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Nano-encapsulation of tea polyphenols/ catechins in poly(D,L-lactic-co-glycolic acid) biopolymer and its biological activity

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Abstract: Tea is the second most consumed beverage in the world. Clinical and epidemiological study has revealed usefulness of green tea ingredients in various medicinal applications. Many reports suggested that the green tea consumers have reduced risk of generating cardiovascular, neurodegenerative and cancer diseases. This can be attributed to some of the potent active metabolites present in the green tea. Fractions of green tea polyphenols called catechins posses higher medicinal values among others. However, polyphenols/catechins are unstable and poorly absorbed. In this work, polyphenols and catechins are encapsulated in the poly (D, L-lactic-co-glycolic acid) nanoparticles and their biological activity after release was tested.

Keywords: *Catechins; Nanoparticles; Polyphenols; PLGA*

Introduction

Tea has been associated with many health benefits which are due to its active ingredients called "polyphenols".[1] These polyphenols form a are part of the flavonoid group and are also found in red wine, black grapes, cocoa, beans and Chinese rhubarb.[2] In green tea, the most common polyphenol is "catechin" which comprises 80% polyphenols. Catechins constitute 20–30% of the dry weight of the tea leaves. Catechins are most active compound found in the leaves of the tea plant, i.e. *Camellia sinensis*.[3] Catechins posses medicinal values and are believed to provide protection against various health issues. The antioxidative property of catechins is useful against a number of diseases such as cancer, cardiovascular and neuro-degenerative diseases.[4] Unfortunately, catechins are poorly sustainable in the circulatory system, and stability of catechins is very low even at normal physiological pH.[5] Since its bio-availability is very less, it eventually fails to protect or cure the disease. So, there is a possibility to protect it from physiological and oxidative damage to increase stability by application of nanotechnology.

Nanoparticles can be prepared from a variety of materials such as protein, polysaccharides and synthetic polymers.[6] The first naturally occurring material used for the preparation of nanoparticles is consist of two proteins, "albumin" and "gelatin".[7] Poly(D,L-lactic-co-glycolic acid), i.e. PLGA have been extensively studied for different therapeutic applications such as sustained drug release and gene delivery.[8] PLGA is a copolymer with a high bio-compatibility and a convenient biodegradability inside the body into its original monomers which are by-products of many metabolic pathways naturally occurring in the human body.[9,10] It is used for therapeutic purposes owing the advantage of being approved by Food and Drug Administration (FDA). Less toxicity, high safety and controlled drug release make PLGA an important polymer for drug delivery. PLGA-based nanoparticles are very versatile and biocompatible.[11] Objectives of the present study are to synthesize nanoparticles using PLGA, carry out their characterization, loading and release studies of polyphenols-loaded and catechins-loaded nanoparticles and to study their biological properties.

Materials and Methods

Preparation of PLGA Nanoparticles

Green tea polyphenol-loaded PLGA nanoparticles were prepared by a water/oil/water (W/O/W) emulsification solvent evaporation method[12] with modifications. Briefly, 500 µg polyphenols or catechins were emulsified with 500 µl of dichloromethane (DCM) solution containing 2.5, 5 and 10 mg PLGA by sonication for 60 sec to form the first emulsion (E1/O). The E1 was poured into 4 ml of 1% PVA solution (E2) and sonicated for 60 sec to form double emulsion (E1/O/E2). The E1/O/E2 double emulsion was poured into 15.9 ml of 0.5% PVA solution and stirred in a shaker at room temperature for 4 hr to evaporate the DCM. The nanoparticles were collected by centrifugation at 12,000 rpm for 30 min. The supernatant was collected in a separate tube for estimation of

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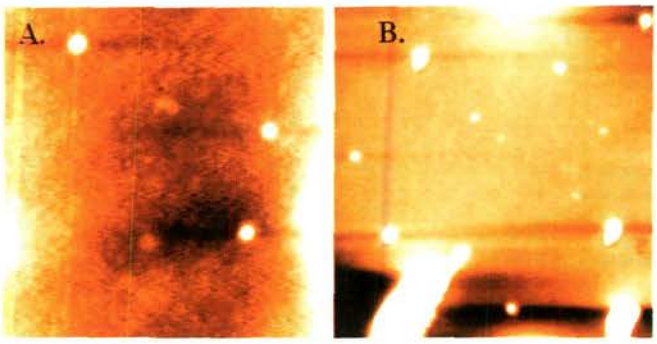

Figure 1 (A, B): AFM images of PLGA nanoparticles.

remaining catechins or polyphenols to find encapsulation efficiency. The nanoparticles were washed thrice by centrifugation.

Characterization of PLGA Nanoparticles

Morphology, polydispersity and particle size of synthesized PLGA nanoparticles were analysed by atomic force microscopy (AFM). About 100 µl of nanoparticles solution was mixed with ethanol, and a thin smear was prepared on a clean glass slide and allowed to dry. The images were taken in AFM (Nano Surf Easy Sccan2, Switzerland). Hydrodynamic diameter and particle size distribution (PSD) of PLGA nanoparticles were measured using dynamic light scattering measurement (DLS).

Determination of Encapsulation Efficiency (EE)

For determination of encapsulation efficiency, the unloaded tea polyphenols and catechins were separated by centrifugation. The loaded polyphenols/ catechins were calculated by subtracting the unloaded polyphenols/ catechins from initially used total feed. Unloaded polyphenols/ catechins concentration was determined by UV absorbance method.

Encapsulation Efficiency (E.E.) (%) = [(Total amount of drug – Amount of drug in supernatant) / Total amount of drug] × 100

In Vitro Release Studies of Catechins or Polyphenols from PLGA Nanoparticles

The tea polyphenols or catechins loaded nanoparticle pellet was dispersed in 50 ml of PBS (pH 7.4) in a tube, incubated at 37°C and continuously shaken at 100 rpm. About 1 ml sample was collected from the tube at regular interval and spinned at 12,000 rpm for 30 min to separate the particles. The supernatant was collected for analysis. The pellet was resuspended in 1 ml of the fresh PBS solution and added again to the tube.

Biological Activity of Catechins or Polyphenols Released from PLGA Nanoparticles

Anti-oxidative Activity

Anti-oxidative activity of the catechins or polyphenols released from PLGA nanoparticles was tested by free-radical diphenylpicrylhydrazyl (DPPH) assay.[13] About 200 µl of samples were taken in clean test tubes and 1 ml of diphenylpicrylhydrazyl (DPPH) was added to them. The test tubes were incubated in a dark room for 30 min, followed by observation of their O.D. values at 517 nm using UV-visible spectrophotometer.

Protection of Human Red Blood Cells

The effect of catechins or polyphenols encapsulated PLGA nanoparticles on human red blood cells (HRBC) against the free-radical induced lysis were tested, following the method of Grinberg *et al.*[14] with modifications. Briefly, serum was removed from blood samples by centrifugation and washing in cold PBS. About one million human red blood cells (HRBC) were used per treatment, and details of different treatments are given in Figure 5. The reaction mixtures were incubated for 3 hr at room temperature, and hemolysis was observed visually.

Results

Preparation and Characterization of PLGA Nanoparticles

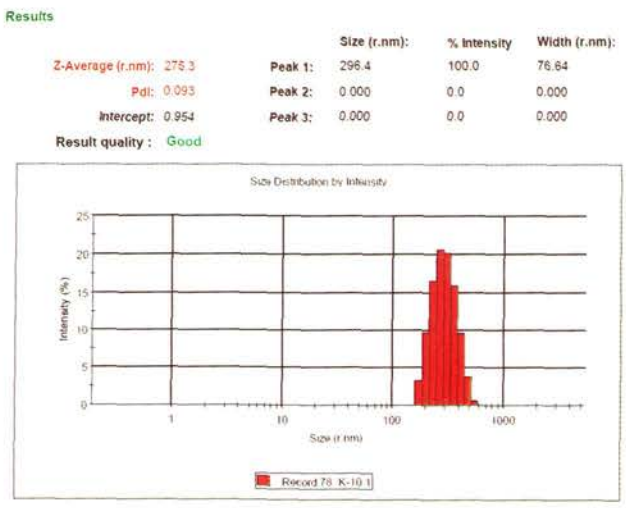

Figure 2: Average size of the PLGA nanoparticles as measured by DLS.

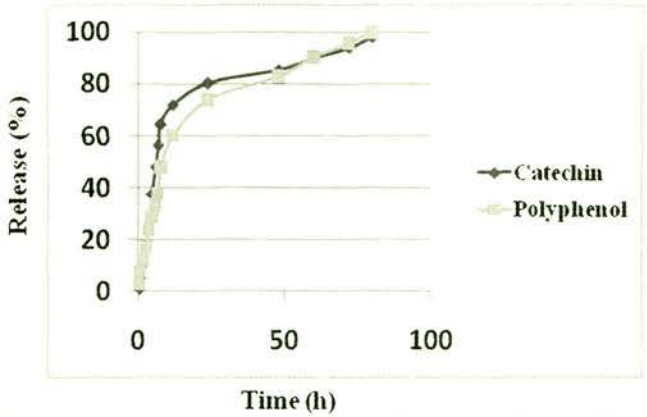

Figure 3: Percentage release of catechins and polyphenols PLGA nanoparticles.

PLGA nanoparticles were prepared by W/O/W emulsification solvent evaporation method. Milky white coloured suspension of PLGA nanoparticles was formed after 4 hr of stirring. AFM picture showed oval-shaped PLGA nanoparticles (Fig. 1). Size of the nanoparticle measured in AFM was lesser than 250 nm. The average polydispersity index of 0.093 was observed, and size distribution was measured by dynamic light scattering (DLS, Fig. 2). The average diameter of PLGA nanoparticles obtained was 275.3 nm.

Encapsulation Efficiency (EE)

After preparation of PLGA nanoparticles, centrifugation was done to separate the nanoparticles, and the supernatant was collected for quantification of residual catechins or polyphenols. In this study, three different quantities of PLGA (2.5, 5 and 10 mg) were tested to optimize EE. EE was increased with increased quantity of PLGA. Only 10 mg PLGA yielded maximum of 38%, and 49.5% EE in case of polyphenols and catechins, respectively (Table 1).

Table 1: Encapsulation Efficiency (EE) of PLGA Nanoparticles with Different Quantity of PLGA (mean ± S.E.)

S. No.	PLGA (mg)	EE (%)	
		Polyphenols	Catechins
1.	2.5	11 ±0.44	13±0.08
2.	5	17 ±0.16	21±0.52
3.	10	38±0.88	49.5±0.16

Release of Catechins or Polyphenols from PLGA Nanoparticles

Release of catechins or polyphenols under *in vitro* conditions is presented in Figure 3. Within 6 hr, 60% of catechins or polyphenols release occurred from the PLGA nanoparticles in PBS (pH 7.4), then rate of release was constant, and it took 16 hr to reach 70% release. More than 90% of drug release was occurred after 72 hr, indicating the sustained release of catechins or polyphenols from the PLGA nanoparticles.

Biological Activity of Catechins or Polyphenols Encapsulated PLGA Nanoparticles

Anti-Oxidative Activity

In case of catechins or polyphenols encapsulated in PLGA nanoparticles, antioxidant activity was high in the beginning and it gradually decreased with time although a substantial antioxidative activity was retained even after 72 hr in the PBS (pH 7.4) at 37°C (Fig. 4). This result indicates that catechins or polyphenols were slowly released from the PLGA nanoparticles for a longer time and maintained their biological activity.

Protection of Human Red Blood Cells

The RBCs treated with catechin or polyphenols remained unaffected, but hemolysis was noticed within 1 hr when RBCs were treated with DPPH. Degree of hemolysis increased with increase in the quantity of DPPH used. Finally, RBCs treated with DPPH along with catechin or polyphenol encapsulated PLGA nanoparticles showed considerable protection of RBCs against DPPH-induced hemolysis (Fig. 5).

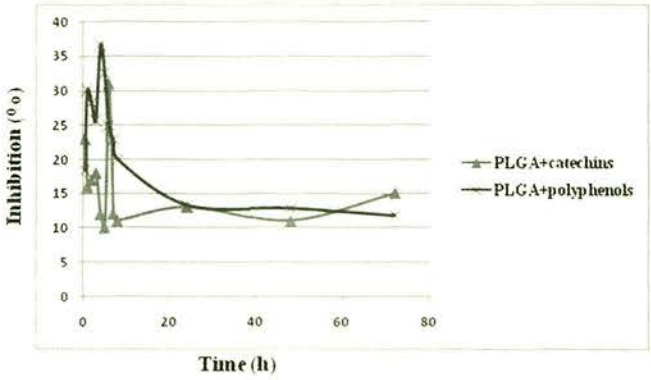

Figure 4: Percentage of inhibition as measured by DPPH assay for released catechins and polyphenols from PLGA nanoparticles.

Discussion

In this study, tea catechins and polyphenols loaded PLGA nanoparticles were successfully prepared by W/O/W emulsion method. Different parameters were set to prepare PLGA nanoparticles; among all, we have considered the one which gave us maximum loading efficiency. The polymer concentration is critical in influencing the nanoparticle size because it determines the viscosity of the PLGA solution and its dispersion in aqueous phase.[15] In our study, 10 mg of PLGA was required to encapsulate maximum amount of catechins or polyphenols. PVA is the most common emulsifier, which tends to be associated with nanoparticles by interconnection at the interface.[16] The prepared nanoparticles encapsulated with catechins or polyphenols were characterized by AFM. The particles were ovoid in shape; few were spherical as well as aggregated (Fig. 1). The particles size was measured by dynamic light scattering (DLS), the average size of the particles was found to be around 275 nm (Fig. 2). The average particle size of PLGA nanoparticles prepared by the same method was around 375 nm incase of experiment done by Van de Ven *et al.*[17] At the same time, the average size of PLGA nanoparticles prepared by Cun *et al.*[18] was ranged from 250 nm to 265 nm. This result is more close to our result. The encapsulation efficiency of catechins or polyphenols in the PLGA nanoparticles varied from 11% to 49.5% (Table 1). Release studies under *in vitro* conditions showed that the encapsulated catechins or polyphenols were slowly released from PLGA nanoparticles. The sustained release of catechins or polyphenols was found even after 72 hr. Within 10 hr, about 60% catechins and polyphenols were released (Fig. 3). Similar pattern of drug release from the PLGA nanoparticles was observed under *in vitro* conditions by others.[15, 19, 20] Free-radical scavenging ability of the catechins or polyphenols that were released from PLGA nanoparticles was tested. The activity depends on the concentration of catechins or polyphenols, the catechins sample (catechins + PLGA nanoparticles) collected after 1 hr showed the highest inhibition in the DPPH assay. Whereas the polyphenols sample (polyphenols + PLGA nanoparticles) collected at 6th hr showed maximum activity (Fig. 4). In another experiment, the ability of PLGA nanoparticles with catechins or polyphenols was tested against free-radicals-induced damage in membrane of HRBC, leading to hemolysis. HRBCs were protected by the PLGA nanoparticles encapsulated with catechins or polyphenols from the attack of free-radicals by scavenging them effectively at constant rate by the sustained release (Fig. 5). Similar results were observed in experiments done by Nakayama *et al.* and Shiraki *et al.*[21, 22]

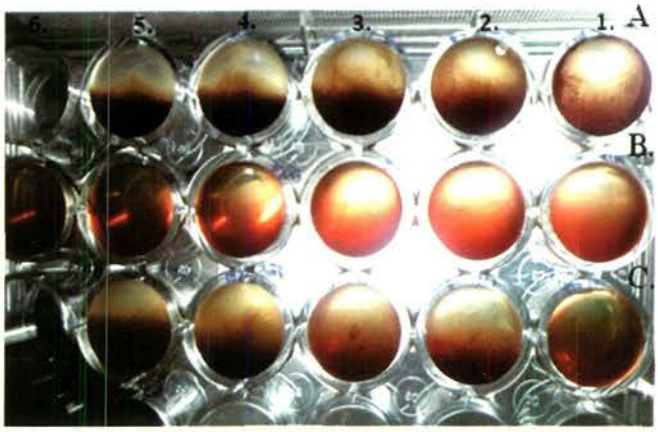

Figure 5: A. (1) HRBC + PBS (pH7.4), (2) HRBC + free polyphenols (250 µg/ml), (3) HRBC + free polyphenols (500 µg/ml), (4) HRBC+ free catechins (250 µg/ml), (5) HRBC + free catechins (500 µg/ml). **B.** (1) HRBC + 5 µM DPPH, (2) HRBC + 10 µM DPPH, (3) HRBC + 25 µM DPPH, (4) HRBC + 50 µM DPPH, (5) HRBC+ 75 µM DPPH, (6) HRBC + 100 µM DPPH. **C.** (1) HRBC + 100 µM DPPH, (2) HRBC + 100 µM DPPH + 100 µl PLGA polyphenol loaded PLGA nanoparticles, (3) RBC+100 µM DPPH+200 µl polyphenol loaded PLGA nanoparticles, (4) HRBC+100µM DPPH+100 µl catechins loaded PLGA nanoparticles, (5) HRBC + 100 µM DPPH + 200 µl catechins-loaded PLGA nanoparticles.

Conclusion

In conclusion, the PLGA nanoparticles encapsulated with catechins or polyphenols were successfully prepared. The sustained release of catechins or polyphenols from PLGA nanoparticles was estimated, and retention of their biological properties like antioxidant activity and anti-hemolytic activity were proved.

Acknowledgements

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Further Reading

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