Research Letter

Synthesis of PET-PLA/Drug Nanoparticles and Their Effect with Gold Nanoparticles for Controlled Drug Release in Cancer Chemotherapy

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Polyethylene terephthalate-poly(lactic acid) copolymer (PET-PLA) was synthesized from bis (2-hydroxyethyl terephthalate) and L-lactic acid oligomer in the presence of manganese antimony glycoxide as a catalyst. The synthesized PET-PLA copolymer was used for controlled drug release systems with gold nanoparticles. Fluorouracil containing PET-PLA nanocapsules was prepared in the presence of gold nanoparticles by solvent evaporation method. The morphologies of the nanocapsules were characterized using scanning electron microscopy and transmission electron microscopy. Controlled release of Fu and Fu@Au was carried out in 0.1 M phosphate buffer (pH 7.4) and 0.1 M HCl solution. The results indicated that the drug release for gold nanoparticles/fluorouracil (Au@Fu) incorporated PET-PLA nanocapsules was controlled and slow compared to Fu incorporated PET-PLA nanocapsules. This may be due to the interaction between the gold nanoparticles and fluorouracil in PET-PLA nanocapsules.

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1. INTRODUCTION

Nanotechnology provides a novel route for many biomedical applications especially in the case of incurable diseases such as cancer, diabetes, and so forth. Cancer can affect just every organ in human body. The various treatments of cancer are chemotherapy, radiation therapy, surgery, biological therapy, hormone, and gene therapies. Chemotherapy uses chemical agents (anticancer or cytotoxic drugs) to interact with cancer cells to eradicate or control the growth of cancer. Depending on the type of cancer and kind of drug used, chemotherapy drugs may be administered differently. 5-Fluorouracil (5-Fu) is one of the oldest chemotherapy drugs and has been used for decades. It is an active medicine against many cancers. Over the past 20 years, increased understanding of the mechanism of action of 5-Fu has led to the development of strategies that increase its anticancer activity. 5-Fu is given for treatment of cancers like bowel, breast, stomach, and gullet cancer [1]. However, anticancer drugs normally attack both normal cells and cancerous cells when the drug was given as an injection or tablet form for a long time. In order to overcome this side effect,

targeting the drug delivery and sustained release of drugs are required. Many research investigations are focused in the preparation of drug encapsulated polymer nanoparticles for the controlled release applications. Biodegradable polymers have become increasingly important in the development of drug delivery systems [2–5]. There are several methods that can be used to make microcapsules [6] from biodegradable polymers. Polyethylene terephthalate (PET) is a semicrystalline polymer with high mechanical strength and an excellent thermal stability. Copolyesters such as PET-PLA are biodegradable materials used for tissue engineering, bone reconstruction, and controlled drug delivery systems [7–9]. Further gold nanoparticles play an important role in cancer therapy to detect or to deliver the drug to the cancerous cell without affecting the normal cells and have good ability to form complex with many drugs through chemical bonding. Nanoparticles have uniform shape and size and are soluble in an aqueous medium. With this view in mind, the present work is undertaken to synthesize PET-PLA copolyester and the preparation of PET-PLA/Fu and PET-PLA/Fu-Au nanocapsules to study their drug release behavior. A comparative study of sustained release of 5-Fu

FIGURE 1: (a) and (b) show the UV-spectrum of Au nanoparticles and Fu@Au nanoparticles.

under different pH conditions was also carried out. The present work is the continuation of our previous work with gold nanoparticles [10, 11].

2. EXPERIMENTAL

2.1. Synthesis of PET-PLA copolyester

Bis (2-hydroxy ethyl terephthalate) and L-lactic acid oligomers (30/70) wt% were reacted in the presence of 10 mg of manganese antimony glycoxide catalyst [12]. The reaction mixture was placed [13] in a 500 mL flask connected to a vacuum line (0.4 kPa) and immersed in an oil bath at 180°C for 6 hours. As the reactants were stirred, glycol and water were slowly distilled out. The copolyesters were dissolved in chloroform, precipitated in methanol, filtered and dried at 70°C. This was used without further purification.

2.2. Preparation of citrate-capped gold nanoparticles

Trisodium citrate (38.8 mM, 50 cm³) was added to a boiling HAuCl₄ solution (1 mM, 500 cm³). As a result, the previously yellow solution of gold chloride turned to wine red color and gave a characteristic absorbance at 518 nm in the UV-vis spectrum.

2.3. Preparation of nanocapsules

Nanocapsules were prepared by the solvent evaporation method similar to that reported previously by Beck et al. [14]. For PET/PLA/5Fu-Au experiment, 5-Fluorouracil (50 mg) was added in 0.5 mL nanogold aqueous suspension (with a 30-minute shaking under ultrasound to help the adsorption of 5-Fluorouracil on gold nanoparticles) and this solution was added to an organic polymer solution (300 mg PET-PLA + 5 mL CH₂Cl₂) under stirring condition. This was continued until the organic solvent was completely evaporated. The suspension became clear after all the nanocapsules precipitated out of the solution. These nanocapsules were collected by filtration and washed with deionized water to remove any undesirable residuals. Finally,

the clean nanocapsules were dried in a vacuum oven at 40°C for 24 hours to ensure a complete removal of the organic solvent and deionized water. All the nanocapsules were stored in a desiccator at 25°C. PET-PLA/Fu nanocapsules were also prepared under similar conditions.

Encapsulation efficiency (%) = [(Mass of drug added during Nanoparticle (NP) preparation – Mass of free drug in supernatant)/Mass of Drug added during NP preparation] × 100.

Drug loading (%) = [Mass of 5-Fluorouracil in NP/Mass of NP recovered] × 100.

3. RESULTS AND DISCUSSION

3.1. UV-vis characterization

Figure 1 shows the UV-visible spectrum of citrate stabilized gold nanoparticles. The plasmon band observed for the wine red colloidal gold at 518 nm is characteristic of the gold nanoparticles. The pure drug shows a maximum at 273 nm, but with the addition of 5-Fu to colloidal gold, both the bands at 273 and 518 nm pertaining to pure drug and Au colloids decrease in intensity steadily with time.

This decrease is accompanied by the emergence of an additional peak at 650 nm (Figure 1(b)), that is, a change from wine red to blue with the addition of drug to colloidal gold. The appearance of the new peak is due to the aggregation of gold nanoparticles and the replacement of citrate by 5-Fu, leading to the formation of gold-drug complex. Citrate ions are readily replaced by the –NH ligand on the surface of gold nanoparticles. This ligand exchange reaction provides an important means for the chemical functionalization of the nanoparticles and greatly extends the versatility of these systems.

3.2. Morphological characterization

PET-PLA/5-Fu-Au and PET-PLA/5-Fu nanocapsules were easily distinguished from PET-PLA/5-Fu nanocapsules by their color. While the color of PET-PLA/5-Fu nanocapsules

FIGURE 2: The TEM images of (a) Au@Fu, and (b) Au-Fu/PET-PLA nanoparticles.

FIGURE 3: The SEM images of (a) Fu/PLA-PET nanoparticles, and (b) Fu-Au/PET-PLA nanoparticles.

was white, PET-PLA/5-Fu-Au nanocapsules was purple/blue in color, due to the presence of the gold nanoparticles.

From the TEM (Figures 2(a) and 2(b)) measurements, the average diameters of the gold nanoparticles were found to be in the range of 18–20 nm.

Morphology of nanocapsules (PET-PLA/5-Fu and PET-PLA/5-Fu-Au) prepared was characterized by the SEM analysis (Figures 3(a) and 3(b)).

From the SEM measurements, the size of nanocapsules was found to be in the range of 230–260 nm. The average sizes of the microcapsules to nanocapsules can be prepared by changing the rotational speed from 2000 to 12000 rpm.

The average size decreased with an increase in the rotational speed, because of the higher shear stress. However, since the size difference was within the limits of statistical error, this statement remained speculative without further investigations. The surface topography of PET-PLA/5-Fu and PET-PLA/5-Fu-Au nanocapsules was smooth as seen in SEM photographs. The SEM micrographs manifested that nanocapsules prepared in the present study possess a nearly spherical shape.

3.3. Encapsulation efficiency

The encapsulations of PET-PLA/5-Fu-Au and PET-PLA/5-Fu nanocapsules were obtained both in the presence and in the absence of gold nanoparticles. For PET-PLA/5-Fu-Au nanocapsules, the adsorption of 5-Fluorouracil on Au

led to a larger particle (Fu-Au complex) size and made the diffusion of 5-Fluorouracil to the external solution less efficient. Gold nanoparticles also hindered the diffusion of 5-Fluorouracil to the external solution because of their insolubility in water and methylene chloride. In addition, each gold nanoparticle was composed of many Au atoms and would adsorb more than one 5-Fluorouracil molecules. As a result, PET-PLA/5-Fu-Au nanocapsules had a higher encapsulation efficiency (70.21%) and a larger average size than PET-PLA/5-Fu nanocapsules (42.56%). The amount of 5-Fluorouracil entrapped within NP was determined by measuring the amount of nonentrapped drug in the supernatant recovered after centrifugation and washing of the nanoparticles, using a UV-visible spectrophotometer [15].

However, PET-PLA/5-Fu-Au nanocapsules had a higher drug loading (4.63%) than PET-PLA/5-Fu nanocapsules (2.46%).

3.4. Drug release study

The release profiles of 5-Fluorouracil from PET-PLA/5-Fu and PET-PLA/5-Fu-Au nanocapsules in the hydrochloric acid (0.1M) and the phosphate buffered saline (pH 7.4) at $37 \pm 0.1^\circ\text{C}$ are shown in Figure 4. Desorption profiles were obtained as follows. 0.03 g of drug encapsulated polymer nanoparticles was mixed with 10 mL of phosphate buffer solution (pH 7.4) in five fractions. Each fraction was centrifuged as a function of time. The absorbance of each solution was monitored at different times. One sample solution was used only once to ensure that there was no change in the concentration of the solution.

The intensity of absorption was plotted against time which gave the desorption profile of fluorouracil. In the present study, fluorouracil encapsulated PET-PLA in the presence of gold nanoparticles was used for controlled drug release. In order to find the drug loading, a standard graph was drawn using known concentration of the drug. Figure 4 shows the drug release at different intervals of time using phosphate buffer solution at pH 7.4. The same procedure has been repeated for the drug encapsulated with gold nanoparticles. From Figure 4, it was understood that the drug release was slow and sustained. The release rate of 5-Fluorouracil for PET-PLA/5-Fu-Au nanocapsules was slower than that of PET-PLA/5-Fu nanocapsules in the two different dissolution media. PET-PLA/5-Fu-Au nanocapsules had a slower release behavior mainly because gold nanoparticle in nanocapsules hindered the diffusion of 5-Fluorouracil away from the nanocapsules. An initial burst effect (i.e., the rapid release of 5-Fluorouracil) of PET-PLA/5-Fu nanocapsules was observed as shown in Figure 4.

The effect of gold nanoparticles on the release rate was particularly important because the release rates of 5-Fluorouracil for PET-PLA/5-Fu nanocapsules were higher than that of PET-PLA/5-Fu-Au nanocapsules in two different dissolution media. Further, the release rates of 5-Fluorouracil from PET-PLA/5-Fu and PET-PLA/5-Fu-Au nanocapsules were relatively higher in 7.4 PBS (0.1 M) than in pH 0.1 M HCl. This observation was attributed to the fact that the

FIGURE 4: The controlled drug release of Fu@Au in 0.1 M HCl, and 0.1 M PBS.

dissolution medium had a strong influence on the solubility of the drug, and the solubility of 5-Fluorouracil in pH 7.4 PBS was higher than that in 0.1 M HCl at the same temperature.

4. CONCLUSION

The binding of fluorouracil to colloidal gold and encapsulation of Fu to polymer nanoparticles were studied using different analytical techniques. The aggregations of gold nanoparticles were ascertained using UV-visible spectroscopy and TEM analysis. Further, it was observed that the drug release was slow and sustained in the case of PLA-PET/Au-Fu compared to that of PLA-PET/Fu nanoparticles due to the interaction between the drug and gold nanoparticles. The combination of gold and polymer with Fu results in a more potent complex compared to the individual parts, since such a complex can release the drug at controlled rate as well as at the targeted places.

REFERENCES

- [1] E. A. Bruijn, A. T. Oosterom, and U. S. Tjaden, "Site specific delivery of 5Fu with 5-deoxy-5-fluorouridine," *Regional Cancer Treatment*, vol. 2, pp. 61–76, 1989.
- [2] J. H. Eldrige, J. K. Staas, J. A. Meulbroek, J. R. McGhee, T. R. Tice, and R. M. Gilley, "Biodegradable microspheres as a vaccine delivery system," *Molecular Immunology*, vol. 28, no. 3, pp. 287–294, 1991.
- [3] D. T. O'Hagan, J. P. McGee, J. Holmgren, et al., "Biodegradable microparticles for oral immunization," *Vaccine*, vol. 11, no. 2, pp. 149–154, 1993.
- [4] T. Uchida, S. Martin, T. P. Foster, R. C. Wardley, and S. Grimm, "Dose and load studies for subcutaneous and oral delivery of poly(lactide-co-glycolide) microspheres containing ovalbumin," *Pharmaceutical Research*, vol. 11, no. 7, pp. 1009–1015, 1994.
- [5] S. Salhi, M. Tessier, J.-C. Blais, R. El Gharbi, and A. Fradet, "Synthesis of aliphatic-aromatic copolyesters by a high temperature bulk reaction between poly(ethylene terephthalate) and cyclodi(ethylene succinate)," *Macromolecular Chemistry and Physics*, vol. 205, no. 18, pp. 2391–2397, 2004.
- [6] M.-K. Lai, C.-Y. Chang, Y.-W. Lien, and R. C.-C. Tsiang, "Application of gold nanoparticles to microencapsulation of thioridazine," *Journal of Controlled Release*, vol. 111, no. 3, pp. 352–361, 2006.
- [7] X. Yuan, A. F. T. Mak, and K. Yao, "Comparative observation of accelerated degradation of poly(L-lactic acid) fibres in phosphate buffered saline and a dilute alkaline solution," *Polymer Degradation and Stability*, vol. 75, no. 1, pp. 45–53, 2002.
- [8] X. Yuan, A. F. T. Mak, and K. Yao, "Surface degradation of poly(L-lactic acid) fibres in a concentrated alkaline solution," *Polymer Degradation and Stability*, vol. 79, no. 1, pp. 45–52, 2003.
- [9] C. F. van Nostrum, T. F. J. Veldhuis, G. W. Bos, and W. E. Hennik, "Hydrolytic degradation of oligo(lactic acid): a kinetic and mechanistic study," *Polymer*, vol. 45, no. 20, pp. 6779–6787, 2004.
- [10] V. Selvaraj and M. Alagar, "Analytical detection and biological assay of antileukemic drug 5-fluorouracil using gold nanoparticles as probe," *International Journal of Pharmaceutics*, vol. 337, no. 1–2, pp. 275–281, 2007.
- [11] V. Selvaraj, M. Alagar, and I. Hamerton, "Analytical detection and biological assay of antileukemic drug using gold nanoparticles," *Electrochimica Acta*, vol. 52, no. 3, pp. 1152–1160, 2006.
- [12] J. F. Kennay, "Reaction products of specific antimony compounds with a carboxylate of zinc calcium or manganese and an alcohol or glycol," US patent 4122107, 1978.
- [13] E. Olewnik, W. Czerwiński, J. Nowaczyk, et al., "Synthesis and structural study of copolymers of L-lactic acid and bis(2-hydroxyethyl terephthalate)," *European Polymer Journal*, vol. 43, no. 3, pp. 1009–1019, 2007.
- [14] L. R. Beck, D. R. Cowsar, D. H. Lewis, et al., "A new long-acting injectable microcapsule system for the administration of progesterone," *Fertility and Sterility*, vol. 31, no. 5, pp. 545–551, 1979.
- [15] R. Pignatello, D. Amico, S. Chiechio, C. Spadaro, G. Puglisi, and P. Giunchedi, "Preparation and analgesic activity of eudragit RS100® microparticles containing diflunisal," *Drug Delivery*, vol. 8, no. 1, pp. 35–45, 2001.