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Biocompatible, pH-responsive, and biodegradable polyurethanes as smart anti-cancer drug delivery carriers

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ABSTRACT

In this study, biocompatible and biodegradable polyurethanes (PUs) were prepared by using different amino acids as chain extenders. Their structure and morphology were determined by fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and X-ray diffraction (XRD) analysis whereas their mechanical properties were evaluated by universal testing machine. These PUs were not only screened for their *in vitro* and *in vivo* cytotoxicity but also evaluated for their water uptake, swelling behavior, hydrolytic degradation and employed as pH-responsive drug delivery carriers. An anti-cancer drug imatinib was loaded to these PUs with 94% of loading efficiency and its release behavior was studied at different pH values. All the PUs showed a persistent local release for > 300 h. The results exhibited that drug released at physiological pH (7.4) is far lesser as compared to the acidic and basic pH of the release media. At acidic pH, 82% of the drug has been released as compared to the 41% at physiological pH. The results clearly demonstrate that these PUs are safe and can be used as intelligent drug release matrices for targeted drug delivery.

1. Introduction

Cancer is considered as a serious life-threatening condition and one of the leading causes of death worldwide (six million deaths *per annum*) with nearly ten million newly identified cases every year. In spite of extensive research, the major treatment methodology is still restricted to conventional chemotherapy. However, the efficacy of most of the anticancer drugs is very limited because of their inability to discriminate between cancerous and normal cells causing severe side effects to healthy organs [1–3]. Importantly though, these problems led the researchers to develop innovative methods for site-specific drug delivery. One such effective methodology is the use of a carrier which can provide site-specific drug release along with an optimum release profile. This targeted release of drugs to receptor site has the potential to reduce side effects against healthy organs while increasing the pharmacological response of drugs over diseased cells. As the pH of tumours is lower than that of blood, therefore this difference in pH is used as a perfect stimulus for the release of anticancer drugs to the targeted sites [4–7]. Recent developments in polymer science have provided great opportunities to fabricate novel PUs to be used as controlled drug delivery systems. For this purpose, biodegradable PUs have attracted considerable attention and are extensively used as the suitable candidates for intelligent drug delivery [8,9]. Most importantly, various anti-cancer drugs are precisely released at the acidic pH of the tumour cells [10,11] using pH-responsive PUs as smart drug delivery materials [12]. Worth highlighting are the doxorubicin, paclitaxel, 5-fluorouracil, dexamethasone, and temozolomide [13–17]. Despite these tremendous applications in anticancer drug delivery, the PUs are still not employed as a carrier for imatinib which is a first line FDA approved the drug with a low rate of toxicity and the extraordinary efficacy [18]. Herein, we report new PUs having amino acids as chain extenders that are not only biocompatible and biodegradable but also stimuli-responsive carriers of the imatinib. Importantly, these PUs have the considerable ability to discriminate cancerous and normal cells and show a persistent local release for > 300 h. Therefore, these new PUs are safe and potential candidates to be used as intelligent and smart drug delivery vehicles of imatinib.

2. Materials and methods

2.1. Materials

Chemicals used for the synthesis of PUs are, hexamethylene diisocyanate HDI (99%, Sigma Aldrich), polyethylene glycol (M.W 2000,

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Sigma Aldrich), lysine (> 98%, Sigma Aldrich), arginine (99%, Sigma Aldrich), glutamine (99%, Sigma Aldrich), dibutyltin dilaurate (DBTDL, 95%, Sigma Aldrich), 1,4-dioxane (99.8%, Sigma Aldrich). For biochemical and *in vivo* studies, deionized water, phosphate buffered saline (PBS tablets, Sigma Aldrich), imatinib (Sigma Aldrich), normal human fibroblast (NHFB) cell lines, (Biological Industries), ethyl alcohol, and Balb c mice were obtained.

2.2. Methods

2.2.1. Synthesis of PUs

Typically, 30 mmol of HDI and 15 mmol of polyol (molar ratio of 2:1) was taken in a reactor equipped with mechanical stirrer and a thermometer. In this mixture, 30 mL of 1,4-dioxane as a solvent and 0.5 mL of DBTDL was added as the catalyst. This mixture was refluxed for 4 h with continuous stirring to prepare pre-PU. The temperature was lowered to 35 °C and 15 mmol of chain extenders was added along with continuous stirring [19,20].

The reaction mixture was heated at 75 °C for another 4 h and then poured into Teflon moulds. The solvent was evaporated at room temperature and then placed overnight at 110 °C [21,22]. The dried PU films, thus obtained were preserved in polyethylene bags and stored in a desiccator for the purpose of characterization and future experiments.

2.2.2. Characterizations

A thermo Nicolet FTIR was used to obtain spectra at the resolution of 2 cm^{-1}, and 32 scans per measurement. Mechanical properties were measured by using the universal testing machine from Zwick GmbH, Ulm Germany. SEM images were taken from Joel scanning electron microscope. Hardness values were obtained by using digital hardness check shore D, Gibitre instruments Sri Lanka. X-ray diffractograms were obtained by an X-ray diffractometer (PANalytical, X' Pert Pro, Almelo, Netherlands) with Cu Kα as a radiation source operated at 40 kV. UV/Visible spectrophotometer (Shimadzu UV-265) was used for determining the concentration of the drug.

2.2.3. Water absorption and swelling behavior

Square samples with dimensions 1 cm × 1 cm × 0.1 cm were cut from dried PU films and incubated at 37 °C in 20 mL of deionized water. These samples were taken out at pre-determined time intervals, surface water was carefully blotted and was weighed. The percentage swelling was calculated by using Eq. (1).

$$\text{Swelling}\ (\%) = \frac{M_2 - M_1}{M_1} \times 100 \quad (1)$$

where M_1 and M_2 are the weights of the PU before and after swelling, respectively. Each measurement was taken in triplicate and the average was reported [23,24].

2.2.4. Hydrolytic and biodegradation

Square samples with 1 cm × 1 cm × 0.1 cm were incubated at 37 °C in 20 mL of phosphate-buffer saline (PBS) in separate glass vials. Each vial was added with 20 mg of sodium azide for inhibiting the bacterial growth and sealed before placing in an incubator. After a predetermined time, samples were taken out, dried and mass loss was determined to measure the percent weight loss by using Eq. (2).

$$\text{Weight loss}\ (\%) = \frac{(M_1 - M_2)100}{M_1} \quad (2)$$

where M_1 and M_2 are the respective masses of samples before and after degradation.

For biodegradation studies, the same method was followed by immersing the PU films in $H_2O_2/CoCl_2$.

2.2.5. MTT assay for in vitro cytotoxicity

All the PUs were subjected to MTT assay for cytotoxicity evaluation against the normal human fibroblast (NHFB) cell lines. For this purpose, 1×10^5 cells were seeded in each well of a 96-well plate, supplemented with 10% FBS. Other conditions of temperature, carbon dioxide, and humidity were maintained *i.e.* 37 °C with 5% CO_2 and > 90% humidity. The cells were treated with different concentrations 0, 10, 20, 40, 80, 160, 320 and 640 μg/mL of all PUs. After 48 h, firstly MTT and then DMSO was added to dissolve the formazan crystals produced because of the mitochondrial expression. The viability was evaluated by measuring the absorbance with a microplate reader (Spectra Max, USA) at 590 nm.

2.2.6. Histological analysis for in vivo cytotoxicity

Balb c mice were acclimatized with laboratory conditions according to the ethical guidelines. PUs were injected subcutaneously and after the trial of one week, mice were dissected for skin tissue histopathology. Briefly, PUs were taken in the solution form, before the film formation and solvent evaporation and a suspension were prepared by adding PBS and then injected subcutaneously. The detailed protocol has been described in our previous work [25].

2.2.7. Drug loading

Imatinib which is an anti-cancer drug was physically loaded to the PU films by employing the solvent evaporation method. For this purpose 500 mg of drug was dissolved in 20 mL of dimethyl sulfoxide (DMSO) and added to the 10 g of PU solution (57% solid contents). The mixture was stirred strongly and poured into the Teflon moulds kept in a vacuum oven at 110 °C till the complete removal of the solvent. It was gently washed with acetone for removal of the residual drug. Percentage loading efficiency and drug loading efficiency was calculated to be 8% (80 mg/g) and 91–94% respectively by using Eqs. (3) and (4). This drug-loaded PUs were kept in sealed polyethylene bags and stored in a desiccator for further use [26].

$$\text{Amount of drug loaded}\ (\%) = \frac{\text{Mass of loaded drug}}{\text{Mass of polyurethane}} \times 100 \quad (3)$$

$$\text{Drug loading efficiency}\ (\%) = \frac{\text{Mass of loaded drug}}{\text{Total mass of drug}} \times 100 \quad (4)$$

2.2.8. Drug release studies

In a clean glass vial, drug-loaded PU film was immersed in 20 mL of PBS solution and incubated at 37 °C. After specific time intervals, 1 mL of this solution was taken out and concentration was determined by using UV/Vis spectrometer. The same amount of fresh PBS was also added to the vial to keep total volume same. All experiments were performed in triplicates and an average value was reported.

3. Results and discussion

3.1. Synthesis biodegradable polyurethane

PUs were prepared by using HDI, PEG, and amino acids as chain extenders (Scheme 1). Their structure was then determined by using Fourier transform infrared spectroscopy. FTIR spectrum of PU3 is shown in Fig. 1(A), and structures of PU1 and PU2 along with their FTIR spectra are provided in the supplementary information. The peaks at 3352 cm^{-1} due to N—H stretching and 1560 cm^{-1} due to the N—H bending are the characteristic peaks of the new urethane bonds. The intense peak at 1730 cm^{-1} corresponds to the carbonyl absorption of the hard segment [27,28]. The asymmetric and symmetric stretching vibrations due to CH_2 are present at 2938 cm^{-1} and 2862 cm^{-1}, respectively. The peak at 1465 cm^{-1} is designated as CH_2 bending vibration. The absence of absorption peaks of OH and NCO near 3600 and 2250 cm^{-1} respectively indicate the complete consumption of reactants. Other significant peaks further support the synthesis, such as absorption peaks at 1644 cm^{-1} is due to urea, at 1240 cm^{-1} and 1105 cm^{-1} are due C—O—C [29–31].