Review Article

Nanomaterials: amyloids reflect their brighter side

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Abstract

Amyloid fibrils belong to the group of ordered nanostructures that are self-assembled from a wide range of polypeptides/proteins. Amyloids are highly rigid structures possessing a high mechanical strength. Although amyloids have been implicated in the pathogenesis of several human diseases, growing evidence indicates that amyloids may also perform native functions in host organisms. Discovery of such amyloids, referred to as functional amyloids, highlight their possible use in designing novel nanostructure materials. This review summarizes recent advances in the application of amyloids for the development of nanomaterials and prospective applications of such materials in nanotechnology and biomedicine.

Keywords: *Nanotechnology*; *self-assembly*; *peptide/protein*; *fibrils*; *tissue engineering*; *stem cells*; *drug delivery*; *nanowires*

Proteins/peptides have the unique ability to self-assemble into a variety of structures that are capable of performing desired functions in the biological world. For example, the biological functions of actin filaments and microtubules depend on their self-assembly, where they provide shape, motility and play an important role in cell division (1). The self-assembly of proteins could also produce unique biomaterials such as silk, whose tensile strength is comparable to steel (2, 3). Considerable efforts are being made in the development of self-assembled protein/peptide based materials with desired physical, chemical and biological applications [For review see (4, 5)]. Recently, it has been suggested that amyloids – a class of protein aggregates, originally associated with human diseases (6) – could be used for the bio-nanotechnological applications (7–9). In this review, we provide an overview of amyloid fibrils and their unique properties favorable for nanotechnology and biotechnology applications.

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Amyloid fibrils

Amyloid fibrils are highly ordered protein/peptide aggregates often characterized by a cross-β-sheet structure (10–12). They are associated with more than two dozen human diseases including Alzheimer's, Parkinson's and Prion diseases (6). Both natively unstructured and structured proteins can self-assemble to these amyloid fibrils via a partially folded intermediate (13). The amyloid fibrils are very stable and resistant to proteases and other harsh environmental conditions. Many recent findings have shown the existence of functional amyloids in nature that play a role in the host organism's survival rather than creating diseases (14–16). Some examples of functional amyloids include curli in *E.coli*, Sup35 in *S.cerevisiae*, and Pmel17 in mammals. Finally, many proteins/peptides can form amyloids under certain experimental conditions which suggests that amyloid formation is a generic property of the polypeptide chain (17).

Characteristics of amyloid fibrils

Amyloids possess distinct biophysical and histological properties; (i) they are characterized by a cross-β-sheet structure, where individual β-strands are perpendicular and each β-sheet is parallel to the fibril axis. The X-ray diffraction of aligned amyloid fibrils yields two characteristic reflections at 4.7 Å and 10 Å, corresponding to the interstrand and stacking distances between individual β-sheets, respectively (11); (ii) amyloids bind to histological dyes such as Congo red (CR) (18) and Thioflavin T (ThT) (19). After binding to CR, it produces an apple-green birefringence under cross-polarized light. CR and ThT, however, do not bind to monomeric proteins/peptides. These dyes fluoresce when they bind to β-sheet-rich fibrils and are therefore useful for spectroscopic monitoring of fibril growth and kinetics; (iii) under the electron microscope (EM), amyloid fibrils appear to be few micrometers long, non-branched filaments with 6–12 nm diameter (Fig. 1C) (11). In a majority of cases, amyloids are composed of 2–4 protofilaments that are either helically twisted or laterally associated with each other forming higher order fibrils; (iv) amyloids are resistant to heat, wide ranges of pH and proteases (20–22). Upon proteinase-K treatments, amyloids most often produce a protease-resistant amyloid core (23). These amyloid cores can be characterized at high resolution by Hydrogen/Deuterium (H/D) exchange experiments coupled with solution NMR spectroscopy (23).

Mechanism of amyloid formation

Amyloid formation is not a simple two state process, where monomeric protein/peptides simply get aggregated into an amyloid state, rather its formation involves several discrete intermediates before converting to mature fibrils

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Dr. Samir K. Maji is currently an Assistant Professor in the Department of Biosciences and Bioengineering, IIT Bombay, India. He obtained his B.Sc. and M.Sc. in Chemistry from Calcutta University, and a Ph.D. in peptide chemistry (2003) from the Indian Association for the Cultivation of Science, Kolkata, India. Dr. Maji performed fundamental work in peptide chemistry and protein design during his Ph.D. Subsequently, he moved to Harvard Medical School and Brigham and Women's Hospital for his postdoctoral studies. As a postdoctoral fellow in the laboratory of Professor David B. Teplow (2002–2005), Dr. Maji was involved in delineating the structural biology of amyloid β-protein (Aβ) fibrillogenesis (relevant to Alzheimer's disease). In 2005, Dr. Maji moved to University of California at Los Angeles (UCLA) with Professor Teplow and continued to study the mechanism of Aβ fibrillogenesis. In 2006, Dr. Maji joined the group of Professor Roland Riek at the Salk Institute for Biological Studies, San Diego, USA where he studied the mechanisms of α-synuclein aggregation (relevant to Parkinson's disease). At the Salk Institute, Dr. Maji also showed that protein/peptide drug could be formulated as amyloids that enhances the duration of action of protein/peptide drugs. After two years research at Salk Institute, he moved to ETH-Zurich, Switzerland with Professor Roland Riek and worked there in the field of functional

(Fig. 1). Amyloid formation is generally considered as a 'nucleation-dependent polymerization' process (24, 25), where soluble native proteins are converted into aggregation-prone 'partially folded intermediates' that subsequently self-assemble into oligomers (nucleus) (Fig. 1B). These oligomers represent a heterogeneous population of different sized species and are highly dynamic in nature. Oligomers further proceed into mature fibrils in a very fast kinetics with monomer addition to the nuclei. Further, amyloid formation can be accelerated by the addition of 'pre-formed nuclei' (fibrils seed), which reduces the lag phase of nucleation (24).

Historically, amyloid fibrils are associated with human diseases

Improper folding or misfolding of native proteins are closely associated with disease-related amyloid formation (6). Presently, many human diseases including Alzheimer's disease (AD), Parkinson's disease (PD), and Type II diabetes are associated with protein aggregation and amyloid formation. For example, in AD, 40/42-residue amyloid β-protein (Aβ) is converted into amyloid fibrils (26). Similarly in PD, the 140 residue α-synuclein protein forms amyloid as intraneuronal inclusions (Lewy bodies) (23). Amyloids can accumulate either outside the cell (extracellular) or inside the cell (intracellular) and may be cytotoxic. Various *in vitro*, *in vivo* and cell biological studies have demonstrated a tight link between amyloid formation and disease phenotypes. Recent evidences suggest that the soluble intermediates (oligomers) are the most plausible cytotoxic species in amyloid diseases (27–29). However, detailed structure-toxicity relationship

amyloid. At ETH Zurich, Dr. Maji made a fundamental discovery that protein/peptide hormones could be stored as amyloid-like structure in secretory granules of pituitary gland. Dr. Maji's current research group at IIT Bombay is involved in unraveling the mechanisms underlying amyloid formation by protein/peptides associated with human diseases and native biological functions (functional amyloids). The laboratory also aims to exploit amyloid material for drug delivery and functional bio-nanomaterials applications.

must be established to fully understand the nature of toxic species responsible for the diseased condition.

Functional amyloids are widespread in nature

Recently, several studies have indicated that amyloid fibrils are abundant in living organisms from prokaryotes to eukaryotes, where they have evolved to perform native functions of the host. Such amyloids are termed as 'functional amyloids'. For example, amyloid fibrils formed from the protein curli is used by *E. coli* to colonize and bind to host surfaces (30). The amyloid forms of Chaplins in filamentous bacterium *Streptomyces coelicolor* support the organism in the development of aerial hyphae and spore dispersal (31). The amyloid form of yeast prions such as Sup35 and Ure2p provide selective advantage to the host instead of causing cell death (16, 32). Furthermore, spider silk has been reported to show an amyloid-like cross-β-structure (33). The formation of functional amyloids has also been observed in mammalian system. For example, Pmel17 protein, which acts as a template for melanin polymerization, has
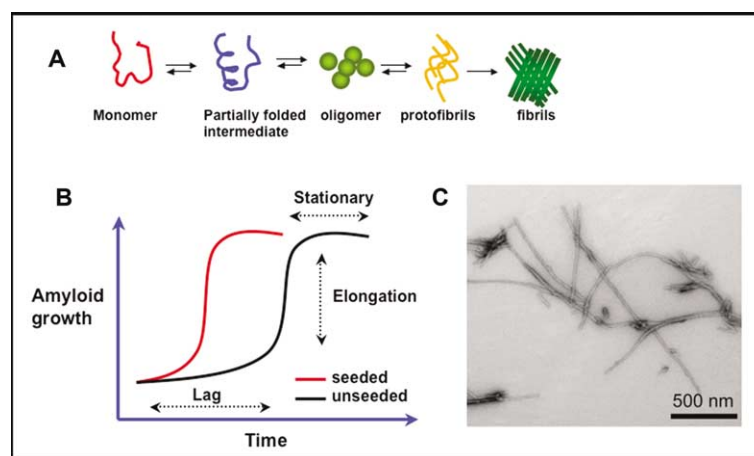

Fig. 1. (A) Schematic representation of amyloid aggregation. The natively folded/unfolded monomeric form of protein slowly transforms to a partially folded intermediate and then gets converted to soluble oligomers and protofibrils. These oligomers eventually form β-sheet-rich fibrils. (B) Amyloid formation by nucleation dependent polymerization mechanism showing an initial lag phase and then elongation followed by a stationary phase. Preformed amyloid fibrils can acts as a 'seed' to accelerate the kinetics of fibril formation by reducing the lag time. Most of the functional and disease amyloid formation follow this mechanism. (C) Typical morphology of amyloid fibrils as observed under electron microscope.

been shown to form amyloid-like fibers within the melanosome (34). Recent studies have revealed that peptide/protein hormones in pituitary secretory granules are stored as amyloid-like aggregates (35). Collectively, these results demonstrate that amyloid formation could be physiologically useful for specific biological functions in organisms where it is highly regulated. The unique biophysical characteristics and discovery of multiple functional amyloids suggest that amyloids could contribute to the development of novel bio-nanomaterials.

Amyloid: a natural nanomaterial

Amyloids are highly organized fibrillar structures that hold great potential to be used as nanomaterials for various technological and biological applications (Table 1). The unique properties that make amyloid fibrils attractive for technological use include: (i) spontaneous formation of amyloids by (m)any protein/peptides under certain given conditions (17, 36, 37); (ii) stability, high mechanical stiffness comparable to silk and steel (2), and ability to form highly ordered structures (11); (iii) nucleation-dependent polymerization process, where preformed amyloid fibrils can act as seed to accelerate the kinetics of fibril formation (24); (iv) ability to tune physico-chemical properties of amyloid by modulation of amino acid sequences (38); (v) ease of functionalization of individual fibrils for specific applications (39). The functional group may be recruited at the amino acid side chain for applications such as receptor-ligand and gold-thiol interactions (8, 39); (vi) higher order amyloid aggregation of fibrils leading to formation of complex networks of filaments, gels and films (38, 40–42) that may be suitable for immobilizing enzymes, small molecules and drugs. Apart from these above-mentioned properties, amyloids display structural plasticity and their formation could be reversible depending upon the condition.

Amyloids: highly stable and comparable to steel

Amyloid fibrils possess robust mechanical properties, which highlights their possible applications in nanotechnology. Smith and coworkers characterized the mechanical properties of individual insulin amyloid fibrils using atomic force microscopy and spectroscopy. Their data revealed that amyloid fibril possess mechanical strength (0.6 ± 0.4 GPa) comparable to that of steel (0.6–1.8 GPa) and silk (1–1.5 GPa) (2). To determine the molecular forces responsible for the stability of different amyloid fibrils, Knowles et al. performed AFM topographical studies with individual amyloid fibrils formed by α- and β- lactalbumin, insulin, and transthyretin 105–115 (3). Intriguingly, the bending rigidity (C_B) of these fibrils varied about four orders of magnitude. The data further suggests that stiffness of individual amyloids is determined by the extent of intermolecular interactions between peptide backbones and is indicative of the common mechanism underlying the mechanical properties of these supramolecular structures. Furthermore, comparison of material properties of amyloid fibrils with other classes of materials of biological origin (for example, tubulin, capture silk and elastin) revealed that amyloid fibrils are stiffer than most intracellular biological filaments (3). Simulations aimed at calculating the elastic constants as a function of the size of amyloid fibril of Aβ40 demonstrated that the length of amyloid fibrils significantly influenced their stability, with the long fibrils found to be more stable and mechanically rigid compared to the shorter ones (43). The self-assembled peptide fibrils are stable over a wide range of pH, salt concentrations (21), pressure (up to 1.3 GPa) (44) and are also resistant to proteolysis and dehydration. The amyloids fibrils were also found to be stable up to 100°C temperature (20). The extensive hydrogen bond network and side-chain interactions in steric-zipper of tightly

Table 1. Amyloids and their potential applications in bio-nanotechnology

Amyloid	Application(s)	Reference
Amyloid peptide (105–115) of transthyretin protein	Functionalized amyloid fibrils for cell adhesion	(8)
Amyloid of Gonadotropin releasing hormone (GnRH) analogs	Depot-formulation of long acting peptide/protein drugs	(38)
Self-assembling decapeptide (killer peptide, KP) from *Candida albicans*	Model for auto-delivering therapeutic peptides	(78)
α-synuclein fibrils	Amyloid hydrogel for enzyme entrapment	(41)
Sup35p NM domain (Yeast)	Development of nanowires	(39)
Bovine insulin fibrils (integrated with semi-conducting oligoelectrolytes)	Nanowire for optoelectronic application	(89)
Insulin fibrils (coated with polymer PPF)	Use of amyloid fibrils in polymer light emitting diodes	(90)
Insulin fibrils (coated with conjugated polymer APFO-12)	Nanowires for optical applications	(91)
Insulin fibrils (coated with PEDOT-S)	Conducting nanowire development	(92)
Hen egg white lysozyme amyloid fibrils	Development of thin films	(42)
β2-microglobulin	Development of nanoporous matrix	(82)

packed cross β-sheet structure could account for this extraordinary stability (45).

Structural and morphological plasticity of amyloid fibrils

The distinct features of amyloid fibrils including size, shape, morphology, and secondary structure could be modulated by varying experimental conditions (such as pH, agitation, temperature, salt concentration etc.) that are employed for the formation of amyloids from its monomeric peptide/protein counterpart. Amyloid-like fibrils have been seen in a range of different shapes and forms including curly twisted fibrils, linear straight fibrils, rods, tapes and spherical clusters (such as spherulites) (40, 46–49). Tycko and coworkers have shown that two different modes of aggregation of Aβ40 lead to morphologically distinct amyloids with different secondary structure and toxicity (50). Recently, studies on the structure and intermolecular dynamics of amyloid fibrils by H/D exchange experiment revealed that monomers of amyloid fibrils are in continuous recycling within the fibrils (51). Amyloid fibrils assembly could also be reversed by changing conditions such as pH and dilution (20, 35, 38, 52). The recycling behavior of amyloid fibrils may be exploited for specific applications. For example, the property of reversible self-assembly has been utilized in formulating long-acting drugs where controlled release of peptide molecule occurs from the fibril termini (38). Furthermore, the reversibility and structural plasticity could play important role in modulating the degradability of amyloid fibrils.

Amyloid technology

Developing novel and biocompatible scaffolds for applications in drug delivery, tissue repair/engineering and other nanotechnological devices is one of the key areas in modern science. Significant attempts have been made using bottom up approach with peptide/protein self-assembly to create functional biomaterials for applications ranging from biotechnology to nanoelectronics. The physico-chemical and mechanical properties of amyloids could be tailored either by modulating amino acid sequence of constituting peptides/proteins or using different experimental parameters such as pH, temperature and pressure (20, 21, 44). Recent advances in computer algorithms/tools that can determine the secondary structure, hydrophobicity and/or aggregation propensity of the protein have made it possible to design amyloid-based materials with desired properties.

Amyloids fibrils as a bioactive matrix for tissue engineering

Development of biocompatible nano-scaffolds providing suitable microenvironment for cell survival *in vivo* holds immense scope in cell-based therapies. Several biocompatible materials and natural polymers have already shown great promise in this area (53). Peptide/protein self-assembly could also produce diverse set of biomaterials mimicking the extracellular matrix that can promote cell adhesion, migration and differentiation (54–56). Zhang and co-workers developed novel self-complementary β-sheet peptides using alternative positive and negative L-amino acids that could self-assemble under physiological conditions and form hydrogels (57–59). These peptide fibrils were able to form extensive networks and support neuronal cell attachments, differentiation and extensive neurite outgrowth (60). Moreover, the scaffolds made by self-assembling peptides functionalized with different motifs (e.g. osteogenic growth peptide ALK in osteoblast tissue culture) served as excellent material for three-dimensional cell culture systems (61, 62). The peptide scaffolds promoted proliferation and osteogenic differentiation of mouse MC3T3-E1 cell, suggesting its application in bone tissue engineering (62). In addition, these materials were useful in vascularization, where it created a cellular milieu within the myocardium for survival and organization of endothelial cells (63). It was also reported that self-assembling peptide KLD-12 hydrogel provides an excellent scaffold for the production and accumulation of a cartilage-like ECM within 3D tissue culture that have application in cartilage tissue repair (55).

Amyloid fibrils can similarly be utilized for the development of scaffolds for tissue engineering applications (Fig. 2A). Amyloid fibrils made from peptide/proteins with or without tagged functional moieties and/or fibrils immobilized with functional protein/peptides (such as laminin or fibronectin) can be used as scaffolds for promoting cell attachment and growth (8). Recently, Gras and co-workers (2008) reported that amyloid fibrils made from the partial amino acid sequence of the transthyretin containing the RGD ligands (adhesion moieties) on the fibril surface provide accessibility for cell adhesion. The design of such functionalized fibrils can be exploited to promote interactions with a wide variety of cell types (8). Amyloid fibrils can also be used in enamel repair *via* bioactive surface groups and hard tissue engineering as proteins from demineralized enamel matrices form filaments with cross-β sheet like structure (64, 65).

Possible applications of amyloids as scaffolds for controlling stem cell fate

Stem cells are defined by their ability to self-renew and differentiate into different types of specialized cells. As a consequence, they represent the most versatile cell source for the regeneration of aged, injured, or diseased cells. However, the major roadblock in using stem cell therapies is due to difficulties in maintaining stem cells in their self-renewing state under *ex vivo* conditions. *In vivo*, stem
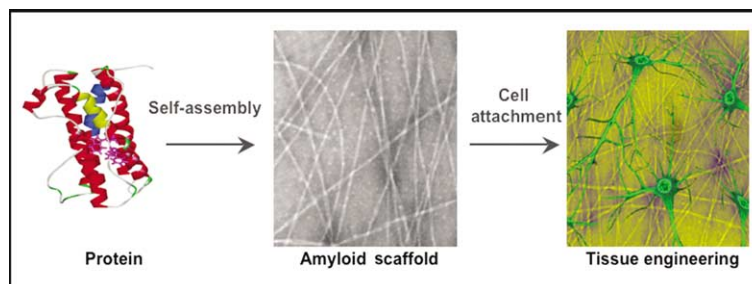
Fig. 2A. Amyloid as a scaffold for tissue engineering. (A) The figure represents amyloid scaffold utilization for neuronal cell attachment, neurite outgrowth and synapse formation.

cells exist in a tissue-specific microenvironment commonly termed as the stem cell niche. This has led to the hypothesis that mimicking the stem cell niche will lead to better control of stem cell self-renewal and differentiation *ex vivo*. The stem cell niche comprises of stem cells, niche cells, growth factors and the extracellular matrix (ECM). Of these, the ECM provides structural support to the cells thereby regulating cell division, cell adhesion, and cell migration. In addition, the ECM also presents biochemical signals to cells in a spatio-temporal manner. Taken together, robust control of stem cell fate requires a precise tuning of each of these factors, both spatial and temporal.

The stem cell niche is very complex to reproduce. Nonetheless, a major emphasis in the field of tissue engineering is to develop natural and synthetic substrates as suitable stem-cell microenvironments for controlling stem cell fate. A lot of these efforts are focused on identifying and understanding the role of the physical and biochemical features of the ECM in modulating stem cell fate. Of these, ECM stiffness and ECM topography have emerged as two key parameters regulating cell function in normal and diseased tissues in a range of different cell types, including stem cells.

Using a polyacrylamide-based hydrogel system, Discher and co-workers (66) demonstrated that human mesenchymal stem cells (MSCs) are exquisitely sensitive to ECM stiffness. Intriguingly, MSCs presented with an ECM of a defined rigidity differentiate towards the cell/tissue type whose *in vivo* stiffness most closely matches that of the ECM. Thus, MSCs became neuron-like on soft gels, myoblast-like on substrates of intermediate stiffness, and osteoblast-like on the stiffest gels. In a separate study, adult neural stem cells cultured on hydrogels of varying stiffness gave rise to glial cells on stiff substrates but became neuronal on soft substrates (67). In order to translate these findings to an *in vivo* setup, one needs to develop natural and biocompatible hydrogels, which can encapsulate the stem cells, maintain their survival and provide the necessary physical and biochemical signals for differentiation.
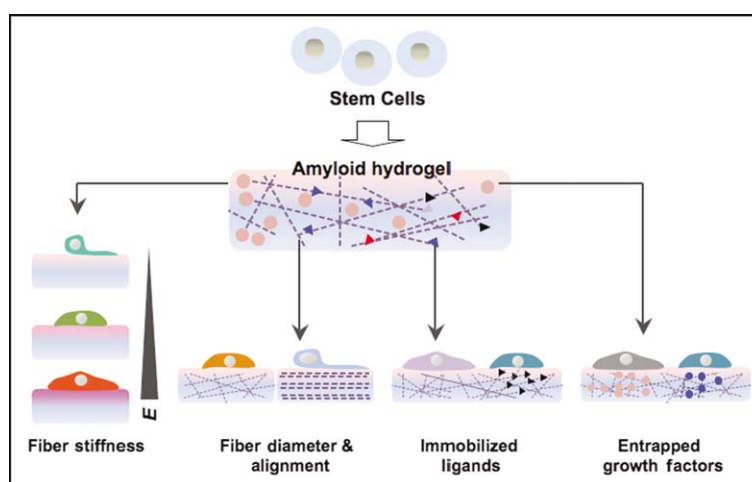

Fig. 2B. Amyloid as a scaffold for tissue engineering. (B) Stem cells may adhere to natural amyloid hydrogels directly, or to those functionalized with different cell adhesion moieties (e.g. RGD). Varying the fiber stiffness or alignment can be tuned to direct stem cell differentiation into different lineages. Furthermore, amyloid hydrogels could be designed with immobilized ligands or entrapped growth factors within, with the action or release kinetics of which tuned to obtain desired stem cell response/behavior.

Cells *in vivo* are exposed to a range of topographies depending on the type of ECM composition. Collagen, the most abundant ECM protein organizes into 3-dimensional fibrils, and plays a significant role in tissue organization by directly modulating cell shape. The success in using electrospun nanofibers as scaffolds for culturing stem cells can be attributed to their morphological resemblance with ECM fibers. Such scaffolds have been successfully employed in achieving chondrogenic, osteogenic and adipogenic differentiation of MSCs *in vitro* (68), several fold expansion of neural and embryonic stem cells (ESCs) *in vitro* compared to tissue culture plastic surfaces (69, 70), and in the *in vivo* repair of articular cartilage defect using MSCs (71).

The ability to tune the microstructure and mechanical strength of amyloid fibrils, ease of attaching any cell adhesion moiety, and the ability to control ligand density on the fibrils, we hypothesize that amyloid-based hydrogels may be an attractive platform for growing and maintaining stem cells *in vitro*, directing stem cell differentiation, as well as using these hydrogels for implanting stem cells *in vivo* (Fig. 2B). For example, while the steel-like stiffness of these fibrils may be ideally suited for differentiating stem cells into osteoblasts, the high degree of order in amyloid fibrils provide a topographic cue for stem cells to differentiate into neurons. However, it is not a priori clear what the stem cells will differentiate into when presented with both the cues simultaneously. Moreover, a new class of fibrils must be developed whose stiffness can be tuned over a wide range for stem cell differentiation, or for maintaining ESC self-renewal where soft substrates are required (72).

Modulation of fibril diameter, fibril alignment, and engineering nanotopography on amyloid fibrils represents another exciting avenue to pursue (Fig. 2B). While fibril diameter will directly influence cell adhesion area, fibril alignment can be particularly suited for differentiating stem cells into skeletal muscle cells, articular cartilage and blood vessels. Moreover, nanotopographic cues can be superimposed on fibrils to further aid in controlling stem cell fate. For example, presence of 350 nm nanogratings was found to be sufficient to induce neuronal differentiation without addition of retinoic acid (73).

Finally, biophysical cues can be combined with chemical cues for regulating stem cell fate using amyloid-based materials. In addition to controlling fibril stiffness, size and roughness, growth factors and cytokines like fibroblast growth factor 2 (FGF2) and leukemia inhibitory factor (LIF) can be entrapped within the fibril network and their release kinetics can be further tuned to have an additional handle towards fine tuning stem cell response and obtaining the desired behavior. Taken together, precise control of stem cell fate requires the presence of multiple factors in the right arrangement and orientation within a well defined scaffold of defined stiffness and topography together with specified biochemical gradients, and amyloid-based hydrogels can potentially serve as good stem cell culture systems.

Amyloids as a novel depot formulation in drug delivery

The protein/peptide drugs often require special means of delivery such as infusion and subcutaneous administration to prevent them from protease degradation (74, 75). Various formulations of peptide/protein drugs have been developed, which aid in achieving controlled release of drugs over an extended period of time thus maintaining a uniform drug concentration. Administration of self-assembled peptide/protein drugs represents one of the effective modes of drug delivery. For example, it was shown that crystallization of insulin and TGF-β3 provides sustained release mechanism, where micro-crystals can serve as a protected reservoir of releasing active drugs (76, 77). Similarly, recent studies have suggested that self-assembly of protein/peptides into amyloid conformation could be used in drug delivery either as drug delivery vehicles or as drugs themselves (38) (Fig. 3). It was suggested that long-acting gonadotrophin releasing hormone (GnRH) analogs are able to form amyloids *in vitro*, which can sustain the release of monomeric drugs *in vitro* and *in vivo*. In contrast, most of the short acting analogs either remain monomeric in solution or form amyloids with high stability (with slow release capability). Additionally, some short acting analogs formed amyloid with very low stability that release monomer instantaneously. The short-acting analogs, which are not able to form amyloids *in vitro*, might form amyloids when incubated for extended periods and showed activity over long durations *in vivo* (38). This study clearly demonstrated that amyloid form could work as a reservoir of drugs. Moreover, the amyloid termini can release the active peptide drug in a controlled manner over a period of several weeks. The advantages of designing amyloid as a depot formulation is that it will maintain convenience, drug dosage within desired range, and patient compliance. The only concern is that amyloids have to be non-toxic and should be able to release the functional monomers. Also, it should not cross-react with other disease related amyloid forming proteins or act as a seed for disease-progression (38). The property of reversible self-assembly of peptides into fibrils encourages a new model of auto-delivering therapeutic peptides as exhibited by a therapeutic antibody-derived decapeptide (killer peptide, KP) from *Candida albicans* (78). The controlled release profile obtained with oligomers made from insulin at pH 7.0 further highlights the potential of using oligomers formulations for long-acting protein/peptide drugs (79).

The self-assembling peptide/protein nanofibrils may also serve as excellent drug carriers. Zhang and
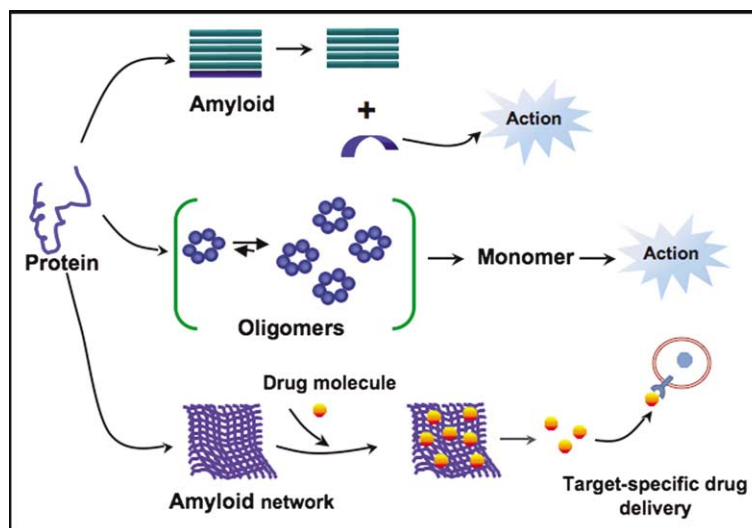
Fig. 3. Schematic representation of the application of amyloid for drug delivery. Protein/peptides drugs under appropriate conditions can form amyloid fibrils. These fibrils could release functional protein/peptides from the fibril termini after administration, allowing a controlled release of monomeric drug (top) (38). Nontoxic protein/peptide oligomers (blue spheres) may similarly be used for auto-delivery of the drugs (middle) as suggested for insulin (79). The monomeric drug released in both the above cases could perform their action at the targeted site. Amyloids also might be utilized as a vehicle for drug delivery, wherein the drug molecules (orange spheres) could be entrapped within amyloid networks (violet mesh) ensuring the slow release of the drugs after administration. The figure shows the drug release occurs at the target site and binds to specific receptors on the cell surface to perform its action (bottom).

co-workers have shown that a self-assembled peptide system of RADA16 hydrogels could serve as a slow delivery carrier of various small molecules as well as variety of proteins such as lysozyme, trypsin inhibitor, bovine serum albumin (BSA) and immunoglobulin G (IgG) (80, 81). Similarly, supramolecular networks of amyloids can entrap small molecules, drugs, protein/peptides and enzymes where it could work as a vehicle for drug delivery. The drug molecules will be protected from heat, enzyme degradation, as they are entrapped in the stable core of the cross-β-sheet-rich structure. Recent work suggests that hydrogels formed from curly-amyloid fibrils of α-synuclein can serve as a nanomatrix for enzyme entrapment (41). Further, their capacity to sustain enzyme activity and act as resistance barrier against the heat treatment highlight their potential to be used in therapeutic delivery (41). Amyloid fibrils of β_2-microglobulin (associated with dialysis-related amyloidosis) were recently utilized to construct nanoporous protein matrix with high mechanical strength and may be used in drug delivery and tissue engineering applications (82).

Development of metal nanowires and biosensors using amyloids

Self-assembly of amyloidogenic peptides into fibril nanostructures can play an important role in building nanowires and nanoelectronic materials. For example, nanotubes made by the self-assembly of Phe-Phe dipeptide from the central region of amyloid β-peptide (Aβ, associated with Alzheimer's disease) was successfully utilized as a template for metal nanowire formation (Fig. 4A) (4, 83–86). The obvious advantages of such dipeptide-based nanotubes are their ease of synthesis and biodegradability. These peptide nanotubes could be produced in large scale without significant cost, and their degradation could be further modified using D-amino acids. Phe-Phe nanotubes can be formed by vapor deposition method and can self-assemble in aqueous solution (87). Therefore, using these kinds of dipeptide templates and their strong tendency to self-associate, it is easy to construct various functional nanomaterials. The amyloid forming capabilities of NM domain of the yeast Sup35p was successfully used for constructing metal nanowires that were able to conduct electricity with low resistance (39). In this study, the genetically engineered cysteine mutant of NM was fibrillized, where surface accessible cysteine was covalently linked to monomaleimido nano-gold. These nano-gold bound NM fibrils were used as promoters for reducing silver ions from the solution and the resulting silver coated fibrils were further used to deposit gold for making metal nanowires (Fig. 4B).

Enhancement of desired properties of conducting materials can also be achieved using peptide nanotubes and fibrils. For example, Yemini et al. reported that the electrochemical properties of graphite and gold electrodes could be improved with the help of peptide nanotubes,
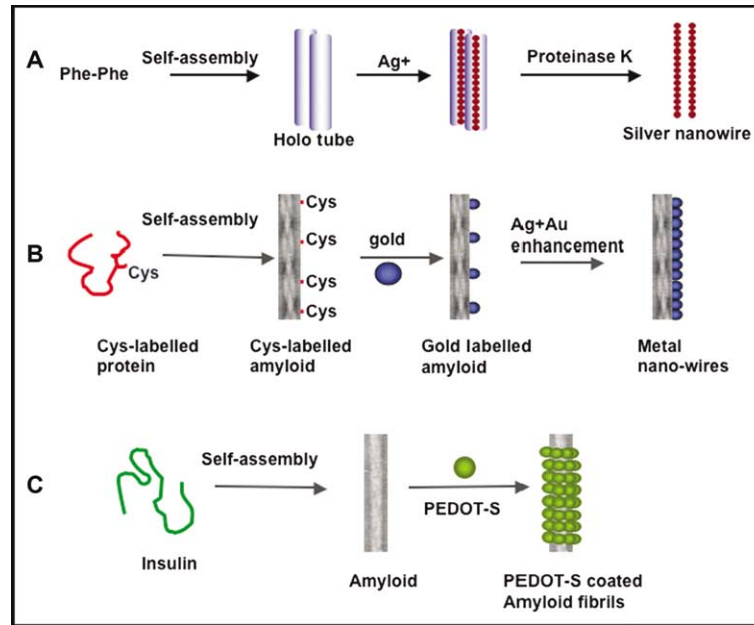
Fig. 4. Nanowire development using self-assembling peptide/proteins and amyloid fibrils. (A) The nanotubes made by the self-assembly of Phe-Phe dipeptide were utilized as a template to form silver nanowires (83). (B) Cysteine labeled amyloid was used to develop metal nanowires, by first covalently linking the nano-gold to surface exposed Cys residues, followed by silver ion reduction in solution to provide a silver coating, and subsequently making deposition of gold (39). (C) Insulin amyloid fibrils coated with alkoxysulphonate PEDOT-S was able to generate conducting nanowires (92).

when they were directly deposited on the electrode (88). This technique could be useful for the development of (bio)sensors with high analytical performances. Integrating amyloid fibers and polymers can lead to novel nanocomposite materials with high performance of material properties. Herland et al. integrated semi-conducting conjugated oligoelectrolytes with bovine insulin where both components co-assembled to form electroactive luminescent fibrillar nanowires (89). It is also important to note that the organization/orientation of the polymers within the amyloid fibrils may influence various material properties (e.g. optoelectronic property in the above study). Amyloid nanofibrils of insulin, when decorated with luminescent polymer PPF resulted in a complex that could successfully be employed as an active layer in light emitting diode (LED) (90). The external quantum efficiency of PPF and PPF-coated amyloid-complex were compared, and it was found that the quantum efficiency of the former was $\sim$0.01% (current density range: 1–100 mA/cm^2), whereas that of the latter device was more than 0.1% (current density range: 0.01–20 mA/cm^2). The PPF-insulin fibrils complex thus exhibited a ten-fold increase in the external quantum efficiency when compared to pure PPF alone (90). Furthermore, when insulin amyloids were coated with a polar, non-charged conjugated polymer APFO-12, the polymer chains were found to align along the fibrils with varying degrees of polarization. The increased anisotropic behavior of the polymer along the fibrils could be utilized for the development of nanowires for optical applications (91). Hamedi et al. demonstrated that conjugated polymer alkoxysulphonate PEDOT could be coated onto insulin amyloid fibrils through self-assembly producing electrically active networks of conducting nanowires (92) (Fig. 4C).

Amyloid can also be utilized for protein immobilization and biosensor development. For example, amyloid surface can be functionalized with ligands such as fluorophores, cytochromes, enzymes and other tags according to the desired application in nanotechnology (8, 9, 93–95). The enzymatic properties and stability of enzymes can be improved by immobilizing them on the surface of amyloid nanofibrils (96). In this aspect, it was reported that organophosphate hydrolase (OPH), when covalently immobilized on bovine insulin fibrils using glutaraldehyde as a cross-linker, resulted in significant increase in the thermal stability of the enzyme compared to the free enzyme (96). Similarly, antibodies could be immobilized on the amyloids for detecting specific antigens.

Other potential applications of amyloid nanofibrils

Recently, Knowles et al. prepared rigid nanostructure thin films from hen egg white lysozyme and bovine β-lactoglobulin amyloid fibrils. These self-assembled macroscopic films can align the unstructured fluorophores within the macroscopic films (42) reflecting the

potential application of amyloids for microbial coating. Nanoscale characterization of amyloid fibrils in natural adhesives of algae by Mostaert et al. revealed the generic mechanism of mechanical strength of the adhesives (97). These bio-adhesives with increased mechanical strength and stability might be useful for application in coating industries. Other prospective applications of amyloid fibrils could be in food industry and in the development of nanofilters and bioseparators.

Conclusions and future directions

Generation of novel biomaterials with diverse structure and functions is one of the upcoming fields in biotechnology. Organic polymers as well as biopolymers have long been exploited to devise biomaterials for various nano- and bio-technological applications including nano-wires/nanotubes development, regenerative medicine and drug delivery (53, 98–101). Engineering nanoscale devices using protein/peptide self-assembly is especially important in this regard as it could adapt lessons from nature that produced numerous biomaterials. In this review, we highlight the various applications of amyloids in nano-biotechnology. Although most of the attention on amyloids has been channeled into studying their role in several human diseases, a paradigm shift in the understanding of amyloid biology has evolved in the last few decades, suggesting the vast potential of amyloid to perform as an elegant biomaterial. The *de novo* design of amyloids and the ability to modify their physicochemical properties, along with the development of computer based algorithms [such as TANGO (102)], has made it possible to utilize the versatile properties of amyloids for developing several new classes of biomaterials.

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There is no conflict of interest in the present study for any of the authors.

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