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Identification of anti-cancer potential of doxazocin: Loading into chitosan based biodegradable hydrogels for on-site delivery to treat cervical cancer

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ABSTRACT

In this study, an effective, biocompatible and biodegradable *co*-polymer comprising of chitosan (CS) and polyvinyl alcohol (PVA) hydrogels, chemically crosslinked and impregnated with doxazocin, is reported. The chemical structural properties of the hydrogels were evaluated by Fourier Transform Infrared spectroscopy (FTIR) and physical properties were analysed by scanning electron microscopy (SEM). The swelling behaviour is an important parameter for drug release mechanism and was investigated to find out the solution absorption capacity of the synthesized hydrogels. MTT assay revealed that doxazocin loaded hydrogels significantly hindered the cell viability. Flow cytometry analysis was performed to analyse the effect of 8CLH and 4CLH on regulation of cell cycle. Moreover, in vivo anti-cancer potential of synthesized hydrogels was assessed by CAM Assay. Results displayed that 8CLH with 1 mg/ml of doxazocin had prominently decreased the angiogenesis and significantly increased the number of cells in G1 phase of cell cycle. These results declared that 8CLH will be a good addition among hydrogels used for treatment of cancer by onsite delivery of drug.

1. Introduction

Cancer is a disorder usually identified by uncontrolled and vigorous proliferation of cells having potential to attack, spread and induce apoptosis to the nearby and distant cells and tissues [1]. Cancer is the prominent reason of mortality in economically developed countries and the second leading cause of death in developing countries [2]. A substantial percentage of the worldwide trouble of cancer could be averted through the application of prevailing cancer control knowledge [3].

Usually, cancer is treated by surgery, radiotherapy, chemotherapy, hormonal therapy and immunotherapy [4,5]. Among all, chemotherapy has been used most commonly to destroy cancerous cells using cytotoxic drugs. However, its use has been declined due to highly toxic and poorly specific drugs, insufficient availability of drugs to the tumor tissue, development of multi-drug resistance, and the dynamic heterogeneous biology of the growing tumors, hair loss, stomach irritation and poor number of blood cells. Due to these side effects of chemotherapy, treatments of cancer have been shifted from chemotherapy to localized drug release [6,7].

For controlled and targeted drug release, polymer-based drug delivery systems have been considered for many years. It includes polymer delivery vehicles, such as, drug-eluting films, gels, wafers, rods, and nanoparticles. It ensures bioavailability of drug to the specific site of disease, increased drug solubility and minimized systemic side effects [8–13].

Among polymer based drug delivery systems, polymeric hydrogels have gained attention as a carrier of drug to specific sites and also used in the field of tissue engineering, regenerative studies and biomedical sciences [14–17]. These hydrogels possess the potential to swell in water without dissolving [18]. Due to their high solution absorption capacity, sometimes their mechanical strength is compromised. To overcome this obstacle, cross linkers have been used to enhance the mechanical properties of hydrogels. Cross linkers highly affect the 3-D structure, porosity, ability of up taking drug solution as well as their affinity for aqueous environment [19,20]. For this purpose, we have used triethyl orthoformate (TEOF) as a crosslinker. From our previous research experience TEOF was proved to be cyto-compatible [21] and made suitable hydrogels for tissue engineering and regenerative purposes [22].

Chitosan, (poly-β(1,4)-D-glucosamine), a cationic polysaccharide,

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has been extensively used as biomaterials in the form of gels, fibers, membranes and in addition used as scaffold for tissue engineering and controlled drug delivery [23,24]. It helps in wound healing and accelerates tissue repair by cell penetration and proliferation [25–27]. It has water high binding capacity, fat binding capacity, bioactivity, biodegradability, nontoxicity, biocompatibility, antifungal activity and antibacterial property [28–31]. It has also been used in treatment and control of various types of cancers for example, ovarian cancer [32], lung cancer [33], breast cancer [34], RIF-1 fibrosarcoma [35], cervical cancer [36], cancers associated with mucin production [37].

PVA possesses good chemical stability, film-forming ability and high hydrophilicity and has been extensively used in the formation of gels and membranes [38–40]. Moreover, PVA is biocompatible and non-toxic, and acquires minimal cell adhesion and protein absorption [41–44]. Combination of chitosan and PVA acquires good mechanical properties [45], and exhibit biodegradable, biocompatible and non-toxic behaviour [46–48]. Due to these properties CS and PVA blends have been employed in controlled drug delivery applications [49].

In this study, doxazocin, which is a quinazoline based α_1-adrenoceptor antagonists drug, has been employed as a potential anti-cancer agent [50]. It is most commonly used as an anti-hypertensive drug and the anti-cancer activity of doxazocin was discovered only recently [50].

According to the research, quinazoline moiety is responsible for apoptic activity through α_1-adrenoceptor-independent-mechanism [51] and suppression of tumor vascularity [52]. Doxazocin can induce apoptosis in prostate cancer cells [53], endothelial and malignant cells [54], cardio-myocytes [55], cardio-myoblasts [56] breast cancer cells [57], bladder smooth muscle cells [58], urothelial cells [59], pituitary adenoma cells [60], colon cancer cells and HeLa cells [61]. Recent studies shows that doxazocin also behaves as anti-angiogenic agent for cancerous tumors [62]. In literature doxazocin was used with two biomaterials that are: carrageenan matrix tablets [63] and cellulose microcrystalline pellets [64].

By keeping in mind, the advantages of onsite drug delivery and disadvantages of chemotherapy treatments, we aimed to synthesize a good biodegradable and biocompatible material which can support on-site delivery of doxazosin. In current research doxazocin loaded hydrogels were prepared from chitosan (CS) and poly vinyl alcohol (PVA) with two different concentrations of triethyl orthoformate (4% & 8%) used as a cross-linker. We prepared **control** hydrogel (without cross-linker), 4% crosslinked hydrogel loaded with doxazocin (**4CLH)** and 8% crosslinked hydrogel loaded with doxazocin (**8CLH**). It is proposed that synthesized hydrogels will inhibit the proliferation of cancerous cells by releasing doxazocin to the selected site. Drug will control the cancer by apoptosis of tumor cells only and later hydrogel will degrade itself without causing any harm.

2. Materials and methods

2.1. Materials

Chitosan (CS) was purchased from Mian Scientific Company, Lahore, Pakistan, and further purified in our laboratories as previously described [65,66] (degree of deacetylation (DD) 84%; MW: 87,047.26 g/mol). Poly (vinyl alcohol) (PVA) (Mw: 72,000, degree of hydrolysis 98%) was purchased from BDH chemical Ltd., Poole England, and hydrochloric acid (HCl) was supplied by RCI Labscan Ltd., Thailand. Sulfuric acid (H_2SO_4) was purchased from Merck (Germany). Triethyl orthoformate (98%) was purchased from Alfa Aesar (Germany). Glacial acetic acid (CH_3COOH) was purchased from AnalaR BDH Laboratory supplies, UK. NaOH was purchased from Sigma Aldrich (Germany). Doxazocin was purchased by Empire Pharmaceuticals (Pvt), Lahore, Pakistan. HeLa cancer cell line MDA-MB-231 was taken to analyse anti-cancer potential.

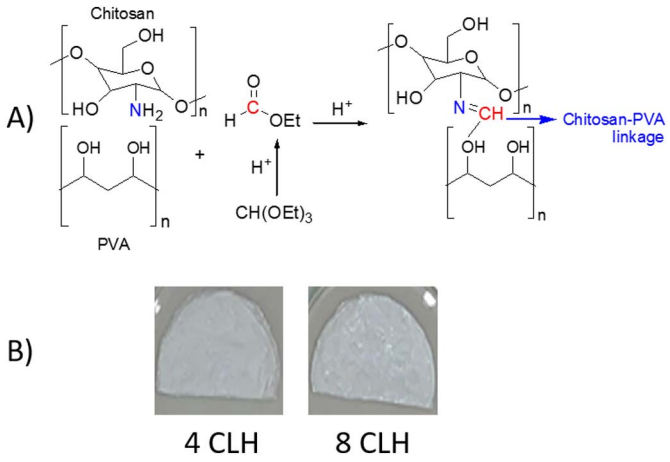

Fig. 1. A) Schematic diagram for the preparation of chitosan and PVA hydrogels. B) Camera photographs of prepared 4CLH and 8CLH hydrogels.

2.2. Experimental procedure

2.2.1. Preparation of triethyl orthoformate crosslinked chitosan and polyvinyl alcohol hydrogels

CS (2.5% *w/v*) was dissolved in acetic acid (1%) solution. To achieve the homogenous and clear solution, it was stirred magnetically for 12 h at room temperature. In another flask, PVA (10% *w/v*) was dissolved in distilled water at 80 °C along with continuous magnetic stirring. After this, the two solutions were mixed together by taking 80:20 ratios (*w/w*) of CS and PVA, respectively, and was subjected to stirring for another 24 h. On completion of 24 h, the solution was poured into separate petri dishes and the dishes were frozen at − 30 °C for 24 h. Lyophilisation of frozen samples was done for next 24 h in a freeze dryer at − 40 °C. Then rehydration of lyophilized hydrogels was done by soaking hydrogels in distilled water which was followed by soaking of samples in different concentration solutions of TEOF (i.e. 4%, 8% *w/v*) in the presence of sulphuric acid (17% *w/v*) for 24 h. The hydrogels were then removed from petri dishes and treated with NaOH (12% *w/v*) for one hour. In the end, samples were washed with distilled water thrice and lyophilized again for 24 h (Fig. 1).

2.2.2. Loading of doxazocin

Synthesized hydrogels were loaded with doxazocin. A solution of doxazocin was prepared having concentration of 1 mg/ml of doxazocin in distilled water. The crosslinked scaffolds (7 × 3 cm) were placed in this solution overnight at room temperature. Scaffolds absorbed almost all the solution. In the end, doxazocin containing scaffolds were frozen at − 20 °C and finally lyophilized at − 40 °C for 24 h.

2.3. FTIR analysis

Structural characterization of prepared hydrogels was analysed by using Fourier Transform infrared (FTIR) spectroscopy, coupled with smart ATR accessory and photo acoustic sampling cells. Spectra were recorded within the wavelength range of 4000–500 cm^{-1}, with average 256 numbers of scans at 8 cm^{-1} resolution on a Thermo-Nicolet 6700P FTIR Spectrometer (USA).

2.4. Scanning electron microscopy (SEM)

The morphology of the hydrogels was assessed with the help of variable pressure scanning electron microscope (Tescan, Vega LMU) at 10 kV under low vacuum mode at 10 Pa. The images were scanned at various magnifications. The average pore diameter was calculated by using image processing software (Image J) by selecting 30 random pores.