Fabrication of Luminescent Hydroxyapatite Nanorods through Surface-initiated RAFT Polymerization: Characterization, Biological Imaging and Drug Delivery Applications

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Abstract

Hydroxyapatite nanomaterials as an important class of nanomaterials, have been widely applied for different biomedical applications for their excellent biocompatibility, biodegradation potential and low cost. In this work, hydroxyapatite nanorods with uniform size and morphology were prepared through hydrothermal synthesis. The surfaces of these hydroxyapatite nanorods are covered with hydrophobic oleic acid, making them poor dispersibility in aqueous solution and difficult for biomedical applications. To overcome this issue, a simple surface initiated polymerization strategy has been developed via combination of the surface ligand exchange and reversible addition fragmentation chain transfer (RAFT) polymerization. Hydroxyapatite nanorods were first modified with Riboflavin-5-phosphate sodium (RPSSD) via ligand exchange reaction between the phosphate group of RPSSD and oleic acid. Then hydroxyl group of nHAp-RPSSD was used to immobilize chain transfer agent, which was used as the initiator for surface-initiated RAFT polymerization. The nHAp-RPSSD-poly(IA-PEGMA) nanocomposites were characterized by means of ^1H nuclear magnetic resonance, Fourier transform infrared spectroscopy, fluorescence spectroscopy and thermal gravimetric analysis in detailed. The biocompatibility, biological imaging and drug delivery of nHAp-RPSSD-poly(IA-PEGMA) were also investigated. Results showed that nHAp-RPSSD-poly(IA-PEGMA) exhibited excellent water dispersibility, desirable optical properties, good biocompatibility and high drug loading capability, making them promising candidates for biological imaging and controlled drug delivery applications.

Key words: Hydroxyapatite nanoparticles, surface initiated RAFT; surface modification; biological imaging; controlled drug delivery

Introduction

Advances of nanotechnology have promoted the development of multifunctional nanomaterials for confederated diagnosis and therapy.^{1,2} Among the diverse nanomaterials, hydroxyapatite nanoparticles ($\text{Ca}_{10}(\text{OH})_2(\text{PO}_4)_6$) have attracted widespread interest for potential applications in the biomedical fields, including biological probes, drug delivery and tissue engineering because of their low cost, biodegradable potential and favorable biocompatibility.³⁻¹² Although hydroxyapatite nanoparticles can be facilely obtained in aqueous solution at large scale, their size and morphology of these hydroxyapatite nanoparticles are also difficult to be controlled. There is no doubt that it will affect their performance for biomedical applications.¹³ Previously, Wang et al have demonstrated that hydroxyapatite nanoparticles doped by lanthanide (Ln) ions such as Eu^{3+} and Tb^{3+} can be obtained via a hydrothermal synthetic route based on the LSS strategy.^{14, 15} Through hydrothermal synthesis, the size and morphology of hydroxyapatite nanoparticles can be well controlled via adjusting the synthesis conditions. However, these hydroxyapatite nanoparticles from hydrothermal route were coated with hydrophobic layers. It is difficult to be dispersed in aqueous solution and utilized for biomedical applications directly.¹⁶⁻²³ On the other hand, the fluorescent intensity of lanthanide ions doped hydroxyapatite nanoparticles is relatively quite weak. Therefore, the development of simple strategies for preparation of hydroxyapatite nanoparticles with controlled size and morphology as well as desirable luminescent properties are quite important.

To overcome the issue mentioned above, some strategies have been developed to transfer the hydrophobic hydroxyapatite nanoparticles into hydrophilic ones in recent years.^{18, 19} The biological imaging applications of these hydrophilic hydroxyapatite nanomaterials have also examined. For example, we have demonstrated that the surfaces of hydroxyapatite nanomaterials can be facilely modified with commercial available and amphiphilic polymers through the hydrophobic interactions between the hydrophobic segments of polymers and the alkyl chain of hydroxyapatite nanomaterials.^{14, 18, 19} Nevertheless, surface coating hydroxyapatite nanoparticles with these polymers through hydrophobic interactions is general weak and lack of designability.^{24, 25} On the other hand, the utilization of these luminescent hydroxyapatite nanoparticles for bioimaging applications is still problematic due to the weak fluorescent intensity of Ln ions doped hydroxyapatite nanoparticles. The surface initiated polymerization is a very promising strategy for surface modification of a large number of materials, which includes the carbon nanotubes, silica nanoparticles, nanodiamond, graphene oxides,

magnetic nanoparticles etc.²⁶⁻³⁷ Different controlled polymerization methods such as atom transfer radical polymerization (ATRP), single-electron transfer living radical polymerization (SET-LRP) and reversible addition-fragmentation chain transfer (RAFT) polymerization have been adopted previously.^{34, 35, 38-42} As compared with ATRP and SET-LRP, the evident advantage of RAFT polymerization is it can be initiated in the absence of metal catalysts.⁴³⁻⁴⁵ This feature makes RAFT polymerization very attractive for preparation of luminescent polymer nanocomposites for biomedical applications because of the simplicity of subsequent purification procedure and good biocompatibility as well as no influence on fluorescent properties.

In this contribution, a simple strategy has been developed for surface modification of hydroxyapatite nanoparticles through combination of surface ligand exchange reaction and RAFT polymerization. The biomedical applications of these hydroxyapatite nanoparticles for bioimaging applications and controlled drug delivery were also examined. As shown in **Scheme 1**, the hydrophobic hydroxyapatite (nHAp) nanorods were first embellished via a ligand exchange reaction whereby RPSSD displaces oleic acid. Then, chain transfer agent (CTA) was immobilized onto nHAp-RPSSD through direct reaction between the carboxyl group of CTA and hydroxyl group of nHAp-RPSSD. In addition, RAFT polymerization was happened using the nHAp-RPSSD-CTA as CTA*, PEGMA and IA as monomers to get nHAp-RPSSD-poly(IA-PEGMA). The characterization results suggested that nHAp can be functionalized by RPSSD and poly(IA-PEGMA) through a ligand exchange reaction and direct surface-initiated RAFT polymerization. Biocompatibility evaluation demonstrated that the nHAp-RPSSD-poly(IA-PEGMA) showed negative toxicity towards cells. Considered the excellent water dispersibility and desirable biocompatibility as well as the superior delivery and slow-release ability, nHAp-RPSSD-poly(IA-PEGMA) should be a desirable biomaterial for different biomedical applications.

Scheme 1 Schematic illustration for the preparation of PEGMA and IA functionalized nHAp by reversible addition-fragmentation chain transfer (RAFT) polymerization.

2 Experiments

2.1 Materials and instruments

nHAp nanorods were synthesized in accordance with our previous reports.¹⁸ Riboflavin-5'-phosphate sodium salt dehydrate (RPSSD, MW: 514.36 Da, 99%) was purchased from Aladdin (Shanghai, China); CTA was synthesized according to our previous report.⁴⁶ Poly(ethylene glycol) methyl ether methacrylate (PEGMA, MW: 950 Da, 98%), itaconic acid (IA, MW: 130.1 Da, 96%), 2, 2-Azodiisobutyronitrile (AIBN, MW: 164.21 Da, 98%) were purchased from Aladdin (Shanghai, China). All other solvents and chemicals were commercial available and used directly without any purification.

¹H nuclear magnetic resonance (NMR) spectroscopy was recorded on Bruker Avance-400 spectrometer using D₂O as the solvent. Fluorescent spectroscopy was obtained from fluorescence spectrophotometer (FSP, model: C11367-11), which was purchased from Hamamatsu (Japanese).

Transmission electron microscopy (TEM) images were recorded on a Hitachi 7650B microscope operated at 80 kV; the TEM specimens were obtained by placing a drop of ethanol nanorod suspension on a carbon-coated copper grid. The Fourier transform infrared (FT-IR) spectra were recorded in a transmission mode on a Nicolet 5700 spectrometer (Waltham, MA, USA). Thermal gravimetric analysis (TGA) was tested on a TA instrument Q₅₀ with a heating rate of 20 °C min⁻¹ using crucibles of aluminum under a N₂ atmosphere.

2.2 Synthesis of nHAp-RPSSD

The synthetic route of nHAp-RPSSD was carried out according to the following ways: nHAp nanorods (254 mg) and RPSSD (72 mg) were dissolved in the 50 mL aqueous/acetone solution. Then, the mixture was deal with ultrasonic treatment for 10 min. After further stirring for about 6-8 h at 60 °C, the desired products (nHAp-RPSSD) were separated by centrifugation at 6000 rpm for 5 min and washed with deionized water and ethanol three times and dried in vacuum drying oven 40 °C to yield nHAp-RPSSD.

2.3 Preparation of nHAp-RPSSD-CTA

260 mg nHAp-RPSSD, 20.5 mg CTA, 25 mg DCC, 10 mg DMAP and 20 mL anhydrous THF were added to a 100 mL flask. Then the polymerization reactor was placed into oil bath under nitrogen atmosphere, and was stirred appropriately by magnetic stirrers at 40 °C for 24 h. Finally, the nHAp-RPSSD-CTA was obtained by centrifugation at 6000 rpm for 5 min and washed with deionized water and ethanol three times and dried in vacuum drying oven 40 °C to yield pure nHAp-RPSSD-CTA.

2.4 Synthesis of nHAp-RPSSD-poly(IA-PEGMA)

The RAFT polymerization was used to synthesize nHAp-RPSSD-poly(IA-PEGMA). 100 mg nHAp-RPSSD-CTA, 200 mg PEGMA, 200 mg IA, 5.1 mg AIBN and 20 mL anhydrous THF were added into a 100 mL polymerization tube. Then the reaction system was put into oil bath, and was stirred at 65 °C under the protection of N₂ for 24 h. In the end, the reaction mixture was separated by centrifuging and the anticipant product was dried in vacuum oven 40 °C.

2.5 Confocal microscopic imaging of cells using nHAp-RPSSD-poly(IA-PEGMA)

The cell images of nHAp-RPSSD-poly(IA-PEGMA) were conducted with excitation wavelength of 458 nm by confocal laser scanning microscopy (CLSM), Zeiss 710 3-channel (Zeiss, Germany). Firstly, HeLa cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) 24 h with a density of 1×

10^5 cells in 37 °C constant temperature incubator containing 95% air and 5% CO₂. Then, the cells were handled with 20 $\mu\text{g mL}^{-1}$ nHAp-RPSSD-poly(IA-PEGMA) nanorods for 3 h at 37 °C. Immediately, cells were washed three times with PBS buffer solution to thoroughly remove nHAp-RPSSD-poly(IA-PEGMA) nanorods without being internalized cells. Last, the assimilation of nHAp-RPSSD-poly (IA-PEGMA) was inquired with CLSM.

2.6 Drug Loading capacity in nHAp-RPSSD-poly(IA-PEGMA).

The drug of CDDP loading procedure was described as follows: First, 30 mg nHAp-RPSSD-poly(IA-PEGMA) was added to 30 mL of phosphate buffered saline (PBS) and ultrasonic treatment for 10 min. Then 8 mg cis-platinum (CDDP) was dissolved into 20 mL of PBS and added into the above preparation of solution as well as stirred at 37 °C 72 h. Afterwards, the reaction system was separated by centrifugation. The sediment and supernatant were preserved in the darkness, specifically. The load rate of CDDP was evaluated at 710 nm by an ultraviolet and visible spectrophotometer (TU-1810) instrument according to our previous reports.³⁶

2.7 Release of CDDP

The release of CDDP from nHAp-RPSSD-poly (IA-PEGMA) was carried out in PBS of different pH values (7.4 and 5.5). Firstly, the 15 mg CDDP-loaded nHAp-RPSSD-poly(IA-PEGMA) nanorods were dissolved into 20 mL PBS and transferred to a dialysis tube with MW cutoff 3500 Da. Next, the dialysis tube was dialyzed in the release buffer (80 mL) in 37 °C with shaking speed of 100 rpm. At predetermined time points, aliquots of 0.1 mL release medium was taken out and added to 5 mL of 1.2 mg/mL pyrocatechol (OPD) and 5 mL of hydrochloric acid (pH = 6.3). The mixture was kept at 100 °C for 10 min. The same volume of fresh PBS was added to maintain the total volume of the release medium. The absorbance of CDDP was evaluated at 710 nm by an ultraviolet and visible spectrophotometer (TU-1810) instrument.

2.8 Cytotoxicity of nHAp-RPSSD-poly(IA-PEGMA)-CDDP

The cytotoxicity of nHAp-RPSSD-poly(IA-PEGMA)-CDDP was assessed applying cell proliferation determination.⁴⁷⁻⁵¹ HeLa cells were cultured in 96-well plates at a density of 2×10^5 cells mL⁻¹ in 200 μL of respective media containing 10% of heat-inactivated fetal bovine serum (FBS) in 5% CO₂ circumstance at 37 °C. After 24 h, 20 μL the suspensions with various nHAp-RPSSD-poly(IA-PEGMA) and nHAp-RPSSD-poly(IA-PEGMA)-CDDP concentrations (10 to 120 $\mu\text{g mL}^{-1}$) were added to well for co-cultivation for 24 h. Each sample was repeated three times. Then, all samples were washed with

PBS to remove the un-internalized nHAp nanocomposites. Finally, CCK-8 dye (10 μ l) was added to each well. Cells were further incubated for 4 h at 37 °C before test. The absorbance of cells were conducted at 450 nm using a microplate reader (VictorIII, Perkin-Elmer).

Results and discussion

The surface modification of nHAp nanorods with polymers through combination of ligand exchange reaction and RAFT polymerization was first characterized by ^1H NMR spectroscopy. As showed in **Fig. 1**, a number of characteristic signals can be found in the resultant products. The ^1H NMR spectrum clearly showed that the proton of aromatic rings can be observed in nHAp-RPSSD-poly(IA-PEGMA), implied that RPSSD has immobilized on nHAp nanorods. On the other hand, the signals located between 3-4 ppm indicated that PEGMA and IA were grafted from nHAp-RPSSD-CTA through RAFT polymerization. The ^1H NMR spectrum confirmed that nHAp nanorods can be modified with poly(IA-PEGMA) through the strategy described in this work. The detailed analysis about the ^1H NMR spectrum was displayed below: a-b: Ph- (6.40-6.85 ppm), g-i: CH- (4.14-4.42 ppm), k: -OH (3.52 ppm), l: -CH₃ (3.45 ppm), m: -CH₂-O- (3.65 ppm), n-p: -CH₂- (3.21-3.32 ppm), q: -OH (2.42 ppm), r: -CH₂-CH- (1.46 ppm), s-t: CH₃-CH- (1.18-1.31 ppm), u: CH₃-CH₂- (1.37 ppm).

Fig. 1 ^1H NMR spectrum of nHAp-RPSSD-poly(IA-PEGMA). A number of characteristic signals indicated that poly(IA-PEGMA) has grafted from nHAp surfaces through combination of ligand exchange reaction and RAFT polymerization.

The size and morphology of nHAp was characterized by TEM. As showed in **Fig. 2**, these nHAp nanorods possess uniform size and shape. The enlarged TEM image showed that the length of nHAp is about 200 nm and their width is about 30-40 nm. After surface modification of nHAp with polymers, the morphology has not obviously changed (**Fig. S1**), implied that the ligand exchange reaction and RAFT polymerization will not destroy the structure of nHAp nanorods. However, we have not found the polymer coating on nHAp through TEM observation. This is likely due to the low contrast between the nHAp nanorods and polymers.

Fig. 2 TEM images of as prepared nHAp nanorods, the scale bar = 500 nm. The inset is the enlarged TEM image of nHAp nanorods, scale bar = 200 nm.

The poly(IA-PEGMA) coated nHAp nanorods were further characterized by FT-IR spectroscopy to verify the formation of poly(IA-PEGMA) coating. As shown in **Fig. 3**, the peaks at 2928 and 2858 cm^{-1} are characteristic of C–H stretching bands of the oleic acid on the nHAp nanorods. Other characteristic bands at 1558, 1447, 1045, 848, 598, and 570 cm^{-1} can be attributed to the characteristic peaks of nHAp nanorods. After surface ligand exchange reaction, the characteristic C–H stretching bands of oleic acid between 2800 and 3000 cm^{-1} were disappeared. On the other hand, the intensity of signals between 1500 and 1800 cm^{-1} was obviously enhanced, which can be attributed to the functional groups of aromatic rings and carboxyl groups of RPSSD. The difference between the nHAp and nHAp-RPSSD indicated that RPSSD has indeed immobilized on nHAp nanorods via ligand exchange reaction. Furthermore, the C–H stretching bands were further emerged in the sample of nHAp-RPSSD-CTA. The

above results indicated that CTA was conjugated with hydroxyl group of RPSSD through esterification reaction. More importantly, as compared with the spectrum of nHAp-RPSSD-CTA, a typical peak at 1732 cm^{-1} assigned to the C=O group was appeared in the sample of nHAp-RPSSD-poly(IA-PEGMA), which indicated that IA and PEGMA were surely grafted from nHAp nanorods through RAFT polymerization. It is well known that RPSSD is a fluorescence molecule containing phosphate and hydroxyl groups. Therefore, RPSSD can act as molecule both for fluorescent signal and immobilization of CTA at the same time. The luminescent properties of nHAp-RPSSD-poly(IA-PEGMA) were examined by fluorescent spectroscopy. As showed in **Fig. 3B**, three peaks ranging from 252–523 nm can be observed in the excitation spectrum of nHAp-RPSSD-poly(IA-PEGMA). The excitation wavelength is similar to RPSSD. On the other hand, the emission peak of nHAp-RPSSD-poly(IA-PEGMA) was located at 537 nm when they were excited at the wavelength 450 nm. Well consistent with the fluorescent spectra, nHAp-RPSSD-poly(IA-PEGMA) water suspensions displayed uniform green fluorescence when they were irradiated with UV lamp ($\lambda = 365\text{ nm}$). These results suggested that nHAp-RPSSD-poly(IA-PEGMA) can be well dispersed in water and possess excellent fluorescent properties. The fluorescence quantum yield (QY) is a very important parameter for their biological applications. Therefore the relative QY of nHAp-RPSSD-poly(IA-PEGMA) was determined using quinine sulfate as the reference dye according to our previous report.⁵² We demonstrated that the relative QY of nHAp-RPSSD-poly(IA-PEGMA) was as high as 25.6% according to the results of UV-vis and fluorescent spectroscopy. The high fluorescence QY is likely due to the nHAp nanorods could effectively avoid the aggregation and aggregation caused quenching effect of RPSSD. Therefore, we believed that the fluorescent intensity of nHAp-RPSSD-poly(IA-PEGMA) is strong enough for biological imaging applications.

Fig. 3 (A) FT-IR spectra of nHAp nanorods, nHAp-RPSSD, nHAp-RPSSD-CTA and nHAp-RPSSD-poly(IA-PEGMA). (B) Fluorescent properties of nHAp-RPSSD-poly(IA-PEGMA). The

fluorescence excitation and emission spectra showed that three excitation peaks were observed in the excitation spectrum. And one emission peak of nHAp-RPSSD-poly(IA-PEGMA) was located at 537 nm when they were excited with the wavelength of 450 nm. Inset is the optical image of nHAp-RPSSD-poly(IA-PEGMA) nanocomposites that were dispersed in aqueous solution and irradiated by UV lamp ($\lambda = 365$ nm).

The surface modification of nHAp with polymers was further confirmed by the TGA and differential thermal analysis (DTA). As illustrated in **Fig. 4A**, a small mass decrease was observed in the sample of nHAp nanorods below 100 °C, that can be attributed to the evaporation of absorbed water. Much greater weight loss was observed between 200-600 °C, which can be ascribed to the degradation of oleic acid of nHAp. The total weight loss of nHAp nanorods is 13.5% at 600 °C. It is obvious that the weight loss of nHAp-RPSSD-poly(IA-PEGMA) was rapidly increased to 26.6% under the identical experimental conditions, symbolizing that poly(IA-PEGMA) copolymers were breezily adhered to the surface of nHAp nanorods by RAFT. In addition, the data of DTA can be used to assist evaluation. First of all, we can observe a gradual peak appeared around 200 °C from the curve of nHAp nanorods. Secondly, moving from left to right across the results presented in the curve of nHAp-RPSSD-poly(IA-PEGMA), we see a decent boost in performance that can be attributed to the poly(IA-PEGMA) grafted onto nHAp nanorods. Therefore the TGA and DTA data powerfully proved that poly(IA-PEGMA-DA) copolymers were potentially coated on the nHAp nanorods. The dispersive capacity of primordial nHAp nanorods and nHAp-RPSSD-poly(IA-PEGMA) in PBS solution was further examined and results were displayed in **Fig. S2**. It should be mentioned that the dispersive capacity of nHAp-RPSSD-poly(IA-PEGMA) in water is obviously enhanced. As showed in **Fig. S2A**, the primordial nHAp nanorods will be quickly precipitated within 10 min. However, nHAp-RPSSD-poly(IA-PEGMA) nanocomposites are still well dispersed in PBS after 12 h (**Fig. S2D**). These results really show that the nHAp nanorods were embellished with poly(IA-PEGMA). It has been demonstrated that PEG is a hydrophilic and biocompatible macromolecule that has been widely used for biomedical applications. Previous reports have demonstrated that surface modification of nanomaterials with PEG or polyPEG could obviously improve their water dispersibility.^{18, 26, 27} On the other hand, the surface PEGylation of nanomaterials has also shown influence on the pharmacokinetic behavior of these modified nanomaterials.⁵³ Therefore the good dispersibility of

nHAp-RPSSD-poly(IA-PEGMA) in PBS implied nHAp-RPSSD-poly(IA-PEGMA) potential for biomedical applications.

Fig. 4 (A) TGA curve of nHAp nanorods and nHAp-RPSSD-poly(IA PEGMA); (B) DTA curve of nHAp nanorods and nHAp-RPSSD-poly(IA-PEGMA).

As the introduction of IA that possesses two carboxyl groups can coordinate with the anticancer agent (CDDP). Therefore the loading and release kinetics of nHAp-RPSSD-poly(IA-PEGMA) towards CDDP was further evaluated. We demonstrated that the drug loading percentage of nHAp-RPSSD-poly(IA-PEGMA) was calculated to be 50.78%. It has demonstrated that the chloride ligands in CDDP can be substituted with carboxyl ligands in PBS. On the other hand, to quantitatively determine CDDP release from the modified nHAp nanorods, CDDP-loaded nHAp-RPSSD-poly(IA-PEGMA) was suspended in either dialysis tubes at pH values of 7.4 and 5.5, respectively. The amount of released CDDP at predetermined time intervals was measured by optical measurement. As illustrated in **Fig. 5**, the release profiles of CDDP show a fast release ratio from polymeric nanocomposites in the first 12 h and then sustain a slow release over a prolonged time. For the nHAp-RPSSD-poly(IA-PEGMA)-CDDP, the cumulative release of CDDP is about 18.7% at pH 7.4. While at pH 5.5, the release ratio of CDDP was increased to about 38.7%. In a word, in the neutral environment, the release of CDDP from nHAp-RPSSD-poly(IA-PEGMA)-CDDP is caused mainly by concentration gradient between nHAp-RPSSD-poly(IA-PEGMA)-CDDP and PBS. However, in an acidic environment, CDDP becomes hydrophilic, which promotes the cumulative release of CDDP. It has been well realized that coordinative bonds are stable under neutral conditions but will be hydrolyzed with the trigger of acid (pH < 5.5). The pH dependent drug release behavior suggested that nHAp-RPSSD-poly(IA-PEGMA) can be potentially used for controlled drug delivery applications.

Fig. 5 pH responsive drug release profiles from the drug-loaded nHAp-RPSSD-poly (IA-PEGMA).

Better cellular uptake of nHAp-RPSSD-poly(IA-PEGMA) can enhance gene and drug delivery. Therefore, we further assessed their cellular uptake behavior by CLSM observation. As shown in **Fig. 6**, strong yellow fluorescence could be observed at the cell location after they were incubated with only $20 \mu\text{g mL}^{-1}$ of nHAp-RPSSD-poly(IA-PEGMA) for 3 h. Furthermore, no significant background interference was detected. The nHAp-RPSSD-poly(IA-PEGMA) mainly located at cytoplasm. The reason that the particles didn't enter the cell nucleus may due to small nucleus pore (less than 30 nm in diameter) or the selectivity of nucleus membrane transport. Although the mechanism about the nHAp-RPSSD-poly(IA-PEGMA) entered into cells was not accurately understand. The nHAp-RPSSD-poly(IA-PEGMA) were cell-permeable and could penetrate living cells without observably cell damage. The cell uptake results indicated that of nHAp-RPSSD-poly(IA-PEGMA) can be used as for tracing the location of drugs and used for intracellular delivery of active components for disease treatment.

Fig. 6 CLSM images of HeLa cells which were incubated with $20 \mu\text{g mL}^{-1}$ of nHAp-RPSSD-poly(IA-PEGMA) for 3 h. (A) excited with 454 nm laser, (B) bright field and (C) merge image of A and B. Scale bar = $20 \mu\text{m}$.

CCK-8 assay was adopted to examine the biological effects of nHAp-RPSSD-poly(IA-PEGMA) and nHAp-RPSSD-poly(IA-PEGMA)-CDDP towards HeLa cells. HeLa cells were treatment with different concentrations of nHAp-RPSSD-poly(IA-PEGMA) and nHAp-RPSSD-poly(IA-PEGMA)-CDDP for 24 h. It can be seen that nHAp-RPSSD-poly(IA-PEGMA) showed almost no toxicity toward HeLa cells after cells were incubated with $10\text{--}120 \mu\text{g mL}^{-1}$ nHAp-RPSSD-poly(IA-PEGMA) for 24 h. The cell viability of nHAp-RPSSD-poly(IA-PEGMA) is still greater than 95% even the concentration is up to $120 \mu\text{g mL}^{-1}$ (**Fig. 7**). The results suggested that nHAp-RPSSD-poly(IA-PEGMA) possess excellent biocompatibility and are promising for drug delivery applications. As compared with nHAp-RPSSD-poly(IA-PEGMA), significant cell viability decrease was observed in the sample of nHAp-RPSSD-poly(IA-PEGMA)-CDDP. Almost all the cells were killed at the concentration of $40 \mu\text{g mL}^{-1}$. The obvious decrease of cell viability of nHAp-RPSSD-poly(IA-PEGMA)-CDDP implied that CDDP can be transported and released in cells. Based on the cell viability results and higher cellular uptake efficiency, we are convinced that the nHAp-RPSSD-poly (IA-PEGMA) will eventually be widely used in various biomedical areas. IA has been reported to design as drug delivery vectors since it can form ionic complexes with drug. Because of the excellent features of PEGMA, such as low cytotoxicity, numerous functional groups amenable to various modification chemistries and high water-soluble, combining PEGMA with IA is a new breakthrough in gene and drug delivery application.

Fig. 7 Cell viability of nHAp-RPSSD-poly (IA-PEGMA)-CDDP to HeLa cells. Cells were incubated with different concentrations (10-120 $\mu\text{g mL}^{-1}$)

4. Conclusion

In summary, we reported a simple and efficient strategy to modify hydrophobic nHAp nanorods. The final nHAp nanocomposites were acted as drug carrier for real-time monitoring of drug release *in vitro*. In particular, the hydrophobic nHAp nanorods were embellished by a ligand exchange reaction with RPSSD. The copolymerization of PEGMA and IA was prepared through RAFT polymerization. The characterization results suggested that we have successfully prepared nHAp-RPSSD-poly(IA-PEGMA). Moreover, nHAp-RPSSD-poly(IA-PEGMA) showed negative toxicity towards HeLa cells and excellent drug delivery behavior. This study provides a novel and efficient strategy to surface modification of nHAp nanorods through combination of surface ligand exchange and subsequent RAFT polymerization. Due to well design capability and monomer applicability of RAFT polymerization, the strategy described in this work should be a general method for fabrication of a number of fluorescent nHAp nanorods based polymer nanocomposites, which should be of great application potential for different biomedical applications.

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