

Preparation and Physicochemical Characterization of Poly (D, L-lactide-co-glycolide) Nanoparticles for Controlled Release of EGCG

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Abstract: Chemoprevention, especially through the use of naturally occurring phytochemicals capable of impeding the process of one or more steps of carcinogenesis, is a promising approach for cancer management. Despite promising results in preclinical settings, its applicability to humans has met with limited success largely due to inefficient systemic delivery and bioavailability of chemopreventive agents. The concept of nanochemoprevention technique has been applied in which drug is encapsulated in polymer nanoparticles to enhance bioavailability and action against cancer. Here, we report the encapsulation of green tea polyphenol epigallocatechin-3-gallate (EGCG) in poly-(D,L-lactide-co-glycolide) or PLGA nanoparticles and its effectiveness as anti-cancer agents. Drug loaded PLGA nanoparticles were prepared by modified emulsification-solvent evaporation technique. The synthesized nanoparticles were characterized to have spherical shape with smooth exterior and an average diameter of 300.8 nm using SEM and DLS techniques, respectively. The *in vitro* release kinetics of drug from the nanoparticles at physiological conditions (pH 7.4, 37°C) followed delayed and controlled release of EGCG. The application of these nanoparticles on cancerous K562 cells showed that EGCG loaded nanoparticles exhibited significantly higher cytotoxicity compared to EGCG alone.

Keywords: EGCG, Nanoparticles, PLGA, *In vitro* release, K562 cells.

1. Introduction

Epigallocatechin-3-gallate (EGCG), a green tea polyphenol (Figure 1), is known to inhibit growth of cancer cells and induce apoptosis in various types of tumor cells due to their pro-oxidant activity [1]–[3]. PLGA is one of the most successfully used biodegradable nanosystem for the development of nanomedicines because it undergoes hydrolysis in the body to produce the biodegradable metabolite monomers, lactic acid and glycolic acid [4], [5]. PLGA have potential because of their size, hydrophobic core with hydrophilic periphery, and biocompatibility [5]. Poor solubility and instability under physiological conditions limit the beneficial properties and potential health benefits of phytochemicals in functional food or pharmaceutical products [6]. Controlled delivery systems such as nanoparticles have shown potential to protect, control the release, and increase the action of different bioactive compounds.

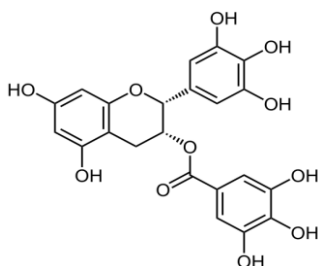


Figure 1: Chemical structure of EGCG

In the present study, EGCG encapsulated PLGA nanoparticles were prepared by modified emulsification-solvent evaporation technique. The synthesized nanoparticles were then characterized by dynamic light scattering (DLS), scanning electron microscopy (SEM), Fourier transform infrared (FT-IR) analysis. *In vitro* release kinetics of EGCG

at physiological conditions was studied. The nanoparticles were tested for anticancer potential in K562 cells to evaluate their cytotoxicity.

2. Materials and Methods

2.1 Chemicals

PLGA, PVA, EGCG were procured from Sigma Aldrich, St. Louis, MO, USA. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was procured from Himedia, Mumbai, India. RPMI 1640 media, fetal bovine serum (FBS), penicillin-streptomycin were obtained from Gibco, Grand Island, NY.

2.2 Preparation of EGCG loaded nanoparticles

Nanoparticles containing EGCG were prepared by using single emulsion-solvent evaporation technique [7]. Briefly, 10 mg of PLGA, and 1 mg of EGCG in 2 ml of 12.5% (v/v) methanol in chloroform was emulsified in 8 ml of 2% (w/v) aqueous solution of PVA to form an oil-in-water emulsion. The emulsification was carried out using a microtip probe sonicator (Hielscher UP100H, Germany) for 2 minutes over an ice bath. The emulsion was stirred for 3 hours on a magnetic stir plate at room temperature to allow formation of nanoparticles. Nanoparticles were recovered by centrifugation at 13800 rpm for 30 minutes at 4°C. The washed formulations were lyophilized (Gemini[®] Heto Maxi Dry Lyo) for 2 days to get the powdered form of nanoparticles.

2.3 Particle Size Analysis

DLS was used to measure the hydrodynamic diameter (d nm) and was performed using Zetasizer Nano ZS (Malvern

Instruments, Malvern, UK). To determine the particle size, a dilute suspension of nanoparticles (100 µg/ml) was prepared in Milli-Q water, sonicated on an ice-bath for 30 seconds and subjected to particle size measurement.

2.4 SEM Studies

The surface morphology of nanoparticles was characterized by SEM (Zeiss EVO 18 Special Edition, Germany). The nanoparticles were sputtered (Quorum Technologies, Q150T ES) with gold to make them conductive.

2.5 FT-IR Spectral Studies

The chemical integrity of the drug and the polymer matrix was investigated using FT-IR spectra (Perkin-Elmer, California, USA).

2.6 In vitro Release Kinetics Study

In vitro release of EGCG was carried out by dissolving nanoparticles in PBS (pH 7.4) at 37°C [7].

2.7 Cytotoxicity Study

The cytotoxicity studies on K562 cells were performed with different concentrations of free drug and nanoparticles formulations containing different ranges of concentrations of drug by MTT based colorimetric assay [8].

3. Results and Discussion

EGCG may be responsible for most of the anticancer activity of tea [9]. Its anticancer properties can be enhanced by successful nanoformulations and efficient drug delivery mechanisms [10]. EGCG loaded PLGA polymer nanoparticles were prepared and DLS analysis revealed that the formulated nanoparticles had an average diameter of 300.8 ± 2.6 nm (Figure 2).

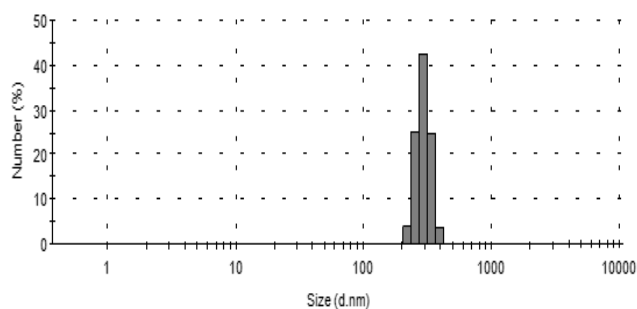


Figure 2: Hydrodynamic size measurement by dynamic light scattering

SEM image identified the surface morphology of drug-loaded nanoparticles to be spherical with smooth exterior (Figure 3).

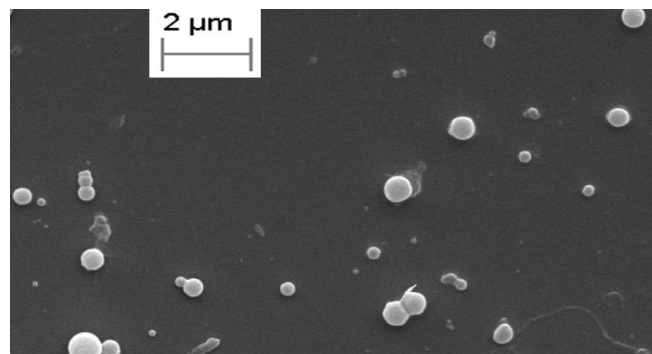


Figure 3: Scanning electron micrograph of EGCG loaded PLGA nanoparticles.

Information on the nature of the molecular interaction within the solid matrix of the nanoparticles was obtained using FT-IR. The spectra for free PLGA nanoparticles showed the characteristic bands of the polymer, $-\text{CH}$, $-\text{CH}_2$, $-\text{CH}_3$ stretching ($2850\text{--}3000\text{ cm}^{-1}$), carbonyl $-\text{C}=\text{O}$ stretching ($1700\text{--}1800\text{ cm}^{-1}$), $\text{C}-\text{O}$ stretching ($1050\text{--}1250\text{ cm}^{-1}$) [11], [12]. FT-IR spectra for EGCG exhibited the characteristic bands corresponding to $-\text{OH}$ stretching ($3600\text{--}3200\text{ cm}^{-1}$), $\text{C}=\text{C}$ stretching ($1600\text{--}1400\text{ cm}^{-1}$), $\text{C}-\text{O}$ stretching of the oxygen in the ring (1272 cm^{-1}). These findings are in agreement with previous studies [13], [14]. In spectra of EGCG loaded nanoparticles, the intensity of $-\text{CH}$, $-\text{CH}_2$, $-\text{CH}_3$ stretching, and $\text{C}=\text{O}$ stretching decreased compared to void PLGA.

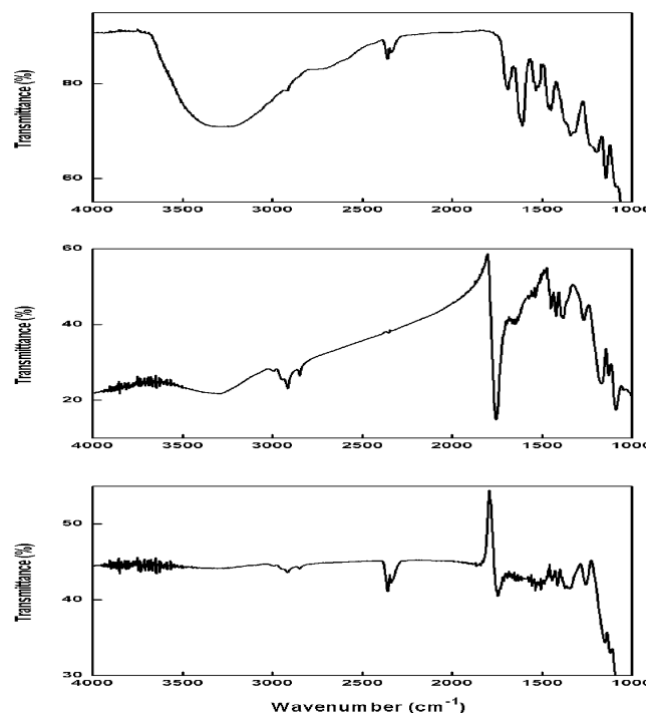


Figure 4: FT-IR spectra of EGCG, void PLGA nanoparticles, and EGCG loaded PLGA nanoparticles. (from top to bottom)

There is also a shift in $\text{C}=\text{O}$ stretching peak position from 1802 cm^{-1} in void PLGA to 1794 cm^{-1} in loaded PLGA suggesting active participation of carbonyl group in the interaction. FT-IR measurement evidenced the encapsulation of EGCG within the polymer nanoparticles (Figure 4).

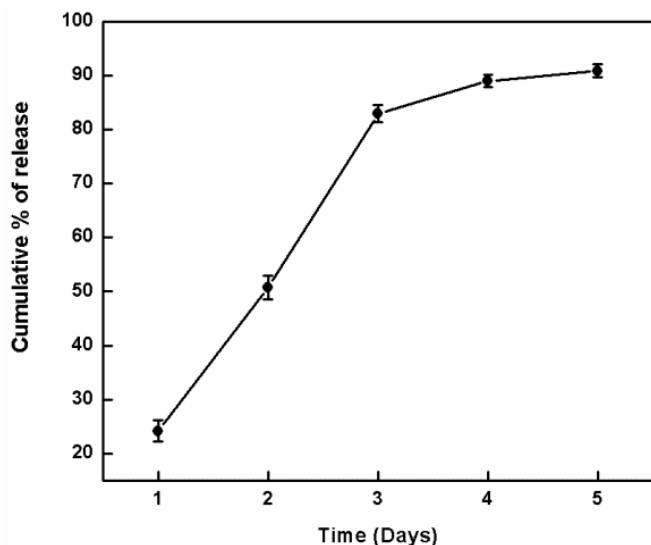


Figure 5: *In vitro* release of EGCG from nanoparticles.

The *in vitro* release of EGCG from PLGA nanoparticles is shown in Figure 5. Around 80% of EGCG is released in first three days, followed by very slow release upto five days and maximum of 90%. Gradual release of drug for longer periods time was achieved by this encapsulation and attempts to increase release time is under process. However, initial burst release was followed by a slower sustained release of EGCG present inside the core of nanoparticles [7], [15].

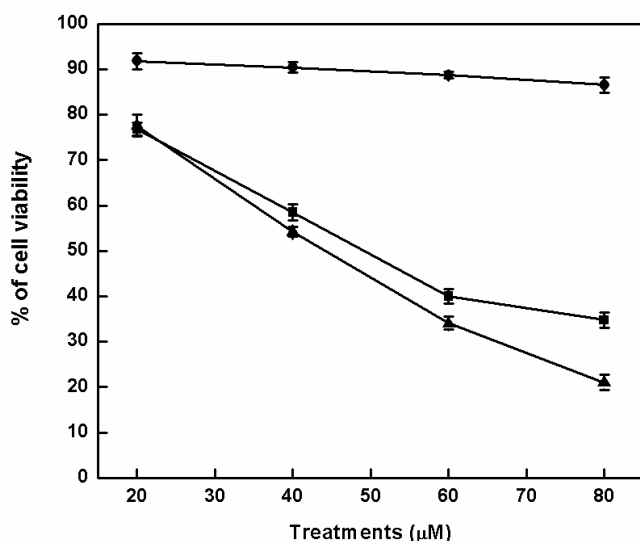


Figure 6: Dose dependent cytotoxicity of different concentration of void PLGA nanoparticles, EGCG, and EGCG loaded PLGA nanoparticles (from top to bottom)

To investigate the therapeutic efficiency of these formulations, K562 cells were treated with different concentrations of nanoparticles for 24 hours, and cell proliferation was measured by MTT assay. It can be seen from Figure 6 that nanoparticles exhibited significantly higher cytotoxicity compared to EGCG in solution. This observation indicates that EGCG loaded nanoparticles enhances the *in vitro* cancer cell death.

4. Conclusion

A novel biodegradable nanoparticle system has been developed for controlled delivery of EGCG, which is more effective in successful killing of cancer cells than EGCG alone. However, more work in this direction is required to promote this formulation as cancer therapeutic nanomedicine. In general, our results have important implications for the design and fabrication of polymeric nanoparticle delivery systems for bioactive compounds with beneficial effects on human health.

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