Original Article

Optimization of Scaffolds for Localized Drug Delivery: An *In vitro* Study

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Abstract

Background: In oncology, major surgical interventions are often followed by an interim period of 3–6 months allowing the patient to recover, following which conventional radiotherapy and chemotherapy are initiated. Lack of therapeutic interventions in the interim period allows proliferation of the residual tumor cells. Thus, introducing a treatment modality in the interim period which inhibits tumor cells without exuberating the postsurgical morbidity is the need of the hour. The objective of the study is to fabricate biodegradable cross-linked scaffolds incorporated with an anticancer drug and optimization of scaffolds for drug release. **Materials and Methods:** Qualitative and quantitative characterization of the drug was done with the help of high-performance liquid chromatography (HPLC) and ultraviolet (UV) analysis. Three-dimensional (3D) discs of biodegradable scaffolds were prepared with anticancer drugs in aqueous solution in different concentrations along with crosslinkers. Discs were studied for their release kinetics with the help of HPLC. **Results:** HPLC analysis of the 3D-discs revealed negative results. There was no sign of cisplatin absorbance after the scaffold immersion in the solution. The results were attributed to the rapid degradation of the drug. **Conclusions:** Although scaffold-mediated local chemotherapy holds a great potential to replace conventional chemotherapy as a postsurgical treatment modality, several practical limitations need to be addressed. Modification in the research methodology including a shorter time for preparing the scaffold and freeze-drying the scaffold material using lyophilization instead of normal drying could prevent degradation of the drug.

Keywords: Cancer, cisplatin, local chemotherapy, scaffold

Introduction

Locoregional recurrence is a major cause for the poor survival rate in oral squamous cell carcinoma.[1-3] 86% of recurrences occur within 24 months of postsurgical interventions.[4,5] Thus, it is essential to revisit the current postsurgical treatment protocols to understand their limitations. At present, conventional chemotherapy and radiotherapy are the major forms of postsurgical interventions in oncology.[6] The major limitation of conventional chemotherapy is the associated morbidity. The high dosage required in chemotherapy can be attributed to the lack of efficiency in the drug delivery system. The chemotherapeutic drugs undergo rapid glomerular filtration. Thus, for sufficient chemotherapeutic action, it is essential to increase both the dosage and duration of chemotherapy which in turn increases the systemic cytotoxicity. To overcome these limitations, recent studies have explored the use of targeted delivery systems wherein the effect of the chemotherapeutic agents is localized which increases the effectiveness of the therapeutic agent at a relatively lower dosage.[7,8] Several means of delivery systems have been suggested for local chemotherapy including nanoparticles and scaffolds. Progress in tissue engineering has led to the emergence of a variety of scaffold biomaterials which are both biodegradable and biocompatible. Most of the scaffold-mediated research is based on common chemotherapeutic agents such as cisplatin. Several formulations of cisplatin have been studied with various scaffold materials including a biodegradable block copolymer, poly-cyclodextrin functionalized porous bioceramics, and

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Quick Response Code:

Website: www.pnrjournal.com

DOI: 10.4103/jpnr.JPNR_22_18

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How to cite this article: Gupta AA, Kheur S, Badhe R, Godse A, Shinkar A, Raj AT. Optimization of scaffolds for localized drug delivery: An *in vitro* study. J Pharm Negative Results 2019;10:XX-XX.

bioresorbable polymersomes.[9-11] Nanoparticle formulations of cisplatin with chitosan and that of paclitaxel have been extensively studied for effective drug release.[12,13] Cytotoxic activity of cetuximab-modified silica nanoparticles and gold nanoparticles has also been explored in epidermal growth factor receptor-mutant lung cancer.[14,15] The major limitation in scaffold-mediated local chemotherapy (SMLC) is the lack of optimal delivery of the drug for the required period at the required dosage. The major reason for this limitation is the lack of sufficient analytical investigation into drug formulations and its subsequent scaffold-mediated delivery on to the targeted site. Thus, in the present study, biodegradable scaffold material chitosan has been prepared and integrated with the anticancer drug cisplatin. The second stage of the study included qualitative and quantitative characterization of drug using ultraviolet (UV) spectrometry and high-performance liquid chromatography (HPLC), following which the scaffold-mediated drug release could be evaluated. The information from the present study could provide us with much insight into the practical application and current limitation in SMLC.

Materials and Methods

Instrument and apparatus: A double-beam UV spectrophotometer with a spectral width of 2 nm, Shimadzu Model 1800 (Japan), was used. The wavelength accuracy of the instrument was 0.5 nm fitted with a pair of 10 mm matched quartz cell to measure the absorbance of the serial dilution solutions.

Cisplatin was taken in solution form. A stock solution of 0.9% sodium chloride was prepared by mixing 0.9 g of sodium chloride in 100 mL of distilled water. A volume of 5 mL of cisplatin drug in the solution form was mixed in 50 mL of stock solution to make the concentration of drug adjusted to 5 µg/mL of stock solution. Serial dilutions of this drug solution were made as 0.5, 1, 2, 3, 4, 5, and 6 mL with the help of 2 mL pipette taking the lower meniscus. The volume was adjusted with the help of stock solution.

The working standard solutions were scanned in the UV-Visible range of 400–800 nm, using the prepared stock solution as blank for obtaining the absorption maxima (λ max). A calibration curve was performed to measure the linearity of the curve in the concentration range using serial dilutions of the solution. Measurements were taken on samples prepared on 3 consecutive days (n =3). The values were reported as the mean ± confidence interval of the calibration curves. The data were analyzed at a wavelength of 254 nm.

High-performance liquid chromatography

Instrument and apparatus included a HPLC system consisting of an HPLC Agilent 1100 Series Diode array and multiple Wavelength Detectors, a rhenodyne injector fitted with a 100 µl sample loop, and an RP 18 end-capped Purospher® STAR Merck column (250 mm × 4.6 mm, 5 µm p) and guard column (4 mm × 3 mm, 5 µm, Hichrom, Kromasil); the mobile phase flow rate was maintained 1 mL/min. The method was validated in accordance with the US Pharmacopoeia and the International Conference on Harmonization.[16] Serial dilutions of 10, 15, and 20 µL of the drug in stock solution were prepared. The mobile phase used is 80:20 ACN: Methanol. Qualitative and quantitative detection of the drug was made with the help of HPLC.

Scaffold preparation

The hydrogel of 2% chitosan with the 5 mL drug was prepared with crosslinkers TPP in increasing concentrations of 5%, 10%, 15%, and 20%. The solution was poured in 24 well plates and kept in an incubator at 37°C for drying. Dried scaffolds were then placed in distilled water for drug release experiment.

Results

Analytical curves were obtained on 3 consecutive days (n =3). The mean of absorbance was plotted at 706 nm against the concentration. The curves were found to be linear for serial dilutions of the drug in the concentration of 5 µg/mL of drug and yielded a correlation coefficient (r) of 0.9738 [Figure 1].

High-performance liquid chromatography analysis

Quantitative analysis

Linearity curve of cisplatin was obtained with increasing concentrations of drug for 25 µL and 50 µL after 12 h. There was no increase in area as the drug concentration increased as shown in Figures 2 and 3.

Qualitative analysis

The presence of the drug in the solution was analyzed with the help of HPLC after 24 h. The results obtained revealed only one peak as shown in Figure 4. Furthermore, it yielded tailing of peak along with the absence of a straight baseline.

Drug release experiments

The scaffold material was placed in a phosphate buffer for 24 h, 48 h, and 72 h to have the drug leached out of the scaffold material. The phosphate buffer solution was then analyzed spectrophotometrically under UV analyzer. However, no peak was observed corresponding to cisplatin absorbance.

Discussion

UV spectrophotometer quantitative analysis of the drug showed a linear curve of increased absorbance with increased

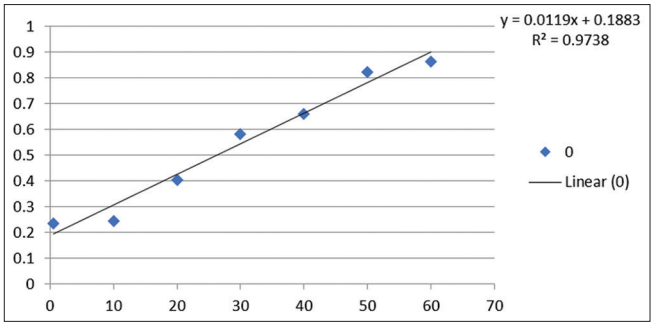

Figure 1: Linearity curve for increased absorbance with an increased concentration of drug

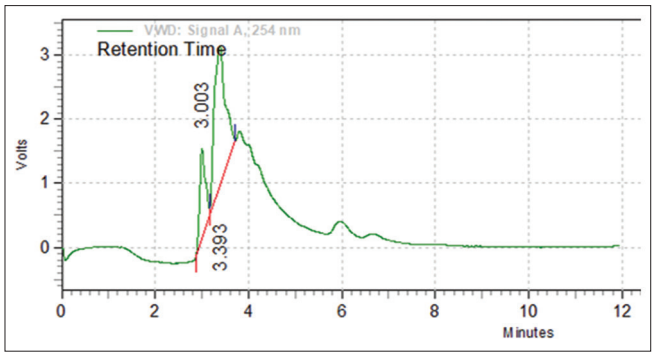

Figure 2: 25 µl of the drug in 80:20 (ACN: Methanol) as mobile phase

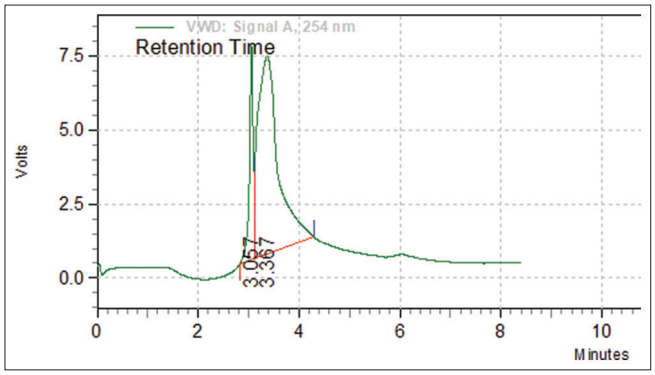

Figure 3: 50 µl of the drug in 80:20 (ACN: Methanol) as mobile phase

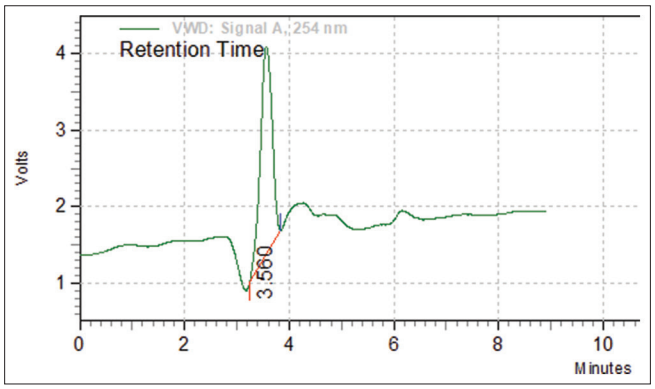

Figure 4: No straight baseline and tailing of the peak of absorption spectra of cisplatin

concentration of the drug. This helped to validate that the solution form of the drug to be used in experiments contain an appropriate amount of cisplatin required to make the scaffolds. The absorbance rate of the drug also increased with the increase in concentration giving a linear curve. However, when the same solution was tested quantitatively for HPLC analysis after 12 h, the area covered by the curve did not show a linear increase with the increased concentration of the drug.[17] It can be explained on the fact that UV analysis may not be an appropriate method for characterization of cisplatin in the solution form.[18] The qualitative characterization of the drug solution was done with the help of HPLC after 24 h. The curve did not reveal any peak corresponding to cisplatin absorbance. Furthermore, the absorbance curve showed the absence of a straight baseline along with its tailing suggesting the degradation of the drug once the solution is opened. The experiments were proceeded further for scaffold preparation by incorporating the crosslinkers and drug solution in the biodegradable scaffold material solution. Dried scaffolds were analyzed for drug release experiments, but the phosphate buffer did not reveal any peak pertaining to cisplatin absorbance after the scaffold immersion in the solution. This can be explained on the grounds of the degraded drug which did not give expected results under HPLC and further gave negative results for drug release experiments in UV analysis. In addition, due to wet weather conditions, the scaffolds took a longer duration to get dried under ambient room temperature. This may also be the reason for the degradation of the drug. The results revealed major limitation in our present methodology. As the drug in solution form degrades faster, it is vital to shorten the scaffold preparation time. In addition to this, it is also important to avoid normal drying of the scaffold material. Instead, the scaffold material should be lyophilized wherein the scaffolds will be freeze-dried as soon as they are formed.[19]

Conclusions

The morbidity associated with conventional chemotherapy has led to the search for localized chemotherapy delivery systems. Recent studies on SMLC have shown success in limiting the proliferation of cancer cells at a relative minimal dosage. The present study was successful in formulating a biodegradable cross-linked scaffold, but the anticancer drug cisplatin which was integrated into the scaffold showed rapid degradation. Thus, to conclude, although SMLC has the potential to replace conventional chemotherapy as the postsurgical treatment of choice in cancer, further research is required to stabilize the drug and to optimize the drug delivery dosage and duration.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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