Songklanakarin J. Sci. Technol.
42 (1), 180-187, Jan. - Feb. 2020

http://www.sjst.psu.ac.th

Original Article

Aptamer-gelatin composite material for prolonging PDGF-BB release

Boonchoy Soontornworajit[1*], Karnjana Kerdsiri[1], Thawatchai Tungkavet[2], and Anuvat Sirivat[2]

[1] *Deparment of Chemistry, Faculty of Science and Technology, Thammasat University, Khlong Luang, Pathum Thani, 12120 Thailand*

[2] *Conductive and Electroactive Polymers Research Unit, The Petroleum and Petrochemical College, Chulalongkorn University, Pathum Wan, Bangkok, 10330 Thailand*

Received: 17 March 2017; Revised: 25 October 2018; Accepted: 2 November 2018

Abstract

Platelet-derived growth factor-BB (PDGF-BB) is a promising molecule with a range of medical applications. However, the very rapid delivery of this growth factor may render treatment ineffective. A delivery system that is able to prolong the release rate is therefore required. In this study, a composite was fabricated from gelatin type IV and an aptamer. The aptamer worked as a binding ligand for PDGF-BB and was shown to slow the release rate. An inverted-tube test showed that the phase transition temperature of the gelatin increased in line with the gelatin concentration and in the presence of the coupling reagents *N,N*'-dicyclohexylcarbodiimide and *N*-hydroxysuccinimide. Binding functionality of the aptamer was confirmed by surface plasmon resonance spectrometry. Rheological measurements indicated that the incorporation had no effect on the mechanical properties of the composite. The degradation of the composite and the kinetic release of the target proteins were investigated. The gelatin was shown to gradually dissolve in the release medium. The rate of PDGF-BB release from the aptamer-gelatin composite was significantly slower than from the native gelatin. This novel composite has potential applications in a range of PDGF-BB-related treatments.

Keywords: gelatin, aptamer, composite, PDGF-BB, protein release

1. Introduction

Platelet-derived growth factor has four subunits: PDGF-A, PDGF-B, PDGF-C, and PDGF-D. These four polypeptide chains are able to form distinct proteins by homo- or heterodimerization via disulfide bonds that yield five different dimeric isoforms: PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC, and PDGF-DD (Fredriksson, Li, & Eriksson, 2004). These five isoforms bind and activate two tyrosine kinase receptors, PDGFR-α and PDGFR-β, which then form either homodimers or heterodimers (e.g., PDGFR-αα, PDGFR-αβ, PDGFR-ββ) (Nazarenko *et al.*, 2012). PDGF-BB has drawn much attention for practical applications because it is able to bind all three of these receptors. It also plays a critical role in many cellular activities including calcium influx, proliferation, and migration (Mazzucco, Borzini, & Gope, 2010). Promising therapeutic applications of PDGF-BB include wound healing, bone and tendon repair, and cancer treatment (Ehnman & Ostman, 2014; Evrova & Buschmann, 2017; Riedel *et al.*, 2006; Sun *et al.*, 2017). However, a number of challenges remain, including the timely delivery of the PDGF-BB growth factor to the target site (Tocco, Zavan, Bassetto, & Vindigni, 2012). Previously proposed delivery strategies have relied on bulk scaffolds (Lee, Olmer, Baek, D'Lima, & Lotz, 2018), electrospun fibers (Yuan, Foushee, Johnson, Jockheck-Clark, & Stahl, 2018), injectable devices (Wu, Liu, Wu, Wan, & Chen, 2016), or nano- or micro-particles (Mohan *et al.*, 2017). The controlled release of PDGF-BB is similar to that of other proteins and can be achieved by a variety of alternative means including diffusion-controlled, environmentally responsive,

*Corresponding author
Email address: sbooncho@tu.ac.th

and affinity-controlled systems (Andreadis & Geer, 2006). In diffusion-controlled systems, the release rate of hepatocyte growth factor from a gelatin matrix could be controlled by varying the level of glutaraldehyde and therefore the crosslink density (Ozeki, Ishii, Hirano, & Tabata, 2001). In environmentally responsive systems, poly (*N*-isopropylacrylamide) has been demonstrated to control the release of bovine serum albumin (BSA) in response to temperature (Yoshida, Sakai, Okano, & Sakurai, 1992). In affinity-controlled systems, poly(ethylene glycol), hydrogel functionalized with a bFGF binding peptide demonstrated a prolonged life of the growth factor and the release rate was shown to be correlated with the affinity of the incorporated peptide (Lin & Anseth, 2009). Aptamer-functionalized hydrogels demonstrated promising functionality as an affinity-controlled system (Soontorn worajit, Zhou, Shaw, Fan, & Wang, 2010).

Aptamer-functionalized hydrogels combine the best properties of both hydrogels and aptamers. Aptamers are single-stranded oligonucleotides that bind tightly and specifically to their target molecules and have been used for molecular recognition in several systems (Proske, Blank, Buhmann, & Resch, 2005). One of the most recognized aptamers binds to the platelet-derived growth factor BB (PDGF-BB) due to its high specificity and affinity (Green *et al.*, 1996). This binding functionality allows the PDGF-BB aptamer to be used in a range of applications including biosensors and diagnostic tools, or as a therapeutic agent (Floege *et al.*, 1999; Jiang, Fang, & Bai, 2004; Zhou *et al.*, 2006). Recently, a PDGF-BB aptamer was incorporated into hydrogels to create controlled-release delivery systems (Soontornworajit, Zhou, Zhang, & Wang, 2010). Uniquely, in these systems, aptamer-protein interactions can be interrupted via hybridization between the aptamer and its complementary oligonucleotide (Rusconi *et al.*, 2002). This results in the dissociation of the protein from the aptamer, thus releasing it from the delivery system (Soontornworajit, Zhou, & Wang, 2010).

Hydrogels are hydrophilic polymer networks that resemble human tissue and are able to maintain the activity of loaded proteins (Kissel, Li, & Unger, 2002). Hydrogels of gelatin have gained attention in the field of biomedical materials because gelatin is a natural, biocompatible, and biodegradable material (Tabata & Ikada, 1998). It is composed of fibril proteins derived from collagen that have been boiled for a certain time. The boiling transforms a coiled structure to a helical structure. The helical region serves as the crosslinking point for molecular entanglement and this gives the gelatin its characteristics as a hydrogel network (Miyamoto, Chinzei, & Komai, 2002). Gelatin has been proposed as the delivery system for a number of molecules including growth factors and plasmid DNA (Fukunaka, Iwanaga, Morimoto, Kakemi, & Tabata, 2002; Ozeki *et al.*, 2001).

The goal of this work was to create a composite material comprised of PDGF-BB aptamer and gelatin that offers a minimal burst release profile. The key hypothesis was that the aptamer in the composite would bind to the PDGF-BB and slowly release this growth factor. The effect of varying the concentration of gelatin and coupling agent on the transition temperature was also investigated, as was the effect of temperature on the binding functionality of the aptamer. The prepared composites were tested for rheological properties, weight loss, and protein release profile.

2. Experimental Method

2.1 Reagents

Streptavidin-coated polystyrene microparticles (1.3 μm) were purchased from Spherotech (Lake Forest, IL, USA). Phosphate buffered saline (PBS), Tween 20, and sodium azide (NaN_3) were purchased from Fisher Scientific (Suwanee, GA, USA). Recombinant human platelet-derived growth factor BB (PDGF-BB) and a human PDGF-BB enzyme-linked immunosorbent assay (ELISA) kit were purchased from R&D Systems (Minneapolis, MN, USA). Bovine serum albumin (BSA) was purchased from Invitrogen (Carlsbad, CA, USA). Gelatin Type B from bovine skin with a bloom value of 225 g, N-ethyl-N-(3-diethylaminopropyl) carbodiimide (EDC), and N-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). DNA molecules were sourced from Integrated DNA Technologies (Coralville, IA, USA). The sequences are listed in Table 1: PDGF-BB aptamer (PDGF-BB Apt) and Scrambled aptamer (S-Apt).

2.2 Phase transition temperature

The temperatures at which the gelatin solution formed a gel and a composite were investigated. Two factors that played a role in the transition temperature were studied. First, to examine the effect of gelatin concentration on the transition point, gelatin solutions of 5, 10, 15, 20, 25, and 30% (w/v) were prepared in PBS. Then, 1000 μL of each solution were transferred to a 1.5 mL microcentrifuge tube and incubated in a water bath for 3 min at controlled temperatures that ranged 10–45 °C (cooling thermostat, LAUDA Alpha RA 8). For the cool down process, the temperature was lowered by 2 °C in each condition. For the heat up process, the temperature was increased by 2 °C in each condition. The transition temperature of the gelatin was investigated using the method of (Y. M. Chung, Simmons, Gutowska, & Jeong, 2002), in which the tube was inverted and the flow behavior was observed. The gelatin was determined to be in the gel state when no flow was observed over 30 s of inversion. Next, to study the effect on transition temperature of the carboxylic group to EDC molar ratio, EDC was added to 30% (w/v) gelatin to final concentrations of 13.00, 6.50, 3.25, 2.17, 1.63, and 1.30 mM. These were equivalent to carboxylic group to EDC molar ratios of 10, 20, 40, 60, 80, and 100, respectively. NHS was also mixed into the reaction mixture using a molar equivalent to EDC.

2.3 Surface plasmon resonance analysis

Surface plasmon resonance (SPR) is a technique for studying and evaluating the interaction of molecules or

Table 1. Sequences of DNA aptamer and oligonucleotide.

Sequence ID	Sequences (5'→3')
PDGF-BB Apt	GCGATACTCCACAGGCTACGGCACGTA GAGCATCACCATGATCCTG
S-Apt	GCGATACTCCATCAATGGACCGCGCAC TCGCCAGTGCTAATGGCAA

ligands. In this study, the effect of temperature on the interaction between PDGF-BB and its aptamer was investigated by SPR spectrometry (SR7000DC, Reichert Analytical Instrument, Depew, NY, USA). The experimental procedures were as follows. First, the PDGF-BB was immobilized on a carboxyl group-functionalized sensor chip via amide bond linkage activated by NHS and EDC. Then, the sensor chip was rinsed with a running buffer (PBS, 0.05% Tween 20) for 30 min. For binding association, the aptamer solutions were flowed over the sensor chip at 30 µL/min for 5 min. For binding dissociation, the flow was switched to a running buffer at 30 µL/min for 5 min. Aptamer was reconstituted in the running buffer at concentrations that ranged 1–30 nM. For each series of aptamer solutions, the system temperatures were set at 17, 25, 30, and 37 °C. The dissociation constant (K_D) of the aptamer at each temperature was determined by fitting an equilibrium response (R_{eq}) to a single site interaction model $R_{eq} = \left[\frac{[apt]}{[apt]+K_D}\right] R_{max}$, where $[apt]$ is the aptamer concentration and R_{max} is the maximum response (Myszka, Jonsen, & Graves, 1998). The experiment was performed in duplicate.

2.4 Preparation of aptamer particle-gelatin composite material

The preparation of the aptamer-gelatin composite material was reported in a previous study (Soontornworajit, Srakaew, & Naramitpanich, 2014). In brief, the aptamer-immobilized particles and control particles were prepared by incubating 2.5 nmol biotinylated PDGF-BB Apt and biotinylated S-Apt with 1 mg streptavidin-coated particles in PBS for 30 min. Next, a 30% (w/v) gelatin solution was reacted with equimolar of EDC/NHS at a carboxylic group to EDC ratio of 20. Consecutively, 120 µg aptamer-immobilized particles were incubated with 4 ng PDGF-BB in 20 µL PBS for 30 min at room temperature. The suspension was then gently mixed with 380 µL of the gelatin solution, which was preheated at 40 °C for 5 min, and aspirated using a micro-pipette for 5 min. The particle-gelatin suspension was transferred to a cylindrical mold and allowed to form a composite at 4 °C. The composite material was cut into small specimens (100 µL) for use in the weight loss and protein release tests.

2.5. Rheology characterization.

The storage moduli of the composite materials were characterized using a rheometer (Rheometric Scientific, ARES), to verify the mechanical properties after gelation. Specimens were prepared by casting 20 mL of 30% (w/v) gelatin solution and 20 mL particle-gelatin suspension onto a petri dish. The hydrogels were set under cold conditions. The specimens were then cut as circular discs (25 mm in diameter). The specimens were placed between two parallel plates covered by a temperature control chamber. A temperature of 25 °C was used in all experiments. A strain sweep was performed by varying the oscillation strain from 0.01 to 10% at a fixed frequency of 6 rad/s to indicate a linear viscoelastic regime. Frequency sweep experiments were then performed from 0.1 to 100 rad/s.

2.6 Weight loss and PDGF-BB release

Next, the release mechanism, weight loss, and PDGF-BB release were investigated. First, 100 µL of native gelatin, control particle-gelatin composite, and aptamer particle-gelatin composite were placed in microcentrifuge tubes containing 500 µL of release medium (PBS, 0.1% BSA, 0.09% NaN_3, and 0.05% Tween 20) and incubated at a temperature of 25 °C. During incubation, the samples were shaken by an orbital shaker (N-Biotek, South Korea). At designated time points, the native gelatin and composite materials were removed from the release medium and the materials were stored at 4 °C and the release medium at −20 °C. In the weight loss study, the native gelatin and composite materials were freeze-dried under vacuum (Flexi-Dry, FTS Systems Inc.) for approximately 18 h. The samples were then weighed using an analytical balance (METTLER TOLEDO) to determine the percentage weight loss. This was calculated as follows:

$$weight\ loss(\%) = \left(1 - \frac{W_t}{W_i}\right) \times 100,$$

where W_t is the weight of the native gelatin and the composites at the specified time point, and W_i is the initial weight, which was approximately 0.120 g. The initial weight of the materials was determined from samples that had not been treated with the release medium. In addition, the kinetics of the weight loss was tested by fitting the data to the zero-order kinetic model $\%W_t = \%W_0 + kt$, where $\%W_t$ is the percentage weight loss at a specified time point, $\%W_0$ is the percentage weight loss at the zero time point, and k is a rate constant of weight loss (%/h) (Guo & Kaletunc, 2016). In the release study, the amount of PDGF-BB in the release medium was quantified using PDGF-BB ELISA. The optical density of the ELISA plates was measured using a plate reader (Plate CHAMELEON, HIDEX, Finland). The percentage cumulative release was calculated as follows:

$$Cumulative\ release(\%) = \left(\frac{P_t}{P_f}\right) \times 100,$$

where P_t is the amount of PDGF-BB released from the composites at each designated time point and P_f is the amount of PDGF-BB released from the native gelatin at the final time point. All experiments were performed in triplicate.

2.7 PDGF-BB ELISA

An ELISA experiment was carried out to determine the amount of PDGF-BB in the release medium. First, 100 µL of anti-hPDGF-BB antibodies at a concentration of 0.4 µg/mL were incubated in each well of ELISA strip plates at 4 °C for approximately 18 h. The wells were washed three times with 300 µL of washing buffer containing PBS and 0.05% (v/v) Tween 20. Next, 300 µL of 1% BSA in PBS was added to each well, blocking the well surface. The blocking step was carried out for 2 h, and the wells were then washed three times using 300 µL of washing buffer. Next, 100 µL of release samples and standard protein solutions, reconstituted in the release media, were incubated in the coated wells for 2 h before being discarded. Washing was carried out three times. An amount of

100 µL of biotinylated antibodies at a concentration of 0.4 µg/mL were added to each well and further incubated for 2 h. After washing, 100 µL of streptavidin-HRP was added to the wells and incubated for 30 min. The streptavidin-HRP was discarded and the wells were washed three times with 300 µL of washing buffer. Finally, 50 µL of substrate was added. The optical density of the solutions was measured using the plate reader. The λ_{max} was set at 450 nm, and the background correction was assigned at 540 nm.

3. Results and Discussion

3.1 Phase transition temperature of gelatin

Upon cooling, the 5, 10, 15, 20, 25, and 30 % (w/v) gelatin underwent sol-gel transitions at 16.0, 18.0, 20.0, 22.0, 24.0, and 24.0 °C, respectively (Figures 1a and 1c). This confirmed that the gelatin concentration had an effect on the sol-gel transition temperature. However the transition reached a plateau at a concentration of 25% (w/v). Under heating, the 5, 10, 15, 20, 25, and 30% (w/v) gelatin underwent gel-sol transitions at 26.0, 28.0, 28.0, 30.0, 30.0, and 32.0 °C (Figures 1b and 1c). These results suggested that the transition temperature increased as the concentration of the gelatin increased. This effect may be due to chain entanglement between the gelatin molecules. Chain entanglement became more dominant as the proportion of gelatin molecules increased. Consequently, molecular mobility was retarded and the hydrogel became set (Zandi, Mirzadeh, & Mayer, 2007). In addition, the gel-sol transition temperature, determined by heating, was higher than the sol-gel transition temperature that was determined by cooling. This may suggest that the gelatin solutions were in a supercooled condition under the cooling rate applied in this experiment since this could outrun the sol-gel transition rate (Guigo, Sbirrazzuoli, & Vyazovkin, 2012). This supercooled condition existed at temperatures below the sol-gel transition temperature because the molecular mobility of the gelatin was slow enough to be maintained (Guigo *et al.*, 2012). However, the gelatin used in this study had transition temperatures lower than body temperature, which would make it impractical in real applications. To increase the phase transition temperature of the materials, a strategy based on increasing the molecular networks was applied. The molecular networks were strategically introduced to the gelatin using a coupling reaction between the amine and carboxylic groups along the gelatin chain triggered by adding EDC and NHS to the 30% (w/v) gelatin solution. The effect on the phase transition temperature of the carboxylic to EDC molar ratio was also investigated. After the coupling reaction had completed, the transition temperature of the modified gelatin was measured. The results are presented in Figure 2. The gelatin formed a permanent solid material when reacted with the coupling agent at a carboxylic group to EDC molar ratio of 10 (Figure 2, vii). It can be clearly seen that the temperature had no effect on the phase transition of this sample. The development of a permanent solid form indicated a large degree of crosslinking. Meanwhile, increasing the molar ratios from 20 to 40 produced a slight decrease in the sol-gel transition temperature of the reacted gelatin from 30.0 °C to 26.0 °C. The temperature then remained constant at molar ratios of 60, 80,

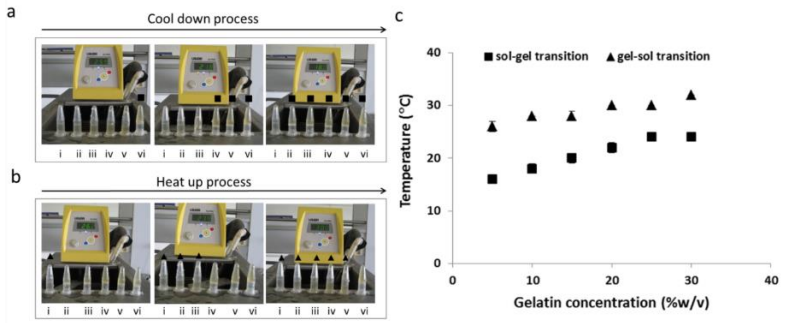

Figure 1. Effect of concentration on gelation temperature; a. images of gelatin solution under cool down process, b. images of gelatin solution under heat up process, c. phase transition plot. The (i), (ii), (iii), (iv), (v) and (vi) tube represent the gelatin solution with concentration of 5, 10, 15, 20, 25 and 30% w/v, respectively. (■): sol-gel transition, (▲): gel-sol transition.

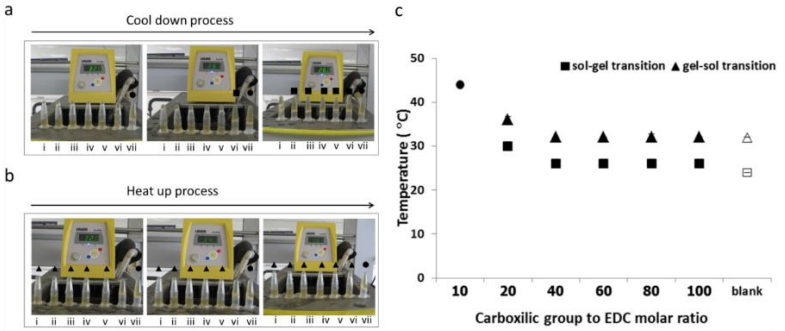

Figure 2. Effect of coupling agent concentration on gelation temperature; a. images of gelatin solution under cool down process, b. images of gelatin solution under heat up process, c. phase transition plot. The molar ratios of carboxylic group to EDC concentration are (i) no EDC, (ii) 100, (iii) 80, (iv) 60, (v) 40, (vi) 20 and (vii) 10.(●): solid gel under all experimental temperature, (■): sol-gel transition, (▲): gel-sol transition, (□, Δ): phase transition of unreacted gelatin.

and 100 (Figure 2c). This may be because the coupling reaction failed to introduce significant molecular networks into the gelatin. The results indicated that the transition temperature was elevated when a significant amount of EDC was used which led to the formation of significant amounts of molecular networks. When a large amount of EDC was added to the gelatin solution, a solid hydrogel formed immediately. Since this would be impractical in material fabrication or drug loading applications, in the rest of this work gelatin reacted with EDC and NHS at a molar ratio of 20.

3.2 Effect of temperature on aptamer-protein interaction

Since PDGF-BB aptamers were screened at 4 °C and 37 °C they were assumed to present the highest binding affinity at the screening temperature (Green *et al.*, 1996). However, the formation of a solid gel at temperatures between 4 and 37 °C observed in this study may affect the binding interaction of the incorporated aptamer. SPR spectrometry was therefore used to investigate the effect of temperature on the aptamer-protein interaction and to confirm the binding functionality of the aptamer. The SPR profiles (Figure 3a) showed the PDGF-BB aptamer binding to the target protein at a temperature of 25 °C, which was different from the screening condition. The binding data were further evaluated to determine the dissociation constant (K_D) using the equilibrium binding analysis method shown in Figure 3b. From the SPR analysis, the K_D values at 17, 25, 30, and 37 °C were 2.13, 2.90, 2.84, and 3.13 nM, respectively (Figure 3c). Since K_D is defined as the ratio between the dissociation rate (k-) and formation rate (k+) of the aptamer-PDGF-BB complex, the small K_D values represent a high binding affinity (Schuck, 1997). The SPR results also indicated that the binding of PDGF-BB aptamer varied inversely with temperature, so that the transition temperature of the gelatin would not compromise the functionality of the aptamer when used in the composite material.

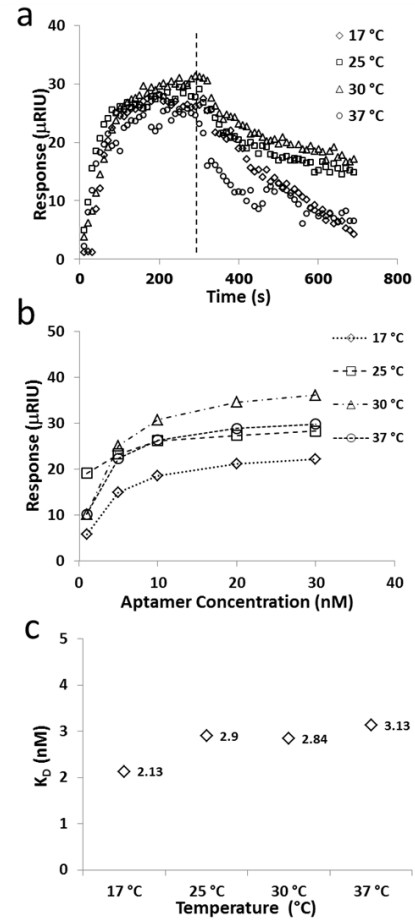

Figure 3. Effect of temperature on aptamer-protein interaction. a. SPR sensorgram: the dashed line indicates time point at which dissociation phase begins. b. Equilibrium binding plot. c. The dependence of dissociation constant (K_D) on temperature. The numbers indicate magnitudes of K_D.

3.3 Aptamer-gelatin composite material

The preparation of the aptamer-gelatin composite material followed the method of a previous study (Soontornworajit *et al.*, 2014). The overall preparation protocol is shown as Scheme1. First, the biotinylated aptamer was immobilized onto streptavidin-coated polystyrene particles. Next, the particles were mixed with gelatin solution at 40 °C and the suspension was cooled for gelation. Gelatin is prone to change its mechanical properties following modification, which would compromise its capability as a drug delivery system (Tungkavet, Pattavarakorn, & Sirivat, 2012). The storage modulus or rigidity of the composite materials after gelation was therefore tested (Figure 4). In a frequency sweep test, the plateaus reached by native gelatin, control composite, and aptamer-gelatin composite in the storage modulus were identified. This indicated the presence of networks produced by molecular entanglement of the gelatin molecules (Y. I. Chung, Lee, & Tae, 2006). The native gelatin, control composite, and aptamer-gelatin composite had average storage modulus values of 300, 286, and 316 kPa, respectively. Since these were of the same order of magnitude, no significant differences were found in their mechanical properties. This showed that the small amount of particles presents had no effect on the mechanical properties of the composites. This result was inconsistent with previous studies, which suggested that particle incorporation enhanced the mechanical properties of composites (Haraguchi, Farnworth, Ohbayashi, & Takehisa, 2003).

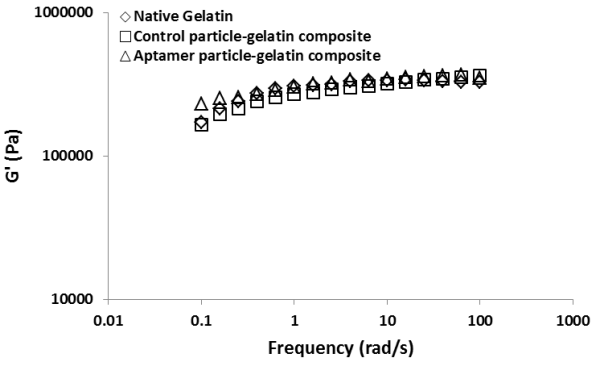

Figure 4. Effect of aptamer-immobilized particles on storage modulus (G') of the composite.

3.4 Weight loss study

To further investigate the role played by the modified particles in the physical property of the materials, a weight loss study in the release medium was conducted under ambient conditions. At one hour of incubation, the native gelatin, control particle-gelatin composite, and aptamer particle-gelatin composite showed weight losses of 24.9%, 26.2%, and 25.4%, respectively (Figure 5). Thereafter, the three materials showed a linear dependence between weight loss and time of incubation with overall rates of 0.28, 0.29, and 0.35 %/hour, respectively. This small difference in the weight loss characteristics of the materials suggested that no interaction had taken place between the naked particles themselves or the aptamer-immobilized particles and the gelatin matrix. These results were consistent with those from the rheological measurements in the earlier study. In addition, the rate of weight loss for each composite material was constant over time (Table 2) and followed a zero-order mechanism similar to that demonstrated for poloxamer-based materials (Liu *et al.*, 2007).

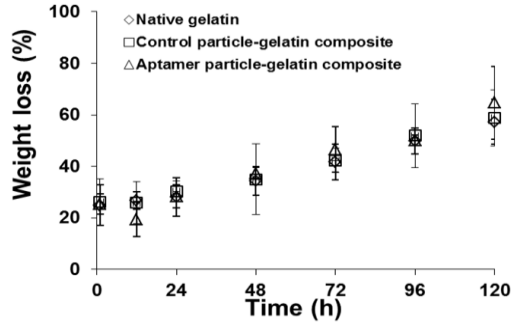

Figure 5. Effect of aptamer-immobilized particles on weight loss of the composite. The error bars indicate one standard deviation (n=3).

Table 2. Rate constant of weight loss for each composite material.

	rate constant (%/h)	R^2
native gelatin	0.2871	0.9875
control particle-gelatin composite	0.286	0.9829
aptamer particle-gelatin composite	0.3491	0.9544

3.5. PDGF-BB Release

The goal of the study was to develop aptamer-gelatin composite materials that are capable of prolonging the release rate through binding interaction between the target protein and a nucleic acid aptamer. The composite materials were prepared simply by mixing aptamer immobilized particles with the modified gelatin. The overall protocol was similar to that reported in a previous study (Soontornworajit, Zhou, & Wang, 2010). A release experiment was performed to test the hypothesis that aptamer could be used as a binding site for a target protein, which in this case was PDGF-BB. This would slow the protein release rate. By the end of the first 24 h, 61% and 52% of the PDGF-BB molecules were released from the native gelatin and the control material (Figure 6). In contrast, only approximately 17% had been released from the aptamer-gelatin-composite material. This suggested that a significant burst release had taken place from the non-aptamer materials and this was significantly reduced by the aptamer-protein interaction. In addition, the composite material prolonged the release period of the PDGF-BB with an average daily release rate of 1.2%. This was attributed to strong binding between the aptamer and the protein. In principle, aptamer-protein interactions are predominated by base pairing and the resulting secondary structure of the aptamer (Jayasena, 1999).

Interpretation of the weight loss and protein release data sheds more light on the protein release behavior. Initially, the native gelatin and the control particle-gelatin composite showed a rapid rate of weight loss and a burst release of protein, suggesting that the protein release was governed simultaneously by material dissolution and protein diffusion caused by the concentration gradient between the gelatin and PDGF-BB protein. In contrast, the aptamer particle-gelatin composite showed a smaller burst release and slower daily release rate. This release profile suggested that two different mechanisms were controlling the PDGF-BB release and clearly identified the role played by aptamer-protein interaction. The controlled release was therefore due to the aptamer-immobilized particles. This aptamer incorporation strategy is flexible and can be applied to other systems such as poloxamer and agarose based materials (Soontornworajit, Zhou, & Wang, 2010; Soontornworajit, Zhou, Zhang, *et al.*, 2010).

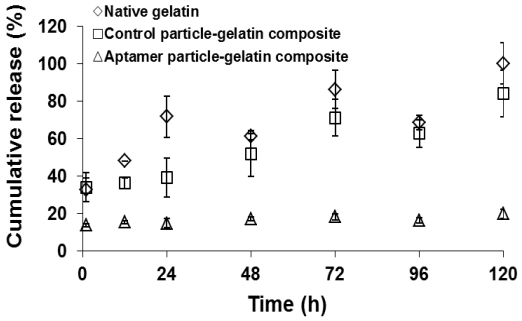

Figure 6. Cumulative release of PDGF-BB from the composite. The error bars indicate one standard deviation (n=3).

4. Conclusions

In this study, gelatin was shown to form a stable solid hydrogel under ambient conditions following the addition of EDC/NHS as the coupling agent. SPR analysis demonstrated that the PDGF-BB interacted strongly with its aptamer and allowed the composite to be prepared and the release experiment to be conducted under ambient conditions. In contrast with the native gelatin, the composite showed no change in rheological properties or rate of weight loss. The PDGF-BB release profile suggested that the slow release was due to strong binding interaction between the protein and its aptamer. Overall, the study demonstrated the successful creation of an aptamer-gelatin composite that was able to slow protein release. This aptamer-based material is a promising delivery tool with a number of biological and biomedical applications.

Acknowledgements

The authors thank Dr. Sitthisuntorn Supothina for use of the lab equipment, Dr. Pichayanoot Rotkrua and Mr. John Winward for comments on the manuscript, and Dr. C. Vijaya Kumar for technical assistance in using the SPR instrument**.** This work was granted by Thammasat University in the fiscal year 2555, BE, and a Research Grant for New Scholar supported by the Office of Higher Education Commission, the Thailand Research Fund and the Thammasat University (MRG5580187).

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