comprising skin tissue, nucleus pulposus in the intervertebral disc, cartilage tissue, synovial fluid and submucosal connective tissue in the bladder neck.

88. The method of claim 86 or 87, wherein said step a) is performed by injecting said hydrogel or a solution of at least one amphiphilic peptide and/or peptoid of any one of claims 1 to 44, preferably of at least one amphiphilic peptide and/or peptoid of any one of claims 40 to 44, at a place in the body of a patient where tissue regeneration or tissue replacement is intended.

89. The method of claim 88, wherein said step a) further comprises the co-injection of a gelation enhancer, preferably of a solution of a salt.

90. The method of claim 85, which is performed *ex vivo*, wherein, in step a) or b), cells from a patient or from a donor are mixed with said hydrogel, and the resulting mixture is provided at a place in the body of a patient where tissue regeneration or tissue replacement is intended.

91. The method of any one of claims 84 to 90, wherein said hydrogel comprises one or more bioactive therapeutics that stimulate regenerative processes and/or modulate the immune response.

92. A method of wound treatment comprising the steps:

a) providing a hydrogel as defined in any one of claims 45 to 56;
b) exposing said hydrogel to said wound.

93. The method of claim 92, wherein said wound is a burn wound.

94. The method of claim 92 or 93, wherein said hydrogel induces autolytic debridement.

95. The method of any one of claims 92 to 94, wherein said hydrogel comprises at least one amphiphilic peptide and/or peptoid of any one of claims 40 to 44.

96. The method of claim 95, wherein said hydrogel functions as a haemostatic agent.

97. The method of claim 95 or 96, wherein said hydrogel functions as an anti-inflammatory agent, which acts by scavenging extracellular nucleic acids that stimulate the innate immune system.

98. The method of any one of claims 92 to 97, wherein, in step a), said hydrogel is mixed with cells that repopulate the wound and expedite wound healing.

99. The method of any one of claims 92 to 98, wherein said hydrogel comprises one or more bioactive therapeutics that diffuse into the wound and facilitate the healing response by modulating the inflammatory response and/or stimulating regenerative processes.