Preparation of Simvastatin Loaded TiO_2 nanotube arrays and its release behavior

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Abstract. TiO_2 nanotube array fabricated by anodization has large specific surface area and good biocompatibility. The simvastatin (SIM), which can promote bone formation, were filled into the TiO_2 nanotube by using step concentration soaking method(SCS).The morphology of TiO_2 nanotube arrays were observed by scanning electronic microscope, and the drug release in phosphate buffer solution (PBS) were determined by visible ultraviolet spectrophotometer. The results show that the simvatatin has been loaded into the TiO_2 nanotube arrays, and could sustain release 15 days.

Introduction

Titanium and titanium alloy are widely applied materials used to replace the bone with lesion due to its excellent mechanical properties, high corrosion resistance and good biocompatibility and so on[1]. However, its bio-inert also made the implant failure easily. Therefore, using anodization to preparation a TiO_2 nanotube layer in the surface of the pure titanium can promote the biocompatibility owing to the large specific surface.

Statins is hydroxymethylglutaryl-coenzyme A reductase inhibitors , which have been widely used for hyperlipidemic patients[2]. Recently, some research shows that simvastatin (SIM), a kind of liposoluble statin, could enhance the expression of bone morphogenetic protein (BMP)-2 mRNA lead to promoted bone formation[3], could encourage the differentiation of osteoblast[4].

Though some animal tests show that systemic administration could facilitate osteogenesis process[5]. However, systemic administration required much higher doses, which is considerable harm to healthy, because of the most of the simvastatin are metabolized in the liver, that would cause a shortage concentration for topical bone to stimulate bone formation[6]. For this reason, topical administration might be a superior way to make local bone for enough drug, and the tubular structure of TiO_2 nanotube array made drug filling possible.. In this paper, I will use four motheds putting simvastatin into the TiO_2 nanotube and selectting optimal measure to load simvastatin by estimating the release of the drug.

Materials and Method

Prepare TiO_2 nanotube array. Titanium foils with an work area of 1cm×1cm, which were polished and sonicated in acetone for 20 minutes, chemical corroded for 15s seconds in 5M HNO_3+4%HF, sonicated in deionized water for 20 minutes and dried in air. The electrolyte consist of 0.50wt% NH_4F+10vol% H_2O in glycerol. Anodization was proceed at a constant voltage of 60V for 24 hours, then took out and dried in air after reaction.

Drug loading and releasing. Titanium foils with an work area of 1cm×1cm, which were polished and sonicated in acetone for 20 minutes, chemical corroded for 15s seconds in 5M HNO_3+4%HF, sonicated in deionized water for 20 minutes and dried in air. The electrolyte consist of 0.50wt% NH_4F+10vol% H_2O in glycerol. Anodization was proceed at a constant voltage of 60V for 24 hours, then took out and dried in air after reaction.

One concentration soaking (OCS). Putting the TiO_2 nanotube array in the bottles which respectively contained 30mg/ml, 60mg/ml, and 100mg/ml of simvastatin solution for 24 hours.

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Step concentration soaking (SCS). TiO_2 nanotube array were soaked in the 30mg/ml simvastatin solution for 8 hours, then take out quickly and put it into 60mg/ml simvastatin solution for 8 hours, following take out and put it into simvastatin solution for 8 hours too. So the TiO_2 nanotube array soaked in the three simvastatin solution for 24 hours in total.

Once the sample were prepared, then putting them in 10ml of PBS at 37^{o}C for 15 days. Taking about 0.5ml PBS for specific intervals time, as well as replace with 0.5ml of fresh PBS to make sure the volume dose is 10ml. Every method were made about four groups, and then averaging over the dates of the four groups.

Characterization. The morphology of TiO_2 nanotube array were observed by Field emission scanning electronic microscope (FESEM, JSM-7500F). The absorbance of the drug release were measured by visible ultraviolet spectrophotometer (UV, TU1900) at 238nm. The composition of the drug in the TiO_2 nanotube were determined by Fourier transform infrared (FTIR).

Results and discussion

The morphology of the samples. Fig.1(a,b) show the SEM images of TiO_2 nanotube arrays fabricated at 60V for 24h at 30^{o}C in a 10 vol% H_2O glycerol electrolytes. The length of TiO_2 nanotube arrays is about 6.7um , and the extent of inner diameter is about 220nm, and the thickness of tube wall is about 44nm. Fig.1(c,d) show that the tube wall became thicker after drug loaded, and didn't made a coating , which were loaded SIM by using SCS method. As well as , the advantage of soak method, is to preserve the topography not to be destroyed.

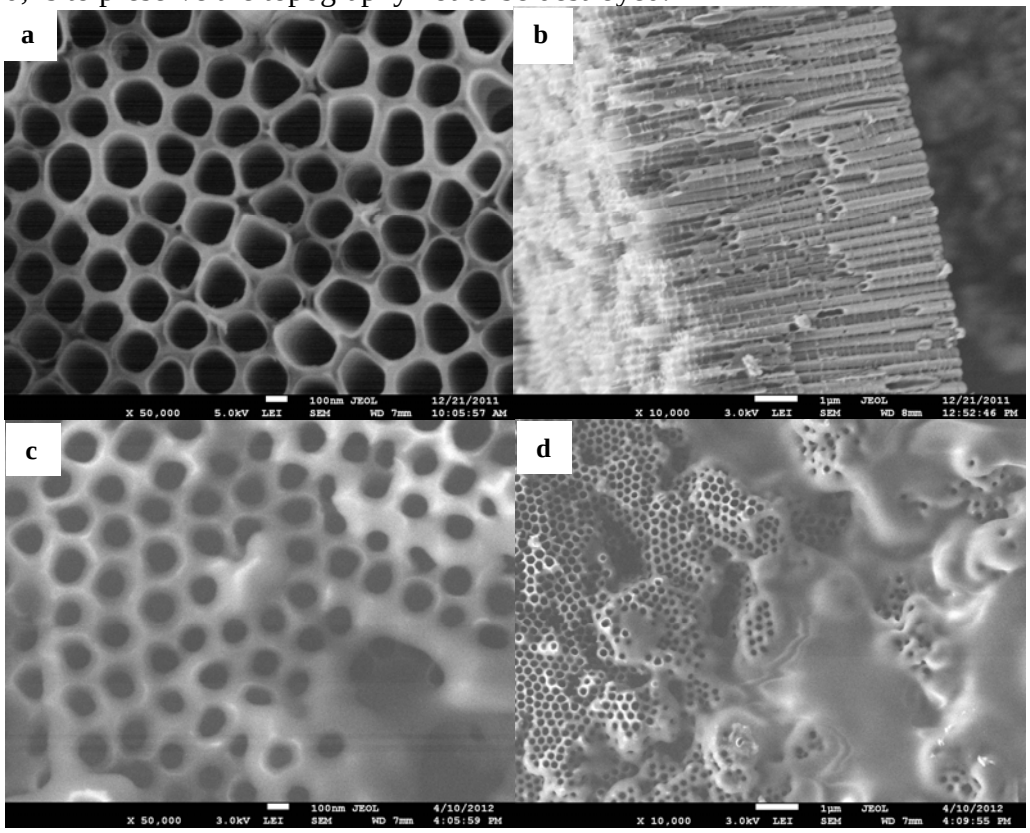

Fig.1 The morphologies of nanotube arrays before loading drug: (a).top-view (b).cross-section, and after loaded SIM by SCS method:(c) high magnification, (d) low magnification.

FTIR of the samples. Fig 2. shows the FTIR spectrum of the TiO_2 nanotube arrays after loading simvastatin. Compare with three curve ,from the Fig.2(b) spectrum we can see that about 2964 cm^{-1}, 2927 cm^{-1} and 2875 cm^{-1} of wave-number is the flex vibration absorption peaks of C-H for methyl or methylene group in simvastatin, about 1718 cm^{-1} of wave-number is the flex vibration absorption peaks of C=O in simvastatim, about 1464 cm^{-1} and 1386 cm^{-1} of wave-number is the angle-change vibration absorption peaks of C-C for methyl or methylene group in simvastatin, and about 1254 cm^{-1},

1158 cm^{-1} and 1053cm^{-1} of wave-number is the vibration absorption peaks of ester group in simvastatin. Therefore, the simvatatin has been loaded into the TiO_2 nanotube arrays.

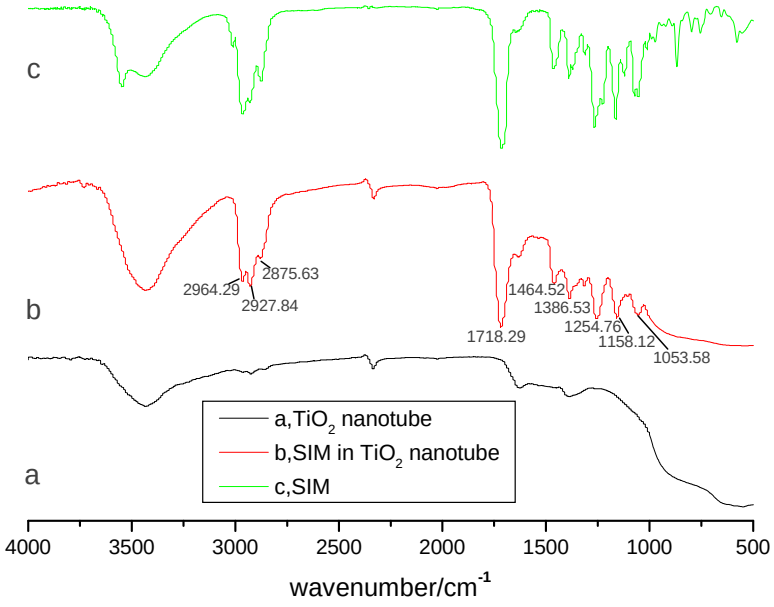

Fig.2 FTIR spectra of the sample:(a). TiO_2 nanotube arrays;(b).the TiO_2 nanotube loading simvastatin;(c).pure simvastatin.

The releases behavior of the samples. Fig.3 shows the drug release both OCS method and SCS method. Curve (a) shows TiO_2 nanotube arrays soaked in 30mg/ml SIM for 24 hours. Curve (b) shows the arrays soaked in 60mg/ml SIM for 24 hours. Curve (c) shows the arrays soaked in 100mg/ml SIM for 24 hours. Curve (d) shows the arrays loaded SIM by SCS method. Compare with fours curve indicate that the SCS method present can load more drug than OCS method.

The reason is probably that TiO_2 nanotube arrays is hydrophilic, but simvastatin is hydrophobic, this characteristic made the drug into the TiO_2 nanotube arrays difficultly. In addition, the higher concentration of simvastatin, the stickier of solution, this is another reason to made loading hardly. Taken measure by step concentration soaking (SCS) method, the TiO_2 nanotube arrays soaked from the low concentration to high concentration of the simvastatin, when the simvastatin concentration is low, nicer hydrophilism of ethanol carry the simvastatin to the internal of TiO_2 nanotube easily, after a time, taken arrays out and soaked in high concentration, this might cause the number of the simvastatin in the outside mouth of TiO_2 nanotube is much more than the internal of TiO_2 nanotube, for this reason, simvastatin molecules will move from the high concentration area to low concentration area. Therefore, we will achieve the goals to filling the simvastatin into the TiO_2 nanotube arrays. Furthermore, from dates above, the order of magnitude for the drug release still remain at 10^{-6}mg/ml, this accord with the reports that a relatively low concentration of simvastatin (10^{-7}mol/L, 10^{-6}mol/L) might be the optimal concentration for promoting bone formation[6]. Moreover, from the dates of drug release, we can see that simvastatin continued to release after 15 days, which approach a purpose to make simvastatin working sustained.

Conclusion

The simvatatin was loaded into the TiO_2 nanotube arrays by step concentration soaking method. The release of simvatatin loaded TiO_2 nanotube arrays in PBS solution could sustain release 15 days.

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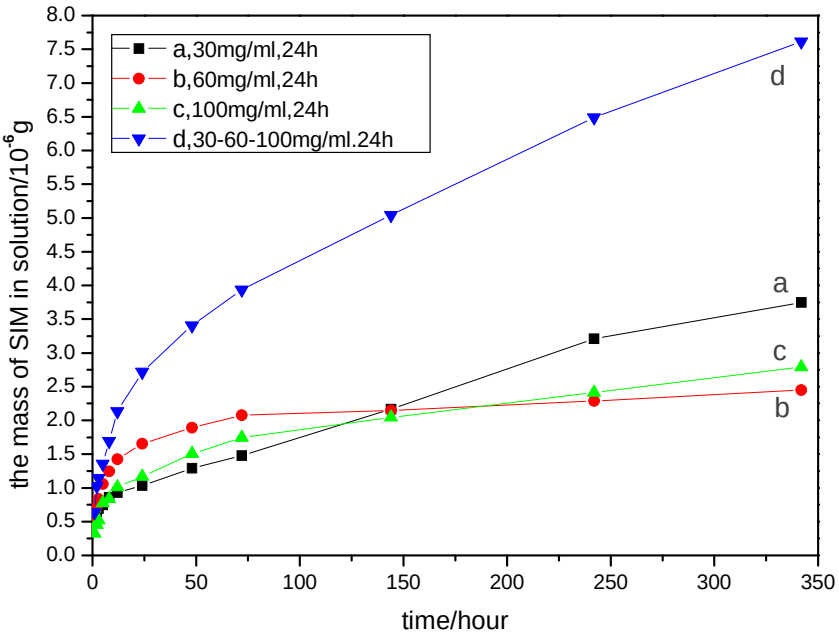

Fig.3 The release curves of the samples:(a) soaked in 30mg/ml SIM for 24h by OCS ; (b) soaked in 60mg/ml SIM for 24h by OCS;(c) soaked in 100mg/ml SIM for 24h by OCS;(d) soaked in 30+60+100mg/ml SIM for 24h in total by SCS method.

Acknowledgements

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