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Biological and biocompatible characteristics of fullerenols nanomaterials for tissue engineering

Yizhe Zhao[1,2,3,4*], Xinyuan Shen[1,3,4*], Ruimeng Ma[1,3,4], Yiting Hou[1,3,4], Yun Qian[1,4] and Cunyi Fan[1,4]

[1]Department of Orthopedics, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, [2]Biomedical Engineering Academy, Shanghai Jiao Tong University, [3]Shanghai Jiao Tong University, School of Medicine and [4]Youth Science and Technology Innovation Studio, Shanghai Jiao Tong University, School of Medicine, Shanghai, China

*These authors contributed equally to this study

Summary. Fullerenes, as hydrophobic molecules, are limited in biomedical function due to their very low solubility. But taking $C_{60}(OH)_x$ as an example, the properties of fullerenols were analyzed. It was found that fullerenols had good stability, water solubility, good biocompatibility and low cytotoxicity by adding a hydroxyl group to carbon atoms. In the biomedical field, it has been found that fullerene C_{60} can be used as a powerful free radical scavenger, with antioxidant activity, with antibacterial and inhibitory effects on cancer cells. Fullerenols inherit the good properties of fullerenes, and are better used in cancer treatment, including loading drug therapy and directly as an anticancer drug. In addition, fullerenols are also used in the repair of myocardial injury, the treatment of myocardial infarction and neuroprotection. With the development of tissue engineering technology, the preparation of nerve scaffolds which can improve ischemia, hypoxia and oxidative stress after nerve injury has become a research hotspot. The electron absorption and reduction characteristics of fullerenols in biomedical research bring new ideas for the treatment of oxidative stress in the repair of peripheral nerve defects. It seems that the research on fullerenols loaded neural scaffold has great prospects.

Key words: Fullerene derivatives, Fullerenols, Biomedicine, Neural scaffold, Oxidative stress, Nerve regeneration

Corresponding Author: Yun Qian, Department of Orthopedics, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai 200233, China. e-mail: lollipopcloudland@foxmail.com

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Introduction

Carbon nanomaterials are attracting more and more attention due to their exceptional and unique properties in various fields of life (Cao et al., 2020; de Oliveira et al., 2020; Duoerkun et al., 2020; Hu et al., 2020; Liu et al., 2020; Wang et al., 2020; Xu et al., 2020; Saadun et al., 2021). Among them, fullerenes are isotopic isomers of carbon nanomaterials, which are famous for their unique cage structures. The prominent representative of fullerenes is C_{60}, which is a spherical cage molecule composed of sp^2 carbon atoms (Kazemzadeh and Mozafari, 2019). Taking C_{60} as an example, due to its strong hydrophobicity, fullerene C_{60} is difficult to use directly in physiological media, and its physiological activities are affected, which limits its application in biomedicine. Through hydroxylation reaction, we can obtain a type of fullerene derivatives, fullerenols, whose water solubility overcomes the property defects of fullerenes.

In this paper, we review the physicochemical and bioactive properties of fullerenols, and describe the application of fullerenols in the biomedical field. At the same time, in view of their applications in neuroprotection and their properties, combined with the current problems in the repair of peripheral nerve injury, the application prospect of fullerenols as water-soluble fullerene derivatives in this field is discussed.

Fullerene C_{60}

C_{60} is the most abundant form of fullerene. The geometric construction of fullerene C_{60}, which is a polyhedral closed cage, is highly symmetric. It is made up of 60 carbon atoms containing 12 pentagon rings and 20 hexagonal rings. Fullerenes in the form of powder or crystal are soluble in halogen and methylbenzene, but cannot be dissolved in water or proton acceptor solvents (Djordjević et al., 2006). The

most representative fullerene C_{60} has been considered as a promising material in various fields of biomedicine, but these hydrophobic molecules have been greatly limited in their biomedical functions due to their extremely poor solubility. Therefore, a great number of fullerene-based compounds have been synthesized and applied in the field of anticancer or antimicrobial therapy, controlled drug delivery, and cytoprotection (Bakry et al., 2007).

Fullerenols $C_{60}(OH)_x$: water-soluble fullerene derivatives

Derivatization has been applied to improve the solubility of fullerenes, as hydrophilization or encapsulation of C_{60} can help to form more stable molecular dispersed suspensions that prevent adjacent fullerene cores from interacting (Injac et al., 2013).

Fullerenols $C_{60}(OH)_x$ are polyhydroxylated derivatives of C_{60} fullerene. They have received much attention due to their bioactive characteristics such as hydrophilic properties and the ability to scavenge free radicals. The solubility of fullerenes in polar solvents as well as their biocompatibility has been increased by attaching hydroxyl groups to C_{60} (Zhu et al., 2016).

Some other biological functions have also made this spherical-shaped fullerene C_{60} a potent free-radical scavenger, a neuroprotective agent and an antiproliferative agent for vascular smooth muscle cells (Jin et al., 2000). It also has the potential of acting as a cytotoxic agent against tumor cells and normal cells, as a protective agent and also serving as a drug carrier due to its hollow spherical shape (Grebowski et al., 2013a).

Physicochemical properties of fullerenols $C_{60}(OH)_x$

The biochemical modification of attaching OH groups onto the hollow carbon spherical surface of fullerenes generates solubility and antioxidant activities of several degrees in aqueous solutions as a result of distinct distributions according to the number and the position of OH groups across the carbon sites on the fullerene surface (Zhu et al., 2016).

It has been reported that $C_{60}(OH)_6$ and $C_{60}(OH)_{12}$ are of the most stable structure with the lowest energy. The 1,2—additional reaction of adding (OH) groups to the neighboring arris of the C_{60} pentacyclic ring and its hexacyclic ring yields the most stable isomer (Wang and Cheng, 1997).

Fullerenols are also considered efficient photosensitizers of reactive oxygen under ultraviolet irradiation due to their unique electronic π-bond system (Fileti et al., 2008).

Meanwhile, fullerenes tend to take up an electron due to the large number of conjugated π bonds with low energy unoccupied molecular orbitals (LUMO) which make fullerenes function as potent antioxidants endowed with radical scavenging ability (Grebowski et al., 2013a).

Bioactive properties of fullerenols $C_{60}(OH)_x$

Like fullerenes, highly hydroxylated fullerenols are also free radical scavengers but with increased solubility and higher efficiency. This property has made fullerenols strong antioxidants (Nielsen et al., 2008). While in the presence of irradiation and oxygen, fullerenols can generate cell-damaging reactive oxygen species, also known as ROS (Markovic and Trajkovic, 2008). The dualistic nature of fullerenols therefore contributes to their characters as both cytoprotector and cytotoxic anticancer/antimicrobial agent.

Fullerenols $C_{60}(OH)_x$ as radical scavengers

Fullerenol $C_{60}(OH)_{24}$ has been well known for its protective function as an antioxidant against ionizing radiation, UVA radiation and other ROS-mediated oxidative damage. For instance, $C_{60}(OH)_{24}$ can mitigate doxorubicin (DOX) toxicity towards the heart by eliminating the H_2O_2 and superoxide anion radical. $C_{60}(OH)_{24}$ also helps DOX inhibit tumor cells by suppressing angiogenesis (Zhu et al., 2016).

Fullerenols exhibit their neuroprotective properties during the common process of biological oxidation both *in vivo* and *in vitro*. Nerve cells are the major target for free radical reactions because of the low activity of ROS eliminating enzymes and high oxygen consumption. Hydrophilic fullerene derivatives like fullerenols exert promising neuroprotective as well as antioxidative effects on neurons. $C_{60}(OH)_{18\text{-}20}$ has been discovered as the antagonist of glutamate receptors to protect neurons and it can also lower glutamate-induced elevation of Ca^{2+} (Jin et al., 2000).

Fullerenols $C_{60}(OH)_x$ as ROS producers

Under irradiation, water-soluble fullerenols generate singlet oxygen via energy transfer from the excited triplet of fullerene to oxygen (Injac et al., 2013). While in the presence of electron donors such as NADH or amines in the solution, the photo-irradiation of fullerenols produces the radical anions followed by the generation of superoxide anion radical and hydroxyl radical through the electron transfer (Markovic and Trajkovic, 2008).

Research progress of fullerenols in the biomedical field

In the biomedical field, the research of fullerenols is mainly focused on the research progress of fullerenes' application in the treatment of cancer. Studies have proved that fullerenols have the properties of inhibiting tumor and anti-metastasis. At the same time, as drug carriers, fullerenols can load anticancer drugs well and act as connectors to fix drugs to the target position. In addition, fullerenols have good biocompatibility and protective activity. Besides being applied in oncotherapy,

fullerenols are also used in the repair of myocardial damage and neuroprotection.

Oncotherapy

Fullerenols as anticancer drugs

Due to the physical and chemical advantages of fullerenes, they have excellent performance in virus inhibition, antibacterial and antioxidant properties (Da Ros et al., 1996; Mashino et al., 1999), and have certain effects on cell protection and organ protection (Yin et al., 2009), and thus they are often used to produce anticancer drugs.

As water-soluble hydroxylated derivatives of fullerenes, fullerenols have a similar main structure to fullerenes, and basically inherit all the excellent properties of fullerenes, and their stability is high. The fullerenols formed by introducing hydroxyl groups onto the carbon atoms of fullerenes also have good water solubility, so they can be made better use of in the biomedical field.

Zhu et al. (2008) found that $C_{60}(OH)_x$ had an inhibitory effect on tumor growth. It was observed that the inhibition rate of tumor was increased and liver injury was mitigated in mice with liver cancer. Histological examination showed that the capsule of fibroblasts and lymphocytes formed around the tumor tissue in the $C_{60}(OH)_x$ treatment group inhibited tumor infiltration into adjacent normal skeletal muscle tissue. At the same time, $C_{60}(OH)_x$ can enhance the phagocytic function of peritoneal macrophages and increase the activities of arginase and acid phosphatase, so that the innate immunity of experimental mice is enhanced and tumor growth is inhibited. Sun et al. (2016) found that $C_{60}(OH)_{22}$ was able to inhibit the invasion and angiogenesis of endothelial cells *in vitro*, and inhibit the activity of MMP2 and MMP9.

In addition to their inhibitory effect, fullerenols have anti-metastasis effects as anticancer drugs. Jiao et al. (2010) found that fullerenols had antitumor and anti-metastatic activities and related mechanisms. The oxidative stress of tumor tissue in mice treated with fullerene $C_{60}(OH)_{20}$ was regulated significantly. Meanwhile, the expression level of various angiogenic factors decreased in the tumor tissue, resulting in the decrease of tumor vascular density and nutritional supply to tumor cells, which may be an important mechanism of fullerenols inhibiting tumor growth and metastasis.

Fullerenols as drug carriers

Functionalized fullerenes can be used as drug delivery nanoparticles. Paclitaxel embedded buckysomes (PEBS), formed by embedding paclitaxel into the hydrophobic sac of amphiphilic fullerene AF-1, demonstrated that water-soluble fullerene derivatives were able to absorb paclitaxel without the need of non-aqueous solvents, which would lead to patients' discomfort and other unwanted side effects (Partha and Conyers, 2009). Therefore, fullerenols can be loaded with anticancer drugs for use.

Grebowski et al. (2013b) preliminarily estimated the properties of fullerenols as potential linkers to transfer compounds (such as anticancer drugs) to the erythrocyte membrane. Fullerenols with hydroxyl groups attached to them can be absorbed by erythrocyte cytoskeleton protein. At the same time, fullerenols slightly change the cell morphology. Therefore, the intracellular tissue of erythrocytes is changed and the drug retention time is prolonged via the combination with cytoskeletal proteins.

Thotakura et al. (2017) explored the use of aspartic acid linked fullerenols. They worked on the characterization of aspartic acid derived fullerenols and docetaxel conjugate by evaluating the cancer cell cytotoxicity, cell uptake confocal laser microscopy and pharmacokinetic curve to study the therapeutic effect of docetaxel transfer into cancer cells. Cytotoxicity studies showed that IC50 decreased by 4.3 times with the increase in cell uptake of conjugates. Compared with pure DTX, the bioavailability (biocompatibility) was increased by 5.8 times. The developed nanostructures are compatible with erythrocytes and reduce protein binding.

Fullerenols in myocardial tissue engineering

In myocardial infarction transplantation, injectable myocardial tissue engineering strategy or three-dimensional construction of myocardial tissue are often used to repair the damaged myocardial tissue. Many kinds of stem cells, biocompatible hydrogels based on polysaccharides and proteins, and natural biomaterials (such as collagen /Matrigel, fibrin and alginate) have been applied. Nakanishi et al. (2014) found that fullerenes, as representative two-dimensional nano carbons, can be used as the basic materials for building high-dimensional or complex materials through nano building technology, and applied to the cell growth of nanostructured scaffolds. Hao et al. (2016) found that fullerene C_{60} improves the MAPK expression level and stem cell survival, proliferation, and cardiomyogenesis. Besides, fullerene C_{60} has no cytotoxic effects on brown adipose-derived stem cells (BADSCs) at an elevated concentration of 100 μg/mL. In addition, fullerene C_{60} promotes the formation of gap junction among cells. These findings have important implications for clinical application of fullerenes in the treatment of myocardial infarction.

As fullerene derivatives, fullerenols have inherited the application of fullerene characteristics. At the same time, in dealing with the myocardial pathological microenvironment, fullerenols have strong electron absorption and reduction characteristics, which enables it to remove free radicals such as reactive oxygen species in organisms and deal with excessive oxygen

free radicals (ROS) in myocardial infarction microenvironment. Matija et al. (2013) found that fullerenol nanomaterials can protect the heart from oxidative stress by scavenging free radicals *in vivo*. Hao et al. (2018) found that fullerenol / alginate hydrogel could effectively remove superoxide anion and hydroxyl radical. On this basis, they studied the brown adipose derived stem cells (BADSCs) with fullerenol / alginate hydrogel as carrier, and found that fullerenol / alginate hydrogel had no cytotoxic effect on BADSCs, which could effectively inhibit BADSC oxidative stress damage and promote the differentiation into myocardium.

In addition, Potonik et al. (2017) found that pretreatment with fullerenol (FRL) had a protective effect on DOX induced cardiotoxicity. In the colorectal cancer model of rats, autonomic nervous regulation was accompanied by the development of DOX induced cardiotoxicity. It was found that in all DOX treated animals, the oxidative imbalance of heart tissue and the early myocardial injury were significantly reduced in FzRL pretreated rats.

Fullerenols in Neuroprotection

Krusic et al. (1991) have verified the role of carboxyfullerene as a neuroprotective agent. Carboxyfullerene is effective on excitatory necrosis and provides protection for two forms of neuronal apoptosis. At the same time, it leads to the view that oxidative stress is the key downstream medium of different necrosis and apoptosis neuron deaths. Therefore, fullerene C_{60} derivatives can be used as neuroprotective drugs *in vivo*.

Fullerenols, C_{60} fullerene derivatives, have proved to be effective free radical scavengers. Jin et al. (2000) found that fullerenols, or cage like fullerene oxide, exerted their neuroprotective function by blocking glutamate receptor and reducing intracellular calcium [$Ca^{(2+)}$]. In neuron culture, fullerenols inhibited glutamate receptor binding in a dose-dependent manner, and reduced glutamate induced neurotoxicity by about 80% at 50 μG. At the same time, the potential mechanism of neuroprotective function of the fullerenols may be due to their ability to block glutamate receptor and reduce [$Ca^{(2+)}$] level.

Application prospect of fullerenols in nerve injury

Problems in the repair of peripheral nerve injury

Peripheral nerve injury is a common clinical injury, and its repair and reconstruction have always been a worldwide problem (Qian et al., 2019a, 2020a, 2021). At present, the main methods of treating peripheral nerve injury are autologous nerve transplantation and the construction of tissue engineered nerve scaffolds (Qian et al., 2018b,c). However, autologous nerve transplantation which is called the gold standard for treatment has problems, such as limited source of nerve donors and size mismatch, so its effect on severe peripheral nerve injury is not good (Qian et al., 2018a,d). With the development of tissue engineering technology, the preparation of nerve scaffolds that can alleviate ischemia, hypoxia and oxidative stress after nerve injury has become a current research hotspot.

In the process of repairing peripheral nerve injury, oxidative stress and inflammatory reaction of nerve cells are the two major factors limiting regeneration (Qian et al., 2019b,c, 2020b). Oxidative stress is due to the imbalance of oxidants and antioxidants in the body, which leads to the imbalance of oxidation and antioxidant activities in the body (Sofroniew, 2005). The imbalance of oxidation and antioxidant activities tends to be oxidation. The negative effect of free radicals in the body is considered to be an important factor leading to aging and disease. In the early stage of nerve injury, the indexes of inflammation and oxidative damage are increased. Oxidative damage and inflammatory reaction are important early pathological changes of nerve injury, which can cause a large number of neuron deaths, and are important therapeutic targets of nerve regeneration (Fitch and Silver, 2008). Effective anti-inflammatory and anti-oxidation treatment can increase the survival of early-stage neurons and achieve good recovery of motor nerve function in later stages.

Oxidative stress after peripheral nerve injury

During the period of ischemia and ischemia-reperfusion, extracellular glutamate increases significantly, resulting in glutamate excitotoxicity, ion homeostasis imbalance, mitochondrial dysfunction, and a large number of reactive oxygen species (ROS) are produced (Stogsdill et al., 2017). Excessive ROS, which is beyond the body's antioxidant capacity, interacts with proteins, lipids, carbohydrates and nucleic acids, induces apoptosis and autophagy through apoptotic protease, lysosomal protease or endonuclease, leading to widespread neuronal apoptosis and aggravating dysfunction after nerve injury (Sofroniew, 2005). Lipid peroxidation can transform ROS into active chemicals, amplify the effect of reactive oxygen species and cause cell death.

Inflammatory response after peripheral nerve injury

After peripheral nerve injury, a large number of inflammatory cells will gather in the injured area, releasing IL-6, IL-1 and other inflammatory factors as well as activating the apoptosis process, which further aggravates tissue edema, causes neuronal apoptosis in a larger range, ultimately deteriorates the nerve function, affects the recovery of motor function, produces neuralgia, and finally influences patient life quality (Schomberg et al., 2012; Wang et al., 2017).

Application prospect of fullerenols in peripheral nerve defect repair

Conduits currently used in tissue engineering of peripheral nerve repair provide mechanical pipeline support for peripheral nerve regeneration, and have certain effects on repairing short-distance nerve defects (Qian et al., 2019d; Cheng et al., 2020; Jiang et al., 2020; Yan et al., 2020). Since the current conduits can barely avoid tissue ischemia, hypoxia and oxidative stress after nerve injury, they have poor efficacy on severe peripheral nerve defects (Nielsen et al., 2008). However, fullerenes and their hydroxylated derivatives fullerenols exhibit electron withdrawing and reducing properties in biomedical research (Yang et al., 2014).

The research suggested that the solubility of fullerenes can be improved by combining fullerenes with amphiphilic substances to form supramolecular complexes or adding surfactants to their aqueous solutions, in order to obtain water-soluble fullerenes (Ros and Prato, 1999). *In vitro* experiments showed that water-soluble fullerenes were able to protect neurons from oxidative stress and glutamate-induced injury. The results further showed that fullerenes restored glutamine synthetase and glutamate transporter expression in astrocytes after suffering inflammatory-related insults (Stogsdill et al., 2017), which brings new ideas for the treatment of oxidative stress and inflammatory response in peripheral nerve defect repair.

Similar to water-soluble fullerenes, fullerenols, as hydroxylated derivatives of fullerenes, have high biocompatibility, low cytotoxicity, good mechanical strength, sustained-release control and drug loading ability according to their properties and applications in various fields like neuroprotection (Kolosnjaj et al., 2007; Kazemzadeh and Mozafari, 2019). They have the properties required for a good nerve scaffold and to promote cell proliferation and adhesion, and enhance the cells' ability to differentiate into bone and nerve-like cells to further improve the nutritional microenvironment of the tissue (Kokubo et al., 2008). However, at present, the research on fullerenols loaded neural scaffold is just at the beginning, and there is still a long way to go.

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Conflict of Interest. The authors declare no conflicts of interest.

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