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Biological 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)$_n$ 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)$_2$ is radical scavenger, while pristine C$_{60}$+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$_{60}$ 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$_{60}$ 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$_{60}$: (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$_n$ n 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]. Multilaminate nanotubes wrap into the giant fullerenes, which will sublime several C$_2$ and twine further to form the more stable C$_{20}$ and C$_{80}$. If the reaction continues, the carbon atoms are removed to form the smaller fullerenes (like C$_{60}$), 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$_n$ ≤ C$_{80}$) 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$_n$ ≥ 76) have at least 2 IPR isomers. The number of IPR isomers increases with the enlargement of the size of fullerene, except C$_{54}$ (24 IPR isomers) and C$_{60}$ (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$^{2.5}$-hybrid transforms to sp$^3$-hybride. 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] cycloadduct on account of [6,6] bond much closer to olefinic bond than [5,6] bond [9]. Various C$_{60}$ adducts can be obtained through Bingel reaction [12-14], Diel-Alder reaction [15], [3+2] cycloaddition reaction, [2+2] cycloaddition reaction [16], SET-promoted photodissociation 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 ($^{1}$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. •OH 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$_{60}$@ in water

Radical scavenging ability is attributed to the high electron affinity, whilst both pristine C$_{60}$ and hC$_{60}$ 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$_{60}$@ 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)$_{2}$ 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, •OH) 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)$_{2}$, 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)$_{2}$
-OHs were introduced on C60 cage in order to improve the hydrophilicity of C60 by oxidative agents. The common agents were H2O2, SO2, Br2, HNO3, O2 and H2O2. The early work was that C60(OH)12-24 expunge O2- and was considered as radical scavenger [27, 28]. Other C60(OH)24 had the same effect to prevent the oxidative damages from DOX, X-ray, H2O2, lead and CCl4. The protective activity of C60(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 CCl4

C60(OH)24 is taken as an antioxidant protector against the cardiotoxicity, pulmonotoxicity, nephrotoxicity and hepatotoxicity induced by DOX [29-33]. C60(OH)24 was obtained by the derivatization of C60Br24, which was afforded by C60 and bromide in the presence of catalytic amount of FeBr3 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 O2, inducing the production of O2- and H2O2 [35]. C60(OH)24 was tested to the healthy rats with the co-treatment of DOX. The DOX alone group led to adrenalin-induced rectal bradycardia and vacuolization of cardiomyocytes. The pre-treated C60(OH)24 group delayed or even diminished these side effects [29]. In addition, 100 mg/kg C60(OH)24 maintained the level of antioxidative enzymes superoxide in cardiomyocytes which would be enhanced by 8 mg/kg DOX. The enzymes contained SOD, catalase (CAT), glutathione peroxidase (GSH-Px) and glutathione reductase (GR) [35]. Lactate dehydrogenase (LDH) and γ-hydroxybutyrate dehydrogenase (γ-HBDH) in cardiomyocytes were marks to evaluate the tissue injuries. Both of them being elevated by DOX decreased by C60(OH)24 [35]. Except the cardioprotection, C60(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 C60(OH)24. Compared with the parameter of cardioprotection, GSH-Px activity in the lung and kidney was decreased by DOX and C60(OH)24 maintained the level as the control [30, 31]. The hepatoprotection of C60(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/C60(OH)24/DOX (8663 ± 100 µg/L) was much higher than the control (970 ± 77 µg/L) and the MNU-DOX group (584 ± 85 µg/L). The plausible reason was the poor water-solubility of C60. About 20% C60(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 alanine aminotransferase (ALT), aspartate aminotransferase (AST), and the ALT/AST ratio as well [32]. 20% more DMSO in the physiological solution of C60(OH)24 could maintain the level of MDA and those enzymes [33]. Besides, less amount of C60(OH)24 can avoid the side effect as well. C60(OH)24 (n = 22.24) at the dose of 5 mg/kg decreased MDA, ALT and AST level. It showed hepatoprotection against CCl4-induced oxidative damage, which was coincident with pristine C60 suspension [36, 147]. The further study showed that C60(OH)24 had the cardioprotection and hepatoprotection against the chronic toxicity induced by DOX as well [33].

C60(OH)24,24-24 not only lowered DOX toxicity towards heart, but also inhibited angiogenesis, which assisted DOX to inhibit tumor cells [37, 38]. PEG-C60(OH)16-24-DOX inhibited the growth of mouse melanoma cell line B16-F10. Both of free DOX and PEG-C60(OH)16-24-DOX decrease the tumor volume in the same level. Free DOX was especially toxic to the spleen and heart, while PEG-C60(OH)16-24-DOX was mainly accumulate in the tumor tissue and liver [37]. Moreover, PEG-C60(OH)16-24-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-C60(OH)16-24-DOX strengthened the tubulogenesis inhibition of either C60(OH)16-24 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

C60(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, C60(OH)24 decreased the effect. Moreover, the cell survival of C60(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 C60(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 C60(OH)24, such as CAT, Mn-SOD, mitc oxide synthase, glutathione S-transferase isofrom GSTA4, glutathione peroxidase and gamma-glutamyltransferase [40].

2.3 Other protection

C60(OH)24 had other protective applications. C60(OH)16-32 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 C60(OH)16-32 inhibited 50% activity of glutamate [41]. C60(OH)24•H2O prevented UV-induced cell injuries to protect human keratinocytes [42]. The mixture C60H2(OH)24 promoted the growth of Escherichia coli [43]. C60(OH)12-12 inhibited the inflammation through reducing expression of interleukin-1β and toll-like receptor 4 [44]. C60(OH)24 lowered the angiogenesis factors, leading the anti-tumor activity [45].

2.4 Radical scavenger and ROS producer

A recent study described that C60(OH)24 acted as both photosensitizer and antioxidative reagent [46]. Folic acid (FA) was introduced to target HeLa cells. DOX-hydrazone-C60(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-C60(OH)24-FA decreased the viability of HeLa cells from 100% to 60%. 1H quantum yield of hydrazone-C60(OH)24-FA was 0.40. Therefore, photodynamic activity strengthened the inhibition of DOX to HeLa cells.
Furthermore, DOX-hydrazone-Cd(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 Cd$_{en}$OH$_2$.[46].

3. Cd$_{en}$ with carboxylic acid

3.1 Radical scavenger

Malonic acid Cd$_{en}$, the mainly used carboxyfullerene (Cd$_{en}$), were synthesized through Bingel reaction and hydrolysis (Fig. 2) [47].

![Synthesis of Malonic Acid Cd$_{en}$ and the structures of C$_3$Cd$_{en}$ and D$_3$Cd$_{en}$](image)

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

Same as Cd$_{en}$(OH)$_2$, Cd$_{en}$(C(COOH)$_2$)$_2$ prevented the oxidative damage. C$_3$Cd$_{en}$ and D$_3$Cd$_{en}$ were regioisomers of Cd$_{en}$(C(COOH)$_2$)$_2$ (Fig. 2). C$_3$Cd$_{en}$ was more effective on antioxidative protection than D$_3$Cd$_{en}$. It was considered as two reasons: (1) C$_3$Cd$_{en}$ had the stronger interaction with the membrane [49, 50]. (2) Because of the dipole structure, the Cd$_{en}$ cage which was adjacent to the malonic acid group was electron-deficient. This area was potent to attract O$_2^\cdot$-. On the contrary, the electron density was even on the cage of D$_3$Cd$_{en}$ owing to the symmetrical distribution of malonic acid groups [51]. Cd$_{en}$ 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 Cd$_{en}$ at the concentration of 20 μM, more than 90% Hep3B cells were protected from apoptosis [50]. 25 μM of Cd$_{en}$ recovered the viability of keratinocytes after UVB irradiation. C$_3$Cd$_{en}$ reduced the activation of caspase-3, -6, -8, -9 and -10, which caused by UVB-induced apoptosis. C$_3$Cd$_{en}$ regulated the molecular level of pro-apoptotic protein Bid, anti-apoptotic protein Bcl-2 and Bad, while it did not work to bcl-1 [52]. Moreover, C$_3$Cd$_{en}$ prevented the neuronal apoptosis through decreasing O$_2^\cdot$- in the mitochondria. NMDA induced excitotoxicity was associated with O$_2^\cdot$- production. 30 μM of Cd$_{en}$ kept the neuronal survival completely from NMDA. C$_3$Cd$_{en}$ 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$_2^\cdot$- was elevated in the old mouse brain. Cd$_{en}$ decreased the oxidative stress from 165% to 37% measured by the fluorescence of oxidative dityhroethidium. C$_3$Cd$_{en}$ enhanced 11% of the median lifespan. Besides, C$_3$Cd$_{en}$ elevated the spatial learning and memory performance of old mouse, which was comparative level as young mouse [53].

3.2 ROS producer

Malonic acid Cd$_{en}$ act as ROS producer when they were dispersed by human serum albumin (HSA) and PEG-modified poly(amideamine) (PAMAM) dendrimer. Both of Cd$_{en}$/HSA complex and malonic acid Cd$_{en}$/PEG-PAMAM were non-toxic in dark. Cd$_{en}$ was monomolecularly encapsulated by HSA. Because O$_2^\cdot$- quantum yield of Cd$_{en}$/HSA complex was 0.46, same level as that of monomeric Cd$_{en}$ (0.48). After the irradiation of 380-600 nm light, Cd$_{en}$/HSA complex (20 μM) induced 57% LY80 tumor cells death [54]. Mono-malonic acid Cd$_{en}$ (MC$_{en}$) and di-malonic acid (DC$_{en}$) were encased by PEG-PAMAM through hydrophobic interaction and electrostatic interaction (the tertiary amine of PAMAM and –COOH group of malonic acid Cd$_{en}$) [55]. MC$_{en}$/PEG-PAMAM was more stable than DC$_{en}$/PEG-PAMAM in physiological pH. MC$_{en}$ was released in the acidic environment. Because of EPR effect and the relatively acidic environment of tumor cells, PEG-PAMAM/MC$_{en}$ accumulated in the tumor cells and decreased the survival of HeLa cells from 80% to 30% under the laser irradiation [56].

4. Cd$_{en}$ with quaternary ammonium salts

These cationic fullerenes were generated via methylation after 1,3-dipolar cycloaddition of Cd$_{en}$ 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 hCd$_{en}$ were as ROS producer and DNA vector.
could prevent the inhibition\[61\]. Compared to mouses possessed inherently antibacterial activity owing [60], had the similar bacteriostatic effect. 1

4.1 O₂ uptake inhibition

The cationic hC₅₀₈₈ 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 C₆₉₈ [59]. Two processes were involved: cationic hC₅₀₈₈ at a low concentration consumed NADH which was indispensable for O₂ uptake; cationic hC₅₀₈₈ 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 C₅₀₈₈ derivatives between the cell and inner-membrane, C₅₀₈₈ derivatives inhibited bacterial growth more effectively than dioxygen uptake [58]. Cationic C₅₀₂ (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 C₅₀₂, 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 (²O₂, ROS, and OH⁻) generation of cationic hC₅₀₈₈ led to bacterial inhibition as well. Both NaN₃ (²O₂ quencher) and mannitol (the scavenger of ROS 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 penetrating 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, 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 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.

5. C60 with peptide

5.1 Radical scavenger

Fig. 4. C60 with peptides
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 –NH2 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•− 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 mostly from oxidative damage induced by H2O2 (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

C60-Phe and C60-Gly generated ROS. After the irradiation of a 25-W incandescent lamp for 30 min, C60-Phe led to 21.8% human breast cancer cell line MCF-7 apoptosis at the concentration of 320 µg/mL, while C60-Gly (300 µg/mL) induced 41.25% cell apoptosis. The two C60-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 C60-Phe and C60-Gly [73].

5.3 ROS producer and Radical scavenger

HSA stabilized C60 in water. C60/HSA was obtained via the exchange reaction between C60/CD derivative and HSA. The stable C60/HSA aqueous solution maintained the size from 160 nm to 200 nm during 15 days. C60 changed the secondary structure of HSA, which was indicated by the decreased Try214 fluorescence. Without light exposure, C60 strengthened the antioxidant ability of HSA. HSA had this protective effect because of cysteine residue. 50% scavenging activity required 16.5 ± 2.81 µM C60/HSA and 22.3 ± 2.25 µM free HSA. Under the irradiation of visible light, C60/HSA produced the comparable amount of O2•− with C60/PNVP and large amount of HO2•− [74, 75].

5.4 Delivery

Highly hydrophobic C60 cage delivered peptides to the internal membranes. C60-alanine and C60-alanyllalanine quenched the erythrosine triplet both outside and inside of artificial membranes. On the contrary, Co2+ erased the phosphorescence of erythrosine outside [76]. Baa was synthesized by C60 and an amino acid (Fig. 4) [77]. Baa-Lys[attached with fluorescein isothiocyanate (FITC)]-Lys2-OH 17 and Baa-Lys(FITC)-Lys2-[nucleic 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 C60, Lys was beneficial to delivery as well. When parts of Lys were replaced with negatively charged Glu, Baa-Lys(FITC)-Glu4-Gly4-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 C60 with peptide as a potential drug delivery [79].

The steric hindrance of C60 prevented tuftsin (Thr-Lys-Pro-Arg) to decompose by leucine aminopeptidase. Tuftsin is an immunostimulating agent. Compared to tuftsin, C60 enhanced the stimulation of phagocytosis and chemotactant effect. C60-tuftsin-COOH 20 at the concentration of 20 µmol/L led to the highest phagocytosis and NH2-tuftsin-C60 at the same concentration reached the best chemotaxis. Both NH2-tuftsin-C60 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

hC60s inhibited both HIV aspartic protease and HIV reverse transcriptase. Bis(phenethylamincucinate) C60 ((K = 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]. C60-Thr-Tyr-Asn-Thr inhibited HIV protease as well, but weakly [82]. Furthermore, C60 with amino acid derivatives 25 and 26 inhibited HIV reverse transcriptase with IC50 value of 0.029 µM and 1.0 µM, respectively. The activity was better than Nevirapine (IC50 = 3.0 µM) and C60 with quaternary ammonium salt 2 [83]. Fmoc-Baa (K = 36 nM) had more potent inhibition against HIV aspartic protease than Baa (K = 120 nM). The possible reason was that Fmoc-Baa possessed more hydrogen bonding and van der Waals interaction with HIV aspartic protease [84].

6. C60 containing sugar

6.1 Radical scavenger

27 and 28 were weak radical scavengers (Fig. 5). The large amount of −OH groups maybe induce C60 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].
5

ROS generated by C40-sugar derivatives inhibited HeLa cells and degraded HIV aspartic protease. C40-monosugars produced more \( ^3\)O\(_2\) 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•, H\(_2\)O\(_2\), and \( ^3\)O\(_2\) scavengers, respectively. 29 (1.5 \( \mu \)M) and 30 (15 \( \mu \)M) 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 (IC\(_{50} = 15.1\ \mu \)M) was weaker than that (IC\(_{50} = 2.25\ \mu \)M) under irradiation. Moreover, 30 inhibited significantly HIV replication in human leukemic Molt-4 T cells and peripheral blood mononuclear cells. 30 (10 \( \mu \)M) 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 \( \alpha \)-mannase, especially, 32 (Fig. 5). However, the corresponding monosaccharide of 32 had better inhibition than 32 against maltase, isomaltase and \( \beta \)-glucase. It was attributed to the different shape of the catalytic sites. \( \alpha \)-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 \( \beta \)-glucase, bore the deep and narrow active sites. Monosaccharides were more efficient than the multivalent system [89, 90]. The similar multivalent system, C40 conjugating with 36 mannoses 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-\( \beta \)-CD conjugates, C40-\( \beta \)-CD micelles and C40-\( \gamma \)-CD complex (Fig 6). \( \alpha \)-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-\( \beta \)-CD conjugates aggregated in aqueous solution, they produced ROS under irradiation. ROS destroyed DNA. The mechanism of C40 cleaving DNA was as follows: (1) \( ^3\)O\(_2\), 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-\( \gamma \) 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 \( ^3\)O\(_2\) to destroy DNA [95]. Another C40-\( \beta \)-CD conjugate 37 confirmed that NADH was indispensable to pBR 322 DNA cleavage. It meant that O\(_2^-\) and \( ^{\cdot}OH \) generated through Type I Electron Transfer played an important role.
Although 33 μmoL^{-1} 37 ripped DNA to small fragments completely, the photodynamic ability to the cells was weak. 400 μmoL^{-1} 37 killed less than 40% SH-SYSY cancer cells [96].

γCD with a big cavity could encapsulate C60 and avoid aggregation [97]. The cell inhibition was dependent on the ability of ROS production (especially, 1O2) and cellular uptake. The quantum yield of 1O2 generated by C60γCD complex (0.78) was much higher than that of C60(OH)2 (0.08) in D2O. C60γCD complex (IC50 = 0.25 μM) showed higher photodynamic ability than C60(OH)2 (IC50 = 15 μM) against human skin keratinocytes (HaCaT) irradiated with UVA (15 J/cm²) [98]. The aggregates in C60γCD solution increased with heating and no 1O2 produced after 150 min. Hence, 2 μM C60γCD complex led to approximately 95% death of human lens epithelial cells, while 30 μM nC60γCD aqueous solution (prepared by heating C60γCD complex aqueous solution for 150 min) had very low effect. The aggregate in nC60γCD aqueous solution was 136-6 nm [99]. Although C60γCD complex was absorbed into cells much more slowly than C60(OH)2 and nC60γCD, it showed the most potent inhibition [98, 99]. However, C60γCD complex did not have strong inhibition against HeLa cells. 10 μM C60γCD had negligible effect either in dark or under 400-500 nm irradiation. C60γCD complex (10 μM) caused more than 60% HeLa cell death under irradiation. The intriguing phenomenon was that C60γCD could squeeze C60 out when pH was lowered from 7.4 to 6.4. It possessed stronger inhibition at pH = 6.4 than at pH = 7.4, although there was a critical aggregate size in the solution with pH = 6.4. The size of colloidal aggregate was small, only 20 nm. It was possible that the small C60 aggregates were absorbed by HeLa cells faster than C60γCD and C60γCD [100].

8. C60 delivered by liposomes

8.1 ROS producer

Lipid membrane-inciporporated C60 (LMIC60) was more stable than γCD/C60 complex in water. LMIC60 was obtained via an exchange reaction between liposomes and γCD/C60 complex by three methods, which were heating, microwave irradiation and photoinduced electron transfer [102, 103]. C60 was released from γCD and encased into liposomes. Because the peak assignable to γCD/C60 complex at 4.19 and 5.05 ppm disappeared. Under the exposure of visible light, cationic 40- incorporated C60 showed 44% DNA cleavage and zwitierionic 41-incorporated C60 converted 24% supercoiled DNA (form I) to nicked DNA (form II) at the same concentration of 20 μM. Anionic 42-incorporated C60 (20 μM) had little DNA cleaving ability (just 2%), even lower than 20μM γCD/C60 complex (6%). It was attributed to the electrostatic repulsions between ‘anionic’ 42-incorporated C60 and ‘anionic’ DNA [104].
Cationic LMICα had stronger inhibition against HeLa cells than anionic LMICα [105]. Because cationic 43-incorporated Cα was relatively easier to bind to the anionic cellular surface and engendered cell death [133]. LMICα 43-41 led to the morphological change of cells and 85% cells were killed, while 1% cells were dead with the treatment of LMICα 42-41 under 350-500 nm exposure [105]. Zwitterionic liposome 41 was used as a matrix here.

Compared to LMICα, the block copolymer micelle-incorporated Cα (BPMICα) aqueous solution was more stable [106]. Cationic BPMICα was absorbed by cells, while anionic and neutral BPMICα can not. BPMICα 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 Cα, Cα solubilized by hydrogenated lecithin 46 and glycine soja sterols was as ROS scavenger. Cα/46/sterol reduced ROS generation and improved the cell viability of HaCaT cells under 10 J/cm² UVA exposure [107]. It repressed the striated skin surface, abnormal scaling of epidermis and dermis. Cα/46/sterol (0.63 μM) suppressed the abnormality of nucleus, such as, condensed chromatin and shrunken nucleus. Cα/46/sterol was nontoxic to the normal tissues. It penetrated into the epidermis and can not arrive at the dermis [108].

Cα/46 inhibited influenza virus (H1N1) in vivo, which was possibly relate to ROS scavenging [109]. The average mouse survival was Cα/46 (3.3 mg/kg/day) > Cα/46 (1.6 mg/kg/day) > rimantadine (90 mg/kg/day) > Cα/46 (0.8 mg/kg/day) > the control without administration. The mice treated with Cα/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 Cα/46 (3.3 mg/kg/day), which was same level as rimantadine. The co-treatment with both Cα/46 and rimantadine lowered the lung index to the normal level as the control group [109].

9. Cα and polymers

9.1 Radical scavenger

Cα/PNVP was Radical Sponge®. It protected HaCaT cells from the oxidative damage induced by UVB and t-BuOOH [110]. Besides, Cα/PNVP decreased UVA-induced ROS level in normal human epidermis melanocytes. The effect was not dose-dependent. Compared to Radical Sponge® 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 50 μM Radical Sponge®, the melanin content induced by 0.1 J/cm² UVA reduced from 190% to 54.6% and tyrosinase activity lowered from 136% to 50%. Radical Sponge® 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 Cα/squalene (a skin emollient) localized in the epidermis and did not penetrate into the dermis. The location of Cα/squalene was consistent with Cα/46/sterol [112].

Although PEG/Cα was not efficient as Cα/PNVP, Cα/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 Cα/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].

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

9.2.1 C60 and PNVP

Radical polymerization is a common method to produce C60-PNVP copolymer (Fig. 8). On one hand, the copolymerization of C60 and N-vinylpyrrolidone (NVP) was carried out directly with 2,2'-azobis(isobutyronitrile) (AIBN, a radical initiator) (Fig. 8) [115, 116]. On the other hand, PNVP linked to C60 through PVAc via cobalt-mediated radical polymerization (CMRP). 2,2'-azobis(4-methoxy-2,4-dimethyl valeronitrile) (V-70) served as a radical generator. PNVP-C60-PVAc-co-bond(II) acrylacetonate (Co(acac)2) was prepared and reacted with C60 (Fig. 8) [117, 118].

\[ C_{60}-\text{PNVP copolymer} \quad \text{and PNVP-C60-PVAc copolymer} \]

C60-PNVP copolymer [49] and PNVP-C60-PVAc copolymer [50] were photosensitizers to cleave DNA and kill cells in vitro, respectively. 49 produced O2•− under the irradiation of visible light, while 1O2 was the main ROS generated by 50. C60-PNVP copolymer [49] (the feed ratio of C60: NVP = 1:200) showed the highest water-solubility (7.8 mM, calculated by C60). 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 C60) 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 1O2 with a common photosensitizer, 4,4′,4′′-terphenyl-5,10,15,20-tetrayltertrakis(benzene)dicarboxylic acid (TPPS, 5.5 μM) [117]. Moreover, 50 decreased human promyelocytic leukemia HL-60 cells more than PVOH-C60 copolymer [51] and poly[(PEG acrylate)-co-(vinyl acetate)]-C60 [52]. It was attributed to relatively high 1O2 quantum yield of 50 (Φ(1O2) = 0.50), compared to that of 51 (Φ(1O2) = 0.12) and 52 (Φ(1O2) = 0.13) [120]. Another 1O2 producer, C60/PNIPAM-co-PNVP, micelle [53] had the DNA-cleaving effect as well [121].

9.2.2 C60 and PEG

The well-dispersed C60-PEG derivatives were innate photosensitizers (Fig. 9). This ability was extended in combination with other biologically effective agents. Although C60-PEG [54] had interaction with A549 (IC50 = 192 μM), the affinity of monosaccharide-C60-PEG [55] was stronger (IC50 = 35 μM) binding ability was enhanced to IC50 ≤ 2 μM under 365nm irradiation. With A549 monomer and oligomer were degraded by 55 upon UV exposure because of 1O2•− and OH• generation [122]. Another example was that C60-iron oxide nanoparticle (INOP)-PEGhematoporphyrin monomethyl ether (HMME) [56] produced more ROS than C60-INOP-PEG and HMME under the exposure of 532 nm laser. The relative tumor volume of C60-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 C60. Gd-DTPA-PEG-C60 had similar level of 1O2•− production and reducing cell viability (40%) with the irradiation of visible light compared to C60-PEG. The R1 relaxivity of Gd-DTPA-PEG-C60 (5.3 mM−1 s−1) was comparable with Magnevist® (5.3 mM−1 s−1). Both of them enhanced MRI signal intensity in tumor, but Gd-DTPA-PEG-C60 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. C60-Fe3O4-PEG5000/diocetaxel-thermosensitive liposome was a negative T1 contrast agent. Besides, this particle released diocetaxel and C60-Fe3O4-PEG5000 after the increasing temperature of tumor issues by 13.56 MHz, radiofrequency. The radiofrequency also led to ROS generation. ROS assisted diocetaxel to inhibit MCF-7 tumor cells [125].

C60 has the highest absorption under UV. However, UV cannot penetrate the skin. Therefore, the agents absorbing the light with long wavelengths (> 620 nm) were utilized. The photodynamic activity was improved when C60-PEGs conjugated with graphite oxide (GO), chlorin e6 (Ce6), and upconversion nanoparticles (UCNP). Among these conjugates, FA was introduced to target tumor cells. FA-GO-PEG-C60 had a synergetic effect on antineoplastic therapy. GO absorbed the energy from 808 nm and released vibrational heat. C60 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].

Ce6-PEG-C60-PEG-FA had both photothermal and photodynamic ability, leading 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, Ce6-PEGs enhanced the bioavailability of polyethyleneimine (PEI), 5-fluorouracil (5-FU), doxorubicin (DOX) and pentoxifylline (PTX). The introduction of Ce6-PEG balanced the toxicity and transfection efficiency of PEI. Ce6-PEG-PEI 57 was as DNA vector. Ce6-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 Ce6-PEI. Because the efficiency of 57 transporting pEYFP-C1 plasmid at the N/P ratio above 60 was comparable with that of Ce6-PEI/pEYFP (N/P ratio = 20) [129]. Other examples showed that Ce6-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 Ce6-PEG protected 5-FU from the degradation by dihydroxyquinidine 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]. Ce6-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 Apo2L-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(OR)3, malonic acid C60H, C60-amino acids, C60-neutral liposomes, C60PEG, C60/PNP, C60-PNIPAM. ROS producers are C60 with quaternary ammonium salts, C60 containing sugars, C60/peptide, C60-β-CD conjugates, C60γ-CD complex, C60/cationic...
lipoosomes, C_{60}−PNVP copolymer, PNVP−co-PVAc copolymer and C_{60}−PEGs. To our knowledge, the preference is dependent to the extent of dispersion. bC_{60} as ROS producer form less aggregates than radical scavenger. The aggregates in aqueous solution will decrease ROS production ([3 Pristine C_{60} and hC_{60}s). Furthermore, C_{60} with quaternary ammonium salts inhibit O_{2} uptake. C_{60} amino acids are HIV inhibitor. C_{60} with quaternary ammonium salts, C_{60}−amino acids, C_{60}−multivalent iminosugars, C_{60}−PEGs are good vectors for DNA or drugs.

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References:


Highlight:

- Hydrophilic C₆₀ derivatives (hC₆₀s) 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 C₆₀s structure.
- High ¹O₂ quantum yield means low aggregation. C₆₀s with aggregates scarcely generate ¹O₂, but O₂⁻.
- The typical reactions to get covalent C₆₀s are oxidation, Bingel reaction, Prato reaction, Diel-Alder reaction and radical polymerization.