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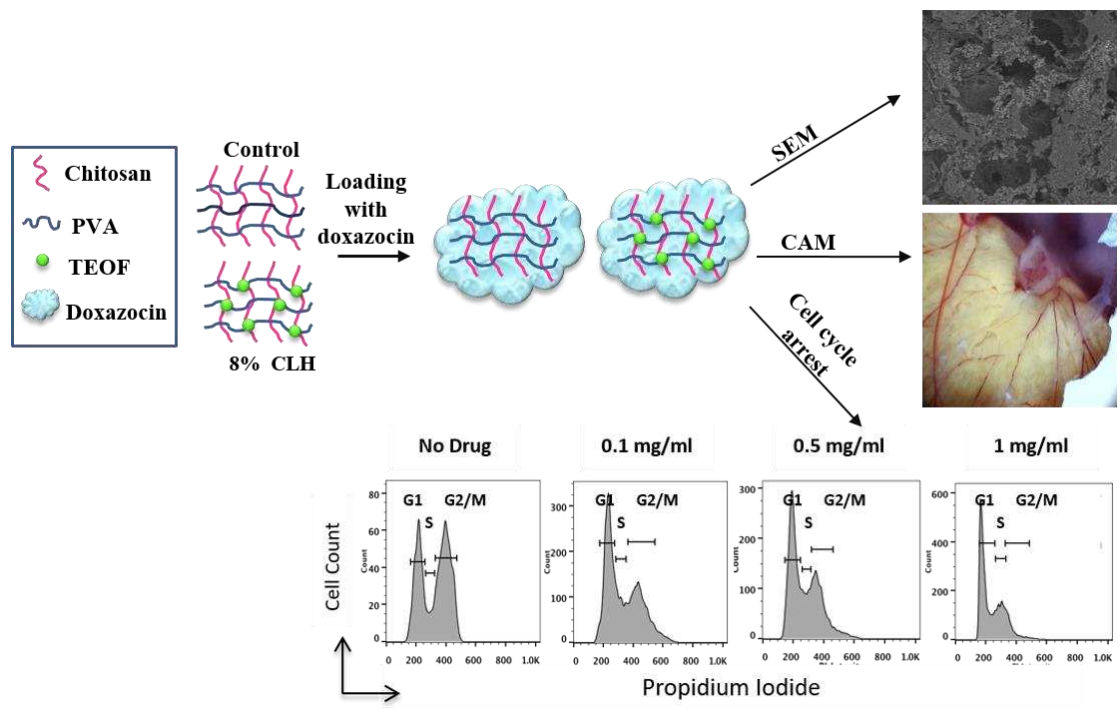
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Graphical Abstract



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 1mg/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 induces 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 tumour tissue, development of multi-drug resistance, and the dynamic heterogeneous biology of the growing tumours, hair loss, stomach irritation and poor number of blood cells. Due to these side effects of chemotherapy, treatments of cancer has 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, 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 nontoxic, 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 nontoxic behavior [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 loaded with doxazocin (**4CLH**) and 8% crosslinked 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: 87047.26 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 (H₂SO₄) was purchased from Merck (Germany). Triethyl orthoformate (98%) was purchased from Alfa Aesar (Germany). Glacial acetic acid (CH₃COOH) 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.

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 hours 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 hours. On completion of 24 hours, the solution was poured into separate petri dishes and the dishes were frozen at -30°C for 24 hours. Lyophilisation of frozen samples was done for next 24 hours 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 hours. 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 hours.

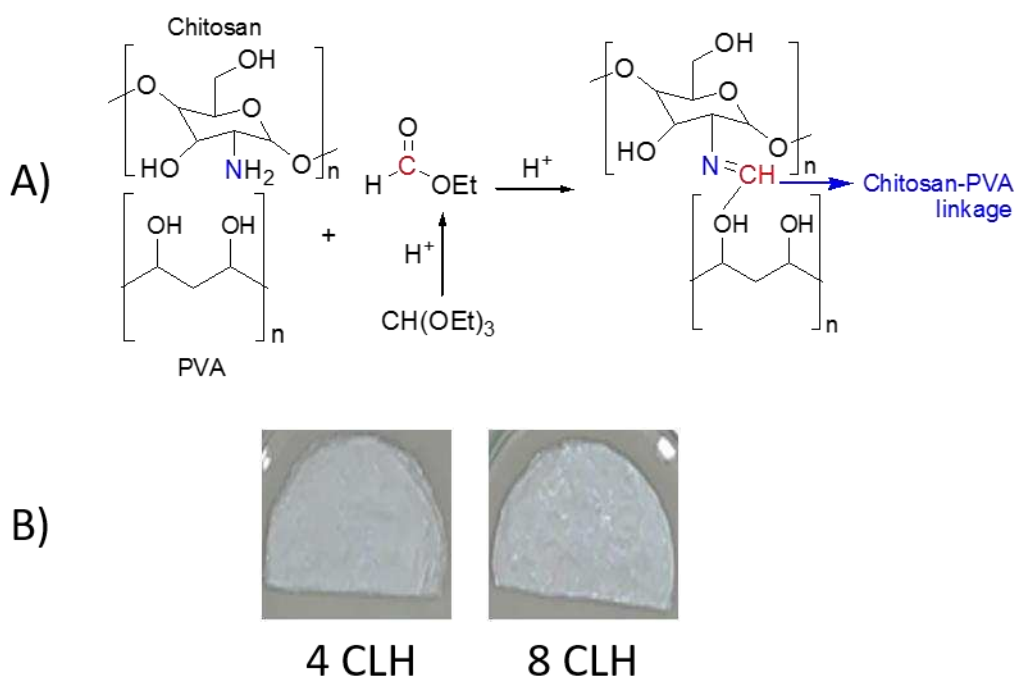


Figure 1: A) Schematic diagram for the preparation of chitosan and PVA hydrogels. B) Camera photographs of prepared 4CLH and 8CLH hydrogels.

2.2.2. Loading of doxazocin

Synthesized hydrogels were loaded with doxazocin. A solution of doxazocin was prepared having concentration of 1mg/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 4000-400 cm^{-1} , with average 256 numbers of scans at 8cm^{-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.

2.5. Swelling studies

Fluid absorption studies are of paramount importance for preliminary analysis of biodegradable materials because it is important factor while preparing biomaterials for tissue engineering applications [67]. For this study, samples were cut to get approximately equal weight of $10 \text{ mg} \pm 2 \text{ mg}$. Samples were submerged in buffer solution of pH 1.2, 4.8 and 7.4 at 37°C . The samples were taken out after the intervals of 15 min, 1, 2, and 3 h. Every sample was tapped gently on blotting paper to remove excessive solution and weighed. To calculate the percentage degree of swelling, following formula was applied:

$$\text{Degree of Swelling (\%)} = \left[\frac{M - M_d}{M_d} \right] \times 100$$

Where M is the weight of sample after submersion in buffer solution and M_d is the weight of sample before submersion in buffer solution, in its dry state.

2.6. Chorioallantoic membrane (CAM) assay to investigate anti-cancer effect of doxazocin

CAM assay was performed under sterilized conditions to analyze the *in vivo* anticancer effects of drug loaded **Control**, **4CLH** and **8CLH**. For this purpose, fertilized chicken eggs were purchased from Big Bird Group (Lahore, Pakistan) and put in humidified egg incubator (HHD 435) from day 0 of fertilization until day 8 at 37°C . At day 8, a square window (1 cm^2) was cut into the egg shell, and a 1 cm^2 hydrogel piece was applied onto the chorionic allantoic membrane. One egg was implanted with only one piece of hydrogel. After implantation, shell window was resealed with parafilm (Bemis Flexible Packaging, USA) and fixed with adhesive tape and eggs were returned to incubator at 37°C in a 55% humidified incubator until day 14. At day 14, angiogenesis was quantified by taking light microscope (Mitotic, China) pictures of the material on the CAM and the eggs were sacrificed.

2.7. Cell Culture

Hela cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco BRL, Rockville, IL, USA) supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin G sodium, 100 mg/ml streptomycin sulphate in an atmosphere of 95% humidified air and 5% CO_2 . Cells were passaged regularly and sub-cultured prior to treatment.

2.8. MTT Cell Viability Assay

Cell viability was quantified using an assay that utilizes the ability of live cells to reduce 3-(4, 5-dimethylthiazol-2-yl)-2, 5 diphenyltetrasodium bromide (MTT) to produce a coloured formazan compound. Prior to cell culture, all the hydrogels (1 cm²) included in this study, (a) control hydrogels, (b) 4CLH and (c) 8CHL were sterilized in 70% ethanol for 30 min. Immediately before cell seeding, the drug loaded hydrogels were washed 2–3 times with PBS and pre-conditioned in DMEM medium for 20 min. Hela cells were seeded in 24-well cell culture plate with 5×10⁴ cells per well with or without hydrogels in 1 ml medium. Cells seeded in 24-plate wells without hydrogels were used as a positive control, and were labelled tissue culture plate (TCP). On day 3, the medium was discarded and cells were washed with 1 ml PBS, 1 ml (0.5 mg/ml) MTT solution was added to each well and the wells were incubated at 37 °C for 3 h. The MTT solution was discarded and the cells (and hydrogels) were washed once with 1 ml PBS. To solubilize the formazan crystals 0.5 ml dimethyl sulfoxide (DMSO) was added to each well and the plate was kept under shaking conditions for 10–20 min. The optical density (OD) of the dissolved crystals was measured by using a microplate reader at 590 nm. The assay was set up in triplicates for each composition. % Viability is represented as the mean ± SD of three independent experiments. % Viability was calculated using the following formula:

$$\% \text{ Viability} = \frac{\text{Absorbance (sample)} - \text{Absorbance (Blank)}}{\text{Absorbance (control)} - \text{Absorbance (Blank)}} \times 100$$

2.9. Fluorocytometric Cell Cycle Analysis

Different phases of the cell cycle were distinguished by flow cytometry [68]. The assay is based on stoichiometric binding of propidium iodide to increasing amounts of DNA in cell cycle phases G0/G1, S, and G2/M. After doxazosin treatment, Hela cells were trypsinized, fixed and permeabilized using ethanol, rinsed in phosphate-buffered saline (PBS), and treated with RNAase to remove RNAs. DNA was then quantitatively stained with propidium iodide for 1 h at room temperature protected from light. Fluorescence was analyzed using a FACSC alibur flow cytometer (Becton Dickinson) and FlowJo software (Treestar, Ashland, OR, USA).

3. Results and discussions

3.1. Chemical structure analysis

The FTIR spectrum of doxazocin drug was matched well with the spectra of doxazocin reported in literature [69]. This proved the chemical identity of the drug.

FTIR spectroscopy analysis was performed to characterize the presence of specific chemical groups and possible interactions between CS, PVA and TEOF in the hydrogel and to analyse any chemical structural change in synthesized hydrogels after drug loading.

In figure 3, all samples showed broad band in the region of $3500\text{--}3200\text{ cm}^{-1}$ that was attributed to NH_2 and OH stretching vibrations [20, 70]. Discrete bands were observed around 2900 cm^{-1} and attributed to CH_2 stretching vibrations [71]. Peaks observed around $1076\text{--}1080\text{ cm}^{-1}$ were assigned to C-O-C stretching [72]. A new band was found around $1635\text{--}1640\text{ cm}^{-1}$ in the spectra of cross linked hydrogels due the interaction of CS, PVA and TEOF which resulted in the formation of (-C=N-) imine group [73]. This band was completely absent in the spectrum of control hydrogel. From figure 3, it was concluded that drug did not affect the chemical composition of hydrogels.

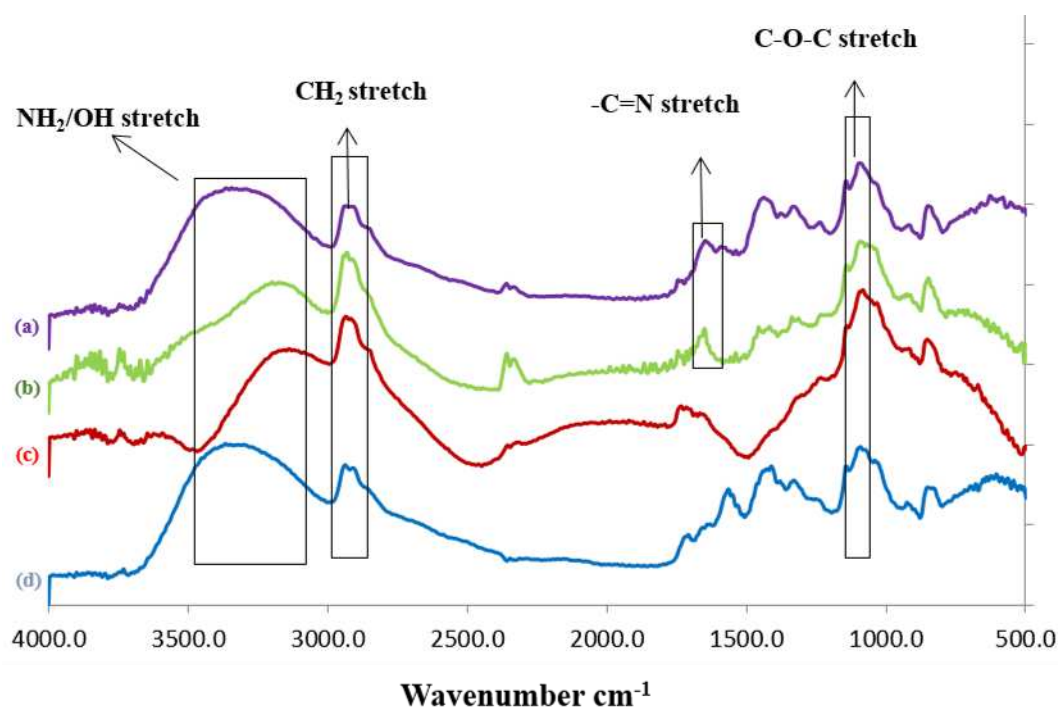


Figure 3: FTIR results of PVA/chitosan/TEOF hydrogels. FTIR spectra in the spectral region ($4000\text{--}500\text{ cm}^{-1}$) for (a) 8CLH with drug (b) 4CLH with drug (c) Control with drug (d) Control without drug.

3.2. Evaluation of the morphology of synthesized hydrogels

Figure 4 is showing scanning electron micrographs of control, 4CLH and 8CLH at different magnifications. These micrographs provided clear idea about porosity and morphology of the samples before and after drug loading.

The SEM micrographs revealed highly porous structure of these hydrogels. The concentration of crosslinker significantly affected the pore sizes which were decreased with the increase in the concentration of crosslinker. Before drug loading, the mean pore size of control was $51.42 \pm 13.31 \mu\text{m}$. After addition of crosslinker, the average pore size of 4CLH significantly decreased to $27.82 \pm 9.12 \mu\text{m}$ ($p=0.0156$) and that of 8CLH was further declined to $7.729 \pm 0.31 \mu\text{m}$ ($p=0.0059$). Un-paired t-test was used to statistically analysed the data.

It was also deduced by analysing micrographs, that interconnected porous structures of the scaffolds were retained even after loading of doxazosin but an increase in pore sizes of all synthesized hydrogels was observed. The drug was loaded using physical loading technique and drug loaded hydrogels were lyophilized again. This could be the reason behind increase in pore size, the re-lyophilisation might have merged the smaller pores thus giving rise to larger pores. The increased pore sizes were as $62.03 \pm 1.98 \mu\text{m}$, $31.96 \pm 12.95 \mu\text{m}$ and $18.40 \pm 11.78 \mu\text{m}$ for control, 4 CLH, and 8 CLH, respectively. Un-paired t-test showed significant difference among pore sizes of drug loaded hydrogels as well ($p < 0.05$).

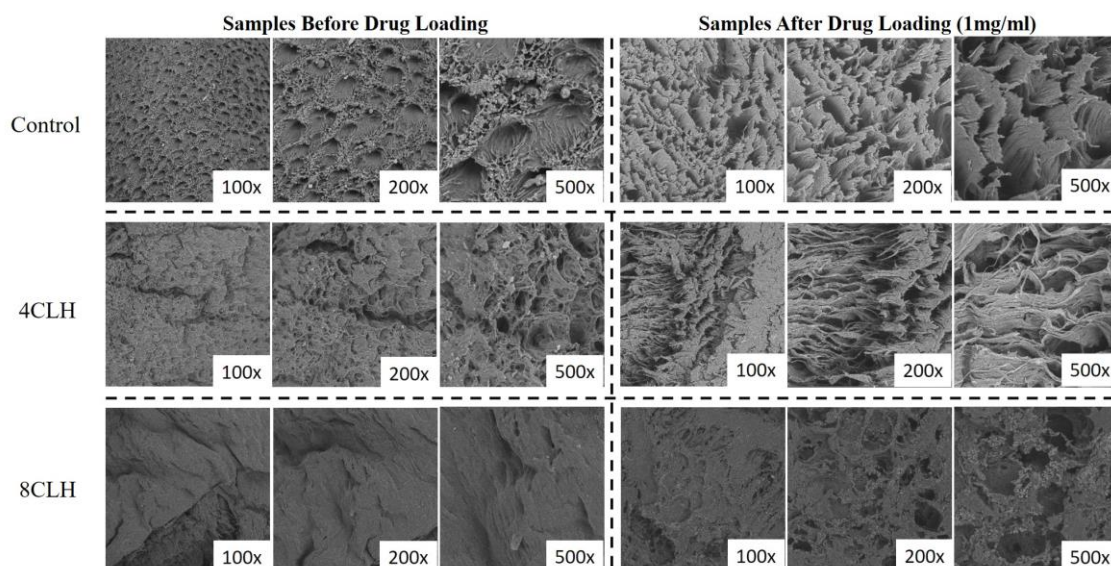


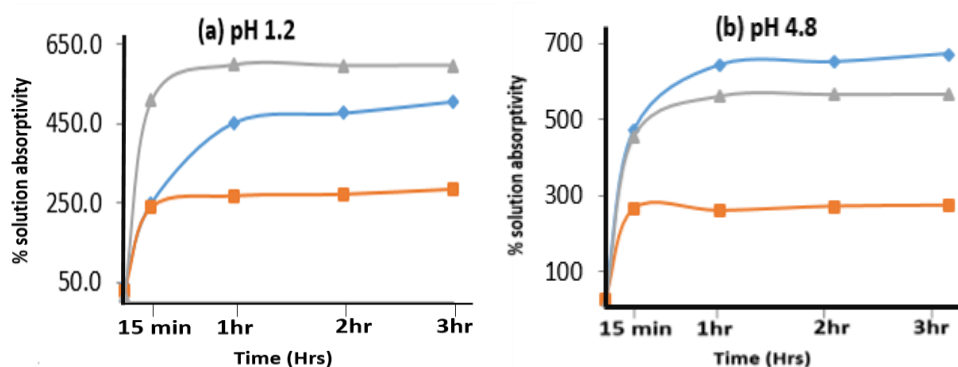
Figure 4: Scanning electron micrographs of hydrogels: (a) Control (b) 4CLH(c) 8CLH (magnification bars are given with each image) before and after drug loading.

3.3. Estimation of solution absorption capacity of materials

This test was performed to evaluate the solution absorption potential of synthesized hydrogels at different pH. Figure 5 is showing the swelling behaviour of hydrogels at different pH values i.e. 1.2, 4.8 and 7.4.

At pH 1.2, 8 CLH showed maximum swelling behaviour among all three hydrogels. Unpaired t-test showed significant difference between control and 4CLH ($p=0.0467$), 4CLH and 8CLH ($p=0.0001$), no significant difference was observed between control and 8CLH ($p=0.0504$). The possible reason behind maximum degree of swelling by 8CLH was attributed to the presence of imine linkage. This imine group might get protonated at high acidic pH which caused increase in intermolecular hydrogen bonding between protonated imine group and water molecules in 8CLH. Overall, the degree of swelling at pH 1.2 was summarized as: 8CLH > control > 4CLH.

At pH 4.8, the trend in degree of swelling was: Control > 8CLH > 4CLH. Unpaired t-test showed statistically significant difference between control and 4CLH ($p=0.0005$), no significant difference between control and 8CLH ($p=0.2747$) and significant difference between 4CLH and 8CLH ($p=0.0001$). The trend in degree of swelling of synthesized hydrogels at pH 7.4 was similar to the trend at pH 4.8: Control > 8CLH > 4CLH. Unpaired t-test showed significant difference between control and 4CLH ($p = 0.0005$), strongly significant difference between control and 8CLH ($p = 0.0190$) and significant difference between 4CLH and 8CLH ($p = 0.0009$).



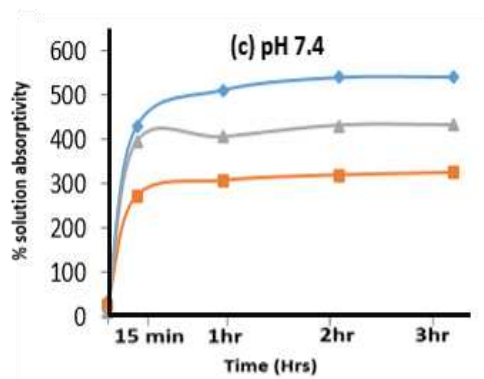


Figure 5: Solution absorption (%) of control, 4CLH, 8CLH. Values shown in the graph are means with 1 standard deviation either side of $n=3$ replicates. Blue = Control, Red = 4 CLH, Grey = 8 CLH.

At pH 4.8 and 7.4, control showed maximum swelling and cross-linked hydrogels showed relatively less degree of swelling. That fact is attributed to a more rigid network formed by the inter-intra polymer chain reactions due to the presence of cross-linker, reducing the flexibility and number of hydrophilic groups of hydrogel which is unfavourable to the swelling behaviour [20]. Control hydrogel showed maximum uptake of water because of presence of larger number of free $-NH_2$ and $-OH$ groups which might enhanced the intermolecular hydrogen bonding with water molecules. From figure 5, it was concluded that synthesized hydrogels were pH sensitive.

3.4. Qualitative and quantitative analysis of angiogenesis using CAM Assay

Angiogenesis, particularly anti-angiogenesis, is an area of therapeutic interest in cancer treatment. Using an established chicken embryo chorioallantoic membrane (CAM) assay, we are reporting that doxazocin has anticancer potential and increase in cross-linker in hydrogels helped to increase the anti-angiogenic activity of the drug loaded hydrogels.

Our studies have shown that control hydrogel (without any drug) delivered normal angiogenesis. In 4 CLH, relatively lower number of blood vessels surrounded the incorporated scaffolds and very few capillaries were seen invading the scaffold. In 8 CLH, the lowest number of blood vessels were seen in the vicinity of hydrogel and no capillaries were seen invading the scaffolds. The reason behind more anti-angiogenic potential of 8CLH was due to the high percentage of cross-linking in it, which might have supported more drug holding capacity. The dense porous network of 8CLH might also have entrapped large amount of drug inside it.

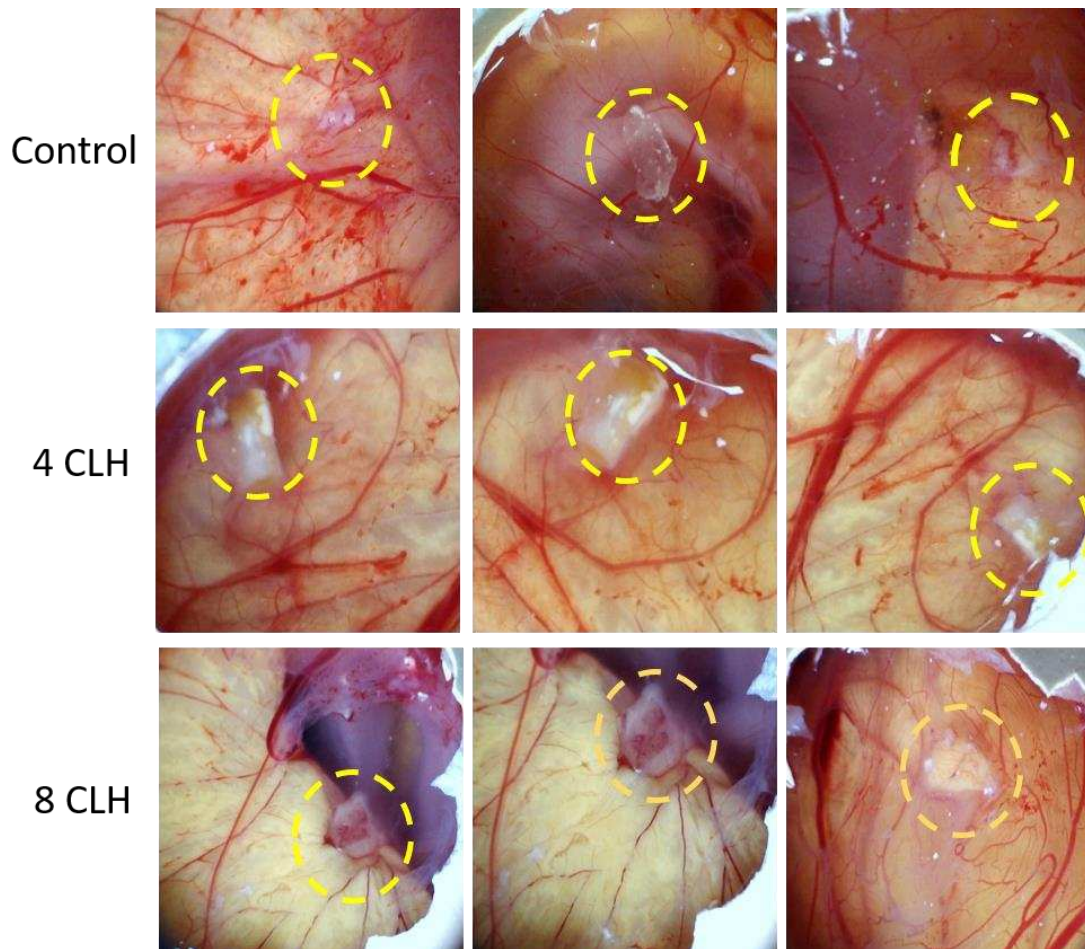


Figure 6: Hydrogels on CAM (a) control (b) 4CLH (c) 8CLH.

Results of CAM assay were found to be statistically significant by applying unpaired t-test to the quantification data in figure 7. It showed significant difference ($p=0.0132$) between the control and 4 CLH hydrogel. Difference in the number of blood vessels was also statistically very significant between control and 8CLH hydrogel ($p=0.0031$) and statistically significant difference was also observed between 4CLH and 8CLH ($p=0.1012$).

The order of anti-angiogenic activity showed by three hydrogels was as following:
 8CLH > 4CLH > control.

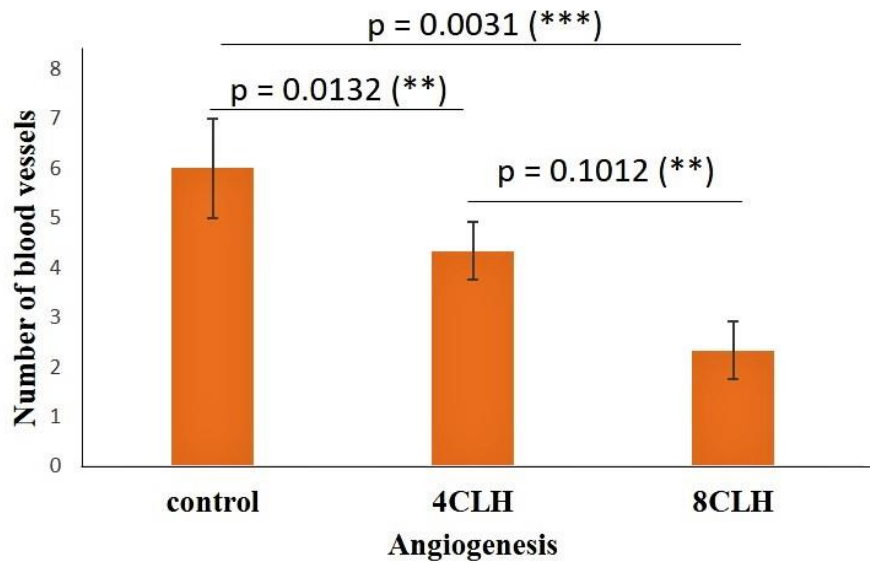


Figure 7: Quantification of blood vessels by blind scoring from images of scaffolds retrieved from CAM assay.

3.5. Cell viability of doxazocin loaded hydrogels against human cervical cancer (Hela) cell line

MTT assay was employed to investigate the anti-proliferative effects of hydrogels mediated doxazocin. Human cervical cancer (Hela) cell line was used for this test. Our results demonstrated that doxazocin loaded hydrogels vigorously hampered the cell viability compared to untreated control and tissue culture plate (TCP) control [74]. The progressive decrease in cell viability can be observed and directly co-related with the gradual increase in quantity of the loaded drug (Figure 8A) [75]. The lowest quantity of drug loaded (i.e., 0.1mg/ml) on both cross-linked (4% & 8% CLH) and non-cross linked hydrogels showed $\leq 80\%$ cell viability compared to TCP control. Similarly, the increase in concentration of loaded drug to 0.5 mg/ml further reduced the mean cell viability to $\leq 70\%$. Moreover, the further increase in concentration of loaded drug to 1 mg/ml revealed robust inhibition in mean % cell viability (i.e., 60% in non-cross linked, 50% in 4% CLH and 35% in 8% CLH).

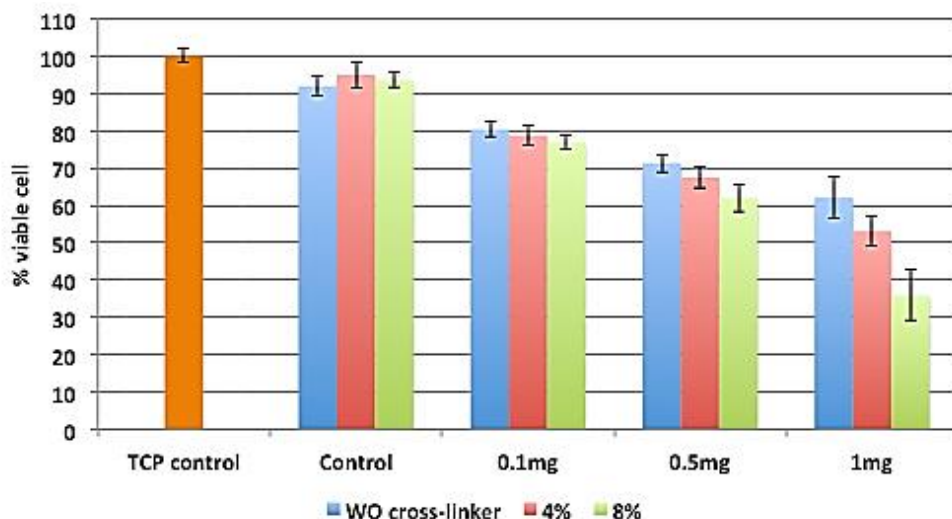
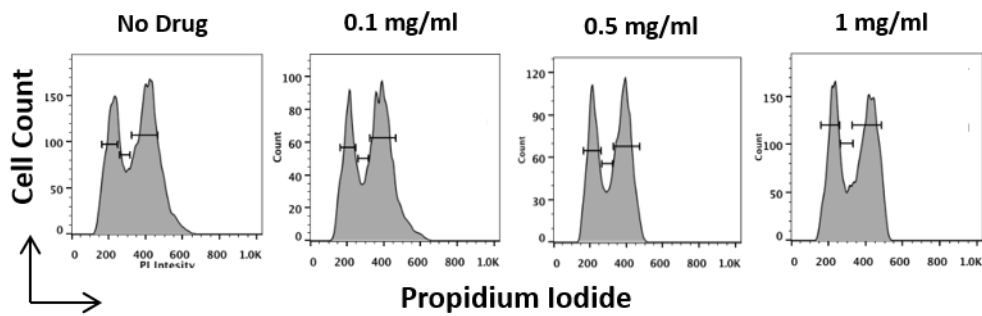


Figure 8A: Doxazocin loaded hydrogels mediated effect on cell viability of human cervical cancer cell line (Hela Cell Line) determined by MTT assay on day 3. Tissue culture plate (TCP) control, control (hydrogels without drug), hydrogels loaded with 0.1, 0.5 and 1 mg/ml drug. Blue bars represent hydrogels with cross linker, red (4HCL) & green (8HCL) show 4 and 8% cross linked hydrogels. Data demonstrate the mean \pm SD of three independent experiments.

3.6. Doxazocin loaded hydrogels causes cell cycle arrest in G1/G0 phase

In addition to anti-proliferative response, we studied the effect of doxazocin on cell cycle regulation of Hela cells [76]. Cell cycle phases were analysed with flow cytometry (FACS) after treatment with the drug-loaded hydrogels. Our FACS (flow cytometry) analysis showed that the control hydrogels loaded with different concentrations of the drug (i.e., 0.1, 0.5 and 1mg/ml) did not show the alteration in cell cycle pattern (Figure 9A). However, the 8CLH loaded with different concentrations of doxazosin (i.e., 0.1, 0.5 and 1 mg/ml), effectively induced an increase in number of cells in G1 phase (Figure 8B). The increase was directly correlated with the concentration of doxazosin and amount of cross-linker in hydrogel. Our findings reflect that the inhibition in viability and cell cycle arrest were effects of doxazocin loaded hydrogels.

LANE A



LANE B

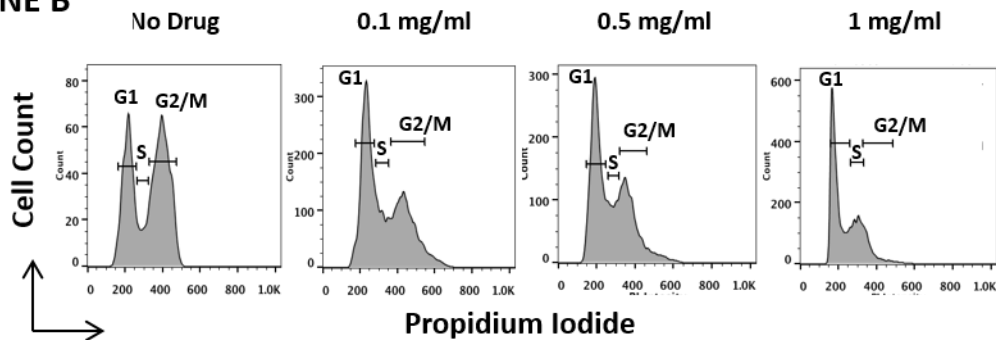


Figure 8B: Cell cycle arrest mediated by doxazocin loaded hydrogels, revealed by Flow Cytometry (FACS). Propidium Iodide fluorescence intensity correlates with the amount of cellular DNA contents. Decreased diploid DNA content reflects a reduced number of cells in G2/M phase after application of hydrogels (8CLH) (Lane B) loaded with different concentrations of doxazocin (i.e., 0.1, 0.5 & 1 mg/ml) compared with non-cross linked hydrogels (Lane A). Data shown are representative of three independent experiments.

Conclusion

In present research chitosan/PVA based hydrogels were successfully crosslinked by using triethyl orthoformate and doxazocin was physically loaded into these hydrogels. The 8 CLH showed ability to hold higher amount of doxazocin as compare to non-crosslinked and 4 CLH. FTIR spectroscopy confirmed that drug did not change its chemical structure in composite materials and SEM showed that hydrogels are porous. For assessment of anti-angiogenic potential of the hydrogels CAM assay was used. This test showed that 8CLH loaded with 1mg/ml of doxazocin was best to inhibit angiogenesis. To further prove the inhibitory potential of 8CLH loaded with 1mg/ml of drug, we performed MTT assay and flow cytometry analysis. MTT assay displayed that

anti-proliferative effect of synthesized hydrogels was directly related to higher amount of cross-linker and drug and declared 8CLH best material to give least %age of viable cells. Flow cytometry analysis was performed to analyse the effect of synthesized hydrogels on cell cycle arrest. 8CLH caused significantly increase in G1 population in cell cycle.

Hence, by gathering all results, it was concluded that 8CLH (1mg/ml of doxazocin) is the most approving hydrogel among all synthesized hydrogels for the onsite delivery of drug to treat cancer cells. 8CLH will find wide interest in biomedical materials research community for further development and for clinical applications.

Acknowledgment

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