Macromolecular Research, Vol. 16, No. 4, pp 303-307 (2008)

Antimicrobial Drug Release Scaffolds of Natural and Synthetic Biodegradable Polymers

Periasamy Prabu and Kwan Woo Kim

Department of Bionanosystem Engineering, Chonbuk National University, Jeonju 561-756, Korea

Nallusamy Dharmaraj

Department of Chemistry, Bharathiar University, Coimbatore 641046, India

Jong Hoon Park, Myung Seob Khil, and Hak Yong Kim*

Department of Textile Engineering, Chonbuk National University, Jeonju 561-756, Korea

Received October 17, 2007; Revised February 18, 2008; Accepted February 20, 2008

Abstract: A series of biodegradable polymeric scaffolds was prepared by using a combination of natural (collagen) and synthetic (poly(caprolactone)) (PCL) polymers in various compositions. These scaffolds were soft, spongy, porous and transparent in nature and were characterized by thermogravimetric analysis (TGA) and Fourier transform infrared (FT-IR) spectroscopy. The entrapment efficiency and drug release activity of the scaffolds were analyzed using penicillin and tetracycline as antimicrobial drugs. The drug release activity of the scaffolds with various combinations of collagen and PCL were studied by measuring the optical density in a spectrophotometer at the following time intervals: 1, 4, 24, 48 and 60 h. These scaffolds showed better and continuous drug release for up to 60 h. Even after such a long duration, a portion of the drug remained entrapped in the scaffolds, indicating that they can be utilized for wound healing applications.

Keywords: biodegradable polymers, poly(caprolactone), collagen, scaffolds, penicillin, tetracycline.

Introduction

Collagen, a well-known protein is a natural biodegradable polymer that has considerable use in medical field as a biomaterial owing to its biocompatibility, non-toxicity and well documented structure, physical, chemical, biological and immunological properties. In particular, collagen based biomaterials find significant place in the field of drug delivery.[1,2] Further, the mechanical stability compliance, cost effectiveness and ease of availability of collagen also facilitates its application as a biomaterial. Hence, collagen based biomaterials used in the preparation of the new scaffolds in combination with PCL are expected to possess good biocompatibility for wound tissue. Earlier, collagen was used in wound healing and skin regeneration.[3] On the other hand, the synthetic biodegradable polymer poly(caprolactone) (PCL) is also used as a biomaterial in combination with collagen for wound dressing. PCL attracts much attention due to its cost-efficiency, flexibility at room temperature, higher crystallinity and lower degradation rate compared to other polymers and it is an FDA approved biodegradable polymer for drug release application. In addition, the higher permeability of PCL for a wide variety of drugs makes it as a potential drug delivery carrier.[4,5]

Drug carriers are being developed in different forms such as scaffold, membrane and porous matrix to encapsulate drugs using wide range of biodegradable polymers of both natural and synthetic origin. Among these, scaffolds have shown promise as drug delivery carrier due to many reasons including biocompatibility and availability.[6-12] To improve wound healing by reducing wound contamination, several antimicrobial drugs have been developed and investigated so far. Penicillin and tetracycline exhibit bacterial activity against a broad spectrum of microorganism including critical clinical microbes especially against both gram (+) ve and gram (-) ve bacteria's.[13,14] From this point of view, penicillin and tetracycline are used in our work to entrap into the scaffolds without loss of its activity. Moreover tetracycline has broad-spectrum range as compared to penicillin. These drugs are selected to use as topical antibacterial drugs because of their low toxicity and their potential to act on microbes.[13,14]

*Corresponding Author. E-mail: khy@chonbuk.ac.kr

Based on the above facts and in continuation of our interest in the formulation of novel drug delivery systems, we present herein the preparation of biodegradable polymeric scaffolds containing collagen and PCL. The drug entrapment efficiency of the prepared scaffolds have been determined by using the antimicrobial drugs penicillin and tetracycline. The *in vitro* release of these drugs from the scaffolds was estimated by using UV spectrophotometer.

Experimental

Materials. PCL (high molecular weight =80,000), dichloromethane (DCM), calcium chloride ($CaCl_2$) and Tritrion X-100 were purchased from Sigma-Aldrich (USA) and pure collagen was extracted from Bovine Achilles Tendon. Pepsin used in the preparation of scaffolds was purchased from Sigma-Aldrich (USA). Antimicrobial agents penicillin and tetracycline were purchased from Chipla Pharm Co. (India). Remi stirrer was used for homogenization of solutions. Phosphate buffer solution (PBS) with a pH of 7.2 was prepared and used in the drug release study.

Thermogravimetric analysis (TGA 2050, TA Instruments Co., USA) was carried out at a heating rate of 10 °C per minute in air. FT-IR spectrum of the sample was recorded (as KBr pellet) using Bio-Rad Win spectrophotometer.

Extraction of Collagen. Pure collagen was extracted from Bovine Achilles tendon tissue and minced with a mincer by following the reported procedure.[15] The minced tissues were treated with surfactant (Tritron-X) and mixed well to remove the fat content present in the tissue and then repeatedly washed with distilled water to remove the surfactant. Then the tissues were treated with alkali to swell and it was stirred occasionally and left at 4 °C to avoid the denaturation of collagen. The swollen tissues were treated with pepsin enzyme to remove antigenic site. After this, glacial acetic acid was used to get the collagen solution and the protein in the solution was precipitated by adding 5% NaCl with continuous stirring. The precipitated protein was filtered, washed with slightly alkaline water to remove excess salt and dissolved in 0.5 M acetic acid and homogenized to get clear collagen solution.

Standardization of Antimicrobial Agents. For the preparation of collagen-PCL scaffolds containing antimicrobial agents, the drugs were standardized from stock solution.

Penicillin and Tetracycline Standardization. Each penicillin and tetracycline vial consisted of 40 mg/mL. Therefore, stock was prepared by taking 1 mL from the vial and made up to 10 mL in distilled water, and different aliquots ranging from 0.5, 1, 1.5, 2.0 mL were taken, and it was made up to 10 mL with distilled water. They were further diluted to half of the concentration. The readings were taken at 257 nm for penicillin and 270 nm for tetracycline. The optical density (O.D.) value ranges from 50-400 μg for penicillin and 1,000-4,500 μg for tetracycline.

Preparation of Collagen-PCL Scaffolds Containing Penicillin and Tetracycline Drugs. 100 mg of PCL was dissolved in 5 mL of dichloromethane and the resulting polymer solution was mixed with 10 mL collagen solution. To this 1 mL of penicillin (15 mg) drug was added and stirred for 2 min. Then the homogenized solution was casted into 55 mm plastic petri plates and dried in air for 3 to 4 days, resulting in the formation of soft, spongy and transparent scaffolds with an average thickness of 50 mm. This scaffold was named as Pc_1. Using the above method, different scaffolds were prepared by keeping constant quantity of collagen (10 mL) and varying the PCL concentration (i.e. 200, 300, 400, 500 mg) and named as Pc_2, Pc_3, Pc_4 and Pc_5.

Using the above procedure, the scaffolds containing tetracycline were prepared by taking 1 mL of tetracycline (25 mg) instead of pencillin. The scaffolds with the average thickness of 50 mm were obtained and named as Tc_1, Tc_2, Tc_3, Tc_4 and Tc_5.

Contact Angle Measurement. The hydrophilicity of the collagen-PCL scaffolds was determined by contact angle measurement to relate the permeating rate of water into the scaffolds. The water contact angle was measured by the video contact analyzer (SEO-330, S.E.O Co., Korea). Deionized water was automatically dropped onto the scaffolds to check the hydrophilicity. The contact angle was measured 5 times from different positions on each scaffold and an average value was calculated.

Drug Release Studies. The drug release activities of the scaffolds containing penicillin and tetracycline were carried out as follows.

A piece of 100 micrograms was weighed from each scaffold (Pc_1, Pc_2, Pc_3, Pc_4 and Pc_5 and Tc_1, Tc_2, Tc_3, Tc_4 and Tc_5) placed in separate test tubes. To this, 3 mL of PBS (pH 7.2) was added and kept in orbital shaker. The amount of penicillin /tetracycline released from the scaffolds was estimated by collecting PBS from the test tubes and replacing by adding fresh 3 mL buffer after 1 h. The same procedure was followed after 4, 24, 48 and 60 h. The concentration of drug release was determined spectrophotometrically at 257 nm for penicillin and 270 nm for tetracycline. The quantity of drug released was calculated from the standard curve.

Results and Discussion

In this study, we estimated the penicillin and tetracycline release efficiency of the collagen and PCL containing scaffolds in DDS. Collagen was chosen as a carrier because of its bioactivity and biocompatibility. Collagen scaffolds are poorly permeable to water vapors and hence they are considered occlusive.[14,16] The use of collagen, which constructs the skeleton system in association with PCL, makes the scaffolds more proximity with the body fluids thereby providing hydrophilic interaction.

FT-IR Spectroscopy. Using FT-IR spectroscopy, the com-

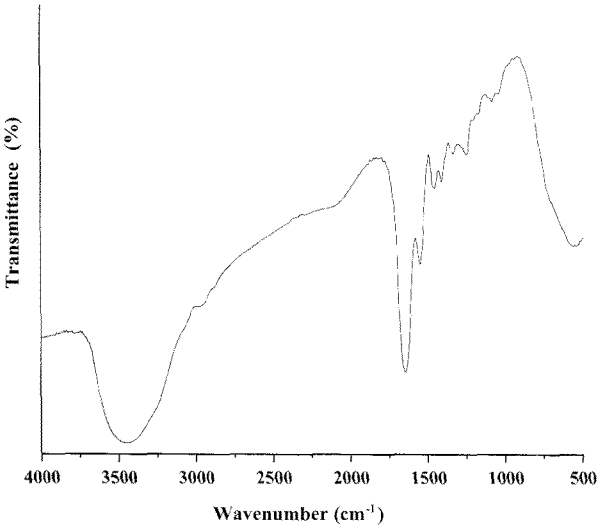

Figure 1. FT-IR spectrum of collagen-PCL scaffold.

posite scaffolds were characterized to find the presence of both collagen and PCL (Figure 1) fractions in the composite. Different modes of vibrations having contributions from both collagen and PCL were found in the spectrum. The characteristic bands of carbonyl and -NH_2 asymmetric stretching vibrations were observed at 1645 cm^{-1} and 1550 cm^{-1}, respectively. A broad band in the region of 3400-3500 cm^{-1} has been assigned as due to that of the combined vibrations of both the amino and hydroxyl groups.

Thermogravimetric Analysis. Figure 2 shows the TGA curve of collagen-PCL scaffolds without antimicrobial drugs. From this figure, we observed that the decomposition temperature of pure PCL and collagen are 388 and 310 °C, respectively. The TGA of the collagen-PCL scaffold showed a monotonic weight loss (10%) at the temperature interval of 350-380 °C due to the decomposition of collagen and a sharp decomposition step with complete weight loss at 430 °C is due to the decomposition of PCL. The increase in the decomposition temperature of collagen and PCL may be attributed to the physical mixing of them.

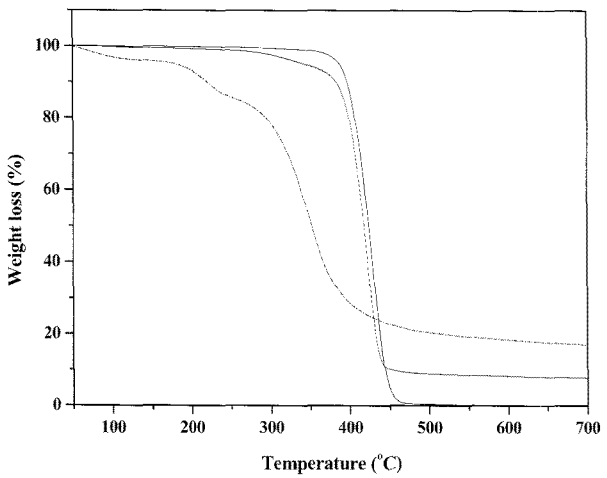

Figure 2. TGA curves of collagen-PCL scaffold (----: collagen, —: PCL, -----: collagen-PCL).

Contact Angle Measurement. The hydrophilicity of the collagen-PCL scaffolds was determined by contact angle measurement. In drug delivery system, the drug delivery rate is related to the permeating rate of water into the scaffolds. Higher hydrophilicity can accelerate the permeation of water and improve drug diffusion rate.

In the present collagen-PCL scaffold, a drop of de-ionized water was dropped on the scaffolds in order to study its hydrophilicity and the results compared with pure PCL scaffolds. It was observed that the diffusion of the drop in collagen-PCL scaffolds is linear with time. The drop was completed absorbed within a minute. However, it was retained on the surface of pure PCL scaffolds for a long time as revealed by contact angle measurement (Figure 3).

Physico-Chemical Activity of Drug Release. It is a well-known fact that the entrapment efficiency of polymeric scaffolds mainly depends upon the nature of polymers. In our present study, the entrapment efficiency has been found to range from 87% to 95% in both the cases (Table I). The highest encapsulation efficiency was observed with low PCL content. This probably results from the hydrophobic nature of PCL. Presumably hydrophobic materials have large inhomogeneity with hydrophilic materials. This can be further stated by the homogeneity of hydrophilic collagen with hydrophilic drug, which in turn encapsulated into PCL.

***In vitro* Release Study.** Figures 4 and 5 shows the release patterns of drugs from collagen-PCL scaffolds of varying PCL concentrations at 1, 4, 24, 48 and 60 h for both penicillin and tetracycline, respectively. In both the cases, the drug

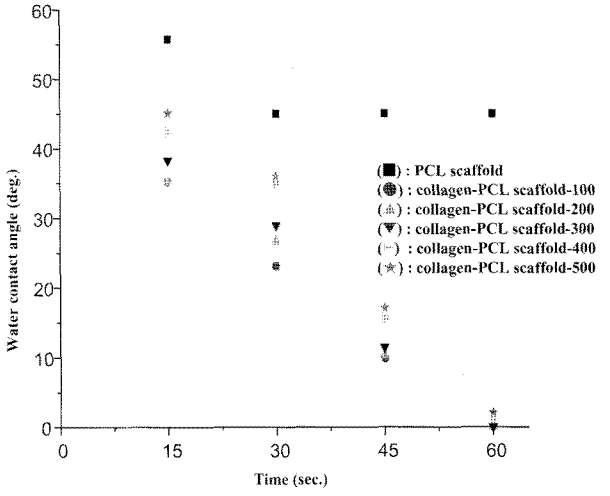

Figure 3. Water contact angles of collagen-PCL scaffolds. (■): PCL scaffold, (●): collagen. PCL scaffold-100, (▲): collagen-PCL scaffold-200, (▼): collagen-PCL scaffold-300, (▶): collagen-PCL scaffold-400, (★): collagen-PCL scaffold-500.

Table I. Physico-Chemical Activity of Drug Release

(a) Collagen- PCL Scaffolds with Penicillin

Formulation	Drug Loading (mg)	Entrapment Efficiency	
		(mg)	(%)
Collagen 10 mL/PCL-100 mg	15	14±1	95±1
Collagen 10 mL/PCL-200 mg	15	14±1	95±1
Collagen 10 mL/PCL-300 mg	15	13±2	87±2
Collagen 10 mL/PCL-400 mg	15	13±2	87±2
Collagen 10 mL/PCL-500 mg	15	13±2	87±2

Mean value ± SD (n=4)

(b) Collagen- PCL Scaffolds with Tetracycline

Formulation	Drug Loading (mg)	Entrapment Efficiency	
		(mg)	(%)
Collagen 10 mL/PCL-100 mg	24	23±1	95±1
Collagen 10 mL/PCL-200 mg	24	23±1	95±1
Collagen 10 mL/PCL-300 mg	24	23±1	95±2
Collagen 10 mL/PCL-400 mg	24	21±3	87±3
Collagen 10 mL/PCL-500 mg	24	21±2	87±3

Mean value ± SD (n=3).

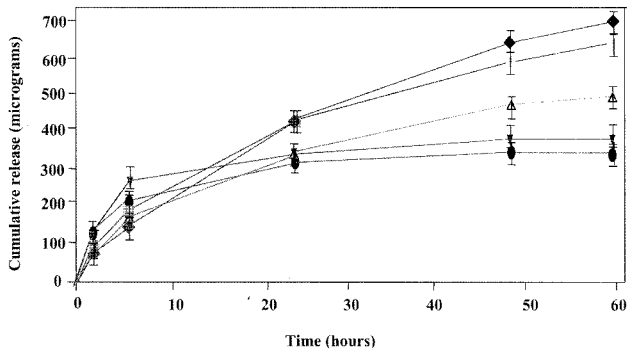

Figure 4. Penicillin release profile of collagen-PCL scaffolds at various time intervals (mean value ± SD (n=4)) (●: Pc_1, ▼: Pc_2, ▲: Pc_3, ■: Pc_4, ◆: Pc_5).

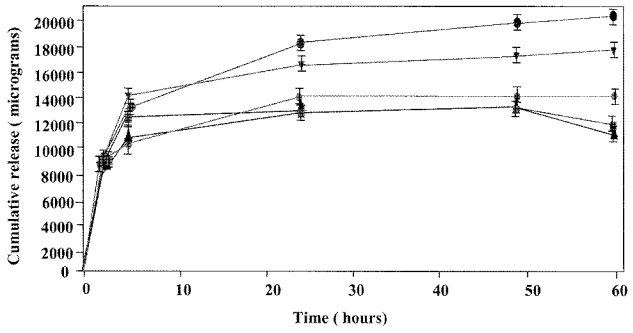

Figure 5. Tetracycline release profile of collagen-PCL scaffolds at various time intervals (mean value±SD (n=3)) (▲ : Tc_1, ■: Tc_2, ●: Tc_3, ▼: Tc_4, ●: Tc_5).

release rate increased because of high PCL concentration. Similarly, drug release profile of both formulations exhibit an initial burst release, followed by a sustained release driven by diffusion of drug through polymer wall and polymer erosion in the scaffolds. The drug release from scaffolds containing tetracycline is significantly higher than that from the scaffolds containing penicillin. For example, the release of drugs from the scaffolds containing tetracycline was 8,500-20,000 μg, but in the case of penicillin showed 70-700 μg.

An initial burst release of penicillin was observed from all the scaffolds containing penicillin, followed by a sustained release from the scaffolds Pc_3, Pc_4 and Pc_5 because of diffusion of drug in polymeric scaffolds. This shows the constant release of drug over the entire period of study (60 h). The concentration of tetracycline released from the scaffolds is much higher than that of penicillin. The slower rate of release found in the case of penicillin is because of the strong interaction between the drug and the polymer than that found in the case of tetracycline. Similarly, in this also the release is found entire over the period of time in the case of Tc_3, Tc_4, Tc_5 scaffolds.

Conclusions

From this study, we observed that the scaffolds containing penicillin may be used as a ordinary wound dressing material, whereas those containing tetracycline possess the potential to be used as deep wound dressing material because of its sustained release over a long period. Hence, these biodegradable polymeric scaffolds have great potential in drug delivery application as wound dressing material.

Acknowledgement. This work was supported by the Korean Research Foundation Grant founded by the Korean Government (MOEHRD) (Through The Center for Health Care Technology Development, Chonbuk National University, Jeonju 561-756 Republic of Korea).

References

(1) W. Y. Chen and G. Abatangelo, *Wound Repair Regen.*, **7**, 79 (1999).
(2) T. Miclau, M. L. Edin, F. E. Lester, R. W. Lindsey, and L. E. Daheners, *J. Orthop. Tramuma.*, **9**, 401 (1995).
(3) J. S. Lee, J. K. Kim, and S. R. Park, *Macromol. Res.*, **15**, 205 (2007).
(4) C. G. Pitt, in *Biodegradable Polymers as Drug Delivery Systems*, M. Chasin and R. Langer, Eds., Marcel Dekker, New York, 1990, pp. 71-120.
(5) H. S. Nam, J. An, and D. J. Chung, *Macromol. Res.*, **14**, 94 (2006).
(6) M. W. Saltzman and S. P. Baldwin, *Adv. Drug. Deliver. Rev.*, **33**, 71 (1998).
(7) J. Sohier, R. E. Haan, K. De Groot, and J. M. Bezemer, *J. Con-*

trol. Release, **87**, 57 (2003).
(8) M. A. Beonit, B. Baras, and J. Gillard, *Int. J. Pharm.*, **184**, 73 (1999).
(9) M. Sivakumar and K. P. Rao, *Biomaterials*, **23**, 3175 (2002).
(10) V. S. Komlev, S. M. Barinov, and E. V. Koplik, *Biomaterials*, **23**, 3449 (2002).
(11) A. Krajewski, A. Ravaglioli, E. Roncari, P. Pinsco, and L. Montanari, *J. Mater. Sci.: Mater. Med.*, **11**, 763 (2000).
(12) H. W. Kim, J. C. Knowles, and H. E. Kim, *Biomaterials*, **25**, 1279 (2004).
(13) S. T. Boyce, A. P. Supp, G. D. Warden, and I. A. Holder, *Antimicrob. Agents Chemother.*, **37**, 1890 (1993).
(14) J. Grzybowski, W. Kolodziej, E. A.Trafny, and J. Struzyna, *J. Biomed. Mater. Res.*, **36**, 163 (1997).
(15) R. Sripriya, M. R. Ahmed, P. K. Sehgal, and R. Jayakumar, *J. Appl. Polym. Sci.*, **87**, 2186 (2003).
(16) S. Sakeil and J. Grzybowski, *Polym. Med.*, **25**, 19 (1995).