

A NEW SUPRAMOLECULAR HYDROGEL BASED ON PHOTOPOLYMER AND PEPTIDE AMPHIPHILE

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Technical Field

The invention relates to a new supramolecular hydrogel based on photopolymer and peptide amphiphile.

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Prior Art

When considered in terms of production techniques, hydrogels are synthesized by covalent cross-linking of biopolymers, by enzymatic polymerization, by free radical polymerization method, by thermal-bonding and by radiation effect. In the supramolecular gelation technique, which has become a popular technique in recent years, it is formed by direct and physical contact of specially designed building-blocks without applying any enzymatic, chemical, thermal or radiation induction. The major disadvantage of supramolecular hydrogels based on such physical interactions is their low mechanical strength. When developing supramolecular hydrogels, hydrogels with poor mechanical strength are formed by bringing electrically oppositely charged building blocks together. In this invention, making use of the fact that GelMA, which is one of the two oppositely charged building blocks (photopolymer -GelMA- and peptide amphiphiles) is also a UV-sensitive biopolymer, supramolecular hydrogels with high mechanical strength occurrence have been achieved with a short-term UV-light effect applied after ensuring electrostatic supramolecular hydrogelation. When the literature and patents are searched, it has been determined that no article or patent has been published on supramolecular hydrogels of a photo-(bio)polymer - and GelMA- and peptide amphiphiles before.

As a result, prior to this invention, different strategies such as covalent chemical bonding, enzymatic reaction, heat/pH/light induction was used for the production of hydrogels; and for the production of supramolecular hydrogels, the method of physical contact of two building blocks with opposite electrical charges was used. In this invention, making use of the ability of one of the building-blocks (GelMA) to polymerize with UV sensitivity, a second cross-linking process was carried out with the application of UV-light after the physical interaction

of the two oppositely charged building-blocks, resulting in the production of supramolecular hydrogels that preserve the nano/microstructure and are mechanically durable.

There are patents in the literature on peptide amphiphile hydrogels and multi-component
5 supramolecular hydrogels. However, it has been determined that there is no patent on the photo-polymer/peptide amphiphile-based supramolecular hydrogels, which is the subject of the proposed patent.

The patents in the literature with a similar theme to the present patent application are given
10 below.

In the patent application numbered WO 2007/148334, the contribution of hydrogels obtained from peptide amphiphiles to bone tissue healing by utilizing their biomineralization enhancing properties is discussed. In this invention, it does not target bone tissue, but contains a neuro-
15 inductive epitope that enables stem cells to differentiate (transform) into nerve cells. Furthermore, the mechanism of hydrogel formation is different, and it is formed as a result of the interaction of peptide amphiphile molecules, which is a new design, with a commercialized biopolymer named GelMA (GelMA used in this invention was not commercially procured and was synthesized within the scope of this invention).

20 Patent application WO 2011/112856 relates to the hydrogel forming abilities of different peptide amphiphile designs. The present invention is a supramolecular hydrogel composed of a photo-(bio)polymer and peptide amphiphiles having a unique design.

25 In the patent application no WO 2013/066274, hydrogels obtained by the interaction of a non-peptidic biopolymer and ultra-short (3-8 amino acid) peptides are discussed. In this invention, besides the development of hydrogels formed as a result of supramolecular interaction of cationic and anionic polymers with ultra-short peptides, the hydrogel formed as a result of the interaction of ultra-short peptides with a photo-sensitive but synthetic polymer
30 such as diethylene glycol diacrylate (DEGDA) is also presented. In this invention, although it is similar in concept, GelMA, a modified form of gelatin, in other words, a hybrid photo-(bio)polymer, was used instead of DEGDA, a synthetic polymer, in the patent proposal. In addition, while ultra-short peptides were used in the patent in question, bio-active (neuro-inductive) peptide ampoules were used in the proposed patent application. Peptide
35 amphiphiles with nano-fiber morphology gave a nanofibrous structure to the developed GelMA/peptide amphiphile supramolecular hydrogels.

The patent application no US 2007/0224273 addresses the hydrogelation of Fmoc-modified amino acids and pamidronate after supramolecular interaction. The biological theme is wound healing. In these aspects, it is quite different from the scope of the present invention.

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In the patent application no WO 2012/078760, hydrogels obtained with specially designed peptide amphiphile molecules were designed to increase the stabilization after hydrogelation.

Until today, many traditional techniques have been used for the production of biomaterials and hydrogels. Techniques such as freeze-drying to produce foamed biomaterials, electrospinning to develop nano-fibrous membrane materials, and gas foaming to develop porous biomaterials have been developed. Although the supramolecular hydrogel developed within the scope of this invention is in nanofibrous structure, the factor that gives this feature to the hydrogel is not the material fabrication technique, but the natural structures of the peptide amphiphiles used.

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Technical Problems the Invention Aims to Solve

Hydrogels formed by supramolecular interactions are mechanically weak. In addition, the macromolecules used are usually synthetic polymers. In this invention, mechanically more durable supramolecular hydrogels were obtained by performing post-interaction secondary photo-crosslinking, by applying UV-light to hydrogels obtained with supramolecular interactions (weak chemical interactions, electrostatic interactions) from peptide amphiphiles and gelatin derivative as a natural polymer, after hydrogelation. These hydrogels have microporous and nanofibrous structures.

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In the process of forming supramolecular hydrogels, generally, only bioactive molecules (such as proteins, biopolymers) are used instead of responsive cross-linking macromolecules. In this invention, the supramolecular interactions of gelatin methacrylate (GelMA), which is also a biopolymer, with peptide amphiphiles are discussed. Thanks to the MA (methacrylic anhydride) group contained in the GelMA biopolymer, hydrogels with high mechanical strength are formed when UV-light is applied to the GelMA biopolymer (for 1 minute). Since GelMA is also a biopolymer with negative zeta potential (at pH=7.4), it can have supramolecular interaction (electrostatic interaction) with peptide amphiphiles designed to have positive zeta potential under the same conditions. In this invention, with a second cross-linking (UV-light treatment, photo-crosslinking) applied to the hydrogel formed after the

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supramolecular interaction of the (-) electrically charged GelMA photo-(bio)polymer and the (+) electrically charged peptide amphiphile molecules for 1 minute, supramolecular hydrogels with high mechanical strength have been developed. The peptide amphiphile molecule is designed with a (+) charge to interact electrostatically and also contains the bioactive epitope
5 necessary to induce differentiation of mesenchymal stem cells into nerve cells. Thus, nanofibrous and microporous, bio-active (neuro-inductive) and photo-polymer-peptide amphiphile supramolecular hydrogel with high mechanical strength was formed.

Description of the Invention

10 The invention; a hydrogel product was formed as a result of the interaction of the UV-sensitive biopolymer GelMA with the peptide amphiphiles followed by the secondary crosslinking process. Also, the method used to create this product has never been reported in the literature before.

Description of the Drawings

Figure 1: TEM views of PA-GSR(-) and PA-GSR(+)

Figure 2: SEM view of GelMA (%10) and PA-GSR(+)-GelMA supramolecular hydrogel

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Figure 3: G'G'' - frequency graphics of PA-GSR(+), PA-GSR(+)-GelMA (- UV) and PA-GSR(+)-GelMA (+UV)

Detailed Description of the Invention

25 Hydrogels that occur only with physical interactions (electrostatic interactions) without the need for processes such as chemical bonding, enzymatic reaction, thermal induction or radiation and in line with the design of the relevant components are called supramolecular hydrogels.

30 In this invention, hydrogel biomaterial obtained by the supramolecular (electrostatic) interaction of gelatin methacrylate (GelMA), which is an (-) electrically charged and photo-sensitive (UV-sensitive) photo-(bio)polymer obtained by the methacrylic acid (MA) modification of gelatin biopolymer that is a biodegradable and low-cost polymer, and bio-active (containing the relevant amino acid sequence -YIGSR- for the differentiation of stem
35 cells into neural cells), nano-fibrillary peptide amphiphile (PA) molecules containing an (+) electrically charged hydrophobic tail (palmitic acid) obtained by solid phase peptide synthesis

method, was discussed. The chemical and physical properties of the components that make up the hydrogel are presented in Table 1.

Table 1. Components of hydrogel and their properties

PA	Sequence	Mw (kDa)*	Zeta Potential (mV)*
PA-GSR(+)	C ₁₅ H ₃₁ CONH-V ₃ A ₃ K ₃ YIGSR-CONH ₂	1727	+26
PA-GSR(-)	C ₁₅ H ₃₁ CONH-V ₃ A ₃ E ₃ YIGSR-CONH ₂	1730	-14
PA-RGDS(+)	C ₁₅ H ₃₁ CONH-V ₃ A ₃ K ₃ RGDS-CONH ₂	1566	+17
GelMA	Gelatin methacryloyl	150-200	-12

The purpose of use of the hydrogel components in Table 1 is as follows:

PA-GSR(+) is a PA designed as positively charged for electrostatic interaction with GelMA biopolymer containing YIGSR amino acid sequence as bioactive epitope. In the design of this PA, the palmitic acid molecule (C16), which provides the formation of the nano-fibrillary structure, the VVVA₃ amino acid sequence which provides the formation and stabilization of the β -layer structure, the KKK amino acid sequence which provides the positive electrical charge, and finally, the neuro-inductive (providing neurogenic differentiation of mesenchymal root cells) YIGSR amino acid sequence are present, respectively.

PA-GSR(-) is an (-) electrically charged PA molecule, which has the same characteristics as PA-GSR(+), but with the EEE amino acid sequence instead of the KKK amino acid sequence. Since this PA molecule is negatively charged similarly to GelMA, it does not have supramolecular interaction.

GelMA is the methacrylate derivative of gelatin biopolymer and is a UV-sensitive photopolymer.

The chemical structure, molecular weight and zeta potentials (pH 7.4) of all 3 components are presented in Table 1.

Transmission electron microscopy (TEM) images of PA-GSR(-) and PA-GSR(+) peptide amphiphiles are presented in Figure 1.

Preparation of supramolecular hydrogels

For the preparation of supramolecular hydrogels, 1% w/v PA (PA-GSR(+)) molecules dissolved in HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer were injected into 100 μ L of GelMA (10% w/v) biopolymer dissolved in DPBS (Dulbecco's Phosphate Buffer Saline) to 50 μ L. Hydrogels with high mechanical strength were formed with the effect of UV-light (23 mW/cm²) applied for 1 minute to the resulting supramolecular hydrogel after a 1-minute incubation period for electrostatic interaction to occur. When the same process was repeated with PA-GSR(-), it was observed that no supramolecular hydrogels were formed.

The physical properties of the resulting supramolecular hydrogels were characterized by scanning electron microscopy (SEM) and the flow properties were characterized with rheometer.

While SEM images of GelMA biopolymer show standard and porous (microporous) hydrogel morphology, nano-fibrous and microporous nano/micro structures were observed in SEM images of the PA-GSR(+)-GelMA supramolecular hydrogel.

Supramolecular hydrogel formation was also characterized by examining the flow properties (rheological properties).

In the G' ω /frequency plot presented in Figure 3, it is seen that only PA-GSR(+) forms weak hydrogel (G' approximately 1kPa), whereas PA-GSR(+)-GelMA supramolecular hydrogel forms stronger and more mechanically stable hydrogel (G' is approximately 2 kPa). It was observed that the mechanical strength of the hydrogel had increased (G' approximately 10kPa) after the UV-light applied to the PA-GSR(+)-GelMA hydrogel formed by supramolecular interactions for 1 minute.

Thanks to this invention, supramolecular hydrogels with higher mechanical strength have been obtained compared to those achieved with previous technologies.

In vitro cell culture studies carried out with supramolecular hydrogels of the invention have shown that the gel in question differentiates human adipose mesenchymal stem cells into nerve cells. The data obtained as a result of gene expression studies carried out after 3D seeding of stem cells into supramolecular hydrogels and culturing for 14 days showed that PA-GSR(+)-GelMA hydrogel is significantly expressed further in the neuro-tissue-related

gene regions such as Nestin, MAP2, SOX2 in mesenchymal stem cells. The invention will benefit users due to its low-cost production, and advanced material technology.

CLAIMS

- 5 1. A supramolecular hydrogel based on photopolymer and peptide amphiphile, characterized in that it is formed with a short-term UV-light effect applied after ensuring electrostatic supramolecular hydrogelation by making use of the fact that GelMA, which is one of the two oppositely charged building blocks (photopolymer -GelMA- and peptide amphiphiles) is also a UV-sensitive biopolymer.
- 10 2. Hydrogel according to Claim 1, characterized in that it is obtained by supramolecular (electrostatic) interaction of bio-active (containing the relevant amino acid sequence -YIGSR- for the differentiation of stem cells into neural cells), peptide amphiphile (PA) molecules having nano-fibrillary structure, containing an (+) electrically charged hydrophobic tail (palmitic acid) obtained
15 by solid phase peptide synthesis method, with gelatin methacrylate (GelMA).
- 20 3. Hydrogel according to Claim 1, characterized in that it comprises a neuro-inductive epitope that enables stem cells to differentiate (transform) into nerve cells.
4. Hydrogel according to Claim 1, characterized in that it is a supramolecular hydrogel composed of peptide amphiphiles having a unique design with a photo-(bio)polymer.
- 25 5. Hydrogel according to Claim 1, characterized in that it is prepared with the method below:
- 100 μ L of GelMA (10% w/v) biopolymer dissolved in DPBS (Dulbecco's Phosphate Buffer Saline) is dissolved in HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer,
 - 30 - 1% w/v PA (PA-GSR(+)) molecules are injected, to 50 μ L,
 - Hydrogels with high mechanical strength are formed with the effect of UV-light (23 mW/cm²) applied for 1 minute to the resulting supramolecular hydrogel after a 1-minute incubation period for electrostatic interaction to occur.