Surface grafting of Eu³⁺ doped luminescent hydroxyapatite nanomaterials through metal free light initiated atom transfer radical polymerization for theranostic applications

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Abstract

We reported a simple and efficient method to prepare the hydrophilic luminescent HAp polymer nanocomposites through the combination of ligand exchange and metal free light initiated **surface-initiated atom transfer radical polymerization (SI-ATRP)** using 10-phenylphenothiazine (PTH) as organic catalyst and 2-methacryloyloxyethyl phosphorylcholine (MPC) and itaconic acid (IA) as monomers. The biological imaging and drug delivery performance of HAp-poly(MPC-IA) nanorods were examined to evaluate their potential for biomedical applications. Results suggested that hydrophilic HAp-poly(MPC-IA) nanorods can be successfully prepared. More importantly, the HAp-poly(MPC-IA) exhibited excellent water dispersibility, desirable biocompatibility and good performance for biological imaging and controlled drug delivery applications. As compared with other controlled living polymerization reactions, the metal free light initiated SI-ATRP displayed many advantages such as easy for handle, mild reaction conditions, toxicity and fluorescence quenching from metal catalysts. Therefore, we believe that this strategy should be a useful and effective strategy for preparation of HAp nanomaterials for biomedical applications.

Key words: Luminescent hydroxyapatite nanomaterials, metal free atom transfer radical polymerization, light initiated, drug

delivery, theranostic applications

Introduction

Thanks to their excellent biocompatible, osteoconductive, nonimmunogenic, bioactive and biodegradable properties, hydroxyapatite ($\text{Ca}_{10}(\text{OH})_2(\text{PO}_4)_6$, HAp) based nanomaterials have drawn more and more attention of the scientists from chemistry, material and other fields.[1-11] HAp nanomaterials are a kind of hosts suitable for rare earth ions doping such as Eu^{3+} and Tb^{3+} by hydrothermal treatment to endow their excellent luminescent properties.[12-16] Furthermore, HAp nanomaterials have also been intensively investigated for many other biomedical applications, such as drug/gene delivery, bone tissue engineering.[17-34] Over the past few decades, HAp nanomaterials have been synthesized with different nanocrystal morphologies through hydrothermal treatment, like rod-like, spherical or needle-shaped crystals.[35-37] Though these HAp nanomaterials possess well controlled size and morphology, they are difficult to maintain good dispersion in water. This makes a great challenge for their biomedical applications, which often required these materials water dispersibility. Therefore, it is a key problem to develop a strategy to tune the surface properties of HAp nanoparticles and endow their water dispersibility.

In recent years, several methods, such as surface initiated atom transfer radical polymerization (ATRP), reversible addition fragmentation chain transfer polymerization (RAFT) and single-electron transfer living radical polymerization (SET-LRP) have been developed to graft with different polymer chains on the surface of materials to enhance their biocompatibility or enhance their dispersibility and stability in hydrophobic or hydrophilic solvents.[38-46] As is known to all, the traditional ATRP usually involves transition metal catalysts and the transition metal complex is hard to remove and will be retained in the final polymeric products. These metal catalysts could not only influence the properties of final polymer products but also toxic to the living organisms. Therefore, it is the main barrier for the use of this process in biomedical applications. On the other hand, these transition metal catalysts will also quench the fluorescence of luminescent materials. Therefore, it is highly desirable to develop novel strategy for the surface functionalization of luminescent HAp nanomaterials. In recent years, an innovative metal free photoinitiated ATRP process was emerged as a novel polymerization method, which doesn't require heavy metal catalysts such as copper ions and expensive organic ligands.[47] It has been reported that the new metal free ATRP process requires an organic-based photocatalyst and is driven by UV irradiation. To the best of our knowledge, there have been only very few reports have investigated the metal free ATRP for luminescent HAp modification.

In this contribution, the luminescent Eu^{3+} doped hydroxyapatite nanorods modified with 2-methacryloyloxyethyl phosphorylcholine (MPC) and Itaconic Acid (IA) [HAp-poly(MPC-IA)] have been synthesized through combination of ligand exchange reaction and the metal free SI-ATRP. The strategy for the hydrophilic and multiple-carboxyl HAp-poly(MPC-IA) depicted in **Scheme 1**. First, the formation of the NH_2 -contained HAp-AMP was carried out through the ligand exchange reaction with adenosine monophosphate (AMP). Then the Br-contatined initiators (HAp-Br) were obtained by amidation reaction. In the end, the polymerization of MPC and IA on the surface of HAp using the metal free surface initiated ATRP using 10-phenylphenothiazine (PTH) as organic photocatalyst. The properties of HAp-poly(MPC-IA) nanorods have been characterized

by a series of characterization methods, including ^1H NMR, TEM, FT-IR, TGA and XPS. The biomedical application of HAp-poly(MPC-IA) has been tested for controlled drug delivery.

Scheme 1 Schematic representation for the preparation of HAp-poly(MPC-IA) nanorods by the combination of ligand exchange reaction and the metal free SI-ATRP.

2. Experiment Section

2.1 Materials

Luminescent Eu^{3+} doped hydroxyapatite nanorods (HAp-Eu^{3+}) were prepared according to previous method. [48] Adenosine monophosphate (AMP) was purchased from Shanghai Sangon Biological Engineering Technology And Service Co., Ltd., MPC (MW: 295.27 Da, 98%), IA (MW: 130.10 Da, 99%), 2-bromoisobutryl bromide (BIBB, 98%) and cis-Diammineplatinum dichloride (CDDP, MW: 300.05 Da) were purchased from Aladdin (Shanghai, China). All other reagents and solvents were obtained from Aladdin and used without further purification.

2.2 Characterization

Transmission electron microscopy (TEM) images were obtained with a FEI Tecnai G2 Spirit microscope operated at 120 kV. The X-ray photoelectron spectra (XPS) were collected on a VGESCALAB 220-IXL spectrometer using an Al $K\alpha$ X-ray source (1486.6 eV). All binding energies were calibrated using the containment carbon ($\text{C1s} = 284.6$ eV). The Fourier transform infrared (FT-IR) spectra for all the samples were carried out using a Nicolet 380 Fourier transform spectrometer instrument by transmission mode ranging from 400 to 4000 cm^{-1} with a resolution of 2 cm^{-1} to detect the characteristic functional group. TGA curves were recorded on a Q50 Thermo gravimetric Analyzer under pure N_2 flow, and the samples were heated from $25\text{ }^\circ\text{C}$ to $800\text{ }^\circ\text{C}$ with the weight of 5-10 mg, applying heating rate of $10\text{ }^\circ\text{C/min}$ under pure N_2 atmosphere. ^1H NMR spectra were recorded on a Bruker Avance-400 spectrometer with D_2O as the solvents.

2.3 Synthesis of amino-contained HAp nanoparticles

The amino-contained HAp (HAp-NH_2) was prepared through ligand exchange reaction and the specific synthetic route can be described as follows: firstly, 200 mg HAp-Eu^{3+} and 100 mg AMP were suspended in the 50 mL aqueous/acetone solution. Then

the mixture was dealt with ultrasonic processing at 150 W for 20 min. The reaction mixture was stirred under reflux condition for 7 h. The obtained solid was collected by centrifugation at 6000 rpm for 10 min and washed with deionized water and acetone several times. The final products were dried at 60 °C overnight.

2.4 Synthesis of HAp-Br initiators

For the in situ polymerization of polymer on the surface of HAp nanorods through ATRP method, the Br-contained initiator was introduced through the amidation reaction. 300 mg HAp-NH₂ nanorods were dispersed in 39 mL anhydrous toluene, then 48 mg TEA was added into the reaction flask. Subsequently, the solution of BIBB (36 mg) dissolved in 1 mL dry toluene was injected slowly at 0-5 °C on ice-water bath. The reaction system was stirred for 2 h at ambient temperature and then the crude HAp-Br initiators were collected by centrifugal machine at 6000 rpm for 10 min. To remove the excess BIBB, the solid was washed with acetone several times. In the end, the product was collected and dried at 60 °C for 12 h.

2.5 Preparation of the hydrophilic HAp-poly(MPC-IA)

The metal free surface initiated ATRP method was used to prepare the hydrophilic HAp-poly(MPC-IA) nanorods. 150 mg HAp-Br and photocatalyst (0.1 mol%) were added into 100 mL dry Schlenk flask which was equipped with a magnetic stir bar. Then 77 mg MPC, 34 mg IA in 20 mL dry DMF were added into the reaction flask under vigorous stir at room temperature. The mixture was degassed by freeze-pump-thaw cycle for three times and the reaction flask was backfilled with N₂. The reaction flask was placed inside a light box illuminate by the 380 nm LEDs light. The reaction was carried out for 4 h at ambient temperature. The reaction mixture was dialyzed with a dialysis bag (MWCO = 3500 Da) against distilled water for 48 h to remove unreacted monomers. The final products were collected with centrifugation at 6000 rpm and dried at 60 °C for 12 h.

2.6 Drug loading capacity in HAp-poly(MPC-IA)

The drug of CDDP loading procedure was described as follows: 30 mg HAp-poly(MPC-IA) was dispersed in 42 mL **phosphate buffer solution (PBS)** (pH = 7.4). Then 8 mL 1 mg/mL CDDP solution dissolved in PBS (pH = 7.4) was added into the above mentioned solution and stirred at 37 °C for 48 h. The HAp-poly(MPC-IA) nanorods loaded with CDDP was separated from the mixture by centrifugation and wash with deionized water. In the end, the CDDP HAp-poly(MPC-IA) system was dried and for further experiment. The concentrations of CDDP were determined using the procedure according to our previous reports.[49, 50] The drug loading efficiency of HAp-poly(MPC-IA) nanorods was also calculated based on the concentrations of CDDP before and after drug loading.

2.7 The release of CDDP

The properties of the release of CDDP from HAp-poly(MPC-IA) was carried out in PBS with different pH values (7.4 and 5.5) by dialysis method. The same amount (15 mg) CDDP HAp-poly(MPC-IA) nanorods were dispersed into 20 mL PBS (pH = 7.4) and PBS (pH = 5.4), respectively. Then the solution was poured into a dialysis tube (MWCO: 3500 Da) and the dialysis tube was placed into a beaker that contained with 80 mL PBS at 37 °C with shaking speed of 100 rpm. At different time, 0.1 mL solution was taken out and mixed with 5 mL 1.2 mg/mL pyrocatechol and 5 mL HCl (pH = 6.3). Then the mixture was heated at 100 °C for

10 min. After taking the sample, the same amount PBS was added into the solution to keep the total volume. The CDDP content in the release solution was measured by an ultraviolet and visible spectrophotometer (TU-1810) instrument at 710 nm.

2.8 Biological imaging capability of HAp-poly(MPC-IA)

The cell uptake behavior of HAp-poly(MPC-IA) nanorods was conducted with excitation wavelength of 405 nm by confocal laser scanning microscopy (CLSM), Zeiss 710 3-channel (Zeiss, Germany) to evaluate their potential for biological imaging applications. HeLa cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with a density of 1×10^5 cells for 24 h in 37 °C constant temperature incubator containing 95% air and 5% CO₂. Then, HAp-poly(MPC-IA) nanorods with final concentration of 100 $\mu\text{g mL}^{-1}$ were added into cell culture plates and incubated for another 3 h at 37 °C. After that, the cells were washed three times with PBS to thoroughly remove HAp-poly(MPC-IA) nanorods without being internalized cells. Finally, the assimilation of HAp-poly(MPC-IA) nanorods was inquired with CLSM.

2.9 Cell viability evaluation of HAp-poly(MPC-IA)

The cell viability of HAp-poly(MPC-IA) nanorods was determined by the cell counting kit-8 (CCK-8) according to our previous reports using HeLa cells.[51, 52] The cell viability results were used to evaluate the preliminary biocompatibility. The experimental procedure was described in the supporting information in detail.

2.10 Therapeutic effects of HAp-poly(MPC-IA)-CDDP

The therapeutic effects of HAp-poly(MPC-IA)-CDDP complex were also evaluated based on the cell viability evaluation. HeLa cells were incubated with different concentrations of HAp-poly(MPC-IA)-CDDP complex for 24 h. Then the cell viability values of cells were calculated based on CCK-8 results. The detailed experiment procedures were displayed in supporting information.

3. Results and discussion

Fig. 1 ¹H NMR spectrum of HAp-poly(MPC-IA) dissolved in D₂O.

The successful surface modification of HAp nanorods with MPC and IA through the combination of ligand exchange reaction and Surface Initiated Metal Free ATRP were first characterized by ^1H NMR spectroscopy. The ^1H NMR spectra of AMP, MPC and IA were displayed in **Fig. S1**. Both of the MPC and IA monomers have two peaks located between 5.5 and 6.3 ppm, which can be attributed to the protons connected to the C=C bond. As shown in **Fig. 1**, a series of characteristic signals can be observed in the sample of HAp-poly(MPC-IA). After the polymerization, the signals located between 5.5 and 6.3 ppm disappeared, which indicated that the MPC and IA were grafted from the surface of HAp-Br by metal free ATRP. As compared with the ^1H NMR spectrum of AMP, the two peaks located between 8.0 and 8.5 ppm can be attributed to the protons of adenine of AMP in the sample of HAp-poly(MPC-IA). In addition, the peak at 5.93 ppm is also caused by the protons of AMP. The detailed analysis about the ^1H NMR spectrum of HAp-poly(MPC-IA) was described as follows: 0.6-1.5 ppm ($-\text{CH}_2-$, $-\text{CH}_3$); 2.0-2.5 ppm ($-\text{CH}_2-\text{N}$); 3.1 ppm ($-\text{N}+(\text{CH}_3)_3$); 3.5-4.6 ppm ($-\text{CH}_2\text{O}$).

Fig. 2 TEM images of HAp nanorods, HAp-AMP, HAp-Br and HAp-poly(MPC-IA).

The TEM images of Eu^{3+} doped HAp nanorods, HAp-AMP, HAp-Br and HAp-poly(MPC-IA) samples are displayed in **Fig. 2**, respectively. As shown in **Fig. 2A-C**, it found that the HAp- Eu^{3+} , HAp-AMP and HAp-Br consist of relatively uniform rod-like particles with the length of 50-80 nm and width around 15 nm. The results indicate that the introduction of small molecules have little influence on the morphology of HAp. Amazingly, the morphology of HAp-poly(MPC-IA) nanorods also has not obvious change and maintains the original characteristic, which can be attributed that the processes of the new metal free surface initial ATRP is rather gentle. On the other hand, the sparse distribution of HAp-poly(MPC-IA) is in good accordance with its dispersibility which are shown in **Fig. S2**. It can be observed that the original HAp- Eu^{3+} nanorods are easily to agglomerate into large ones and precipitate at the bottom of the bottle. On the contrary, the HAp-poly(MPC-IA) nanorods show an excellent colloidal stability, which suggesting that the grafting of MPC and IA on the surface of HAp is an efficient strategy to enhance the dispersibility of HAp nanorods.

Fig. 3 FT-IR spectra of HAp nanorods, HAp-AMP, HAp-Br and HAp-poly(MPC-IA).

Fig. 3 shows the FT-IR spectra of HAp nanorods, HAp-AMP, HAp-Br and HAp-poly(MPC-IA). Compared with HAp-Eu³⁺ nanorods, there are some peaks disappeared on the HAp-AMP, such as the peaks at 2925 and 2854 cm⁻¹ which can be attributed to the symmetry stretching CH₂ vibration and the asymmetry stretching CH₂ vibration, respectively. The result suggested that the AMP have successfully immobilized onto the surface of HAp nanorods by ligand exchange reaction with oleic acid. However, after the introduction of BIBB, the vibration bands of alkyl C-H stretching vibration located between 2960 and 2850 cm⁻¹ have re-emerged in the sample of HAp-Br. In addition, the HAp grafted with MPC and IA also exhibited some new peaks, such as the stretching C=O vibration at 1723 cm⁻¹, the stretching C-N vibration at 1246 cm⁻¹. The absorption peak at 1040 cm⁻¹ could be caused by the superposition of the stretching C-O-P vibration in MPC and absorption peak of HAp nanorods. All these results showed that the MPC and IA have been successfully grafted onto the surface of HAp.

Fig. 4 TGA curves of HAp nanorods and HAp-AMP, HAp-Br and HAp-poly(MPC-IA).

The grafting amount of polymers on the surface of HAp nanorods was determined by TGA. And **Fig. 4** shows the TGA curves of HAp samples with temperature increasing from room temperature to 800 °C. When the sample was heated to 550 °C, the weight loss of HAp-Eu³⁺ can be calculated to be about 15.7%, which was caused mainly by oleic acid on the surface of HAp. However, there was no further weight loss in the temperature range of 550-800 °C, which suggests the high thermal stability of sample. After reaction with AMP, the TGA curves showed a two stage weight loss at the temperature range of 25-150 °C and 250-500 °C. The first stage showed about 3.6% weight loss caused by the adsorbed and lattice water. The second stage showed about 11.0% weight loss caused by the AMP exchanged onto the surface of HAp. Due to the dosage of BIBB used in the reaction is very little, there was no obvious weight loss could be observed in the sample of HAp-Br. For the HAp nanorods grafted with MPC and IA, the weight loss in the range of 200-500 °C was due to the decomposition of the grafted with MPC and IA. From these TGA analyses, two important conclusions may be obtained. First, the MPC and IA can be grafting from the surface of HAp by metal free ATRP. Second, the weight percentages of polymers grafted on the surface of HAp could be calculated to be 34.4%.

Fig. 5 the XPS full scan spectra of HAp-Eu³⁺, HAp-AMP, HAp-Br and HAp-poly(MPC-IA) ranging from 0 to 1200 eV.

To further investigate the composition on the surface of HAp nanorods, XPS measurement was employed. **Fig. 5** shows the XPS full scan spectra of the samples of HAp nanorods, and the existence of these elements including Br, C, Ca, N, O, P and Eu were confirmed. The high resolution XPS spectra of O1s, C1s, N1s and Br3d were shown in **Fig. 6A-D**. After the ligand exchange reaction, the appearance of the N1s peak located at 399.2 eV, which can be attributed to the N-H bond, indicating that the AMP was successfully immobilized onto the surface of HAp nanorods. On the other hand, the intensity of O1s signal (530.9 eV) was decreased. **Fig. 6D** presents the Br3d signal. There was no Br3d signal to have been detected. However, a new peak between 66 and 72 eV relating to Br3d can be observed in HAp-Br, indicating that the BIBB has successfully introduced into the HAp-AMP by amidation reaction. After grafting with MPC and IA, the intensity of Br3d signal was decreased greatly. Furthermore, careful peak fitting on the C1s peak resolves a series of peaks representing different carbons (**Fig. S3**). The intensity of -O-C=O (288.6 eV), C-O (286.5 eV) and C-N (285.7 eV) were increase, which suggested that the presence of poly(MPC-IA) on the surface of HAp.

Fig. 6 the representative XPS spectra of HAp nanorods. (A) O1s region, (B) C1s region, (C) N1s region and (D) Br3d region.

Table 1 Element contents (%) of HAp samples based on XPS analysis.

Samples	Br3d	C1s	Ca2p	N1s	O1s	P2p	Eu3d
HAp-Eu ³⁺	0	41.42	12.48	0	35.93	7.87	2.30
HAp-Amp	0	43.82	8.68	4.49	35.33	6.08	1.60
HAp-Br	0.23	37.26	9.91	6.20	37.23	6.99	2.18
HAp-poly(MPC-IA)	0.07	56.79	4.00	5.03	29.19	4.15	0.77

Based on the XPS analysis, the weight percentages of elements including Br, C, Ca, N, O, P and Eu in the samples of HAp were calculated and listed in the **Table 1**. It can be seen that the intensity of N1s signal was increased from 0 to 4.49% in HAp-AMP and the intensity of Br3d signal was increased from 0 to 0.23% in HAp-Br, which indicated that the HAp-AMP and HAp-Br have been successfully prepared, respectively. After grafting with MPC and IA, the intensity of C1s was increased greatly from 37.26% to 56.79%. And these weight percentages of other elements were decreased correspondingly. These results further demonstrated that the MPC and IA can graft from the surface of HAp by combination of ligand exchange reaction and metal free surface initiated ATRP.

Fig. 7 the CDDP release profiles from the drug loaded HAp-poly(MPC-IA).

Based on the calculation results before and after drug loading, the drug loading efficiency of CDDP on HAp-poly(MPC-IA) nanorods is as high as 142.4 mg g^{-1} . The CDDP release profiles of drug loaded HAp-poly(MPC-IA) nanorods at 37°C in PBS were shown in Fig. 7. It can be seen that the release profiles of CDDP show a fast release rate both of PBS ($\text{pH} = 7.4$) and PBS ($\text{pH} = 5.5$) in the first 20 h and followed by a slow release rate. The slow release effect is helpful to ease the toxic and side effect. The difference is that the release rate in PBS ($\text{pH} = 5.5$) is significantly greater than in PBS ($\text{pH} = 7.4$), which is conducive to cancer therapy. It is anticipated that the drug delivery system will release a small amount of CDDP in the circulation of the blood. After internalization by cancer cells, it will release a lot of CDDP, which can improve the drug utilization rate. It is therefore this drug delivery system can be applied for targeted drug delivery applications after their surface was functionalized targeted agents or through the enhanced permeability and retention (EPR) effect, thus improving the drug therapeutic index and reducing their side effects.

Fig. S4 shows the emission spectrum of the HAp-poly(MPC-IA) nanorods, recorded under excitation at 396 nm. There are two main peaks located at 591 and 615 nm, which can be attributed to $^5\text{D}_0 \rightarrow ^7\text{F}_1$ and $^5\text{D}_0 \rightarrow ^7\text{F}_2$, respectively. The result suggests the potential application of HAp-poly(MPC-IA) nanorods in biomedical field. For example, the HAp-poly(MPC-IA) nanorods can be used as probes for monitoring or tracking the drug release based on their luminescence. The biological imaging performance of HAp-poly(MPC-IA) nanorods was evaluated based on the cell uptake behavior of HAp-poly(MPC-IA) nanorods. As shown in Fig. 8A, the fluorescent signals could be clearly observed at the cell locations when cells were irradiated with 405 nm. This implied that the luminescent HAp-poly(MPC-IA) nanorods can be internalized by cells through non-specific endocytosis due to the HAp-poly(MPC-IA) nanorods lack of targeting agents. More importantly, the areas of weak fluorescent signals are surrounded by the areas with intensive fluorescent signals, suggesting that the HAp-poly(MPC-IA) nanorods were mainly distributed in the cytoplasm and could not enter into cell nucleus. Fig. 8B shows the bright field optical imaging of cells after they were incubated

with $100\ \mu\text{g mL}^{-1}$ of HAp-poly(MPC-IA) nanorods. It can be seen that cells were adhered to cell plates very well and still kept their normal morphology. This indicated that HAp-poly(MPC-IA) nanorods possess good biocompatibility. **Fig. 8C** showed the overlap image from **Fig. 8A** and **Fig. 8B**. It can be seen that the areas with fluorescent signals are exactly covered on the location of cells. This further confirmed HAp-poly(MPC-IA) nanorods were indeed internalized by cells. The locations of cell nucleus at the center of cells could also be clearly observed from **Fig. 8C**. All of the above results suggested that the fluorescence of HAp-poly(MPC-IA) nanorods is strong enough for biological imaging applications.

Fig. 8 Optical CLSM imaging of HeLa cells incubated with $100\ \mu\text{g mL}^{-1}$ of HAp-poly(MPC-IA) nanorods for 3 h. (A) fluorescent imaging under the excitation with 405 nm laser, (B) bright field and (C) merged image. Scale bar = $20\ \mu\text{m}$.

The biocompatibility of HAp-poly(MPC-IA) nanorods was preliminarily evaluated using CCK-8 assay. The HeLa cells were incubated with different concentrations of HAp-poly(MPC-IA) nanorods for 12 and 24 h. As shown in **Fig. S5**, the CCK-8 assay results demonstrated that the cell viability values of HAp-poly(MPC-IA) nanorods in all the tested concentrations are obviously greater than 95% even the sample concentrations are as high as $300\ \mu\text{g mL}^{-1}$. The high cell viability values give the preliminary conclusion that HAp-poly(MPC-IA) nanorods possess excellent biocompatibility. On the other hand, it is well known that the compositions of HAp are similar to the inorganic components of bone. Therefore, these multifunctional HAp-poly(MPC-IA) nanorods should also be decomposed *in vivo*. In other word, they can be degraded after they were accumulated in living organisms. The therapeutic effects CDDP loaded on HAp-poly(MPC-IA) nanorods were further evaluated through CCK-8 assay. As displayed in **Fig. 9**, the cell viability values were rapidly decreased after HeLa cells were incubated with various concentrations of HAp-poly(MPC-IA)-CDDP complexes for 24 h. It can be seen that about 50% cells were killed when the concentrations of HAp-poly(MPC-IA)-CDDP is $20\ \mu\text{g mL}^{-1}$. When the concentrations of HAp-poly(MPC-IA)-CDDP up to $40\ \mu\text{g mL}^{-1}$. Almost all of the cells were killed. The above results clearly confirmed that HAp-poly(MPC-IA) nanorods are good biocompatibility and can be used for intracellular delivery of CDDP for efficient cancer treatment.

Fig. 9 Cell viability of HeLa cells after they were incubated with different concentrations of HAp-poly(MPC-IA)-CDDP complexes for 24 h.

Conclusion

In conclusion, a simple and efficient method to synthesize hydrophilic and multiple-carboxyl HAp nanorods has been reported. In this experiment, the HAp-poly(MPC-IA) nanorods were prepared by metal free surface initiated ATRP using PTH as organic catalyst. A series of characterization techniques have been employed to demonstrate the successful preparation of HAp-poly(MPC-IA). The final product showed significantly improved dispersibility in aqueous solution. These results suggested that the HAp-poly(MPC-IA) nanorods are potentially useful as a drug carrier. On the other hand, the anticancer drug CDDP can be effectively loaded onto HAp-poly(MPC-IA) nanorods and released from the HAp-poly(MPC-IA)-CDDP complexes in a pH dependent manner. Furthermore, the luminescent HAp-poly(MPC-IA) nanorods showed desirable biocompatibility and can be used for cell imaging. More importantly, the HAp-poly(MPC-IA)-CDDP still kept their therapeutic effects towards HeLa cells. All of the above properties indicated that HAp-poly(MPC-IA)-CDDP are promising multifunctional nanocomposites with great potential for biomedical applications. Finally, As compared with other controlled living polymerization reactions, the metal free surface initiated ATRP displayed more facile and effective. Therefore, the strategy described in this work could be used for the surface modification of other materials and expanded their application in biomedicine fields.

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