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Advancements in amyloid-based biological materials for healthcare, environmental and sensing applications

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Amyloids are insoluble accumulations of fibrils formed by various proteins such as A β 1–40, lysozyme, α -synuclein, insulin and peptides associated with several protein aggregation-related diseases. The self-assembling property of amyloid fibrils has been demonstrated to be a powerful characteristic for creating protein-based materials for various applications. Biomaterials based on amyloid fibrils have recently gained momentum due to their superior mechanical strength, exceptional self-assembly and stability in various matrices. Owing to their versatile structural characteristics, amyloid fibrils can have remarkable potential for application in medicine, materials, packaging, tissue engineering, sensors and drug delivery. Here, the recent advancements in the structural characterization, synthesis, classification and the emerging applications of amyloid fibrils in healthcare, sensing and the environment have been reviewed. The biocompatibility, biodegradability and structural stability of amyloid fibril-based biomaterials open up new avenues for sustainable and eco-friendly alternatives to the conventionally used synthetic materials.

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1. Introduction

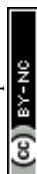
Globally, industrial and consumer preferences are shifting towards green, sustainable and eco-friendly alternatives to conventionally used synthetic materials.¹ Utilizing renewable agro-resources to develop biodegradable materials has gained significant attention. Various biopolymers are found in natural sources, such as proteins, starch, cellulose fibres and lipids.^{2,3} Proteins and peptides are functionally important biomolecules performing several functions in the maintenance of cellular homeostasis.⁴ Proteins have shown to provide the molecular basis for the development of various biomaterials.⁵ Natural protein-based biomaterials such as silk proteins and egg hen lysozyme demonstrate the ability of proteins to self-assemble and aggregate to form functional materials.^{6,7} The biocompatibility and structural characteristics of proteins and peptide assemblies have tremendous potential in biomaterial applications. Under physiological conditions, proteins fold to their

native three-dimensional structure; however, exposure to various stress conditions they may misfold and aggregate due to non-native interactions and form amyloid fibrils.^{4,8} The formation of amyloid fibrils is related to proteins with amyloid cores and has a self-assembling and aggregation propensity.⁹ The amyloid protein aggregates were first identified as disease hallmarks associated with several protein aggregation-related diseases, including Alzheimer's and Parkinson's disease.¹⁰ Structurally, amyloid fibrils are composed of β -sheets formed by the structural transition of the helical structure and are categorized as supramolecular polymers of β -strands stacked perpendicularly to the fibrils on their long axis.¹¹ Amyloids are insoluble accumulations of fibrils formed by various independent proteins such as A β 1–40, α -syn, insulin and peptides.^{12,13} Apart from pathologically associated proteins such as amyloid β (A β 1–40), tau and α -synuclein (α -syn), several other proteins also form amyloid fibrils at high concentrations and under destabilizing conditions.^{8,13} The protein aggregates and amyloids are formed when the soluble physiological native form of protein undergoes misfolding and forms a nucleus for aggregation that further elongates by the addition of misfolded monomers.^{14,15} The protein aggregates formed thus include small oligomeric precursors, amorphous aggregates and mature fibrils, each exhibiting distinct structural and morphological characteristics.¹⁶

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Several functional forms of amyloids have been identified and characterized in plants and animals, showing potential for diverse applications.^{17,18} The self-assembling property of amyloid fibrils has been demonstrated to be a powerful characteristic for creating protein-based materials for various applications. Functional amyloids have numerous applications in metal–organic frameworks (MOF) as sensors for amyloid diseases, whey protein isolate amyloid fibrils in combination with carboxymethyl cellulose as a drug delivery vehicle, membrane formation, nanosensor applications, and as membranes for packaging and healthcare applications.^{19–21} The biocompatibility, biodegradability and structural stability of amyloid fibrils can present a sustainable and eco-friendly alternative to the conventionally used synthetic materials.²² The stable proteinaceous biostructures formed by amyloid fibrils are structurally flexible, allowing modification using chemical modification, denaturation, enzymatic modification, nanomaterials and bioactive molecules to develop sensors, embedded nanomaterials and membranes.^{23,24}

In the present review, the recent advancements in structural characterization, synthesis, classification, and the emerging applications of amyloid fibrils in healthcare, sensing and the environment have been reviewed. Further, an overview of the protein-based materials available from natural sources and their role in drug delivery and sensor applications has been provided. Biomaterials based on amyloid protein aggregates have several advantages due to their superior mechanical strength, exceptional self-assembly and stability in various matrices. The biocompatible and biodegradable nature of amyloid fibril-based biomaterials and their versatile structural properties can provide a sustainable and eco-friendly alternative to conventionally used synthetic materials.

2. Structural characteristics of amyloid fibrils

2.1 Formation of amyloid fibrils

The application of amyloid fibrils in developing functional materials and membranes has gained interest due to their

extended, highly stable β -sheet structures and β -turns.²⁵ Here, partially misfolded monomers result in the assembly of oligomers, ultimately leading to the formation of fibrils as shown in Fig. 1.²⁶ The transition state of aggregation is represented by oligomer formation, where the metastable β -sheet conformations dominate and gradually result in the formation of β -sheet rich structures. The formation of oligomeric aggregates further leads to rapid nucleation of monomers to oligomers or fibrils.²⁷

The fundamental structure of amyloids was first identified in the 1950s with the advancement in electron microscopy techniques and X-ray fiber diffraction.^{28,29} The distinguishing elements of the fibrillar structure of amyloids are present at the different structural levels, such as tertiary and quaternary, which can be easily identified using nuclear magnetic resonance (NMR), cryo electron microscopy (cryo-EM) and solid-state nuclear magnetic resonance (SSNMR).³⁰ Amyloid fibrils have a needle-like and unbranched morphology consisting of several bundled protofilaments. Each protofilament comprises cross- β structure sheets that are parallel to the fibrillar axis and stacked perpendicular to the main axis.²⁸ The fibrillar arrangement depends upon the exposure to different conditions such as agitation, salt concentration, protein concentration, temperature, pH, and the degree of folding and unfolding during fibril formation.³¹ Various peptides frequently assemble β -sheets by stacking elongated β -strands, and a steric zipper is formed when two β -sheets are strongly interdigitated with adjacent chains.³² The intricate structure and high organization of amyloid fibrils prevent their breakdown by proteases.³³ These characteristics of amyloid fibrils are due to a dense backbone of hydrogen bonding network in cross- β structures, that provides stiffness up to the gigapascal (GPa) level.²⁸ Various studies have also reported diversity in amyloid structure formation from a single protein, referred to as amyloid polymorphism. Depending on different fibril growth conditions such as temperature, solvents, protein concentrations and agitation, it results in the generation of amyloid polymorphs that are produced *in vitro* and are also related to protein aggregation diseases.²⁸

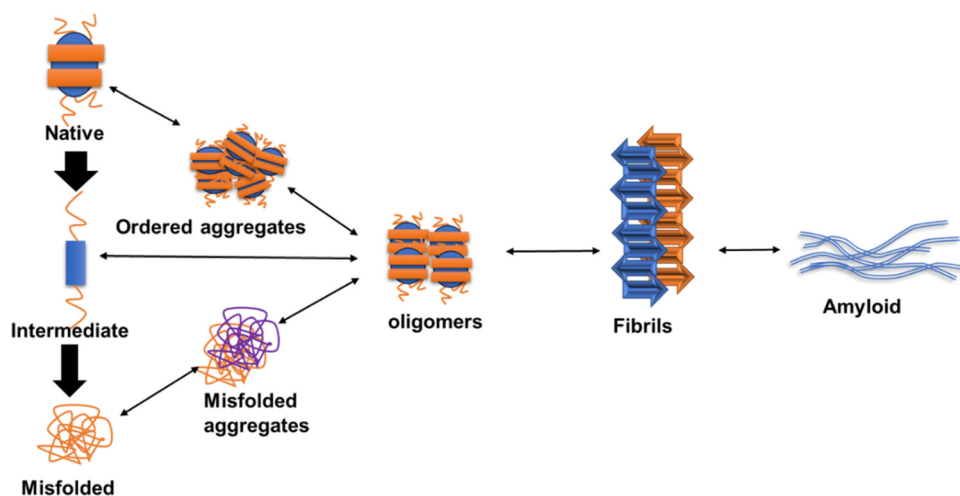


Fig. 1 The process of amyloid formation is initiated with protein misfolding, oligomer formation and fibril accumulation leading to amyloid formation.



2.2. Structural characterization of amyloid fibrils

Various biophysical approaches can be employed to characterize amyloids based on their structure and stability. Among these, X-ray diffraction is one of the standard and classical methods that investigates the formation of cross- β structures.³⁴ The widely used method to determine the structure and track the formation of amyloid aggregation is the thioflavin T (ThT) assay, which binds specifically to the β -sheet structures formed in the amyloids.³⁵ Various other biophysical methods are used to determine the presence of β -sheet rich structures in amyloid fibrils, such as Fourier transform infrared (FTIR) spectroscopy and circular dichroism (CD) for analyzing secondary structures, transmission electron microscopy (TEM), scanning electron microscopy (SEM) and atomic force microscopy (AFM) for structure visualization, and super-resolution microscopy (SRM) and light/neutron/X-ray scattering for microscopic analysis of amyloid structures.³⁶ The molecular and atomic scale investigation of the amyloid morphology and structural studies done by hydrogen-deuterium (H/D) exchange and cryo-EM techniques are alternatives to conventional techniques such as X-ray crystallography and NMR.³⁷ Other methods, such as dynamic light scattering (DLS) and quartz crystal microbalance (QCM), are used to monitor misfolding, fibril elongation and aggregation.³⁸ Characterization of protein-based membranes by X-ray diffraction reveals that the membranes possess a high level of ordered organization ranging from the nano to micrometer scale. X-ray diffraction can analyze the structure specific diffraction patterns, providing information about individual β -strands and protein nanofibrils.³⁹ As a single biophysical technique cannot provide enough information on the detailed structure of amyloid fibrils, multiple techniques are employed to minimize the possibility of artefacts. Among the diverse techniques available, the most used techniques for characterizing amyloid fibrils are explained below.

ThT fluorescence. Thioflavin T (ThT), a fluorescent dye, is an amyloid formation indicator commonly used for staining and identification of amyloid fibril formation. ThT intercalates within the β -sheet upon aggregation, releasing a strong fluorescence signal at 450 nm excitation and 485 nm emission.³⁵ ThT fluorescence has been used extensively to study the kinetics of amyloid formation.⁴⁰

CD and FTIR spectroscopy. CD and FTIR spectroscopy help to determine the secondary structure of the protein, including α -helices, β -sheets, turns and random coils. Amyloid fibrils having β -sheets as the dominant secondary structure (intermolecular β -sheets) display a strong peak at ~ 1611 – 1630 cm^{-1} in the FTIR spectrum and a characteristic negative peak at ~ 215 – 218 nm in the CD spectrum.^{41,42}

Hydrogen-deuterium exchange. Hydrogen-deuterium (H/D) exchange is used for fibril characterization of A β , β 2-microglobulin and yeast prion Sup35 proteins.³⁰ The technique is based on the exchange of hydrogens (especially NH amide hydrogen) of protein with the solvent (usually D₂O) deuterium.³⁰ The rate of hydrogen exchange can also be easily monitored *via* mass spectrometry, referred to as hydrogen/deuterium exchange-mass spectrometry (HDX-MS), or through NMR, which can be related to the respective secondary structure of proteins.⁴³

Cryo-electron microscopy (cryo-EM). The structures of amyloids can be determined by the cryo-EM technique through the exposure of flash-frozen amyloids with high-speed electrons and recording the resulting images. Cryo-EM is a suitable alternative to NMR as it does not require isotope labelling. The structures of various amyloid conformations are provided by cryo-EM, which uses electron density to create a detailed image.³⁰ The assembly and structural details of a wide range of amyloid fibrils, generated by different amyloid proteins, including tau and α -syn, have been studied using single-particle cryo-EM, contributing toward the understanding of these complex structures and their polymorphism.^{44,45}

3. Amyloid-based materials

Highly stable amyloid fibrils are currently being used to develop biocompatible and biodegradable materials. These properties have increased scope for the development of functional amyloid materials such as hydrogels, scaffolds, membranes and nanomaterials for application in therapeutics, electronics, food packaging and the environment.⁴⁶ Amyloid fibrils are assembled and modified to produce amyloid membranes that can be utilized for water purification, conductive three-dimensional aerogel biomaterials with sensing properties and drug-releasing vehicles.^{20,47} The amyloid protein-based membranes are being developed as hybrid materials with desired functional properties for use in drug delivery, water purification and nanofiltration. Hybrid amyloid membranes prepared from amyloids of β -lactoglobulin and porous carbon hybrids are used for removing arsenic contamination from water.⁴⁸ Further, modified protein amyloid fibril/zirconium oxide (ZrO₂) nanoparticles are utilized for removing fluorides from water.⁴⁹ A hybrid membrane synthesised from carboxymethyl cellulose (CMC) and whey protein isolate amyloid fibril (WPI-AF) *via* chemical crosslinking coupled with phase inversion has been used as a drug delivery vehicle for methylene blue (MB) and riboflavin.²⁰ Similarly, graphene oxide (GO)/amyloid-based membranes are also being used in water purification.⁵⁰ The detailed applications of various amyloid-based materials are discussed in Sections 5.1–5.3.

4. Natural protein-based materials

4.1. Concept of protein membranes

Proteins exist in various distinct structural conformations, such as monomers, small oligomers (homo/hetero-compositions), elongated fibrils and rods. Structural conformers of proteins such as lysozyme and β -lactoglobulin amyloid fibrils undergo polymerization, resulting in the formation of membranes and functional materials.⁵¹ The multi-level organisation of the protein structure allows interaction and intermolecular binding with different amino acids resulting in the formation of several linkages.²³ Protein structure and stability modified using methods such as denaturation, chemical hydrolysis and crosslinking provides strength and reduces flexibility and permeability to



different gases, vapours and liquids, required for membrane formation.^{23,52} Efforts are being made for environment-friendly approaches to create sustainable packaging materials from naturally occurring biopolymers.⁵³ The application of membranes developed from proteins is being explored in food packaging to enhance the shelf life of food commodities.⁵⁴ Protein-based membranes that are either animal- or plant-derived are becoming popular as compared to synthetic biopolymers due to their relative abundance in nature, ability to form membranes, biodegradability and nutritive value.⁵⁵ Protein-based membranes have better gas and aroma-barrier properties along with the desired flexibility and mechanical strength as compared to lipid- and polysaccharide-derived membranes. Proteins isolated from animal sources such as collagen, gelatin, chitosan, and whey, and proteins from plant sources such as starch, gluten, zein, and gelatin are being used for the preparation of membranes.^{56–61}

4.2. Major sources of proteins for biomaterial synthesis

Proteins, based on their structure, are classified into two categories: fibrous and globular proteins. Fibrous proteins such as collagen are water-insoluble and stabilized by intermolecular hydrogen bonds, whereas globular proteins such as wheat gluten, corn zein and soy proteins are water-soluble, and their structure is maintained by covalent (disulfide) bonds, hydrogen bonds, ionic and hydrophobic interactions.⁶² Their high abundance in nature and ease of processing, along with structural complexity and a higher tendency for intramolecular interactions, help in providing better mechanical properties, making proteins a suitable candidate for the synthesis of biomaterials.^{61,63} Therefore, several animal and plant proteins such as collagen, wheat gluten, corn

zein, and soy proteins are highly used in the preparation of edible membranes,⁶¹ where the properties of the developed biomaterial or membrane depend on the nature of the protein, degree of chain extension and the sequence of amino acids. The developed biomaterials have desired flexibility, gas and liquid permeability, mechanical strength, shelf life and opacity,⁶⁰ which can be enhanced using various additives such as plasticizers, vitamin E, nisin and cellulose.^{23,64} Thus, protein-based biomaterials are promising alternatives to synthetic packaging materials, and the natural sources of proteins utilized for the development of various functional materials are discussed in the following sections.

4.3. Natural sources of protein membranes

Biomolecules such as proteins, carbohydrates and lipids have the potential to produce multifaceted biomaterials and membranes owing to their biodegradability and sustainability.^{65,66} Various animal and plant proteins such as soy protein, milk whey, casein, wheat proteins, corn zein, collagen, silk and oilseed proteins from different natural sources have been explored and studied for membrane production as listed in Tables 1 and 2.⁶⁷ These biodegradable protein-based materials are used in medicine, biotechnology and packaging applications.⁶⁸ Protein-based membranes and biomaterials are strong; however, non-plasticized membranes are brittle.^{69,70} To address this issue, various chemical and physical modifications of protein components are required during membrane production to enhance the mechanical properties and functional applications.⁷¹

4.4. Plant-based sources for biomaterials

Plant-based proteins derived from peas, beans, kernels, legumes and agro-waste have been used for preparing membranes and

Table 1 Plant-based protein membranes and their applications

Protein group	Plant source	Features of the membrane				Applications
		Mechanical strength	Water vapour permeability	Thermal stability	Antioxidant activity	
Legume proteins	Kidney beans ⁸¹	Enhanced	Not reported	Not reported	Not reported	Antimicrobial food packaging
	Mung bean ⁸²	Enhanced	Enhanced	Not reported	Enhanced	Edible coating
	Faba bean ⁸³	Enhanced	Low	Enhanced	Not reported	Biodegradable packaging
	Soy bean ⁸⁴	Enhanced	Low	Enhanced	Not reported	Bio-based packaging
	Horse gram ⁸⁵	Enhanced	Enhanced	Not reported	Not reported	Antimicrobial food packaging, biomedical materials
	Fenugreek ⁸⁶	Enhanced	Low	Moderate	Not reported	Bio-based packaging
	Red bean ⁸⁷	Enhanced	Low	Not reported	Not reported	Packaging materials
Nut and oilseed proteins	Peanut ⁸⁸	Low	Low	Not reported	Enhanced	Antimicrobial food packaging
	Sesame ⁸⁹	Enhanced	Low	Enhanced	Not reported	Food packaging
	Canola ⁹⁰	Enhanced	Not reported	Enhanced	Not reported	Food packaging
	Sunflower ⁹¹	Enhanced	Low	Not reported	Enhanced	Fresh fruit packaging
	Flax seed ⁹²	Enhanced	Low	Enhanced	Not reported	Antimicrobial food packaging film
	Rape seed ⁹³	Enhanced	Not reported	Not reported	Not reported	Edible food coating
	Castor bean ⁹⁴	Enhanced	No change	Not reported	Not reported	Seedling packaging bags
Sorghum ⁹⁵	Enhanced	Enhanced	Not reported	Enhanced	Antimicrobial food packaging	
Cereal grain proteins	Wheat ⁹⁶	Enhanced	Low	Enhanced	Not reported	UV-resistant edible coating
	Oat ⁹⁷	Enhanced	Low	Enhanced	Not reported	Biodegradable packaging
	Corn ⁹⁸	Enhanced	Not reported	Enhanced	Not reported	Scaffolds, biomaterials
	Rice ⁹⁹	Enhanced	Low	Enhanced	Enhanced	Antimicrobial food packaging
	Barley ¹⁰⁰	Enhanced	Low	Enhanced	Not reported	Biodegradable packaging
Pseudo-cereal proteins	Quinoa ¹⁰¹	Enhanced	Low	Not reported	Enhanced	Antimicrobial active packaging
	Amaranth ¹⁰²	Enhanced	Low	Not reported	Not reported	Edible food coating

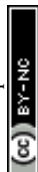


Table 2 Animal-based protein membranes and their applications

Source	Protein	Advantages	Limitations	Applications
Animal meat	Gelatin ^{105–108}	Gas barrier properties, antioxidant and anti-bacterial activity	Weak water vapor barrier properties, moderate mechanical properties	Active food packaging, edible coating
Animal meat	Collagen ^{109,110}	Antioxidant and anti-bacterial properties, barrier for moisture, high mechanical strength, thermostability	High water vapour transmission rate	Active edible food packaging
Animal meat	Myofibrillar protein ¹¹¹	High tensile strength	Highly sensitive to water vapour	As a plasticizer in packaging
Milk protein	Caseins ^{112–114}	Low oxygen permeability, moderate mechanical strength	Highly sensitive to water vapour, low elasticity	Packaging for cheese and food products
Milk protein	Whey protein ¹¹⁵	High mechanical strength, improved barrier properties, antibacterial and antioxidant properties	High vapour permeability, low tensile strength	Packaging

coatings.^{67,72–74} The presence of various amino acids and active binding sites in proteins helps to form molecular networks, providing desired elasticity or plasticity for preparing biopolymer membranes.⁷⁵ Plant protein-based biopolymer membranes have issues with limited elongation properties, leading to brittleness in the developed materials and coatings.⁷⁶ The structural characteristics and functional groups of the protein are exploited for enzymatic, chemical and physical modifications.⁷⁷ Such modifications enhance their intrinsic barrier, plasticity, antimicrobial, antioxidant, thermal, mechanical and other biophysical properties.^{75,78,79} The use of plasticizers (*e.g.*, glycerol, fatty acids and nanocomposites) significantly improves the functionality and bioactivity of the membranes.⁸⁰ Various sources of plant protein-based materials and their applications are listed in Table 1.

4.5. Animal-based sources

Various animal-derived biomolecules such as keratin, gelatin, collagen, silk fibroin/sericin and myofibrils have potential for synthesis of value-added biopolymer-based materials. Biopolymers derived from the waste by-products generated at slaughterhouses and meat processing industries can also be repurposed for developing biomaterials.^{103,104} Some of the widely used animal protein-based materials are described in Table 2.

4.5.1. Silk protein-based membranes. Silk proteins are derived from silkworm protein fibres and are widely utilized in various biomaterial applications. Silk fibres comprise a fibroin core that gives them strength and a glue-like casing of sericin protein helps in maintaining their structural integrity as illustrated in Fig. 2.^{116,117} Sericin is a globular protein having a molecular weight ranging from 20 to 400 kDa and consists of up to 18 amino acids.¹¹⁸ Sericin is highly hydrophilic, making it suitable for application in a vast arena of biological applications. The presence of multiple polar chemical groups and structural makeup of amino acids not only offer features such as antioxidant, anti-tumor, anti-inflammatory, antibacterial and wound healing activities, but also assist in binding and crosslinking with other polymers.¹¹⁸ Sericin is rich in random coils and β -sheets. The random coils convert into β -sheets in response to heat stress, chemical reactions and mechanical stretching. This conversion facilitates gelation and formation of sericin gel when sericin is exposed to suitable temperature

and chemical treatments.^{116,119} The hydrogen bonds are responsible for the glue-like properties of silk fibres, allowing their use in cosmetics, food, and other biomedical applications in hydrogels, tissue engineering and drug delivery.^{120–125} Fibroin is a glycoprotein that consists of two subunits of proteins: heavy chains having repetitive hydrophobic blocks and light chains comprising non-repetitive hydrophilic blocks in an equimolar ratio connected by disulfide bonds.¹²⁶ Fibroin consists of repetitive motifs that are mainly dominated by GAGAGS motifs (Fig. 2).¹²⁷ The heavy chains of the glycine-rich crystalline portion of fibroin and discrete β -sheets in the fibres are responsible for forming the structure and providing mechanical properties.^{128–130} Silk fibroin presents outstanding mechanical strength, thermal stability and break-strain index as compared to synthetic fibres.¹³⁰ Owing to its unique structural characteristics, silk fibroin protein can be used in sponges, scaffolds for the adhesion of cellular models, hydrogels for drug delivery, *etc.*¹³¹

4.5.2. Silk fibroin- and sericin-based materials. Silk-based biopolymers are excellent for developing fibres, 3D scaffolds, sponges, biomedical prosthetics, gels and other biomaterials. This is mainly due to their exceptional strength, biodegradability, biocompatibility, immune-compatibility and adjustable mechanical characteristics.^{131–136} Silk-based membranes are also used as food coatings, preventing water and oxygen permeability in the food products resulting in an extension of their shelf-life.¹³⁷ Silk fibroin electrospun mats and nanofiber matrices are some examples of biomaterials formed from silk fibroins.¹³⁸ Sericin-based hydrogels and scaffolds have been used in skin regeneration owing to their cell adhesion properties, proliferation and antioxidant properties.¹³⁹ Sericin can also be used to produce foams, sponges, and membranes by incorporating silver or zinc oxide nanoparticles, which enhance its antimicrobial applications.^{139–141} Some of the silk protein-based materials are discussed below with their applications.

Sponges. Silk fibroin sponges are macroporous biomaterials used in tissue engineering. These porous sponges are prepared by mixing the silk solution through freeze-drying, gas foaming and addition of suitable porogens (polymers, sugar, salt crystals) through gelation.¹⁴²



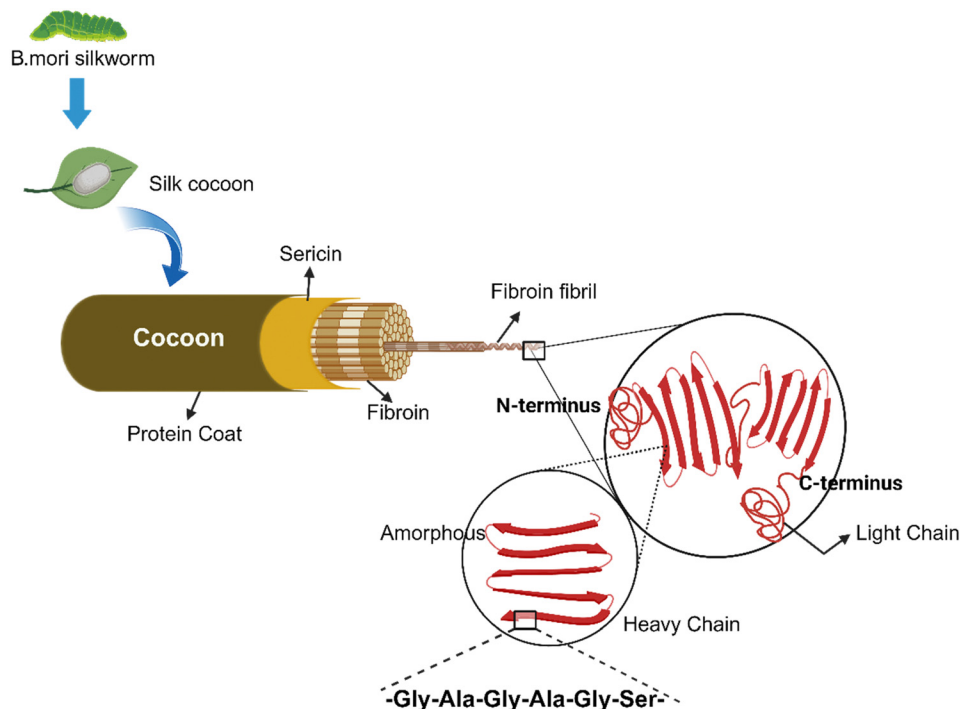


Fig. 2 The structural organization of fibroin silk protein.

3D printed structures. Silk-based bio-inks and filaments are used as structural materials for the 3D bioprinting of custom geometric structures. Employing different 3D printing methods, complex and difficult-to-design customized biological constructs can be fabricated for biomedical applications.¹⁴³

Electrospun mats. Electrospinning technology is used to create non-woven mats by spinning a polymeric solution in an electric field. Silk fibroins are used to produce nanofibrous mats by electrospinning the polymer solution through a needle and an electric force. These electrospun nanomats are used in grafts, implants, tissue scaffolds and other crucial healthcare applications.^{138,144}

Silk-based hydrogels. Silk-based hydrogels and scaffolds are developed through chemical modifications *via* cross-linking of silk proteins for application in wound healing and medication.¹⁴⁵ Hydrogels prepared using sericin–sodium alginate and silver nanoparticles (AgNPs) can potentially help in preventing bacterial infection.¹⁴⁶ Similarly, hydrogels prepared by incorporating lysozyme, sericin and agarose are used to enhance antibacterial and water absorption properties.¹⁴⁷ Sericin hydrogels prepared by crosslinking of glutaraldehyde are photoluminescent, enable cell adhesion, migration, and proliferation, and also help in long-term survival.¹⁴⁸ Sericin and genipin cross-linked hydrogels have potential applications in the treatment of ischemic stroke and ischemic myocardial infarction,¹⁴⁹ and also facilitate *in vitro* neuron attachment and neuroprotection.¹⁵⁰

5. Application of amyloid-based functional materials

5.1. Amyloid-based materials in therapeutics and health

Amyloid fibrils have distinct features of self-assembly, structural stability and mechanical stiffness, making them promising candidates for healthcare interventions.^{151,152} *In vitro* studies have revealed that amyloid fibrils derived from milk protein and plants are non-toxic towards Caco-2 and Hec-1a cell lines.¹⁵³ Studies also reported an enhancement in the anti-inflammatory activity and bio-adhesivity of skin drugs used for topical treatments utilizing amyloid fibrils derived from milk protein and β -lactoglobulin.¹⁵⁴ Amyloid-based hydrogels are dynamic structures and biocompatible, allowing fine-tuning of drug release in a controlled manner with high efficiency.¹⁵⁵ A lysozyme-based amyloid microgel has been developed with potential drug-carrying and delivery properties through amyloid fibril networks,¹⁵⁶ where ThT, Remazol Brilliant Blue R (RBBR), tetracycline and penicillin V have been demonstrated for their controlled release.¹⁵⁷ Due to the physical cross-linking of amyloid-fibrils, hydrogels are formed that possess water-holding capability, biocompatibility and resilience to chemical and physical alterations while preserving their structural integrity.^{158,159} Injectable hydrogels have gained attention for administering various therapeutic molecules to the target organs owing to their compatibility with hydrophobic and hydrophilic drug molecules, peptides and other therapeutic compounds for targeted delivery as shown in Fig. 3.¹⁵⁹ An example of such a system is the amyloid hydrogel derived from hen egg peptides, where an injectable drug carrier hydrogel synthesized using amyloid fibrils and peptide fragments



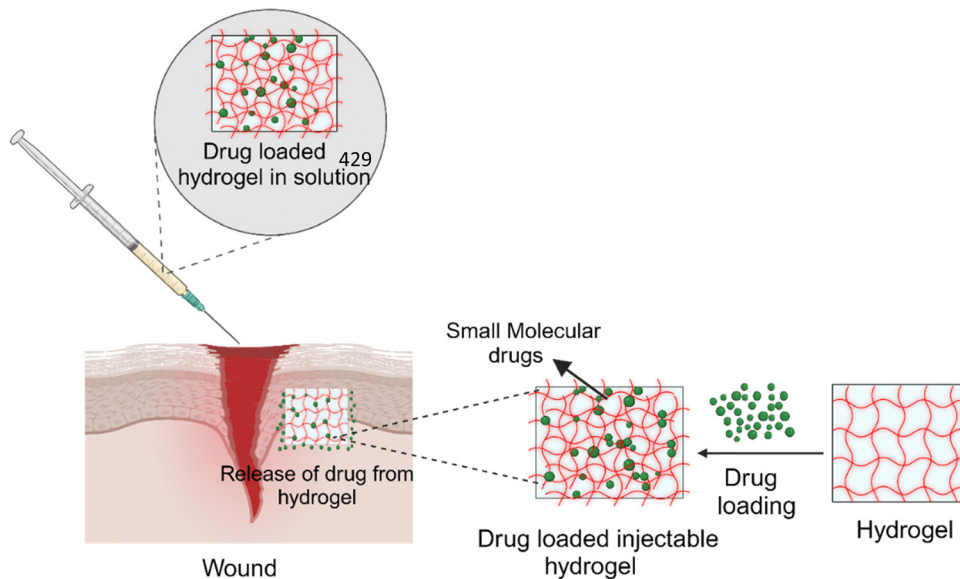


Fig. 3 Schematic representation of amyloid-based hydrogels in drug loading and administration (created with BioRender.com).

obtained from hen egg white lysozyme were used.¹⁵⁹ Doxorubicin, a chemotherapeutic agent, was used as a model compound to show the effectiveness of the developed injectable drug carrier system. The amyloid hydrogel exhibited low cytotoxicity and thixotropic properties.¹⁵⁹

5.2. Amyloid-based materials

Amyloid-based materials are being widely used in medical applications such as tissue repair, scaffolds for cell culture and drug delivery. Recent reports have shown that amyloid fibril scaffolds generated from various proteins enhanced cell growth and proliferation of mammalian cells by interacting with the cell membrane and activating the cellular adhesion machinery.^{152,160,161} It also helps in the transportation of positively charged and hydrophobic therapeutic molecules such as methylene blue and riboflavin.¹⁶² Recently, aloe vera and bovine serum albumin (BSA) have been used for the development of a composite hydrogel. The developed composite hydrogel was tested *in vitro* and *in vivo* for its high efficiency in wound healing. Along with the development of the composite hydrogel, 3D printed conformation and bio-ink have been developed for wound dressing and tissue engineering applications.^{163,164} Also, macroscopic materials are being synthesized using amyloid fibrils, where the robust mechanical characteristics and chemical stability of amyloids have been harnessed to create macroscale materials such as hydrogels, macro-fibres, composites, sensors and matrices.^{152,165} The fibrillar structure of amyloid allows modification for the development of conductive materials that can be used as biodegradable and biocompatible conductive wires.¹⁶⁶

Amyloid fibrils can also be used as catalytic materials by introducing specific sites within the amyloid fibril structure that helps in providing a suitable microenvironment for catalytic reactions.¹⁶⁷ Tyrosine and histidine side chains show esterase activity when they get a favourable microenvironment

of self-assembled cross- β fibrils (HYHYHYHY). Histidine, owing to its potential for non-covalent interactions and hydrolyase activity, plays a key role in esterase activity. Further, by modulating the activity through the tripeptide His-^DPhe-^DPhe, a thermally reversible hydrogel was formed with esterase activity. Also, an octapeptide-based hydrogel having hydrophobic activities of leucine and isoleucine has been reported for enhanced catalytic activities.¹⁶⁵

5.3. Amyloid-based materials in sensors

Self-assembled modified amyloid-based fibrils as nanomaterials have gained attention in various sensor applications.^{168–173} Amyloid-based sensors detect response due to alterations in amyloid conformation induced by the target analytes. These responses can be optical, electrochemical or mechanical and can be used for real-time detection and quantification of the target analytes (Fig. 4(a)). The application of biotin-streptavidin functionalized amyloids has been reported for the development of glucose sensors.¹⁷⁴ Biotinylated whey protein-based amyloid fibrils have been prepared using succinimide-based biotin conjugates. The modified amyloids interacted with streptavidin-modified nanoparticles, quantum dots and glucose oxidase (GOx), where cyclic voltammetry was used to monitor glucose in the samples (Fig. 4(a)).¹⁷⁵ The applications of amyloid fibrils in the development of colorimetric sensors has also been explored, where a colorimetric sensor was developed using an external stimuli-responsive fluorescence reporter molecule, 10,12-pentacosadiynoic acid, with α -synuclein amyloid fibrils.¹⁷⁶ Also, an amyloid fibril-based colorimetric nanosensor has been reported for the detection of chromium(vi) using hen lysosome fibrils and acidified diphenylcarbazide as the colorimetric probe.¹⁷⁷ The hydrogel of α -synuclein amyloid fibrils immobilized with horseradish peroxidase (HRP) enhanced the activity of immobilized HRP by four-fold, suggesting applications of enzyme-amyloid conjugates.¹⁷⁸



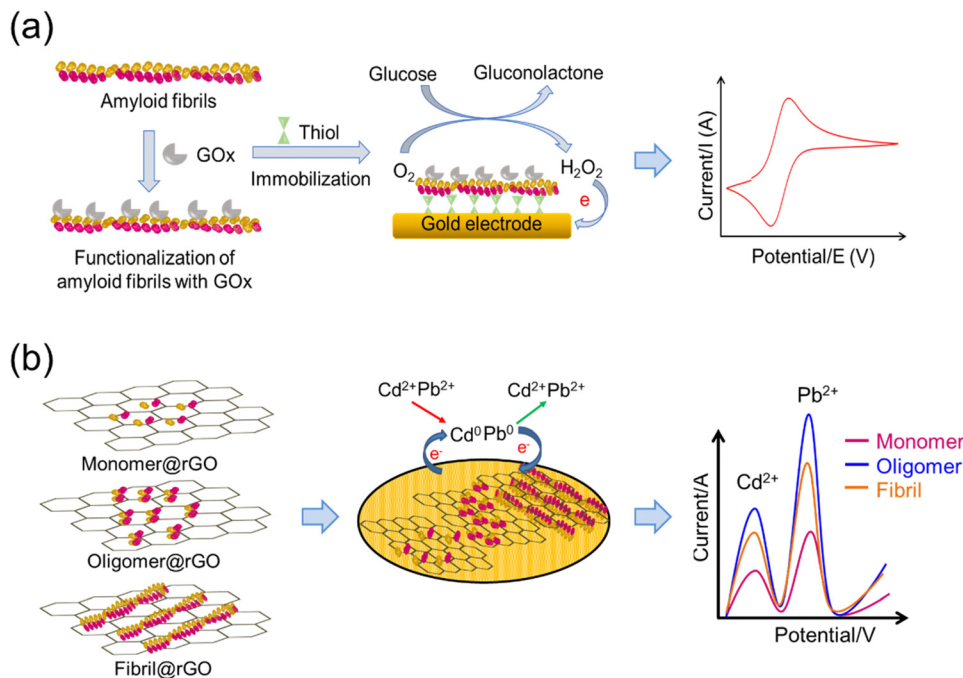


Fig. 4 Application of amyloid-based fibrils in sensors: (a) immobilization of glucose oxidase for the development of the electrochemical glucose biosensor and (b) amyloid-rGO composite (ARGOC) based amperometric sensor for detection of heavy metals.

A gas sensor based on amyloid-reduced graphene oxide (rGO) nanocomposite materials has been developed and applied to e-textile yarns. The rGO/amyloid nanofibril/cotton yarns were used for the detection of NO_2 gas owing to quick detection and high sensitivity and selectivity, which led to an e-textile wearable gas sensor.¹⁷⁹ These studies suggested that amyloid nanofibrils can be used as molecular adhesives in the fabrication of sensitive sensors for personal healthcare or industrial purposes. A nanocomposite material based on an amyloid oligomer-reduced graphene oxide composite (AOrGOC) for developing an amperometric sensor has been reported to detect heavy metals using the stripping voltammetry technique (Fig. 4(b)).^{180,181} The sensor response of the amyloid fibrils generated from different proteins like lysozyme, bovine serum albumin and lactoglobulin has been investigated to determine the reactivity between amyloids and various heavy metal ions.

5.4. Nanofibrils as water purification materials

The problem of water contamination due to heavy metals is a global concern specifically for developing countries, necessitating the development of efficient and eco-friendly purification methods.¹⁸² The use of amyloid-based chelation for water purification has the potential to mitigate health risks associated with metal exposure and contribute to sustainable water treatment strategies.¹⁸³ Due to distinct structural features, such as β -sheet rich conformations and exposed functional groups, amyloid fibrils have been reported for potential application in water purification.^{48,184-188} The ability of amyloids to sequester metals can be explored further for their potential as novel chelating agents and applications in environmental settings for the removal of metals.^{48,184} For effective and specific metal removal, the metal-

binding properties of amyloid-derived peptides can be enhanced through modification of the amino acid sequences or introduction of specific functional groups. Hybrid materials along with amyloid-based chelators are being developed that can be incorporated into porous matrices or membranes for efficient removal of metal contaminants from water.¹⁸⁹ For example, whey protein, a waste by-product from the cheese-processing industry, was used for preparing a functional scaffold for the β -lactoglobulin amyloid fibril/ZIF-8 (zeolitic imidazolate framework) based metal-organic framework for water purification and wastewater treatment.^{183,190} Owing to their hierarchical porous structure, the resultant aerogels based on amyloid fibrils with the ZIF-8 hybrid can successfully eliminate various types of heavy metals from water. Importantly, through adsorption-regeneration cycles, the hybrid aerogels can efficiently remove Hg^{2+} and Pb^{2+} from water.¹⁹⁰ The reusable and sustainable materials from soy protein-based amyloids can also be used for manufacturing carbon aerogels having high removal efficacy for metals.¹⁹¹ The amyloid fibrils and their hybrids with modified properties can be a promising choice for efficient wastewater treatment.^{105,191}

Challenges and future directions. The demand for amyloid fibril-based biomaterials is gaining importance in various applications owing to their exceptional self-assembling capability and stability in diverse matrices. The attractive properties and utility of amyloid fibril-based biomaterials make them suitable candidates for application in therapeutic, health and materials research.^{192,193} For the successful application of amyloid fibril-based biomaterials, it is necessary to develop methods for preparation of controlled amyloid fibrils and the identification of various protein species or conformers other than amyloids. However, it is still challenging to control the



final homogenous amyloid structures precisely by steering the design at the molecular level, necessitating technological manipulations through structural fine-tuning to assure accurate performance of the composite materials.¹⁹⁴ The toxic oligomeric species are the starting nuclei for fibril formation and are produced during the fibrillization process, where these oligomeric species might have prion-like properties that can result in detrimental consequences upon long-term exposure. Thus, an in-depth understanding of the safer use of amyloid fibril-based biomaterials can provide further advancements for acceptability of such materials.

Focused interventions to improve the elasticity, permeability and strength of amyloid fibril-based biomaterials by incorporating plasticizers, cross-linkers or polymers can further expand the field of their application.⁶⁸ The major applications of amyloid-based biomaterials are in the fields of healthcare and sensing, where amyloid-based polymeric membranes are produced at an industrial scale for commercialization.¹⁵² It is essential to evaluate their stability and functionality to ensure effectiveness for safe use in patient-specific therapies by designing and incorporating specific drugs.¹⁹⁵ Balanced production costs, optimal performance and safety considerations are critical to achieve sustainable and widely acceptable amyloid fibril-based biomaterials, as compared to conventionally used biomaterials.¹⁹⁶ The emerging advances in the preparation and characterization of biocompatible and biodegradable amyloid fibril-based materials will open up new avenues for future applications of sustainable materials in healthcare and sensing.

Author contributions

Smriti Singh Yadav: writing of the original draft, conceptualization, visualization, writing – review & editing. Prabeen Kumar Padhy: visualization, writing – review & editing. Ashish Kumar Singh: visualization, writing – review & editing. Supriya Sharma: writing – review and editing. Tanu: writing – review & editing. Siraj Fatima: writing – review & editing. Anurag Sinha: writing – review & editing. Ramsha Tariq: writing – review & editing. Varsha: writing – review & editing. Sandeep Kumar Sharma: conceptualization, supervision, project administration, funding acquisition, writing – review & editing. Smriti Priya: conceptualization, supervision, project administration, funding acquisition, writing – review & editing.

Conflicts of interest

The authors declare no conflicts of interest.

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