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Carbon nano-onions as potential nanocarriers for drug delivery

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Nanocarriers are nano-sized delivery vesicles that can transport desired molecules to a specific location. The utilisation of nanocarriers for targeted drug-delivery is an emerging field that aims to solve certain disadvantages of free drug delivery; including premature drug degradation, non-specific toxicity, lack of tissue penetration, undesired side-effects, and multi-drug resistance. The nanocarrier approach has proven effective in this regard, with some examples of FDA approved nanocarrier systems available on the market. In this perspective, we investigate the potential of carbon nano-onions (CNOs) as nanocarriers for drug delivery. The various criteria and considerations for designing a nanocarrier are outlined, and we thoroughly discuss how CNOs fit these criteria. Given the rapidly developing interest in CNOs, this perspective provides a baseline discussion for the use of this novel carbon nanomaterial as a potential nanocarrier for drug delivery.

1 Introduction

Cancer is a lead actor in the theatre of premature mortality. With an incidence rate of over 10 million, it is the leading cause of premature death in most developed countries worldwide, preceded only by heart disease in certain countries.[1,2] There are many approaches to treating this disease, including chemotherapy, immunotherapy, radiotherapy, and surgery, or most typically, a combination of these. A recent approach to anti-cancer therapy is through the use of nanocarriers.[3,4] Nanocarriers are nano-sized (1-100 nm in at least one dimension) materials that, in essence, act as targeted delivery vesicles for other substances, such as anti-cancer drugs.[5]

The popularity of the *nanocarrier* approach can, justifiably, be ascribed to the benefits it offers over free-drug administration. An ideal nanocarrier tackles the various disadvantages associated with free drug delivery, including premature drug degradation, non-specific toxicity, lack of tissue penetration, undesired side-effects, and multi-drug resistance. As nanoparticle-based drug delivery has demonstrated its capacity to successfully alleviate these issues, several such formulations have been developed and FDA approved, with many more undergoing clinical trials.[6] For instance, Myocet is a liposome-based nanocarrier system for the delivery of doxorubicin (DOX) in combinatorial anti-cancer therapy.[7] Abraxane is another FDA approved nanocarrier system comprising albumin-bound paclitaxel nanoparticles for the treatment of metastatic breast cancer.[8]

In fact, many types of different nanoparticles (NPs) have been used as nanocarriers for drug delivery applications.[3,4] The most prominent NPs used for this purpose include liposomes,[9,10] micelles,[11] dendrimers,[12] inorganic nanoparticles,[13] and polymeric nanoparticles.[14,15] Various carbon nanomaterials (CNMs) have also been utilised as nanocarriers; including nanotubes[16] and graphene oxide (GO).[17] Topically, an interesting CNM has presented itself as a potential nanocarrier; the carbon nano-onion.

Carbon nano-onions, also commonly referred to as onion-like carbon (OLC) or carbon onions (COs), are concentric, multi-layer fullerenes consisting predominantly of carbon, with an sp^2 hybridised surface. The interlayer distance in CNOs is typically 0.334 nm, and the shell series & respective carbon atom number can be described by the series (Eq. 1):[18,19]

$$\overbrace{C_{60}}^{\text{Layer 1}} @ \overbrace{C_{240}}^{\text{Layer 2}} @ \overbrace{C_{540}}^{\text{Layer 3}} \ldots \overbrace{C_{60\times n^2}}^{\text{Layer } n} \quad (1)$$

The specific morphological characteristics of the material depend on the method of preparation.[19] The most common method is thermal annealing of detonation nanodiamonds (DNDs),[20–22] which results in small (< 10 nm), spherical, dense-core CNOs. However, other preparation methods can yield larger CNOs that can be polygonal, have a hollow core, or a core filled with different metals.[19]

This curious member of the CNM family was first seen by

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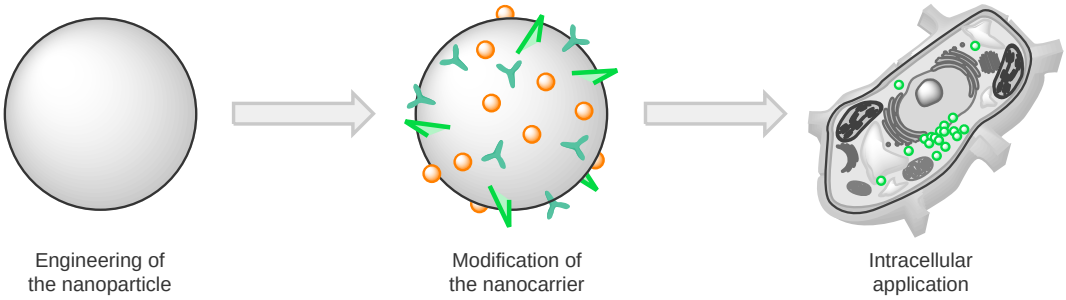

Fig. 1 The three stages in the rational design of a nanocarrier for targeted drug delivery purposes.

Ugarte in 1992.[23] Notwithstanding their early discovery, around the same time as the nanotube,[24,25] CNOs have only recently amassed considerable academic interest, with many emerging applications in the chemical, physical and biomedical fields.[19,26–30] The recently emerging applications of CNOs are grand in scope, ranging from their use as photothermal agents in photothermal cancer therapy,[31] to their use as lubricant additives given their interesting tribological properties.[32,33] In fact, one of the most recent applications of CNOs under investigation, is their use in nanocarrier-based drug delivery systems.[34–39]

The recent interest in utilising CNOs as a nanocarrier arises from numerous benefits this curious CNM offers over other drug delivery systems. The most substantial of which include its biocompatibility, which has been revealed through numerous *in vitro*, *in vivo* & *ex vivo* studies.[37,39–48] Another benefit is their relatively simple method of preparation—the thermal annealing of detonation nano diamonds—which results in a material of high purity and narrow polydispersity.[20–22] Organic and inorganic nanomaterials which involve multi-step preparation processes, often suffer from low yield, high cost, a difficult scale-up. A further benefit of CNOs is their stability; thanks to which they do not require specialised storage conditions. Also, the intrinsic sp^2 surface of CNOs allows for easy covalent and non-covalent functionalisation, enabling the attachment of multiple functional moieties.[28,49] Though, this interesting nanomaterial is not without fault—a well known issue with CNOs, like with other CNMs, is their lack of dispersibility. Though, with the use of specific solvents and surfactants, this issue may be alleviated.[18,50,51] These benefits and drawbacks, among others, have been discussed (Sec. 2) in further detail.

In this perspective, we cover the potential application of CNOs as a novel nanocarrier for drug delivery applications. We outline and assess the various rational considerations and criteria that need to be made when designing a novel CNO-based nanocarrier system under three topics; the engineering of the nanoparticle, the modification of the nanocarrier, and the intracellular applications (Fig. 1). We review how CNOs behave under each of these topics, within the scope of targeted drug delivery. Also, we discuss various literature examples where CNOs have been utilised within this area of interest.

2 CNOs as nanocarriers for drug delivery: rational design

Nanocarrier design and engineering is a complicated process that involves many variables, each of which can alter the ultimate physio-chemical properties and function of final product. The design of a nanocarrier can be split into three main stages; the engineering of the nanoparticle, the surface modification of the nanoparticle, and the intracellular applications of the nanocarrier (Fig. 1). At each stage of the design of a novel conceptual nanocarrier system, a number of considerations need to be made. These considerations need to be properly assessed and addressed early on, as to ensure the most effective translation of the novel system from concept to clinic. In this section, we discuss the various rational considerations relevant to CNOs and outline how CNOs fit these criteria.

2.1 Engineering of the nanoparticle

Nanoparticle engineering governs specific physical & chemical properties of the nanomaterial, such as shape, morphology, composition, surface porosity, surface charge, oxygen content, density, surface area, and conductivity.[26] The specific approach to the engineering of a nanoparticle provides a strong foundation for type of properties a resulting nanocarrier will possess. In terms of carbon nano-onions (CNOs), this approach needs to be carefully considered, as there are a vast array of various methods for the production of this interesting nanomaterial. These include the preparation of CNOs by ball-milling of graphite,[52] underwater arc discharge,[53] chemical vapour deposition,[54,55] electron beam irradiation,[56] ion-implantation,[57] through various plasma processes & pyrolysis,[58–60] and most commonly, through thermal annealing.[20–22] Each of these methods and the respective variances in their variables, such as the specific annealing time and temperature, results in CNOs of different physiochemical properties, including variances in size, shape (spherical / polyhedral), oxygen content, density, shell-count, surface area, sp^2 carbon ordering, core type, contaminant content, and conductivity.[19,26]

The method most commonly utilised for the production of CNOs is the thermal annealing of detonation nano diamonds (DNDs)

under an inert atmosphere.[20–22] The popularity of this method owes particularly due to the low cost of DND precursor material, the large quantity of material produced, and the high purity and narrow polydispersity of the resulting CNOs.[26] Simply, this approach involves the annealing of small DNDs under vacuum or an inert gas at high temperatures.[19,26] The variables of this approach include the size and purity of the DNDs utilised, which are typically 5-10 nm in size; the atmosphere under which the process is carried out, which is either vacuum or a positive pressure of nitrogen, hydrogen, argon or helium; the temperature of annealing, which can range anywhere from 1200 to 1800^{o}C; and duration for which the DNDs are annealed. All these variables have been optimised to produce CNOs with desired properties in high purity.[26,61]

Ultimately, depending on the particular approach for the preparation of carbon nano-onions, the resulting material will have different physio-chemical properties. However, the resulting nanomaterial can be further modified, which can alter the intrinsic properties of the material.

2.2 Modification of the nanocarrier

The sp^2 hybridised surface of CNOs affords an ease of functionalisation, with many approaches reported; both covalent and non-covalent.[28,49] However, the core of CNOs cannot be modified post-preparation of the nanomaterial, given its concentric nature. As such, if a diamond / hallow / or co-doped type core is desired, one must consider the approach with which they prepare CNOs.

The covalent bond is moderately strong under standard conditions—this strength positions it as an ideal mode for linking various moieties to the nanomaterial surface, as it prevents undesired release. In fact, covalent linkers are commonly utilised for the attachment of targeting, bioimaging and biosensing moieties directly to the surface of nanocarriers, or through spacer groups.[4,28] The sp^2 surface of CNOs has been covalently functionalised through many various approaches, including cyclopropanation,[20] 1,3-dipolar cycloaddition,[21,62] radical addition,[20,63] fluorination,[64] reduction and alkylation,[65] and most commonly, oxidation.[19–21,28,66] Moreover, the functional groups introduced through the aforementioned methods allow for further functionalisation of the nanomaterial. For instance, the carboxylic acid moieties introduced to the CNO surface through an oxidation process can undergo further amidation and esterification reactions.[21,67] As another example, a molecule with an acetylene group introduced to the CNO surface can undergo further functionalisation through a *click* reaction with a molecule functionalised with an azide.[63]

In contrast to covalent binding, a palette of various non-covalent interactions exist, ranging from the extremely weak Casimir force,[68] to ionic binding.[69] Non-covalent interactions of interest to CNO-based nanocarriers include electrostatic interactions, which extend to charge-charge interactions, halogen bonding, and hydrogen bonding;[69–71] van der Waal forces, which extend to charge-dipole interactions, dipole-dipole interactions, and dipole-induced dipole interactions;[72] various π interactions, including π-π stacking, charge-π, lone pair-π, polar-π, and dipole-π interactions;[72–74] and hydrophobic interactions.[72] These interactions are typically weak and reversible; as such, they can be preferential to covalent bonding in certain cases. Whilst the targeting agent and imaging moiety may be bonded covalently to the CNO surface to avoid dissociation, non-covalent binding may be a desirable choice for loading the drug molecules onto the nanocarrier system. In this way, non-covalent interactions may be used to create a CNO-based supramolecular system that will selectively dissociate the drug at the specific target site.[49,75]

The first supramolecular CNO-based system was reported 2008, by Echegoyen *et al.*[76] Since then, non-covalent binding has become increasingly popular as a mode of moiety-attachment to the CNO surface.[29,49,50,77–86] Moreover, in the recent years, an increasing interest has been observed in utilising non-covalent functionalisation for drug delivery related applications.[37,38]

As the primary function of a nanocarrier is to deliver and release a drug payload at the target site, one must consider an appropriate approach for appending the drug onto the nanomaterial surface. There are many approaches for loading a drug payload onto/into a nanocarrier. However, given their closed-shell graphitic structures, CNOs are limited to surface loading approaches; they cannot act as a traditional reservoir or matrix system for sustained drug release—a contrast to other types of nanocarriers, such as liposomes, where the active pharmaceutical can be embedded inside the nanoparticle.[87] However, depending on the method of preparation, CNOs can posses a certain degree of micro- and mesoporosity.[22,26] In this case, small drug molecules can embed within the pores on the CNO surface, as discussed in Sec. 3.[38] In terms of surface loading, the drug of interest can be coupled onto a CNO nanocarrier either through non-covalent means or covalently, using a linker (Fig. 2). The non-covalent approach typically involves appending the drug onto the CNO surface using π-π stacking interactions, provided the drug has an adequate aromatic system, or the drug can be non-covalently incorporated into other functionalities present on the CNO surface, such as a polymer matrix. In regards to covalent binding, the drug can be attached using a linker agent that is designed to cleave at the specific target site, thus releasing the drug.

As the drug payload is loaded onto the CNO nanocarrier system, the drug loading efficiency and stability needs to be considered. Literature cases where the drug loading efficiency has been investigated in CNO-based nanocarrier systems have reported excellent results, namely for ibuprofen (IBU) & paracetamol (PA),[38] 5-fluorouracil (5-FU),[34] and DOX.[35,36] The high drug loading efficiency reported is accredited to the large surface area of CNOs, their surface porosity, the favourable interactions between a drug molecule and the CNO surface (such as π-π stacking), and the incorporation of other functionalities into the system, such as poly-

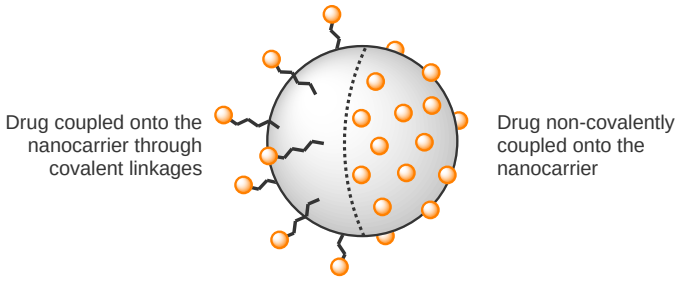

Fig. 2 The drug payload, or other functional moieties, can be loaded onto the CNO nanocarrier either covalently or non-covalently; each of which has its benefits and drawbacks.

mer matrices.[34–36,38] In a physical study, which investigated the diffusivity dynamics of phenanthrenequinone on the surface of CNOs, a high surface coverage of the molecule has been reported, at 0.60 molecules / nm^2 of a CNO surface.[78] Together, these studies indicate that CNOs offer a high drug loading capacity, and that the favourable CNO-drug interactions, such as π-π stacking, allow for good loading stability. In terms of drug release efficiency, 5-FU was found to release most efficiently at basic pH[34]—a contrast to DOX, for which the most efficient release stimuli was an acidic environment.[35,36] These findings indicate that the nanocarrier system can be tailored to be stable in desired environments. Further discussion on these specific examples is given in (Sec. 3).

In relation to other moieties, targeting and imaging agents are typically appended to the CNO surface through covalent means, such as to afford a high degree of stability. However, there are cases where theses moieties have been successfully attached to CNOs through non-covalent interactions.[37,81] Furthermore, the different moieties can either be appended directly to the CNO surface,[30] or to each other in a bioconjugate fashion. For instance, in 2020, our group successfully conjugated a targeting polymer with a fluorophore moiety.[37] Although appending moieties directly to the surface of CNOs is simpler in principle, there are benefits to the bioconjugate approach. For instance, the degree to which the targeting polymer is functionalised can be controlled to a higher precision than the functionalisation of the CNO surface itself—which is due to the polydisperse nature of nanomaterials. Furthermore, a targeting polymer can be saturated with more imaging moieties than the CNO surface itself, allowing for better quality emission analyses. The conjugate approach also leaves more CNO surface area for the targeting agent and the drug molecules to bind to, further increasing the loading capacity of the nanocarrier.

One recognised complication with CNOs is their tendency to aggregate.[18,28] Like with other carbon nanomaterials,[88] CNOs aggregate in polar and non-polar solvents and biological fluids due to intermolecular van der Waal and π-interactions.[18,37] However, this issue is assuaged in biomedical applications of the material—the introduction of various moieties and functionalities to the nanomaterial surface, particularly carboxylic acid groups, significantly improves the dispersibility of CNOs; as evidenced by DLS studies.[27,30,39,84] Furthermore, the use of various biocompatible surfactants for the purpose of dispersing CNOs has been investigated, with positive results reported, especially with anionic surfactants.[50,51] Moreover, the Hansen solubility parameters of various common solvents have also been assessed, with the purpose of identifying ideal solvents for dispersing CNOs. Although the following would not be utilised for bio-applications; acetone, THF, DMF, NMP, chloroform, acetonitrile, R-130, and benzyl alcohol have been identified as good solvents.[18]

2.3 Intracellular application

When designing a drug formulation, it is essential that each inactive ingredient and excipient is biocompatible. The FDA provides a database of such approved inactive ingredients.[89] However, when designing a new drug-delivery system, with novel, unapproved components, it is essential to test various biological aspects of the material on various cell lines and animal models. Thus, biocompatibility can be regarded as an essential criteria that CNOs have to match in order to be utilised as nanocarriers.

To date, biological studies on pristine & functionalised CNOs have been carried out *in vitro*, *in vivo* and *ex vivo* on a vast array of various normal & cancer cell-lines and animal models. Reported *in vitro* studies involving CNOs involved the following cell lines: human breast adenocarcinoma cell line MCF-7;[28,39,66] mouse embryonic fibroblast cell line NIH 3T3;[39] human breast cancer cell line MDA-MB-231;[37] human ovarian cancer cell line A2780;[37] human cervical cancer cell line HeLa[30,40,81,90] & HeLa Kyoto;[41] murine mammary carcinoma cell line 4T1;[85] and the KB cell line (a subline of the HeLa line).[40] *In vivo* studies involved a number of animal models, including common fruit flies (*Drosphila melanogaster*);[42] fresh-water polyps (*Hydra vulgaris*);[43] and zebrafish (*Danio rerio*),[37,44,45] a commonly utilised vertebrate model. *Ex vivo* studies involving CNOs have also been conducted on a number of murine models.[46–48] In mutual agreement, these biological studies report that CNOs have low toxicity and good biocompatibility. Presently, there are no reports of any significant toxicity posed by CNOs. As biocompatbility is a barrier for the use of a material for bio-applications, the good biocompatibility of CNOs is a key enabling property that allows for their prospective application as nanocarriers.

An important factor to consider in the design of a nanocarrier is the mode of drug release. Once the nanocarrier reaches its target site, the release of the drug payload can be achieved in many ways. Stimuli-responsive, or '*smart*' nanocarriers are those which are able to react to various specific exogenous and endogenous stimuli to release the drug.[3,15] These stimuli are site-specific, allowing the nanocarrier to take advantage of the conditions present at the target site, releasing the drug only when such a stimuli is applied. Exogenous stimuli are those which are externally applied, and include heat,[91–93] ultrasound,[94] magnetism,[95] electric fields, and light.[96] Endogenous stimuli are site-specific

variations in pH,[97,98] redox-potential,[99] or the presence of various enzymes.[97] Together, there are many approaches which can be utilised to achieve targeted drug-payload release (Fig. 3).

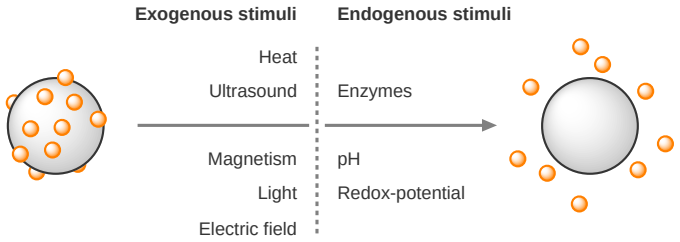

Fig. 3 The various types exogenous and endogenous stimuli for responsive, 'smart', drug release.

One prominent benefit of the nanocarrier approach for drug delivery applications is that the surface of the carrier vesicle can be multifunctionalised with different moieties. This can be used to expand the functionality of the nanocarrier beyond that of a therapeutic, into a theranostic system; a system that possesses both therapeutic and diagnostic capabilities. One moiety commonly appended to the CNO surface for this purpose is a fluorophore/contrast moiety, which is utilised for imaging purposes.[30,37] This moiety enables various emission-related bioanalyses, the most prominent of which are confocal microscopy, emission spectroscopy, and flow cytometry. The presence of a fluorophore tag on the nanocarrier enables biodistribution studies through confocal microscopy, both at the cell level, and at organism level (Fig. 4).

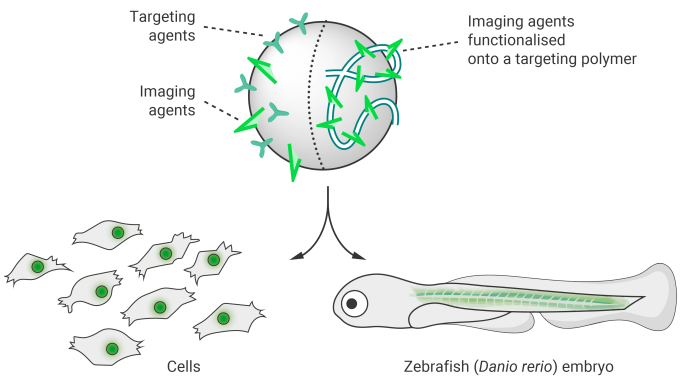

Fig. 4 CNOs functionalised with a fluorophore moiety can be used for bioimaging both *in vitro* and *in vivo*; such as in cellular localisation studies (left), or for biodistribution studies in a vertebrate model (right).

3 Targeted delivery applications of CNOs

Since they were first discovered in 1992, carbon nano-onions have been utilised in a variety of applications, given their interesting physio-chemical properties.[23] The most recent of these applications is their use as a nanocarrier. Herein are outlined literature cases where CNOs have been utilised as a carrier in the scope of targeted delivery.

The blood-brain barrier (BBB) consists of tight intercellular junctions specialised in limiting the molecular transit between the brain and capillaries.[100] The extremely low permeability of this barrier is a significant limiting factor in central nervous system (CNS) drug development. However, certain nanocarriers, such as liposomes, have demonstrated an inherent ability to cross this membrane.[101] In 2016, Sarkar *et al.* investigated the crossing of onion-like carbon through the BBB in two murine models; CADASIL (cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy) mice and GBM (glioblastoma multiforme) induced mice. They prepared onion-like carbon through the pyrolysis of wood-wool, and fragmented the resulting nanomaterial into a smaller size (approx. 15 nm) through nitric acid treatment. The resulting nanoparticles were water soluble and fluorescent. The group administered a 1 mg/mL water dispersion of this nanomaterial using tail vein injection and imaged the brain *in vivo* over time. By monitoring the fluorescence using 380, 488 & 560 nm excitation lines, the group observed that the nanoparticles cross the BBB smoothly without causing any perfusions. Furthermore, the group observed that they are readily excreted within a few days. Although further studies are necessary involving onion-like carbon of different size and structure, this study outlines the potential use of this nanomaterial as a nanocarrier for the targeted delivery of therapeutics across the BBB.[47]

In 2019, our group investigated the potential of CNOs as a carrier for the cellular delivery of a glycopeptides and proteins. In this investigation, we conjugated a fluorescent synthetic glycopeptide onto CNOs (Gly-CNOs) and a bovine serum albumin (BSA) onto CNOs (BSA-CNOs) (Fig. 5). For each bioconjugation, the CNOs were decorated with maleimido groups, which then underwent a maleimide-thiol chemoselective reaction with the cysteine moieties present on both the glycopeptide and the BSA protein. The Gly-CNO and BSA-CNO systems were then analysed for their cytotoxicity and cellular uptake mechanism. Cytotoxicity analysis on normal (NIH 3T3) and cancer (MCF7) cells presented good biocompatibility. The cellular uptake mechanism revealed that the nanocarriers are internalised via an endocytosis pathway, and then translocate into lysosomes within 16-24 hours where they are metabolised without inducing apoptosis or any adverse effects on the cells. Notably, the synthetic glycopeptide alone was not able to internalise into cells, whereas the Gly-CNO conjugate was internalised readily. Similarly, BSA alone was internalised with a lower efficacy when compared to the BSA-CNO conjugate. Good cellular uptake is an essential parameter necessary for a nanoparticle to be utilised as a nanocarrier. The findings for both Gly-CNO & BSA-CNO systems suggest the viability of CNOs as a nanocarrier for intracellular delivery of glycopeptides and proteins.[39]

In 2019, Mamidi *et al.* developed zein protein hydrogels doped with poly 4-mercaptophenyl methtacrylated CNOs (f-CNOs) for pH-responsive drug release. The composites were prepared by functionalising oxidised CNOs with 4-mercaptophenol, followed by a methacrylation and polymerisation, resulting in f-CNOs. This nanomaterial was then incorporated into a zein protein matrix

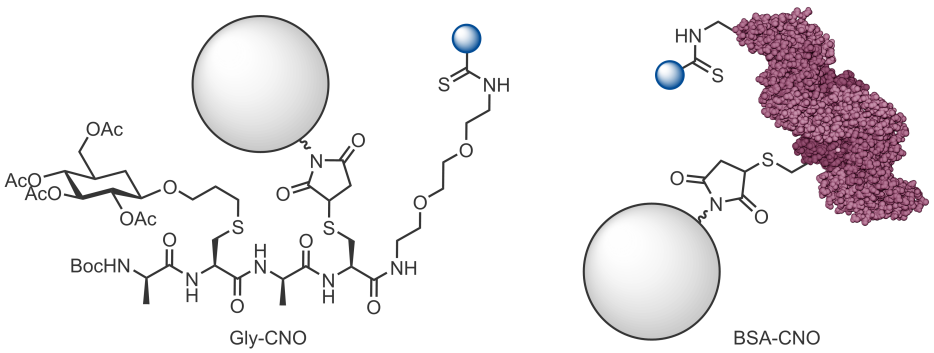

Fig. 5 CNOs covalently functionalised with a glycopeptide (Gly-CNOs; left) and with bovine serum albumin (BSA-CNOs; right).[39] The blue spheres represent the imaging moiety; fluoresceinamine isomer I.

through an acoustic caviation method. As the drug of choice, 5-FU (a chemotherapeutic used for colorectal carcinoma) was used. 5-FU was incorporated into the hydrogel composite prior to crosslinking, which was then achieved with glutaraldehyde. The hydrogel composite was investigated for its physiochemical and mechanical properties, as well as drug release and cytocompatibility properties, as a potential drug delivery system. Mechanical measurements showed improved tensile properties of the hydrogel when doped with the f-CNOs. The drug release studies indicated that the system exhibits a pH-sensitive release, with the quickest release at pH 7.4 - 9.0. Furthermore, cytotoxicity studies on osteoblast cells underlined the good biocompatibility of the system. It is also of note that the f-CNOs formed a stable dispersion in physiological buffer, as analysed over a 12 month period. This further underlines the possibility of improving CNO dispersibility through surface functionalisation. Overall, the good cytocompatibility and pH-sensitive drug release position f-CNO/zein composites as potential nanocarriers for the oral delivery of colorectal carcinoma therapeutics.[34]

In 2020, our group developed a supramolecular hybrid CNO-based system as a nanocarrier for targeted delivery.[37] The nanocarrier system composed of CNOs non-covalently functionalised with hyaluronic acid (HA) 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine (DMPE) (Fig. 6). The resulting CNO:HA-DMPE system showed a marked improvement in dispersibility in biological fluids over the pristine CNOs—an effect mediated by the hydrophilic nature of HA, which was bound to the system through the non-covalent interaction of the hydrophobic DMPE chains with the CNO surface. *In vitro* toxicity and cellular uptake studies on two cancer cell lines ($CD44^+$ & $CD44^-$ lines; MDA MB231 & A2780, respectively), and *in vivo* toxicity and biodistribution studies on zebrafish larvae (*Danio rerio*) were carried out on this nanocarrier. Both *in vitro* & *in vivo* studies indicated a good biocompatibility of the hybrid system. The results also indicated a strong targetability of the system towards CD44 receptor overexpressing cancer cells, as facilitated by the HA targeting agent. Lastly, the *in vivo* biodistrubution analysis showed a specific localisation of the nanocarrier system in the digestive tract of the zebrafish larvae. Combined, these results indicate the promising potential of a CNO-based supramolecular system for targeted delivery purposes.[37]

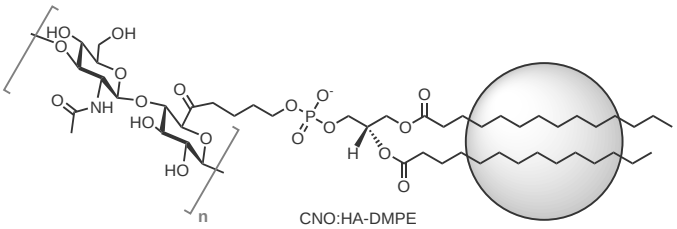

Fig. 6 CNO:HA-DMPE supramolecular system for $CD44^+$ cancer cell targeting.[37] The DMPE chains non-covalently interact with the CNO surface through hydrophobic interactions.

In 2020, Ye *et al.* investigated the viability of partially graphitised nanodiamonds as a porous carrier for oral drug delivery. Through the thermal annealing of nanodiamonds at a low annealing temperature (1100°C), they created NPs with a diamond core and graphitic layers, with a high surface porosity. A cytotoxicity assay of this material on human colon carcinoma cells (Caco-2) revealed their low toxicity. Using PA & IBU as model drugs, they adsorbed the drugs into the porous surface of the aggregates to produce PA/NPs & IBU/NPs with a loading efficiency of 11.5% & 20.7%, respectively. Overall, Ye *et al.* demonstrated the viability of CNOs as an amorphous drug carrier—the nanomaterial did not affect the chemistry of the drugs, complete drug release was achieved, and the drugs were released into solution faster than the pure crystalline drugs alone.[38]

In 2020, Mamidi *et al.* developed a dual stimuli responsive CNO-based nanocarrier for anticancer drug delivery. The group functionalised CNOs with poly 4-hydroxyphenyl methacrylate (f-CNOs) through an EDC/NHS mediated coupling reaction. They then non-covalently loaded the f-CNOs with DOX through π-π stacking (f-CNOs/DOX). Subsequently, they incorporated the resulting material into BSA through forcespinning in a spinneret. The resulting BSA/f-CNOs/DOX drug delivery nanohybrid was investigated for its physical and biological properties. These studies revealed that the nanohybrid can release the drug through two types of stimuli; the exogenous application of heat and an en-

dogenous increase in pH. Furthermore, biological studies showed that the BSA/f-CNOs nanohybrid possesses good cell adhesion, proliferation, and cell viability. This study underlines the potential use of CNOs as nanocarriers.[36]

In 2020, Mamidi *et al.* conducted a further study involving the use of CNOs for drug delivery purposes. In their research, the group developed a CNO-reinforced composite thin film for the stimuli responsive release of DOX. Poly (*N*-(4-aminophenyl) methacrylamide)-carbon nano-onions (f-CNOs) were synthesised, which were then used to reinforce an anilinated-poly (ether ether ketone) (AN-PEEK) polymer matrix. The final thin film, AN-PEEK/f-CNOs, was developed through layer-by-layer self-assembly; a by-layer deposition of material through non-covalent interactions. An *in vitro* cytotoxic evaluation of this system showed good surface biocompatibility. When loaded with DOX, the system showed a pH-responsive drug release, with the best response present at acidic conditions. Aside from the good surface biocompatibility and stimuli-responsive DOX release, the AN-PEEK/f-CNOs drug delivery system showed exceptional tensile strength, Youngs modulus, and toughness. The group hypothesises that the excellent physical properties stem from the use of CNOs, which allow for π-π stacking and hydrogen-bonding interactions with the AN-PEEK polymer matrix. Overall, the group presented the potential use of CNOs in a thin film type nanocarrier-based drug delivery system.[35]

Presently, the number of studies where CNOs have been used in the scope of nanocarrier-based drug delivery is relatively low. However, majority of the existing studies have been carried out in recent years, which indicates a growing interest in the use of this promising nanomaterial for targeted drug-delivery purposes. Furthermore, literature applications for which CNOs have been used in this subject area are of good variety; with such examples as delivery across the BBB,[47] pH-sensitive delivery of colon cancer chemotherapeutics,[34] cellular delivery of glycopeptides and proteins,[39] targeted delivery to CD44 receptor overexpressing cells,[37] as a carrier for amorphous drug delivery,[38], for dual-stimuli responsive delivery of DOX,[36] and in reinforcing a polymeric thin film pH-responsive DOX delivery system.[35] This variety of applications and the growing recent interest shows that CNOs present promising viability as a nanomaterial of choice for nanocarrier applications.

4 Conclusions

Cancer poses a significant global burden. There are many approaches to treating this disease, of which the novel nanocarrier approach has gained a significant interest in recent years. The use of nanocarriers has been explored with a number of nanomaterials, both inorganic and organic. Recently, interest has amassed in the use of carbon nano-onions (CNOs) as a choice material for targeted delivery applications. The recent interest in nanocarrier technology is justified as they alleviate a number of issues present with the standard drug-only approach, which include: premature drug degradation, non-specific toxicity, lack of tissue penetration, undesired side-effects, and multi-drug resistance.

When engineering nanocarrier for drug delivery purposes, a number of aspects need to be considered. These aspects fall under the headings of: engineering of the nanoparticle, modification of the nanomaterial, and intracellular applications. The behaviour of CNOs with respect to these headings has been outlined, and the various considerations and important criteria in regard to CNOs has been discussed.

Engineering of the nanomaterial covers its intrinsic properties, including morphology, composition and size. CNOs are multi-layer fullerenes with an interlayer distance of approx. 0.344 nm and an sp^2 hybridised surface. The specific physio-chemical properties, including their size, layer count, and type of core, can be fine-tuned through the approach taken to prepare them. There are many methods of CNO preparation, the most popular being the thermal annealing of detonation nanodiamonds under an inert atmosphere, which results in CNOs of low polydispersity and high purity.

Modification of the nanoparticle is the next step in the preparation of a CNO nanocarrier. The CNO surface can be modified with the drug payload itself, and any desired functionalities, including targeting agents, fluorophores, and biosensing moieties (Fig. 7). This can be achieved either through covalent means or non-covalently. Furthermore, the moieties can be appended either directly onto the CNO surface, or through spacer groups. Alternatively, a bioconjugate approach can be taken to appending different functionalities onto CNOs, whereby two different moieties are conjugated together; for instance, a targeting polymer with an imaging moiety. Having functionalised CNOs, the question of dispersibility needs to be considered. Although pristine CNOs, like other carbon nanomaterials, suffer with the problem of low dispersibility, the functional groups appended to the CNO surface can impart improved dispersibility properties. Also, careful choice of biocompatible surfactants can further improve the dispersibility of the material.

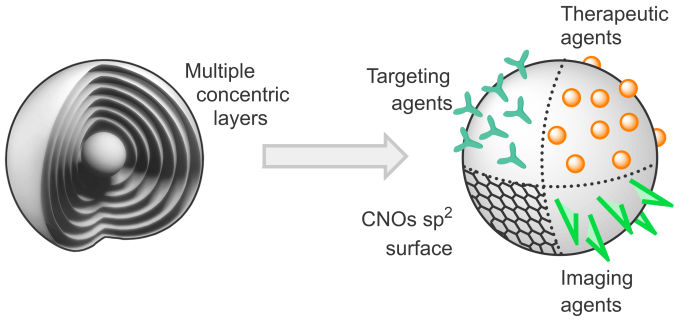

Fig. 7 General schematic depicting the interlayer structure of CNOs (left), and the components of a CNO-based nanocarrier system (right).

At this stage, the intracellular applications of the CNO-based nanocarrier may be considered. The most important aspect of a nanocarrier is its biocompatibility. CNOs have shown to have

good biocompatibility through numerous studies *in vitro* on normal and cancer cell lines, *in vivo* on various animal models, and *ex vivo* on various murine models. Next, once a nanocarrier reaches the target site, the mode of drug release needs to be considered. CNOs can be engineered into smart nanocarriers that release their payload in a stimuli responsive manner. For instance, the drug payload can be appended to the CNO surface through a linker that will be selectively cleaved through enzymatic activity at the tumour site. The CNO nanocarrier also holds the potential to be engineered beyond that of a therapeutic system into a theranostic system. Imaging and sensing moieties can be appended to the CNOs, thus expanding its functionality.

In essence, CNOs offer many advantages over other organic and inorganic drug delivery systems. Their robust preparation process allows for the generation of a high-purity and narrow polydispersity material, without the need for the removal of catalysts, as is the case with nanotubes. CNOs are highly stable, expelling the need for specialised storage conditions, as needed with antibodies. Pristine and oxidised CNOs have shown great biocompatibility; a contrast to certain carbon nanomaterials. CNOs are also easily functionalised; their large sp^2 hybridised can be multifunctionalised with different moieties, enabling their use in a range of versatile applications. As discussed in Sec. 3, there are a number of reported cases where CNOs have been utilised in the scope of targeted delivery. These include the targeted delivery across the blood-brain barrier, cellular delivery of glyopeptides and proteins, targeted delivery to CD44 receptor overexpressing cancer cells, and as a nanocarrier for amorphous drug delivery. It is of note that majority of the literature encompassing this subject area is very recent, indicating a developing interest in utilising CNOs as nanocarriers. The literature examples are also of good variety, indicating that there are many applications that CNOs can be utilised for, in the scope of targeted drug delivery.

To summarise, through careful consideration of all aspects involved in the engineering of CNOs, their modification, and their intracellular applications—CNOs can be tailor-made into nanocarriers with desired physio-chemical and biological properties.

Conflicts of interest

There are no conflicts to declare.

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