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Preparation and characterization of caffeic acid grafted chitosan/CPTMS hybrid scaffolds

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ABSTRACT

In this study, preparation and characterization of caffeic acid grafted chitosan (CTS)/(3-chloropropyl)trimethoxysilane (CPTMS) hybrid scaffolds were investigated. The grafting of caffeic acid onto CSC1.0 (CTS/CPTMS hybrid scaffold prepared in weight ratio of 1:1) not only increased its compressive strength but also enhanced its antibacterial, antioxidant and anti-cancer property. The caffeic acid grafted scaffolds exhibited higher anti *Staphylococcus aureus* and scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH) property than CSC1.0 did. The compressive strength of the caffeic acid grafted scaffolds is 0.54∼0.65 MPa, which was higher than that of CTS scaffold (0.14 MPa) and CSC1.0 (0.33 MPa). In culturing human osteosarcoma UMR-106 cells, the caffeic acid grafted scaffolds showed inhibitions on cell growth, alkaline phosphatase activity and cell attachment of UMR-106 cells. Caffeic acid grafting approach could be very promising for chitosan scaffolds to be used in hard-tissue engineering application.

1. Introduction

In recent years, the application of degradable polymer for tissue engineering purposes is growing very fast. Many biodegradable polymers had been chemically or physically modified to meet the requirement for scaffolds (Jagur-Grodzinski, 2006). Among them, much attention has been given to a naturally occurring biopolymer, chitosan (CTS). Chitosan is derived by partial deacetylation of chitin, a second abundant natural polysaccharide from crustacean shells. It is renewable, biocompatible, biodegradable, antibacterial, and non-toxic to human tissue. It can be easily manufactured into desired shapes, and can be molded into porous structures. It also contains amino and hydroxyl groups that can be easily reacted and functionalized (Jayakumar, Prabaharan, Reis, & Mano, 2005; Joshi & Sinha, 2007; Mu & Fang, 2008).

For using as scaffold, the CTS matrix should have a three-dimensional structure with high porosity. The porous morphology can provide more surface area for cell to seed and attach, as well as sufficient space for extracellular matrix regeneration and minimal diffusion constraints during the in vitro culture (Wang, Cai, & Bei, 2003). Despite its successful applications, the mechanical properties of CTS remained a main concern when it is used in porous form (Madihally & Matthew, 1999; Suh & Matthew, 2000; Karageorgiou & Kaplan, 2005). Although increased porosity and pore size facilitate cells ingrowth, but the mechanical properties of CTS scaffolds are weakened. Hence, efforts to improve the mechanical properties of CTS are essential for using as hard-tissue engineering scaffold. Cross-linking on CTS is both convenient and effective way to improve its physical and mechanical properties. The organic–inorganic hybrid materials which combine both the functionality of organic compounds and the stability of inorganic compounds, is another approach to improve the mechanical strength of porous CTS scaffolds. If a cross-linking reaction forms an organic–inorganic hybrid composite, the physico/chemical properties of CTS are improved. To impart strength, many silylating agents such as tetraethoxysilane (Uragami et al., 2004), vinyltriethoxysilane (Airoldi & Monteiro, 2000), 3-(trimethoxysilyl) propylamine (Silva et al., 2005), 3-isocyanatopropyl triethoxysilane (Chen et al., 2007), 3-aminopro-pyltriethoxysilane (Yeh, Chen, & Huang, 2007) and γ-glycidoxypropyl trimethoxysilane (Chao, 2008) have been used as cross-linking agents in the formation of chitosan–silane hybrid materials.

Infection can cause implant failure, prolong times and costs of hospitalisation, and sometimes lead to the patient's death (Campoccia, Montanaro, & Arciola, 2006). The incidence of

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infection typically approximates 1%, but has been reported to be as high as 16% (Engelsman, Van der Mei, Ploeg, & Busscher, 2007). The causative infection includes bacterial contagions, surgical technique, medical history and comorbidities of patient, and properties of the implant (Cordero, Munuera, & Folgueira, 1994; Mankin, Hornicek, & Raskin, 2005; Simison, Noble, & Hardinge, 1986). Sterilisation of implant is difficult as tissue is involved. Generally, there are two primary ways to keep the patient away from infections. One is to make the patient take antibacterial medicine before the operation. The other is to make improvement on the implant materials with an antimicrobial property.

Oxidative stress have been implicated in a wide array of human diseases, including cancer, neurodegenerative diseases, diabetes, inflammatory joint diseases, cardiovascular dysfunctions as well as ageing. Some observations indicated that oxidative stress is able to inhibit normal cell differentiation (Bai et al., 2004; Mietus-Snyder, Gowri, & Pitas, 2000; Nair & Olanow, 2008). Many studies show polyphenols can reduced oxidative stress and possesses anti-tumorigenic ability (Feng et al., 2007; Fujii, Yokozawa, Kim, Tohda, & Nonaka, 2006; Park, Han, Park, & Park, 2005). However, the incorporation of scaffold with polyphenols to improve anti-tumorigenic function had never been mentioned.

Caffeic acid, a natural polyphenols, has antibacterial property (Elegir, Kindl, Sadocco, & Orlandi, 2008) and potential efficacy against oxidative stress (Dastmalchi et al., 2008). It has a vinyl bond which can be grafted with CTS (Zohuriaan-Mehr, 2005). The aim of this study is to enhance the mechanical strength, the antibacterial, antioxidant, and anti-cancer property of CTS membrane scaffolds. A novel porous chitosan/(3-chloropropyl)trimethoxysilane hybrid membrane scaffold is prepared, and its properties are investigated after caffeic acid is grafted.

2. Experimental

2.1. Materials

Chitosan (CTS, molecular weight 400 kDa, deacetylation of 85%), caffeic acid (purity ⩾ 95%) are produced from Fluka. (3-Chloropropyl)trimethoxysilane (CPTMS, purity ⩾ 98%), potassium persulfate (purity ⩾ 99%), calcium chloride (purity ⩾ 97.0%), sodium phosphate (purity ⩾ 99.0%) are obtained from Aldrich. NaCl particles (purity > 99.5%) is from Taiwan salt industrial corporation (Taiwan). All other chemicals are of reagent grade or higher grade and used without further purification.

2.2. Preparation of macroporous CTS/CPTMS and CTS membrane scaffolds

CTS was dissolved in a 5 wt.% acetic aqueous solution to form a CTS solution of 4 wt.%. The solution was stirred at room temperature for 24 h. Then 1 g of CPTMS was added to 25 g of this solution. The mixture was stirred for 1 h, and 25 g sieved NaCl particles (size ranging 150–250 μm) were added, and then homogenized. The homogenized mixture was cast in a 6 cm Teflon dish, and then dried in an oven at 70 °C. The dried membrane was then immersed in a 20 ml, 1 N aqueous NaOH solution for 24 h to neutralize the residual acid. The wet membrane in basic state was dried at 70 °C to complete the nucleophilic aliphatic substitution. Finally, the membrane was immersed in a water bath to dissolve the NaCl particles, and to remove the remaining NaOH. The CTS/CPTMS hybrid membrane formed was then dried in vacuum (15 Pa) and stored in a dehumidifying cabinet for future use.

CTS membrane scaffold was prepared with procedure to produce CTS/CPTMS scaffolds except CPTMS was not added.

2.3. Caffeic acid grafted onto CTS/CPTMS hybrid scaffolds

One gram of dry CTS/CPTMS hybrid membrane was first mixed with 30 ml of water in stopper flask, then the initiator and caffeic acid which dissolved in 30 ml of acetone were added in order. The flask was kept at 40 °C in a thermostat bath, and the reaction mixture was shacked occasionally under nitrogen atmosphere. After 24 h, the product was filtered, washed with acetone to remove the unreacted monomer. Exhaustive extraction of the product with acetone in a soxhlet allowed the separation of polycaffeic acid homopolymer formed during the grafting reaction. The caffeic acid grafted CTS/CPTMS hybrid membrane was thoroughly washed with water, followed by drying in vacuum (15 Pa) to constant weight w_g and stored in a dehumidifying cabinet for future use.

The graft weight% of these samples was calculated using the following equation:

$$\text{Graft weight}\% = \frac{w_g - w_0}{w_0} \times 100$$

where w_0 is the weight of CTS/CPTMS hybrid membrane before grafting reaction.

2.4. Scaffold characterization

Approximately 1 mg of freeze-dried sample was pressed into a pellet with 100 mg of potassium bromide, and FTIR spectra were recorded with a Jasco FT/IR-470 plus Fourier Transform Infrared Spectrometer (FTIR). The compressive strength of the scaffolds was measured by a universal testing machine (AI-7000S, Gotech, Taiwan) at a crosshead speed of 0.5 mm/min using a screw driven load frame equipped with a 500 N load cell.

2.5. In vitro enzymatic degradation

First the absolute-dry weight (w_0) of the scaffolds were measured, and the samples were placed into 37 °C PBS buffer solution (0.1 M at a pH of 7.3), simultaneously add in 5 μg/ml of Lysozyme. Then, the samples were placed in an Orbital Shakers with temperature set at 37 °C, to do the time course weight-change experiment of enzymatic degradation. After the samples were washed with distilled water and vacuum-dried, their weight (w_t) were measured. The weight loss ratio of these samples was calculated using the following equation:

$$\text{Weight loss}(\%) = \frac{w_0 - w_t}{w_0} \times 100$$

2.6. Antibacterial test

Testing of antibacterial activity of the scaffolds was performed according to the method described by Grzybowski, Antos, and Trafny (1996). The samples used for the antibacterial assay were sterilized at 121 °C by an autoclave for 30 min. In the antibacterial assay, 16-mm-diameter test samples cut from the sterilized samples were placed on the bottoms of the wells in a 24-well plate (the diameter of each well is about 16 mm). Subsequently, 50 μl of bacterial broth culture was seeded onto the scaffolds (10^6 CFU/ml), respectively. The bacterial broth cultured in the well without containing any scaffold was used as control. The bacteria used for the test were *Staphylococcus aureus*. Subsequently, the scaffolds were put in the moisture incubator and incubated at 37 °C. After 6 h of incubation, each scaffold was placed into test tubes containing 1 ml PBS and sonicated for 75 s in an ultrasonic washer (64 kHz). Subsequently, 50 μl of the incubated medium taken out from each tube was seeded on agar plates containing

nutrient broth and incubated at 37 °C for 18 h. Finally, the units of colony formation in each agar plate were calculated to examine the antibacterial ability of each scaffold.

$$\text{Antimicrobial activity}(\%) = \frac{CFU_b - CFU_s}{CFU_b} \times 100$$

where CFU_b and CFU_s represent a colony-forming unit (CFU) of the blank and sample, respectively.

2.7. Antioxidant activity measurement

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging ability was assayed to assess the antioxidant activity of the scaffolds. A modified procedure according to the method proposed by Jung, Chung, and Lee (2006) was used. DPPH was dissolved in a mixing solution composed of deionized water (100 mL) and ethanol (100 mL). The scaffold ($1.5 \times 1.5\ cm^2$) was immersed in DPPH (8 mL). The reaction mixtures were continuously shaken in the dark, until the absorbance of the solution was measured. The absorbance of the resulting solutions was analyzed at wavelength of 515 nm with a UV/visible spectrometer (Hitachi U-2001, Japan) against blank in which DPPH was absent. The percentage of inhibition was calculated as follows:

$$\text{Inhibition ratio}(\%) = \frac{(A_b - A_s)}{A_b} \times 100$$

where A_b and A_s are the absorbance of blank without the scaffold and the measured absorbance with the scaffold at 90 min, respectively.

2.8. Biological assessment

2.8.1. Culture of cells with scaffolds

The scaffold was first placed in the dishes, and two or three spots of silicone gel were then applied around the scaffold. A coverslip was placed on the top of the scaffold and adjacent silicone gel spots. Silicone gel was used to glue together the coverslip and the culture dish. Cells used in this study were human osteosarcoma UMR-106. The cells were cultured in minimal essential medium (αMEM), supplemented with 10% FBS and 100 U/ml penicillin–streptomycin–amphotercin, at 37 °C in 5% CO_2. UMR-106 cells suspended in the culture medium (5×10^4 cells/ml) were then added to the dishes to allow the ingrowth of cells onto the scaffolds which were 5 mm in length, width and height. The culture medium was replaced with serum-free αMEM medium containing 10 mM sodium β-glycerophosphate, 50 μg/ml ascorbic acid, and 10^{-7} M dexamethasone. The medium was changed every 2 days. After incubation for 7 days, cells on the scaffolds were harvested for cell attachment, cell viability, and alkaline phosphatase (ALP) activity analysis.

2.8.2. Cell attachment analysis

The morphology of the cells attached and grown on each sample with culturing 7 days was observed by SEM. For SEM analysis, scaffolds with UMR-106 cells were fixed with a 2 wt.% glutaraldehyde solution and dehydrated in ethanol aqueous solutions. Drying with supercritical CO_2 was performed to prevent deformation of the cells attached to the scaffolds. The resultant samples were gold sputtered in a vacuum and were then viewed with a Jeol 5600 SEM.

2.8.3. Cell viability

Reduction of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrasodium bromide (MTT) to a purple formazan reaction product by living cells, was used to estimate cell viability. Control cultures and the seeded specimens were incubated with 0.5 mg/ml of MTT during the last 5 h of the culture period tested; the medium was then decanted, formazan salts were dissolved with dimethylsulphoxide and the absorbance of the resulting solution was measured using a spectrophotometer at a wavelength of 570 nm.

2.8.4. Alkaline phosphatase (ALP) activity

The ALP activity was measured colorimetrically using *p*-nitrophenyl phosphate as the substrate. The enzyme ALP expressed by the cells hydrolyzes the substrate to *p*-nitrophenol and an inorganic phosphate. Under alkaline conditions, the *p*-nitrophenol was converted to a yellow product, and its absorbance was subsequently measured at 405 nm using a spectrophotometer. The absorbance was directly converted to ALP activity.

3. Results and discussion

3.1. CTS/CPTMS hybrid scaffolds preparation

CTS was cross-linked with CPTMS. The hydration of the trimethoxy groups on the CPTMS formed silantriol pendent through an acid catalyzed reaction, then in basic state completed the nucleophilic aliphatic substitution between the methyl chloride of CPTMS and the amino group of CTS. Using this sol–gel route, a macroporous CTS/CPTMS hybrid scaffold without shrinkage was obtained.

When more than 25 g of NaCl particles (for 1 g of dry chitosan) was used, cracks appeared on CTS/CPTMS hybrid membrane. Therefore 25 g of NaCl particles in 1 g of CTS was used to prepare CTS/CPTMS scaffolds. As weight ratio of CPTMS/CTS was greater than 1:1, the hybrid membrane disintegrated, so the added CPTMS was 0.2, 0.6, and 1.0 g in 1 g of CTS (abbreviated as CSC0.2, CSC0.6, and CSC1.0, respectively) to prepare the CTS/CPTMS hybrid scaffolds.

3.2. Caffeic acid grafted onto CSC1.0 scaffolds

CSC1.0, has the highest compressive strength among CTS/CPTMS hybrid scaffolds (discussed later), was employed as the trunk matrix for grafting of caffeic acid. Its characteristics are: mean pore size 125.3 ± 6.2 μm, porosity 78.5 ± 3.4%, diameter 4.95 ± 0.08 cm and thickness 5.06 ± 0.04 mm.

Hsu, Don, and Chiu (2002) indicated a free radical degradation of chitosan by potassium persulfate. The free radical results in the breakage of the glycosidic C–O–C bond in the CTS main chain. Mochalova et al. (2006) found that as concentration of ammonium persulfate was higher than 10^{-2} M, the rate of chitosan macrochain degradation is high; on the contrary, as the concentration of ammonium persulfate is around 10^{-4} M, the chitosan macrochain degrades insubstantially. Therefore, to avoid serious chain breakage of CSC1.0 by free radical, low concentration of potassium persulfate was used in this study. Potassium persulfate aqueous solution (2.5, 5.0, 7.5, and 10 mM) was used to initiate the graft polymerization of 0.5 g caffeic acid (products were abbreviated as KPS2.5, KPS5.0, KPS7.5, and KPS10, respectively). And 0.75, 1.0, and 1.25 g of caffeic acid was initiated by 2.5 mM potassium persulfate (products were abbreviated as CA0.75, CA1.0, and CA1.25, respectively), at 40 °C for 24 h. As shown in Table 1, the caffeic acid graft weight% increased with the increasing dosage of potassium persulfate and caffeic acid.

3.3. Scaffold characterization

The FTIR spectra of the selected scaffolds, using the potassium bromide (KBr) disk sandwiched technique (1 mg sample in 100 mg KBr), are shown in Fig. 1. The absorption bands in FTIR spectra at 1655, 1550 and 1310 cm^{-1} were characteristic of the *N*-acetylglucose amine residues. The absorption band of the

Table 1
The caffeic acid graft weight% for various scaffolds.[a]

Reagent used							
KP[b] (mM)/CFA[b] (g)	2.5/0.5	5.0/0.5	7.5/0.5	10.0/0.5	2.5/0.75	2.5/1.0	2.5/1.25
Product name	KPS2.5	KPS5.0	KPS7.5	KPS10	CA0.75	CA1.0	CA1.25
CFA graft weight%	3.8	7.2	10.9	16.0	5.7	7.1	8.8

[a] One gram of CSC1.0 was used to prepare caffeic acid grafted scaffolds.
[b] KP, potassium persulfate; CFA, caffeic acid.

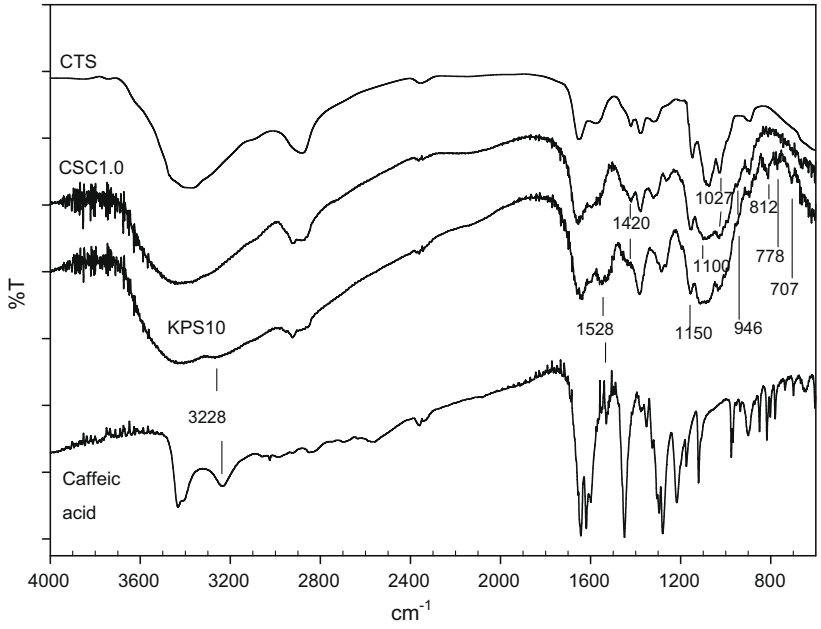

Fig. 1. FTIR spectra of CTS, CSC1.0, KPS10, and caffeic acid.

CSC1.0 decreased in intensity at 1420 cm^{-1}, and the absorption band of KPS10 decreased even further. The decrease of the peak at 1420 cm^{-1} of CSC1.0 could have been indicative of a loss of NH_3^+ groups, and that revealed the expense of amine groups in chitosan when CTS cross-linked by CPTMS and caffeic acid grafted onto CSC1.0. The absorption bands of CSC1.0 and KPS10 at 946 cm^{-1} was the Si–O–H stretching. The band broaden around 1027–1100 cm^{-1} and showed intensity increase was owing to the absorption from the Si–O–C bonds. The intensity increase at 1150 cm^{-1} can be attributed to the stretching vibration of Si–O–Si groups. At 707, 778, and 812 cm^{-1} bands of KPS10 showed multi-substituted benzene, 1503 cm^{-1} showed C=C benzene ring stretch, and 3228 cm^{-1} showed O–H stretching vibration of phenol, respectively. Thus the FTIR results provided evidence that a CTS–silane matrix had formed in CSC1.0, and caffeic acid had been grafted in KPS10.

Desirable hard tissue scaffolds should have mechanical strength strong enough to maintain a fixed shape. Compression test is widely accepted to evaluate the mechanical strength of scaffolds (Jiao, Liu, & Zhou, 2007). The compressive strength of the test scaffolds is shown in Fig. 2. The compressive strength of CSC1.0 is about 2.3 times as that of CTS scaffold. After caffeic acid grafting, the compressive strength of CSC1.0 was increased further, however, the compressive strength was decreased with the increasing dosage of potassium persulfate. Muzzarelli and Ilari (1994) found the grafting of benzene ring moiety onto CTS film could improve CTS film with mechanical strength. Caffeic acid grafting, gives benzene ring pendant in CSC1.0, imparts the mechanical strength of pore wall, so increased compressive strength in KPS10 and CA1.25. Though there were more benzene ring pendants in KPS10 than in CA1.25, the high level of potassium persulfate decreased compressive strength in KPS10 (because of chain breakage), so CA1.25 exhibited the highest compressive strength.

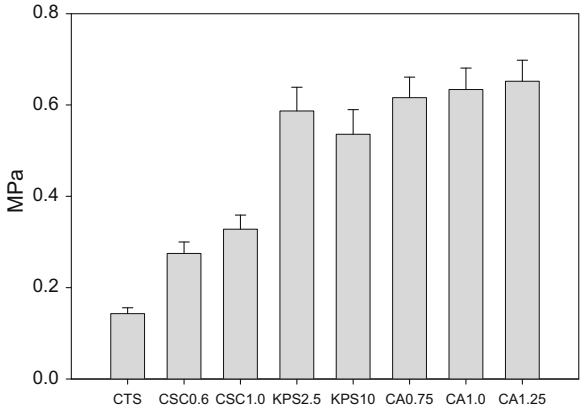

Fig. 2. The compressive strength of the various scaffolds measured at a crosshead speed of 0.5 mm/min using a screw driven load frame equipped with a 500 N load cell.

3.4. In vitro biodegradation

The ultimate goal for scaffolds is to let it disintegrate naturally along the cells growth. To evaluate the degradation kinetics of the formed scaffolds, samples were incubated in lysozyme containing PBS. Fig. 3 shows the enzymatic degradation experiments for

various scaffolds. Scaffolds incubated in lysozyme showed weight reduction in 14 days. CTS and CSC1.0 present fast degradation rate, nearly in 40° angle, as compare to the slow degradation rate nearly in 30° angle of others. After caffeic acid grafting, the scaffolds increased in hydrophobic property, and decreased in degradation rate by lysozyme (a hydrophilic enzyme), but still kept in an acceptable degradation rate. The weight loss of CSC1.0 is less than that of CTS, due to the cross-linked structure in CSC1.0. In first 3 days the weight loss in KPS10 was faster than others. As afore indicated, KPS10 had sustained more free radical degradation by potassium persulfate than others, so it had a faster initial degradation rate by lysozyme.

3.5. Antioxidant activity

The model of scavenging stable DPPH free radicals can be used to evaluate the antioxidant activities in a relatively short time compared to other methods (Prior, Wu, & Schaich, 2005). In this study, the antioxidant activity of the scaffolds was determined by this method. As shown in Fig. 4, the scavenging activity order of the test scaffolds was CA1.25 > KPS2.5 > KPS5.0 > KPS10 > CSC1.0 ≈ CTS. CTS and CSC1.0 exhibited almost no antioxidant activity. The effect of caffeic acid on DPPH radical scavenging was thought to be due to their hydrogen donating ability (Leopoldini, Marino, Russo, & Toscano, 2004). Thus, the scavenging activity should increase with the caffeic acid graft weight%. However, sulfate radical anion capture hydrogen of caffeic acid and reduced its antioxidant activities (Swaraga, Charitha, & Adinarayana, 2005). High level of potassium sulfate was used to prepare KPS10, thus it reduced the antioxidant activities of the grafted caffeic acid. In preparation of CA1.25, low level of potassium persulfate and high level of caffeic acid was used, so it could keep higher antioxidant activities than KPS10.

3.6. Antibacterial test

When using CTS derivatives as scaffolds, it is important to prevent incidence of infection. In this study Gram-positive organism *S. aureus*, a human pathogen of significant importance, was used as the test bacteria to examine the antibacterial property of the scaffolds. The antibacterial activity (%) of the test scaffolds are shown in Fig. 5. It was noted that the antibacterial activity (%) of CTS was decreased after cross-linked by CPTMS. There were reported that the cationic amino group of CTS can associate with anions on the bacteria wall to suppress its biosynthesis and disrupt the mass transport across the wall, and hence cause the death of the bacteria (Jung, Kim, Choi, Lee, & Kim, 1999; Li, Wang, Chen, Huangfu, & Xie, 2008). CSC1.0 has less amino groups such that had lower antibacterial activity (%) than CTS. The antibacterial activity (%) was enhanced after caffeic acid grafting, and had value of antibacterial activity (%) higher than 96% in KPS2.5 and CA1.25. According to the hypothesis postulated by Ultee, Bennik, and Moezelaar (2002), the hydroxyl group of caffeic acid, act as a proton exchanger, can reduce the pH gradient across the cytoplasmic membrane. The resulting collapse of the proton motive force and depletion of the ATP pool eventually lead to the death of *S. aureus*. Although

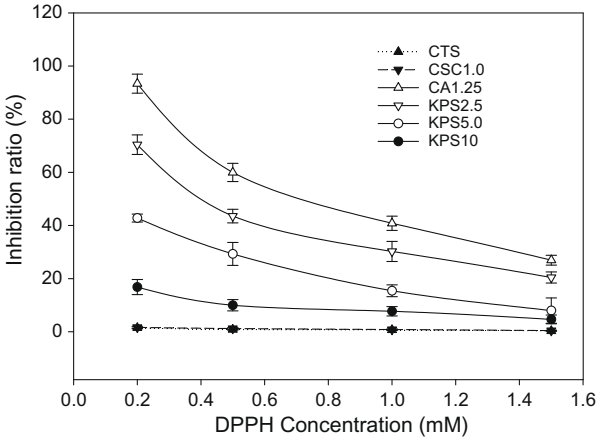

Fig. 4. Antioxidant activity of various scaffolds in different DPPH concentration.

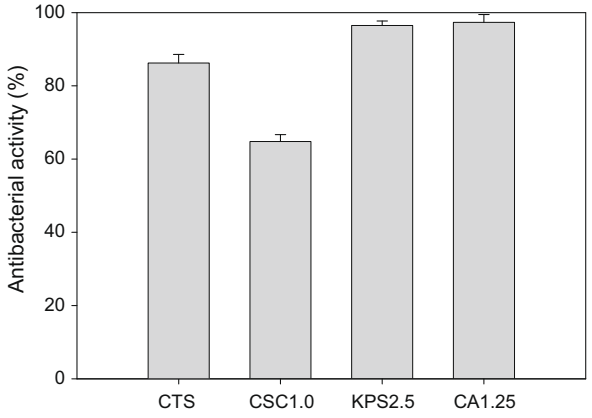

Fig. 5. The results of antibacterial test for CTS, CSC1.0, KPS2.5, and CA1.25.

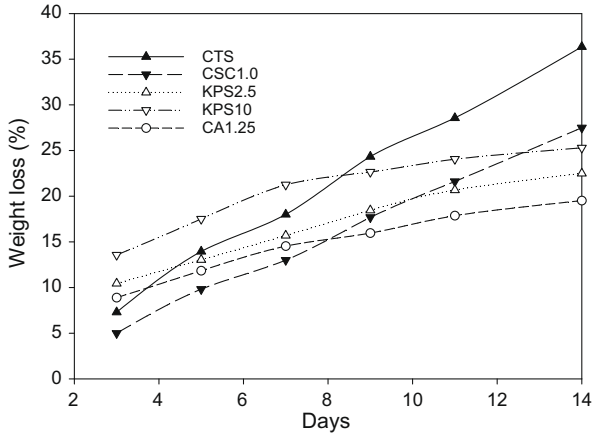

Fig. 3. Enzymatic degradation profiles of CTS, CSC1.0, KPS2.5, KPS10, and CA1.25 in 0.1 M PBS at pH 7.4, 37 °C by lysozyme.

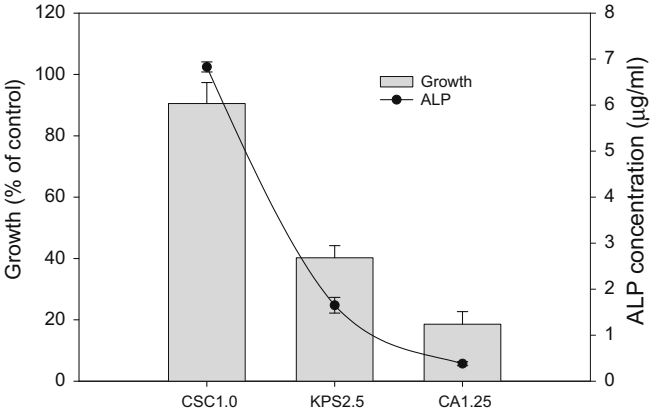

Fig. 6. Effects of scaffolds on cell growth and ALP activity in UMR-106 cells.

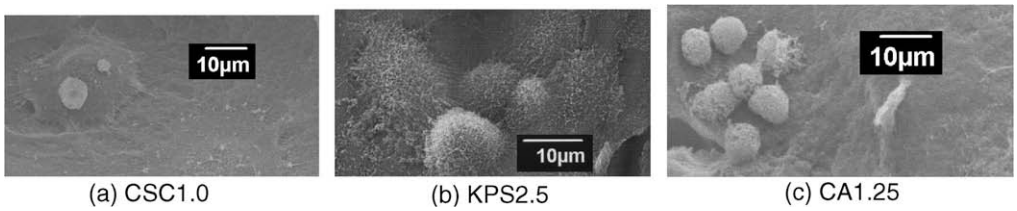

Fig. 7. Cell morphology of UMR-106 cultured on CSC1.0, KPS2.5, and CA1.25. Photographs obtained by SEM, 7 days after cell seeding.

some hydroxyl groups of the grafted caffeic acid might lose proton due to attack by sulfate radical anion, still, the remaining active hydroxyl groups in scaffolds were strong enough to defeat *S. aureus* under the environment of this study.

3.7. Biological assessment

MTT assay was used to estimate cell growth and ALP activity assay was employed to measure cell differentiation. CA1.25 (had highest antioxidant activity among the scaffolds) and KPS2.5 was selected as model scaffold to test the effect of the grafted caffeic acid on culturing human osteosarcoma UMR-106 cells. As shown in Fig. 6, KPS2.5 and CA1.25, both exhibited significant inhibitions of the UMR-106 cell growth and ALP activity. CA1.25 had higher antioxidant activity, and showed better inhibition activity than KPS2.5.

The SEM images of Fig. 7 shows the growth of UMR-106 cells on the scaffolds. The cells cultured on CSC1.0 were polygonal shaped and presented a completely spread morphology. They migrated into the pores and extended to contact each other could already be observed. On KPS2.5 scaffold, cells spreading were lower, but the fibrils of the cells can clearly be observed. That indicated the cells could still attach to KPS2.5. The cells of CA1.25 displayed a spherical morphology and rough dorsal surface, being attached to the pores walls through short filopodia. The SEM morphology indicated a caffeic acid graft weight%-dependent detachment of UMR-106 cells, and was in agreement with the differential cellular responses on scaffolds. It is conceived that the Si–OH and –Si–O–Si– groups provide a good osteoblastic cells attachment (Shirosaki et al., 2005). So the incorporation of CPTMS in CSC1.0 favored cells attachment. The cells attachment might be promoted by the presence of positively charged groups, such as amino groups on chitosan chains (Liu, Jiao, Zhang, & Zhou, 2007). After caffeic acid grafting, not only the amino groups residue of the scaffolds decreased, but also the inhibition ability on UMR-106 cells increased, resulted in the potency for cells attachment was in the order of CSC1.0 > KPS2.5 > CA1.25.

4. Conclusions

Biodegradable hybrid polymer, KPS2.5 and CA1.25, obtained by grafting caffeic acid onto CSC1.0 have higher antioxidant, antibacterial, and mechanical property than CSC1.0 does. Moreover, the two scaffolds showed caffeic acid graft weight%-dependent inhibitions on cell growth, alkaline phosphatase activity and cell attachment of human osteosarcoma UMR-106 cells. This meant the caffeic acid grafting approach provides a novel chitosan scaffold with anti-cancer property and enhanced antibacterial, antioxidant properties. The improved mechanical property made the scaffold more suitable to be using in hard-tissue engineering.

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