ELSEVIER

Short to ultrashort peptide hydrogels for biomedical uses

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Hydrogels can offer cells an extracellular matrix-like growth environment which traditional culture methods cannot provide. Hydrogels therefore have great value in tissue engineering and regenerative medicine applications. In this article, we pay special attention to peptide-based hydrogels, which we further classified into short (≤20 residues) or ultrashort (≤7 residues) peptides. The design principles of these peptides are presented, and, based on their technical advantages and potential, biomedical applications are discussed.

Introduction: the wonderful world of hydrogels

Hydrogels have existed for more than half a century, with Wichterle and Lim providing one of the earliest records of crosslinked hydroxyethyl methacrylate (HEMA) hydrogels [1]. This heralded the development of contact lenses which is an invention we now take for granted. Today, hydrogels still fascinate material scientists and biomedical researchers and great strides have been made in terms of their formulations and applications [2].

As a class of material, hydrogels are unique – they consist of a self-supporting, water-swollen three-dimensional (3D) viscoelastic network which permits the diffusion and attachment of molecules and cells. This is reminiscent of the landscape – more specifically, the extracellular matrix (ECM) – which most cells in our body grow in and offers a native culture condition which traditional 2D surfaces cannot reproduce. This has been shown to be crucial as cells are known to sense the topography of their microenvironment [3,4], which in turn influences their cellular phenotype (e.g., extend of spreading), differentiation and gene expression profile [5,6]. Thus, in acknowledgment of its biomedical value, hydrogels have been explored as scaffolds for tissue engineering [7] for over 35 years [6,8]. Specific applications include cell encapsulation [9], wound healing [10,11], cartilage repair [12], recovery after injury to the spine [13] and heart [14] and fillers after surgery [15], just to select some prominent examples. Other applications include bioprinting [16] and the use as matrices for the delivery of cargoes such as drugs [17], nucleic acids [18], proteins [19], antibodies [20] and growth factors [21]. Recently, the field of hydrogel has even been broadened to include microgels [22] and nanogels [23].

There is great diversity in hydrogel formulations where both synthetic and natural materials have been used. These include silicone [24], gelatin [25], hyaluronic acid [26], fibrin [18], chitosan [27], collagen [28], curcumin [15], alginate [29] and dextran [30]. Polymers based on ethylene glycol [31], ε-caprolactone [32], organophosphazene [33], vinyl alcohol [12], hydroxyethyl acrylamide [34] and HEMA [35] have also been used and it is common to create hybrids with different materials. This review, however, focuses only on short peptide-based hydrogels which, for the sake of classification, is defined here to be ≤20 amino acids (AA) in length. Therefore, hydrogels based on polypeptides or proteins [36,37], while important, fall outside of our scope.

Peptide-based hydrogels derived from naturally occurring AA offer key advantages such as being biocompatible, biodegradable and generally non-immunogenic. Advances in solid phase peptide synthesis have also significantly lowered production costs although, currently, peptide synthesis is still more expensive relative to some polymeric hydrogels. However, AA coupling is precisely controlled in terms of sequence, reproducibility and polydispersity. It can also be automated and does not require complicated work-ups – for example, complex chemistry, harsh reaction conditions, harmful/expensive catalysts/enzymes and lengthy purification procedures – that other hydrogel systems require. Being chemically defined further means that peptide-based hydrogels are safer for bioapplication, unlike animal-derived matrices – for example, Matrigel™, which is a basement membrane obtained from murine tumors

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GLOSSARY

THE 20 NATURAL AMINO ACIDS IN THEIR SINGLE-LETTER CODE

G	GLYCINE
P	PROLINE
A	ALANINE
V	VALINE
L	LEUCINE
I	ISOLEUCINE
M	METHIONINE
C	CYSTEINE
F	PHENYLALANINE
Y	TYROSINE
W	TRYPTOPHAN
H	HISTIDINE
K	LYSINE
R	ARGININE
Q	GLUTAMINE
N	ASPARAGINE
E	GLUTAMIC ACID
D	ASPARTIC ACID
S	SERINE
T	THREONINE

where large batch variability and the presence of residual soluble factors may ultimately prove to be limiting [6]. Moreover, bioactive motifs can be facilely appended to peptide sequences [38]. Being assembled in a piecemeal bottom-up fashion also means that a modular approach can be rigorously employed to study the sequence–function relationship of a particular peptide. Clearly, a short peptide sequence (≤20 AA) confers even more savings in terms of production time, effort and cost. Here, we further categorize a subclass of short peptides, that is, ultrashort peptides with ≤7 AA. The term 'ultrashort' was initially coined to describe a class of rationally designed aliphatic peptides containing three to seven AA and will be discussed in the following section [39]. Together with hybrid-peptide systems, these classes of peptides form the backbone of this review. Nonetheless, we note that the delineation between short and ultrashort peptides is based, subjectively, on an empirical survey of the literature and does not reflect their relative scientific worth.

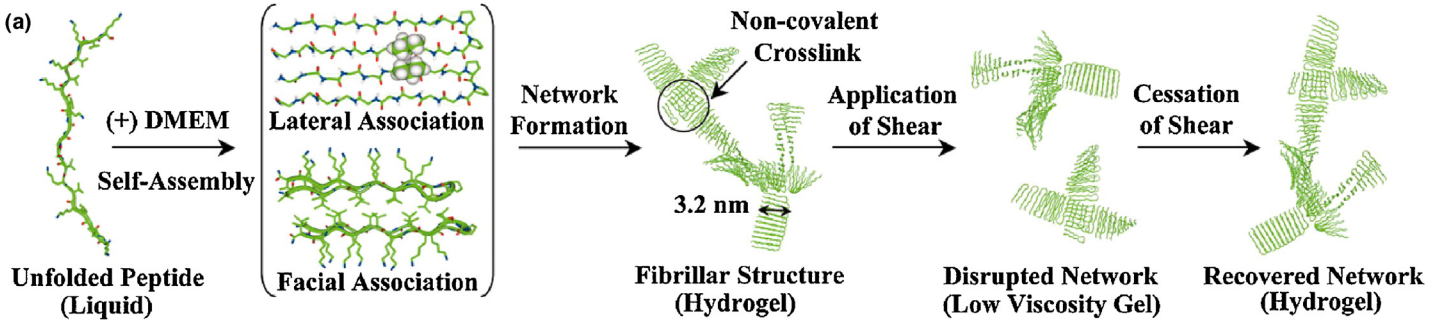
Although much heterogeneity exists, some generalizations still hold true for most of the peptide sequences studied to form hydrogels. (1) Most peptide hydrogels consist of β-structures (sheets, hairpins, turns), although α-helical gels are available [40]. (2) The peptide sequence is usually amphiphilic, although a ready exception is the fluorenylmethoxycarbonyl-protected diphenylalanine (Fmoc-FF) developed by Ulijn and colleagues [41]. Which was, in fact, inspired by Gazit and co-workers who had earlier studied the propensity of the FF motif to encourage amyloid fibrils formation [42,43]. (3) Self-assembly is the most popular mechanism for gel formation and is usually driven by one of the following physical forces: ionic interactions, hydrophobic forces or π–π stacking of aromatic groups. (4) Gelation is often triggered or enhanced by ions. These points and more will now be discussed in the following sections.

Peptide-based hydrogels

Short peptide hydrogels (AA ≤ 20)

Ionic self-complimentary self-assembling peptides

Zhang and colleagues serendipitously discovered several self-assembling peptide sequences more than 20 years ago [44]. Prominent members, given in their single-letter AA code (see glossary) include: Ac-(AEAEAKAKAEAEAKAK)-NH_2 (EAK16) [44], Ac-(KLDLKLDLKLDL)-NH_2 (KLD12) [45], Ac-(RADARADARADARADA)-NH_2 (RAD16-I) [46] and Ac-(RARADADARARADADA)-NH_2 (RAD16-II) [14]. Features of these peptides include regular alternating of hydrophilic/hydrophobic AA where positive charges are juxtaposed to negative ones. Due to ionic self-complementary encouraged by the presence of salts, these peptides then self-assemble into nanofibers that interweave to finally form β-sheet hydrogels [47]. These gels have been evaluated for different applications. For instance, chondrocytes encapsulated within KLD12 maintained their phenotype after a four-week culture period and secreted ECM which stiffened the gel over time [45]. This suggests that it may be useful for application in cartilage repair. However, the most extensively studied member has to be RAD16-I, which is



(b)

MAX1: VKVKVKVKV^{D}P^{L}PTKVKVKVKV-NH_2

MAX8: VKVKVKVKV^{D}P^{L}PTKVEVKVKV-NH_2

FIGURE 1

(a) Scheme showing the self-assembly and shear-thinning property of β-hairpin peptides. Growth media (DMEM) can be added to the buffered peptide solution to trigger the lateral and facial association of peptide fibers into hydrogel. The application of shearing forces disrupts the hydrogel network, which recovers upon cessation of shear. Such shear-thinning and healing properties make the peptide suitable for injection-based therapies where the hydrogel has to reform after extrusion from a syringe. (b) AA sequence of MAX1 and MAX8. Figure reproduced from [53]. Copyright 2007 National Academy of Sciences, U.S.A.