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A Brief Review on Hydrophobic Modifications of Glycol Chitosan into Amphiphilic Nanoparticles for Enhanced Drug Delivery

(Suatu Ulasan Ringkas pada Pengubahsuaian Hidrofobi Glikol Kitosan kepada Zarah Nano Amfifili untuk Penambahbaikan Penghantaran Dadah)

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ABSTRACT

Glycol chitosan (GC) is the chitosan derivative that is capable of forming amphiphilic nanoparticles upon structure modifications at the reactive functional amine group on the polymer sugar backbone. Owing to the hydrophilic feature of GC and hydrophobic moieties that can be added to the GC structure, modifiable nanosystems were constructed to entrap poorly soluble drugs, mostly chemotherapeutic agents and several anti-inflammatory, anaesthetic, immunosuppressant, and antifungal drugs for more efficient delivery of the payload to the target site and improving the intended therapeutic effects. This review highlights the various hydrophobic molecules used in the chemical modification of GC to create amphiphilic nanoparticles for hydrophobic drug delivery, along with the summary of their physicochemical criteria and successful therapeutic enhancement achieved with the application of the drug-loaded amphiphiles. The biodegradable, GC-based nanoparticles particularly having the inner hydrophobic core and outer hydrophilic shell are an efficient system for drug entrapment, protection and targeting to improve the bioavailability and safety of the drug, in particular for cancer treatment purposes. The significant drug delivery enhancements achieved by these various hydrophobically-modified GC nanoparticles may provide the insights for their further use in nanomedicine.

Keywords: Amphiphiles; drug delivery; glycol chitosan; hydrophobic drugs; nanoparticles

ABSTRAK

Glikol kitosan (GC) adalah bahan terbitan kitosan yang berupaya membentuk zarah nano amfifili dengan pengubahsuaian struktur kumpulan amina berfungsian reaktif pada tulang belakang gula polimer. Disebabkan sifat hidrofili GC dan moieti hidrofobi yang boleh ditambah pada struktur GC, nanosistem boleh ubah suai telah dicipta untuk memerangkap dadah kurang larut, kebanyakannya agen kemoterapi dan beberapa dadah antiradang, anestetik, imunosupresan dan antikulat untuk penghantaran bahan muatan tersebut ke tempat sasaran dengan lebih berkesan dan menambah baik kesan terapeutik yang dikehendaki. Ulasan ini menekankan pelbagai molekul hidrofobi yang digunakan dalam pengubahsuaian kimia pada GC untuk mencipta zarah nano amfifili dalam penghantaran dadah hidrofobi, berserta ringkasan kriteria fizikokimia dan penambahbaikan terapeutik yang berjaya dicapai dengan aplikasi amfifil berisikan dadah. Amfifil terbiodegradasikan berasaskan GC ini membentuk zarah nano secara khususnya mempunyai teras hidrofobi dan cangkerang hidrofili untuk pemerangkapan, perlindungan dan penyasaran dadah untuk menambah baik ketersediaan bio dan keselamatan dadah tersebut, secara khususnya untuk tujuan rawatan kanser. Penambahbaikan ketara dalam penghantaran dadah yang dicapai oleh pelbagai pengubahsuaian hidrofobi pada zarah nano GC ini mungkin dapat memberikan satu wawasan untuk penggunaannya yang lebih mendalam dalam bidang nanoperubatan..

Kata kunci: Amfifil; dadah hidrofobi; glikol kitosan; penghantaran dadah; zarah nano

INTRODUCTION

Hydrophobicity has been a main factor for the inefficient delivery of many drugs to the target sites. About forty percent of the marketed drugs are poorly soluble in aqueous solution, whereas ninety percent of drugs in the development stage can be classified as poorly soluble (Loftsson & Brewster 2010). Hydrophobic drugs (high log *P*) that have low solubility in water are not fully dissolved in the gut for absorption in the stomach upon oral administration. These hydrophobic drugs are also less soluble in the blood plasma thus affecting its transport via the blood stream to the tissues. The limited solubility in the gastrointestinal tract (GIT) and blood circulation could

lead to the low bioavailability of the drug which can also cause unnecessary toxicity to the body as higher dose need to be administered to reach the therapeutic concentration. Higher drug concentration being exposed to cells could lead to an increased possibility for drug resistance. The unabsorbed drugs would be eliminated from the body, thus reducing the residence time of the drug in the body. Most anticancer drugs in the market are hydrophobic and this has been hugely affecting their potency in the action against the cancerous tissues, as well as causing adverse effects to the patients.

Formulation of these poorly soluble marketed drugs into nano-sized particles or structures could enhance their solubility in aqueous media. The drug-loaded nanoparticles (NPs) offer protection to the entrapped drug molecules from premature degradation, as well as better drug targeting as the NPs could be designed to have surface features that enables targeting of selected cells and tissues. The use of biodegradable chitosan-based NPs is of preference as it is biocompatible with the microenvironment in the human body, non-toxic and non-carcinogenic (Karlsson et al. 2018; Nair & Laurencin 2007; Wang et al. 2011). Chitosan is also permeable through the epithelial layers thus enabling drug delivery into the targeted tumour tissues (Vivek et al. 2013).

In the recent years, a water-soluble chitosan derivative known as glycol chitosan (GC) has been used in many nanoparticle formulations as a drug carrier. The production of GC involved the reaction of chitin with ethylene oxide to incorporate the hydrophilic glycol group, followed by deacetylation process (Choi et al. 2019). The hydrophilic ethylene glycol (EG) group on the GC sugar backbone contributes to the steric stabilization and good solubility in broad range of pHs (Ghaz-Jahanian et al. 2015; Trapani et al. 2009). The free amine and hydroxyl groups on the GC structure have been mostly utilized for chemical modifications of the polymer to suit the need of the intended NPs (Kim et al. 2017).

For the purpose in improving the formulation of hydrophobic drugs, GC polymer was modified in ways to form NPs that able to increase the bioavailability of hydrophobic drugs for various therapeutic purposes. Most common GC modification is by the attachment of hydrophobic moiety to the hydrophilic GC sugar backbones, mainly to the free amine groups. This leads to the formation of amphiphilic NPs that are capable to self-assemble in aqueous solution. The amphiphiles, which can be in the form of polymeric micelles, tubules or vesicles, form a structure of outer hydrophilic shell and inner hydrophobic core. The presence of the hydrophobic moiety at the core of the amphiphiles attracts the hydrophobic drugs to reside at the inner part structure of the NPs. This allows the amphiphilic GC NPs to encapsulate and protect the hydrophobic drugs for the transport of the drug load to the intended sites.

This review focuses on the reported GC-derived amphiphiles that were capable of self-assemble in aqueous solution into nanometre-range particles. The GC amphiphiles were produced from the attachment or conjugation of various hydrophobic moieties to the hydrophilic GC structure (Figure 1). In this review, the criteria of the GC-based NPs before and after the incorporation of various hydrophobic drugs were elaborated, along with the improvement of therapeutic effects following the formulation of the associated drugs into the GC-based NPs.

HYDROPHOBIC MODIFICATIONS OF GC POLYMER

PALMITIC ACID

Attachment of the palmitic acid chains (palmitoylation) for the creation of hydrophobic moieties on the GC backbones was attempted (Uchegbu et al. 2001). The conjugation of the hydrophobic 16-carbon palmitic chains to the amino groups on the sugar chain of GC involved the reaction with palmitic acid N-hydroxysuccinimide (PNS). The resulted chitosan-based amphiphilic polymers known as quaternary ammonium palmitoyl glycol chitosan (GCPQ) consists of hydrophobic domains contributed by the palmitoyl chain, while the hydrophilic domains contributed by quaternary ammonium and ethylene glycol units (Ahmad et al. 2010). Increased GCPQ molecular weight was reported to produce more stable micelles as the lengthy GCPQ chain provides more hydrophobic intra and inter-molecular contacts. The self-assembled GCPQ micelles without encapsulating any substances was between 20-30 nm (Qu et al. 2006). The GCPQ aqueous dispersions displayed viscoelastic behaviour at high concentration due to the inter-micellar network formations, which is influenced by the molecular weight and hydrophobicity of the polymer (Chooi et al. 2014).

Critical micelle concentration (CMC) is a crucial indicator for the NPs stability in diluted media. The low CMC prevents the NPs from disaggregating upon dilution in the GIT following oral administration or in blood circulation upon intravenous administration. The CMC for GCPQ was reported at ~20 μM (~0.2 to 0.4 mg mL^{-1}) (Chooi et al. 2014). The higher level of hydrophobic substitutions (more hydrophobic regions) in GCPQ has led to a lower CMC, whereas low molecular weight GCPQ had higher CMC value (Siew et al. 2012).

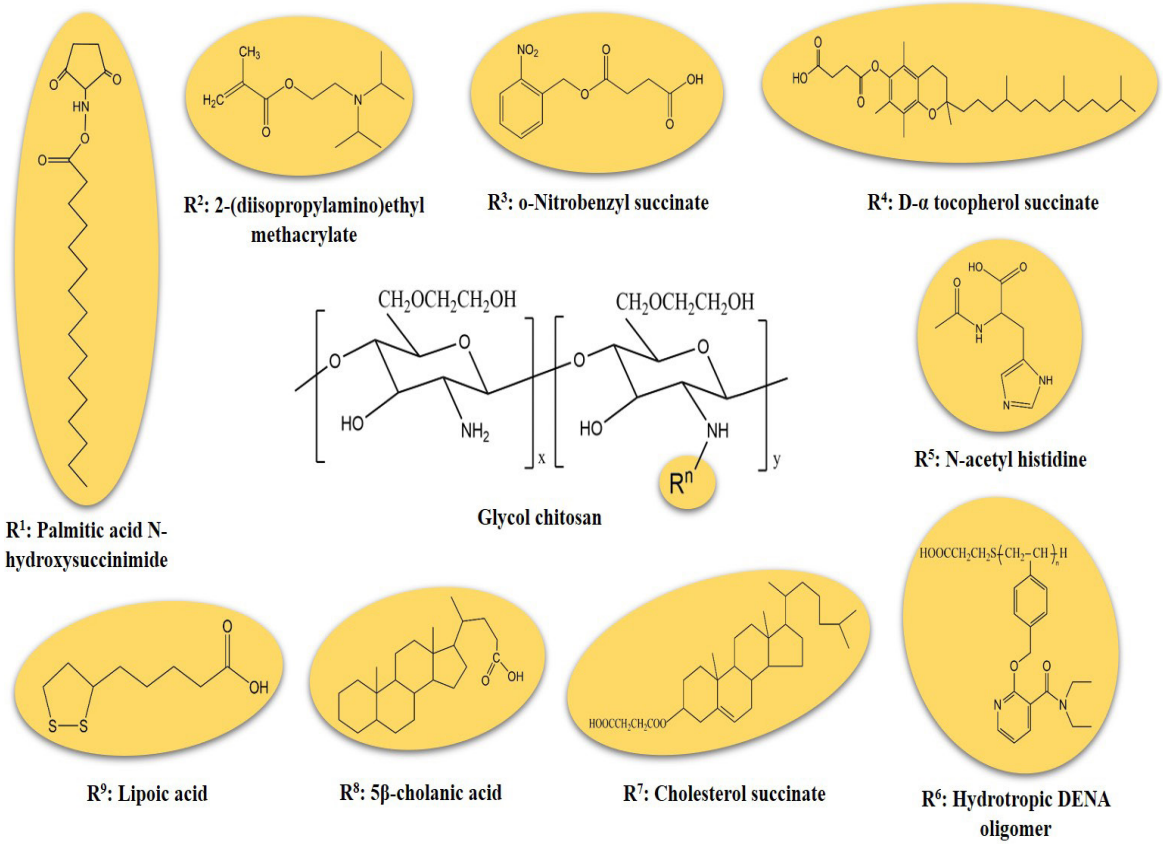

FIGURE 1. The schematic diagram shows the chemical structure of GC and various hydrophobic molecules that were utilized in hydrophobic modification of GC. R^n represents the different hydrophobic moeities of either R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9 or the reduced albumin (not included in the figure) attached at the amine group on GC to form the amphipilic NPs (adapted from Duhem et al. (2012), Kim et al. (2017), Lee et al. (2010), Meng et al. (2013), Razi et al. (2019), Saravanakumar et al. (2009), Uchegbu et al. (2001), Yang et al. (2018), Yu et al. (2008) and Zhou et al. (2016))

The presence of hydrophobic drugs influences the GCPQ polymer aggregates to form dense NPs rather than micellar cluster and encapsulate greater amount of drug due to the formation of more apolar domains. This supports the later findings of the increment in degree of palmitoylation of the GC polymer enables encapsulation of higher drug concentration due to high hydrophobicity in the inner core of the self-assembled NPs (Chooi et al. 2014). Ocular anti-inflammatory drug prednisolone and the intravenous anaesthetic propofol were encapsulated with GCPQ. Propofol (4.4 mg mL^{-1}) was encapsulated inside the hydrophobic inner core of GCPQ (5 mg mL^{-1}) and produced NPs at average size of 50-200 nm. On the other hand, 1 mg/mL^{-1} prednisolone was encapsulated with GCPQ and formed a mixture of micellar clusters and dense larger particles. The GCPQ has increased the concentration of drugs being transported across the cornea and to the brain hence increased the bioavailability of the drugs (Qu et al. 2006). Coarse-grained molecular dynamics simulations (MARTINI force field) used to model GCPQ in aqueous media showed that the hydrophobic drug propofol was found to be residing at the interface between the hydrophilic and hydrophobic portions in the micelles and distributed heterogeneously within the micelles (Ahmad et al. 2010).

The formulation of cyclosporin A and griseofulvin (low water solubility, high gut permeability) with GCPQ increased the oral bioavailability of both drugs (Siew et al. 2012). As reported by the same authors, GCPQ was able to promote absorption of hydrophobic drugs, as well as the hydrophilic ones, by enhancing the dissolution of the hydrophobic drugs, bioadhesion of the NPs to the GIT

and promote transcellular transport. The bioadhesion of the NPs causes prolonged contact between the NPs with absorptive enterocytes in the GIT, thus, allowing the transport of the drug into the epithelial cells through the mucous layer. Stable GCPQ NPs that encapsulated hydrophobic drugs also have high surface areas for faster drug dissolution.

Mennini et al. (2014) reported GCPQ formulations with anti-inflammatory drug celecoxib had strongly improved the drug solubility by 60-fold, as well as shortened the pain alleviation onset upon administration of the micellar dispersion to the mice. The bioavailability of anticancer drug doxorubicin (DOX) was improved upon incorporation with GCPQ, as well as increasing the drug accumulation in the skin tumour xenografts due to the small particle size and colloidal stability of the nanoformulation. Slow release of DOX from the GCPQ NPs was attributed to the higher drug accumulation at the tumour site (Kanwal et al. 2018).

Formulation of lomustine with GCPQ into Molecular Envelope Technology (MET) was developed for brain delivery. The MET formulations were able to improve the lomustine level in the brain by 2-fold, suggesting the transport of lomustine-contained MET across the blood-brain barrier (BBB). The effective MET-lomustine formulations had also increased the survival time in mice with glioblastoma multiforme tumours (Fisusi et al. 2016).

5β-CHOLANIC ACID

5β-cholanic acid is a type of bile acid that is known for its importance in the emulsification of lipophilic vitamins, cholesterol and fats for absorption. The attachment of 5β-cholanic acid to the GC backbone via the formation of amide bond was introduced by Kwon et al. (2003). The attachment was done with the presence of 1-ethyl-3(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS). The resulted hydrophobically modified GC (HGC) was able to self-aggregate in aqueous solution and later was used to encapsulate various types of hydrophobic drugs, which were mainly the anticancer drugs such as paclitaxel (PTX), camptothecin (CPT), docetaxel (DTX) and cisplatin (CDDP).

Kim et al. (2006) formulated HGC with PTX to produce PTX-HGC NPs that recorded 90% drug loading efficiency at the average particle size of 400 nm. PTX-HGC showed a sustained release of loaded PTX for 8 days at 37 °C in phosphate buffered saline (PBS). The PTX-HGC however, showed lower cytotoxicity against the MCF-7 breast cancer cells compared with cells treated with PTX formulated with Cremophor EL. Nevertheless, the administration of PTX-HGC at 50 mg kg^{-1} was able to reduce the volume of melanoma tumours in mice at faster rate compared to the reduction by 20 mg kg^{-1} Cremophor EL-based PTX.

In a later PTX-HGC study by Kim et al. (2010), the HGC was labelled with near-infrared fluorescent dye Cy5.5 prior to the formulation with 10 wt.% PTX to produce PTX-encapsulated-chitosan-based NPs (PTX-CNPs). The drug loading efficiency and average particle size of the Cy5.5-labelled PTX-CNPs was at 92% and approximately 260 nm, respectively. The PTX-CNPs was able to keep the desired physicochemical criteria of the HGC-based NPs whilst having the added theragnostic function that allows non-invasive monitoring and localization of the NPs in the tumour.

Water-insoluble CPT was encapsulated into HGC NPs (CPT-HGC) to produce self-aggregated NPs in aqueous medium (Min et al. 2008). The NPs were recorded at the size range of 280-330 nm with drug loading efficiency of more than 80%. CPT-HGC showed a sustained CPT release for 1 week. The *in vivo* study showed that Cy5.5-labelled CPT-HGC was able to reduce tumour volume and growth rate of the subcutaneous MDA-MB231 xenografts.

Hwang et al. (2008) encapsulated DTX with HGC to form self-assembling DTX-HGC NPs at the size of 350 nm in aqueous solution. The NPs were reported stable in physiological conditions for 2 weeks and in the presence of excess bovine serum albumin (BSA). The DTX-HGC formulation showed anti-tumour efficacy in A549 lung cancer cells-bearing mice by reducing the tumour volume and improved the animal survival rate.

HGC was also used to encapsulate CDDP to form CDDP-HGC NPs. Drug loading efficiency of the NPs was recorded at about 80% with mean particle diameter of 300 to 500 nm. The CDDP also recorded sustained drug release for over a week. The Cy5.5-labelled CDDP-HGC were seen accumulated in the squamous cell carcinoma (SCC7) cell-derived tumours and therefore could have been the reason of the significant antitumour efficacy in the cancer-bearing mice (Kim et al. 2008).

CHOLESTEROL

Yu et al. (2008) incorporated cholesterol succinate to GC with the presence of EDC and NHS to prepare cholesterol-modified GC (CHGC) conjugates with succinyl linkages. The CHGC conjugates were able to self-aggregate into amphiphilic, monodisperse and roughly spherical CHGC NPs in aqueous medium. Indomethacin (IND) was used as the model hydrophobic drug in the formulation of IND-

loaded CHGC (IND-CHGC) NPs. The loading capacity of IND was reported at 7.14 to 16.2% for 1/10 to 6/10 IND/CHGC weight ratios. The size of the IND-CHGC was increased towards the higher drug content (275 to 384 nm). The NPs showed 60% *in-vitro* drug release within 2 h and up to 98% in 12 h.

Yu et al. (2009) later developed doxorubicin-loaded CHGC NPs (DCNs) that self-assembled in aqueous solution, forming particles with mean diameter of 237 to 336 nm for DCNs with 9.36% drug content (DCN-16). An increase in drug release was noted in more acidic PBS medium (pH 5.5). DCN-16 exhibited delayed blood clearance as its circulation time in rat plasma is prolonged, as well as causing suppression of murine sarcoma S180 tumour growth in comparison to the free drug treatment.

The CHGC was later conjugated with prostate cancer-binding peptides (PCP) into PCP-CHGC micelles for targeting of prostate-specific membrane antigen (PSMA) (Xu et al. 2014). The degree of substitution for PCP and cholesterol groups per 100 GC sugar residues was 5.2 and 5.8, respectively. The PCP-CHGC was loaded with DOX and recorded more than 80% drug encapsulation efficiency with particle size at 293 nm. The drug release behaviour of the NPs showed a biphasic pattern in PBS which demonstrated initial rapid release in the first 6 h, followed by sustained release up to 72 h. The DOX-PCP-CHGC was taken up by the PSMA-positive, prostate cancer cell lines LNCaP cells through endocytosis, proving that the PCP-conjugated CHGC micelles helped in the cellular uptake of the NPs. The NPs were also seen accumulated in the LNCaP cancer xenografts and caused significant inhibition to the growth of the tumour.

TOCOPHEROL SUCCINATE

Duhem et al. (2012) conjugated D-α tocopherol succinate (TOS) to the GC structure via the formation of amide bond between the carboxyl groups of TOS with the primary amine group of GC, also with the presence of EDC and NHS to form GC-TOS NPs. GC-TOS was reported as non-cytotoxic at the concentration of 10 mg mL^{-1} and capable of increasing the solubility of hydrophobic antifungal drugs itraconazole and ketoconazole with loading efficiency of 0.14 and 2.4%, respectively. The ketoconazole encapsulated with GC-TOS was subjected for cytotoxicity assay and permeation study using Caco-2 cell monolayers and showed low toxicity against the cells as proven by the low release of lactate dehydrogenase (LDH) (<4%), as well as causing 3.4-fold increase in the drug absorption through the cell monolayers.

N-ACETYL HISTIDINE

Park et al. (2006) attached hydrophobic moiety of fusinogen N-acetyl histidine (NAcHis) to the hydrophilic GC structure to create amphiphilic NAcHis-GC NPs at the size range of 150 to 250 nm. The NPs internalized by the cell via adsorptive endocytosis were either exocytosed or localized in the endosomes. In acidic endosomes, the NPs disassembled to release the drug load directly into the cytosol due to the loss of hydrophobicity caused by protonation of the imidazole groups. This mechanism of action suggests the release of incorporated PTX from the NAcHis-GC NPs into the cancer cells and induced cell growth arrest.

In another study wherein the NAcHis-GC was loaded with DOX, the loading efficiency was reported at 47 to 64% with NP mean diameter of 220 to 292 nm. The DOX-loaded NPs also exhibited triggered drug release in the weakly acidic condition (pH 6.4). The xenograft animal model used to study the efficacy of the NPs showed better tumour targeting and therapeutic effect on the human colon cancer HT29 tumours by the drug-loaded NPs compared with the free DOX treatments. The results suggested that the hydrophobic core stability of the NPs determines their targeting efficiency to the tumours (Lee et al. 2010).

REDUCED ALBUMIN

The negative charge of albumin interacts with positively charged chitosan, leading to the fabrication of reduced BSA and GC nanocomplexes (rBG-NPs) (Razi et al. 2019). The reduced state of albumin increases its hydrophobicity, as well as the availability of the free thiol groups leading to higher hydrophobic interactions between the GC and BSA. The thiol cross-linking has also contributed to the high stability of rBG-NPs in neutral pH environment. Upon encapsulation of PTX, the size of rBG-NPs was recorded at approximately 400 nm with encapsulation efficiency as high as ~90% with low polydispersity index (PDI) value of 0.2. Following the endocytosis of rBG-NPs into the HeLa cells, *in vitro* cytotoxicity on the cancer cells was observed after a 48 h treatment.

O-NITROBENZYL SUCCINATE

The hydrophobic o-nitrobenzyl succinate (NBS) is a light-sensitive chromophore used in the formation of amphiphilic glycol chitosan-o-nitrobenzyl succinate conjugates (GC-NBSCs) (Meng et al. 2013). The GC-NBSCs was further stabilized with crosslinking of the GC shells with acid-labile glutaraldehyde to form

crosslinked GC-NBSC polymeric micelles. The light-responsive NBS undergoes photolysis upon exposure to near-infrared light or UV light, whereas glutaraldehyde is cleaved in the acidic environment. These dual stimuli responsive behaviour of the NPs allowed the controlled drug release under stimulation of pHs and lights. The GC-NBSCs incorporated with CPT showed a rather low drug loading efficiency of 5%. Nevertheless, the *in vitro* drug release study showed higher CPT release during the first hour of simultaneous exposure to pH 5.0 and UV irradiation compared to the release upon only either one stimulus was applied. The cumulative release of the NPs was recorded at 81.68% after 12 h. The blank NPs were also found biocompatible as the cell viability was at 75% for NP concentration up to 1 mg mL^{-1}. The CPT-loaded GC-NBSC polymeric micelles showed better cytotoxicity against MCF-7 cancer cells under UV irradiation compared with the nonirradiated ones.

LIPOIC ACID

Lipoic acid (LA), a hydrophobic vitamin analogue, has been used in the synthesis of glycol chitosan-lipoic acid conjugates (GC-LA) via formation of amide bonds between the amino group of GC and carboxyl groups of the lipoic acid (Zhou et al. 2016). The substitution degree of GC-LA was 8.3 lipoic acid groups per 100 sugar units of GC with CMC for GC-LA at 0.081 mg mL^{-1}. The disulphide-containing lipoic ring of LA enabled the GC-LA for further cross-linking using dithiothreitol (DTT) to form stimuli responsive GC-LA/cc micelles.

The micelles were loaded with DOX to form DOX-GC-LA/cc and recorded a size of 408 nm upon drug loading with 10.74% of loading content. The DOX-GC-LA/cc micelles destabilize in the presence of reducing agent such as glutathione (GSH) to release the drug load. This was proven as the DOX-loaded crosslinked micelles demonstrated 45.5% and full DOX release at 12 and 96 h timepoints, respectively, upon exposure to PBS containing 20 mM GSH. The DOX release by the crosslinked micelles was higher than the release from non-crosslinked GC-LA micelles in PBS without the GSH. It was postulated that in the presence of GSH such as in the reducing environment of the cancerous cells, the polydisulfide crosslinking disconnected to release the drug load and therefore enabling the targeted drug release function of the NPs.

The GC-LA was also attached with PCP to create PGC-LA micelles and later core-crosslinked PGC-LA micelles (PGC-LA/cc) with the catalysis by DTT (Feng et al. 2019). The degree of substitution for PCP and lipoic acid groups per 100 GC sugar residues was 5.2 and 10.7, respectively. The crosslinked PGC-LA/cc were loaded with DTX and exhibited PSMA-targeting and better growth inhibition of the LNCaP tumour xenograft compared with the inhibition by other DTX-loaded micelles in the study.

N,N-DIETHYLNICOTINAMIDE

Saravanakumar et al. (2009) uses a water-soluble compound known as hydrotropic agent to increase water solubility of hydrophobic substances in the production of GC-based polymeric NPs. A nicotinamide derivative known as N,N-diethylnicotinamide (DENA) was used as the hydrotropic agent for dissolution of PTX, which also act as the hydrophobic cores in the making of hydrotropic DENA oligomer-conjugated GC (HO-GC) nanoparticles. The HO-GC self-assembled to form particles with the size of ±300 nm in aqueous solution. The structure of HO-GC remained unchanged up to 50 days in PBS. PTX was loaded into the HO-GC to form HO-GC-PTX that had 94.7% drug-loading efficiency when the feed weight ratio of PTX to HO-GC was at 20 wt.%. The HO-GC-PTX reduced the viability of human breast cancer cells MDA-MB231 and was seen dispersed in the cytoplasm parts of the cell. *In vivo* study using mice xenograft model showed accumulation of the NPs in subcutaneous tumours induced by the inoculation of squamous cell carcinoma (SCC7). Koo et al. (2013) reported that HO-GC-PTX was able to move from the tumour vessels to the surrounding tumour cells, suggesting the enhanced permeability and retention effect of the NPs in living tissues.

2-(DIISOPROPYLAMINO)ETHYL METHACRYLATE (DPA)

The 2-(diisopropylamino)ethyl methacrylate (DPA) was used in the preparation of GC-based poly-DPA (PDPA) grafted copolymers via free radical copolymerization process (Yang et al. 2018). The GC-PDPA-grafted copolymers (GCNP) exist in the form of core-shell NPs that spontaneously self-assemble in aqueous solution. The PDPA is hydrophobic at pH 7.4, such as in the physiological environment, but turns hydrophilic at pH lower than 6.3, such as in the cellular microenvironment of the tumours. This enabled the NPs to maintain their structure during the transport and dissociate only in the appropriate pH in cancer cells to release the drug load. The GCNP was conjugated with estrone (ES) at the amino group of GC to form GCNP-ES that is able target the breast cancer cells. GCNP-ES was significantly taken up more by the estrogen receptor-positive MCF-7 cells compared to GCNP, suggesting that the ES modification increased

the NP cellular uptake via endocytosis. Both GCNP and GCNP-ES recorded average particle size between 110 and 180 nm with narrow PDI of less than 0.3. As the NPs were loaded with PTX, the size had increased to 220 to 265 nm with PTX/GCNP-ES showed higher encapsulation efficiency than PTX/GCNP (92.4 and 85.4%, respectively). Both PTX/GCNP and PTX/GCNP-ES prolonged the circulation and improved bioavailability of PTX with the latter NPs caused accumulation of the drug in the breast cancer xenografts and causing superior antitumour activity against the tumour growth.

TABLE 1. Overall properties of hydrophobically modified GC-based amphiphilic nanoparticles upon incorporation with hydrophobic drugs

Hydrophobic molecules	Incorporated drug	Drug loading efficiency	Particle size	Drug release	*In vitro* cellular study	*In vivo* effects	Reference
Palmitic acid	Prednisolone	<5%	10-100 nm	-	-	Rabbits; facilitated drug absorption across the cornea to the aqueous humor	Qu et al. (2006)
	Propofol	44%	50-200 nm	-	-	Mice; drug delivery to the central nervous system	
	Cyclosporin A	~14%	100-500 nm	60% enhancement of tablet dissolution rate	No paracellular permeability across Caco-2 cell mono layer, no opening of tight junctions	Rats; enhanced oral absorption of drugs	Siew et al. (2012)
	Griseofulvin	~4%		-			
	Celecoxib	15-47%	200-300 nm	-	No cytotoxicity against SH-SY5Y cell line	Mice; shortened pain alleviation onset, prolonged pain reduction effect	Mennini et al. (2014)
	Doxorubicin	80%	98 nm	Sustained	Cytotoxic against RD cells	Accumulation of NPs in skin xenografts	Kanwal et al. (2018)
	Lomustine	-	50-600 nm	-	Slower NPs uptake by macrophages	Mice; reduced myelosuppression, increased survival time	Fisusi et al. (2016)
5β-cholanic acid	Paclitaxel	90%	400 nm	Sustained	Cytotoxic against MCF-7 cells	Mice; reduced melanoma tumour volume	Kim et al. (2006)
	Paclitaxel	92%	310 nm	Sustained	Cytotoxic against SCC7 cells	Mice; reduced SCC7 tumour volume	Kim et al. (2010)
	Camptothecin	>80%	280-330 nm	Sustained	Cytotoxic against MDA-MB231 cells	Mice; reduced xenograft MDA-MB231 tumour volume and growth rate	Min et al. (2008)
	Docetaxel	90%	350 nm	Sustained	-	Mice; reduced xenograft A549 tumour volume, increased survival rate	Hwang et al. (2008)
	Cisplatin	80%	300-500 nm	Sustained	Cytotoxic against SCC7 and A549 cells	Mice; reduced xenograft SCC7 tumour volume, low toxicity	Kim et al. (2008)

Cholesterol	Indomethacin	7-16%	275-384 nm	Sustained	-	-	Yu et al. (2008)
	Doxorubicin	1.7-9.4%	237-336 nm	Sustained	-	Rat; prolonged plasma circulation time Mice; suppressed sarcoma S180 tumour growth	Yu et al. (2009)
	Doxorubicin (in PCP-conjugated NPs)	>80%	293 nm	Sustained	Cytotoxic against the LNCaP cells	Mice; inhibited LNCaP xenograft tumour growth	Xu et al. (2014)
Tocopherol succinate	Itraconazole	0.1%	101 nm	-	-	-	Duhem et al. (2012)
	Ketoconazole	2.4%		Sustained	Low cytotoxicity against Caco-2 cells	-	
N-acetyl histidine	Paclitaxel	61-64%	-	Sustained	Cytotoxic against A549 and MDA-MB231 cells	-	Park et al. (2006)
	Doxorubicin	47-64%	220-292 nm	Sustained at pH 7.4, rapid in acidic pH 6.4 medium	-	Mice; reduction in HT29 tumour volume	Lee et al. (2010)
Reduced albumin	Paclitaxel	84-96%	349-421 nm	-	Cytotoxic against HeLa cells	-	Razi et al. (2019)
O-nitrobenzyl succinate	Camptothecin	5%	15-71 nm	Triggered upon UV irradiation in pH 5.0 medium	Biocompatible with NIH/3T3 cell, cytotoxic against MCF-7 cells	-	Meng et al. (2013)
Lipoic acid	Doxorubicin	60-64%	305-408 nm	Sustained at pH 7.4, rapid upon presence of glutathione	Cytotoxic against A549 cells	-	Zhou et al. (2016)
	Docetaxel (in PCP-conjugated NPs)	61%	397 nm	Sustained	Cytotoxic against LNCaP cells	Mice; inhibited LNCaP xenograft tumour growth	Feng et al. (2019)
N,N-diethylnicotinamide	Paclitaxel	94.7%	331-363 nm	Sustained	Cytotoxic against MDA-MB231 cells	Mice; accumulation of NPs in MDA-MB231 xenografts	Saravanakumar et al. (2009)
	Paclitaxel	78.4-96.8%	343 nm	Sustained	Cytotoxic against MDA-MB231 cells	Mice; accumulation of NPs in SCC7 xenografts	Koo et al. (2013)
2-(diisopropylamino) ethyl methacrylate	Paclitaxel (in ES-conjugated NPs)	92.4%	220-265 nm	Sustained	Cytotoxic against MCF-7 cells	Mice; inhibited MCF-7 xenograft tumour growth	Yang et al. (2018)

PCP = prostate cancer-binding peptides, ES = estrone

Conclusion

From the review, it can be summarized that GC is a unique polymer that can be modified with various hydrophobic molecules for the formation of GC-based amphiphilic NPs (Table 1). The GC amphiphiles helped in improving the efficacy of chemotherapeutic drugs, as well as anti-inflammatory, anaesthetic, immunosuppressant and antifungal drugs. The preference of improving the therapeutic effect of anticancer drugs is related to the issues involving inadequate chemotherapeutic drug concentration in the systemic circulation due to premature degradation and metabolism, non-specific distribution of drugs to other places other than the target tumour site and unnecessary cytotoxicity to the non-cancerous cells (Amreddy et al. 2018). The increased hydrophobic regions on the GC structure had been linked to the increased encapsulation of hydrophobic drugs and improved stability of the GC NPs in aqueous solutions. The low CMC values of the GC amphiphiles ensured the stability of the NPs upon dilution in the blood circulation. Most of the GC amphiphiles were capable of encapsulating high amount of drug at the centre hydrophobic core of the NPs while maintaining the size at the nanometre range. The GC amphiphiles also displayed controlled drug release behaviour upon stimulations, such as by the light exposure for the NPs aided with light-sensitive chromophore, as well as upon presence of reducing agent GSH and acidic pH environment. The GC NPs were also being delivered to and accumulated at the targeted cancerous tumours with or without conjugation with cancer-specific peptides. The increased efficacy of the anticancer drugs upon formulation with GC amphiphiles was mostly due to the internalization of the NPs into the cancerous cells *in vitro* and *in vivo*.

In conclusion, the hydrophobic modifications of the hydrophilic GC structure had allowed the formation of GC-based amphiphiles capable of encapsulating hydrophobic drugs for protection and targeted delivery of the active ingredients. The GC amphiphilic NPs are therefore proven to have the criteria suitable for improving the therapeutic efficacy of hydrophobic drugs via enhancement of the drug delivery system. These compiled and summarized findings may provide a strong background for further research and development of GC-based amphiphiles for wider application in nanomedicine.

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References

Ahmad, S., Johnston, B.F., Mackay, S.P., Schatzlein, A.G., Gellert, P., Sengupta, D. & Uchegbu, I.F. 2010. *In silico* modelling of drug-polymer interactions for pharmaceutical formulations. *Journal of the Royal Society Interface* 7(4): S423-S433.

Amreddy, N., Babu, A., Muralidharan, R., Panneerselvam, J., Srivastava, A., Ahmed, R., Mehta, M., Munshi, A. & Ramesh, R. 2018. Recent advances in nanoparticle-based cancer drug and gene delivery. *Advances in Cancer Research* 137: 115-170.

Choi, Y., Lim, S., Yoon, H.Y., Kim, B.S., Kwon, I.C. & Kim, K. 2019. Tumor-targeting glycol chitosan nanocarriers: Overcoming the challenges posed by chemotherapeutics. *Expert Opinion on Drug Delivery* 16(8): 835-846.

Chooi, K.W., Carlos, M.I.S., Soundararajan, R., Gaisford, S., Arifin, N., Schätzlein, A.G. & Uchegbu, I.F. 2014. Physical characterisation and long-term stability studies on quaternary ammonium palmitoyl glycol chitosan (GCPQ) - A new drug delivery polymer. *Journal of Pharmaceutical Sciences* 103(8): 2296-2306.

Duhem, N., Rolland, J., Riva, R., Guillet, P., Schumers, J.M., Jérome, C., Gohy, J.F. & Préat, V. 2012. Tocol modified glycol chitosan for the oral delivery of poorly soluble drugs. *International Journal of Pharmaceutics* 423(2): 452-460.

Feng, X., Zhou, Y., Xie, X., Li, M., Huang, H., Wang, L., Xu, X. & Yu, J. 2019. Development of PSMA-targeted and core-crosslinked glycol chitosan micelles for docetaxel delivery in prostate cancer therapy. *Materials Science and Engineering: C* 96: 436-445.

Fisusi, F.A., Siew, A., Chooi, K.W., Okubanjo, O., Garrett, N., Lalatsa, K., Serrano, D., Summers, I., Moger, J., Stapleton, P. & Satchi-Fainaro, R. 2016. Lomustine nanoparticles enable both bone marrow sparing and high brain drug levels - A strategy for brain cancer treatments. *Pharmaceutical Research* 33(5): 1289-1303.

Ghaz-Jahanian, M.A., Abbaspour-Aghdam, F., Anarjan, N., Berenjian, A. & Jafarizadeh-Malmiri, H. 2015. Application of chitosan-based nanocarriers in tumor-targeted drug delivery. *Molecular Biotechnology* 57(3): 201-218.

Hwang, H.Y., Kim, I.S., Kwon, I.C. & Kim, Y.H. 2008. Tumor targetability and antitumor effect of docetaxel-loaded hydrophobically modified glycol chitosan nanoparticles. *Journal of Controlled Release* 128(1): 23-31.

Kanwal, U., Bukhari, N.I., Rana, N.F., Rehman, M., Hussain, K., Abbas, N., Mehmood, A. & Raza, A. 2019. Doxorubicin-loaded quaternary ammonium palmitoyl glycol chitosan polymeric nanoformulation: Uptake by cells and organs. *International Journal of Nanomedicine* 14: 1-15.

Karlsson, J., Vaughan, H.J. & Green, J.J. 2018. Biodegradable polymeric nanoparticles for therapeutic cancer treatments.

Annual Review of Chemical and Biomolecular Engineering 9: 105-127.

Kim, J.H., Kim, Y.S., Park, K., Lee, S., Nam, H.Y., Min, K.H., Jo, H.G., Park, J.H., Choi, K., Jeong, S.Y. & Park, R.W. 2008. Antitumor efficacy of cisplatin-loaded glycol chitosan nanoparticles in tumor-bearing mice. *Journal of Controlled Release* 127(1): 41-49.

Kim, J.H., Kim, Y.S., Kim, S., Park, J.H., Kim, K., Choi, K., Chung, H., Jeong, S.Y., Park, R.W., Kim, I.S. & Kwon, I.C. 2006. Hydrophobically modified glycol chitosan nanoparticles as carriers for paclitaxel. *Journal of Controlled Release* 111(1-2): 228-234.

Kim, K., Kim, J.H., Park, H., Kim, Y.S., Park, K., Nam, H., Lee, S., Park, J.H., Park, R.W., Kim, I.S. & Choi, K. 2010. Tumor-homing multifunctional nanoparticles for cancer theragnosis: Simultaneous diagnosis, drug delivery, and therapeutic monitoring. *Journal of Controlled Release* 146(2): 219-227.

Kim, S.E., Kim, H.J., Rhee, J.K. & Park, K. 2017. Versatile chemical derivatizations to design glycol chitosan-based drug carriers. *Molecules* 22(10): 662.

Koo, H., Min, K.H., Lee, S.C., Park, J.H., Park, K., Jeong, S.Y., Choi, K., Kwon, I.C. & Kim, K. 2013. Enhanced drug-loading and therapeutic efficacy of hydrotropic oligomer-conjugated glycol chitosan nanoparticles for tumor-targeted paclitaxel delivery. *Journal of Controlled Release* 172(3): 823-831.

Kwon, S., Park, J.H., Chung, H., Kwon, I.C., Jeong, S.Y. & Kim, I.S. 2003. Physicochemical characteristics of self-assembled nanoparticles based on glycol chitosan bearing 5β-cholanic acid. *Langmuir* 19(24): 10188-10193.

Lee, B.S., Park, K., Park, S., Kim, G.C., Kim, H.J., Lee, S., Kil, H., Oh, S.J., Chi, D., Kim, K. & Choi, K. 2010. Tumor targeting efficiency of bare nanoparticles does not mean the efficacy of loaded anticancer drugs: Importance of radionuclide imaging for optimization of highly selective tumor targeting polymeric nanoparticles with or without drug. *Journal of Controlled Release* 147(2): 253-260.

Loftsson, T. & Brewster, M.E. 2010. Pharmaceutical applications of cyclodextrins: Basic science and product development. *Journal of Pharmacy and Pharmacology* 62(11): 1607-1621.

Meng, L., Huang, W., Wang, D., Huang, X., Zhu, X. & Yan, D. 2013. Chitosan-based nanocarriers with pH and light dual response for anticancer drug delivery. *Biomacromolecules* 14(8): 2601-2610.

Mennini, N., Furlanetto, S., Bragagni, M., Ghelardini, C., Di Cesare Mannelli, L. & Mura, P. 2014. Development of a chitosan-derivative micellar formulation to improve celecoxib solubility and bioavailability. *Drug Development and Industrial Pharmacy* 40(11): 1494-1502.

Min, K.H., Park, K., Kim, Y.S., Bae, S.M., Lee, S., Jo, H.G., Park, R.W., Kim, I.S., Jeong, S.Y., Kim, K. & Kwon, I.C. 2008. Hydrophobically modified glycol chitosan nanoparticles-encapsulated camptothecin enhance the drug stability and tumor targeting in cancer therapy. *Journal of Controlled Release* 127(3): 208-218.

Nair, L.S. & Laurencin, C.T. 2007. Biodegradable polymers as biomaterials. *Progress in Polymer Science* 2(8-9): 762-798.

Park, J.S., Han, T.H., Lee, K.Y., Han, S.S., Hwang, J.J., Moon, D.H., Kim, S.Y. & Cho, Y.W. 2006. N-acetyl histidine-conjugated glycol chitosan self-assembled nanoparticles for intracytoplasmic delivery of drugs: Endocytosis, exocytosis and drug release. *Journal of Controlled Release* 115(1): 37-45.

Qu, X., Khutoryanskiy, V.V., Stewart, A., Rahman, S., Papahadjopoulos-Sternberg, B., Dufes, C., McCarthy, D., Wilson, C.G., Lyons, R., Carter, K.C. & Schätzlein, A. 2006. Carbohydrate-based micelle clusters which enhance hydrophobic drug bioavailability by up to 1 order of magnitude. *Biomacromolecules* 7(12): 3452-3459.

Razi, M.A., Wakabayashi, R., Goto, M. & Kamiya, N. 2019. Self-assembled reduced albumin and glycol chitosan nanoparticles for paclitaxel delivery. *Langmuir* 35(7): 2610-2618.

Saravanakumar, G., Min, K.H., Min, D.S., Kim, A.Y., Lee, C.M., Cho, Y.W., Lee, S.C., Kim, K., Jeong, S.Y., Park, K. & Park, J.H. 2009. Hydrotropic oligomer-conjugated glycol chitosan as a carrier of paclitaxel: Synthesis, characterization, and *in vivo* biodistribution. *Journal of Controlled Release* 140(3): 210-217.

Siew, A., Le, H., Thiovolet, M., Gellert, P., Schatzlein, A. & Uchegbu, I. 2012. Enhanced oral absorption of hydrophobic and hydrophilic drugs using quaternary ammonium palmitoyl glycol chitosan nanoparticles. *Molecular Pharmaceutics* 9(1): 14-28.

Trapani, A., Sitterberg, J., Bakowsky, U. & Kissel, T. 2009. The potential of glycol chitosan nanoparticles as carrier for low water soluble drugs. *International Journal of Pharmaceutics* 375(1-2): 97-106.

Uchegbu, I.F., Sadiq, L., Arastoo, M., Gray, A.I., Wang, W., Waigh, R.D. & Schätzleinä, A.G. 2001. Quaternary ammonium palmitoyl glycol chitosan - A new polysoap for drug delivery. *International Journal of Pharmaceutics* 224(1-2): 185-199.

Vivek, R., Babu, V.N., Thangam, R., Subramanian, K.S. & Kannan, S. 2013. pH-responsive drug delivery of chitosan nanoparticles as Tamoxifen carriers for effective anti-tumor activity in breast cancer cells. *Colloids and Surfaces B: Biointerfaces* 111: 117-123.

Wang, J.J., Zeng, Z.W., Xiao, R.Z., Xie, T., Zhou, G.L., Zhan, X.R. & Wang, S.L. 2011. Recent advances of chitosan nanoparticles as drug carriers. *International Journal of Nanomedicine* 6: 765.

Xu, J., Yu, J., Xu, X., Wang, L., Liu, Y., Li, L., Zhao, J. & He, M. 2014. Development, characterization, and evaluation of PSMA-targeted glycol chitosan micelles for prostate cancer therapy. *Journal of Nanomaterials* 2014: Article ID. 462356.

Yang, H., Tang, C. & Yin, C. 2018. Estrone-modified pH-sensitive glycol chitosan nanoparticles for drug delivery in breast cancer. *Acta Biomaterialia* 73: 400-411.

Yu, J.M., Li, Y.J., Qiu, L.Y. & Jin, Y. 2009. Polymeric nanoparticles of cholesterol-modified glycol chitosan for doxorubicin delivery: Preparation and *in-vitro* and *in-vivo* characterization. *Journal of Pharmacy and Pharmacology* 61(6): 713-719.

Yu, J.M., Li, Y.J., Qiu, L.Y. & Jin, Y. 2008. Self-aggregated nanoparticles of cholesterol-modified glycol chitosan conjugate: Preparation, characterization, and preliminary assessment as a new drug delivery carrier. *European Polymer Journal* 44(3): 555-565.

Zhou, Y., Yu, J., Feng, X., Li, W., Wang, Y., Jin, H., Huang, H., Liu, Y. & Fan, D. 2016. Reduction-responsive core-crosslinked micelles based on a glycol chitosan-lipoic acid conjugate for triggered release of doxorubicin. *RSC Advances* 6(37): 31391-31400.

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