ISSN : 2249 - 8877 Volume 2 Issue 2

Trade Science Inc.

Research & Reviews On Polymer

Full Paper

RRPL, 2(2), 2011 [100-105]

In-vitro release test of hydroxyapatite/collagen/polylactide reservoir controlled-release drug carrier

Zhenlin Wang[1]*, Yuhua Yan[2], Tao Wan[2]

[1]School of Materials and Engineering, Chongqing University of Technology, Chongqing, (CHINA)

[2]Research Centre of Biomaterials and Engineering, Wuhan University of Technology, Wuhan, (CHINA)

E-mail: wzl@cqut.edu.cn

Received: 21st June, 2011 ; Accepted: 21st July, 2011

ABSTRACT

In order to explore the characteristics of release rate of Hydroxyapatite/collagen/polylactide (HAp/Col/PLLA) reservoir controlled-release drug carrier, biomimetic bone-like hadroxyapatite/collagen (HAp/Col) composite was prepared by co-precipitation, 3-D porous reservoir control-released drug carrier was further synthesized with polylactide by thermal-induced phase separation plus non-solvent extraction pore-leaching technique. In vitro control release test was performed using bromothymol blue (BTB) as model compound and phosphate buffer as stimulated body fluid, the release mechanism is demonstrated through an analysis of a drug-release model. The results show that the pore size of the carrier was in the range of 50-100μm in diameter; BTB released at a stable rate during the 80% releasing period, the release process can appropriately be regarded as zero order release and the as-prepared carrier principally achieved a consistent controlled release state.

KEYWORDS

Hydroxyapatite;
Collagen;
Polylactide;
Reservoir;
Controlled-release carrier;
In-vitro release.

INTRODUCTION

Many skeletal diseases are systemic in nature, the pathology is often expressed at localized sites. One of the therapeutic challenges is to deliver pharmaceutical agents to these sites[1]. In order to tackle the problem of the conventional drug administration including oral taking and injection for the treatment of human's bone disease such as osteocarcinoma or bone tuberculosis that drug transfer to either the abnormal tissue or the other parts and therefore results in toxic reactions or adverse effect on liver and kidney, it is necessary to prepare a kind of bioresorbable reservoir control-released drug carrier, which must consistently release drug only to the defective parts after implantation and fixation by polylactide plate and bone nails, and is also in vivo degradable and facilitates the formation and repairing of bone tissue for avoidance of secondary surgery. Figure 1 demonstrates the possible application scheme and mechanism thereof. In recent years, various biodegradable delivery systems have been developed and evaluated for local delivery of antibiotics in the treatment of bone infection. Biodegradable implants could provide high local bactericidal concentrations in tissue for the prolonged time needed to completely eradicate the infection and the possibility to match the

rate of implant biodegradability according to the type of infection treated. Biodegradation also makes surgical removal of the implant unnecessary. The implant can also be used initially to obliterate the dead space and, eventually to guide its repair[1].

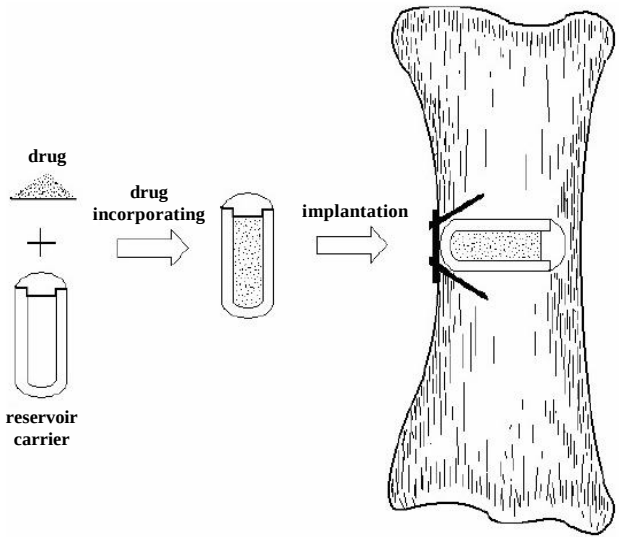

Figure 1 : Schematic illustration for reservoir drug carrier implanted in focal osseous tissue.

The synthetic bioceramic hydroxyapatite (HAp) has achieved an extensive application in medical field as a substitute of nature bone thanks to its biocompatibility, bioresorbability, osteoconductivity and nontoxicity[2]. Polylactite is one of biodegradable polymers, which has been widely researched in the field of tissue engineering and drug delivery, it has gained the approval of American Food and Drug Administration (FDA) for clinical applications[3,4]. However hydroxyapatite cannot be used in human's load bearing parts for its poor mechanical performances, and poly (L-lactic acid) (PLLA) is easy to degrade to acid byproducts. Composites made from HAp and PLLA are supposed to combine their advantages and also to offset the disadvantages, Inorganic-organic composites aiming to mimic the composite nature of real bone combine the toughness of the polymer phase with the compressive strength of an inorganic one to generate bioactive materials with improved mechanical properties and degradation profiles. For such composites, the alkalinity of the inorganic particle as hydroxyapatite neutralizes acidic autocatalytic degradation of polymers such as PLA, exploiting a bioactive function[5].

Nature bone contain small amounts of organics such as collagen (Col), glycoproteins or glycosaminoglycans in addition to main inorganic component i.e. hydroxyapatite, The hybrid of HAp crystals and Col fibers by self- assembling process is believed to give peculiar mechanical properties and biological functions to bone[6], therefore it is vital to biomimeticly prepare HAp/Col bone-like composite with promising similarities of both composition and configuration to nature bone as substitute of traditional bioceramic HAp[7,8], the manufactured HAp/Col will be combined with PLLA to improve the biomechanical property of implant and tissue regeneration of post-implantation. In this study, for the first step biomimetic bone-like HAp/Col composite was prepared by imitating in vitro biomineralization and self-assembly process.

Drug control-release system operates by diffusing of incorporated drug physically integrated with polymer matrix, which required to be endued with porous structure[9]. The succeeding work in this study involved the fabrication of 3-D porous HAp/Col/PLLA reservoir control-release drug carrier by modified thermal-induced phase separation (TIPS) technique, and in vitro release test of model compound bromothymol blue (BTB) was carried out to investigate the control-releasing behavior.

The objective of the prepared carrier is to achieve a consistent and controlled release in the defective part. Obviously the diffusion-induced drug release process is motivated by apparent directional move of the drug molecules resulting from thermal movement (buraun movement) driven by concentration gradient. The drug release characteristics correlate much to the properties and structure of the carrier materials in addition to the qualities of the applied drug such as solubility, molecule weight. In this study in vitro release test of model compound bromothymol blue (BTB, molecule weight 624.4) was carried out to investigate the release rate characteristics of control-releasing behavior, the releasing kinetics based on experimental data in line with an established model was tentatively proposed.

EXPERIMENTAL

Preparation of hydroxyapatite/collagen bone-like composite

Rat tail tendon collagen was extracted from 4 frozen albino rat tails, which skin was removed and ten-

don isolated from other tissue, tendon fibers were washed with 70% alcohol solution, finely cut and rinsed with deionized water, then the fine rat tail tendon fibers were immersed in 1000ml 0.5mol/L acetic acid for 3-4 days at 4°C, their telopeptide was removed by pepsin treatment, 200ml 0.1mol/L NaOH solution was added and collagen fibers precipitated, the mixture were centrifuged at 4000r/min and precipitate was collected then freeze- dried[10,11].

The detailed preparation and characterization of HAp/Col had been described elsewhere[12]. An aqueous suspension was made by ultrasonicating 4.46g finely grounded CaO powder in distilled water and transferred completely to a tap funnel, 2g freeze-dried rat-tail tendon collagen was dissolved in 400ml 0.15mol/L acetic acid, 5.4ml H_3PO_4 was added and thoroughly stirred; the mixture was transferred to another tap funnel. Both solutions were gradually added through respective tap funnel into a central reaction vessel with 1000ml distilled water previously added and vigorously stirred. The reaction temperature was controlled by a thermostatic water bath and the pH of the reaction solution by a set of pH metric. The temperature was set at 40°C and pH at 9, the resultant products was aged for 12h, the supernatant was removed and the precipitation was repeatedly washed with deionized water, then suction filtrated and naturally dried at room temperature.

Preparation and characterization of HAp/Col/ PLLA 3-D porous reservoir drug carrier

The fabrication and in vitro simulate test of the prepared carrier had been depicted in detail in previous report[13]. 10% (w/v) PLLA solution was prepared by dissolving a 2g PLLA (M_w: 400,000) in 20ml 1, 4-dioxane, a known amount of finely ground HAp/Col composite powder (with particle size=100μm) was simultaneously added, the mixture was magnetic stirred to be homogeneous solution, then quickly cast into a steel mold, the cast mold was quenched to -20°C in a refrigerator and maintained for 5h. After that the mold was removed and the sample was rapidly immersed in -20°C ethanol aqueous solution for 12h, then rinsed in 4°C distilled water for 1h, the sample was naturally dried in the air and residual solvent was removed by subsequently extraction under vacuum for 24h. The flow chart for the process is given as figure 2 shows.

The morphology of the carrier was investigated by JSM5610-LV scanning electronic microscopy (SEM), fresh cross-sections of the carrier were coated with Au and examined.

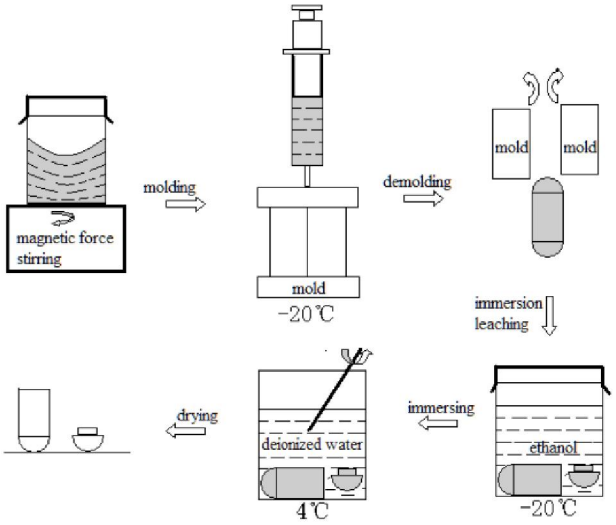

Figure 2 : The flow chart for preparing HAp/ Col/ PLLA reservoir drug carrier

In vitro control release test

In vitro control release test was performed using bromothymol blue (BTB, molecule weight 624.4) as model compound, 0.1g BTB was added to the capsule of carrier, the carrier was sealed with its cap by a little ethyl acetate, the BTB incorporated carrier was immersed in a beaker filled with 40ml 0.1mol/L phosphate buffer (PB, pH7.4, used as stimulated body fluid), the beaker was covered and placed in a thermostatic water bath at 37°C, oscillated at intervals. During the first 48h, for every 3h the phosphate buffer in the beaker was renewed and the recycled PB impregnant was analyzed by a type751 spectrophotometer for the measurement of absorbance (A_j) at wavelength of 616nm, which concentration (C_j) was determined by standard curve accordingly. After that, repeated foregoing experiments daily. The instant release rate (dM_j/dt) of model compound BTB released to PB from the drug carrier at every time t_j were calculated by 1:

$$dM_j/dt=C_jV/t_j \quad (1)$$

where dM_j/dt is the release rate for the j-th time test, C_j is the concentration of BTB while renewing PB for the j-th time, V(=40ml) is the renewed volume of BTB, t_j is the release time for every duration.

RESULTS AND DISCUSSION

Structrure of HAp/Col/PLLA reservoir drug carrier

From the micrographs exhibited in Figure 3(a) observed by JSM5610-LV scanning electron microscope, it can be seen clearly that HAp/Col bone-like composite particles dispersed evenly and were embedded in the polymer matrix, interconnected pores distributed uniformly in HAp/Col/PLLA drug carrier with connective mini- channels through the rigid pore walls, the pore size was in the range of 50-100μm in diameter, which was suitable for application to control-release of drug. Figure 3(b) gives the photograph of the resultant reservoir drug carrier which is a sort of capsule-like drug container.

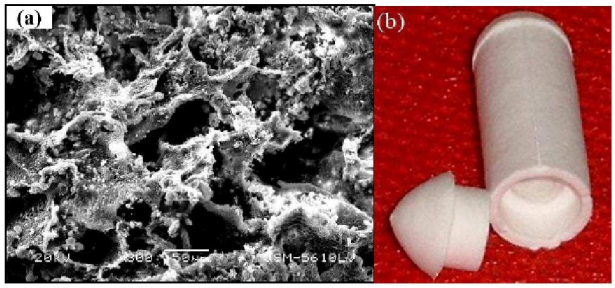

Figure 3 : (a) SEM images of the cross section of HAp/ Col/ PLLA drug carrier (b) Profile photo of HAp/Col/PLLA reservoir drug carrier.

Drug release characteristics of drug carrier

Figure 4 reveals the plots of BTB release rate determined by equation 1 versus time $t_n(=\Sigma t_j, j = 1, 2,\ldots, n)$, it can be clearly found that the release of model compound is generally divided into three stages: the release rate increases drastically during the original 96h owing to the bursting release of BTB resulting from a higher internal- to-external concentration gradient during the initial stage after the model compound's dissolving. During the period of 120h starting from time 960h, the release rate slow down gradually and a stagnant stage follows due to the weakened diffusion drive caused by a decreased BTB concentration gradient. In the course of 864h intermediate stage (equals to 80% total releasing duration), the release rate tolerates in the range of 0.1-0.2mg/h that can be consider approximately a constant rate and the release behavior would be regarded as an approximate zero order release. The characteristics of the release rate can be interpreted that the lasting saturation state of BTB in the carrier stabilized the concentration gradient for long time and thus ensures a stable consistent release at a constant rate. However in the later stage, on one aspect the release rate slows down because of the lowered concentration gradient due to the exhausting BTB in the carrier, on the other aspect the release rate will speed up to some degree ascribing to a more open porous structure on account of materials biodegrading, the two converse functions can also synergistically attain a steady constant-rate release. The prepared drug carrier can generally achieve a steady controlled release.

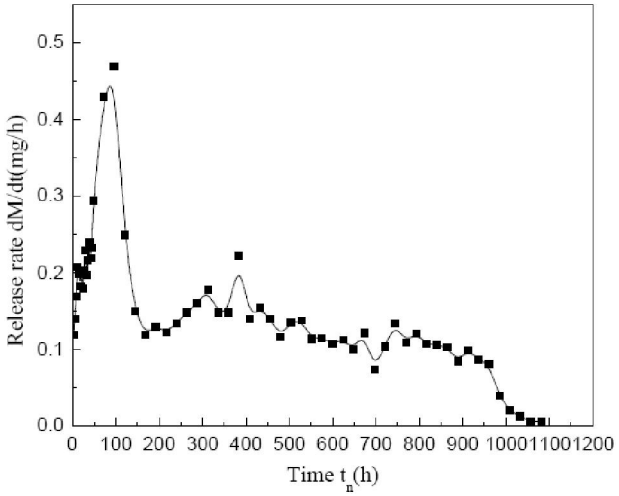

Figure 4 : Release rate of BTB in the carrier versus time.

Release kinetics model for the reservoir carrier

Various mathematic models are established to depict drug release[14]. In accordance with the characteristics of the prepared carrier materials, the controlled release mechanism of the studied carrier is normally adapt to physical controlled release mechanism i.e. diffusion controlled drug-release. In this investigation, in vitro release test using BTB as model compound was performed to explore the common characteristics of the controlled release for reservoir drug carrier. A release kinetics model for the prepared capsule-like reservoir carrier is presented based on related theories. The hypothesis of the model include: (i) the diffusion coefficient during releasing time remains constant; (ii) drug powder distributes uniformly in the capsule cavity of the carrier, the release rate is diffusion-controlled; (iii) the release course is a quasi steady state process.

The resulting reservoir drug carrier consists of the cavity and porous periphery wall made of HAp/ Col /

PLLA composite. The structure demonstration diagram in cross sectional view of the carrier is displayed in Figure 5. The cavity of the carrier is fulfilled with drug powder, and the thickness of the capsule wall is R_1-R_0. The drug diffuses from the cavity into the ambient at a controlled rate which kinetically complies with Fick's diffusion laws[15], which depict the penetration speed of substance through a specific area in a steady diffusion state.

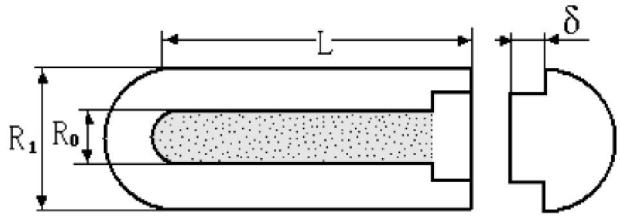

Figure 5 : The structure illustrative diagram in across sectional view of the carrier.

In accordance with Fick's first law, the drug releases through wall of the carrier at a rate of

$$\frac{dM_i}{dt} = -K_{p/s} \cdot S \cdot D_p \cdot \frac{dc}{dr} \quad (2)$$

For a capsule wall, according to Fick's second law the drug release rate through an arbitrary periphery cylinder wall with area of $S=2\pi rL$ can also be expressed as

$$2\pi rL \cdot \frac{dc}{dt} = \frac{d(2\pi rL \cdot K_{p/s} \cdot D_p\, dc/dr)}{dr} \quad (3)$$

$$\frac{dc}{dt} = \frac{K_{p/s} \cdot D_p}{r}\left(\frac{dc}{dr} + r\frac{d^2c}{dr^2}\right) \quad (4)$$

Where M_i is drug amount passing through a cylinder wall; t is time; $K_{p/s}$ is the partition coefficients of drug in the porous polymer cylindrical wall and ambient solution; S is the surface area of cylinder with arbitrary radius; D_p is the diffusion coefficient of drug in the porous wall; c is the drug concentration; r is the position in the wall drug arrives; L is the length of the capsule; c_o, c_s is the drug concentration in the cavity and ambient solution respectively; R_0, R_1 is the outer and inner radius of the cylindrical carrier respectively; δ is the thickness of lid of the capsule. In a steady state Eq. 4 equals to zero, that is

$$\frac{dc}{dr} + r\frac{d^2c}{dr^2} = 0 \quad (5)$$

The solution of the differential equation is

$$c = C_1 \ln r + C_2 \quad (6)$$

in which C_1, C_2 is integral constants, Solution of C_1 can be obtained using boundary conditions: $c=c_0$ if $r=R_0$; $c=c_s$ if $r=R_1$.

$$C_1 = \frac{c_0 - c_s}{\ln(R_0/R_1)} \quad (7)$$

Equtions 6, 7 are put in 2 to get the drug release rate of cylindrical part of the carrier as:

$$\frac{dM_i}{dt} = \frac{2\pi LD_pK_{p/s}(c_0 - c_s)}{\ln(R_0/R_1)} \quad (8)$$

Similarly the release rates at the other parts of the carrier such as a hemispherical end and a plane cap can also be expressed respectively as

$$\frac{dM_j}{dt} = \frac{2\pi D_pK_{p/s}(c_0 - c_s)}{1/R_1 - 1/R_0} \quad (9)$$

$$\frac{dM_k}{dt} = \frac{\pi R_0^2 D_pK_{p/s}(c_0 - c_s)}{\delta} \quad (10)$$

Accordingly the total release rate can be obtained by superimposing 8, 9 and 10 as

$$\frac{dM}{dt} = \frac{dM_i}{dt} + \frac{dM_j}{dt} + \frac{dM_k}{dt} =$$

$$\left\{\frac{2\pi LD_pK_{p/s}}{\ln(R_0/R_1)} + \frac{2\pi D_pK_{p/s}}{1/R_1 - 1/R_0} + \frac{\pi R_0^2 D_pK_{p/s}}{\delta}\right\}(c_0 - c_s) \quad (11)$$

Where parameters such as L, δ, R_0, R_1, D_p and $K_{p/s}$ can all be regarded as constants, let

$$k = 2\pi LD_pK_{p/s}/\ln(R_0/R_1) + 2\pi D_pK_{p/s}/(1/R_1 - 1/R_0) + \pi R_0^2 D_pK_{p/s}/\delta \quad (12)$$

$\Delta c = c_0 - c_s$, so the total release rate equation of the reservoir carrier simplifies to $dM/dt = k\Delta c$ (in which k is a constant related to structure dimensions of the carrier). While the carrier is in the body fluid, the solution permeates the porous wall from the outside and dissolves the drug promptly revealing a short bursting release followed by a steady diffusion attributing to the unchanging saturated drug concentration. With the diffusion process goes on, the drug concentration decreases gradually until finally the drug undergoes an unstable diffusion course. The foregoing release test of model compound BTB has evidently indicated a steady release process for 80% total release period of time. In accordance with the resulting equation $dM/dt = k\Delta c$, drug release rate depends on the concentration gradient between the inner and outer carrier,

which is the impelling force for drug to diffuse into body fluid. Δc can be regarded as a constant in a steady release state, thus the release rate dM/dt will approximately keep constant implying a zero order release process. Nevertheless, concentration gradient Δc differs remarkably in the initial and final stage and is a function of time $\Delta c = f(t)$ indicating an unstable release process which is referred as "bursting effect" and "hysteresis effect" respectively.

CONCLUSIONS

The pore size of the reservoir carrier is in the range of 50-100μm in diameter which is suitable for application to control-release of drug; BTB released at a constant rate during the 80% releasing period of time, the release process can appropriately be regarded as zero order release and the prepared carrier principally achieved a consistent controlled release state.

REFERENCES

[1] S.K.Nandi, P.Mukherjee, S.Roy, B.Kundu, D.K.De, D.Basu; Mater.Sci.and Eng.C, **29**, 2478-2485 **(2009)**.
[2] M.Wang; Biomater, **24**, 2133-2151 **(2003)**.
[3] F.J.Hua, T.G.Park, D.S.Lee; Polym., **44**, 1911-1920 **(2003)**.
[4] L.C.Lu, J.P.Susan, D.L.Michelle; Biomater, **21**, 1595-1605 **(2000)**.
[5] I.Armentano, M.Dottori, E.Fortunati, S.Mattioli, J.M.Kenny; Polym.Degrad.Stab., **95**, 2126-2146 **(2010)**.
[6] A.Ficai, E.Andronescu, G.Voicu, C.Ghitulica, B.S.Vasile, D.Ficai, V.Trandafir; Chem.Eng.J., **160**, 794-800 **(2010)**.
[7] C.Hellmich, F.J.Ulm; J.Biomechanics, **35**, 1199-1212 **(2002)**.
[8] M.Swethaa, K.Sahithi, A.Moorthi, N.Srinivasan, K.Ramasamy, N.Selvamurugan; Int.J.Biol. Macromol., **47**, 1-4 **(2010)**.
[9] P.Sher, G.Ingavle, S.Ponrathnam, A.P.Pawar; Microporous Mesoporous Mater, **102**, 290-298 **(2007)**.
[10] F.Y.Hsu, S.C.Chueh, Y.J.Wang; Biomater., **20**, 1931-1936 **(1999)**.
[11] D.L.Christiansen, E.K.Huang, F.H.Silver; Matrix Biol., **19**, 409-420 **(2000)**.
[12] Z.L.Wang, Y.H.Yan, T.Wan; Acta Materiae Compositae Sinica, **22**, 83-86 **(2005)**.
[13] Z.L.Wang, T.Wan, Y.H.Yan; Chinese Journal of Clinical Rehabilitation, **10**, 198-201 **(2006)**.
[14] Y.Xing, X.B.Yuan, J.Chang, S.H.Wang, J.Sheng, X.Hou; Polym.Bull., **12**, 22-30 **(2004)**.
[15] S.P.Li; 'A An Introduction to Biomaterials', Press of Wuhan University of Technology, Wuhan, China, **(2000)**.