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Adsorption and Drug Release Based on β-Cyclodextrin-Grafted Hydroxyapatite Composite

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ABSTRACT: In this study, β-cyclodextrin (β-CD) was covalently grafted on hydroxyapatite (HA) using a coupling agent to improve the drug loading capacity and prolong the drug release. The binding of β-CD on the HA surface was confirmed by Fourier transformation infrared spectroscopy, thermal gravimetric analysis, and X-ray powder diffraction. The adsorption capacity of ofloxacin on β-CD-grafted hydroxyapatite (β-CD-*g*-HA) composite was found to be 30 mg g^{-1} at 37°C and 24 h. The adsorption process is spontaneous, given the negative values of free energy change. Compared with the release of ofloxacin loaded on HA, the release of ofloxacin loaded on β-CD-*g*-HA was slowed down 28% and 21% in pH 2.0 and pH 7.4 buffer media at 2 h, respectively. Biocompatibility of β-CD-*g*-HA was assessed by MTT assay, and the result showed that it had no cytotoxicity. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

KEYWORDS: composites; drug release systems; adsorption

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INTRODUCTION

Hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$), an interesting biomaterial with potential orthopedic, dental, bone regeneration, and maxillofacial applications due to its excellent biocompatibility, bioactivity, and osteoconductivity,[1,2] is native to the human body. HA crystals have been widely used in delivery systems for antibiotics,[3–5] genes,[6] proteins,[7] and various drugs,[8,9] as have been experimentally confirmed by recent reports.[10–12] However, HA crystals have a limited drug loading capacity on hydrophobic drugs, and the release of drug from HA has been proven to be initially very fast, owing to the weak interaction between the drugs and HA particles.[13] Thus, modifications of HA particles become necessary by developing polymer/HA composites.[14] β-Cyclodextrin (β-CD) has a hydrophobic inner cavity and a hydrophilic outer surface[15] exhibiting the ability to form reversible complexes with drugs, and has been used as a promising carrier for antibiotics.[16,17] It is considered as biocompatible material and, in general, it does not elicit an immune response and has low cytotoxicity.[18]

To our best knowledge, β-CD was immobilized in the cavity of HA by mechanical blocking and physical interactions, which could adsorb and release vancomycin hydrochloride and ciprofloxacin hydrochloride, respectively.[19] The study[20] attempted incorporating hydroxypropyl-β-CD polymer into microporous HA via impregnating either in a CD monomers mixture solution or a pre-synthesized CD polymer solution, followed by thermal fixation processing and sustained release of ciprofloxacin. In this study, β-CD-grafted hydroxyapatite (β-CD-*g*-HA) used a chemical synthesis method through adding silane coupling agent (KH-560), which was different from the physical blending. Compared with the functionalized yield (7%) of the earlier articles, the β-CD-*g*-HA gained higher grafted yield, which could load more guest drug molecules. The β-CD-*g*-HA composite was characterized by Fourier transformation infrared (FTIR) spectroscopy, thermogravimetric analysis (TGA), and X-ray powder diffraction (XRD). Adsorption experiments of this composite were measured using ofloxacin as the model drug. The drug release profiles were carried out in buffer solution at 37°C. In addition, the cytotoxicity was assessed by detecting the cell relative proliferation rate of L929 mouse fibroblast in extracted liquid of β-CD-*g*-HA.

MATERIALS AND METHODS

Materials

Hydroxyapatite (HA, Ca/P-ratio: 1.667) was obtained from Emperor Nano Material (Nanjing, China); β-CD was purchased from Medical and Chemical Works (Shanghai, China), which was distilled in Sodium hydroxide solution and precipitated from methanol before use; 2-(2,3-epoxypropoxy) propyltrimethoxysilane (KH-560) was received from multidimensional bridge chemical (Jinan, China); N, N-dimethyl formamide (DMF)

was obtained from Fu Yu Fine Chemical (Tianjin, China), which was dried by anhydrous $MgSO_4$ before use; Ofloxacin from Pharmaceutical Factory (Nanjing, China) was selected as model drug; L-929 cell and DMEM cell cultures were received from the fourth military medical university (Xi'an, China); 3-(4,5)-dimethylthiahiazo(-z-yl)-3,5-di-phenytetrazoliumromide (MTT) was purchased from Hao Ran Biological Technology (Shanghai, China); All chemicals were analytical grade.

Methods

Synthesis of β-CD-g-HA. The β-CD-*g*-HA composite was prepared according to the following procedure. Briefly, the pretreated β-CD sodium salt (8.0 g) was dissolved in anhydrous DMF (50 mL) in three-neck flask (250 mL) where a dispersion of KH-560 (1.5 mL) in dry DMF was added dropwise at 90°C. The mixture was allowed to react and stir for 12 h. Then, HA (7.0 g) was added to the previous solution with a maintained stirring at 90°C for 12 h. The β-CD-*g*-HA mixture was filtered, and washed with methanol and distilled water by the Soxhlet extraction in sequence. Finally, the β-CD-*g*-HA composite was dried at 80°C for 12 h and dried at 45°C under vacuum for 48 h to constant weight. Preparation of β-CD-*g*-HA is illustrated in Scheme 1.

The grafted yield of β-CD-*g*-HA composite was calculated according to the weight increasing of the composite and using the following equation as

$$\beta - CD\%Wt = [(m_i - m_f)/m_f] \times 100 \quad (1)$$

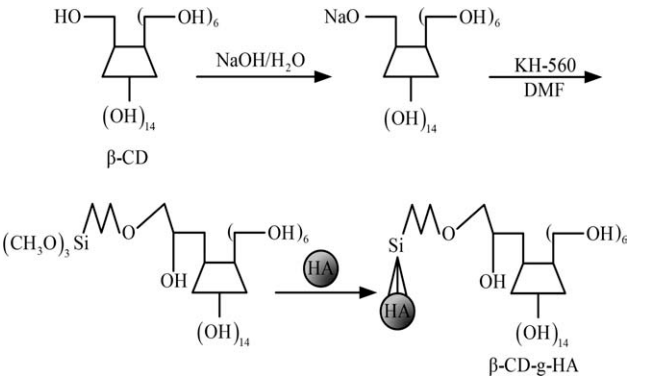
where m_i and m_f were the mass of the β-CD-grafted HA before and after, respectively. The grafted yield was 28% by eq. (1).

Characterizations of β-CD-g-HA. The synthesized β-CD-*g*-HA composite was characterized by (FTIR) spectrometer, TGA, and XRD. The FTIR spectra of the samples as solid were recorded with a FTIR Tensor 27 spectrometer (Bruker, Germany) in the range between 4000 and 400 cm^{-1} using KBr pellets. The thermogravimetric analysis (STA 449C, Netzsch, American) was recorded in the 30–700°C temperature range with a heating rate at 10°C/min under N_2 atmosphere and the amount of the surface-grafted polymer was determined by weight loss. XRD measurement was performed with X-ray powder diffraction (D8 Advance, Bruker, Germany) in the 2θ range from 10° to 60°.

Adsorption Properties of HA and β-CD-g-HA. Adsorption experiments of HA and β-CD-*g*-HA were carried out under the condition of different initial concentrations of ofloxacin and different temperatures. Sorption rate was studied by keeping HA and β-CD-*g*-HA as 0.1000 g and volume of solution as 10.00 mL. The solution was reacted in the condition of shaking at a speed of 100 rpm for 24 h and dealt with centrifugation. The supernatant was diluted and measured by UV–Vis spectrometer. Calibration curves were plotted between absorbance and the concentration of ofloxacin solution. The amount of ofloxacin adsorbed onto the β-CD-*g*-HA and raw HA was calculated by a mass balance relationship.

$$Q = (C_0 - C_t)\mathrm{V/m} \quad (2)$$

where V (mL) is the volume of solution, C_0 and C_t (mg mL^{-1}) are the initial and final solution concentrations of ofloxacin, respectively, and m (g) is the dry mass of raw HA or β-CD-*g*-HA.



Scheme 1. Schematic representing formulation of β-CD-*g*-HA.

***In Vitro* Drug Release in Agitated Vials.** Ofloxacin loaded HA and β-CD-*g*-HA were placed into vials which were filled with 15.00 mL buffer solution pH 2.0 and 7.4, respectively. Then, the vials were shaken in water bath under the speed of 100 rpm and 37°C. At pre-determined sampling time points, the release medium was replaced with fresh buffer media. The amount of ofloxacin released was determined by UV–Vis spectrophotometry at $\lambda = 287$ nm and $\lambda = 292$ nm, respectively. The drug release studies were performed in triplicate for each of the samples.

Cytotoxicity Test of β-CD-g-HA Composite. The cytotoxicity of β-CD-*g*-HA composite was assessed by the MTT assay. L929 fibroblasts were cultured in DMEM medium filling with 10% fetal calf serum at 37°C with 5% CO_2 and 100% relative humidity. Then, 1.0×10^4 cells/well were added in a 96-well plate and treated with the β-CD-*g*-HA for 24 h. 0.5% MTT solution was added to the medium and incubated for 4 h at 37°C. After discarding the culture medium, 0.2 mL of DMSO was added to dissolve the precipitates, and the resulting solution was measured for absorbance at 492 nm. The relative cell growth was calculated by the absorbance value.

RESULTS AND DISCUSSION

Characterizations of β-CD-g-HA

FTIR Analysis of β-CD-g-HA. The FTIR spectra of HA and β-CD-*g*-HA between 4000 and 400 cm^{-1} are given in Figure 1. As shown in Figure 1a, the absorption band at 1639, 1038, 956, and 561 cm^{-1} are assigned to PO_4^{3-} group, the —OH absorption peak at 3439 cm^{-1} was also observed, which are characteristic peaks of HA. Moreover, comparing with HA, Figure 1b showed an addition —CH_2— at 2924 cm^{-1} and some absorption peaks at 710, 750, and 810 cm^{-1} due to introduction of β-CD. These changes suggest that β-CD-*g*-HA composite was successfully synthesized.

Thermogravimetric Analysis of β-CD-g-HA. The grafting ratio determined by TGA method is shown in Figure 2. The amount of β-CD grafted on the surface of HA is calculated by eq. (3), w_1 and w_0 are the weight loss of HA and β-CD-*g*-HA, respectively.

$$\text{Grafting ratio} = w_1(\%) - w_0(\%) \quad (3)$$

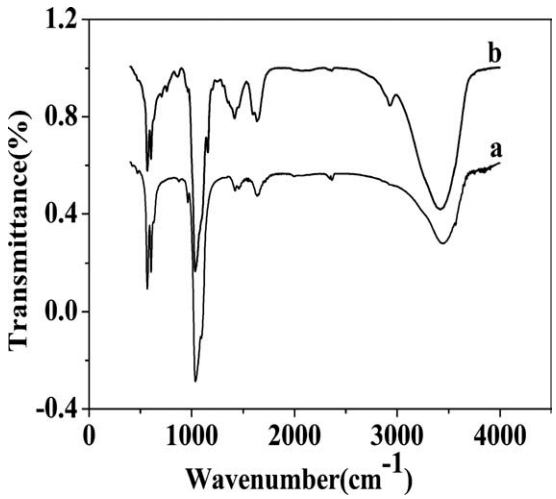


Figure 1. FTIR spectra of (a) HA and (b) *β*-CD-*g*-HA.

The TGA graph showed that the organic polymer on the surface of HA was about 28% by weight loss, which was in accordance with the grafted yield of eq. (1).

X-ray Powder Diffraction Analysis of *β*-CD-g-HA. XRD patterns confirmed successfully grafting on the surface of HA. XRD patterns of the HA and *β*-CD-*g*-HA were depicted in Figure 3. The sharp peaks of HA at 2θ = 25.8°, 31.8°, 46.5°, and 49.5° without other phases were detected in Figure 3b, which are consistent with the crystalline of HA. Figure 3c appeared the same position as HA particles, and this confirmed that the grafting reaction did not cause any crystalline change and secondary phase formed.[21] Furthermore, some addition peaks at 2θ = 10.6°, 12.5°, 14.5°, 17.3°, 19.6°, 20.8°, and 22.9°, which are the characteristic peaks of *β*-CD (Figure 3a). These changes proved that *β*-CD was grafted on the surface of raw HA rather than in the cavity of HA, which was differ from the previous study.[19,20] It is considered as a further proof *β*-CD-*g*-HA composite.

Adsorption Thermodynamic Studies

Adsorption isotherms of ofloxacin on *β*-CD-*g*-HA and HA were shown in Figure 4. The adsorption quantities of ofloxacin on *β*-CD-*g*-HA and HA were obtained as 30 mg g^{-1} and 15 mg g^{-1} for 2.000 mg mL^{-1} initial ofloxacin concentration at 37°C, respectively. Comparable with the result of HA, the quantity of ofloxacin on *β*-CD-*g*-HA increased by 50% for 24 h at 2.000 mg mL^{-1} and 37°C, which was attributed to entrap efficiently within the *β*-CD. It indicated that the adsorption capacity of ofloxacin on *β*-CD-*g*-HA for ofloxacin was higher than that of raw HA.

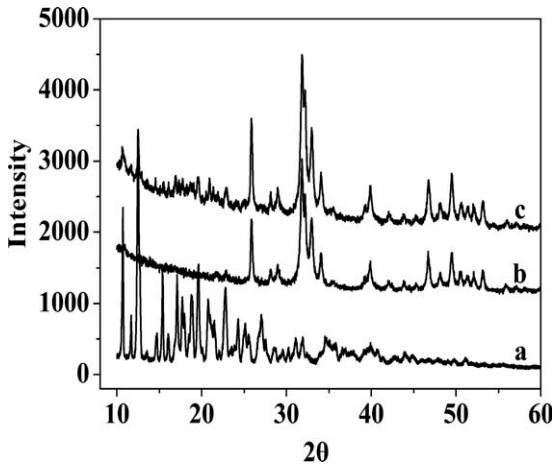


Figure 3. XRD spectra of (a) *β*-CD (b) HA and (c) *β*-CD-*g*-HA.

Adsorption isotherms are used for understanding the mechanism and quantifying the distribution of adsorbate between the liquid phase and solid adsorbent phase at equilibrium during the adsorption process. The Freundlich[22] isotherm model is an empirical equation and is generally represented as follows:

$$\log Q_e = \log K_F + \frac{1}{n}\log C_e \tag{4}$$

where Q_e is the equilibrium adsorption capacity of the *β*-CD-*g*-HA(mg g^{-1}), K_F is the empirical constant of Freundlich isotherm(L mg^{-1}) and C_e is the equilibrium concentration of the ofloxacin solution(mg L^{-1}). The constant n is the empirical parameter related to the intensity of adsorption.

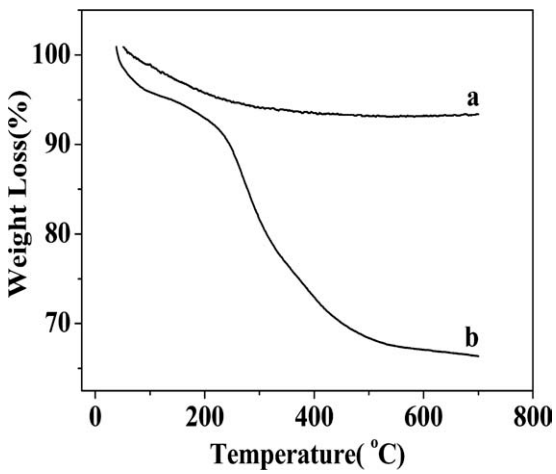


Figure 2. TGA curves of (a) HA and (b) *β*-CD-*g*-HA.

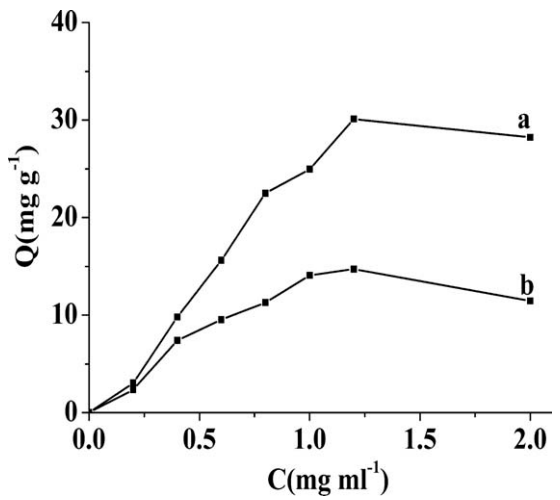


Figure 4. Adsorption isotherms of (a) *β*-CD-*g*-HA and (b) HA at 37°C.

Table I. Freundlich Isotherms of **β**-CD-*g*-HA Composite at Different Temperatures

T (K)	Freundlich equation	1/*n*	K_F (mg g^{-1}) (L mg^{-1})$^{1/n}$	R^2
298	Log Q_e = 0.55 log C_e + 1.1	0.55	12.5	0.9527
313	Log Q_e = 1.30 log C_e + 1.4	1.30	25.1	0.9821
333	Log Q_e = 1.60 log C_e + 1.4	1.60	39.8	0.9547

The adsorption isotherm was listed in Table I. It indicated the applicability of Freundlich isotherm. The estimated thermodynamics parameter value of *β*-CD-*g*-HA was elucidated according to the study.[23] The standard free energy change (ΔG°), standard enthalpy change (ΔH°), and standard entropy change (ΔS°) were calculated and presented in Table II. The thermodynamic treatment of the sorption data indicated that ΔG° values were negative at all temperatures. The negative values of ΔG° confirmed the spontaneous nature of sorption of ofloxacin by *β*-CD-*g*-HA. The negative value of ΔS° indicated that the freedom of ofloxacin is restricted in the *β*-CD-*g*-HA. The adsorption of the surface of HA is exothermic process, and the entrapping of *β*-CD is endothermic process, so the negative estimated value of ΔH° indicated that sorption of HA is mainly dominated in the sorption process. The heat of sorption has become slightly exothermic, which means that the introduction of *β*-CD may induce some changes of the adsorption mechanism to some degree.

Adsorption Kinetics Studies of β-CD-g-HA

The *β*-CD-*g*-HA (0.1000 g) composite was added to 10.00 mL of ofloxacin solution (1.000 mg mL^{-1}), which was treated centrifugation and the supernatant was analyzed by using UV–Vis spectrometer. The adsorption kinetics curve of *β*-CD-*g*-HA was shown in Figure 5, and the result proved that the adsorption capacity became higher with the time increasing, but until 48 h, the adsorption process gradually tended to balance state. Then, in the following 24 h, the amount of adsorption remained unaltered value.

Table II. Thermodynamic Parameters for Sorption on **β**-CD-g-HA Composite at Different Temperatures

	ΔG° (kJ mol^{-1})		ΔH° (kJ mol^{-1})	ΔS° (kJ mol^{-1})
298 K	303 K	333 K		
−3.10	−2.35	−0.637	−21.0	−60.5

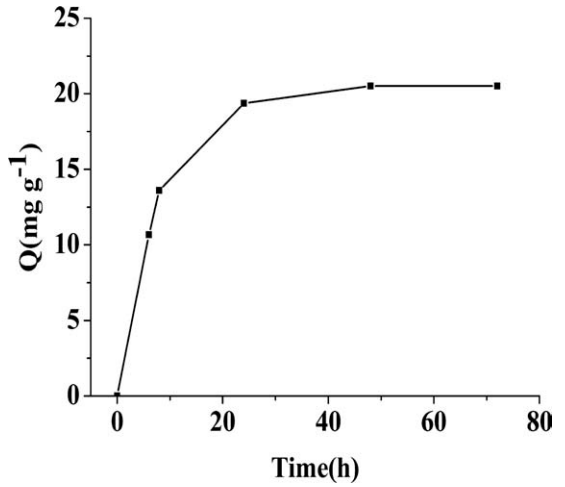


Figure 5. The adsorption kinetics of *β*-CD-*g*-HA.

In Vitro Cumulative Release Studies

In vitro release of ofloxacin was measured using the agitated vial method to mimic *in vivo* conditions. The release profiles at different interval of time were shown in Figure 6, and we can see that the cumulated liberation rate of ofloxacin from *β*-CD-*g*-HA was significantly slower than that of nongrafted HA in buffer solution. Concerning the prolonged drug release effect for ofloxacin (pH 2.0), Figure 6(a) showed that after 1 h, approximated 37% of ofloxacin was released from HA, while

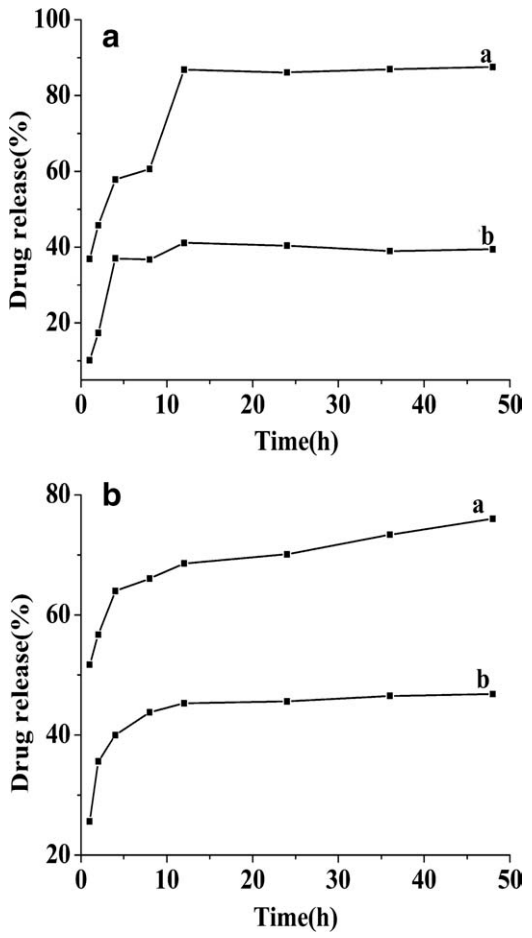


Figure 6. Drug release of HA and *β*-CD-*g*-HA composite at 37°C. (a) pH 2.0 (a: HA; b: *β*-CD-*g*-HA) (b) pH 7.4 (a: HA; b: *β*-CD-*g*-HA).

Table III. The Linear Regression Equation of Ofloxacin Release from Raw HA and β-CD-g-HA in Different pH at 37°C

Materials	Fitted equation	R^2
HA (7.4)	$\text{Log}[M_t/M_\infty] = 0.15 \log t - 0.29$	0.9994
β-CD-g-HA (7.4)	$\text{Log}[M_t/M_\infty] = 0.33 \log t - 0.61$	0.9309
HA (2.0)	$\text{Log}[M_t/M_\infty] = 0.45 \log t - 0.34$	0.9668
β-CD-g-HA (2.0)	$\text{Log}[M_t/M_\infty] = 0.25 \log t - 0.66$	0.8298

the grafted sample had released only 10% of ofloxacin loaded β-CD-g-HA. The cumulative release amount of ofloxacin (pH 7.4) was expressed in Figure 6(b), which showed that the cumulative release rate of ofloxacin loaded HA was about 51% at first 1 h, but the release rate of ofloxacin loaded β-CD-g-HA was 25%. The cumulative release rate of ofloxacin was slowly increased after 12 h, and then a progressive slow down of the release up to 48 h.

For a better understanding of the release mechanism, the linear regression equations of ofloxacin release from raw HA and β-CD-g-HA were showed in Table III. The release rate could be expressed by the Peppas-Korsmeyer equation, which indicated drug release mechanism was controlled mainly by Fick diffusion. As we know, one of the goals in development of drug delivery systems was to reduce the initial burst and achieve a constant release rate. *In vitro* release of ofloxacin indicated that β-CD-g-HA composite showed significant slower drug release and lower initial burst as a great potential carrier in drug delivery system.

Cytotoxicity Evaluation of β-CD-g-HA Composite

For the concerns of drug delivery and tissue engineering applications, the materials should exhibit minimal cytotoxicity. To investigate potential cytotoxicity effect of β-CD-g-HA composite, the viability of L929 cells was tested in the different percent extracted liquid of β-CD-g-HA composite using MTT assay. In the presence of the β-CD-g-HA composite, the growth rate of the L929 cells was not significantly inhibited. This result showed that there was no obvious cytotoxicity of β-CD-g-HA composite against the L929 cells. However, when increasing the concentration of extracted liquid, the number of cell was decreased. The relationship between cell relative proliferation rate of L929 fibroblasts and cytotoxicity grade was showed in Figure 7, the relative growth rates (RGR) of 25, 50, and 100% extracted liquid of β-CD-g-HA composite were 88, 83, and 81%, respectively, which demonstrated that the cytotoxicity was one grade according to the last standard of USP, meaning that the grafting material had no cytotoxicity.

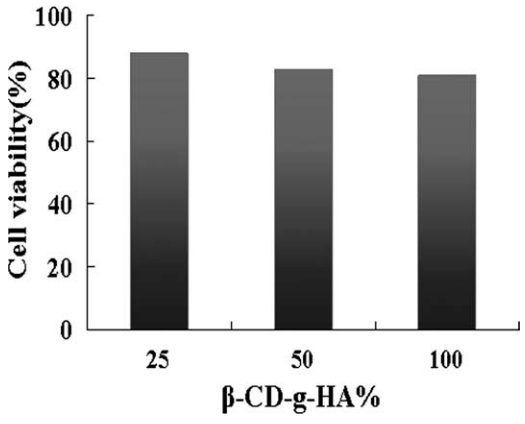


Figure 7. Relationship between cell relative proliferation rate (RGR) and grade of cytotoxicity.

CONCLUSIONS

The β-CD-g-HA composite was an excellent biomaterial combining biodegradability of β-CD with biocompatibility of HA. In this investigation, FTIR spectra and XRD confirmed the grafting reaction between β-CD and HA, and TGA showed the grafted yield of β-CD was 28%. The β-CD-g-HA could further strengthen the drug loading efficiency and form prolonged drug release. The grafting material had no cytotoxicity by MTT assay. This study well indicated that the β-CD-g-HA composite could be as an excellent carrier for antibiotics drug delivery system, so further studies are necessary to evaluate for clinical application and human health care.

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