SHORT PEPTIDE-BASED POLYSACCHARIDE HYDROGELS FOR TISSUE ENGINEERING: A MINI REVIEW

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The usage of short peptide-based polysaccharide hydrogels for tissue engineering was discussed in this review. It explained the drawbacks of employing short peptide-based polysaccharide hydrogels as tissue regeneration scaffolds, while highlighting their benefits. In this review, we first gave a brief overview of short peptide-based polysaccharide hydrogel design process. Then, we provided additionally detailed information of the hydrogels with categorized polysaccharides (hyaluronic acid, dextran, chitosan, alginate, and agarose). We also explained the bioactive short peptides Arg-Gly-Asp (RGD), Ile-Lys-Val-Ala-Val (IKVAV), and Tyr-Ile-Gly-Ser-Arg (YIGSR) that were used to modify these polysaccharide hydrogels in order to enhance cell behaviors, including survival, adhesion, proliferation, and migration. Their applications in tissue engineering were also demonstrated and summarized in this review.

Keywords: hydrogel, peptide, hyaluronic acid, dextran, chitosan

INTRODUCTION

As an important component of organs and body structures, tissue plays a functional role between cells and organs.[1,2] Disruptions to the tissue, which can be induced by either soft or hard injuries, often result in the loss of specific bio-functions.[3] Soft tissue injuries commonly happen throughout the body, and include tendon rupture, ligament tear, nerve distortion, muscle strain and blood vessel burst.[4] Hard tissue injuries are also universal, and include fractures of bone, enamel, dentin and cementum.[5,6]

One approach to treating such injuries is to use hydrogels fabricated by biomaterials, as scaffolds, for regeneration of injured tissue, because it can release bioactive molecules and regulate the living cell behaviors to the desired purposes.[7,8] So far, there are several kinds of hydrogels based on distinct types of biomaterials, such as polysaccharide, polyethylene glycol, zwitterionic, *etc*.[9-14] Even if there are some issues related to their use, including low viability and negative effect of degraded biomaterial residuals, the modification of biomaterials using short peptides is encouraging.[15]

Grafting short bioactive peptides to these hydrogels is a promising way to offset the drawbacks of biomaterial hydrogels.[16,17] Grafted short bioactive peptides, such as Arg-Gly-Asp (RGD), Ile-Lys-Val-Ala-Val (IKVAV) and Tyr–Ile–Gly–Ser–Arg (YIGSR), can efficiently interact with cells, and lead to improvement of cells viability, attachment, proliferation and migration.[9,18]

This review mainly focused on discussing the use of short peptide-based polysaccharide hydrogels for tissue engineering. Despite the abundance of top-notch review publications that provide in-depth and thorough analysis of the use of biomaterials as hydrogels for tissue engineering,[19-22] to the author's knowledge, none of them categorizes the short peptides and polysaccharides used for this purpose. Also, the publications encountered to this point do not provide excessively detailed information on each type of short peptide-based polysaccharide hydrogel for tissue engineering as a result. Here, we not only provided the design strategy of short peptide-based polysaccharide hydrogels, but also gave a more categorized perspective, with a focus on the use of short peptides as hydrogels for tissue engineering applications, which are produced from

extracellular matrix (ECM) components and are based on five most prevalent physiologically significant polysaccharides.

DESIGN OF PEPTIDE-BASED POLYSACCHARIDE HYDROGELS

Short peptide-based polysaccharide hydrogels have been designed using various techniques. The design of bio-functional hydrogels consists of methods of conjugating short peptides to polysaccharides and gelation of short peptide-based polysaccharides. The primary method of conjugating short peptides to polysaccharides is to functionalize polysaccharides via the N-ethyl-N′-(3-(dimethylamino)propyl)carbodiimide /N-hydroxysuccinimide (EDC/NHS) system, though occasionally initiators are used.[23] Gelation theories divide hydrogels mainly into two groups: chemically crosslinked hydrogels and physically crosslinked hydrogels.[24,25]

Conjugation of short peptides to biomaterials using EDC/NHS system

Polysaccharides have carboxylic acid groups, which can react with EDC to form an O-acylisourea active ester that reacts with primary amino groups on short peptides due to the displacement of nucleophilic attack.[26] NHS combined with EDC can efficiently enhance the yield of conjugation of short peptides to polysaccharides due to the creation of relatively dry stable amine-reactive NHS ester.[27]

Gelation of polysaccharide hydrogels

Although there are various kinds of polysaccharide hydrogels – such as thermosensitive, enzymatic, pH-sensitive, ionic crosslinked hydrogels – crosslinked according to different approaches, gelation is theoretically mainly based on chemical crosslinking or physical crosslinking.[28-30] In reality, for short peptide-based polysaccharides' applications, the functional groups between the polysaccharides/polysaccharide and short peptides, or the initiator can also contribute to gelation.[23,31]

BIO-FUNCTIONALIZATION OF SHORT PEPTIDE-BASED POLYSACCHARIDE HYDROGELS

Short peptide-based polysaccharide hydrogels are excellent candidates for the design of tissue engineering scaffolds due to the unique nature of hydrogels.[32] A hydrogel is composed of crosslinked polymer chains that are hydrophilic, and water contained in the hydrogel network structure.[33] The flexibility characteristic of hydrogels mimics that of natural tissue.[34] Still, polysaccharide hydrogels are highly qualified for tissue engineering applications due to their specific chemistry, structure, bio-functionalized polymeric surface, and biocompatibility.[35-37] Before applying to clinic situations, however, a lot of defects of these hydrogels have to be solved, including imperfect degradation, unregulated transportation of bioactive molecules and difficult control of cell behaviors, such as low viability, detachment, random differentiation and migration.[38] The solutions to deal with these problems are urgently needed, and these could include modification of hydrogel polymer chemistry, structure, and conjugation of short peptides.

Hyaluronic acid

Hyaluronic acid (HA) is a unique component in the ECM and plays a basic functional role in a variety of biological processes.[39] Before incorporating HA in a number of biomaterials or scaffold systems, the modification of HA is necessary, to reverse its negative biological functions. After chemical modification, HA can get many desirable physical forms: viscoelastic solutions, soft or stiff hydrogels, nanofibers and so on.[40] An RGD-HA hydrogel crosslinked by Michael-type addition modulated its mechanical property by varying the molecular weight of hyaluronic acid and the weight percent of the hydrogel.[41] The tunable stiffness of the RGD-HA hydrogel platform, which mimics key biochemical and biophysical characteristics of the brain matrix, demonstrated its utility in elucidating ECM mechanobiological regulation of glioma cell morphology, motility, and 3D invasion.[42] A photo-crosslinked RGD-HA hydrogel with high DM had robust mechanical property and maintained cytocompatibility and double cell adhesion, compared to that with low DM, in an *in vitro* study.[43] Interestingly, the fibrous RGD-HA hydrogel significantly affected human mesenchymal stem cell interactions, including spreading, proliferation, and RGD density is related to focal adhesion formation. The chondrogenic signal level was influenced by fiber mechanics and adhesivity, in which softer fiber and lower RGD density generally enhanced chondrogenesis.[44]

One promising method to promote robust neurite outgrowth is the fabrication of the HA hydrogel.[7] An implanted RGD-HA hydrogel in the defects of cortex in rats supported cells infiltration and angiogenesis, simultaneously inhibited the

formation of glial scar, and promoted neurites extension after 6 and 12 weeks,[45] although there was no obvious benefit from this hydrogel in a rodent spinal cord injury model.[46]

The synthesized IKVAV-HA hydrogel, as a scaffold for axonal regeneration, had a suitable size of the open porous structure and a large surface area available for cell interaction, which promoted tissue repair and axonal regeneration.[47] After implanting in the lesioned rat cerebrum, the hydrogel repaired the tissue defect and formed a permissive interface with the host tissue.[48] Another research group crosslinked an IKVAV-HA hydrogel using metalloproteinase peptide incorporated brain-derived neurotrophic factor (BDNF), which induced neuronal differentiation of human mesenchymal stem cells (hMSCs) *in vitro*. The injected hydrogel in rat SCI model inhibited the inflammatory reaction and exerted a positive effect on the regeneration of motor functions *in vivo*.[49]

Matrix metalloproteinase (MMP) sensitive HA hydrogel was prepared by conjugation with two different peptides: cell adhesion peptide containing integrin binding domains RGD and a cross-linker with MMP degradable peptide. This functionalized hydrogel degraded faster and improved spreading of mesenchymal stem cells (MSCs), which kept round.[50] Thermos-sensitive RGD-Pluronic-HA hydrogel successfully produced a tissue containing cartilage-like components, such as Glycosaminoglycans (GAGs) and type II collagen, in an *in vitro* study.[51]

Dextran

Dextran, which is a straight chain of glucose molecules linked by α-1,6 glycosidic bonds, has a primary candidate for tissue engineering application due to its excellent biocompatibility.[52,53] Modifying a dextran hydrogel using short peptides, such as RGD and IKVAV, can functionalize its properties.[54] Due to its nature, dextran is difficult to degrade under physiological conditions.[55] The nature of the pristine dextran hydrogel, which is similar to many other hydrogels, is non-cell-adhesive.[56] There are many methods to functionalize these non-cell adhesive dextran hydrogels. One popular method is to graft ECM-derived peptides to these hydrogels.[57] Molly S. Shoichet *et al*. modified a dextran hydrogel with the combination of YIGSR and IKVAV for axonal regeneration *in vivo*.[58] The functionalized hydrogel exhibited improvement of cell adhesion and neurite outgrowth, and dorsal root ganglia (DRG) penetrated within the first 600 μm in the hydrogel.[59] Another research group incorporated RGD into the dextran hydrogel using a liquid-handling robot.[60] Though the viability of human colon carcinoma cells HCT-116, which were embedded in this automatically fabricated hydrogel, is similar to that seeded in a manually fabricated hydrogel, the automatically fabricated hydrogel is preferable for drug development because its network is highly flexible.[61]

Interestingly, Gang Cheng *et al*. synthesized a novel enzyme-degradable peptide based zwitterionic biomaterial hydrogel: the carboxybetaine was grafted onto dextran, which was then functionalized with RGD. This research group demonstrated this hydrogel is low toxic and can be degraded within 8 hours when exposed to 0.1 mg mL^{-1} of collagenase. Further, the immobilized RGD helped more NIH-3T3 to adhere to the dextran hydrogel.[62]

Chitosan

Chitosan, deacetylated from chitin, is a linear polysaccharide, composed of randomly distributed β-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine.[63] The functionalization of a chitosan scaffold with RGD occurs through the imide-bond forming reaction between the amino groups in chitosan and the carboxyl groups in peptides.[64] An immobilized RGD chitosan hydrogel enhanced attachment and proliferation of rat osteosarcoma (ROS) cells, in contrast to the RGES chitosan hydrogel and non-peptide chitosan hydrogel.[65] An *in vitro* study also showed deposition minerals are more abundant on the immobilized RGD chitosan hydrogel than on the unmodified chitosan hydrogel.[66] Ki Dong Park *et al*. complexed an RGD-chitosan hydrogel using Pluronic *via* the same imide-bond forming reaction. The relatively lower storage modulus and thermosensitive nature of this hydrogel make it suitable for supporting articular cartilage regeneration. After 2-week tests, the viability and proliferation of bovine chondrocyte and extracellular matrix expression were improved in the RGD-Pluronic-alginate hydrogel, in contrast to those of the control, *i.e.* the pristine alginate hydrogel. A thermoresponsive chitosan hydrogel was developed by conjugation to peptide glutanine-histidine-arginine-glutamic acid-aspartic acid-glicine-serine (QHREDGS) and Collagen I, for cardiac cell culture and delivery. The morphology, viability, and metabolic activity of cardiac myocytes (CM) in the high-peptide gel were better than in the low peptide gel and in the control. CM were not significantly different

between the groups, however, the success rate of obtaining a beating construct was improved in the hydrogel with the high amount of QHREDGS immobilized peptides, compared to the low and control groups. After 1 week, the injected hydrogel in the Lewis rats successfully localized at the site of injection, retained cells, and identified CM contractile apparatus.[67] Another example of complexing an RGD-chitosan hydrogel is the RGD-Chitosan/hydroxyapatite scaffold. The grafted RGD was through physical adsorption, and this hydrogel promoted initial cell adhesion, spread and differentiation toward an osteogenic phenotype.[68]

Chitosan hydrogels can also be prepared via freeze-drying and UV crosslinking.[69,70] Post-crosslinking increased its size and mechanical strength. The incorporation of RDG to chitosan improved the viability of rat primary osteoblasts. Calcium deposition is also twice greater than in the control.[71] Amsden *et al*. also photo-crosslinked N-methacrylate glycol chitosan (MGC) hydrogel, as a scaffold for repairing cartilage, and encapsulated bone morphogenetic protein 6 and transforming growth factor-β3. The grafted RGD on MGC improved the viability of adipose-derived stem cells (ASCs) and kept a survival rate of more than 90% over 2 weeks. At the beginning stage of BMP-6 and TGF-β3 stimulated ASCs, chondrogenic markers expressed more obviously. Further, the expression of GAGs and collagen type II protein was stronger than that of the control. ASC chondrogenesis was rapidly inducted and enhanced, and differentiated even without growth factor when controlling release.[72]

Interestingly, Ki Dong Park *et al.* fabricated peptide grafted chitosan derivative hydrogels via enzymatic crosslinking. Tyramine-terminated polypseudorotaxane-Gly-Arg-Gly-Asp-Ser and 4-hydroxylphenylacetamide chitosan solution gelled fast in the presence of horseradish peroxidase and hydrogen peroxide. *In vitro* tests exhibited that the attachment of L929 mouse fibroblasts onto the RGD-chitosan is better, compared to that without RGD.[73]

Alginate

Alginate is an anionic polysaccharide, which is composed of (1-4)-linked β-D-mannuronate and C-5 epimer α-L-guluronate residues, with widely varying compositions and sequential structures.[65] Aqueous carbodiimide chemistry crosslinked RGD-alginate hydrogel illustrated the achievement of cellular interaction, such as attachment, proliferation and differentiation of mouse skeletal myoblasts, with the otherwise non-adhesive hydrogel substrate.[74] For instance, the immobilized RGD-alginate hydrogel, as scaffold for cardiac tissue regeneration, supported the adherence of neonatal rat cardiac cells, prevented its apoptosis and regenerated its tissue. The RGD-alginate hydrogel supported CM, which reorganized and reconstructed myofibers. Non-myocytes surrounding the myofibers also improved CM.[75] It was demonstrated that the RGD-alginate hydrogel inhibited chondrogenesis of BMSCs.[76]

YIGSR is another popular peptide, which is always grafted to alginate hydrogel.[77] It was demonstrated that the YIGSR-alginate hydrogel elicited a five-fold increase in numbers of NB2a neuroblastoma cells attachment in contrast to the non-peptide alginate hydrogel and promoted neurite outgrowth.[78] Though there were no obvious differences in blood vessel density, scar thickness, myofibroblast or macrophage infiltration or cell proliferation between the functionalized alginate hydrogel and the control, utilizing the calcium ions crosslinked RGD/YIGSR-alginate hydrogel reduced the effects of therapy, including the extent of scar thickness, left ventricle dilatation and function.[77] Interestingly, a peptide Gly–Arg–Glu–Asp–Val (GREDV), compared with RGD or YIGSR, conjugated to an alginate hydrogel, as a scaffold for inducing angiogenesis, attached HUVECs selectively and led to their superior proliferation. The GREDV-alginate promoted to form new vessels, and the density of vessels was 1.5 times higher than in the case of other peptides conjugated alginate hydrogels.[79]

An innovative application of the RGD-alginate hydrogel was meant to improve cochlear implant performance.[80] This hydrogel coating consisted of RGD-alginate hydrogel and poly(3,4-ethylenedioxythiophene) conductor. The functionalized coating is non-cytotoxic and reduced electrode impedance, enhanced charge delivery, and provided trophic factor into cochlear fluids in *in vitro* and *in vivo* assays.[81]

Agarose

Agarose is a linear polymer, which consists of alternating D-galactose and 3,6-anhydro-L-galactopyranose.[82] The nature of agarose – its stable physical, chemical and thermal properties, and lower degree of chemical complexity – makes it inert in communicating with biomolecules.[83]

P. Aerbischer *et al*. is the first group that bound peptide to agarose to develop a hydrogel, as a

scaffold for regeneration of the nervous system. This group functionalized different agarose hydrogels with several kind peptides using coupling agent 1'1, carbonyldiimidazole. Neurite outgrowth from DRG improved in the PEPMIX (Tyr-Ile-Gly-Ser-Arg, IKVAV and Gly-Arg-Gly-Asp-Ser-Pro)-agarose hydrogel, whereas neurite extension was limited in the IKVAV-agarose hydrogel. Interestingly, the IKVAV-agarose hydrogel, however, stimulated neurite outgrowth from PC12 cells.[84] Using extra coupling agent for gelation, in some conditions, is toxic to cells. Another group functionalized an agarose hydrogel with sulphydryl group that was bound to the peptide without coupling chemical.[85]

Another hot topic of binding peptides is to complex the agarose hydrogel with (polyethylene glycol) PEG. M. S. Detamore *et al*. bound RGD to a PEG-agarose hydrogel. The complexed hydrogel did not present weaker mechanical strength. Furthermore, chondrocyte viability and performance enhanced when the concentration of RGD was 100 μg/mL. It might stimulate cell migration or local proliferation because of clustered cells in the interpenetrating network and higher concentration of aggrecan. The migration stimulated proliferation and synthesis of matrix, which facilitated integration with surrounding cartilage *in vivo*.[86]

TISSUE ENGINEERING APPLICATIONS OF PEPTIDE-BASED POLYSACCHARIDE HYDROGELS

Short peptide-based polysaccharide hydrogels have been applied for tissue regeneration in the most recent ten years.

Table 1
Short peptide-based polysaccharide hydrogel scaffolds for tissue engineering applications

Peptide	Polysaccharide	Polysaccharide derivative	Cell used	Application
RGD	HA	HA-g-Pluronic	Chondrocytes	Cartilage tissue
		HA-methacrylate	Glioma cells	Malignant brain tumors
	Dextran	Dextran-acrylate acryloyl-PEG	Human embryonic stem cells	Vascular healing
	Chitosan	Azido-chitosan	Osteoblasts	Bone tissue
		Chitosan/hydroxyapatite	Bone marrow stromal cells	Bone tissue
		Chitosan	Rat osteosarcoma cells	Bone tissue
		Chitosan-Pluronic	Chondrocyte	Articular cartilage regeneration
		N-methacrylate glycol chitosan (MGC)	Adipose-derived stem cells	Cartilage repair
	Alginate	Sodium alginate/hyaluronate	Primary chondrocytes/ NIH3T3 cells	Regenerate cartilage tissues
		Sodium alginate	Human umbilical vein endothelial cells	Stimulating new vessel formation
		Sodium alginate	Human mesenchymal stem cells	Chondrogenesis
		Sodium alginate	Cardiac cells	Cardiac tissue
	Agarose	Agarose-PEG	Chondrocyte	Surrounding cartilage
IKVAV	Dextran	Methacrylated dextran copolymerized with aminoethyl methacrylate	Primary embryonic chick dorsal root ganglia	Axonal regeneration
	HA	HA sodium salt	Glial cells	Tissue repair and axonal regeneration
YIGSR	Alginate	Sodium alginate	NB2a	Neurite outgrowth

The three-dimension network, flexible stiffness, regulated cell behavior, and controlled transportation of bioactive molecules support polysaccharide hydrogels as promising candidates for tissue engineering applications. Table 1 summarizes the data from recent experiments on short peptide-based polysaccharide hydrogels that have been used as scaffolds for tissue engineering. Each of these experiments is related to a specific tissue or application based on 1) binding a specific short peptide, 2) polysaccharide or its derivative, 3) specific cells used, 4) most *in vitro* applications.

CONCLUSION

We made an effort to provide a detailed overview of short peptide-based polysaccharide hydrogels for tissue regeneration in this paper. We categorized the hydrogels based on the type of polysaccharide used in their fabrication. Tissue engineering, as a promising alternative method for regenerating damaged or degenerated tissues, has many advantages compared to traditional methods of treatment. For stimulating the formation of new tissue, a desirable matrix has at least two functions: attachment of cells to the surface of the matrix and suitable space for cells proliferation and migration. Functionalized biomaterial hydrogels with short peptides, derived from components of ECM, are desirable materials for assembling structures that satisfy both requirements. Though there are many investigations on tissue regeneration applications using short peptide-based polysaccharide hydrogels as scaffolds, only a few of them were applied in *in vivo* studies. The future challenges of short peptide-based polysaccharide hydrogels in the clinical tissue regeneration application are mainly based on filtering more useful short peptides from ECM, using innovative methods of conjugating short peptides to polysaccharide hydrogels, and meanwhile, maintaining "reasonable" production costs.

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