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Drug-eluting coating of ginsenoside Rg1 and Re incorporated poly(lactic-*co*-glycolic acid) on stainless steel 316L: Physicochemical and drug release analyses

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Abstract:
Active ingredients of ginsenoside, Rg1 and Re, are able to inhibit the proliferation of vascular smooth muscle cells and promote the growth of vascular endothelial cells. These capabilities are of interest for developing a novel drug-eluting stent to potentially solve the current problem of late-stent thrombosis and poor endotheliazation. Therefore, this study was aimed to incorporate ginsenoside into degradable coating of poly(lactic-*co*-glycolic acid) (PLGA). Drug mixture composed of ginseng extract and 10% to 50% of PLGA (*x*PLGA/g) was coated on electropolished stainless steel 316L substrate by using a dip coating technique. The coating was characterized principally by using attenuated total reflectance-Fourier transform infrared spectroscopy, scanning electron microscopy and contact angle analysis, while the drug release profile of ginsenosides Rg1 and Re was determined by using mass spectrometry at a one month immersion period. Full and homogenous coating coverage with acceptable wettability was found on the 30PLGA/g specimen. All specimens underwent initial burst release dependent on their composition. The 30PLGA/g and 50PLGA/g specimens demonstrated a controlled drug release profile having a combination of diffusion- and swelling-controlled mechanisms of PLGA. The study suggests that the 30PLGA/g coated specimen expresses an optimum composition which is seen as practicable for developing a controlled release drug-eluting stent.

Keywords: Dip coating; drug release; ginsenoside; poly(lactic-*co*-glycolic acid); stent

1. Introduction

In the South Asia region, the high incident of morbidity and mortality caused by cardiovascular disease (CVD) is expected to increase even more due to high prevalence of diabetes, hypertension and smoking [1]. Statistical data from the Ministry of Health Malaysia in 2013 show that diseases of the circulatory system are the number one cause of death in public and private

hospitals with 24.38% and 27.73% of total deaths, respectively [1]. Besides, the Global Atlas on cardiovascular disease prevention and stroke report states that low and middle income countries projected to have more than 80% of CVD deaths [2]. The World Health Organization predicts more than 23.3 million of people would die annually from CVDs by 2030 [3]. Therefore, it is vital to take active prevention and offer more affordable treatment of CVDs due to increasing number of cases in the low and middle income countries.

Atherosclerosis has been the leading cause of CVDs created by the accumulation of fatty plaque and cholesterol that later thicken and narrow coronary arterial blood vessels [3]. One of the current most used treatments for CVD is the implantation of stent that acts as a scaffold to mechanically widen the narrowed blood vessel [4]. The high incident of restenosis associated with stent's post-implantation has led to the development of second generation of stent, the drug-eluting stent, which is coated with drug to prevent the proliferation of vascular smooth muscle cells [5-7]. However, most of drug-eluting stents face the main problem of delayed endotheliazation due to rapid release of anti-proliferative drug and polymer hypersensitivity leading to late-stent thrombosis [7]. In order to overcome this problem, development of a multifunctional drug coating is crucial to promote the growth of vascular endothelial cells while inhibiting the proliferation of vascular smooth muscle cells.

Ginseng root has been used since five centuries ago as a medicinal herb in traditional Chinese medicine and still in use as a health supplement worldwide [8]. Ginsenosides are the active ingredient of Asian ginseng, scientifically known as Panax Ginseng and has been studied intensively for its effect on cardiovascular system such as improving circulation and antioxidant activity, modulating vascular function, improving cardiac function, inhibiting platelet aggregation and adjusting lipid profile [8]. In CVD treatment, ginsenoside Rg1 suppress the mitogen-activated protein kinases (MAPK) pathway to serve as an anti-inflammatory agent and to inhibit a shear-induced inflammation [9]. This ginsenoside can also stimulate nitric oxide synthase expression, nitric oxide production and reduce oxidative stress resulting into inhibition of neointimal hyperplasia [10]. While ginsenosides Rb3, Rp1 and Rp4 are significantly important in the treatment of CVD as a therapeutic agent for myocardial ischemia-reperfusion injury [11]. Certain types of ginsenosides such as Rb1, Rg1, Rg3 and Re are able to inhibit the proliferation of vascular smooth muscle cells and promote the growth of endothelial cells [8, 12-14] which beneficial to be used as herbal-based drug to develop a novel drug-eluting stent.

Among the polymers that have been used as coating on drug-eluting stents are poly(lactic acid), poly(glycolic acid), poly(D,L-lactide acid), poly(caprolactone) and poly(lactic-*co*-glycolic acid) or PLGA [15]. PLGA is a copolymer of lactic and glycolic acid that degrades by hydrolysis of its ester linkages in the presence of water [15]. Since its approval by the United States Food and Drug Administration, PLGA has been utilized in many biomedical devices such as graft, sutures, implants and nanoparticles [16]. In this study, PLGA was chosen as the matrix to carry and deliver the ginsenosides Rg1 and Re by coating selected PLGA/ginsenoside mixtures on a stainless steel 316L (SS316L) substrate using a dip coating technique. The physicochemical properties of the coating were characterized principally by using attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR), scanning electron microscopy (SEM) and contact angle analyses. A quadrupole time-of-flight liquid chromatography mass spectrometry instrument (Q-TOF LC/MS) was then used to identify the drug release profile over a one month immersion period.

2. Materials and Methods

2.1 Materials preparation: All chemical reagents in the sample preparation process except for PLGA and ginseng extract were purchased from Quality Reagent Chemical (New Zealand). Thin foil medical grade SS316L (Goodfellow Cambridge Limited, Huntingdon, England) with a thickness of 0.25 mm was cut into 10 mm square shape specimens. They were first pre-treated by ultrasonic cleaning in acetone, deionized water and methanol for 10 minutes to free the metal surface from organic contaminants. They were then electropolished in an electrolyte consisted of 50% glycerol, 35% phosphoric acid and 15% distilled water to remove the original oxide layer and macro level surface defects [17]. The electropolishing was performed at 80^{o}C for 6 minutes under 1.5 A and 6.0 V direct current voltage. The mirror-shined electropolished specimens were then rinsed with deionized water and dried in a desiccator until further used as coating substrate.

PLGA (LA:GA ratio of 85:15 and inherent viscosity of 0.63 dL/g, LACTEL Absorbable Polymers, USA) and ginseng extract or ginsenoside (Dalian Hongjiu, Biotech, China) were dissolved at a final concentration of 5% (w/v) and a total volume of 10 mL. The PLGA was firstly dissolved in 10 mL of dimethyl sulfoxide (DMSO) with various ratio between 10 - 50% (w/w) and then the ginsenoside was added to the PLGA solution as detailed in Table 1. The composition of ginsenosides Rg1 and Re in the ginseng extract was verified by liquid

chromatography-mass spectrometry as given in Supplementary file, Fig. S1. The dark brown drug mixture was stirred at 600 rpm for 16 hours for complete blending and viscosity of the mixture was measured by a rotation viscometer (Brookfield DVII Pro viscometer, Brookfield AMETEK Inc., USA) using a CPE41 spindle. Five readings were taken with an increment speed of 5 rpm for each measurement. Deposition amount of the coating was calculated using the following equation (1) where w_f is the final weight after coating, w_i is the initial weight and A is the total surface area [18].

$$Deposition\ amount\ (mg/cm^2) = (w_f - w_i) / A \qquad (1)$$

Table 1 Composition of PLGA/ginsenoside mixtures in 10 mL DMSO for coating application

Sample	PLGA		Ginsenoside	
	Weight (g)	Composition (w/w%)	Weight (g)	Composition (w/w%)
Ginsenoside	0	0	0.50	100
10PLGA/g	0.05	10	0.45	90
30PLGA/g	0.15	30	0.35	70
50PLGA/g	0.25	50	0.25	50

The drug mixture was coated on the electropolished metal specimens by using a desktop dip coater (HTWL-01 Desktop Dip Coater, MTI Cooperation, USA) at room temperature. The dip coating was done at dipping time and withdrawal rate set at 120 seconds and 200 mm/min, respectively [18]. The coated metals were dried for 5 minutes to allow coating attachment. The coating process was then repeated for five times to obtain thicker and evenly coating. Three group of coated metal specimens were obtained namely 10PLGA/g, 30PLGA/g and 50PLGA/g, while bare electropolished SS316L specimen and 100% ginsenoside coated served as the control and for comparison purposes. The final coated metals were air-dried and kept in a desiccator for further analyses.

2.2 Surface characterization: Chemical composition of the coating was analyzed by using an ATR-FTIR instrument (Nicoler iD5, Thermo Scientific, USA) with a diamond crystal. The scanning resolution was set at 1.0 cm^{-1} with 32 average scans within 400-3500 cm^{-1} frequency range. SEM observation was done to visualize microstructure, morphology and thickness of the coating. The coating thickness was visualized and measured at an operating voltage of 15 kV using a tabletop SEM (TM300, Hitachi, Japan) at COMPO mode, while the morphology of the coating was further viewed using a higher resolution SEM (XL40, Philips, Netherland) at an operating voltage of 25 kV. Finally, the contact angle analysis was performed to measure the wettability property of the coating by using a contact angle instrument (VCA Optima, AST product inc., USA). A 23 gauge needle was used to dispense 2 µL of deionized water on the coating surface. The readings were recorded for three times to obtain an average data. Additional analyses using XRD (Siemens-D500, Germany), with CuKα line generated at 40 kV and 35 mA, and DSC (STA8000, Mettler, USA) were performed to verify any change on the physicochemical property of the coating in-term of crystallinity and state of transition.

2.3 Drug release test: Drug release test of the coating was done by ageing the ginsenoside coated and the 10PLGA/g, 30PLGA/g and 50PLGA/g coated specimens in 3 mL of Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin (Thermo Scientific, Massachusetts, USA). The DMEM solution was chosen to closely imitate the drug release condition in a physiological environment of blood vessel. The coated metals were incubated at 37°C with the supplement of 5% CO_2 and 95% humidity to mimic a human physiological environment. The drug release test was performed for one month by collecting the aged coated metals and aged media at day 1, 4, 14, 21 and 30.

The aged specimens were observed under SEM to measure the thickness of the remaining coating film. While the samples of aged media were subjected to Q-TOF LC/MS analysis using an Agilent 1200 Series liquid chromatograph system (Agilent Technologies, Palo Alto, USA) equipped with a binary pump, an online degasser, an auto-sampler and a thermostatically controlled column compartment. A total of 10 µL of the aged media were injected into Agilent Zorbax Eclipse Plus C18 Rapid Resolution HT column (2.1 mm x 100 mm x 1.8 µm) and the separation was run at 30°C. The mobile phase consisted of a mixture of solvent A (0.1% formic acid) and solvent B (acetonitrile with 0.1% formic acid) using an isocratic elution (5% B at 0 to 2 minutes; 98% B at 20 to 22 minutes; and 5% B at 25 to 30 minutes) was set at a flow rate of 0.5 mL/min.

Mass spectrometry analyses were finally performed using 6540 UHD Accurate-mass Q-TOF LC-MS equipped with dual AJS ESI ionization source system operating in a negative mode. The mass range was set between m/z 100 and 3000. The condition of ionization source was: gas temperature of 300°C; drying gas (N_2) flow rate of 8 L/min; nebulizer of 35 psig; sheath gas temperature of 350°C; sheath gas flow of 11 L/min; capillary voltage of 3500 V in a negative mode; fragmentor of 175 V; skimmer voltage of 65 V; OCT RF V of 750 V; TOF vacuum of 2.2×10^{-7} Torr; and Quad vacuum of 4.08×10^{-5} Torr. The energy for collision-induced dissociation was set from 0 to 40 V for

precursor ion. Agilent LC-MS-Q-TOF MassHunter data acquisition and analysis software B.06.00 were controlling the acquisition and data analysis, respectively.

3. Results and Discussion

Table 2 shows the properties of coated metal specimens at different coating composition. Increased amount of PLGA resulted in higher viscosity of coating mixture, higher amount of coating deposition and thicker coating. The viscosity of coating mixture affected the coating mixture retention during the withdrawal stage of dip coating and eventually the coating thickness. According to Grosso [19], dip coating setting that follows the Landau-Levich equation has a coating thickness (h), dependent on mixture viscosity (η), withdrawal speed (v), surface tension (γ), density (ρ), and gravitational acceleration (g) as shown in the following equation (2). Therefore, as shown in Table 2 the coating thickness was directly proportional to the increase of mixture viscosity as withdrawal speed and gravitational acceleration remained constant. The coating thickness for 30PLGA/g and 50PLGA/g falls within the typical range of the commercial drug-eluting stent which is between 5 and 16 µm.

$$h = 0.94\,[(\eta \times v)^{2/3} / \gamma^{1/6}(\rho \times g)^{1/2}] \qquad (2)$$

The most common method and technique used for coating a metal stent with drugs or degradable polymers include dip coating, ultrasonic spray and electrophoretic deposition [20-23]. Among them, dip coating is considered as the most low-cost and simplest technique for organic and inorganic coating materials with high coating quality without the involvement of high processing temperature [21]. Dip coating has been applied for production of drug-eluting stents due to its simplicity, ability to coat complex strut design and high productivity in processing large number of stents efficiently [24]. However, align with the focus of this study, we performed the coating of PLGA/ginseng mixture on flat metal substrate instead on a stent prototype for the ease of characterization and testing.

Table 2 Properties of drug mixture solution and coating of ginsenoside coated, 10PLGA/g, 30PLGA/g and 50PLGA/g coated specimens

Sample	Viscosity (mPa.s)	Deposition amount (mg/cm²)	Thickness (µm)	
			As deposited	After 1 month immersion
Ginsenoside	1.32	0.06 ± 0.01	1.1 ± 0.2	0.0 ± 0.0
10PLGA/g	1.40	0.07 ± 0.02	2.3 ± 0.3	0.3 ± 0.1
30PLGA/g	3.34	0.09 ± 0.02	10.0 ± 0.4	1.5 ± 0.1
50PLGA/g	4.26	0.11 ± 0.01	11.9 ± 0.5	5.2 ± 0.3

3.1 FTIR analysis: Figure 1 shows ATR-FTIR spectra of bare SS316L control, ginsenoside powders, PLGA, 10PLGA/g, 30PLGA/g and 50PLGA/g coated specimens. The control produced no identical peaks indicated that the metal surface was free of hydroxide layer after been electropolished. While for all 10PLGA/g, 30PLGA/g and 50PLGA/g coated specimens, the ATR-FTIR spectra demonstrated strong peaks of PLGA profile between 1730 and 1750 cm^{-1} due to carbonyl C=O stretching of its aliphatic ester group.

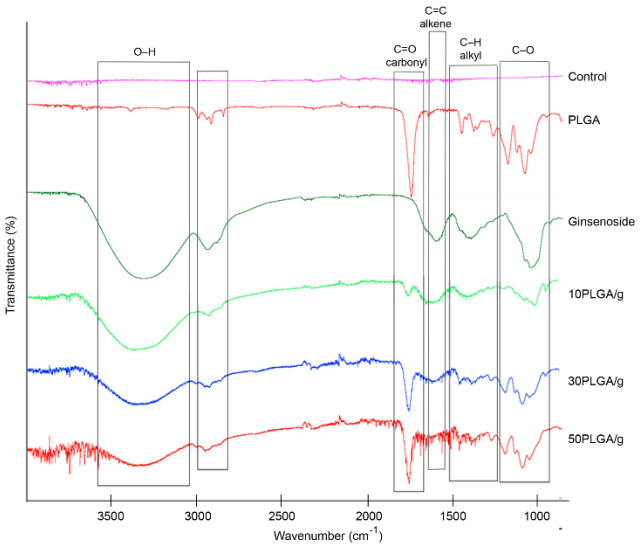

Figure 1 ATR-FTIR spectra of control (bare SS316L substrate), ginsenoside powders, PLGA, 10PLGA/g, 30PLGA/g and 50PLGA/g coated specimens.

Strong and broad peaks of O–H stretch and medium peaks of C=C stretch of alkene were noticed at 2850 - 3500 cm^{-1} and 1620 - 1680 cm^{-1}, respectively, validating the presence of ginsenoside. Furthermore, the C–O stretch at 1050 - 1250 cm^{-1} and other peaks of alkyl group consisted of alkyl CH_3 bond at 1380 - 1385 cm^{-1} and alkyl CH_2 bond at 1450 - 1470 cm^{-1} were also found on the spectra which arisen from the combination PLGA and ginsenoside composition. The intensity of PLGA determinant on the 50PLGA/g was higher than the 30PLGA/g and 10PLGA/g specimens which was identical to the total amount of PLGA composition. Similar condition was observed on the intensity of ginsenoside determinant. All these ATR-FTIR findings semi-quantitatively confirm the presence of ginsenoside in all coating which later will be quantitatively validated by mass spectrometry analysis in the drug release profile determination.

3.2 SEM and contact angle analysis: Figure 2(a) shows SEM images of bare SS316L control, 10PLGA/g, 30PLGA/g and 50PLGA/g coated metal specimens. The control's surface contained few pits left by inclusions

that were removed during the electropolishing. The 10PLGA/g specimen showed thin, homogenous and less coverage coating as indicated by the presence of few dark spots as observed on the control. The 30PLGA/g specimen showed thicker, more coverage and moderate homogenous coating whereas the 50PLGA/g specimen displayed the thickest, full coverage and non-homogenous coating as indicated by the bright clumps on the surface.

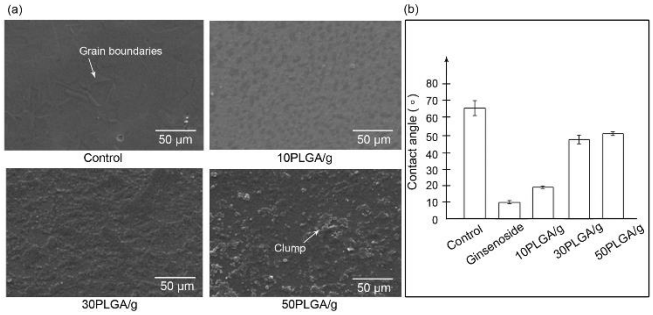

Figure 2 (a) SEM images of control, 10PLGA/g, 30PLGA/g and 50PLGA/g coated specimens and (b) contact angle data of control, ginsenoside coated, 10PLGA/g, 30PLGA/g and 50PLGA/g coated specimens.

The less coverage coating observed on the 10PLGA/g specimen is due to surface energy and surface tension between the metal and the PLGA/ginsenoside drug mixture solution. Based on the contact angle data in Figure 2(b), the bare SS316L control has the most hydrophobic surface indicating low surface energy compared to the surface of ginsenoside coating. Large gap in the surface energy between the metal and the drug mixture solution cause difficulties for the metal surface to overcome the surface tension of the solution, thus making it harder for the solution to adhere on the metal surface [25].Besides, the first layer of coating film of 10PLGA/g specimen was washed away during the subsequent dipping as it inclined to adhere to the bulk drug mixture solution than the metal surface.

For the 30PLGA/g and 50PLGA/g specimens, the difference surface energy was reduced, thus resulting into better adhesion and better coverage of the coating. However, the SEM images showed a non-homogenous coating at higher PLGA content (50PLGA/g specimen) due to drug mixture solution cohesiveness and solidification period during the dipping process [25]. Even though the 30PLGA/g and 50PLGA/g specimens have better coating coverage than the 10PLGA/g specimen, the non-homogenous coating was still observed after the first dipping cycle due to natural dip coating “wedge effect” caused by gravity force [26]. This effect made variation to the film thickness from top to bottom producing a thin coating at the top of specimen and thick coating at the bottom [26]. High viscosity drug mixture solution produced more “wedge effect” to a point where the solution’s cohesion was stronger than its adhesion to the metal surface [26]. This cohesive strength was derived by several molecular forces such as chemical bonds within the PLGA, intermolecular interaction, mechanical bonds and interaction between the PLGA molecules that all affected consistency of drug mixture solution, flow property and viscosity.

Higher cohesive than adhesive force resulted in poor coverage of the first coating layer where some volume of drug mixture solution spread and adhered to the metal surface while some other with strong cohesive force clustered on the surface. The solidification time of the first layer also varies where the upper thin film solidified faster than the bottom thick film. The changes of cohesiveness occurred when the drug mixture solution underwent solidification through a formation of new bonds, thus strengthening the existing bonds that built the crosslinking of short chain molecules to form longer chains [25]. These longer chains formed a three dimensional network of molecular orientation, making the solidified coating more cohesive than in the liquid form [25]. The poor coverage combined with the incomplete solidification created variety of surface energy on the first coating layer’s surface that affected the adhesion of subsequent layers. At the second dip, the drug mixture solution has weaker cohesion compared to the solidified first layer, possessed higher adhesive force due to small difference in the surface energies between the solidified first layer film and the mixture solution, thus forming a better coverage coating [25].

From a wettability perspective, a hydrophobic surface is preferred for stent implant as it can avoid the adhesion of inflammatory molecules, such as leukocyte, platelet, and growth factors from the blood stream, on the luminal side of new implanted stent which can further aggravate inflammation [27]. However, extremely hydrophobic polymer surface is not preferred as it induces hypersensitivity to the lumen and causes restenosis as observed in the case studies of non-degradable hydrophobic polymers used in the first generation of drug-eluting stents [27].

The additional XRD analysis (Supplementary file, Fig. S2a) shows that only peaks correspond to the metal substrate are observed with an indication of non-identical XRD pattern between the ginseng coated and 30PLGA/g specimens. Meanwhile, the DSC thermogram of 30PLGA/g specimen (Fig. S2b) indicates an unclear phase transition of the coating. These findings could be due to the fact that the coating process did not involve high temperature, thus the crystallinity of the PLGA remained low.

Table 3 Release kinetics data of ginsenosides Rg1 and Re for ginsenoside coated, 10PLGA/g, 30PLGA/g and 50PLGA/g coated specimens

Sample	Zero order		First order		Higuchi		Hixson-Croswell		Korsmeyer-Peppas	
	r^2	$K_0(h^{-1})$	r^2	$K_1(h^{-1})$	r^2	K_H	r^2	$K_{HC}(h^{-\frac{1}{3}})$	r^2	n
Rg1										
Ginsenoside	0.55	0.11	0.65	25.33×10^{-4}	1.00	1.19	0.86	0.50×10^{-2}	1.00	0.11
10PLGA/g	0.86	0.12	0.96	27.64×10^{-4}	0.96	3.24	0.93	0.32×10^{-2}	0.99	0.45
30PLGA/g	0.88	0.06	0.96	16.12×10^{-4}	0.96	2.66	0.93	0.21×10^{-2}	0.94	0.56
50PLGA/g	0.88	0.06	0.92	6.91×10^{-4}	0.94	1.65	0.91	0.11×10^{-2}	0.93	0.56
Re										
Ginsenoside	0.49	0.11	0.48	23.03×10^{-4}	1.00	0.80	0.81	5.2×10^{-2}	1.00	0.07
10PLGA/g	0.86	0.13	0.96	32.24×10^{-4}	0.96	3.43	0.93	0.36×10^{-2}	0.98	0.45
30PLGA/g	0.91	0.10	0.98	18.42×10^{-4}	0.98	2.90	0.96	0.24×10^{-2}	0.97	0.58
50PLGA/g	0.87	0.07	0.92	9.21×10^{-4}	0.94	1.93	0.90	0.31×10^{-2}	0.92	0.57

3.3 Drug release analysis: The coating thickness before and after the one month immersion test is listed in Table 2. After a month of immersion, the ginsenoside coated and 10PLGA/g coated specimens loss more than 95% of their coating thickness whereas the 30PLGA/g and 50PLGA/g specimens loss 85.0% and 56.3%, respectively. These results clarified that the PLGA was able to control the release of ginsenoside. The hydrophobicity of PLGA reduced the penetration of DMEM media to dissolve the coating film as higher dissolvability was found on the specimens with less PLGA amount. There are several other factors that need to be considered such as polymer swelling and deposition of halite or other debris on the coating that may contributed to the measured thickness [28]. However, decreased in coating thickness after the immersion testified that there was no large formation of new materials that can increase or expand the coating more than its initial thickness.

The drug release data from the aged DMEM media were fitted to five mathematical models to predict the release mechanism. The release profile of ginsenosides Rg1 and Re for all coated specimens is shown in Figure 3. The ginsenoside Rg1 release profile shows that within 30 days, the amount of drug release was 1.25, 1.03, 0.92 and 0.52 g/L for the ginsenoside coated, 10PLGA/g, 30PLGA/g and 50PLGA/g coated specimen, respectively. Those amounts are higher than the minimum amount of ginsenoside Rg1 (0.020 g/L) required to inhibit the proliferation of vascular smooth muscle cells [14, 29]. While the ginsenoside Re release profile shows that within 30 days, the amount of drug release was 1.26, 1.15, 0.97 and 0.57 g/L for similar respected specimens. These values are also higher than the minimum amount of required ginsenoside Re (0.03 g/L) to promote the growth of vascular endothelial cells [30]. The initial burst release of drugs was due to the hydrophilicity of ginsenoside that might be beneficial for rapid wound healing of endothelial cells within 24 hours of lesion caused by balloon inflation [31, 32].

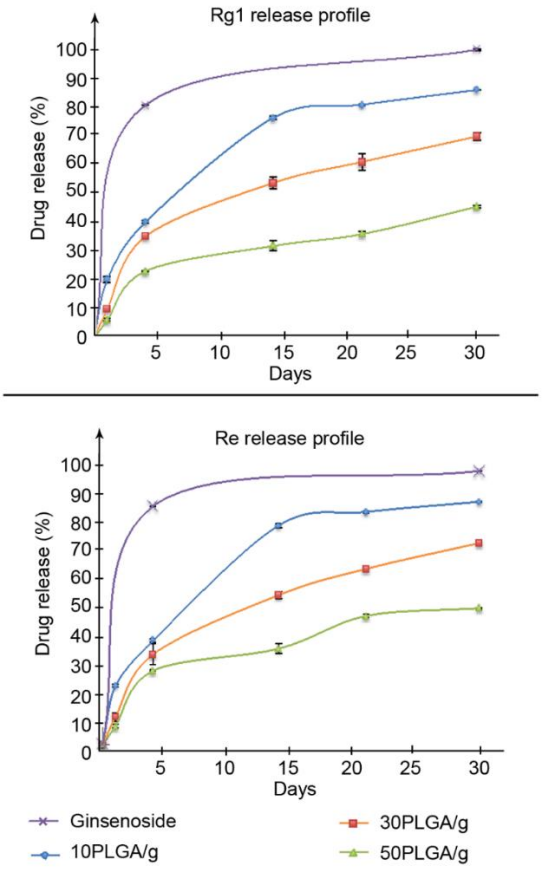

Figure 3 Ginsenoside Rg1 and Re release profiles for ginsenoside coated, 10PLGA/g, 30PLGA/g and 50PLGA/g coated specimens.

The ginsenoside Rg1 and Re were continuously released until day 30 by all coated specimens. However, the ginsenoside coated specimen was not able to produce a gradual drug release compared to the 10PLGA/g, 30PLGA/g and 50PLGA/g specimens. These drug release profiles clearly demonstrate that the PLGA played a role in maintaining a gradual and continuous release of ginsenosides Rg1 and Re. Gradual and continuous release of both active ingredients are very important to maintain its significant advantages on vascular tissues, thus preventing future late thrombosis. Furthermore, all drug release data were best fitted the Higuchi release model as shown by the comparison of kinetics released in Table 3. However, the usage of Higuchi equation in this drug system is not suitable as the matrix consisted of polymer that undergo swelling when degraded, thus causing variable diffusion coefficient. The second best fitted graph was the Korsmeyer-Peppas model that consider the polymer swelling and it is used to explain the drug release mechanism. Only the 30PLGA/g and 50PLGA/g specimens showed a combination of diffusion-controlled and swelling-controlled releases ($0.5 < n < 1$) whereas the 10PLGA/g and ginsenoside coated specimens followed a Fickian diffusion ($n < 0.5$) of thin film. This analysis suggested that a minimum of 30% PLGA is required to obtain a controlled drug release coating.

It is worth noted that numerous studies focusing on the most common ginsenosides (Rb1, Rd, Rg3 (protopanxadiol type) Rg1 and Re (protopanaxatriol type)) have confirmed their therapeutic effects on CVD such as antioxidant activity, vascular function modulation, vasomotor functions adjustment, platelet aggregation inhibition, lipid profile adjustment and Ca^{2+} channels regulation [12]. Ginsenoside Rg1 selectively inhibits tumor necrosis factor (TNF-α)-induced vascular smooth muscle cells in a dose-dependent manner [33-35]. It will directly retard the TNF-α-induced vascular smooth muscle cells by causing cell cycle arrests at G1 phase via inactivation of MAPK and PI3K/Akt pathways [36], and it may also protect the endothelial cells that being stimulated by the same cytokines. Ginsenoside also showed anti-proliferative activity towards vascular smooth muscle cells by regulating the production of nitric oxide [37]. The nitric oxide has an ability to inhibit platelet aggregation, induce endothelial cells growth, retard smooth muscle cells proliferation and delay leukocyte chemotaxis [38-40]. Therefore, the successful incorporation of ginsenosides Rg1 and Re on PLGA coating portrays a potential for developing a novel herbal-based drug-eluting stents.

Conclusion

Ginsenosides Rg1 and Re have the ability to promote the growth of vascular endothelial cells and to inhibit the proliferation of vascular smooth muscle cells, the events desirably occurred during coronary stent implantation. The successful development of optimal PLGA/ginsenoside coating on stainless steel substrate brings up a potential alternative to the current drug-eluting stent development. Among all studied PLGA/ginsenoside coating composition, the 30% PLGA/70% ginsenoside composition produces a homogenous and well coverage coating with 10 μm thickness that falls within the range of current commercial drug-eluting stent, and acceptable wettability identical to ginsenoside and PLGA determinants. This composition also demonstrates a drug release profile governed by the combination of diffusion- and swelling-controlled of PLGA with 10% drug release in a month which is desirable to provide optimum ginsenoside dose over a period of time. This study suggests the potential application of PLGA/ginsenoside coating for developing a novel herbal-based drug-eluting stents.

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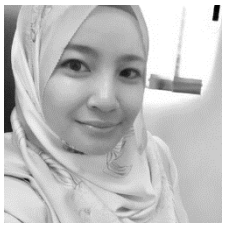

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