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2023-02

Sedighi , M , Mahmoudi , Z , Ghasempour , A , Shakibaie , M , Ghasemi , F , Akbari , M , Abbaszadeh , S , Mostafavi , E , Santos , H A & Shahbazi , M-A 2023 , ' Nanostructured multifunctional stimuli-responsive glycopolypeptide-based copolymers for biomedical applications ' , Journal of Controlled Release , vol. 354 , pp. 128-145 . https://doi.org/10.1016/j.jconrel.2022.12.058

http://hdl.handle.net/10138/356338
https://doi.org/10.1016/j.jconrel.2022.12.058

Journal of Controlled Release 354 (2023) 128–145

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Nanostructured multifunctional stimuli-responsive glycopolypeptide-based copolymers for biomedical applications

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ARTICLE INFO

Keywords:
Glycopolypeptide
Nanocarriers
Drug delivery
Regenerative medicine
Self-assembly

ABSTRACT

Inspired by natural resources, such as peptides and carbohydrates, glycopolypeptide biopolymer has recently emerged as a new form of biopolymer being recruited in various biomedical applications. Glycopolypeptides with well-defined secondary structures and pendant glycosides on the polypeptide backbone have sparked lots of research interest and they have an innate ability to self-assemble in diverse structures. The nanostructures of glycopolypeptides have also opened up new perspectives in biomedical applications due to their stable three-dimensional structures, high drug loading efficiency, excellent biocompatibility, and biodegradability. Although the development of glycopolypeptide-based nanocarriers is well-studied, their clinical translation is still limited. The present review highlights the preparation and characterization strategies related to glycopolypeptides-based copolymers, followed by a comprehensive discussion on their biomedical applications with a specific focus on drug delivery by various stimuli-responsive (*e.g.,* pH, redox, conduction, and sugar) nanostructures, as well as their beneficial usage in diagnosis and regenerative medicine.

1. Introduction

Nature, which applies fundamental building blocks including saccharides, peptides, and nucleobases to create biochemical macromolecules, has inspired the creation of new classes of self-assembled molecular conjugates, to form various nanostructures for therapy, diagnosis, or monitoring of diseases within the human body [1–5]. Among these, glycopolypeptides with well-defined secondary structures have attracted great interest owing to their potential biomedical applications, including drug delivery, tissue engineering, and imaging. These are stemmed from the complex intervention ability of carbohydrates in various biomolecular processes, such as cancerous cell metastasis, adhesion, cell-cell recognition, pathogen invasion, toxin mediation, and many other events mediated by glycoproteins [6–11]. It is proved that protein lifetime and structure can be affected by the glycosylation process due to the protection against proteases, and the introduction of charge or affecting folding, respectively [12]. Although the term glycopeptide is usually used to address polyamino acids/

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https://doi.org/10.1016/j.jconrel.2022.12.058
Received 3 June 2022; Received in revised form 22 December 2022; Accepted 28 December 2022
Available online 6 January 2023

polypeptides linking with sugar moieties, there are other structures, including chemical conjugates of peptides with natural saccharides or glycopolymers with different linear or dendritic topologies, which can also be referred to as glycopolypeptides [13]. They can mimic the function of natural glycoproteins involved in a large number of biological processes, including inflammatory reactions, protein folding, recognition events, signal transmission, and many more [13–15]. Having a similar structure to natural glycoproteins has resulted in the synthetic glycopeptides enjoying biological properties similar to these compounds. They also present the advantages of facile preparation procedures and more regular structures, making them a reliable candidate for scale-up production [16,17]. These structures are a novel class of small molecules with the ability to form reversible supramolecular gels in solvents with different linear or branched peptide molecules *via* van der Waals interactions, non-covalent hydrogen bonding, and π-π stacking [18,19]. Glycopolypeptides consist of pendant sugar on a polypeptide backbone that can mimic proteoglycan composition and fold into secondary structures such as α-helix with the ordered presentation of sugar moieties on the surface. Integration of sugar moieties to the termini or side chains of the polypeptides can provide some new features such as specific biomolecular recognition, as well as cell adhesion characteristics, or improve the existing characteristics, such as biodegradability and the ability of self-assembly [20]. It has been demonstrated that the supramolecular structures of glycopolypeptides as micelles or vesicles have revealed better mimics of glycoproteins on the cell surface than the individual glycopolypeptides [7,21,22].

Glycopolypeptides can self-assemble into macromolecular ordered and stable assemblies, and their geometry is dependent on some parameters, including chain length, the situation of sugar residue, and backbone conformation (helicity content) [6]. These structures give rise to applicable biomimetic conformations with unique medicinal values different from synthetic polymers [22]. In fact, glycopolypeptides-based nanostructures can benefit from different characteristics of both carbohydrates and polypeptides in biomedical applications. Here, we aim to review glycopolypeptide structure, formation mechanisms, and characterization methods. Their applications are also discussed as stimuli-responsive assemblies in drug delivery, imaging, and regenerative medicine.

2. Fabrication of glycopeptides-based supramolecular structures and copolymers

Block copolymers have shown a great capability to produce a large variety of nanostructures applied in therapeutic and diagnostic applications [23]. The self-assembled nanostructures developed by numerous approaches have attracted great attention, especially in drug delivery systems, to provide a safe hydrophobic harborage for small hydrophobic molecules and to protect the core from an aqueous microenvironment with the hydrophilic shell [24–26]. Among them, biocompatible, biodegradable, safe, and non-immunogenic natural blocks, including polysaccharides and polypeptides, have shown great potential in nano-biomedical applications. Polysaccharides with many functional groups are prone to chemical modification to form amphiphilic structures [27].

Glycopeptides copolymer hydrogels are a new type of nanostructures to develop more successful drug delivery vesicles with effective carrier properties. It is shown that the balance between the dissolution and precipitation of amphiphilic glycopeptides in aquatic buffer tends to prepare supramolecular hydrogels [1]. In recent years, peptide-based hydrogels synthesized by supramolecular self-assembly procedure have appeared as an important class of biomimetic materials because they are intrinsically biocompatible and biodegradable, and morphologically similar to fibrous proteins in extracellular matrix (ECM) [3,18,28–32]. Since the hydrogel derived from the natural component of ECM possesses some drawbacks such as difficult purification and probability of immunogenicity or pathogen transmission, it is needed to mimic desirable characteristics from ECM with the fabrication of hydrogels by synthetic polymers like glycopeptides [33]. The advantages of synthetic *co*-polypeptides include precise control over chain length, biocompatibility and feasible surface functionalization, minimum toxicity, and the absence of immunogenicity [34,35], which makes them perfect building blocks to synthesize supramolecular nanostructures.

Xu *et al.* have shown the formation of core-shell micelles by an amphiphilic polypeptide copolymer, which was capable of carrying different drugs due to their flexible chemical structures and the availability of functional groups [36]. Polypeptide-based polymersomes similar to liposomes can encapsulate both hydrophilic and hydrophobic small molecules in the aqueous interior and hydrophobic part of the membrane, respectively, and protect the drugs from *in vivo* and *in vitro* environment for the targeted and controlled release [37,38]. It is proven that different morphologies of the self-assembled glycopolypeptides are affected and controlled by the solvents, the order of solvent addition, and the conformation of hydrophilic glycopolypeptides [38]. For instance, the self-assembly of amphiphilic glycopeptide dendrimers resulted in different nanostructures including worm-like micelles, glycospheres, and fibers [39]. It was proven that the subtlest change in peptide sequence isomerism significantly influences the designed structure of glycopeptide by self-assembly. As an example, switching the position of the Ala residue under the same condition created different self-assembled morphologies of azobenzene-glycopeptide, including nanotwists, nanoribbons, and nanofibers [15]. Furthermore, the saccharide moiety characteristics (the hydrophobicity and steric demands) were reported to be decisive in the self-assembly and aggregation process [40].

Glycomics is considered to be one of the main biological challenges due to the lack of a biological template to have a controlled polysaccharide biosynthesis. Carbohydrate moieties have attracted more research interest for studying the multivalent carbohydrate-protein (called lectin) or -biological targets (cells-viruses *etc.*) interaction as well as their use in tissue engineering and drug delivery [13]. Glycoproteins are often formed by the attachment of carbohydrates to proteins through an enzymatic reaction. For glycopeptides to be utilized as drug carriers, it would be superior if they could be gathered into supramolecular self-assemblies with the tunable and spatial arrangement of carbohydrate moieties on their surfaces [6,13,14,16,41,42].

The glycosylation process provides the possibility of active targeting with minimum side effects by which other characteristics, such as the bio-stability, solubility, and bio-adhesion of glycopeptides-derived nanostructures have also been improved [43,44]. In particular, multivalence phenomena in glycopolypeptides demonstrated higher affinity to carbohydrate-binding receptors in comparison to individual carbohydrate molecules. It has been proved that the carbohydrate-lectin interactions are quite weak. To circumvent this limitation, they can be improved *via* a multivalent procedure of glycopolypeptides making self-assembled nanostructures with better biological binding [45]. According to previous research, glycopolypeptide architectures and the amount and type of carbohydrate units can determine the efficiency and kinetics of receptor binding [38]. Despite the importance of glycopeptides and glycoproteins in different physiological activities, there are no more reports from a structural and functional point of view due to the difficulties in their extraction and purification [13]. Therefore, synthetic copolymers can be promising to stimulate their physiological or biological properties.

Surfing previously published studies, researchers illustrate that there are various methods to prepare glycopolypeptides [16,36,46,47]. Bioactive peptides are found in different sources, and they can be produced from extracted proteins by enzymatic procedures [48–51]. In the case of amphiphilic peptide copolymers, the self-assembly process is intrinsically the main technique to generate well-defined and high-ordered nanostructures [16] affected by three energetic factors, including interfacial tension, corona chain crowding, and core chain stretching. The self-assembled polypeptide copolymers have attracted

tremendous interest, especially in the fabrication of stimuli-responsive nanostructures (pH, salt, temperature, and so on) that possess the ability to change their secondary structure in response to external and internal stimuli. This class of copolymers called "pepsome", are promising nanostructures in biomedical applications introducing many advantages such as biocompatibility and biodegradability [37,52]. The self-assembly mechanism is introducing a versatile way to form micellar or vesicular nanostructures from their precursors and the cross-linking approach enhances the stability of micelles and prolongs their blood circulation. It also provides the possibility to apply several different smart groups with responsiveness to various triggers [53].

There are two classifications for the development of glycopolypeptides, including the direct synthetic procedure by which sugars are conjugated to monomers during the polymerization process; and the post-synthetic grafting procedure in which sugars are grafted into the side chains of polypeptides through coupling reactions like click chemistry (Fig. 1A) [54]. The self-assembly of glycopolypeptides in supra-structures can be controlled by changing some physicochemical properties consisting of molecular weight, chemical composition, surface chemistry, and functional groups, as well as the construction of the copolymers [7]. For instance, bioactive glycopolypeptide polyion complexes were efficiently synthesized through electrostatic interactions by self-assembly of glycopolypeptide-based positively charged polyamino acids (catiomer) with polyethyleneglycol (PEG)-based negatively charged polyamino acids (aniomer) without the utilization of organic solvents in an aqueous medium [7]. Meantime, amphiphilic structures are so reminiscent of biological assemblies and promising in studies on different glycopolypeptide-based supra-molecular structures. In this regard, the self-assembly of amphiphilic glycopolypeptides with carbohydrate moieties on their surface is essential for receptor recognition and cell-cell interactions [53].

Synthetic polypeptides have attracted more attention in scientific communities, which can order in different types of secondary conformations, including helices, sheets, and turns relying on their amino acid composition [14]. The most favorite route to synthesize polypeptides is the ring-opening polymerization (ROP) of glycosylated α-amino acid N-carboxyanhydrides (NCAs) monomers with simultaneously possessing the activated C═O group and the protected amino group ROP [37], as a renowned technique, is used to polymerize cyclic esters, carbonates, or anhydrides, introduced around 1930 by Hasan *et al.* [55]. High sensitivity to moisture and low stability are enumerated as the disadvantages of structures synthesized by this method. In addition, there are still some challenges to obtaining desired purity in the glycosylated NCA monomers to control their polymerization. Recently, there has been tremendous interest in the preparation of α-amino acid NCA containing reactive functional groups, such as alkyne, alkene, and azido functional groups with the ability to undergo highly effective "click" reactions, such as thiol–ene, thiol–yne reactions, and azide-alkyne cycloadditions, to prepare glycopolypeptides [56]. Click chemistry reaction has also been proven as a highly efficient approach to obtaining glycopeptides. As an example, an amphiphilic glycopolypeptide analog, lactosylated pullulan-graft arginine dendrons (LP-g-G3P) was fabricated using azide-alkyne click chemistry between hydrophilic lactosylated pullulan and hydrophobic generation 3 arginine dendrons [57].

In an attempt to overcome some of the limitations, Kapetanakis and Heise presented the synthesis of a linear glycopolypeptide *via* ROP and click chemistry with combined lectin recognition and well-defined thermo-responsive functionality [58]. It was demonstrated that the hydrophilicity and the cloud point temperature (T_{cp}) could be modified by changing the ratios of azide functional galactose and 1-azido-2-(2-methoxyethoxy) ethane on the glycopolypeptide backbone. In a related example, a self-assembled amphiphilic homoglycopolypeptide with an amphiphilic carbohydrate on its side chain was synthesized using a combination of NCA polymerization and click chemistry. Rod-like copolymers were prone to form multimicellar spherical aggregates in an aqueous solution triggered by hydrophobic interactions of the aliphatic chains characterized by Dynamic Light Scattering (DLS), Transmission Electron Microscopy (TEM), and Atomic Force Microscopy

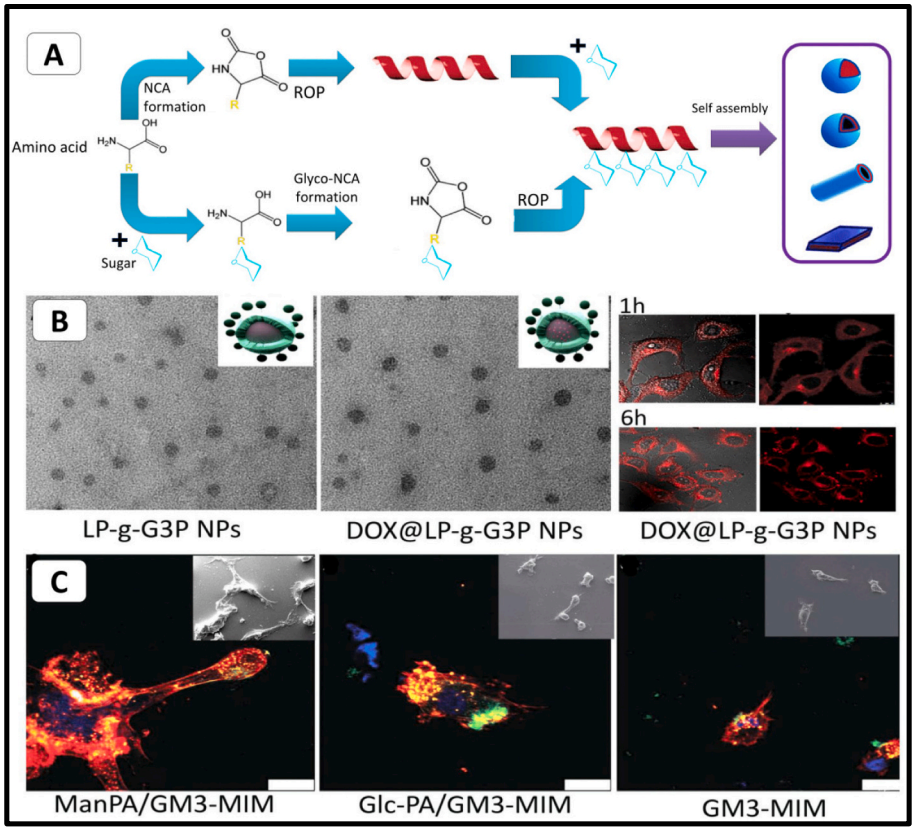

Fig. 1. (A) Schematic representation of glycopolypeptides synthesis pathways. (top) Synthesis of polypeptide and subsequent glycosylation; and (down) ring-opening polymerization (ROP) of glycol-N-carboxyanhydrides (glycol-NCA). The as-prepared glycopolypeptides can be self-assembled in different supramolecular structures as shown in the box. The core-shell nanocarriers are synthesized by self-assembly of lactosylated pullulan-graft-arginine dendrons (LP-g-G3P). **(B)** Transmission electron microscopy (TEM) images of nanocarriers before and after doxorubicin (DOX) loading to show size distribution and morphology of structures as well as fluorescence microscopy images of DOX-loaded LP-g-G3P after 1 and 6 h incubation on HepG2. Reproduced with permission from ref. [57]; Copyright 2017, Elsevier. **(C)** Dendritic uptake studies of GM3-MIM and morphological changes treated by Man-PA/GM3-MIM, Glc-PA/GM3-MIM, and only GM3-MIM for 24 h (scale bars are 20 μm). The morphological studies using fluorescent imaging of each treatment were shown as inset images (scale bars are 30 μm). Reproduced with permission from ref. [59]; Copyright 2017, American Chemical Society.

(AFM). These structures had both hydrophobic and hydrophilic domains confirmed by the incorporation of hydrophobic (Nile red) and hydrophilic (calcein) dyes visualized by confocal microscopy. Using this methodology, it is possible to synthesize amphiphilic random polypeptides including 10% and 20% α-D-mannose along with amphiphilic glucose unit and hydrophobic alkyl chain and assembled into spherical nanostructures similar to the homoglycopolypeptide. These nanostructures were found to interact with the lectin Concanavalin A (Con-A), and their hydrophobic and hydrophilic domains seem to provide a reservoir for different drugs for targeted drug delivery [54]. This group previously reported that tuning the hydrophobicity and hydrophilicity could affect self-assembled structures. Multiple topologies, including nanorods, micelles, and organogels were prepared for the first time from glycopeptides-dendron conjugates, which are synthesized by self-assembly dependent on glycopolypeptide chain length, dendron generation (the size of dendron), the type of sugar residue, and secondary structure of polypeptide backbone [6]. Synthesized glycopeptides by NCA polymerization were attached to hydrophobic dendrons by click chemistry connected through a PEG linker to prepare amphiphilic anisotropic polymer.

3. Glycopolypeptide-based drug delivery systems

Drug delivery to a specific target site has endured systemic side effects derived from fast or premature release in non-target sites and/or slow-release reducing the drug efficacy and increasing drug resistance. To overcome these drawbacks, the design and fabrication of multifunctional smart polypeptide-based polymers are highly motivated for smart drug delivery under controlled conditions [12,37]. Targeting delivery of therapeutics is one of the most important fields of biomedical applications that can be achieved by active or passive pathways. For this purpose, modification of a ligand to the nanoparticles specified for a receptor on the target cell is the widely used approach. Glycopeptide-based copolymers with specific oligosaccharide sequences are known as targeting nanocarriers in biomedical applications [60]. In this regard, an amphiphilic glycopeptide consisting of lactobionolactone as a target ligand for hepatocarcinoma cancer cells, HepG2 cell line, and poly(L-lysine) was reported by Huang *et al.* [61], which are self-assembled in acidic and neutral conditions to create vesicles. It is previously reported that target saccharide ligands can enhance the efficiency of drug delivery to desired target cells with attachment to polymers. The functionality of glycopeptides vesicles stabilized *via* genipin cross-link was evaluated as a targeted carrier loaded by a model hydrophilic small molecule, doxorubicin (DOX), and affected on HepG2 cell line. The regulation of the amphiphilic nature and chain conformation of the designed glycopolypeptide was revealed by the experimental data. The stable structure, sensitivity to pH changes, and cell-targeting ability of the glycopolypeptide make it interesting to employ for DOX encapsulation with 45 wt% loading degree, noticeable pH-responsive behavior, and cytotoxic on HepG2 in comparison to free DOX.

Amphiphilic chitosan(CHI)-based glycopeptide was synthesized through hydrophilic carboxylmethylation and ROP of NCA using short-chain CHI and self-assembly to form vesicles in water which can be prepared by double emulsion method in the size range of 140 to 250 nm. The applicability of as-prepared vesicles as encapsulants or nanocarriers was examined with encapsulation of Fluorescein isothiocyanate (FITC)-dextran (loading content of 20%) to achieve the sustained *in vitro* release over two weeks. To improve the stability of the designed nanostructure, amine-functionalized PEG was further used to complex with the negatively charged glycopeptides vesicles at pH 7.4. According to this study, these biodegradable graft copolymers were introduced as a great nanocarrier for protein encapsulation and drug delivery [62].

Pati *et al.* demonstrated that glycopolypeptide-based vesicles (≤100 nm) can enter *via* receptor-mediated endocytosis into MCF-7/MDA-MB-231 breast cancer cells. They offered the possibility of receptor-mediated drug delivery to cancer cells by these nanostructures with recognition of an overexpressed mannose receptor C-type 2 (MRC2) receptor [63]. An amphiphilic polysaccharide-based graft copolymer was generated with a straightforward synthesis method that covalently attached the hydrophobic polypeptides to the hydrophilic polysaccharide [57]. It was shown that this structure (lactosylated pullulan-graft-arginine dendrons) was able to self-assemble spontaneously in water to produce novel core-shell nanocarriers used in anticancer therapy. Since both lactose and pullulan could actively target asialoglycoprotein receptor (ASGPR) abundantly expressed on hepatocytes and hepatoma cells of the liver, this structure could be used as liver targeted drug delivery system to physically entrap 16.89 ± 2.41% DOX in the hydrophobic core through π-π stacking, hydrophobic and hydrogen interactions with desirable stability and sustained release affected by pH changes. The *in vitro* cytotoxicity assessment of the designed structure was performed on HepG2 and Mouse embryonic fibroblasts cells (NIH3T3) as ASGPRs and RCA_{120} positive and negative cells, respectively, and illustrated the higher dose- and time-dependent toxicity in HepG2 compared to NIH3T3 due to the higher affinity to HepG2 cells (Fig. 1B).

The administration of nanostructures for antigen delivery would be advantageous in comparison to using free soluble antigens due to simulated cellular uptake and targeted delivery. The amphiphilic mannosylated peptide nanofibers prepared by self-assembly were designed to load by an immunogenic melanoma-associated GM3 lactone antigen and applied in the activation and maturation of dendritic cells *via* targeting DC-SIGN receptors. The results (Fig. 1C) confirmed that the treatment of dendritic cells with nanocarriers increased significantly antigen internalization and surface expression of the target markers such as CD86, CD83, and HLA-DR, and the nanocarriers based on mannosylated polypeptide are promising candidates for targeted antigen delivery [59].

Hyaluronan, a natural polysaccharide, has a relative affinity to CD44 receptors up-regulated on several cancerous cells, and it can be a promising choice to design biodegradable glycopolypeptides assemblies such as hyaluronan-b-poly(γ-benzyl-L-glutamate) copolymer. This nanostructure efficiently targeted lung tumor cells with overexpressed CD44 receptors on their surface. It was also used for the co-delivery of vorinostat and gefitinib in lung cancer cells [64,65].

MRP@DOX is a co-assembled glycopeptide nanotransforrs consisting of glycopeptide, cationic peptide, and DOX with the ability to induce transformation by legumain cleavage. It was reported recently by Kong *et al.* [66] to apply as an effective and safe drug nanocarrier (about 150 nm with a surface charge of +14.2 mV) to the fundus. It was demonstrated that this nanocarrier has the potential to highly penetrate through the ocular surface and target M2 macrophages in the fundus. Then phagocytosis was mediated by the M2 macrophage-specific mannose receptor and legumain could induce the transformation of the nanoparticles to nanofibers contributing to a 44.7% DOX retention at 24 h in cells. *In vivo* experiments on the mouse OIR model indicated the complete restoration of the physiological angiogenesis and reduction of pathological neovascularization by MRP@DOX.

Another interesting use of glycopolypeptides as a drug carrier was reported by Li *et al.* [67]. Aiming to develop a DOX carrier for targeting hepatic carcinoma, a glycopolypeptide block of poly(LP-*co*-L-lysine) (P(LP_{10}-*co*-LL_{11}) was modified with lactose (Lac) for specific targeting of hepatic cells and self-assembled in micelles. Lac moiety served as a ligand for the ASGP receptors and resulted in entering cells by receptor-mediated endocytosis. This designed nanocarrier demonstrated admirable characteristics in comparison to free drugs, including greater accumulation in the tumor site, improved DOX loading content due to the π-πstacking effect, and targeted delivery of DOX to hepatic cells by Lac-ASGP R interaction. Moreover, *in vivo* biodistribution experiment revealed that less DOX-nanocarrier was found in the heart and kidney compared to free DOX, and a great deal of nanocarrier was accumulated at tumor sites rather than other organs.

3.1. Stimuli-responsive glycopolypeptide-based drug delivery systems

Stimuli-responsive nanostructures are defined as intelligent systems such as liposomes, dendrimers, and micelles that enable them to form and deform based on self-assembly which is a consequence of the conformational change in their polymeric chain. There have been enormous efforts to use conventional hydrophobic blocks including polycarbonates, aliphatic esters, and aliphatic chains as well as hydrophilic blocks such as poly (ethylene glycol) (PEG; or PEO) as stimuli-responsive building blocks. Nevertheless, polypeptides which are composed of repeating amino acids and present 3D structures, such as α-helices and β-sheets, still seem to be superior candidates due to their purely physical forces, enabling them to achieve more functionality *via* self-organizing into various lengths from a few angstroms up to a few microns. Polypeptides have the advantage of being composed of much simpler components than natural proteins but do not present the complex biological activity of a protein, resulting in the formation of stimuli-responsive nanostructures with conformational alteration without loss of functionality in response to different external or internal stimuli. As carbohydrates play an important role in cell-cell recognition, and by considering their overexpression on cancer cells, conjugating saccharide-based chemical moieties to the stimuli-responsive polypeptide nanostructures recently appeared as a promising way to design ligand-directed, stimuli-responsive nanostructures [68]. Glycopolypeptide-based nanostructures have opened up new perspectives in biomedical applications due to their stable three-dimensional (3D) structures, high drug loading efficiency, excellent biocompatibility, biodegradability, tunable internal and external features during polymerization, and importantly feasible modification routes to prepare stimuli-responsive hydrogels [69,70]. They have grabbed more attention to design responsive nanocarriers due to their reversible conformational alteration without loss of functionality in response to different external or internal stimuli and they can be applied to improve the limitations of enhanced permeability and retention (EPR) effects [71,72]. Here, stimuli-responsive glycopeptide-based drug delivery systems have been reviewed for pH, redox, temperature, and sugar-responsive as well as multi-responsive carriers.

3.1.1. pH-responsive glycopeptide-based carriers

Polypeptide-based block or graft copolymers that can self-assemble to form various structures such as micelles are considered as a promising candidate for application in stimuli-responsive drug delivery systems. The conformational adaptation such as α helices or β-sheets would influence the amphiphilic nature and morphology of the assemblies. While the normal physiological pH of blood stream is 7.4, the high amounts of lactic acid produced during the glycolysis procedure in cancer cells lead to the formation of a hypoxic microenvironment in solid tumors cytoplasm with pH around 6.5–7.2 which even reaches 4.5–6.8 in endolysosomes owing to the vacuolar proton ATPase-mediated influx. One way to design pH-responsive peptide-based nanostructures is to introduce ionizable groups in polypeptides sequence. When accumulated in tumor tissues, the low pH of the tumor microenvironment leads to pH-responsive nanostructures disassembly due to their higher pKa compared to intracellular pH, resulting in the controlled release of their cargo.

Poly(L-histidine) (Phis), a polypeptide with hydrophobic and pH-responsive nature, has recently grabbed huge attention in studies of pH-responsive actuators [73]. Benefited from Phis with pH-responsive nature and by incorporation of hyaluronic acid (HA), a polysaccharide with affinity to CD44 receptors, targeted, pH-responsive micelles without any additional linkages was developed. It was demonstrated that the formed micelles can be destabilized in lysosomes by pH-induced protonation of the imidazole ring, followed by lysosomal membrane disruption and release of the incorporated drug into the cytoplasm [74,75]. *In vitro* drug release studies confirmed pH-dependent DOX release from HA-PHis micelles. MTT assay against MCF-7 cells (overexpressed CD44 receptors) showed that DOX-loaded micelles with a low PHis DS were highly cytotoxic [75].

Acid-labile bond incorporation in polypeptide sequence is also another way to design pH-responsive polypeptide nanostructures. The tertiary amine and the Schiff base are typical acid-sensitive materials to fabricate pH-sensitive nanomaterials. Related to this, Chen *et al.* partially substituted alkyl chains including hexanoyl, decanoyl, and tetradecanoyl groups into poly (L lysine) (PLL), to remit self-assembly ability, leading to the formation of vesicles-formed nanostructures. The results indicated that the design of chain conformations and nanovesicles sizes and subsequently their membrane permeability could be tuned *via* the degree of substitution and alkyl chain length. The carbohydrate−lectin binding measurements were first performed to evaluate the bioactivity of the saccharide-conjugated vesicles. Conjugation with lactobionolactone stabilized by genipin cross-linking led to the efficiently entering of the LacPLD4 vesicles to HepG2 liver cells using galactose-specific receptors [76].

Wang *et al.* also succeeded in the synthesis of self-assemble lactobionolactone-conjugated poly (L-glutamic acid)-b-poly (*L*-phenylalanine) amphiphilic block *co*-polypeptides (Lac-PGA-b-PPhe). These amphiphilic glycopolypeptides self-assembled to form nanomicelles loaded with DOX. The results of selective lectin binding analysis confirmed the galactose-specific receptor binding. Drug release experiments demonstrated that DOX was released faster from saccharide-conjugated micelles under acidic pH in comparison to neutral conditions. The higher cytotoxicity of DOX-loaded saccharide-conjugated micelles toward HepG2 tumor cells compared to free DOX and saccharide-free DOX-loaded micelles revealed saccharide-conjugated micelles' efficient binding to the cells through specific recognition [77].

Recently, the development of pH-responsive di/tri-block copolymers of poly N-(2-hydroxypropyl)methacrylamide–b-polyhistidine/poly leucine (pHPMA-pHis/pLeu) was reported by Abbasi *et al.* [78] synthesized *via* sequential reversible addition-fragmentation chain-transfer (RAFT) polymerization and ROP. The DLS results revealed the self-assembly capability of copolymers into nano-micelles dependent on the pH of media, proved by AFM. The prepared nano-micelles were efficiently loaded by Paclitaxel (PTX, 75% loading efficiency) affected by *co*-polypeptide composition, and the PTX-loaded micelle constructed by tri-block copolymer 5–5-3 (HPMA:His:Leu ratio of 35:32:23) revealed the higher release in endosomal pH *in vitro*. As a result, the optimum PTX-loaded micelles were introduced as a well-defined pH-sensitive nanocarrier for solid tumors (Fig. 2A).

Ding *et al.* reported a series of galactopeptides synthesized *via* ROP of NCA, deprotection of the benzyl group. Targeted delivery of fluorescent glycopeptide is possible due to the interaction of galactose ligand with ASGPR on HepG2 cells. The two-stage physical interactions were applied to fabricate micellar nanoparticles by cooperative self-assembly of galactopeptide, and DOX showed pH-triggered release in cancerous acidic microenvironments and significantly promoted the receptor-mediated endocytosis into HepG2 cells with recognition of ASGPR by galactose ligands [79].

It is assumed that full-branched synthetic glycopeptide nanostructure can manifest a stronger potential in terms of pH responsiveness and efficient drug delivery compared to simple linear or linear-branched building blocks structures which usually leads to the formation of zero-dimensional structures such as micelles and vesicles [9,80]. In this regard, Bi *et al.*, fabricated a library of dynamic glycopeptide amphiphilic glycopeptide dendrimers using 14 hydrophilic saccharides (located as dendrons), and 7 hydrophobic peptides (located as arms), to be conjugated to β-cyclodextrin (as the core of dendrimers) *via* an acylhydrazone dynamic covalent bond. Based on the TEM results, dendrimer glycopeptide could form various self-assembly morphologies including glycospheres, worm-like micelles, and fibers based on their sugar to amino acids units' ratio in an aqueous solution. The dye-loaded glycopeptide dendrimers manifested a pH-controllable release behavior around the physiological and acidic tumor environment [39].

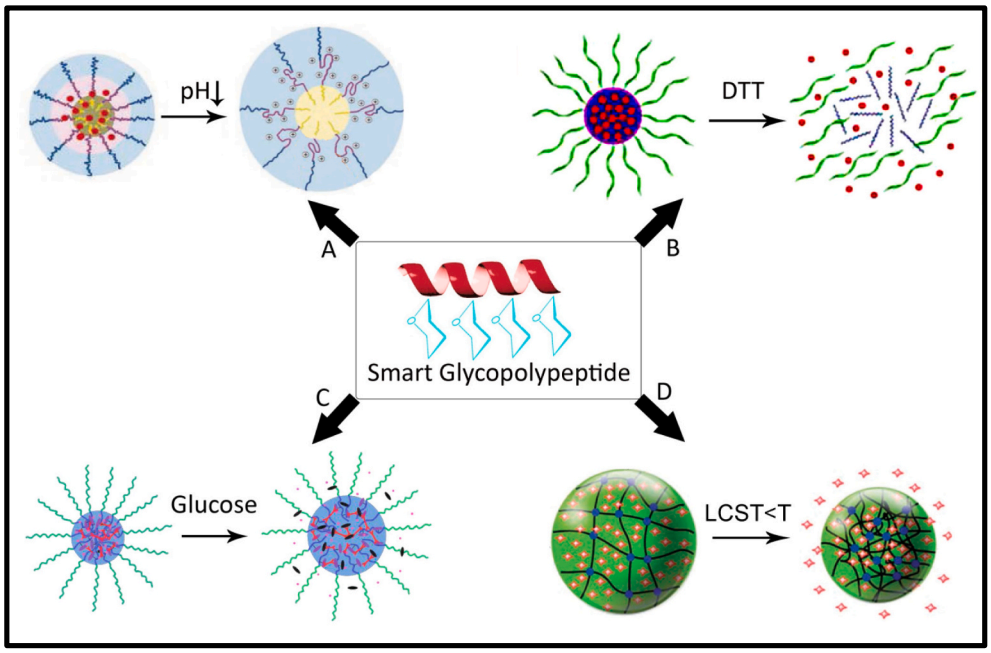

Fig. 2. Schematic illustration of stimuli-responsive glycopolypeptide-based drug delivery systems. **(A)** The pH-responsive carrier is synthesized *via* self-assembly of hybrid copolymers; drug release is induced at specific pH after protonation or charge reversal of the glycopeptide leading to structural transformation or disassembly. The material swelling and/or dissolution and ionization disrupt the hydrophobic core structure to release therapeutics in pH-sensitive drug release. **(B)** The redox-responsive carrier with the structural transition of micelles in response to the redox microenvironment; After internalization by endocytosis, the redox-responsive agent broke down under the presence of high redox potential and the nanostructure will be disassembled. The reduction of disulfide bonds results in the cleavage of the amphiphilic monomers and disassembly. **(C)** Sugar-responsive carrier for insulin release in response to glucose (black ellipse) concentration. Glucose swells the sugar-sensitive hydrogel allowing for the release of loaded therapeutic directly. These carriers induce insulin release by glucose-stimulated swelling/contraction, pore size change, dissolution, polymer deterioration, and charge reversal. **(D)** Temperature-sensitive carrier and release of the payload during LCST behavior. In a temperature higher than the transitional temperature, the solubility and volumetric properties of the carrier are decreased by which the release of the payload will be achieved.

3.1.2. Redox-responsive carriers

Taking advantage of the reducing intracellular microenvironment, an amphiphilic glycopeptide conjugate was developed for the first time as a redox-responsive carrier containing the disulfide bond [81]. It was obtained by the ROP of benzyl glutamate NCA in the presence of (propargyl carbamate) ethyldithio ethylamine and click reaction with α-azido dextran (Fig. 2B). It is shown that the micelles could successfully encapsulate the most widely studied hydrophobic anticancer drug, Methotrexate (MTX), with a 45.2% loading degree and significantly accelerated and triggered release in the presence of 10 mM 1,4-dithio-DL-threitol. Based on this study, glycopolypeptides conjugates have been paid extensive attention to selective and intracellular drug delivery into cancer cells.

Recently, Wang *et al.* prepared a series of redox-responsive amphiphilic glycopolypeptide analogs, poly (6-O-methacryloyl Dgalactopyranose)–SS–poly (g-benzyl-L-glutamate), by the combination of RAFT polymerization, ROP, click chemistry, and following trifluoroacetic acid-mediated deprotection. Further, cellular experiments were performed on HepG2 assessed by CCK-8 and Lactate dehydrogenase (LDH) assays and suggested the low cytotoxicity and biocompatibility of synthesized glycopeptides. The stabilized micelles with uniform size obtained from the glycopolypeptide self-assembly could act as multifunctional vehicles for lectin recognition, DOX encapsulation (>90% loading degree), and redox-responsive release as well as hepatoma cell receptor targeting [82].

Sequential ROP of benzyl-L-glutamate and *L*-phenylalanine NCAs can be applied to synthesize the block *co*-polypeptides, poly(γ-benzyl-L-glutamate)-b-poly-(*L*-phenylalanine) self-assembled in an aqueous solution with spherical micelle-like structure. To conjugate a saccharide moiety, click chemistry was used to click sugar azides to the alkyne side chains of polypeptides. In a study, the polypeptide assemblies introduced a model target ligand on their surface, lactobionolactone consisting of galactose, to target liver cells. The presence of poly(L-glutamic acid (PGA) in this nanostructure made it prone to pH changes. So, it was illustrated that designed glycopolypeptides micelles can use as pH-responsive carriers for a model drug, DOX, and the cells with overexpressed ASGPR were recognized by aiming saccharide moieties on the micelles. High uptake of the micelles resulted in high drug release and subsequently higher cytotoxicity in HepG2, due to acidic conditions [83].

3.1.3. Sugar-responsive carriers

Zhao *et al.* [84,85] developed glucose-sensitive core-shell nanogels prepared by cross-linking glycopolypeptides through adipoylamidophenylboronic acid (Fig. 2C), which was loaded with insulin. The incorporation of phenylboronic acid endowed the sugar sensitivity to nanogels in PBS buffer, resulting in increased hydrodynamic radius (84 ± 1.5 nm to 110 ± 1.9 nm in glucose concentration from 0 to 3.0 mg. mL^{-1}) in response to high glucose. The *in vitro* drug release behaviors triggered by glucose at physiological pH in a competitive binding mechanism with the structural glucose moiety revealed the increased release profile as the concentration of glucose increased in PBS. Additionally, Methytethrazolum (MTT) and hemolysis assays confirmed the non-toxic and biocompatible nature of nanogel. In this regard, this group has previously reported another polypeptide-based nanogel synthesized by one-pot thiol-ene click chemistry of poly(ethylene glycol) diacrylate (PEDGA), poly(ethylene glycol) diacrylate (mPEGA), and pentaerythritol tetra(3- mercaptopropionate) (QT) using 4-dimethylaminopyridine (DMAP) as a catalyst. It was also loaded with insulin, and alizarin red S, and the release profile was investigated by DLS and fluorescence microscopy in the presence of glucose so that the fluorescence intensity decreased with increasing glucose concentration. In this case, the biocompatibility and non-cytotoxicity of nanogel were confirmed *via* MTT, LDH, and hemolysis assays. Therefore, the prepared nanogels can introduce as a self-regulated diabetic therapeutic delivery system due to their high glucose sensitivity.

3.1.4. Temperature-responsive carriers

Among various triggering agents, the temperature can be considered as a unique one as it can be dually categorized into internal and external

stimuli. Temperature-responsive nanocarriers are officially assumed as efficient tools among stimuli-responsive nanocarriers, which easily start to release their encapsulated drug by triggering relatively small variations in temperature (Fig. 2D). Temperature-responsive hydrogels and polymers usually undergo a volume phase transition at a certain temperature, leading to alteration in their solvation state. Polymers with lower critical solution temperatures (LCST) become insoluble upon heating, and polymers with an upper critical solution temperature (UCST) start to become soluble upon heating [86,87].

Poly (N-isopropylacrylamide) (pNIPAM) is a block polymer with water solubility at room temperature while precipitates above the LCST. Mokrus *et al.* grafted pNIPAM to a glycopeptide block using N-carboxyanhydride polymerization and subsequent deprotection to yield thermoresponsive poly(l-glutamic acid)-b-poly(N-isopropylacrylamide) block copolymers (pGA-b-pNIPAM). The 6.7:1 ratio for pGA: pNIPAM showed the possibility of particle formation. 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholin-4-ium chloride (DMT-MM) mediated coupling method was performed for the amino sugars functionalization with Glu-NH_2, Mal-NH_2, and Glu-CAr-NH2 pGA blocks with various degrees of substitution (DS). Glycosylated pGA-b-pNIPAM was also shown to be capable of forming particles. Quantitative lectin interaction assays indicated nonaggregated and aggregated glycosylated pGA-b-pNIPAM can efficiently bind to lectin. The interaction with lectin showed to be independent of the degree of substitution in the non-aggregated state while in the aggregated state increasing the degree of substitution made stronger interactions [40].

Tedious and expensive chemical synthesis methods of glycopeptides have seriously limited the robust application of these structures. Related to this, Ayanda *et al.*, used temperature-sensitive recombinant elastin-like polypeptides (ELPs) with methionine residues to enable post-synthetic modification *via* thioether alkylation using alkyne functional epoxide derivatives. Then azido-functionalized monosaccharides were conjugated to ELP to obtain temperature-sensitive glycoconjugate ELP with periodic saccharide functionality and uniform length and precise carbohydrate spacing by a robust procedure. Temperature-dependent solution properties of ELPs were then used to successfully control the interaction of multivalent galactose-functionalized ELPs with the lectin *Ricinus communis* agglutinin (RCA120) [88].

3.1.5. Multi-responsive carriers

There are some drug carriers designed to respond to multiple stimuli [89]. In recent years, alginate-based polymers have attracted tremendous attention due to their great advantages, such as biocompatibility, stability, cost-effectiveness, non-immunogenicity, *etc.* In contrast, they have intrinsically poor mechanical strength and high hydrophilicity. Chemical modification of alginate polymer was recommended through the graft copolymerization technique to alter the mechanical strength and hydrophilicity. It was shown that the grafting of N-Acryloyl-*L*-phenylalanine (APA) on sodium alginate has the potential to improve its mechanical characteristic. The APA monomer contains an essential and optically active *L*-phenylalanine with the ability to form secondary structures like helices. APA-containing copolymers can construct physical crosslinks through H-bonds leading to higher toughness and strength of the designed samples [90,91]. In this regard, the synthesis, and characterization of dual-responsive polymeric hydrogels were performed to control the release of a model anticancer drug, imatinib mesylate, in response to pH and temperature changes. To prepare the hydrogels, the synthesis of *N*-acryloyl-*L*-phenylalanine grafted sodium alginate copolymer (NaAla-g-PAPA) was carried out *via* free radical polymerization followed by cross-linking with *N,N′*-methylenebisacrylamide. The *in vitro* drug release was conducted at pH 1.2 (real stomach gastric) and pH 7.4 buffer solution at 25 °C and 37 °C. The results indicated that the maximum drug release (98%) within 48 h at pH 7.4 and temperature of 37 °C. It is reported that these smart hydrogels can be utilized as controlled drug delivery systems [92]. In another study, Kranning *et al.* [71] investigated the pH- and temperature-responsivity of secondary structures in glycopolypeptides derived from poly(L-glutamate-*co*-allylglycine or propylglycine). The glycopeptides adopted random coil conformation at pH 6.5 or higher, and α-helix structures were formed at pH 6 or lower affected by the number and configuration of allylglycine defects that participated in the stability of structures. The stability of α-helix structures was studied by CD at pH 3.5 in the temperature range between 15 and 90 °C and confirmed the reversible denaturation and the decreasing of the helix content from 77% at 15 °C down to 39% at 90 °C.

For pH-responsive nanostructures, having the optimum dimensions (around 100 nm) is of great importance to escape the blood compartment, accumulate in the pathological cells, and effectively release their payload. In this regard, How *et al.*, used cationic lactobionolatone/lipoic acid-modified poly (L-lysine), (PLL-g-(Lipo–Lac)), and anionic poly (acrylic acid) (PAA), to yield reduction/pH-responsive nanogels which efficiently was loaded with DOX. The formation of interchain disulfide bonds led to the fabrication of cross-linked and helical nanogels with a size smaller than 150 nm. Based on *in vitro* drug release studies, acidic conditions and/or the presence of disulfide cleaving agents significantly enhanced DOX release from the nanogels. The results of carbohydrate-lectin binding and cellular uptake analysis revealed that Lac-conjugated nanogels could effectively afford cell internalization *via* cells bearing ASGPR on the HepG2 cell surface, manifesting efficient cell proliferation inhibition toward HepG2 cells [93].

It is proved that dynamic covalent bonds and possessing reversible features are of great importance in the creation of stimuli-responsive nanostructures. Schiff base linkages, disulfide bonds, boronic esters, and acylhydrazone bonds are common covalent bonds used in pH-responsive glycopolypeptide nanostructures. Profited from their dynamic covalent bonds, phenylboronic acids (PBAs) can form reversible covalent boronic ester bonds with diols in pH ranges greater or equal (7.8–8.6) to their pKa value. On the other hand, a pH value lower than their pKa leads to boronic ester hydrolysis, making them a promising candidate to be used in pH-responsive glycopeptide structures. Wu *et al.*, recruited phenylboronic acid-oxidized dextran and caffeic acid grafted ε-polylysine to construct pH/reactive oxygen species (ROS) dual responsive injectable glycopeptide hydrogels to improve the healing process in diabetic wounds mice models. To do so, pH-responsive nanomicelles were synthesized through grafting 2-(diisopropylamino) ethylamine (DIP) group onto mPEG45-PBLA60-PPhe30 triblock copolymer. Mangiferin (MF), a natural angiogenesis promoting agent, was encapsulated into nanomicelles core using hydrophobic interactions (MIC@MF). MIC@MF and diclofenac sodium (DS) were then incorporated into the hydrogel system. Cleavage of the boronic ester bonds in the acidic environment of the infected diabetic wound model led to the continuous release of MF. *In vitro* cell culture analysis and *in vivo* evaluation of hydrogels on diabetic wound mice models indicated biocompatibility with effective anti-infection, anti-oxidant, and anti-inflammatory effects that could also promote angiogenesis for accelerated wound repair [94].

To target hepatocarcinoma cells, amphiphilic star copolymers were synthesized standing on [$(PCL_{50})_2$-b-Pr-gly_6-b-GP_{40}] containing galactose for targeting galactose binding over-expressed ASGPR on liver cancer cells. This designed nanostructure acted as a dual-stimuli-responsive carrier (redox- and enzyme-responsive) due to the presence of polycaprolactone (PCL) as a biodegradable enzyme-responsive polymer and a disulfide bond (*viz.* bis-(azidoethyl) disulfide) for redox-responsivity. The synergistic and programmed impact of both triggers resulted in significantly higher drug release compared to designed micelles with only one stimulus [53].

Considering the potential of glycopolypeptides in stimuli-responsive drug delivery, a smart nanocarrier was designed for insulin and glucose oxidase delivery consisting of phenylboronic acid-containing homopolymer PAAPBA and glycopolypeptide PEG-*b*-P(Asp-*co*-AGA) with dual responsivity to glucose and H_2O_2. The cycloborates in this structure could be demolished through the replacement of glycosyl groups with

glucose and oxidized by H_2O_2. A commendable hypoglycemic effect was inspected with the delivery of insulin/glucose oxidase-loaded nanoparticles to diabetic mice in comparison to insulin-loaded nanoparticles [95]. Recently, a dual-sensitive glycopolypeptide analog nano-prodrug was synthesized *via* ROP of a furan-containing NCA and functionalized by maleimide-modified prodrugs. The results revealed a high drug encapsulation and loading efficiency and the inhibition of the burst release due to the chemical trapping of drugs. After taking up by the breast cancer cells, the drug release was observed in the lower pH and higher glutathione (GSH) contents of tumors [72]. Table 1 summarizes some of the recent studies on drug delivery applications of self-assembled glycopeptide structures.

3.2. Glycopolypeptide-based diagnostics

In the early detection and treatment of various diseases, non-invasive imaging has held a great opportunity to develop a cost-effective and non-painful method for patients [100]. In this regard, molecular probes are introduced on nanocarriers with minimum toxicity and the ability to target specific cell types. [37]. Imaging technologies have attracted growing attention because of their non-invasive property to visualize and monitor biological processes and allow real-time monitoring of patients' responses to therapy [101]. Using glycopeptide nanostructures in diagnostic and molecular imaging demonstrates some advantages related to either saccharide or peptide compartments. It is proved that peptides have several advantages including small size, rapid distribution, low immunogenicity, ease of synthesis, scaling-up potential, and cost-effectiveness [102]. To achieve targeting and intracellular delivery of peptide-base nanostructure, glycosylation can be useful due to its inherent characteristics in lectin recognition, targeting disease-specific receptors, and facilitating cellular internalization [56,103]. These features can be helpful to improve the EPR effect by which the nanocarriers can passively accumulate into the tumor. Adding polysaccharides on the surface of nanocarriers is a common way to design stealthy nanostructures evading rapid clearance and improving the efficiency of EPR. [104]. It was previously reported that the glycopeptide of chitosan and glutamate peptide has the potential to target tumors through glutamate transporters and easily be labeled with radioisotopes like ^{99m}TC [105]. Glycopeptides can interact with cellular targets and have several carboxylic and amino groups to allow the conjugation of therapeutic agents and diagnostic probs for targeted therapy and imaging, providing a selective, region-specific, and controlled delivery [106,107]. Furthermore, good biocompatibility and the outstanding intracellular retention of designed glycopeptide-based nanostructures present promising potential in diagnostic applications [108]. There is an increased interest to investigate magnetic nanoparticles (MNPs) and their assemblies which are capable to target specific cell types for image-guided treatment. In a study, the novel combination of synthetic glycopolypeptide and highly crystalline superparamagnetic MNPs was described to prepare biocompatible and monodisperse carriers for the first time with high monosaccharide density on the surface, which exhibits well-defined water dispersibility, optimal T_1-weighting properties, and targeting characteristics. This research noted that GP-MNPs could be used in stimuli-responsive T_2-weighted MRI with signal suppression following molecular recognition and subsequent controlled MNP aggregation [109].

Reduction-sensitive amphiphilic poly(3-caprolactone)-b-glycopolypeptides (PCL–SS–GPPs) diblock copolymers were synthesized *via* ROP and click chemistry of propargylgalactose/propargyllactose units to prepare PCL-SS-glycopolypeptides as DOX and SPION nanocarrier for controlled intracellular drug delivery and MRI imaging (Fig. 3A). Owing to a hydrophobic PCL block and hydrophilic glycopolypeptides, these copolymers could self-assemble into spherical nano-sized micelles in an aqueous medium. The results revealed lectin-recognition properties, intracellular DOX release, and high efficient uptake through HepG2 tumor cells, as well as their growth inhibition. The Superparamagnetic iron oxide nanoparticles (SPIONs)-loaded PCL–SS–GPPs micelles enabled excellent MRI contrast enhancement followed by T_2 relaxivity increasing (Fig. 3B), thus confirming their effectiveness for MRI imaging in comparison to Feridex® as a commercial MRI agent [56].

In a study, the ordered helical glycopolypeptide-b-poly(propylene oxide) was used as an amphiphilic block to form bioactive spherical polymersomes (~50 nm) by self-assembly and investigated for encapsulation of dual-dye, hydrophilic donor (Calcein), and hydrophobic acceptor (Rhodamine B octadecyl ester perchlorate) by which it is possible to carry out Fluorescence resonance energy transfer (FRET) [110]. It was illustrated that visualization of single polymersomes, probing the dyes' colonization, and investigation of the variation in dual-dye encapsulation is possible. Also, galactose moieties on polymersomes surface were able to recognize lectin RCA_{120} and cause it to be biologically active. Then, the energy-mapped fluorescence imaging and spectrally-resolved microscopy were done on as-prepared polymersomes to prove the simultaneous encapsulation of dyes.

Over the last decade, molecular imaging by radiolabeled peptides has been discovered as a potent and non-invasive method for clinical translation to visualize and monitor any response to therapy. It is investigated that glycosylation results in improved pharmacokinetic properties of radiolabelled peptides. Some researchers have recently summarized the developments in radiolabelled peptides to detect cancerous cells by positron emission tomography (PET) imaging [101,103,111,112]. More recently, a widely used radionuclide, fluorine-18, is applied to label peptide-based tracers. For example, [^{18}F] fluoroglycosylation of neuropeptide Y analog has been developed by Hofmann and co-workers [107] for PET imaging of Y_1R expression in MCF-7 breast tumor-bearing nude mice. The Y_1 receptor subtype is categorized in the human neuropeptide Y receptor system, which has high pathophysiological relevance in breast cancer. *In vitro* autoradiography showed high specific binding for receptor Y_1R and moderate uptake of 1.8% ID/g at 20 min post-injection in Y_1R expressing MCF-7 tumors *in vivo* as well as decreased kidney uptake (**Fig. 4A** and **B**).

It should be noted that the pharmacokinetics and biodegradation of polypeptides are improved by the glycosylation process. Accordingly, Maschauer *et al.* [113] dedicated the preparation of ^{18}F-Glyco-Arginyl-glycylaspartic acid (^{18}F-Glyco-RGD) peptides using efficient radiosynthesis by click chemistry method for targeting the integrin-receptor and PET imaging of integrin expression (Fig. 4C). They generated different RGD glycopeptides with changing saccharides to investigate the yield of ^{18}F-Glyco-RGD peptides and their impacts on biokinetics. It was shown that all prepared glycopeptides exhibited high affinity to integrin receptors positive U87MG cells, and after radio-synthesis, the glycopeptides [^{18}F]6Glc-RGD and [^{18}F]Mlt-RGD were selected due to their high yield in ^{18}F-labeling, which applied for biodistribution studies and PET imaging in the mouse model. Both cyclic RGD-glycopeptides displayed specific tumor uptake while [^{18}F] Mlt-RGD showed a suitable balance between tumor uptake (1% ID/g at 120 min) and clearance as well as high tumor retention of 60%. It is noteworthy that [^{18}F] Mlt-RGD can be a very promising alternative radiotracer for PET imaging of integrin expression in (pre)clinical studies. In another study by this group, [114] the first ^{18}F-labeled neurotensin receptor 2 (NTS2) selective ligand was created. [^{18}F]Fluoroglycosylation of the peptide sequence N-Me-Arg-Lys-Pro-N-homo-Tyr-Ile-Leu-OH displayed high affinity to NTS2 has been reported using click chemistry for PET imaging (Fig. 4D). The results confirmed the high selectivity of developed radiolabeled glycopeptides for NTS2 and revealed high renal and moderate tumor uptake with specific binding to NTS2-positive tumors.

Chitosan plays an important role in angiogenesis and can make a prominent advantage in cancer treatment. Nevertheless, the lack of cellular targeting is one major limitation of chitosan. Since it is proven that radiolabeled glutamate peptide improved intracellular uptake, conjugation of chitosan with glycopeptide would be ideal. It has been implemented by Tsao *et al.* [115], who have developed ^{68}Ga-glycopeptide comprised of glutamate peptide and chitosan, as a PET imaging

Table 1
Recent studies conducted on self-assembled glycopeptide structures for targeted drug delivery to cancer tissue and stimuli-responsive targeted delivery.

	Anchored Sugar	Size	Structural feature	Highlighted result	Cargo	Application	Trigger Agent	Ref
Drug Delivery	Mannose	~37 nm	Co-assemble nanoparticles (eye drop structure)	Higher DOX retention was reported in fundus cells after 24 h compared to the control group, leading to a significant reduction of pathological neovascularization in a mouse *in vivo* model	DOX	Non-invasive drug delivery to fundus (eye)	–	[66]
	9G-A7R Glycopeptide	~50 nm	Lipodisks	Glycopeptide-modified disks demonstrated an enhanced anti-glioma effect both *in vitro* and *in vivo*, significantly prolonging the survival time of glioma-bearing mice	Melittin and PXT	Co-delivry for Glioma-targeted- therapy	–	[96]
	Lactose	53 nm	Micelles	Using ASGP internalization, micelles showed much higher drug accumulation in tumor tissue using *in vitro* and *in vivo* models	DOX	Hepatoma cancer targeting Drug delivery	-	[97]
	Glucose	50 nm	Nanodisk	Modification with glycopeptide could significantly enhance the uptake of nanodisks by brain cells and a higher accumulation in glioma tumors was reported using *in vitro* and *in vivo* mice models	PTX	Glioma-targeted drug delivery	-	[98]
	Chitosan	140 to 250 nm	Vesicles	The *in vitro* two weeks-release was achieved for FITC-dextran	FITC-dextran	Sustained drug delivery	-	[62]
Stimuli-responsive Drug Delivery	Dextran	94 nm	Micelles embedded in injectable hydrogel	Spatiotemporal release of Mangiferin (MF) Encapsulated in micelles and Diclofenac sodium (DS) embedded in hydrogels significantly promoted angiogenesis and accelerated wound repairing using *in vitro* and *in vivo* models	DS /MF	Treatment of chronic diabetic wounds	pH/ROS	[94]
	Lactose	114 nm	Micelle	Compared with neutral pH, glycopeptide micelles had faster DOX release in acidic pH with specific recognition by HepG2 model tumor cells *in vitro,* leading to higher cytotoxicity	DOX	HepG2 tumor-targeted drug delivery	pH	[77]
	Dextran	109.5 ± 07 nm	Micelle	The presence of 10 mM DTT significantly accelerated the release rate of encapsulated MTX	MTX	Triggered release of an anticancer drug	Redox	[81]
	Galactose		Micelle	Glycopeptide micelles exhibited obvious redox-responsive features in the presence of GSH. They also showed good lectin recognition properties, DOX delivery, and human hepatoma cell receptor targeting	DOX	Hepatoma cancer targeting Drug delivery	Redox	[99]
	Mannose	~80 ± 5 nm and ~ 205 ± 15 nm	Micelle	Being recognized by mannose-6-phosphate (M6P) receptors, micelles could selectively target lysosomes in cancerous cells such as MCF-7 and MDA-MB-231 *in vitro* models triggering the release of cargo by acidic pH	rhodamine-B	Lisosomal cargo delivery	pH	[60]
	Seven different saccharide	74 to 350 nm	Glycospheres, Worm-like micelles, Fibers	Being recognized by C-type mannose-specific lectin, the glycodendrons showed a pH-controllable release behavior around the physiological and acidic tumor environment	NR	Targeted delivery	pH	[39]
	galactose	30–50 nm	Micellar Nanoparticle	Using the ASGP receptor, the galactose-decorated nanoparticles retained a much higher antitumor activity toward HepG2 cells in contrast to the nanomedicine without galactosyl group *in vitro* and *in vivo*	DOX	Hepatoma cancer targeting Drug delivery	pH	[79]
	Hyaluronic acid	154–215 nm	Nanomicells	Benefited HA affinity to CD44 receptors, pH-responsive HA-	DOX			[75]

(*continued on next page*)

Table 1 (*continued*)

	Anchored Sugar	Size	Structural feature	Highlighted result	Cargo	Application	Trigger Agent	Ref
				PHis micelles taken up in great amounts by receptor-mediated endocytosis and DOX were efficiently delivered into cytosol using MCF7 *in vitro* cell culture		Tumor-target, controlled drug delivery	pH	
	Lactobionolactone (Lac)	150 nm	Nanogel	Interchain disulfide cross-linking and helical PAA/PLL complexes led to small-size nanogels. Lac-conjugated nanogels effectively bound to the ASGPR and exhibited efficient cell proliferation inhibition toward HepG2 cells *in vitro*	DOX	Dual reduction and pH-controlled, targeted tumor drug delivery	reduction- and pH-	[93]
	Lactobionolactone (Lac)	88–230 nm	Nanovesicles	Chain conformations and nanovesicles sizes could be tuned *via* the degree of substitution (DS) and alkyl chain length. Lac-conjugated nanovesicles effectively bonded to the ASGPR toward HepG2 cells *in vitro* cross-linking by genipin also led to tunable membrane permeability in HepG2 cells *in vitro*	DOX	Targeted drug delivery	pH	[76]
	Glucose	80 ± 4.0 nm	Nanogel	Having high glucose-sensitivity and good biocompatibility, nanogels are a great candidate for self-regulated drug release	Insulin	Sugar-sensitive drug release	Sugar	[89]
	Glucos and Maltose	225–250 nm	Nanoparticles	When the glycosylated pGA-b-pNIPAM was aggregated, interaction of the different saccharides with ConA was possible, which evidenced that the saccharides were indeed located on the particle surface and were thus capable of targeting		Temperature risponsivetargeted drug delivery	Temperature	[40]
	Galactose	–	–	Selective lectin binding and sorting were achieved galactose functionalized temperature-sensitive elastin-like polypeptides (ELPs)	–	Selective lectin binding	Temperature	[88]

probe for tumor angiogenesis. In this regard, tumor uptake was analyzed by PET dynamic scanning for up to 45 min that showed fast tumor uptake by New Zealand white rabbit bearing VX2 tumor and no intestine uptake confirmed the stability of ^{68}Ga-glycopeptide *in vivo*. The ^{68}Ga-glycopeptide clearance and half-life were determined in blood samples collected from 10 s to 20 min. As a result, tumor uptake of developed ^{68}Ga-glycopeptide was proved by PET images in rat and rabbit models and its blood clearance curve determined the half-life of 1.84 h and the elimination rate constant of 0.377 h^{-1}.

Apart from the reports above, in the field of microbiology, a glycopeptides antibiotic, Vancomycin (Van), has attracted tremendous attention due to its vigorous diagnostic and therapeutic activity against gram-positive bacteria [101]. Oosten *et al.* [116] took advantage of fluorescently labeled Van (vanco-800CW) for *in vivo* targeting and detection of the infection caused by G^+-bacteria in mice and *ex vivo* human post-mortem implant models using real-time imaging. Both Van and associate dye (IRDye800CW) have been extensively used in the clinic. Despite the drawback of the penetration to deeper infections, the antibiotic-based imaging agent allowed specific infection detection in a mouse model and the lower leg of a human cadaver (Fig. 5A).

In another *in vitro* study [117], Van was labeled with Cy5.5 to construct a fluorescent probe for the detection of *Mycobacterium tuberculosis* with a strong affinity to the Mtb cell wall. Due to the slow growth of these bacteria on the agar plate, the conventional detection method took a long time, while the near-infrared imaging technique can quickly quantify tubercle bacilli. The fluorescence signal correlated directly with the colony-forming unit of bacteria and can successfully identify critical genes involved in cell invasion.

Among imaging techniques, there are huge challenges in accurate diagnostic methods for bacterial infections and their real-time monitoring under antibiotic therapy [118]. Given Van's unique advantages, it was linked to Rhodamine and iodine-125 as fluorescent- and isotopic probes, respectively, allowing effective imaging of the methicillin-resistant *staphylococcus aureus (*MRSA) in muscle and lung-infected murine models (Fig. 5B and C) that showed significantly increased fluorescence and strong radioactive signal in the infected site. This study anticipated that the novel dual fluorescent and the nuclear probe will attain a place in day-to-day clinical trials in the detection of severe bacterial infections [119]. However, considering their infancy and limitations in the targeted delivery due to the possible protein corona formation and distribution to other parts of the body rather than the infected site, further investigations and improvements are needed to overcome the obstacles of progress toward clinical translation of such systems. In a recent publication by Li *et al.*, a self-assembling peptide and Van molecule were co-modified by gadolinium to detect bacterial infections *in vivo*. The designed probe had the potential to specifically target bacteria and increase the longitudinal relaxivity rate. As a result, this system showed high specificity and accurately detected bacterial cells and it monitored the efficiency of the antimicrobial drug in infected mouse models [118]. Despite the great advancements in imaging techniques by peptide-based probes, there are still several challenges in their clinical translation including poor stability, rapid renal clearance, and inferior *in vivo* selectivity and sensitivity, which urgently need to be solved in future studies [120].

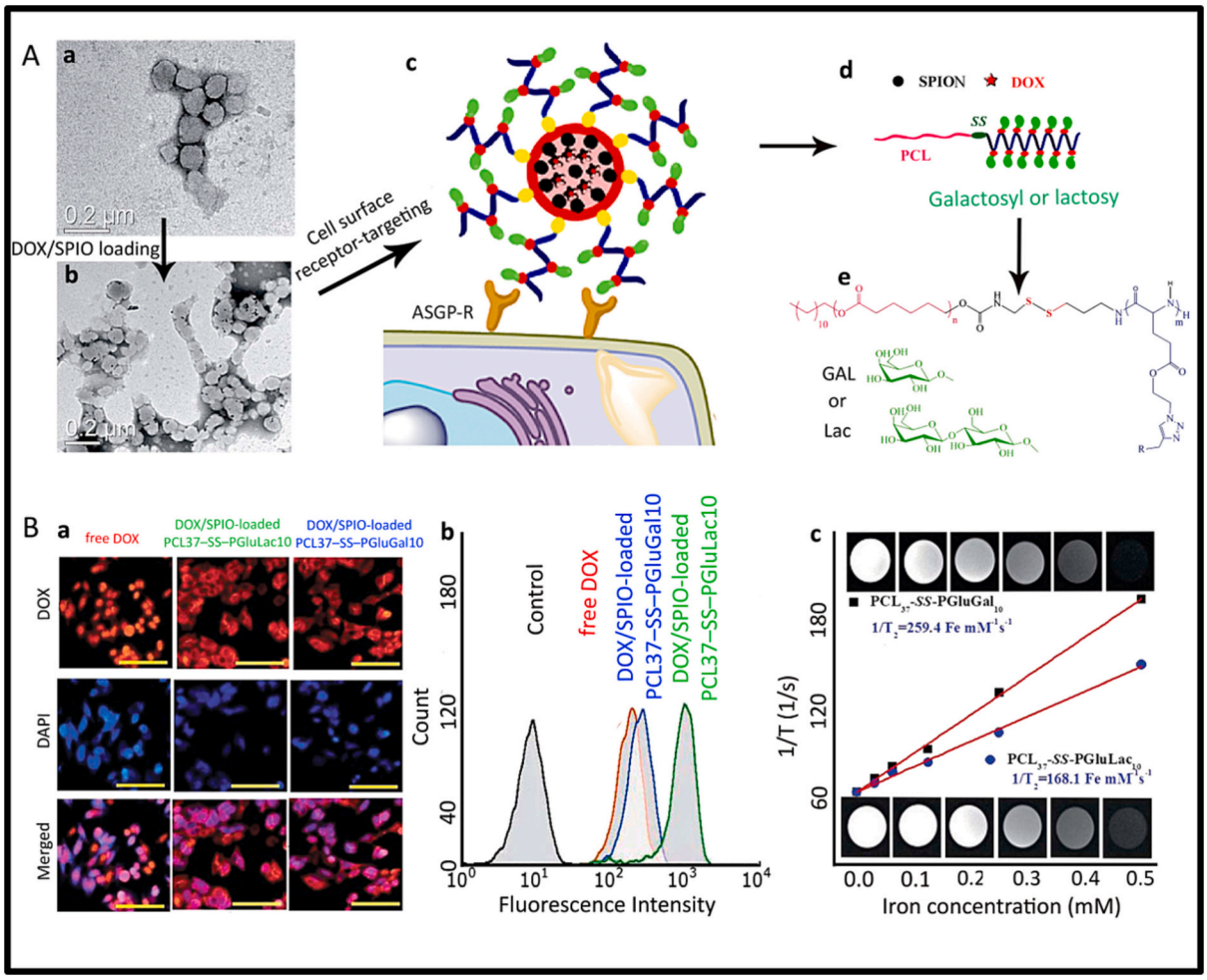

Fig. 3. The reduction-responsive poly(3-caprolactone)-b-glycopolypeptides (PCL-SS-GPPs) nanocarriers for drug delivery and imaging applications. **(A)** The synthesis process of micelles and cell recognition: TEM images of the blank PCL_{37}–SS–$PGluGal_{10}$ (a) and the DOX/SPION-loaded PCL_{37}–SS–$PGluGal_{10}$ micelles (b); Schematic representation of DOX/SPIO-loaded PCL_{37}–SS–$PGluGal_{10}$ micelles binding to ASGP receptors (c); Schematic representation of glycopolypeptide structure (d); and Chemical structure of PCL-SS-GPPs diblock copolymer (e). **(B)** Fluorescence microscopy of HepG2 cells incubated by free DOX, DOX/SPIO-loaded PCL_{37}–SS–$PGluLac_{10}$, and DOX/SPION-loaded PCL_{37}–SS–$PGluGal_{10}$ micelles for 4 h at 37 °C (Scale bars represent 200 mm) (a); Flow cytometry analysis for the control, free DOX, DOX/SPIO-loaded PCL_{37}–SS–$PGluLac_{10}$, and DOX/SPIO-loaded PCL_{37}–SS–$PGluGal_{10}$ micelles in HepG2 cells for 4 h at 37 °C (b); and the relaxation rates of T2 as a function of iron concentration (mM) for the DOX/SPIO-loaded PCL_{37}–SS–$PGluLac_{10}$, and PCL_{37}–SS–$PGluGal_{10}$ micelles along with their T2-weighted MRI images (water was used as control) (c). Reproduced with permission from ref. [56]; Copyright 2011, Royal Society of Chemistry.

Although Van is known as a potent antibiotic in G+ infections, bacterial resistance to Van due to a point mutation has recently appeared as a serious threat to public health. This is the reason that the search for alternative therapy is still needed in the case of Van-resistant bacteria. One introduced alternative approach in recent publications involves the use of photosensitizers inducing ROS generation with effectiveness against G+ and G- bacteria. Accordingly, a multivalent antibiotic was constructed based on Van as an affinity ligand and porphyrin as a photosensitizer and linker to produce dimeric Van conjugates [121]. The Van-porphyrin construction exhibited high affinity to the bacterial cell wall and strong photodynamic antimicrobial chemotherapy (PACT) activities against Van-resistance and -sensitive bacteria. It should be mentioned that it is possible to perform an imaging study on living bacteria using this approach. Meanwhile, Feng *et al.* [122] reported a light-up probe comprising Van and Aggregation-induced emission fluorogenes (AIEgen) for selective imaging and treatment of G+ bacteria, particularly the resistant ones, such as *Enterococcus* strains. The conjugation of Vans by AIE resulted in an enhanced fluorescence signal after binding to G+ *B. subtilis*, and ROS generation by AIE caused irreversible selective damage to the G+ bacteria cell wall. AIE-2Van also showed a significant potential for effective treatment of Van-resistant bacteria over 70% and 90% for *Enterococcus faecium* and *Enterococcus faecalis*, respectively.

Recently, bioimaging and tracing of live cells were reported using Tetraphenylethylene (TPE)-based amphiphilic glycopolypeptides. In this method, the AIE phenomenon was applied due to its remarkable optical properties. According to this phenomenon, fluorescent emissions are enhanced *via* the aggregation of TPE in water, while it is not water-soluble. Self-assembly of designed glycopolypeptides in different supramolecular structures, such as vesicles and spindles count on the water fraction. It is a matter of fact that increasing the water content in dimethyl sulfoxide (DMSO)/water mixture causes the transformation of vesicles into spindles and both structures have the potential to internalize into macrophages. It was shown that the changes in water content could change fluorescence properties due to the restriction of molecular motions. This proposed bioimaging method has captured considerable attention due to the great retention time of the designed nanostructure in stained cells, which was reported to be >7 days [108].

Fluorescent emission dependent on pH changes has been used in cancer imaging. The alteration of charge as a result of pH fluctuation causes the aggregation of designed glycopeptide and can induce nanostructure transformation and molecular emission [123]. Glycopeptide

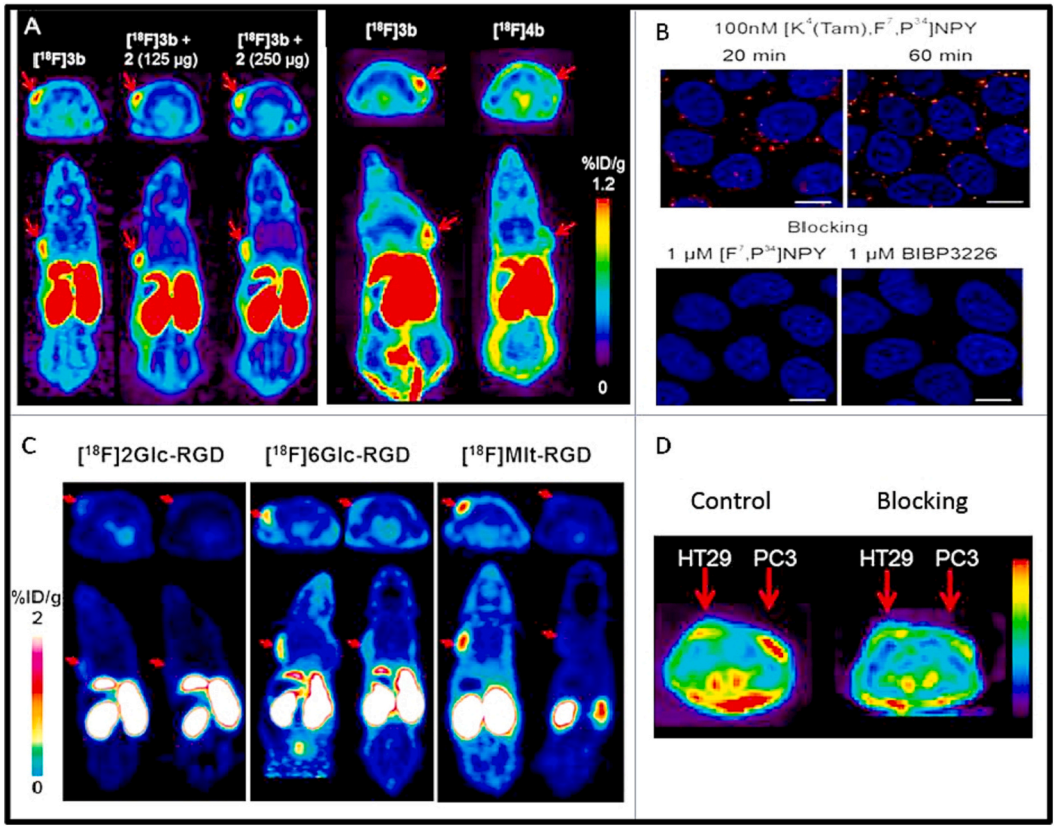

Fig. 4. Positron emission tomography (PET) imaging by the radiolabeled peptide. **(A)** Transaxial (bottom) and coronal (top) μPET images of MCF-7 (red arrows) tumor-bearing nude mice after administration of 2–5 MBq [^{18}F]3b with blocking conditions (left), and μPET images of animals injected with [^{18}F]3b in comparison with the nonbinding peptide [^{18}F]4b at 45–60 min post-injection (right); **(B)** Receptor internalization in MCF-7 cells stimulated by 5(6)-carboxytetramethylrhodamine (Tam)-labeled peptide ligand. 1 μM of nonlabeled agonist 2 or BIBP3226 antagonist used for blocking experiments; Reproduced with permission from ref. [107]; Copyright 2015, American Chemical Society. **(C)** PET images of U87MG-bearing mice using [^{18}F]2Glc- Arginylglycylaspartic acid (RGD), [^{18}F]6Glc-RGD, and [^{18}F]MIt-RGD; Reproduced with permission from ref. [113]; Copyright 2014, American Chemical Society. **(D)** Representative coronal PET images of HT29 and PC3 tumor-bearing nude mice injected with ^{18}F-4. Reproduced with permission from ref. [114]; Copyright 2015, Elsevier B.V. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

structures were introduced as a potent system to detect pH changes with the capability to be employed for monitoring diseases such as cancer, infectious disease, *etc.* A pH-sensitive glycopeptide-modified aggregation-induced emission probe (TGO) was prepared by a solid-phase peptide synthesis approach by which the change to amphipathicity balance occurred as a response to pH alteration. It was demonstrated that changing the pH from 5.5 to 8 could increase the fluorescence intensity due to the adverse protonation of the peptide and the transformation of the nanostructure from nanolamellae to nanomicelles. This recent study provided a novel strategy to design fluorescent probes that can be useful for biological assays and bioimaging [123]. Considering all these, glycopeptides have demonstrated great potential as carriers for imaging and diagnosis during treatment. Using biocompatible and biodegradable glycopolypeptides can benefit this area of science to prepare theranostic devices.

3.3. Glycopolypeptides in regenerative medicine

During the last decades, tissue engineering ushered a new perspective for the regeneration of damages in cartilage by which smooth motion is possible in diarthrodial joints [124]. In this approach, a three-dimensional scaffold as the temporary ECM is used to provide the structural support for cell (chondrocytes or progenitor cells) attachment and tissue development.

Recently, injectable hydrogels have attracted tremendous attention as 3D scaffolds for cartilage regeneration with excellent permeability for nutrients and metabolites, great biocompatibility, and the retainability of a large amount of water [125–128]. Among variously reported hydrogels, the glycopeptide-based hydrogels may serve as 3D scaffolds for tissue engineering and similarly act to proteoglycans present in ECM of native cartilage. Table 2 has summarized recent studies on the applications of glycopeptide-based structure in regenerative medicine. It was shown that the glycopeptides hydrogels were rapidly synthesized *in situ* during an enzymatic crosslinking reaction catalyzed by horseradish peroxidase and hydrogen peroxide [129]. Glycopeptides copolymer was synthesized *via* click chemistry by the conjugation of poly(γ-propargyl-Lglutamate) (PPLG) with azido-modified mannose and 3-(4-hydroxyphenyl) propanamide (HPPA). The cytocompatibility of glycopeptides copolymer and hydrogels was investigated through an MTT assay against L929 cell lines. The *in vivo* studies were performed in rats to find the biodegradability and biocompatibility of injected hydrogels as well as in rabbits to prove the ability of hydrogels to act as a scaffold for chondrocytes. It was shown that the hydrogels were cleared from the body 4 weeks post-injection, which is attributed to the esteric hydrolysis of carbohydrates and enzymatic degradation of polypeptide backbones. It is noteworthy that the degradation of the hydrogels reduced the inflammatory cells in the initial stage. The hybrid system of incorporating chondrocytes to glycopeptides hydrogel had been found to maintain chondrocytes' phenotype and led to the formation of the cartilaginous specific matrix.

The generation of a new kind of hydrogel was reported by the supramolecular assembling of a synthetic glycopeptide consisting of a naphthyl group, a tetrapeptide segment (Phe-Phe-Asp-Tyr(H2PO3)), and a sugar moiety (D-glucosamine) on the side chain of Asp. Here,

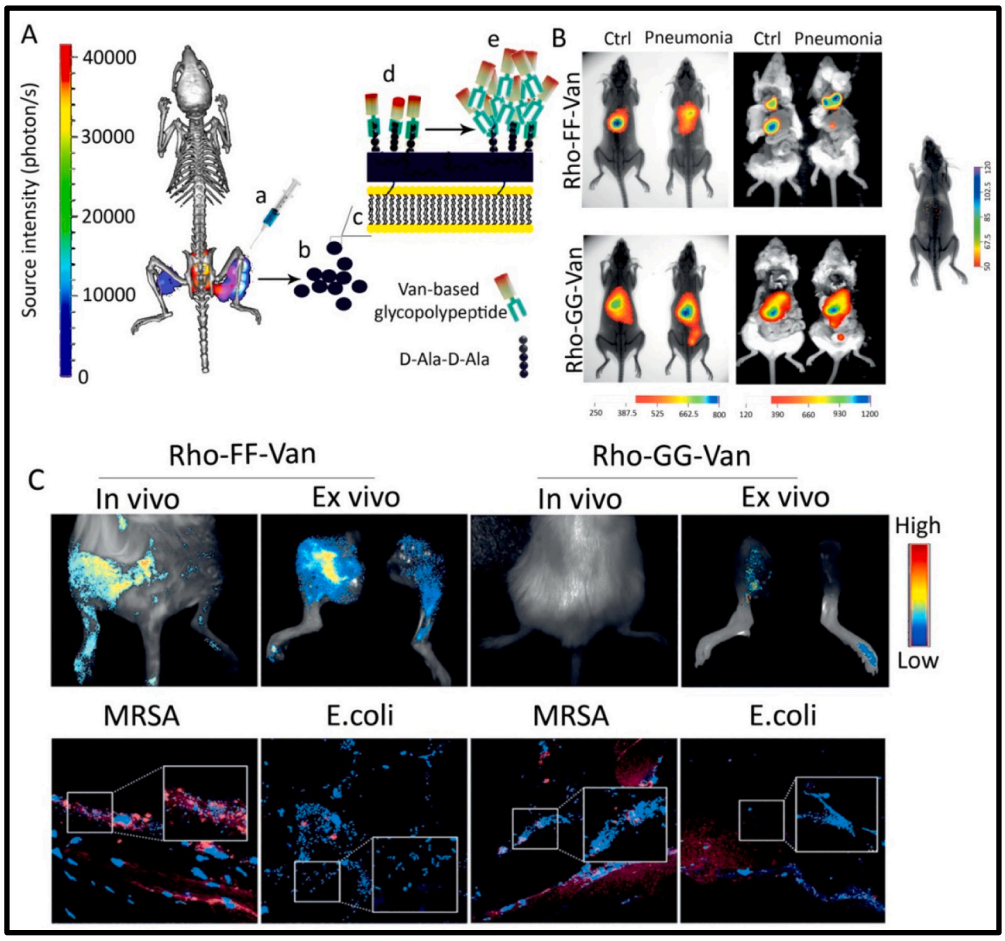

Fig. 5. Fluorescently labeled vancomycin (Van) for targeting and imaging of the infection by gram positive-bacteria. **(A)** Schematic representation of the experimental approach for Van-based (a) imaging mice infected with gram-positive cocci (b), and subsequent detection of the bacterial cell wall (c) with vanco-800CW binding to D-Ala-D-Ala site (d) on the surface and subsequent bacterial surface-induced self-assembly (e). A Micro-computed tomography (CT) imaging of the mouse used for schematic drawing with a detectable fluorescent signal from the bladder behind the spine; **(B)** *In vivo* imaging of mice with MRSA, (the images are including the overlay of X-ray and isotope signals (left) and the overlay of bright field and isotope signals after the opening of the thoracic and abdominal cavity (right)). The elimination of Rho-FF-Van from the mice is shown in the single figure at right. **(C)** *In vivo* and *ex vivo* imaging of infected mouse with *MRSA*-induced myositis after injection of Rho-FF-Van or Rho-GG-Van at upper row and CLSM images of infected tissue slices. Blue DAPI, Red rhodamine. Reproduced with permission from ref. [119]; Copyright 2017, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Glycopeptide-based applications in tissue engineering and wound healing.

Peptide moiety	Anchored Sugar Moiety	Final Feature	Hydrogel Method of Synthesis	Highlighted Results	Application	Ref.
Polylysine	Dextran	Injectable hydrogel embedded with micelles	self-assembly	Possessing stable rheological property, the hydrogel could realize the spatiotemporal delivery of DS and MF	Wound Healing	[94]
Peptide amphiphile (PA)	Series of Mono-saccharides	Nanofibers	self-assembly	The glycopeptide nanostructures amplified the signaling of bone morphogenic protein2 (BMP2) significantly more than the natural sulfated polysaccharide heparin, and promoted regeneration of bone in the spine with a protein dose that is 100-fold lower than that of required in the *in vivo* model	Multipotent protein activation	[131]
PA	Glucose	Supramolecular GAG-like nanofibers	self-assembly	Recognized through CD44 receptors, the supramolecular nanofibers promoted chondrogenesis differentiation in both *in vitro* and *in vivo* models	Cartilage regeneration	[132]
PPLG	Mannose	Injectable hydrogels	self-assembly	*In situ* formation of glycopeptide hydrogel were reported after subcutaneous injection into rats, cartilaginous specific matrix also were formed	Cartilage regeneration	[129]
Phe-Phe-Asp-Tyr	Glucose	Supramolecular hydrogel	self-assembly	Working as an effective matrix and releasing the DFO in sustain manner *in vitro*, the subcutaneous injected hydrogel could trigger the generation of new blood capillaries *in vivo*	Vascularization	[33]
Phe-Phe-Asp-Asp	Glucose	Supramolecular hydrogelators of glycopeptide	self-assembly	Exhibited great potentials to work as new biomimetic scaffolds using *in vitro* models	Scaffold for cell adhesion	[133]
Phe-Phe-Ser-Tyr	Mannose	Supramolecular nanofibers and hydrogels	self-assembly	The injectable hydrogel showed excellent antibacterial and wound healing using *in vitro* and *in vivo* skin defect mice model.	Wound healing	[134]

naphthyl moiety and Phe-Phe dipeptide were used to drive self-assembly, Asp amino acid was applied for sugar decoration and to help cell attachment and growth, and Tyrosine-phosphate was alkaline phosphatase substrate to mimic the glycosylated microenvironment of ECM (Fig. 6A). This glycopeptide gelator, which was prepared by a solid-phase approach, tended to form nano-filament structures with a high

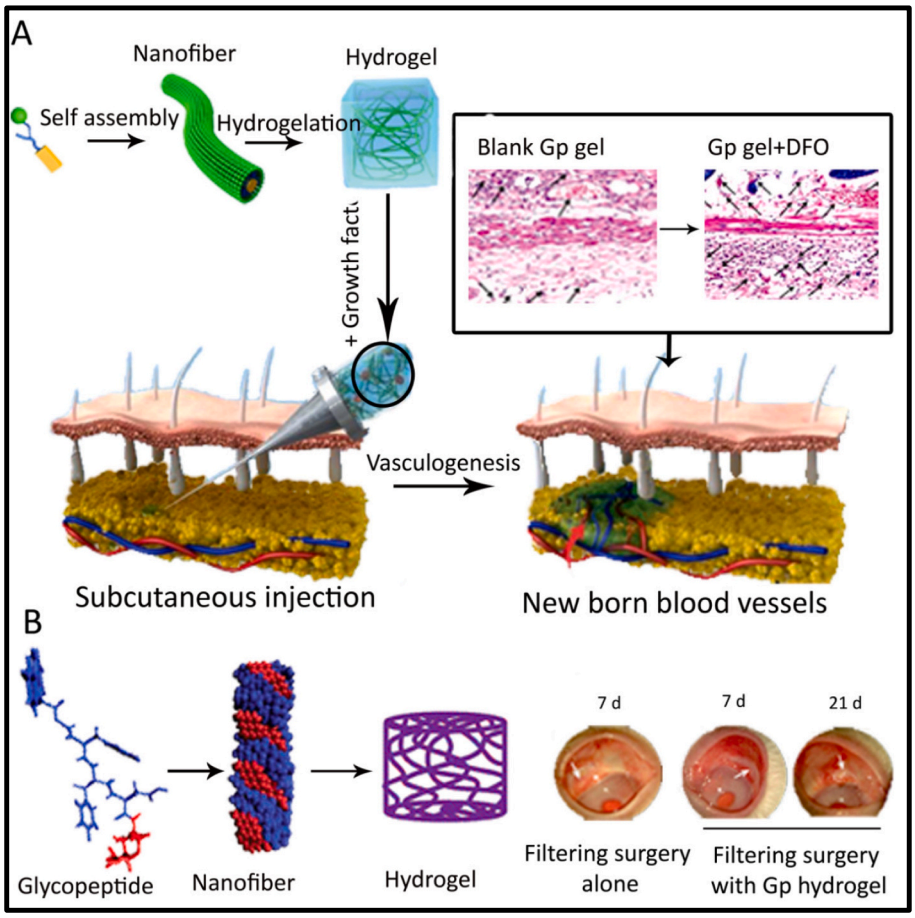

Fig. 6. Glycopolypeptide-based structures in tissue engineering. **(A)** Illustration of the self-assembly process of a glycopeptide to generate a supramolecular hydrogel with glucose moieties capable of inducing angiogenesis *in vivo*. The images in the box show hematoxylin and eosin-stained sections of the implanted glycopeptides gel without (left) and with (right) loading deferoxamine (DFO) on day 10. Arrows: Blood vessels; Reproduced with permission from ref. [33]; Copyright 2018 American Chemical Society. **(B)** The self-assembly illustration of glycopeptides to form hydrogel and photographic images of rabbit eyes with filtering surgery alone at 7 days and intraoperatively received the hydrogel at 7 and 21 days post-surgery. Arrows: the formation of blebs after surgery. Reproduced with permission from ref. [130]; Copyright 2012, Royal Society of Chemistry.

density of glucose moieties on the surface for adhesion and proliferation of human umbilical vein endothelial cells (HUVECs) and could serve as an efficient reservoir for a vascularization-promoting drug, Deferoxamine (DFO), to induce angiogenesis *in vitro* and *in vivo* [33].

Xu *et al.* [130] detailed the preparation of therapeutic glycopeptides hydrogels *via* self-assembly of the glycopeptides comprised of an N-fluorenyl-9-methoxycarbonyl phenylalanine-phenylalanine-aspartic acid (FMOC-Phe-Phe-Asp) sequence and glucosamine moiety. It was observed that the glucosamine moieties inhibited fibrosis, blebs, and filtration fistula after the administration of hydrogels in filtration surgery of rabbit eyes and decreased the intraocular pressure of the eyes 21 days after surgery. The developed hydrogels were characterized by TEM, AFM, CD, FTIR, and fluorescence spectroscopy to evaluate the interior morphology and 3D fiber network and the molecular arrangement of the glycopeptides (Fig. 6B).

Li *et al.* constructed a novel hydrogel composed of carboxyl pullulan and human-like collagen connected by 1,4-butanediol diglycidyl ether that made it possible to be used as soft fillers for tissue engineering. Given their highly tunable properties, they were also incorporated with hyaluronic acid to prepare a highly microporous structure that affected cell adhesion and biodegradability *in vivo*. It was shown that the cell attachment, anti-inflammatory responses, and biodegradability of the hydrogel made them a suitable choice for the treatment of skin defects [34]. In another study, site-specific glucose decoration was performed to prepare novel glycopeptide hydrogelators and elucidate how the self-assembly process is affected by glucose modification and the generation of as-prepared nanomaterials. Five molecules were designed and synthesized with a naphthyl moiety, a tetrapeptide segment (*e.g.*, Phe-Phe-Asp-Asp), and different glucose residues (*e.g.*, 2- amino-D-glucose). Results showed the high water content, ECM-like morphology and desirable biocompatibility. In addition, cell adhesion and proliferation behavior were obtained by sugar-lectin, and sugar-sugar interactions, which were investigated on NIH3T3 and HUVEC cell lines [32].

They expected that peptide glycosylation could offer an alternative way for peptide modification and generation of novel supramolecular hydrogelators and hydrogels with improved biostability, thermostability, and cell adhesion properties for biomedical applications. This group previously designed and synthesized a glycopeptides derivative including an aromatic moiety (*i.e.*, 9- fluorenylmethoxycarbonyl group), a carbohydrate head residue (*i.e.*, D-glucosamine) linked to the side chain of FmocAsp-OH, and a tert-butyl (Boc) group at the C terminal, to make self-assembling designs in different solvents *via* π-π stacking, hydrogen bonding, and van der Waals interactions.

4. Future perspectives and conclusions

The synthesis of a new version of biocompatible and biodegradable glycopolypeptide copolymers with self-assembly capability is a promising research area in biomaterial science and nanomedicine. The design of glycopolypeptide-based copolymers has become possible through impressive developments in polymer synthesis to create adjustable physicochemical, pharmacological, and biological characteristics. This field of study is still in its infancy, and there are many unknowns to be addressed for translation. Among various types of glycopeptide structures, glycopeptide-based tumor vaccines, designed against specific tumor-associated carbohydrate antigens (TACAs) have attracted special attention and are entering into several ongoing clinical trials [135]. Injectable glycopeptide hydrogels loaded with specific tumor antigens can be recruited as effective cancer vaccines due to wide antigen-adaptive and mild gelation conditions, effectively activating the

immune system to attract cancer cells. The mucin 1 (MUC1) family, for example, is the most widely studied in the context of cancer. PankoMab-GEX, a humanized glyco-optimized monoclonal antibody to a novel tumor-specific MUC1 glycopeptide epitope was introduced into the clinical trial phase for patients with advanced carcinomas [136]. Another fully synthetic glycopeptide human tumor vaccine named MAG-Tn3 has recently been introduced. MAG-Tn3 is a multiple antigenic glycopeptide dendrimer displaying a Tn trimer linked to short tetanus toxoid-derived P2 peptide that is already known to be universally immunogenic in mice and humans [137]. However, other glycopeptide-based products still faced limitations in translation to the clinical phase. To meet these limitations, applying cross-linkers or targeting ligands may be useful to enhance stability and blood circulation time, and improve accumulation at the target sites. Nature can be helpful to develop functional glycopolypeptides by studying the structures and functions, as well as synthesis routes of natural glycopeptides/proteins. In this regard, the synthesis of smart monomers utilizing natural resources, as well as the development of controllable polymerization procedures in environmentally friendly reaction mediums, is a necessity that should be explored. On the other hand, the biosafety of glycopolypeptide-based copolymers should be considered primary along with long-term toxicity and immunogenicity, especially in systemic administrations. So, it needs comprehensive information about the biocompatibility of building blocks and the resulting products after degradation. Moreover, the compatibility and the physicochemical properties of glycopolypeptides and their self-assembly behavior are a subject that has to be elucidated. Furthermore, most studies are performed under relatively static circumstances considering a limited number of variables *in vitro* compared with the dynamic condition of the body. To achieve a targeted smart carrier, multiple requirements should be considered including targeting ligands, high biostability, long blood circulation, triggered release in response to different stimuli, and biodegradability. In this regard, both the formulation behavior against specific stimuli and the distribution and concentration at the target site must be investigated in detail. Using controllable supra-molecular synthesis methods and self-assembled functional units of glycopeptides, it can be possible to produce biomimetic glycopolypeptides nano-assemblies with high performance for biomedical applications. Last but not least, a simple straightforward manufacturing process is always needed for translation and it takes a lot of effort in the design, fabrication, characterization, and optimization of copolymers.

Declaration of Competing Interest

The authors declare no conflict of interest in the present study.

Data availability

Data will be made available on request.

Acknowledgment

Mahsa Akbari acknowledges financial support from the School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran, under the framework of the thesis project (No. A-12129-69) with the ethical code of IR.ZUMS.REC.1400.438. Dr. Ebrahim Mostafavi would like to acknowledge the support from the National Institute of Biomedical Imaging and Bioengineering (5T32EB009035). Prof. Hélder A. Santos acknowledges financial support from UMCG Research Funds. Prof. Shahbazi acknowledges financial support from the Incentive Fund of the University of Groningen.

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