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Self-assembling peptide-based building blocks in medical applications☆

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ABSTRACT

Peptides and peptide-conjugates, comprising natural and synthetic building blocks, are an increasingly popular class of biomaterials. Self-assembled nanostructures based on peptides and peptide-conjugates offer advantages such as precise selectivity and multifunctionality that can address challenges and limitations in the clinic. In this review article, we discuss recent developments in the design and self-assembly of various nanomaterials based on peptides and peptide-conjugates for medical applications, and categorize them into two themes based on the driving forces of molecular self-assembly. First, we present the self-assembled nanostructures driven by the supramolecular interactions between the peptides, with or without the presence of conjugates. The studies where nanoassembly is driven by the interactions between the conjugates of peptide-conjugates are then presented. Particular emphasis is given to *in vivo* studies focusing on therapeutics, diagnostics, immune modulation and regenerative medicine. Finally, challenges and future perspectives are presented.

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1. Introduction

1.1. Peptides in medicine

Amino acids are like the letters of the alphabet. They are the building blocks of peptides and proteins in the way letters are the building blocks of words and sentences. In this sense, they convey information about structure and interactions. Peptides and proteins perform a wide range of functions within biological systems, including communication between cells. By tuning the amino acid sequence through the nucleic acid sequence of their genes, proteins fold in different conformations that alter their activities. For these reasons, peptides made of natural and synthetic building blocks are an increasingly popular class of biomaterials. Recent decades have witnessed a steep increase in the popularity of peptide-based targeting and therapeutic agents. Unlike small molecules (<550 Da) and biologics (>5000 Da), peptides offer a distinctive class of therapeutics with greater or equal specificity and potency as biologics but are more accessible for development akin to small molecules [1]. Because of this and recent advances in peptide production costs, efficiency, and use of non-natural amino acids, the market for therapeutic peptides is on the rise [1–3]. From 2009 to 2011 the US Food and Drug Administration (FDA) approved 76 new therapeutics, 58 molecular and 18 biologics (proteins, monoclonal antibodies, and enzymes) [4–7]. As of 2015, there were more than 60 FDA approved peptide therapeutics and this number is only expected to grow [3,8]. There are currently ~140 peptide-based drugs in clinical trials and more than 500 in preclinical development, reflecting predicted growth of a global market for peptide drugs from $14.1 billion (US dollars) in 2011 to $25.4 billion in 2018 [3]. The rise in demand for peptide drug development has spurred new mechanisms to develop feasible biological peptides beyond traditional methods.

Peptides used for pharmacological intervention have been traditionally derived from natural products isolated from plants, animals or humans (as is the case with hormone-based peptide agonists) [9,10]. However, biologically-potent peptides are increasingly mined from genetic or recombinant libraries as well as chemical and peptide screens [11]. Peptides have wide-ranging applications in medicine because they can target proteins more selectively than small molecules, thus decreasing potential off-target side effects [12]. Their small size compared to proteins and antibodies allows peptides to better penetrate into tissues and solid masses such as tumors [12]. Peptides also have a lower immunogenicity profile than proteins and antibodies endowing them with greater potential for stable clinical therapeutic windows, predictable metabolism, and ability for repeat dosing. Peptides also offer several advantages over small molecules given that they are traditionally constructed in the likeness of the smallest functional part of a target protein. This fact potentially endows them with greater efficacy, selectivity, and specificity [12–14].

1.2. Limitations of peptide-based structures in medical applications

Despite recent successes, peptide-based therapeutics have been fraught with difficulties that limit their clinical translation including short circulation half-lives, poor chemical and physical stability in serum, bioavailability limitations *via* oral delivery, along with poor biodistribution, poor cellular penetration, high conformation flexibility limiting protein binding selectivity, and inability to home to diseased areas or target cell populations [12]. Innovative approaches have been proposed to tackle these issues. Not surprisingly, a major focus of translational chemical biology is to devise synthetic strategies to recreate the architecture of biologically active structures for both basic research and medicinal purposes [15–17]. For example, a considerable amount of effort has been directed toward preserving the peptide secondary structure, combating enzymatic degradation, and improving peptide half-lives [18]. One strategy, proposed by Verdine and coworkers, relies on hydrocarbon stapling the peptides by α,α-di-substituted non-natural amino acids bearing olefin tethers in optimal length and stereochemistry for ruthenium-catalyzed ring-closing metathesis (RCM) across one or two α-helical turns [15].

Hydrocarbon stapling was specifically developed to investigate and target α-helical interactions *in vitro* and *in vivo* [19,20]. Substitution/insertion of non-natural amino acids with olefin tethers at positions spanning either one (i,i + 4) or two (i,i + 7) turns of an α-helix followed by RCM crosslinks, or "staples", effectively linking the non-natural amino acids to one another on one face of the helix. Stapled peptides can endow α-helical peptides with improved pharmacologic properties such as cellular penetration, protease resistance, and increased binding affinity. Although these peptide therapeutics can localize to the cytoplasm and nuclei of diseased cells, this and other methods to stabilize the natural secondary structure of peptide elements apart from the parent protein do not always guarantee cellular permeability, exact recapitulation of the natural secondary structure, non-interference at the protein binding interface, or cellular homing [21,22].

1.3. Self-assembling peptide-based structures

To address many of the limitations of peptide-based medicine, self-assembled nanostructures have emerged in recent years. The nanostructures protect the peptide against protease degradation and preserve the functionality of the individual peptides. Likewise, the sizes of the structures can be precisely controlled to provide optimal passive targeting abilities. At an optimal size (10–200 nm), self-assembled, peptide-based nanostructures can penetrate leaky tumor vasculature owing to the enhanced permeability and retention (EPR) effect [23,24]. Nanostructures enter cells through endocytosis and can increase the intracellular accumulation of the drug [25], do not affect healthy tissue, and are eventually removed from the body *via* renal clearance [26]. Furthermore, the peptide sequence can be designed for disease-specific enzymatic activity to control self-assembly [27] or disassembly [28], allowing for active targeting of various diseases.

Peptides can form various secondary structures, such as α-helices, β-strands, β-turns, and random coils, and they can self-assemble into a variety of structures including micelles, fibers, ribbons, tapes, and vesicles [29]. Typically driven by non-covalent supramolecular interactions (electrostatic, hydrogen bonding, hydrophilic and hydrophobic forces, van der Waals interactions, π–π stacking, *etc.*), well-controlled assembly is a combination of repulsive and attractive interactions between constituents of the system [30,31]. The repulsive forces are essential on the bio-functional sides of the peptide-based products to prevent the undesirable precipitation or steric hindrance through the structure [32].

1.4. Peptide-conjugates and synthesis strategies

Peptide-conjugates are expanding the ability to manipulate forces that drive self-assembly toward a desired direction [33,34] and already proving far more effective than naturally occurring peptides in medical applications [18]. For instance, conjugation of hydrophobic moieties to peptides, creating peptide amphiphiles (PAs), can initiate self-assembly driven by the aggregating tendencies of the hydrophobic non-polar groups, dictating the local structure. PAs provide an alternative method of intracellular delivery and stabilization of bioactive peptides. As reviewed in the conjugation-driven self-assembly section of this review, PAs consist of a biofunctional peptide headgroup linked to a hydrophobic alkyl lipid-like tail to create molecules with distinct hydrophobic and hydrophilic ends, akin to natural lipids [16]. PAs self-assemble into a variety of nanoscale structures, including rod-like and spherical micelles, based upon charges in the peptide portion of the molecule and the nature of the hydrophobic tail. The hydrophobic tails of PAs promote cellular membrane anchoring and internalization and because of their dynamic structure, individual monomers can escape and insert their tails into other hydrophobic compartments [35,36].