IOSR Journal of Applied Chemistry (IOSR-JAC)
e-ISSN: 2278-5736.Volume 13, Issue 12 Ser. I (December. 2020), PP 16-22
www.iosrjournals.org

A low cytotoxicity sustained release system of captopril loaded on large-size graphene oxide

Tianxiong Liu, Zhuoliang Liu, Yujiao Li, Jianfang Wang*
College of Liberal Arts and Science, National University of Defense Technology, Changsha, 410073, China;

Abstract: *In this paper, a sustained-release system of captopril (CAPT) loaded on large-size graphene oxide (GO) carrier was prepared. The cytotoxicity of GO with large-size and different concentrations and GO-CAPT to human colorectal adenocarcinoma cell line (Caco-2) and primary mouse hepatic fibroblast cell lines was investigated by using three different kits from three aspects: cell proliferation, cell structural integrity and cell apoptosis and necrosis. The results showed that CAPT was successfully loaded onto GO through hydrogen bonding. Compared with the control group of deionized water, the cytotoxicity of GO-CAPT and deionized water is not much different. GO and GO-CAPT did not cause obvious cell apoptosis or necrosis. This indicates that GO and GO-CAPT have low cytotoxicity and are suitable for further biological applications.*

Keywords: *Graphene oxide; Captopril; low cytotoxicity; sustained–release system*

Date of Submission: 27-11-2020 Date of Acceptance: 11-12-2020

I. Introduction

Hypertension is a common chronic disease and a major risk factor of cardiovascular and cerebrovascular diseases. It needs long–term use of oral drugs to maintain human health. Captopril (CAPT) is a commonly used antihypertensive drug. Generally, captopril takes effect 15 minutes after ingestion and reaches its peak in about 1-2 hours. However, the duration of captopril is short. Its chemical structure determines its low solubility and can not be effectively used by human body [1-2]. Therefore, it is necessary to load and slow release the drug through the carrier to improve its hydrophilicity and make the drug's efficacy reach the optimal level.

Graphene oxide (GO) has a very high specific surface area [3], which makes it have a high drug loading. There are many active groups on the surface of GO, such as carboxyl group and hydroxyl group [4], which greatly increases its water solubility and shows good biological affinity. At the same time, many active groups such as carboxyl and hydroxyl groups and many conjugated π bonds on the framework of six membered rings make graphene oxide form hydrogen bonds and π-π bonds with drug molecules. Therefore, graphene oxide based drug carriers have broad application prospects [5]. However, its cytotoxicity and biocompatibility must be considered.

Dai's group [6] reported for the first time that the derivatives of anticancer drug camptothecin (SN38) was connected on the surface of PEGylated nano graphene oxide through π-π conjugation and other physical actions to form complex (NGO-SN38). The results show that graphene oxide has good biocompatibility under physiological conditions such as serum. NGO-SN38 complex has good water solubility, can increase the solubility of insoluble drugs, and SN38 still has high activity in the complex. Chen's group [7] studied the high-efficiency loading and controlled release of DOX on NGO, and found that the release behavior of NGO has pH response characteristics, which provides an important basis for the controlled release of graphene oxide. We also found that the release behavior of captopril loaded with graphene oxide was pH sensitive [8]. The research results of Zhang's group [9] on controllable joint drug loading of nano graphene oxide show that nano graphene oxide is an ideal drug carrier, which can be absorbed by cells without obvious cytotoxicity, and has super adsorption capacity for some aromatic small molecule drugs, which is far higher than that of general nano material carriers. The comprehensive study of GO cytotoxicity showed that GO neither exhibited significant cellular uptake nor significantly affected cell morphology, viability, mortality, and membrane integrity in human lung basal epithelium (A549) cells [10]. Other studies have shown that GO has good biocompatibility [11-13]. At present, there are many studies on graphene oxide drug loading [14-20]. However, there are few reports on the loading and release of drug molecules without benzene ring on graphene oxide, such as captopril. There are few studies on graphene oxide with large size and high concentration and its biocompatibility after drug loading.

In this paper, we constructed a new low toxicity drug system using GO loaded CAPT (GO-CAPT). The cytotoxicity of large-size and high concentration GO and GO-CAPT on human colorectal adenocarcinoma cell (Caco-2) and primary mouse hepatic fibroblast were investigated from three aspects of cell proliferation, cell structural integrity and apoptosis and necrosis.

II. Materials and Methods

2.1. Materials

The natural graphite powder (300 mesh) and CAPT were obtained from Aladdin Co. Ltd (Shanghai, China). The human colorectal adenocarcinoma cell (Caco-2) were obtained from the American Type Culture Collection (ATCC, USA). Fetal bovine serum and Dulbecco's modified Eagle's medium (DMEM) were purchased from HyClone, Logan, UT, USA. Other chemicals were purchased from conventional reagent companies and do not require further refinement.

2.2. Fabrication of the GO-CAPT

In the experiment, GO and CAPT of a certain concentration were prepared respectively. The two solutions were mixed with the same volume and slowly shook overnight to form GO-CAPT complex, then centrifuged at 8000 rpm for 30 min to remove free CAPT. The GO-CAPT complex samples were lyophilized and preserved.

2.3. Cell culture

The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 20% fetal bovine serum. The cells were grown in a humidified incubator with 5% CO_2 at 37°C. The primary mouse hepatic fibroblast were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 1% pen/strep. The cells were grown in a humidified incubator with 5% CO_2 at 37°C.

2.4. Cytotoxicity test of lactate dehydrogenase (LDH) leakage

The Lactate Dehydrogenase (LDH) Cytotoxicity Assay Kit (Beyotime Institute of Biotechnology, Jiangsu, China) was used to test the structural integrity of cells. The experiment was carried out in full accordance with the kit instructions. The data was measured using an automated microplate reader (Bio–Rad 5 Model 550, Bio–Rad, Hercules, CA, USA). Cytotoxicity percentage was determined by the equation: Cytotoxicity percentage(%)=(Absorbance of treated samples–Absorbance of control group)/(Maximum enzyme activity absorbance– Absorbance of control group).

2.5. Cell viability assays

The WST-1 Cell proliferation and cytotoxicity assay kit (Beyotime, Hangzhou, China) was used to test the cell viability. The experiment was carried out in full accordance with the kit instructions. The data was measured using an automated microplate reader (Bio–Rad 5 Model 550, Bio-Rad, Hercules, CA, USA). Cell viability percentage was determined by the mean optical density (OD) of one experimental group divided by the mean OD of the untreated group × 100%.

2.6. Flow cytometry

The Annexin V–FITC apoptosis detection kit (C1063, Beyotime, China) was used to test the apoptotic cell. The experiment was carried out in full accordance with the kit instructions. Flow cytometry data were collected with a BD LSRFortessa cell analyzer (BD Biosciences, Franklin Lakes, NJ, USA) and analyzed by FlowJo software (Tree Star, Ashland, OR, USA).

2.7. Characterization of GO-CAPT

The atomic force microscope AFM adopts the model Nanomanvs from the American Veeco company. X-ray photoelectron spectroscopy (XPS) was performed on a K-AlpHa 1063 spectrometer (Thermo Scientific), and the Software (XPSPEAK v4) was used to fit the results. Fourier transform infrared spectroscopy (FTIR) was accomplished using a Tensor 27 (Germany) spectrometer, and the wave number range was 400~4000 cm^{-1}.

III. Results and Discussion

3.1. Characterization of GO-CAPT

A drug-loaded complex GO-CAPT with captopril (CAPT) loaded on graphene oxide (GO) was prepared. In our previous study, we obtained the loading and release laws of GO-CAPT sustained-release system [21]. When the ratio of CAPT to GO was more than 8:1, there is a maximum load of 2.418mg/mg. It has the best controlled release effect under acidic conditions. When pH=2, the release rate can reach about 49%, and the release time will increase slowly after 22 hours, until the balance is basically reached around 38 hours. This is closely related to the process mechanism of GO loading CAPT and their structural characteristics. This indicates that GO-CAPT will have a good effect when used as an oral drug for controlled release in the acidic environment of the gastrointestinal tract.

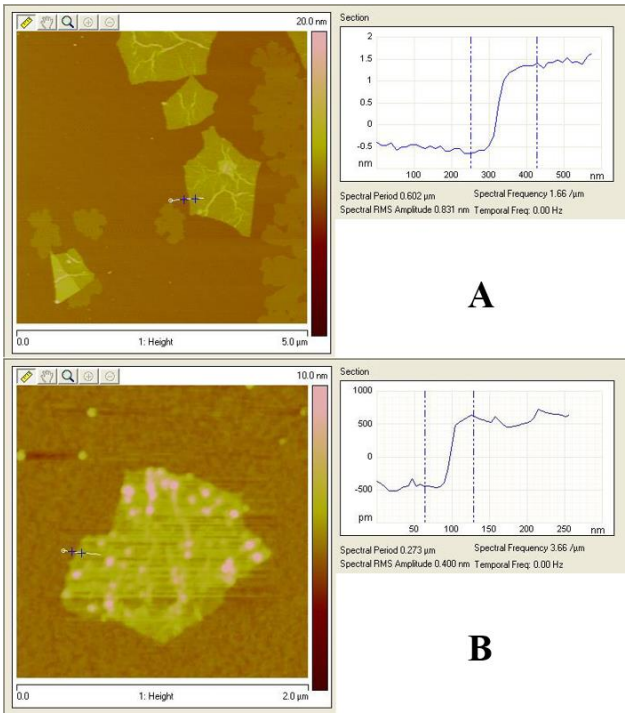

Figure 1. AFM test of GO-CAPT (A) and GO (B)

Atomic Force Microscope (AFM) was used to scan GO-CAPT and GO, and the microscopic morphology is shown in Figure 1. It can be seen that GO-CAPT (A) and GO (B) are both nano-level lamellar structures, and both two-dimensional plane dimensions are relatively large, about 1-2μm. Comparing the thickness of the two lamellae, it can be seen that the thickness of the lamellae is significantly increased after GO is loaded with CAPT, and the thickness of GO and GO-CAPT are about 1nm and 2nm, respectively. This indicates that the load has been successful.

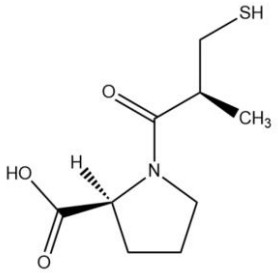

Figure2. Structure of captopril (CAPT)

According to the chemical structure diagram of captopril (CAPT) (Fig. 2), CAPT does not have benzene ring structure, but contains -COOH group, which can form hydrogen bond with oxygen-containing group on graphene oxide. Therefore, it is feasible for captopril to use go as carrier for loading.

The infrared spectra of GO, CAPT and GO-CAPT are shown in Figure 3.The stretching vibration of N–H of CAPT was reflected in the absorption peak at 2989 cm^{-1}. Various CH_2 groups in CAPT was reflected the peaks between 2486 cm^{-1} and 2798 cm^{-1}. Because of the different functional groups that are adjacent to each other their frequencies are going to shift. There are four strong absorption peaks within the range of 1449-1866 cm^{-1} correspond to the benzene ring C atoms. The strong absorption peak at 1133 $cm^{-1}$of GO-CAPT is the characteristic peak of the drug and GO. The peak at 1620 $cm^{-1}$of GO moves to 1715 $cm^{-1}$of GO-CAPT due to the emergence of new specific reactions and hydrogen bond interactions in GO-CAPT. The shift from peak to peak confirms that GO and CAPT did not exist only as physical mixtures. The formation of hydrogen bond interactions makes this complex structure possible.

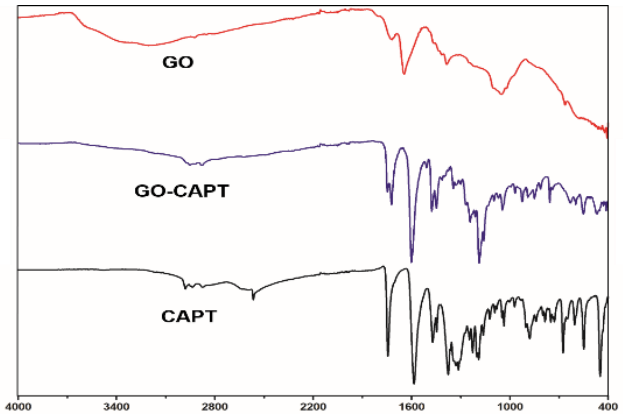

Figure 3. Infrared spectra of lyophilized GO carrier and GO-CAPT composites.

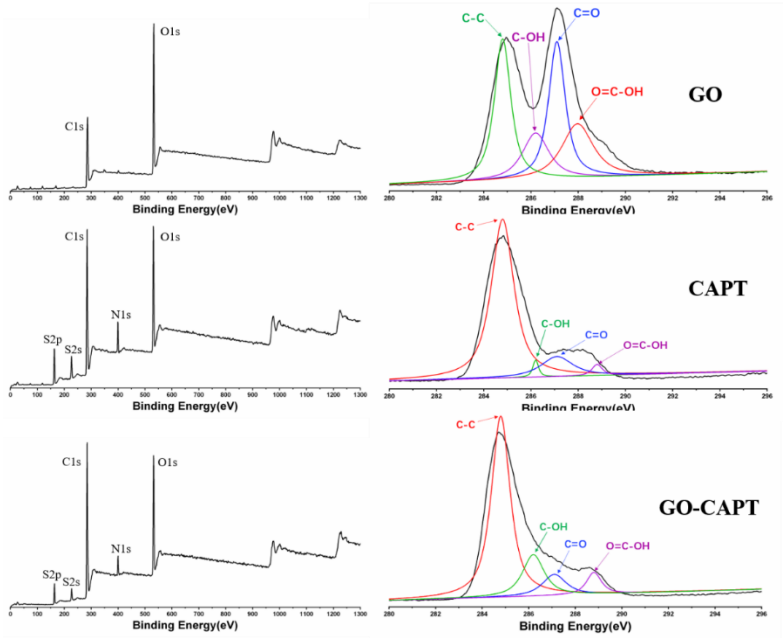

Figure 4. XPS spectra of GO, CAPT andGO-CAPT.

Table 1 The fitting proportion of functional groups in GO, CAPT andGO-CAPT

Peak/ eV	Perssad	Ratio		
		GO	CAPT	GO-CAPT
C–C (~284.8 eV)	Carbon	33%	80%	66%
C–OH (~286.2 eV)	hydroxyl, epoxy group	16%	2%	17%
C=O (~287.1 eV)	Carbonyl	23%	14%	9%
COOH (~288.8 eV)	Carboxyl	28%	4%	8%

XPS characterization of GO, CAPT and GO-CAPT freeze-dried samples is shown in Figure 4. It can be seen that its constituent elements are mainly carbon and oxygen, and the atomic ratio of C1s/O1s are 0.3962 (GO), 4.630 (CAPT) and 4.5995 (GO-CAPT), respectively. The infrared spectrum results (Figure 2) show that these three samples mainly contain functional groups such as epoxy (C-O-C), carbonyl (C=O), carboxyl (-COOH) and hydroxyl (-OH). After integrating the peak data in Figure 4, the specific types and contents of various functional groups on the surface of GO, CAPT and GO-CAPT are obtained, which are listed in Table 1. It can be seen that the content of carboxyl (-COOH) in the functional group of GO is 28%, while the content of carbonyl (C=O), epoxy (COC) and hydroxyl (-OH) are 23% and 16%, respectively. This proves the chemical basis of GO as a drug carrier, and also explains the good solubility of GO. The change of the proportion of functional groups in GO-CAPT loaded with drugs is closely related to the proportion of functional groups contained in CAPT, which indicates that the loading has been successful.

3.2. Effects of GO and GO-CAPT on cell proliferation and cell integrity

Different concentrations of GO and GO-CAPT solutions were added to human colorectal adenocarcinoma cells (Caco-2) and primary mouse liver fibroblasts cultured in vitro, and the WST-1 kit was used to test the effect on cell proliferation to study the cytotoxicity. As shown in Figure5A, the cell survival rate on Caco-2 cells of deionized water (DIW) in the control group was 99.58%, and the cell survival rate of GO solution with concentration of 1, 5, 10, 20 and 50 mg/L were 99.01%, 97.89%, 96.07%, 94.55% and 92.38%, respectively, and that of GO-CAPT was 96.99%. With the increase of GO concentration, the cell survival rate decreased slowly. However, even when the concentration of GO was as high as 50 mg/L, the cell survival rate is only 7.20% different from DIW group, which could be considered as little effect. Compared with DIW group, the cell survival rate of GO-CAPT group only differed by 2.59%, which had no significant effect. The same trend was also observed in primary cells (Fig.5B). According to the principle of WST-1 kit, it can be considered that GO solution with a large size of 1-2μm and a concentration of up to 50 mg/L will not cause much damage to cell proliferation, and the drug delivery system GO-CAPT prepared in this paper has no obvious damage to cell proliferation.

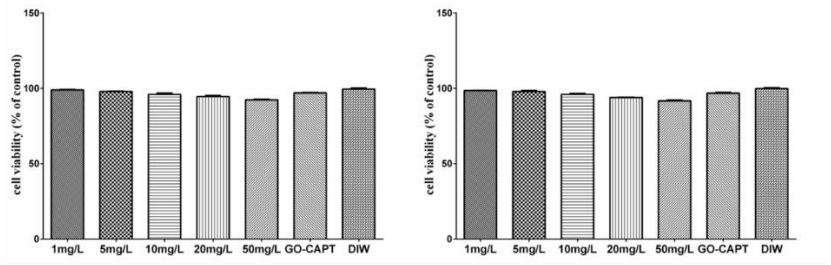

Figure 5. Cells proliferation of different concentrations (1, 5, 10, 20 and 50 mg/L) of GO and GO-CAPT on Caco-2(A) and primary mouse hepatic fibroblast (B). Deionized water (DIW) was used for the control group.

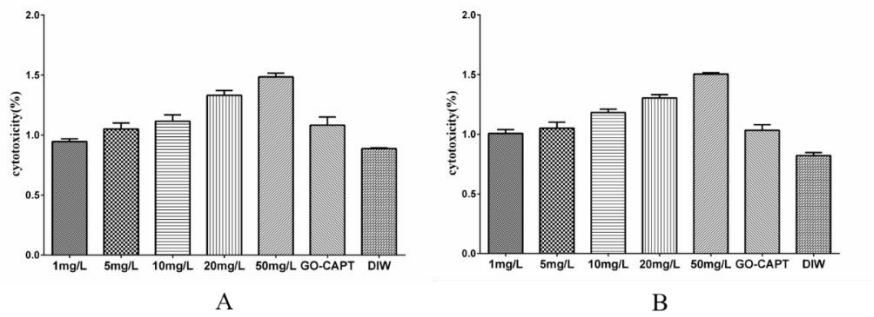

Figure 6. Cellular integrity of different concentrations (1, 5, 10, 20 and 50 mg/L) of GO and GO-CAPT on Caco-2 (A) and primary mouse hepatic fibroblast (B). Deionized water (DIW) was used for the control group.

The LDH kit was used to study the effects of different concentrations of GO and GO-CAPT on the cell structure integrity of Caco-2 cells and primary mouse liver fibroblasts cultured in vitro. As shown in Figure6A, the cytotoxicity of DIW (control group) to Caco-2 cells was 0.89%, and that of GO at concentrations of 1, 5, 10, 20, 50 mg/L and GO-CAPT were 0.95%, 1.05%, 1.16%, 1.33%, 1.48% and 1.08%, respectively. When the GO concentration was increased to 50 mg/L, the cytotoxicity was the greatest, but it was only 0.59% different from the DIW group. The difference between GO-CAPT and DIW group was only 0.19%.A similar trend was also observed in primary cells (Fig.6B). According to the principle of LDH kit, it can be considered that GO and GO-CAPT have almost no damage to cell integrity.

3.3. Effects of GO and GO-CAPT on cell apoptosis and necrosis

In order to verify the cytotoxicity, Annexin V-FITC kit was used to test the effects of GO and GO-CAPT at different concentrations on the apoptosis and necrosis of Caco-2 and mouse primary hepatic fibroblasts cultured in vitro. As shown in Figure 7, in the DIW control group, 95.0% of Caco-2 cells are in the normal cell stage, 0.14% in the early apoptotic stage, and 2.94% in the late apoptotic or necrotic stage. GO at concentrations of 1, 5, 10, 20, 50 mg/L had little effect on late apoptosis or necrosis of Caco-2 cells, which were 8.88%, 5.70%, 3.95%, 5.92% and 6.22%, respectively, and the concentration has no obvious relationship with late cell apoptosis. After GO-CAPT treatment, only 3.75% of Caco-2 cells developed late apoptosis. The same trend was also observed in mouse primary hepatic fibroblasts (Fig.8). The results show that the large size and

high concentration of GO and GO-CAPT prepared therefrom have little effect on the late apoptosis/necrosis state of cells, and had no obvious effect on the early apoptosis state.

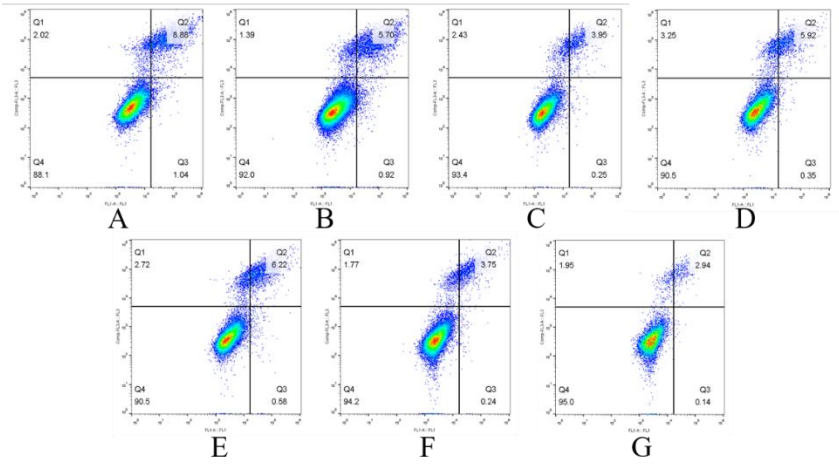

Figure 7. Flow cytometry analysis on cell apoptosis of Caco-2 incubated for 36 h with different concentrations (1, 5, 10, 20, and 50 mg/L) of GO (A~E), GO-CAPT (F) and deionized water (G).

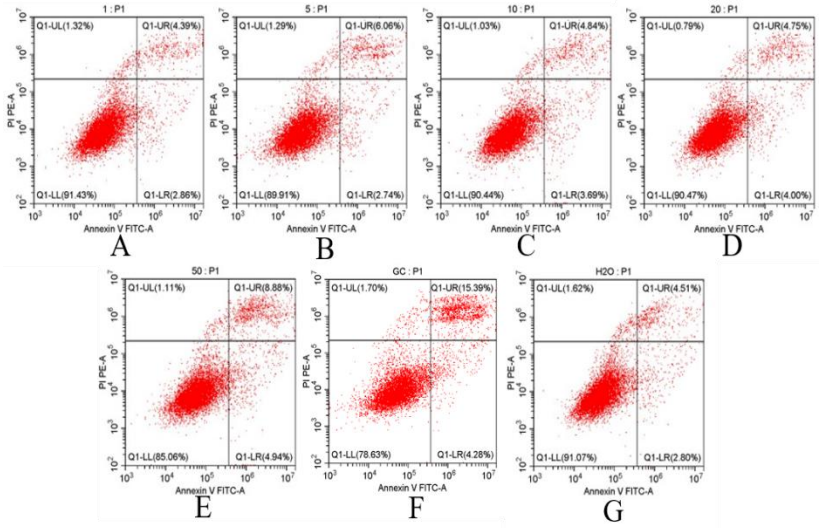

Figure 8. Flow cytometry analysis on cell apoptosis of primary mouse hepatic fibroblast incubated for 36 h with different concentrations (1, 5, 10, 20, and 50 mg/L) of GO (A~E), GO-CAPT (F) and deionized water (G).

According to the cytotoxicity test results of cell proliferation, cell structure integrity and cell apoptosis and necrosis, GO with a large size up to 1-2μm still has low cytotoxicity. It expands the range of the largest GO size studied in this field, which is 205.8nm in the research of Zhang Y, et al. [23-25]. It also provides a basis for the study of graphene oxide in a larger size range.

IV. Conclusions

In this paper, a sustained-release system of captopril (CAPT) loaded on large-size graphene oxide (GO) carrier was prepared. The cytotoxicity of GO with large-size and different concentrations and GO-CAPT to Caco-2 and primary mouse hepatic fibroblast cell lines was investigated from three aspects: cell proliferation, cell structural integrity and cell apoptosis and necrosis. The results showed that CAPT was successfully loaded onto GO through hydrogen bonding. The cytotoxicity of GO-CAPT and the control group of deionized water is not much different, GO and GO-CAPT did not cause obvious cell apoptosis or necrosis. This indicates that the GO and GO-CAPT prepared in this work has low cytotoxicity and are suitable for further biological applications.

Acknowledgments:

We thank college students' scientific research platform of Xiangya Medical College, Central South University for their generous help in flow cytometry analysis.

References

[1]. Ying Chen, Chih–Hsueh Lin, Mei–Jy Jeng. Effects of intratracheal captopril on severely meconium–injured piglet lungs[J]. J Chin Med Assoc, 2019, 82(6): 505-509.
[2]. Swathi N, Jayaprakash D. Formulation Development and Evaluation of Captopril Mouth Dissolving Films[J]. International Journal of ChemTech Research, 2019, 12(3): 17-27.
[3]. McAllister M J, Li J L, Adamson D H, et al. Single sheet functionalized graphene by oxidation and thermal expansion of graphite[J]. Chem Mater, 2007, 19(18): 4936-4404.
[4]. Li D, Muller M B, Wallace G G, et al. Processable aqueous dispersions of graphene nanosheets[J]. Nat Nanotechol, 2008, 3(2): 101-105.
[5]. Shen H, Zhang L M, Liu M, et al. Biomedical applications of graphene[J]. Theranostics, 2012, 2: 283~294
[6]. Liu Z, Robinson J, Sun X M, et al. PEGylated nanographene oxide for delivery of water–insoluble cancer drugs[J]. J. Am. Chem. Soc., 2008, 130: 10876-10877
[7]. Yang X Y, Zhang X Y, Liu Z F, et al. High–efficiency loading and controlled release of doxorubicin hydrochloride on graphene oxide[J]. J. Phys. Chem. C., 2008, 112: 17554-17558
[8]. Tianxiong Liu, Zhouhao Wu, Ning Wang, Cheng–an Tao, Yujiao Li, Jianfang Wang. A pH–sensitive graphene oxide carrier loaded with Captopril as a sustained–release system. Journal of Pharmacy and Biological Sciences, 2020, 15(5): 40-44.
[9]. Zhang L M, Xia J G, Zhao Q H, et al. Functional graphene oxide as a nanocarrier for controlled loading and targeted delivery of mixed anticancer drugs[J]. Small, 2010, 6: 537-544
[10]. Chang Y, Yang ST, Liu JH, et al. In vitro toxicity evaluation of graphene oxide on A549 cells[J]. Toxicol Lett, 2011, 200(7): 201-210.
[11]. Andreeva TD，Svetozar S，Taneva, Stefka G, et al. Hybrid graphene oxide/polysaccharide nanocomposites with controllable surface properties and biocompatibility[J]. Carbohydrate Polymers, 2017, 10(53): 1-16.
[12]. Santos CIM， GOnsalves G， Cicuéndez M, et al. Biocompatible hybrids based on nanographene oxide covalently linked to glycolporphyrins: Synthesis, characterization and biological evaluation[J]. Carbon, 2018, 135: 202-214.
[13]. Zhang Y， Zhang H， Wang Z, et al. pH–Sensitive graphene oxide conjugate Purpurin–18 methyl ester photosensitizer nano complex in photodynamic therapy[J]. New Journal of Chemistry, 2018, 42(16): 3272-13284.
[14]. Wei Y, Zhou F, Zhang D, et al. A graphene oxide based smart drug delivery system for tumor mitochondria–targeting photodynamic therapy[J]. Nanoscale, 2016, 8(6): 3530-3538.
[15]. Hou L, Shi Y, Jiang G, et al. Smart nanocomposite hydrogels based on azo crosslinked graphene oxide for oral colon–specific drug delivery[J]. Nanotechnology, 2016, 27(31): 305-315.
[16]. Mahdavi M, Rahmani F, Nouranian S. Molecular simulation of pH–dependent diffusion, loading, and release of doxorubicin in graphene and graphene oxide drug delivery systems[J]. Journal of Materials Chemistry B, 2016: 7441-7451.
[17]. Puvirajesinghe T M, Zhi Z L, Craster R V, et al. Tailoring drug release rates in hydrogel–based therapeutic delivery applications using graphene oxide[J]. Journal of the Royal Society Interface, 2018, 15(139): 20170949.
[18]. Ali Pourjavadi, Shadi Asgari, Seyed Hassan Hosseini,et al. Codelivery of Hydrophobic and Hydrophilic Drugs by Graphene–Decorated Magnetic Dendrimers[J]. Langmuir, 2018, 34(50): 145-155.
[19]. Xu Y, Hu X, Guan P, et al. A novel controllable molecularly imprinted drug delivery system based on the photothermal effect of graphene oxide quantum dots[J]. Journal of Materials Science, 2019, 54(12): 9124-9139.
[20]. Zahra, Abdollahi, Asghar, et al. PEGAylated graphene oxide/superparamagnetic nanocomposite as a high–efficiency loading nanocarrier for controlled delivery of methotrexate[J]. Journal of Biotechnology, 2019, 298: 88-97.
[21]. Tianxiong Liu, Jianfang Wang, et al. A pH–sensitive graphene oxide carrier loaded with Captopril as a sustained–release system[J]. Journal of Pharmacy and Biological Sciences, 2020, 15(5): 40-44.
[22]. Yang X Y, Zhang X Y, Ma Y F, et al. Superparamagnetic graphene oxide–Fe3O4 nanoparticles hybrid for controlled targeted drug carriers[J]. J Mater Chem, 2009, 19(18): 2710-2714.
[23]. Lv M, Zhang Y, Liang L, et al. Effect of graphene oxide onundifferentiated and retinoic acid-differentiated SH-SY5Ycells line. Nanoscale, 2012, 4: 3861-3866.
[24]. Makharza S , Cirillo G , Bachmatiuk A , et al. Size-dependent nanographene oxide as a platform for efficient carboplatin release. Journal of Materials Chemistry B, 2013, 1:6107-6114.
[25]. Lin Z , Guangxin D , Zaixing Y , et al. Particle Size-Dependent Antibacterial Activity and Murine Cell Cytotoxicity Induced by Graphene Oxide Nanomaterials. Journal of Nanomaterials, 2016, 6(2): 1-9.

Tianxiong Liu, et. al. "A low cytotoxicity sustained release system of captopril loaded on large-size graphene oxide." *IOSR Journal of Applied Chemistry (IOSR-JAC)*, 13(12), (2020): pp 16-22.