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Biochemical Applications of Hydrophilic C_{60} Derivatives (hC_{60}s) - a structural perspective

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Abstract:

Reactive oxygen species (ROS) generation and radical scavenging are dual properties of hydrophilic C_{60} derivatives (hC_{60}s). hC_{60}s eliminate radicals in dark, while they produce reactive oxygen species (ROS) in the presence of irradiation and oxygen. Compared to the pristine C_{60} suspension, the aqueous solution of hC_{60}s is easier to handle in vivo. hC_{60}s are diverse and could be placed into two general categories: covalently modified C_{60} derivatives and pristine C_{60} solubilized non-covalently by macromolecules. In order to present in detail, the above categories are broken down into 8 parts: C_{60}(OH), C_{60} with carboxylic acid, C_{60} with quaternary ammonium salts, C_{60} with peptide, C_{60} containing sugar, C_{60} modified covalently or non-covalently solubilized by cyclodextrins (CDs), pristine C_{60} delivered by liposomes, functionalized C_{60}-polymer and pristine C_{60} solubilized by polymer. Each hC_{60} shows the propensity to be ROS-producer or radical scavenger. This preference is dependent on hC_{60} structures. For example, major application of C_{60}(OH) is radical scavenger, while pristine C_{60}/γ-CD complex usually serves as ROS producer. In addition, the electron acceptability and innate hydrophobic surface confer hC_{60}s with O_2 uptake inhibition, HIV inhibition and membrane permeability. In this review, we summarize the preparation methods and biological applications of hC_{60}s according to the structures.

Keywords: Hydrophilic C_{60} derivatives; ROS generation; Radical scavenger; Biological applications

1. Introduction

Hydrophilic C_{60} derivatives (hC_{60}s) serve as either photosensitizer or radical scavenger. The intriguing co-existence of two opposite capacities leads to in-depth study of hC_{60}s. The photodynamic ability has applications on DNA cleavage, antitumor, antibacterial activities etc., while the ability of absorbing radical causes hC_{60}s to be antioxidant agents. In most of state-of-the-art research, the native C_{60} is produced by two most common ways, Krätschmer-Huffman method and combustion of laminar flames of benzene and oxygen [1-3]. Both of the two methods generate C_{60} by-product, which is removed through chromatography [4]. The pristine C_{60} with poor hydrophilicity limits its further development. Three strategies are adopted to get biocompatible hC_{60}s: (1) the introduction of head-top groups on C_{60} cage, such as hydroxyl, carboxyl, quaternary ammonium salts, (2) the conjugation of small hydrophilic molecules (saccharides, peptides) via different linkers, (3) the encapsulation of macromolecules (CDs, liposomes and polymers). In this review, we present hC_{60}s, their biological applications and try to explain them based on structures.

1.1 Physicochemical property of C_{60}

Fullerene (C_{60} is an even number) is a spheroid made of at least 20 carbon atoms. The formation of the peculiar spheroid structure has been explained by a ‘shrink-wrapping’ mechanism [5]. Multiwall nanotubes wrap into the giant fullerenes, which will sublime several C_{60} and twine further to form the more stable C_{70} and C_{82}. If the reaction continues, the carbon atoms are removed to form the smaller fullerenes (like C_{20}), which are instable and prone to open and disappear irreversibly [6]. All of the fullerene members contain different number of hexagons and 12 pentagons which are essential to constitute the spheroid. Small fullerenes (C_{20} ≤ C_{60} ≤ C_{82}) have been predicted to possess narrow HOMO-LUMO gaps and high reactivity owing to the adjacent pentagons, which violate isolated pentagon rule (IPR) [7]. C_{60} is the first fullerene to conform to IPR and without any other IPR isomers, so is the second abundant fullerene C_{70}. Larger fullerenes (C_{70} ≥ 76) have at least 2 IPR isomers. The number of IPR isomers increases with the enlargement of the size of fullerene, except C_{60} (24 IPR isomers) and C_{82} (19 IPR isomers) [8].

C_{60}, constituted by 60 sp^2-hybridized carbon atoms, is an icosahedron of 12 pentagons which are separated by 20 hexagons [9]. Each carbon atom connects with each other by three non-planar σ bonds, which leads to the angle strain and a π orbit forming a large π electron cloud. The angle between the π orbital and σ bond is 116.6°, while the angels of normal alkene and alkyl are 0° and 19.47°, respectively [10]. The way to alleviate the angle strain is that sp^3-hybrid transforms to sp^3-hybrid. These carbon atoms compose [6,6] bond (located between two fused 6-membered rings) and [5,6] bond fused by 5- and 6-membered rings. The pristine C_{60} is prone to produce [6,6] cyclodextrin on account of [6,6] bond much closer to olefinc bond than [5,6] bond [9]. Various C_{60} adducts can be obtained through Binger reaction [12-14], Diel-Alder reaction [15], [3+2] cycloaddition reaction, [2+2] cycloaddition reaction [16], SET-promoted photoaddition reaction [17] and other different addition patterns [18-20].
1.2 ROS producer and Radical scavenger

C_{60} behaves like an electron-deficient olefin attributed to poor electron delocalization. It could accept at most 6 electrons, which has been confirmed by 6 measured potentials [21, 137]. The high electron affinity endows C_{60} with radical scavenging ability [138].

C_{60} generates ROS under UV irradiation, even under white light (Fig. 1). The dominant one is single oxygen (O_2), quantitatively produced by oxygen accepting energy from C_{60} (Type II Energy Transfer). C_{60} with lower energy (37.5 kcal/mol) is obtained via intersystem crossing from C_{60} with relatively high energy (46.1 kcal/mol) (Fig. 1) [22]. If there are electron donor (such as, triethylamine and NADH) in the solution, C_{60} accepts an electron to form C_{60}^{•−} (Type I Electron Transfer). O_2 obtains the electron from C_{60}^{•−} to get O_2^{•−}, which is followed by disproportionation catalyzed by superoxide dismutase (SOD) and Fenton reaction. O_2^{•−} is generated [23-25]. ROS is applied for tumor inhibition, antibacterial, DNA cleavage, delay of arthritic progress, etc..

1.3 Pristine C_{60} suspensions and hC_{60}s in water

Radical scavenging ability is attributed to the high electron affinity, whilst both pristine C_{60} and hC_{60}s are irradiated to produce ROS. Both pristine C_{60} and hC_{60}s do not have to affect the biological activities through the direct interaction with the target (protein, DNA, etc.) [139]. In fact, the inherent aggregation or cluster hinders pristine C_{60} from interacting with the target by a single C_{60} molecule. It is attributed to the poor solubility of pristine C_{60} in aqueous solution or polar solvents [140, 141]. hC_{60}s not only improve the water-solubility of pristine C_{60}, but also decrease the C_{60} aggregation. Furthermore, the only exception is C_{60} derivatives as HIV inhibitors. The C_{60} cage binds directly to the big hydrophobic pocket of HIV aspartic enzyme. However, no paper has reported that pristine C_{60} shows HIV inhibition [81-84].

The pristine C_{60} suspensions can be obtained by simple solvent extraction, sonication or long-term stirring [142]. Although these preparation methods increase pristine C_{60} concentration in aqueous solution, large aggregates still exist. ROS producing ability will decrease with the growing aggregation. C_{60}, the indispensable intermediate to produce ROS, is sensitive to the outer environment. C_{60} could be quenched by the surrounding C_{60} and another C_{60} among C_{60} aggregates [26]. Besides, the aggregation reduces the diffusion rate of O_2 [143]. The life time of C_{60} is from tens to a hundred of microseconds in the solution of monomeric C_{60} analogues, while it lowers to less than 0.1 µs in a C_{60} cluster [143]. In addition, the decreased area of the conjugated C_{60} reduces O_2 quantum yield [145]. Hence, ROS producing ability is dependent on low aggregation and relatively intact conjugated surface.

On the contrary, high degree of aggregation does not reduce the radical scavenging ability obviously. Fullerenol and C_{60}(COOH)_{20}, with large aggregation erase radicals [29-33, 144]. Their main ability is radical scavenging. Even if fullerenol is irradiated, its ROS producing ability is much weaker than that of C_{60}/CD complex [98]. Besides aggregation, the large amount of substitutions (such as, O_2^{•−}) on C_{60} cage disturb the conjugated system, causing weak ROS production [145].

The reminder of this review is organized as follows: Section 2 presents C_{60}(OH)_{14}, which are mainly as radical scavenger; Section 3 summarizes C_{60} with carboxylic acids, which are radical scavenger and weak ROS producer. Section 4 takes a look at C_{60} with quaternary ammonium salts, which are ROS producer. O_2 uptake inhibitor, DNA or drug vector; in Section 5, we give a detailed study of C_{60} containing peptides, serving as radical scavenger, ROS producer, vector and HIV inhibitor; Section 6 is a brief introduction to C_{60} with sugar as radical scavenger, ROS producer and drug vector; Section 7 shows CD-C_{60} conjugates and CD/C_{60}, which are ROS producer; Section 8 summarizes cationic liposome/C_{60} as ROS producer and neutral liposome/C_{60} as radical scavenger; Section 9 gives a deep analysis of C_{60}-polymer conjugates and C_{60}/polymer complex. In conclusion, we will review all the state-of-the-art of hC_{60} on biology.

![Fig. 1. ROS Generation](image)

2. C_{60}(OH)_{14}
-O·H were introduced on C_{60} cage in order to improve the hydrophilicity of C_{60} by oxidative agents. The common agents were H_2SO_4, SO_3, HNO_3, O_2 and H_2O_2. The early work was that C_{60}(OH)_{15-24} expunge O_2·− and was considered as radical scavenger [27, 28]. Other C_{60}(OH)_{24} had the same effect to prevent the oxidative damages from DOX, X-ray, H_2O_2, lead and CCl_4. The protective activity of C_{60}(OH)_{24} was related to anti-aging, anti-inflammation and promoting bacterial growth [134-136].

2.1 Radical scavenger: Prevent oxidative damage from DOX and CCl_4

C_{60}(OH)_{24} is taken as an antioxidant protector against the cardiototoxicity, pulmototoxicity, nephrotoxicity and hepatotoxicity induced by DOX [29-33]. C_{60}(OH)_{24} was obtained by the derivatization of C_{60}Br_{24}, which was afforded by C_{60} and bromide in the presence of catalytic amount of FeBr_3, with the yield of 98% [29, 34]. The above toxicities were attributed to free radicals generated by DOX in vivo. DOX was especially harmful to heart owing to the abundant mitochondria in cardiomyocytes. NADH dehydrogenase contained in the mitochondria reduced DOX to the semiquinone compound and the latter offered an electron to O_2·−, inducing the production of O_2·− and H_2O_2 [35]. C_{60}(OH)_{24} was tested to the healthy rats with the co-treatment of DOX. The DOX alone group led to adrenalin-induced reflex bradycardia and vacuolization of cardiomyocytes. The pre-treated C_{60}(OH)_{24} group delayed or even diminished these side effects [29]. In addition, 100 mg/kg C_{60}(OH)_{24} maintained the level of antioxidative enzymes superoxide in cardiomyocytes which would be enhanced by 8 mg/kg DOX. The enzymes included SOD, catalase (CAT), glutathione peroxidase (GSH-Px) and glutathione reductase (GR) [35]. Lactate dehydrogenase (LDH) and γ-glutamyltransferase (γ-HBDH) in cardiomyocytes were marks to evaluate the tissue injuries. Both of them being elevated by DOX decreased by C_{60}(OH)_{24} [35]. Except the cardioprotection, C_{60}(OH)_{24} prevented the oxidative injury from the lung and kidney as well. The rats received 1-methyl-1-nitrosourea (MNU, a carcinogen) were treated with 8 mg/kg DOX and 100 mg/kg C_{60}(OH)_{24}. Compared with the parameter of cardioprotection, GSH-Px activity in the lung and kidney was decreased by DOX and C_{60}(OH)_{24} maintained the level as the control [30, 31]. The hepatoprotection of C_{60}(OH)_{24} showed that it downregulated the level of enhanced SOD, GSH-Px, GR, CAT and total antioxidant status (TAS). Nevertheless, MDA level of the group treated with MNU/C_{60}(OH)_{24}/DOX (8663 µg/L) were much higher than the control (970.7 µg/L) and the MNU−DOX group (584.3 µg/L). The plausible reason was the poor water-solubility of C_{60}(OH)_{24}. About 20% C_{60}(OH)_{24} remained on the ventral surface of the liver, pancreas and spleen of the rat after the intraperitoneal injection. It caused the increasing exudates in the abdomen and chest, leading to the significant change of MDA, ALT and AST level. It showed hepatoprotection against CCl_4-induced oxidative damage, which was coincident with pristine C_{60} suspension [36, 147]. The further study showed that C_{60}(OH)_{24} had the cardioprotection and hepatoprotection on the chronic toxicity induced by DOX as well [33].

C_{60}(OH)_{24} not only lowered DOX toxicity towards heart, but also inhibited angiogenesis, which assisted DOX to inhibit tumor cells [37, 38]. PEG-C_{60}(OH)_{14-24}−DOX inhibited the growth of mouse melanoma cell line B16−F10. Both of free DOX and PEG-C_{60}(OH)_{14-24}−DOX decreased the viability of HeLa cells at the concentration of 1 µM [38]. PEG-C_{60}(OH)_{14-24}−DOX was mainly accumulate in the tumor tissue and liver [37]. Moreover, PEG-C_{60}(OH)_{14-24}−DOX completely inhibited angiogenesis at the concentration of 100 µM (calculated by DOX) and DOX alone did not show any inhibition. In other model to detect endothelial tubulogenesis, PEG-C_{60}(OH)_{14-24}−DOX strengthened the tubulogenesis inhibition of either C_{60}(OH)_{14-24} or DOX at the concentration of 1 µM and it did not bear the cytotoxicity [38].

2.2 Radical scavenger: Protect cells from the radiation

C_{60}(OH)_{24} improved the cell survival suffered from X-ray damage. X-ray of high dose (24 Gy) lowered the cell viability of human erythroleukemia K562 cells, C_{60}(OH)_{24} decreased the effect. Moreover, the cell survival of C_{60}(OH)_{24}−incubated group without the irradiation was higher than that of control group [39]. With the irradiation of lower dose X-ray (2 Gy), 10µM C_{60}(OH)_{24} did not enhance obviously the cell survival. But the expression of anti-apoptotic and cytoprotective genes was modulated. Similarly, most of the cytoprotective genes were elevated with the pre-treated C_{60}(OH)_{24}, such as CAT, Mn-SOD, miric oxide synthase, glutathione S-transferase isoform GSTA4, glutathione peroxidase and gamma-glutamyltransferase [40].

2.3 Other protection

C_{60}(OH)_{24} had other protective applications. C_{60}(OH)_{14-24} served as the antagonist of glutamate receptor to protect the nerve cells. It did not work with other receptors, such as N-methyl-D-aspartate (NMDA) and kainate receptor. 50µM C_{60}(OH)_{14-24} inhibited 50% activity of glutamate [41]. C_{60}(OH)_{14-24}·H_2O prevented UV-induced cell injuries to protect human keratinocytes [42]. The mixture Cu_{2}(H_2O)_{2} promoted the growth of Escherichia coli [43]. C_{60}(OH)_{14-24} inhibited the inflammation through reducing expression of interleukin-1β and toll-like receptor 4 [44]. C_{60}(OH)_{24} lowered the angiogenesis factors, leading the anti-tumor activity [45].

2.4 Radical scavenger and ROS producer

A recent study described that C_{60}(OH)_{24} acted as both photosensitizer and antioxidative reagent [46]. Folic acid (FA) was introduced to target HeLa cells. DOX-hydrzone-C_{60}(OH)_{24}·FA was well dispersed in water and the aggregate was 135 nm. 100 nm – 200 nm was the best range of nanoparticles as drug attributed to the enhanced permeation and retention (EPR) effect [46, 119]. After the exposure of 460–485 nm light, hydrazone-C_{60}(OH)_{24}·FA decreased the viability of HeLa cells from 100% to 60%. _1^2O quantum yield of hydrazone-C_{60}(OH)_{24}·FA was 0.40. Therefore, photodynamic activity strengthened the inhibition of DOX to HeLa cells.
Furthermore, DOX-hydrazone-C_{60}(OH)_2-FA was less toxic to HeLa cells than free DOX without the light irradiation. It was explained by the radical scavenging effect of C_{60}(OH)_2 [46].

3. C_{60} with carboxylic acid

3.1 Radical scavenger

Malonic acid C_{60}, the mainly used carboxyl fullerene (C_{60}C), were synthesized through Bingel reaction and hydrolysis (Fig. 2) [47].

![Fig. 2. Synthesis of Malonic Acid C_{60} and the structures of C_{60}C and D_{60}C](image)

C_{60}[C(COOH)_2] had the innate radical scavenging ability, which was utilized to detect protease. Tri-malonic acid C_{60}[C(COOH)_2] at the concentration higher than 5 μM quenched gradually the bioluminescence of the humanized *Gaussia* luciferase (bGluc). C_{60}[C(COOH)_2] was linked with His-tagged bGluc through α-thrombin cleavable sequence. If there was protease in the solution, α-thrombin sequence was cleaved and bioluminescence of bGluc was recovered [48].

Same as C_{60}(OH)_2, C_{60}[C(COOH)_2] prevented the oxidative damage. C_{60}C and D_{60}C were regiosomers of C_{60}[C(COOH)_2] (Fig. 2). C_{60}C was more effective on antioxidative protection than D_{60}C. It was considered as two reasons: (1) C_{60}C had a stronger interaction with the membrane [49, 50]. (2) Because of the dipole structure, the C_{60}C cage which was adjacent to the malonic acid group was electron-deficient. This area was potent to attract O_2^--. On the contrary, the electron density was even on the cage of D_{60}C owing to the symmetrical distribution of malonic acid groups [51]. C_{60}C reduced ROS and prevented the apoptosis caused by transforming growth factor-β (TGF-β) and UVB. Both of TGF-β and UVB enhanced ROS in human hepatoma Hep3B cells and keratinocytes, respectively. After the treatment with C_{60}C at the concentration of 20 μM, more than 90% Hep3B cells were protected from apoptosis [50]. 25 μM of C_{60}C recovered the viability of keratinocytes after UVB irradiation. C_{60}C reduced the activation of caspase-3, -6, -8, -9 and -10, which caused by UVB-induced apoptosis. C_{60}C regulated the molecular level of pro-apoptotic protein Bid, antiapoptotic protein Mrcl-1 and Bad, while it did not work to bcl-1 [52]. Moreover, C_{60}C prevented the neuronal apoptosis through decreasing O_2^- in the mitochondria. NMDA induced excitotoxicity was associated to O_2^- production. 30 μM of C_{60}C kept the neuronal survival completely from NMDA. C_{60}C had stronger effect than Vitamin E (less than 25% cell survival) [51]. The further study showed that the neuroprotection was beneficial for prolonging lifespan and ameliorating cognition of aged mouse [53]. Compared to the young mouse, O_2^- was elevated in the old mouse brain. C_{60}C decreased the oxidative stress from 165% to 37% measured by the fluorescence of oxidative dihydroethidium. C_{60}C enhanced 11% of the median lifespan. Besides, C_{60}C elevated the spatial learning and memory performance of old mouse, which was comparative level as young mouse [53].

3.2 ROS producer

Malonic acid C_{60}C acts as ROS producer when they were dispersed by human serum albumin (HSA) and PEG-modified poly(amideamine) (PAMAM) dendrimer. Both of C_{60}C/HSA complex and malonic acid C_{60}C/PEG-PAMAM were non-toxic in dark. C_{60}C was monomolecularly encapsulated by HSA. Because of O_2 quantum yield of C_{60}C/HSA complex was 0.46, same level as that of monomeric C_{60}C (0.48). After the irradiation of 350-600 nm light, C_{60}C/HSA complex (20 μM) induced 57% LY80 tumor cells death [54]. Mono-malonic acid C_{60} (MC_{60}) and di-malonic acid (DC_{60}) were encased by PEG-PAMAM through hydrophobic interaction and electrostatic interaction (the tertiary amine of PAMAM and -COOH group of malonic acid C_{60}) [55]. MC_{60}/PEG-PAMAM was more stable than DC_{60}/PEG-PAMAM in physiological pH. MC_{60} was released in the acidic environment. Because of EFR effect and the relatively acidic environment of tumor cells, PEG-PAMAM/MC_{60} accumulated in the tumor cells and decreased the survival of HeLa cells from 80% to 30% under the laser irradiation [56].

4. C_{60} with quaternary ammonium salts

These cationic fullerenes were generated via methylation after 1,3-dipolar cycloaddition of C_{60} and azomethine ylides, synthesized by the amino acids and aldehydes, or aziridines (Fig. 3) [57]. Through Prato reaction, the different isomers were obtained and they showed the similar activity [58]. Therefore, the mixture of isomers was used to further biological applications. These cationic bC_{60}s were as ROS producer and DNA vector.
4.1 O₂ uptake inhibition

The cationic hC₄₉₈S possessed inherently antibacterial activity owing to the acceptability of electrons. The inhibition of O₂ uptake was on the inner-membrane [58-60]. This effect was more potent than that of di-malonic acid C₄₉₈S [59]. Two processes were involved: cationic hC₄₉₈S at a low concentration consumed NADH which was indispensable for O₂ uptake; cationic hC₄₉₈S at a high concentration were oxidized by H₂O₂ which was produced by O₂. Both of the two processes restrained O₂ absorption on the inner-membrane. Because of the different accumulation of C₄₉₈ derivatives between the cell and inner-membrane, C₄₉₈ derivatives inhibited bacterial growth more effectively than dioxygen uptake [58]. Cationic C₄₉₈ (a mixture) showed a completely bacteriostatic effect on gram-negative bacterium E. coli at the concentration of 5 µM and lowered saliently the dioxygen uptake at the concentration of 50 µM [59]. The further study showed that compound 4 and 5, the regioisomers of cationic C₄₉₈ 2, had the similar bacteriostatic effect. 1 µM of compound 5 completely inhibited E. coli, while the same effect needs 0.7 µM of compound 4 [58].

4.2 ROS producer

ROS (‘O₂, ‘O₂−, and ‘OH) generation of cationic hC₄₉₈ led to bacterial inhibition as well. Both NaN₃ (‘O₂ quencher) and mannitol (the scavenger of ‘O₂− and ‘OH) could prevent the inhibition [61]. Compared to mouse L929 fibroblasts, the inhibition was less than the microbes under the same incubation time [62]. Compound 2 at the concentration of 1 µM killed 4–5 logs gram-positive bacteria S. aureus with 2 J/cm² of visible light (400-700 nm) [62]. 4 and 6 logs gram-negative bacteria E. coli with less easily permeable outer-membrane were dead after the treatment of compound 2 (10 µM) under 2 J/cm² irradiation. With the irradiation of 16 J/cm², compound 2 engendered 3–5 logs bacterial death to gram-negative bacterium P. aeruginosa which was more resistant. Both compound 2 and 3 were more potent than compound 1 attributed to the cationic numbers. More cationic charges were beneficial for binding microbial membrane with negative charges [62]. The further study corroborated this result: compound 7 (with 6 cations) > compound 6 (with 2 cations), two regioisomers 9 and 10 (with 2 cations) > compound 8 (with 1 cation), compound 13 (with 2 cations) > compound 12 (with 1 cation) > compound 11 (without cation) [63, 64]. Although compound 3 with 3 cations had stronger inhibition against S. aureus than compound 2 with 2 cations, compound 2 and 3 showed the similar efficiency to E. coli and P. aeruginosa attributed to the relatively poor cellular uptake [62]. The gram-positive bacteria S. aureus absorbed compound 2 and 3 more easily than the
gram-negative bacteria *E. coli* and *P. aeruginosa*. The gram-negative bacteria had the different constituents from gram-positive bacterium. The outer layers of gram-positive bacteria were consisted of peptidoglycan and lipoteichoic acid or β-glucan, cationic hC₆₀ penetrated easily into the bacterial cytoplasm. Nevertheless, gram-negative bacteria with the double membrane structure showed the diffuse barrier. The way to enter was “self-promoted uptake”, that was, cationic hC₆₀ replaced with some necessary ions (such as Mg²⁺, Ca²⁺), attached lipopolysaccharide of the outer membrane and penetrated into the cells [65].

4.2 Drug and DNA vectors

Cationic hC₆₀ facilitated quinazolinone to approach and traverse the cell wall of mycobacteria so that they enhanced the efficiency of quinazolinone [66, 67]. Quinazolinone inhibited the indispensable enzymes for DNA replication. 12, with the minimum inhibitory concentration (MIC) 1.562 µg/mL against *Mycobacterium tuberculosis*, was much more potent to disturb the cell growth of mycobacteria than the contrast 15 with MIC of 200 µg/mL. Because of the introduction of C₆₀, 14 (MIC = 6.25 µg/mL) can sneak into the cytoplasm and facilitate the quinazolinone to inhibit the enzymes. Additionally, the cations of 14 interacted with the carboxylic groups of mycolic acid in the cell envelope of mycobacterium cell wall [66]. From molecular docking, 14 was possible to inhibit hypoxanthine-guanine phosphoribosyltransferase. C₆₀ part of 14 was in a pocket of charged amino acid, containing Lys66, Glu122, Leu123, Asp126, Lys154, Asp182 and Asp188 [68].

Cationic hC₆₀ delivered DNA through hydrophobic interaction and electrostatic attraction [69]. 16 bound to pBR322 DNA minor groove via hydrophobic interaction. Its C₆₀ cage bound to guanosines, which were G81, G83 and G85 at the forward strand as well as G33 at the reverse strand. Furthermore, the side chain of 16 had the H-bond, π-π stacking and electrostatic interaction with DNA as well [70].

5. C₆₀ with peptide

![Diagram of C₆₀ with peptide interactions](image)

5.1 Radical scavenger
The less substitution on C60 cage led to stronger antioxidant protection. 22 (135.8 nm) and 23 (376.9 nm) were much bigger than 21 (9.5 nm) attributed to the self-assembly of 22 and 23 through bidentate hydrogen bonds (Fig. 4). –COOH interacted with –NH2 of another molecule and –NH bound to –COOH of another molecule. The cellular permeability was 21 > 22 > 23 owing to the easy penetration into cells with the small aggregate sizes. Although all of them showed the similar efficiency on scavenging •OH, O2\(^\cdot−\) scavenging efficiency was 22 > 21 > 23 attributed to the number of C=C. The most cell survival was shown by the protection of 22. 21 decreased the amount of apoptotic cells most efficiently from oxidative damage induced by H\(_2\)O\(_2\) (800 µM) [71]. Other research indicated the cytoprotective effect of 24 (more than 100% of the cell viability) was slightly better than 21 (less than 90%) and 22 (less than 100%) at 50 µg/mL. 24 at the same concentration reduced malondialdehyde amount (caused by lead-induced oxidative stress) from 0.50 nmol•mg\(^{-1}\) to 0.21 nmol•mg\(^{-1}\) protein [72].

5.2 ROS producer

C_{60}-Phe and C_{60}-Gly generated ROS. After the irradiation of a 25-W incandescent lamp for 30 min, C_{60}-Phe led to 21.8% human breast cancer cell line MCF-7 apoptosis at the concentration of 320 µg/mL, while C_{60}-Gly (300 µg/mL) induced 41.25% cell apoptosis. The two C_{60}-amino acids caused a significant decrease on cell amount in G2/M and S phase. The pre-incubation of NAC (a radical scavenger) attenuated the cell apoptosis to 9.47% and 8.79%, respectively. Furthermore, NAC lowered the damaged DNA and p-p38 level caused by C_{60}-Phe and C_{60}-Gly [73].

5.3 ROS producer and Radical scavenger

HSA stabilized C_{60} in water. C_{60}/HSA was obtained via the exchange reaction between C_{60}/CD derivative and HSA. The stable C_{60}/HSA aqueous solution maintained the size from 160 nm to 200 nm during 15 days. C_{60} changed the secondary structure of HSA, which was indicated by the decreased Tryp214 fluorescence. Without light exposure, C_{60} strengthened the antioxidant ability of HSA. HSA had this protective effect because of cysteine residue. 50% scavenging activity required 16.5 ± 2.81 µM C_{60}/HSA and 22.3 ± 2.25 µM free HSA. Under the irradiation of visible light, C_{60}/HSA produced the comparable amount of O2\(^•−\) with C_{60}/PNVP and large amount of 'O2 [74, 75].

5.4 Delivery

Highly hydrophobic C_{60} cage delivered peptides to the internal membranes. C_{60}-alanine and C_{60}-alanylalanine quenched the erythrosine triplet both outside and inside of artificial membranes. On the contrary, Co\(^{3+}\) erased the phosphorescence of erythrosine outside [76]. Baa was synthesized by C_{60} and an amino acid (Fig. 4) [77]. Baa-Lys (attached with fluorescein isothiocyanate (FITC))-Lys-OH 17 and Baa-Lys (FITC)-nuclear localization sequence (NLS) 18 passed through the cells membranes (human embryonic kidney epithelial cell line, HEK-293), while Lys(FITC)-Lys4 and Lys(FITC)-NLS cannot [78]. Except the hydrophobic effect of C_{60}, Lys was beneficial to delivery as well. When parts of Lys were replaced with negatively charged Glu, Baa-Lys-(FITC)-Glu4-Gly, Ser-OH showed relatively weak cellular uptake. It was attributed to the electrostatic interaction of positive charge of Lys with the negatively charged phospholipid membrane [78]. The further study was explored. 18 (20 µM in 1% PBS) penetrated epidermis and localized within the intercellular spaces of the stratum granulosum after flexing the skin for 90 min. The permeable ability made C_{60} with peptide as a potential drug delivery [79].

The steric hindrance of C_{60} prevented tuftsin (Thr-Lys-Pro-Arg) to decompose by leucine aminopeptidase. Tuftsin is an immunostimulating agent. Compared to tuftsin, C_{60} enhanced the stimulation of phagocytosis and chemotaxis effect. C_{60}-tuftsin-COOH 20 at the concentration of 20 µmol/L led to the highest phagocytosis and NH2-tuftsin-C_{60} at the same concentration reached the best chemotaxis. Both NH2-tuftsin-C_{60} 19 and 20 stimulated the expression of major histocompatibility complex class II (MHC II), which was expressed against antigens. Tuftsin was not able to affect MHC II expression. In addition, 19 and 20 improved cell proliferation approximately 30% and 45%, respectively. The control group increased 12%. More cells led to more stimulation of immune cells against antigens. Moreover, both of 19 and 20 did not bear the innate toxicity towards murine peritoneal macrophages [80].

5.5 HIV inhibitor

hC_{60}s inhibited both HIV aspartic protease and HIV reverse transcriptase. Bis(phenethylaminuccinate) C_{60} (K\(_i\) = 5.3 µM) was the first one which reported to bind the large hydrophobic pocket of HIV aspartic protease through van der Waals force [81]. C_{60}-Thr-Tyr-Asn-Thr inhibited HIV protease as well, but weakly [82]. Furthermore, C_{60} with amino acid derivatives 25 and 26 inhibited HIV reverse transcriptase with IC\(_{50}\) value of 0.029 µM and 1.0 µM, respectively. The activity was better than Nevirapine\(^\circ\) (IC\(_{50}\) = 3.0 µM) and C_{60} with quaternary ammonium salt 2 [83]. Fmoc-Baa \((K_i = 36 \text{ nM})\) had more potent inhibition against HIV aspartic protease than Baa \((K_i = 120 \text{ nM})\). The possible reason was that Fmoc-Baa possessed more hydrogen bonding and van der Waals interaction with HIV aspartic protease [84].

6. C\(_{60}\) containing sugar

6.1 Radical scavenger

27 and 28 were weak radical scavengers (Fig. 5). The large amount of –OH groups may induce C\(_{60}\) aggregation and quenched radicals. Both of 27 and 28 absorbed the peroxyl radicals. Their activity was comparable with phenolic antioxidant compounds, but weaker than vitamins E and C and β-carotene [85].
6.2 ROS producer

ROS generated by C₄₀-sugar derivatives inhibited HeLa cells and degraded HIV aspartic protease. C₄₀-monosugars produced more ¹⁰₂ than C₄₀-bissugars. Therefore, C₄₀-monosugars had more potent inhibition against HeLa cells than C₄₀-bissugars under UV exposure [86]. 29 and 30 generated ROS upon the irradiation of both UV and visible light (Fig. 5). ROS led to the degradation of HIV aspartic protease. The photodynamic ability decreased with the co-treatment of DMSO, KI, and histidine, which were HO•, H₂O₂, and ¹⁰₂ scavengers, respectively. 29 (1.5 µM) and 30 (15 µM) induced the complete degradation of HIV aspartic protease. Compared to 29, 30 did not show inhibition against HIV reverse transcriptase. 30 had the inherent ability against HIV aspartic protease. However, the inhibition without light (IC₅₀ = 15.1 µM) was weaker than that (IC₅₀ = 2.25 µM) under irradiation. Moreover, 30 inhibited significantly HIV replication in human leukemic Molt-4 T cells and peripheral blood mononuclear cells. 30 (10 µM) decreased p24 amount (a marker of HIV replication) obviously [87, 88].

6.3 Drug vector

hC₄₀, with multiple reactive sites can form multivalent monosugar systems. Compared to the corresponding monosaccharides analogues with biological activities, 31, 32, 33 and 34 loading 12 monosaccharides were more efficient to α-mannase, especially, 32 (Fig. 5). However, the corresponding monosaccharide of 32 had better inhibition than 32 against maltase, isomaltase and β-glucase. It was attributed to the different shape of the catalytic sites. α-mannase possessed the shallow and long active site and allowed several monosaccharides to bind it simultaneously. Therefore, the multivalent 32 enhanced the inhibition 557-folds compared with the corresponding monosaccharide. On the contrary, other glycosidases, maltase, isomaltase and β-glucase, bore the deep and narrow active sites. Monosaccharides were more efficient than the multivalent system [89, 90]. The similar multivalent system, C₅₆ conjugating with 36 mannosides through long linkers, inhibited pseudotyped Ebola virus to enter into cells. Martin N. et al. used the multivalent system with 12 and 24 monosaccharides to block the bacterial adhesion to the cell surface [91, 92].

7. C₄₀ and Cyclodextrins (CDs)

CDs with inherent hydrophilicity and big cavities are good tools to enhance hydrophilicity of C₄₀. The common derivatives are C₄₀-β-CD conjugates, C₄₀-β-CD micelles and C₄₀/γ-CD complex (Fig 6). α-CD was not applied because of the relatively small cavity. All of them are radical producer, serving as DNA cleavage and cells inhibition.

Although C₄₀-β-CD conjugates aggregated in aqueous solution, they produced ROS under irradiation. ROS destroyed DNA. The mechanism of C₄₀ cleaving DNA was as follows: (1) O₂⁻, produced via Type II Energy Transfer, oxidized the guanosine to 8-Oxo-guanine (8G). It was suggested by the majority; (2) If DNA strands contained guanosine stacks which were liable to oxidation, C₄₀ and O₂⁻ accepted the electrons from guanosine (Type I Electron Transfer), successively. 8G was obtained. The further oxidation of 8G will generate an alkali-labile site, causing DNA cleavage [93]. 35 was the first reported conjugate to rip DNA. During the cleavage, the characteristic peak 343 nm of C₄₀ was weakened [94]. 36 was an example that it produced O₂⁻ to destroy DNA [95]. Another C₄₀-β-CD conjugate 37 confirmed that NADH was indispensable to pBR 322 DNA cleavage. It meant that O₂⁻ and •OH generated through Type I Electron Transfer played an important role.
Although 33 μmol/L 37 ripped DNA to small fragments completely, the photodynamic ability to the cells was weak. 400 μmol/L 37 killed less than 40% SH-SYSY cancer cells [96].

\[ \text{Fig. 6. Cagβ-CD conjugates and Cagβγ-CD complexes} \]

Cag2/2-hydroxypropyl-β-CD (HP-β-CD) nanoparticles produced ROS and killed HeLa cells. The small aggregate led to the effective photodynamic ability because of attenuating the radical self-quenching. Besides, the large Cag aggregate decreased the surface of exposed light, which was possible to lower the efficiency of ROS production [74]. CagHP-β-CD nanoparticle with the size of 90 nm was obtained by codoping Cag and HP-β-CD for 3h at 4 °C under reduced pressure. CagHP-β-CD colloidal solution was stable and maintained the similar size of aggregate during 28 days. It was more potent to produce ROS than Cag alone solution at the same concentration (40 μM). Because the aggregate in Cag alone solution was 427 nm. There was scarcely \(^1\text{O}_2\) production in Cag alone solution, while CagHP-β-CD colloidal solution generated \(^1\text{O}_2\), \(\text{O}_2^-\) and •OH. Hence, CagHP-β-CD colloidal solution (40 μM) killed 75% cancer cells under the visible light irradiation, while Cag alone solution showed no inhibition. Both Cag(HP-β-CD colloidal solution and Cag alone solution were non-toxic in dark [74].

γ-CD with a big cavity could encapsulate Cag and avoid aggregation [97]. The cell inhibition was dependent on the ability of ROS production (especially, \(^1\text{O}_2\)) and cellular uptake. The quantum yield of \(^1\text{O}_2\) generated by Cagγ-CD complex (0.78) was much higher than that of Cag(HP)cag (0.08) in D₅O. Cagγ-CD complex (IC₅₀ = 0.25 μM) showed higher photodynamic ability than Cag(HP)cag (IC₅₀ = 15μM) against human skin keratinocytes (HaCaT) irradiated with UVA (15 J/cm²) [98]. The aggregates in Cagγ-CD solution increased with heating and no \(^1\text{O}_2\) produced after 150 min. Hence, 2 μM Cagγ-CD complex led to approximately 95% death of human lens epithelial cells, while 30 μM nCagγ-CD aqueous solution (prepared by heating Cagγ-CD complex aqueous solution for 150 min) had very low effect. The aggregate in nCagγ-CD aqueous solution was 136-6 nm [99]. Although Cagγ-CD complex was absorbed into cells much more slowly than Cag(HP)cag and nCagγ-CD, it showed the most potent inhibition [98, 99]. However, Cagγ-CD complex did not have strong inhibition against HeLa cells. 10 μM Cagγ-CD had negligible effect either in dark or under 400-500 nm irradiation. Cag6-aminog-γ-CD (10 μM) caused more than 60% HeLa cell death under irradiation. The intriguing phenomenon was that Cag6-aminog-γ-CD could squeeze Cag out when pH was lowered from 7.4 to 6.4. It possessed stronger inhibition at pH = 6.4 than that at pH = 7.4, although there was colloidal aggregate in the solution with pH = 6.4. The size of colloidal aggregate was small, only 20 nm. It was possible that the small Cag aggregates were absorbed by HeLa cells faster than Cag6-aminog-γ-CD and Cagγ-CD [100].

The pristine Cag can not absorb the long wavelength light (610-780 nm), but the functionalized Cag derivatives could generate \(^1\text{O}_2\) under this range of irradiation. The photodynamic ability against HeLa cells was 1γ-CD complex (IC₅₀ = 0.47 μM) > 39γ-CD complex (IC₅₀ = 0.95 μM) > 38γ-CD complex > Cagγ-CD complex. The clinical photosensitizer Photofrin inhibited HeLa cells with IC₅₀ = 2 μM. The photodynamic activity decreased with the co-treatment of \(^1\text{O}_2\) quencher 1-histidine, while the addition of α-mannitol (O₂− quencher) did not lower cell viability. 38γ-CD complex was a weak photosensitizer attributed to electron-transfer quenching caused by the long pair electrons on the amine. 39γ-CD complex was quenched before it produced \(^1\text{O}_2\) via energy transfer. 39γ-CD complex with the –Ac on amine weakened the quenching effect. Cationic 1γ-CD complex had the best inhibition owing to the electrostatic interaction with the anionic surface of HeLa cells [101].

8. Cag delivered by liposomes

8.1 ROS producer

Lipid membrane-incorporated Cag (LMICag) was more stable than γ-CD/Cag complex in water. LMICag was obtained via an exchange reaction between liposomes and γ-CD/Cag complex by three methods, which were heating, microwave irradiation and photoinduced electron transfer [102, 103]. Cag was released from γ-CD and encased into liposomes. Because the peak assignable to γ-CD/Cag complex at 4.19 and 5.05 ppm disappeared. Under the exposure of visible light, cationic 40- incorporated Cag showed 44% DNA cleavage and zwiterionic 41- incorporated Cag converted 24% supercoiled DNA (form I) to nicked DNA (form II) at the same concentration of 20 μM. Anionic 42- incorporated Cag (20 μM) had little DNA cleaving ability (just 2%), even lower than 20μM γ-CD/Cag complex (6%). It was attributed to the electrostatic repulsions between ‘anionic’ 42- incorporated Cag and ‘anionic’ DNA [104].
Cationic LMIC<sub>60</sub> had stronger inhibition against HeLa cells than anionic LMIC<sub>60</sub> [105]. Because cationic 43-incorporated C<sub>60</sub> was relatively easier to bind to the anionic cellular surface and engendered cell death [133]. LMIC<sub>60</sub> 43-44 led to the morphological change of cells and 85% cells were killed, while 1% cells were dead with the treatment of LMIC<sub>60</sub> 42-44 under 350-500 nm exposure [105]. Zwitterionic liposome 41 was used as matrix here.

Compared to LMIC<sub>60</sub>, the block copolymer micelle-incorporated C<sub>60</sub> (BPMIC<sub>C60</sub>) aqueous solution were more stable [106]. Cationic BPMIC<sub>C60</sub> was absorbed by cells, while anionic and neutral BPMIC<sub>C60</sub> can not. BPMIC<sub>C60</sub> 45 with the proper feed ratio of the fragments (n/p = 53:47) possessed the best water-solubility and photodynamic ability. It induced 98.7% HeLa cells death under 350-500 nm irradiation. No cytotoxicity was observed in dark.

8.2 Radical scavenger

Depending on low dispersion of C<sub>60</sub>, C<sub>60</sub> solubilized by hydrotreated lecithin 46 and glycine soja sterols was as ROS scavenger. C<sub>60</sub> 46/sterol reduced ROS generation and improved the cell viability of HaCaT cells under 10 J/cm<sup>2</sup> UVA exposure [107]. It repressed the striated skin surface, abnormal scaling of epidermis and dermis. C<sub>60</sub>/sterol (0.63 μM) suppressed the abnormality of nucleus, such as, condensed chromatin and shrunken nucleus. C<sub>60</sub> 46/sterol was nontoxic to the normal tissues. It penetrated into the epidermis and can not arrive at the dermis [108].

C<sub>60</sub>/46 inhibited influenza virus (H1N1) in vivo, which was possibly relate to ROS scavenging [109]. The average mice survival was C<sub>60</sub>/46 (3.3 mg/kg/day) > C<sub>60</sub>/46 (1.6 mg/kg/day) ≫ ramitadine (90 mg/kg/day) > C<sub>60</sub>/46 (0.8 mg/kg/day) ≫ the control without administration. The mice treated with C<sub>60</sub>/46 (3.3 mg/kg/day) survived for 16 days, which was longer than 9 days of the control group. The viral yield decreased from 19.3 to 8.6 with the administration of C<sub>60</sub>/46 (3.3 mg/kg/day), which was same level as ramitadine. The co-treatment with both C<sub>60</sub>/46 and ramitadine lowered the lung index to the normal level as the control group [109].

9. C<sub>60</sub> and polymers

9.1 Radical scavenger

C<sub>60</sub>PNVP was Radical Sponge<sup>®</sup>. It protected HaCaT cells from the oxidative damage induced by UVB and t-BuOOH [110]. Besides, C<sub>60</sub>PNVP decreased UVA-induced ROS level in normal human epidermis melanocytes. The effect was not dose-dependent. Compared to Radical Sponge<sup>®</sup> at 75 μM, the lower concentration (25 μM) engendered higher ROS inhibition. 68% intracellular ROS was reduced in human malignant melanoma cell line. With the treatment of 30 μM Radical Sponge<sup>®</sup>, the melain content induced by 0.1 J/cm<sup>2</sup> UVA reduced from 190% to 54.6% and tyrosinase activity lowered from 136% to 50%. Radical Sponge<sup>®</sup> was more effective than arbutin (a tyrosinase inhibitor, preventing the formation of melanin) and L-ascorbic acid (an antioxidant agent) at 500 μM [111]. Another study showed that C<sub>60</sub>/squalene (a skin emulfluent) localized in the epidermis and did not penetrate into the dermis. The location of C<sub>60</sub>/squalene was consistent with C<sub>60</sub>/40/sterol [112].

Although PEG/C<sub>60</sub> was not efficient as C<sub>60</sub>PNVP, C<sub>60</sub>/PEG was a good radical scavenger and delivery [110, 113]. 47 and 48 decreased the innate toxicity of DOX attributed to the ability of radical scavenging and slow-release of DOX (Fig. 9). The urethane bonds linking DOX and C<sub>60</sub>/PEG were degraded in vivo. Both 47 and 48 possessed no antineoplastic effect against MCF-7 cells at the concentration less than 1.5 μM, while free DOX reduced cell viability to 40% at the same concentration. Because of the slow cellular uptake, 47 and 48 did not show stronger inhibition than DOX alone until 72h. Free DOX entered into the nucleus after 15 min, while 47 and 48 were localized in the nucleus after 72h. Both of 47 and 48 formed aggregation in water, which were 143 nm and 147 nm [113].

C<sub>60</sub>PNIPAM copolymer with large aggregates (1000 nm) was a radical scavenger as well. It (1.25 mg mL<sup>−1</sup>) enhanced the fibroblasts viability inherently and prevented the oxidative damage from NOR-3 (NO<sup>·</sup> producer) [114].
9.2 ROS producer

9.2.1 Cao and PNVP

Radical polymerization is a common method to produce Cao-PNVP copolymer (Fig. 8). On one hand, the copolymerization of Cao and N-vinylpyrrolidone (NVP) was carried out directly with 2,2′-azobisisobutyronitrile (AIBN, a radical initiator) (Fig. 8) [115, 116]. On the other hand, PNVP linked to Cao through PVAc via cobalt-mediated radical polymerization (CMRP). 2,2′-azobisis(4-methoxy-2,4-dimethyl valeronitrile) (V-70) served as a radical generator, PNVP-co-PVAc-cobalt(II) acrylacetone (Co(acac)₃) was prepared and reacted with Cao (Fig. 8) [117, 118].

Fig. 8. Synthesis of Cao-PNVP copolymers and Representatives

Cao-PNVP copolymer 49 and PNVP-co-PVAc copolymer 50 were photosensitizers to cleave DNA and kill cells in vitro, respectively. 49 produced O₂⁻ under the irradiation of visible light, while O₂⁻ was the main ROS generated by 50. Cao-PNVP copolymer 49 (the feed ratio of Cao: NVP = 1:20) showed the highest water-solubility (7.8 mM, calculated by Cao). The particle size of 49 in water was 19.5 nm. 49 had the molecular weight of 39 kDa, which was more than 20 kDa, accumulated selectively in tumor cells owing to EPR effect [119]. 49 (1 mM, calculated by Cao) cleaved pBR322 DNA (Form I) to nicked form II in the presence of 10 mM of NADH under irradiation of visible light [115]. On the contrary, no O₂⁻ was produced by 50 [80]. 50 at the concentration of 58 μM produced comparative amount of O₂⁻ with a common photosensitizer, 4,4',4''- (porphine-5,10,15,20-tetrayl)tetraakis(benzene sulfonic acid) (TPPS, 5.5 μM) [117]. Moreover, 50 decreased human promyelocytic leukemia HL-60 cells more than PVHO-Cao copolymer 51 and poly[(PEG acrylate)-co-(vinyl acetate)]-Cao 52. It was attributed to relatively high O₂⁻ quantum yield of 50 (Φ(O₂⁻) = 0.50), compared to that of 51 (Φ(O₂⁻) = 0.12) and 52 (Φ(O₂⁻) = 0.13) [120]. Another 1O₂ producer, Cao/PNIPAm₆b-PNVP₆ micelle 53 had the DNA-cleaving effect as well [121].

9.2.2 Cao and PEG

The well-dispersed Cao-PEG derivatives were innate photosensitizers (Fig. 9). This ability was extended in combination with other biologically effective agents. Although Cao-PEG 54 had interaction with Aβ (IC₅₀ = 192 μM), the affinity of monosaccharide-PEG-Cao 55 was stronger (IC₅₀ = 35 μM). The binding ability was enhanced to IC₅₀ = 2 μM under 365nm irradiation. Both Aβ₄₀ monomer and oligomer were degraded by 55 upon UV exposure because of O₂⁻ and OH⁻ generation [122]. Another example was that Cao-iron oxide nanoparticle (INOP-PEG)hematoporphyrin monomethyl ether (HMME) 56 produced more ROS than Cao-INOP-PEG and HMME under the exposure of 532 nm laser. The relative tumor volume of Cao-INOP-PEG-treated (V/V₀ = 5.96±0.79) and HMME-treated (V/V₀ = 6.45±0.81) mice increased more obviously than 56-treated group (V/V₀ = 2.72±0.55) [123]. The further studies applied magnetic resonance imaging agents Gd-DTPA to enhance the efficiency of Cao. Gd-DTPA-PEG-Cao had the similar level of Gd³⁺ production and reducing cell viability (40%) with the irradiation of visible light compared to Cao. The relaxivity of Gd-DTPA-PeP-Cao(5.3 mM⁻¹s⁻¹) was comparable with Magnevist® (5.3 mM⁻¹s⁻¹). Both of them enhanced MRI signal intensity in tumor, but Gd-DTPA-PEG-Cao maintained in the tumor tissues much longer in a relatively high level than Magnevist® [124]. Except Gd-DTPA, Fe₃O₄ visualized the tumor tissues as well. Cao-Fe₃O₄-PEG₃₂₀/doxetaxel-thermosensitive liposome was a negative (T₂) contrast agent. Besides, this particle released doxetaxel and Cao-Fe₃O₄-PEG₃₂₀ after the increasing temperature of tumor issues by 13.56 MHz. radiofrequency. The radiofrequency also led to ROS generation. ROS assisted doxetaxel to inhibit MCF-7 tumor cells [125].

Cao has the highest absorption under UV. However, UV can not penetrate the skin. Therefore, the agents absorbing the light with long wavelength (> 620 nm) were utilized. The photodynamic activity was improved when Cao-PEGs conjugated with graphene oxide (GO), chlorin e₆ (Ce₆), and upconversion nanoparticles (UCNP). Among these conjugates, FA was introduced to target tumor cells.

FA-GO-PEG-Cao had a synergistic effect on antineoplastic therapy. GO absorbed the energy from 808 nm and released vibrational heat. Cao produced 1O₂ under the irradiation of 532 nm. Both of the released heat and ROS inhibited HeLa cells. The combination of 808 nm and 532 nm exposures led to the cell viability of 3.1% after
treated with FA-GO-PEG-C60 (10 µg/mL). GO lowered the cell viability to 80.4% after 808 nm exposure and FA-C60 decreased the cell survival to 72.4% with 532 nm irradiation. The cellular uptake to FA-GO-PEG-C60 was stronger than GO and FA-GO [126].

**Fig. 9. Ce6-PEGs**

Ce6-PEG-C60-PEG-FA had both photothermal and photodynamic ability, led to the inhibition of human nasopharyngeal epidermal carcinoma KB tumor and arthritic progress. The photosensitizer Ce6 got energy from 670 nm and provided the photon to C60. Under 670 nm irradiation for 7 days, Ce6-PEG-C60-PEG-FA kept the tumor volume at the same level (from 47 mm³ to 54 mm³), while the tumor volume treated by C60-PEG-FA increased from 58 mm³ to 150 mm³. Besides, the arthritic foot surface temperature of the mice increased to 45°C after 2h injection. The generated ROS efficiently inhibited arthritic progress after 5 days irradiation [127].

UCNPs transferred the photon to C60(COOH)2 upon the exposure of near-infrared light (NIR, ~980 nm). PEG-succinimidyl carbonate (SC)-UCNPs-C60(COOH)2 possessed better solubility in water. The particles generated ROS. The phototoxicity to HeLa cells was dose-dependent and the inhibition was obvious at the concentration of 800 µM with the exposure of 980 nm (the cell viability < 30%). On the contrary, PEG-SC-UCNPs-C60(COOH)2 had a lower toxicity without 980 nm irradiation (approximately 90% of the cell viability). Besides, UCNPs emitted multicolor in the visible spectral region, which was applied on NIR imaging [128].

### 9.3 Drug delivery

As a biocompatible linker, C60-PEGs enhanced the bioavailability of polyethyleneimine (PEI), 5-fluorouracil (5-FU), doxorubicin (DOX) and pentoxifylline (PTX). The introduction of C60-PEG balanced the toxicity and transfection efficiency of PEI. C60-PEG-PEI 57 was as DNA vector. C60-PEI and free PEI blocked the cell proliferation, while the cell amount increased to twice with the incubation of 57. More cells meant more expression of enhanced yellow-green Aequorea victoria fluorescent protein (EYFP). This compensated the effect that 57 was less efficient vehicle than C60-PEI. Because the efficiency of 57 transporting pEYFP-C1 plasmid at the N/P ratio above 60 was comparable with that of C60-PEI/pEYFP (N/P ratio = 20) [129]. Other examples showed that C60-PEGs improved effective dosage of 5-FU and PTX. The retention time of 5-FU-PEG-C60 58 was longer than free 5-FU in vivo. Because C60-PEG protected 5-FU from the degradation by dihydropyrimidine dehydrogenase. 58 released 5-FU slowly to cells and liver. 30% MCT-7 cells were inhibited with the incubation of 58 at 3.8 µM, while 5-FU at the same concentration inhibited less than 10% cells [130]. C60-PEG facilitated PTX to pass through the blood-brain barrier attributed to the mechanism of disruption of the tight junctions of brain microvessels endothelial cells [131]. The cell decreased to 67.9% owing to the suffering of ApoEKO. PTX-PEG-C60 59 enhanced the cell viability to 82.7%, which was more potent than PTX (71.0%) [132].

### 10. Conclusion

The main applications of hC60 are radical scavenger and ROS producer. Although each hC60 derivative has the potential to be both, they show a preference: hC60 derivatives, which are usually applied for radical scavengers, are C60(OH)2, malonic acid C60, C60-amino acids, C60-neutral liposomes, C60/PEG, C60/PNVP, C60-PNIPAM. ROS producers are C60 with quaternary ammonium salts, C60 containing sugars, C60/peptide, C60/β-CD conjugates, C60/γ-CD complex, C60/cationic...
lipoosomes, C<sub>60</sub>-PNVP copolymer, PNVP-co-PVAc copolymer and C<sub>60</sub>-PEGs. To our knowledge, the preference is dependent to the extent of dispersion. bC<sub>60</sub> as ROS producer form less aggregates than radical scavenger C<sub>60</sub> as ROS producers. The aggregates in aqueous solution will decrease ROS production (1.3 Pristine C<sub>60</sub> and bC<sub>60</sub>). Furthermore, C<sub>60</sub> with quaternary ammonium salts inhibit O<sub>2</sub> uptake. C<sub>60</sub>-amino acids are HIV inhibitor. C<sub>60</sub> with quaternary ammonium salts, C<sub>60</sub>-amino acids, C<sub>60</sub>-multivalent iminosugars, C<sub>60</sub>-PEGs are good vectors for DNA or drugs.

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References:


Highlight:

- Hydrophilic C₆₀ derivatives (hC₆₀) serve as reactive oxygen species (ROS) producer, radical scavenger, O₂ uptake inhibitor, HIV inhibitor and vectors for DNA or drugs.
- Although each hydrophilic C₆₀ derivative has the potential to be both, they show a preference. The propensity is dependent on C₆₀ structure.
- High O₂ quantum yield means low aggregation. C₆₀ with aggregates scarcely generate O₂, but O₂⁻.
- The typical reactions to get covalent C₆₀ are oxidation, Bingel reaction, Prato reaction, Diel-Alder reaction and radical polymerization.