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REVIEW

Self-Assembling Peptide-Based Hydrogels in Angiogenesis

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Abstract: Ischemic diseases, especially in the heart and the brain, have become a serious threat to human health. Growth factor and cell therapy are emerging as promising therapeutic strategies; however, their retention and sustainable functions in the injured tissue are limited. Self-assembling peptide (SAP)-based hydrogels, mimicking the extracellular matrix, are therefore introduced to encapsulate and controllably release cells, cell-derived exosomes or growth factors, thus promoting angiogenesis and tissue recovery after ischemia. We will summarize the classification, composition and structure of SAPs, and the influencing factors for SAP gelation. Moreover, we will describe the functionalized SAPs, and the combinatorial therapy of cells, exosomes or growth factors with functionalized SAPs for angiogenic process as well as its advantage in immunogenicity and injectability. Finally, an outlook on future directions and challenges is provided.

Keywords: self-assembling peptide, hydrogel, angiogenesis, survival, retention

Introduction

Ischemic diseases in various tissues, such as heart, brain, limb and skin, have severely threatened the life quality and health conditions of human beings, and brought huge economic burdens worldwide. Therapeutic strategies focus on the acceleration of tissue repair by promoting new vessel formation, the process of which consists of stimulation of endothelial cells (ECs) by angiogenic factors; degradation of extracellular matrix (ECM) via extracellular proteinases such as matrix metalloproteinases (MMPs); capillary sprouting and EC migration via integrins; and vessel maturation mediated by growth factors.[1–3] Currently, stem cells and growth factors are proven to be effective in promoting angiogenesis and tissue recovery after ischemia despite the low retention rate.[4,5] In addition, ECM plays an important role in the angiogenic process through sequestering growth factors and mediating signal transduction.[6] It is imperative to replenish a huge amount of ECM that is seriously destructed in ischemia. Facing these obstacles, biomaterials are specifically designed to promote angiogenesis, mimicking the natural features of ECM on the one hand, and encapsulating and sustainably releasing the bioactive agents on the other.[7,8]

Hydrogels are widely used in the therapeutics of ischemic diseases experimentally and clinically.[7,9] Different hydrogelators can form hydrogels, including natural biological molecules (chitosan, hyaluronic acid, collagen, fibroin or acellular ECM), synthetic molecules (polyethylene glycol, polyacrylamide, polymethacrylamide) and supramolecular hydrogelators (self-assembling peptide, nucleobases or

monosaccharide).[10,11] Self-assembling peptide (SAP) hydrogels, as a supramolecular hydrogelator, have attracted much attention for their bioactivity, biocompatibility, biodegradability and tunable mechanical stability.[12–14] SAPs, as a tailor-made material, can be designed with angiogenic bioactivity aiming to enhance neovascularization.[15–17] More importantly, the structurally changeable properties of SAPs make them compatible to encapsulate cells, exosomes and growth factors.[18–20] In our review, we summarize the classification, composition and structure, and the influencing factors for SAPs. Moreover, we will describe the functionalized SAPs, the combinatorial therapy of cells, exosomes or growth factors with functionalized SAPs and the advantage of using SAPs for angiogenic process as well as its advantage in immunogenicity and injectability.

Self-Assembling Peptide Hydrogels

With building blocks including the natural amino acids and/or hydrophobic fatty acid chain, self-assembling peptides, as one of the supramolecular hydrogelators, can be self-organized into ordered nanofibers and further into well-ordered scaffolds in water. Since their discovery, diverse classes of short SAPs have been invented with a wide range of applications, including 3D tissue cell culture, reparative and regenerative medicine, tissue engineering and slow drug release.[21] We will elaborate the physical features of SAPs, which lays the foundation of SAP bioactivity, biocompatibility and biodegradability.

Classification of Self-Assembled Peptides

Depending on different chemical or biological modifications, SAPs can be categorized into native peptides, peptides with terminal acetylation (or formylation) and amidation, peptides modified with an alkyl chain, peptide derivatives containing an aromatic group (fluorene-based, naphthalene-based[22] and pyrene-based[23]) and peptide derivatives containing a photosensitive group. Chemical modification of peptides, such as terminal acetylation and amidation, or attaching an alkyl chain, could change the solubility and stability of SAPs. Self-complementary ionic peptides are peptides with N-terminal acetylation (or formylation) and C-terminal amidation; peptide amphiphiles are hydrogels modified by an alkyl chain. Many native peptides consist of different numbers of amino acids, from dipeptide and tripeptide to hexadecapeptide. The length of the peptides is correlated with the strength of intermolecular interactions. Currently, the longest synthesized peptide consists of 30 amino acids (CKQLEDKIE ELLSKAACKQLEDKIEELLSK).[24] In our review, we focus on two classes of SAPs, peptide amphiphiles and self-complementary ionic peptides, which are broadly used in angiogenesis research.[25]

Peptide amphiphiles (PA) and self-complementary ionic peptides are the major classes of self-assembling peptides, sharing many similarities in their composition and structure.[26] Peptide amphiphiles can be designed with four domains as shown in Figure 1. The first domain is a hydrophobic moiety, which is usually an unbranched alkyl group, such as palmitic acid. Occasionally, fluorenylmethoxycarbonyl (Fmoc) and 2-(naphthalen-2-yl) acetic acid are used as the alternative hydrophobic moiety. The second domain is the β-sheet-forming peptide sequence, which is the central part for nanostructure morphology and can be adjusted to tune the mechanical properties and gelation kinetics. The third domain often consists of one to three charged amino acids to improve aqueous solubility of the PA hydrogel. The fourth domain typically introduces a bioactive signaling peptide, such as the cell-adhesive peptide arginine-glycine-aspartic acid-serine (RGDS). In some instances, PA hydrogels are absent of the third and fourth domains, conferring them enhanced gelling properties as pro-gelators. The four PA domains play different roles in the gelation process (Figure 1). The first domain forms an internal hydrophobic core, while the fourth domain forms an external active group, with the second and third domains adjusting the stability of the hydrophobic core and the solubility of the peptide in aqueous solution, which is then extended to form a 3D nanofiber in water. Subsequently, the nanofibers become longer and interact with the H_2O to form into hydrogel through the modification of salt, temperature, Gibbs free energy etc.

Self-complementary ionic peptides are another common type of self-assembling peptide, and consist of 50% charged residues.[27] These peptides are periodic repeats of alternating ionic hydrophilic and hydrophobic amino acids, forming a β-sheet with two distinct polar and non-polar surfaces. The polar surface of the β-sheet consists of both positive and negative amide acids, while the non-polar surface is composed of hydrophobic amide acids.[28,29] The hydrophobic side forms a double sheet on the inside of the fiber to keep away from water, and the hydrophilic side faces outwards interacting with water molecules. For example, RADA16-I ($_{Ac}$-RADARADARADARADA-$_{NH2}$) can form

stable β-sheet structures in water and spontaneously assemble into nanofiber scaffolds with the aid of hydrophobic interaction and hydrogen bonding (Figure 1). Short bioactive signaling peptides can be added to the middle or the end of self-complementary ionic peptides to emphasize the application related with their respective bioactivities; thus synthesized peptides are called as multidomain peptides (MDP).[30]

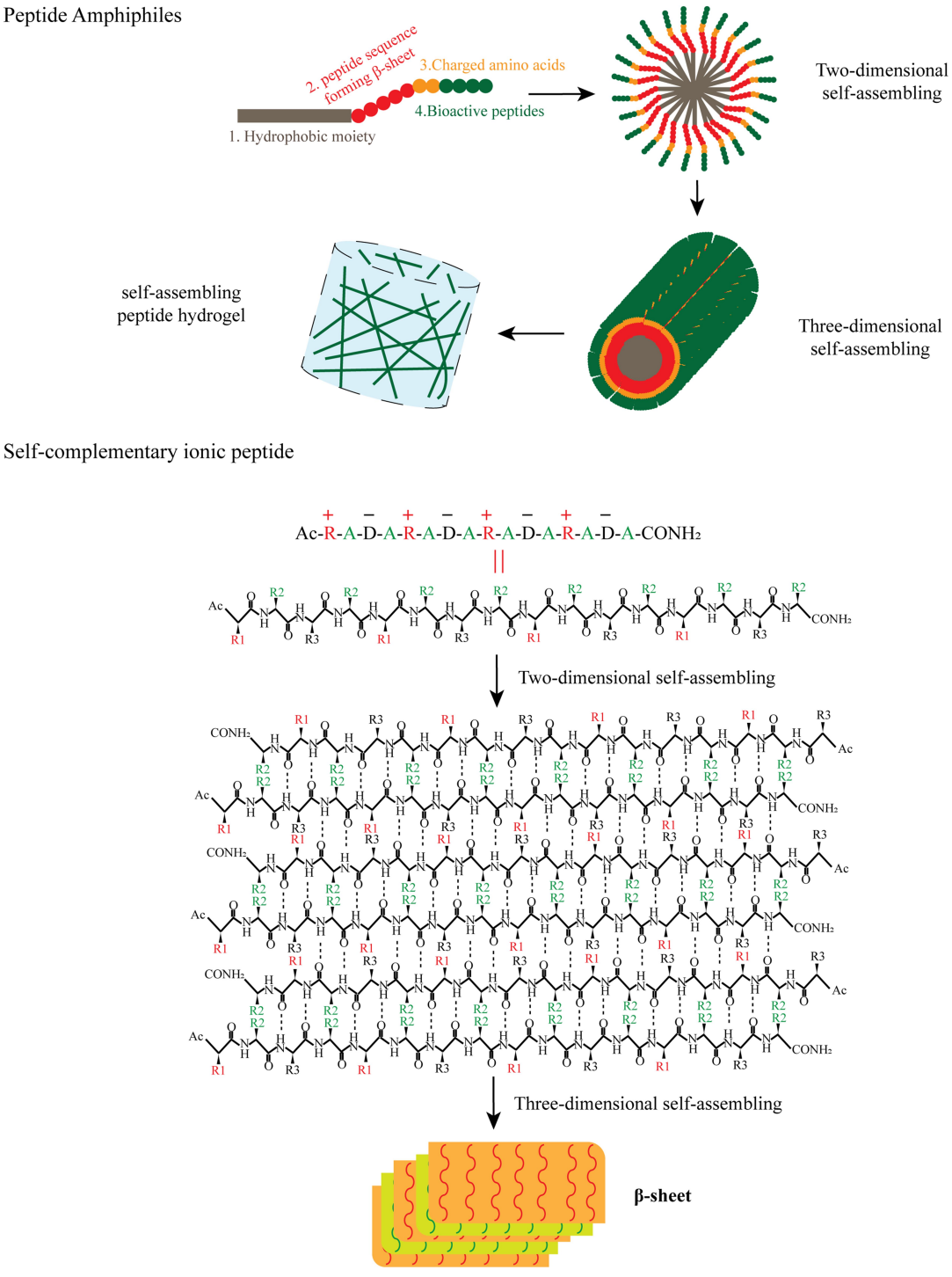

Figure 1 Self-assembling of peptide amphiphiles and RADA16-I. Peptide amphiphiles, hydrophobic moiety including palmitic acid, Fmoc and 2-(naphthalen-2-yl) acetic acid can react with the N terminal of peptide to form the peptide amphiphiles. Peptide amphiphiles self-assemble into nanofibers and hydrogels through hydrophobic interaction and hydrogen bonding. Repeats of alternating ionic hydrophilic and hydrophobic amino acids are distributed in RADA-I. RADA-I self-assembles to form β-sheets and nanofibers, with the hydrophobic alanine sandwiched inside and hydrophilic residues on the outside. R, arginine; A, alanine; D, aspartate; R1, R2, R3, the side chains of arginine, alanine and aspartate.

Ultrashort peptides (< 8 amino acids) are more appealing in biomedical applications owing to their easy synthesis, relatively low sensitivity to enzymatic digestion and high biocompatibility.[25] Normally, hydrogen bonding, π–π, van der Waals, electrostatic and hydrophobic interactions are responsible for the self-assembly of ultrashort peptides and also long peptides (> 8 amino acids).[25] Ultrashort peptides self-assemble into hydrogels depending on their amphiphilicity and/or self-complementarity. Heptapeptide, EDLIIKG and MNFGAFS, and its derived peptide, DLII and NFGAF, can form self-supportive translucent hydrogels with a clear secondary structure change from α-helix to β-sheet during the self-assembly process.[31]

Multiple Factors Influence Gelation of the Self-Assembling Peptides

The gelation process of SAPs can be affected by two types of factors, including the basic chemical features of the peptides and the environmental factors. We summarize all the factors that influence the gelling ability, degradation rate and mechanical properties of SAPs (Table 1), providing an insight on designing personalized hydrogels. The basic chemical features of the peptides greatly depend on the peptide design itself. The composition and arrangement of amino acids determine the hydrophobicity and solubility of the peptides. Peptides with high hydrophobicity could easily form nanofibers with superior mechanical strength.[32] While high percentage of hydrophobic residues, alanine (Ala), valine (Val), isoleucine (Ile), leucine (Leu), tyrosine (Tyr), phenylalanine (Phe), tryptophan (Trp) decreases the solubility of the peptide, charged amine acids are therefore introduced at the end of the SAP to improve its solubility.[33,] Switching the position of I and L in the hydrophobic tail of an ultrashort peptide (changing LIVAGKC into ILVAGKC) resulted in a gel that demanded a higher peptide concentration for gelation and became less stiff.[34] The length of the peptide is another determinant factor for gelation. The intermolecular interactions and physical cross-linking are enhanced for elongated peptides, especially for self-complementary ionic peptides.[35] Conformational change or modification of amino acids and peptides is the most common way to increase the stability of the hydrogels. Natural L-amino acids could be replaced by synthetic D-amino acid counterparts, and the resultant D-peptide-based hydrogels proved to be more stable in vivo since D-form peptide bonds resist natural L-enzyme degradation. Capping the N-terminal of SAPs by an acetyl or the C-terminal by an amide, or both ends, could also enhance the self-organization into hydrogels in water.[36]

Various environmental factors can stimulate the hydrogelation of the SAPs. The gelation process starts when Gibbs free energy turns negative under the stimulation of either physical or chemical methods. Modulating the ionic strength, changing the temperature and applying ultrasound are commonly used methods.[12] Changing the ionic strength by adding polyvalent cations or polyvalent anions, such as Ca^{2+}, Mg^{2+}, or PO_4^{3-}, can induce the gelation of SAPs.[37,38] For example, addition of PO_4^{3-} to the $_{Ac}$-KKSLSLSLSLSLSLKK-$_{NH2}$ solution increased the length of the nanofibers and the storage modulus.[12] In another example, PELELELELELEP peptide, containing only negatively charged residues, formed hydrogels with an almost 10-fold increase of storage modulus in the presence of calcium ion.[12,] In addition, temperature obviously affects the structure and mechanical properties of hydrogels. It was reported that peptide nanofibers assemble faster and more strongly at 25°C than at 5°C.[39] Application of sonication is another way to stimulate gelation. The disarranged nanofibers could be disrupted by sonication, and form arranged and longer nanofibers energetically favorable and further gelate.[40,41,] A pH change could also stimulate the gelation by changing the protonation/deprotonation of basic or acidic groups.[30,42] The gelation of smart hydrogel, with the photo-reactive or enzyme-sensitive peptide introduced inside, could be stimulated by treatment with photochemicals or enzymes.[43,44] Thus, the designable and adjustable mechanical properties make the self-assembling peptide a better alternative in tissue engineering and angiogenesis.

Self-Assembling Peptides in Angiogenesis

Angiogenesis is a complicated process involving several chronological steps. Angiogenic factors, MMPs and MMP-induced ECM degradation play important roles in EC migration and vessel maturation. Molecular manipulation of SAPs could mimic the biological feature of ECM and facilitate these steps: integration of MMP-sensitive motifs increases the biodegradability of SAPs; employment of cell-adhesive ligands facilitates EC adhesion and migration; introduction of growth factor-mimicking peptides initiates angiogenesis and accelerates vessel maturation. Another well-established approach is the

Table 1 Summary of Influencing Factors for SAP Gelation

Influencing Factors	Mechanisms	References
Peptide-related factors		
Peptide Hydrophobicity	Peptide with high hydrophobicity is difficult to dissolve, but easier to gelate.	(Banwell et al, 2009)[32]
Composition of Amino Acids	Peptides with high ratio of hydrophobic residues form hydrogels with better mechanical properties.	(Lutolf et al, 2003)[33]
Peptide Concentration	Higher concentration benefits gelling.	(Hauser and Zhang, 2010[21]; Du et al, 2015[12])
Peptide Length	Longer peptide benefits gelling.	(Fletcher et)[35]
Amino Acid Chirality	D-peptide-based hydrogel is more stable than natural L-amino acid-based peptide.	(Schutz et al, 2015)[12]
Peptides with Capped N- and C-Terminals	Peptides with capped N- and C-Terminals benefit gelling.	(Solaro, 2010)[36]
Environment-related factors		
Salt	Salt changes the ionic strength, thus inducing noncovalent interactions among peptides.	(Ozbas et al, 2004[38]; Feng et al, 2012[37])
Temperature	Heating and cooling achieve highly ordered hydrogel, especially entropy-driven assembled hydrogel.	Du et al, 2015[12]
Sonication	Ultrasound breaks self-locked intramolecular hydrogen bonds or π stacking, and interlocked structures between peptide and water molecule are formed.	(Yokoi et al, 2005[41]; Pappas et al, 2015[40])
pH	pH can affect protonation/deprotonation of basic or acidic groups in peptide.	(Hutchinson etal, 2019[42]; Lopez-Silva etal, 2019)[30]
Photochemical	Photo affects the gelling ability inhibited by photo-reactive groups.	(Collier et al, 2001)[43]
Enzyme	Enzyme cleaves the enzyme-sensitive peptide to remove peptide-inhibited gelation and accelerates the degradation of SAP hydrogel.	(Lian et al, 2016)[44]

encapsulation of cells, cell-derived exosomes or growth factors by SAP hydrogels (Figure 2), which sustains the constant release of cell secretome in situ to promote angiogenesis.

Molecular Modification of Self-Assembling Peptides

A dominant feature of SAPs is the ability to append functional domains, such as MMP-sensitive peptides, cell affinity peptides and proangiogensis peptides, onto their termini without disrupting the assembly properties of the original peptide (Table 2). In this way, SAPs appended with functional domains can provide scaffolds mimicking the native ECM structure and enhancing specific cellular responses as well (Figure 2).

MMPs are multifunctional enzymes capable of cleaving the ECM components including collagens, laminin, fibronectin and many others. The gelatinase MMP2 can recognize and cleave sequences at a defined motif with three prioritized amino acids, with the first amino acid hydrophobic, the second amino acid either hydrophobic or basic and a small residue (alanine, glycine or serine) at the third position.[45] LRG in KSLSLSLRGSLSLSLKG, LIG in GTAGLIGQ and KLDLPVGLIGKLDL, and PVG in KLDLPVGLIGKLDL are in line with the requirement of the prioritized amino acid motif and are introduced in many cases as the MMP2-sensitive sequence. For instance, appending the MMP-sensitive sequence, like LIG or LRG, to the middle of SAPs can accelerate the degradation of hydrogels facilitating the cell migration.[46]

Cell-adhesive ligand Arg-Gly-Asp-Ser (RGDS) is identified as the key sequence in the ECM proteins including fibronectin, vitronectin and laminin. RGDS is sufficient and indispensable for cell membrane binding by interacting with membrane protein integrins.[47] Cell affinity peptides containing the RGD motif conjugated

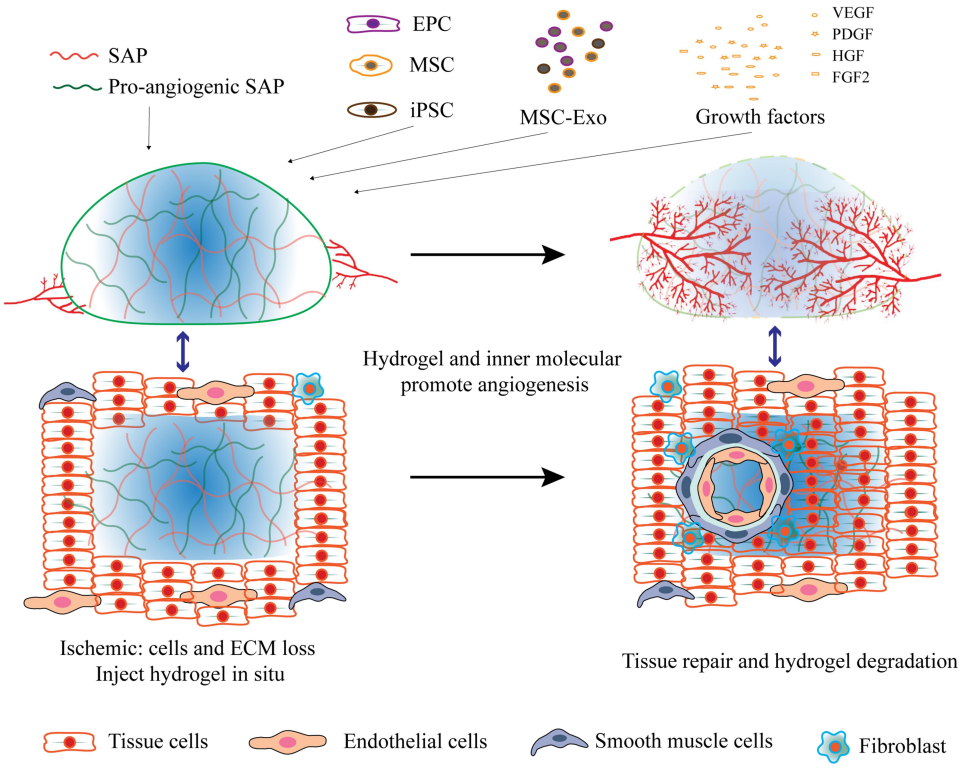

Figure 2 Self-assembling peptides in angiogenesis. Hydrogels formed by pro-angiogenic SAPs, or combined with pro-angiogenic cells, exosomes and growth factors promote angiogenesis.

with SAPs can strengthen the binding of peptides to endothelial cells, therefore enhancing capillary sprouting as ECM is degraded. For example, RGDS-modified hydrogels could greatly promote cell adhesion and survival.[19] In addition, the functional motif PRGDSGYRGDS, containing two similar RGD motif

Table 2 Summary of Pro-Angiogenic Modifications in SAP Hydrogels

Peptide Sequences	Pro-Angiogenic Modifications	Applications	References
RADA16-I and RADA16-I -SVVYGLR (10:1)	SVVYGLR	Facilitate angiogenesis and neurogenesis at the brain injury site	(Wang et al, 2017)[77]
RADA16-II andRADA16-II-substance P (200:1)	Substance P	Promote angiogenesis	(Im et al, 2018)[63]
RADA-I-GPRGDSGYRGDS or RADA-I-KLTWQELYQLKYKGI	PRGDSGYRGDS, KLTWQELYQLKYKGI	Improve angiogenesis in chicken embryo chorioallantoic membrane	(Liu et al, 2012)[49]
KKSLSLSLSLSLSLKK		Highly vascularized after subcutaneous injection	Du et al, 2015[12]
KSLSLSLRGSLSLSLKGRGDS or KSLSLSLRGSLSLSLKGKLTWQELYQLKYKGI	LRG, RGDS, KLTWQELYQLKYKGI	Promote tissue regeneration in ischemic tissue disease	(Kumar et al, 2015)[16]
KSLSLSLRGSLSLSLK–G–KLTWQELYQLKYKGI	LRG, KLTWQELYQLKYKGI	Promote recovery from traumatic brain injury	(Ma et al, 2020)[15]

Abbreviations: SVVYGLR, high affinity for integrin α9β1 and α4β1; PRGDSGYRGDS, cell adhesion peptide; KLTWQELYQLKYKGI, pro-angiogenic sequence; LRG, MMP2-sensitive peptide.

sequences PRGDS and YRGDS, has been shown to distinctly promote the survival, proliferation, migration and morphological differentiation of human umbilical vein endothelial cells (HUVECs).[48,49]

Pro-angiogenesis peptides, such as QHREDGS, SVVYGLR and KLTWQELYQLKYKGI, can also conjugate with SAPs, promoting growth and migration of ECs via transferring of pro-angiogenic signals. For example, the QHREDGS sequence, as the integrin-binding site in angiopoietin-1, could enhance EC survival, tube formation and cell barrier functionality.[50,51] QHREDGS-RADA16-I hydrogel was reported to promote angiogenesis, thereby reducing scar size and restoring cardiac function after MI.[50] Another reported sequence, SVVYGLR, derived from osteopontin, could also stimulate angiogenesis in vitro and in vivo.[52] The SVVYGLR motif conjugated to the terminal of RADA16 increased the adhesion, migration and tube formation of ECs.[53] Notably, VEGF-mimicking peptide KLTWQELYQLKYKGI-modified RADA16 scaffolds could increase HUVEC survival and attachment when compared to pure RADA16 and induce morphologies similar to Matrigel and collagen.[49] GHRPS, belonging to the group of growth hormone-releasing peptides, was also reported to activate pro-survival pathways such as PI-3K/AKT1, increase angiogenesis and reduce inflammation by inhibiting the NF-κB pathway.[54] The last example discussed here is multidomain peptides. A single SAP could be functionalized with multiple peptide domains, like C16-GTAGLIGQ-RGDS, KSLSLSLRG SLSLSLKGRGDS, or KSLSLSLRGSLSLSLKG-KLTW QELYQLKYKGI, conferring on them enhanced angiogenic capabilities.[8]

Encapsulation of Cells and Exosomes by Self-Assembling Peptide Hydrogels

The crucial roles of various cell types in angiogenesis under different ischemic conditions are well established.[8] Endothelial progenitor cells (EPCs) improve local angiogenesis through direct contribution to neovascularization and releasing paracrine factors.[55,56] Mesenchymal stem cells (MSCs) can also improve neovascularization in a paracrine stimulation of angiogenesis and regulation of angiogenic molecules.[57] Induced pluripotent stem cells (iPSCs) showed substantial beneficial effects on the angiogenic process through differentiation into endothelial cells or mesenchymal stem cells. Exosomes, as important secretes vesicles, exhibited similar therapeutic effects compared to their derived cells.[58–61] Importantly, the exosomes do not trigger immune responses in the host, avoiding the risk of rejection in stem cell transplantation. However, both cells and exosomes only demonstrate moderate angiogenesis in vivo due to the low retention rate in ischemic tissue and the high clearance rate caused by activated systemic immune response.[4,5] Currently, SAP hydrogels have attracted much attention since they could encapsulate and protect the cells from a harsh microenvironment, significantly improving the cell viability and the retention time in vivo, and ensuring the continuous and steady release of cells and their derivatives (Figure 2).

Among the SAPs for cell encapsulation, the most widely studied are self-complementary ionic peptides RADA16-I and RADA16-II (Table 3). RADA16-I hydrogel-encapsulated microvascular cells promoted angiogenesis in spinal cord injury by reducing inflammation and glial scar formation.[17] Combinatorial therapy with adipose-derived stem cells (ADSCs) and RADA16-II hydrogel demonstrated a three-fold increase in survival and retention of transplanted cells, a 54.25 ± 4.42% increase in the ejection fraction, and more established vascular networks than did administration of ADSC alone.[62] Moreover, RADA16 is easily functionalized using biologically active epitopes. The functionalized RADA16 scaffolds encapsulating BMSCs demonstrated beneficial effects in both myocardial infarction and skin defect.[50,53,63] For instance, BMSCs encapsulated in QHREDGS-modified RADA16-I hydrogel effectively reduced cell apoptosis and improved cardiac function in the post-MI heart by activating the miR-21-related signaling pathway.[50]

Peptide amphiphile hydrogels are also used to encapsulate and protect transplanted cells (Table 3). Notably, mESCs-derived cardiomyocytes encapsulated in C16-GTAGLIGQ-RGDS demonstrated approximately 3-fold higher engraftment in hearts, and sustained better cardiac function after MI for 12 weeks (Figure 3).[64] C16-GTAGLIGQ-RGDS hydrogel could also protect hiPSC-derived ECs under oxidative stress and improve structural neovascularization for up to 10 months (Figure 3).[19] In addition, C16-GTAGLIGQ-RGDS hydrogel was also reported to induce the osteogenic differentiation of engrafted hMSCs (Figure 3).[65] As a carrier, PA hydrogels have also been used to deliver exosomes. NapFF and PA-GHRPS hydrogels were able to effectively deliver exosomes and sustain the stable release of exosomes, thus promoting angiogenesis in MI hearts.[66]

Table 3 Summary of Cell, Exosomes and Growth Factors Encapsulated by SAP Hydrogels

Peptide Sequences	Pro-Angiogenic Modification	Pro-Angiogenic Factors	Application	References
Cells encapsulated in hydrogel				
RADA16-I		Microvascular cells	Promote repair of spinal cord injury	(Tran et al, 2020)[17]
RADA16-I and QHREDGS-modified RADA16-I (1:1)	QHREDGS	Rat BMSCs	Improve angiogenesis and cardiac function after MI	(Cai et al, 2019)[50]
RADA16-I -SVVYGLR	SVVYGLR	Rat BMSCs	Promote angiogenesis and cardiac repair after MI	(Gao et al, 2017)[53]
RADA16-II		ADSCs	Promote angiogenesis and preserve cardiac function in MI	(Kim et al, 2017[62]
RADA16-II and RADA16-II-substance P (200:1)	Substance P	Human dermal fibroblasts	Promote angiogenesis and recovery of skin defect	(Im et al, 2018)[63]
C16-GTAGLIGQ-RGDS	LIG, RGDS	CMs from mESCs	Promote cardiac repair	(Ban et al, 2014)[64]
C16-GTAGLIGQ-RGDS	LIG, RGDS	hPSC-CDH5^{+} cells	Promote vascular regeneration in hindlimb ischemia	(Lee et al, 2017)[19]
Exosomes encapsulated in hydrogel				
Ac-KLDLPVGLIGKLDL-CONH2	LIG	Exosomes from mouse BMSCs	Decrease chronic renal fibrosis in I/R mice	(Zhou et al, 2019)[20]
NapFF and C16-GTAGLIGQ -GG-GHRPS	LIG, GHRPS	Exosomes from human UMSCs	Promote cardiac repair after MI	(Han et al, 2019)[66]
Proteins/Peptides encapsulated in hydrogel				
Attaching the LRKKLGKA to RADA16-I	LRKKLGKA	VEGF	Improve cardiac function after MI	(Guo et al, 2012)[18]
RADA16-I-GGQQLK or RADA16-I-GGLRKKLGKA	LRK	VEGF and HGF	Promote angiogenesis in minimally invasive surgery, ischemic tissue disorders and chronic wound healing	(Huang et al, 2019)[69]
M-RADA16-II (H2N-RARADADARARADADA-OH)		Notch ligand Jagged-1 mimics	Improve cardiac function after MI	(Boopathy et al, 2015)[67]
KSLSLSLRGSLSLSLKGRGDS	LRG, RGDS	TGFβ1, FGF2, VEGF	Promote regeneration of endodontics	(Galler et al, 2012)[68]

Notes: In this Table, green color labels bioactive peptides, and red color labels MMP2-sensitive peptides.

Abbreviations: RADA16-I, AcN-RADARADARADARADA-CONH$_2$; RADA16-II, AcN-RARADADARARADADA-CONH$_2$, C16, palmitic acid; QHREDGS, prosurvival peptide; SVVYGLR, high affinity for integrin; Substance P, an 11-amino acid neuropeptide extensively found in nervous systems, RPKPQQFFGLM; RGDS, high cell adhesion peptide; LRG and LIG, MMP2-sensitive peptide; LRKKLGKA, heparin-binding; LRK, affinity to proteoglycan heparan sulfate; Notch ligand Jagged-1 mimic, H2N-CDDYYYGFGCNKFCRPR-OH; GHRPS, prosurvival peptide; BMSCs, bone marrow-derived mesenchymal stem cell; ADSC, adipose-derived stromal cells; UMSCs, umbilical cord mesenchymal stem cell; CMs, cardiomyocytes; mESCs, mouse embryonic stem cells; hPSC-CDH^{5+} cells, human pluripotent stem cell-derived endothelial cells with CDH^{5+} expression; EV, extracellular vesicle; TGFβ1, transforming growth factor beta 1; FGF2, basic fibroblast growth factor; VEGF, vascular endothelial growth factor; MI, myocardial infarction; HI, ischemic hindlimbs.

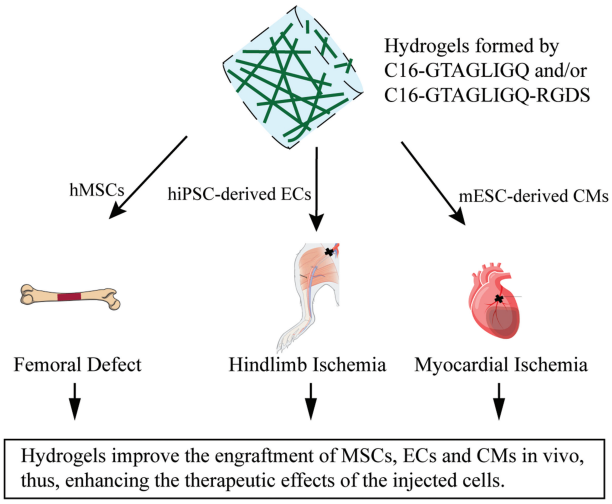

Figure 3 Hydrogels improved the engraftment of hMSCs, ECs or CMs and enhanced their therapeutic effects in ischemia.
Abbreviations: C16, palmitic acid; hMSCs, human mesenchymal stem cells; ECs, endothelial cells; mESCs, mouse embryonic stem cells; CMs, cardiomyocytes.

Encapsulation of Growth Factors by Self-Assembling Peptide Hydrogels

The loss of extracellular matrix and growth factors in an ischemic region makes the adjacent cells lose cell–cell contact and changes the microenvironment. A SAP hydrogel could act as a scaffold for stable release of growth factors. Owing to their tunable physical properties, hydrogels can provide spatial and temporal control over the release of various growth factors, such as VEGF, VEGF165, VEGF121, FGF, HGF, HIF and PDGF (Figure 2). VEGF and HGF delivered by functionalized RADA16-I, RADA16-II or KSLSLSLRGSLSLSLKGR GDS could enhance angiogenesis and functional recovery in ischemic diseases (Table 3).[67–69] For example, a LRKKLGKA-conjugated RADA16 hydrogel provided the constant release of VEGF for up to one month, thus greatly improving cardiac function after MI.[18] Interestingly, the SAP hydrogel with two sequences of RADA16-GGQQLK (QLK) and RADA16-GGLRK KLGKA (LRK) can be crosslinked and strengthened by transglutaminase to prolong the degradation rate of VEGF and HGF.[69] An ultrashort peptide, naphthyl group-linked Phe–Phe dipeptide (NapFF), being introduced with RGD domain and followed by encapsulation with VEGF, greatly improved cell adhesion and cell growth and triggered angiogenesis in vivo when subcutaneously injected in mice.[70] It was also reported that when insulin-like growth factor-I (IGF-1)-derived peptide, GYGSSSRRAPQT, was introduced into the ultrashort peptide NapFF, the β-sheet structure of the combined peptide efficiently activated the IGF-1 downstream pathway and significantly promoted angiogenesis.[71]

Advantages and Application of SAP Hydrogels in Angiogenesis

Self-assembling peptides possess designable immunogenicity and minimally invasive injectability, which make SAP-based hydrogel a better candidate in biomedical application. Amino acid composition, peptide modification as well as surface charge are the most important factors for the immunogenicity of SAPs. Self-assembling peptide OVA-Q11 caused strong T-cell-dependent antibody responses in mice. However, when OVA-Q11 was conjugated to non-antigenic peptides RGD, the immune responses were substantially decreased. Another study also showed that a peptide amphiphile hydrogel induced no inflammation or autoimmune response after modification with IKVAV in mice spinal cord injury.[72,73] Change of surface charge provides a strategy to switch off potentially problematic immunogenicity into nonimmunological application.[74] For instance, negative surface charge of fibrillized SAP completely abolished T-cell responses in mice. Anionic aspartic acid and glutamic acid-based SAP surface provokes a low inflammatory response, cationic lysine-based SAP surface elicits a mild inflammatory response, while cationic arginine-based SAP surface provokes a stronger inflammatory response.[29] The immunogenicity of self-assembling peptide hydrogel is adjustable according to different applications such as vaccine adjuvant and angiogenesis. Naphthalene peptide Nap-GFFY has been used as the adjuvant to induce $CD8^+$ T-cell response. Moreover, Nap-$G^DF^DF^DY$, formed by D-type amino acids, is able to induce a stronger $CD8^+$ T-cell response compared with.[75] Nap-GFF^{p}Y-OMe (naphthylacetic acid-modified phosphorylated tetra-peptide of GFF^{p}Y with C-terminal methyl ester group) has been used to co-assemble with HIV DNA molecules and enhance both humoral and cellular immune response against HIV.[76] When D-tetrapeptide ($G^DF^DF^DY$)-based supramolecular hydrogelators are linked with different hydrophobic domains, such as flurbiprofen (Fbp), carprofen (Car), naproxen (Npx), Fbp- and Car-gels, they exhibit excellent tumor elimination properties in both protective and therapeutic immune assays.[77]

A better understanding of the chemical and physical properties of SAPs is imperative for developing the delivery method of hydrogels. Hydrogen bonding and hydrophobic interactions are the main driving forces for SAP gelation. Peptides can easily associate and disassociate based on temperature and pH. Therefore peptide fibers are able to easily break and re-form.[12] This equilibrium allows the preformed hydrogels to shear thin and shear recover readily, such that preformed hydrogels can be injected by application of shear stress (during injection) and quickly self-heal after removal of shear. Besides, some injectable liquid SAPs can form into hydrogels upon stimulation by salt ions in the physiological microenvironment of the target tissue. Interestingly, smart hydrogel has been designed to adapt to complex repair processes. For instance, adding aniline into ultrashort peptide Fmoc-FF can make the hydrogel conductive,[78] which supports cardiomyocyte organization into a spontaneously contracting system.[78] A multiphase transitioning peptide hydrogel, with the sequence of $_{Ac}$-VKVKVKGKVDPPTKXEVKVKV$_{-NH2}$ (X stands for photocaged MNI-glutamic acid), was reported to have been injected into the lumen of vessels to facilitate suturing. The multiphase transitioning peptide forms solid gel in a syringe and can be delivered to the lumen of collapsed vessels to distend the vessel by shear-thin force, and the space between two vessels to approximate the vessel ends. After exposing the small vessel to light, the hydrogel network in the lumen will be disrupted, leading to gel-sol phase transition, gel removal and blood flow resumption.[79] In a word, the injectability of SAP hydrogels makes them the superior therapeutic scaffolds with minimal invasiveness and applicable in a variety of clinical applications.[8,12,16,80]

Currently, many peptide-based hydrogels such as Puramatrix (RADA16-I) are commercially available and used for 3D cell culture to support stem cells in the repair of tissue injury.[81] Some hydrogels have been proved in preclinical use; for example, direct myocardial injection of 1% SAP ($_{AcN}$-RARADADARARADADA-$_{CNH2}$) with PDGF-BB decreased infarct size after ischemia/reperfusion through activating Akt phosphorylation in cardiomyocytes.[82] Moreover, some hydrogels are in clinical trial. For instance, P11-4 ($_{Ac}$-QQRFEWEFEQQ-$_{NH2}$), a synthetic α-peptide that can be self-assembled into β-sheet amyloids with a hydrogel appearance at low pH, is being used in biomimetic mineralization, enamel regeneration and oral care agent.[83]

Summary and Perspective

In conclusion, SAPs hold great promise owing to their bioactivity, biocompatibility and biodegradability. Their tunable physical properties make them injectable, functional and compatible for encapsulation and controlled release of cells, exosomes and growth factors. Smart hydrogel, which is responsive to the in vivo environment through internal or external stimuli, might represent a future direction. Besides, it is promising to design a complex scaffold system that is suitable for heterogeneous cell populations, and able to dynamically change the composition and physical properties. Furthermore, a molecularly sophisticated SAP hydrogel has been developed to direct migration of stem cells to damaged areas, but the desired homing and in situ assembly still need to be explored. Overall, self-assembling peptide-based hydrogel has a bright future to serve as an effective treatment for a broad range of ischemic diseases. Since the research on the self-assembling peptide hydrogel in angiogenesis is in its infancy, many challenges need to be overcome. The main challenges are how to match the mechanical properties of hydrogel to the natural ECM, and how to precisely control degradation speed of the hydrogel and release speed of the internal molecules or cells. There is still a long way to transform the self-assembling peptide-based hydrogels into clinical applications.

Author Contributions

All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published and agree to be accountable for all aspects of the work.

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