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Inkjet printing of antiviral PCL nanoparticles and anticancer cyclodextrin inclusion complexes on bioadhesive film for cervical administration

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ABSTRACT

Personalized medicine is an important treatment approach for diseases like cancer with high intrasubject variability. In this framework, printing is one of the most promising methods since it permits dose and geometry adjustment of the final product. With this study, a combination product consisting of anticancer (paclitaxel) and antiviral (cidofovir) drugs was manufactured by inkjet printing onto adhesive film for local treatment of cervical cancers as a result of HPV infection. Furthermore, solubility problem of paclitaxel was overcome by maintaining th`is poorly soluble drug in a cyclodextrin inclusion complex and release of cidofovir was controlled by encapsulation in polycaprolactone nanoparticles. *In vitro* characterization studies of printed film formulations were performed and cell culture studies showed that drug loaded film formulation was effective on human cervical adenocarcinoma cells.

Our study suggests that inkjet printing technology can be utilized in the development of antiviral/anticancer combination dosage forms for mucosal application. The drug amount in the delivery system can be accurately controlled and modified. Moreover, prolonged drug release time can be obtained. Printing of anticancer and antiviral drugs on film seem to be a potential approach for HPV-related cervical cancer treatment and a good candidate for further studies.

1. Introduction

Although several risk factors are associated with cervical cancer, the major factor is an infection due to Human Papilloma Virus (HPV), especially with subtypes 16 and 18 (Bosch et al., 2002). HPV-related cervical cancer still constitutes a high incidence and mortality among women in developing countries despite the fact that early diagnosis and preventive medical methods such as vaccination may reduce incidence (Aggarwal, 2014; Sherris et al., 2001). Surgery, radiotherapy, chemotherapy or combination of these approaches are used in the treatment of HPV-related cancer in clinics, but the stage of cancer and patient's genetic and biological characteristics are important parameters to select a successful treatment method. Generally, radiotherapy and surgery are preferred to treat early stage of cervical cancer. On the other hand, systematic chemotherapy and pelvic irradiation are used for patients who have advanced stage of cervical cancer (Ordikhani et al., 2016). Nowadays, novel treatment approaches and chemotherapeutics are needed in consideration of the conditions of the patients to maintain social productivity and the stage of disease for the effective treatment of cervical cancer.

Chemotherapy drugs such as paclitaxel (PCX) that are known to be effective against HPV-induced cancer are very poorly soluble in water (<0.3 μg/mL) (Konno et al., 2003) and need to be solubilized to allow i.v. administration to the patient. Several solubilizers are used for this purpose, which are associated with toxicity problems. The long-term use of conventional chemotherapeutic drugs adversely affects patients due to solvent-induced toxicity (Guenard et al., 1993; Lee et al., 2005; Lorusso et al., 2014; Park et al., 2004).

Local chemotherapy can be helpful in overcoming toxicity or ineffectiveness problems caused by systemic chemotherapy, since it is possible to administer the drug to the cancer tissue without any systemic exposure. Intravesical administration for bladder

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tumors or cervical administration of chemotherapeutics is practiced in clinics for local chemotherapy. Intrauterine drug carrier systems, developed especially for contraception and prevention of infections, have been used in clinics for a long time. Currently, it is important to develop intrauterine drug delivery systems for the treatment of gynecological cancers by simulating these systems (Ordikhani et al., 2016). Although this approach may overcome problems that occur at systemic chemotherapy, it does not provide a complete solution. Development of novel drug carrier system is quite crucial to overcome solubility problems and non-selective cytotoxicity of anticancer drugs, increase the cellular penetration and protect the drugs against enzymatic and physiologic degradation. Furthermore, these systems optimally make it possible to reduce health expenditure, which is a result of ineffective and long-term therapy (Brigger et al., 2002).

Overcoming the solubility and toxicity problems of anticancer drugs is critical for the success of chemotherapy regardless whether local or systematic administration is practiced. Nanoparticulate drug carrier systems are promising dosage forms to overcome these problems. Nanoparticles can increase the solubility of encapsulated drugs and also protect them against physiological conditions such as pH or enzymatic degradation. Furthermore, size and surface properties of nanoparticulate drug carrier systems can be manipulated and cellular penetration can be improved according to polymer properties. In this way, problems in conventional chemotherapy can be eliminated, side effects and repeated dose administration can be reduced, bioavailability of drugs can be improved and unnecessary or over-dose usage can be prevented (Brigger et al., 2002; Hans and Lowman, 2002; Kumari et al., 2010; Soppimatha et al., 2001).

Cyclodextrin (CD), which is a multifunctional biocompatible excipient frequently used for preparation of nanoparticles, is a non-toxic, biocompatible and biodegradable natural oligosaccharide characteristic of some advantages such as solubility or stability enhancement of drugs and mucosal penetration enhancement. Besides that, CDs can form molecular inclusion complex especially with hydrophobic drugs through their unique geometry. They can encapsulate high amount of drug and protect this drug against biodegradation. Some CD derivatives also form nanoparticles spontaneously (Bilensoy and Hincal, 2009). On the other hand, another frequently used polymer for preparation of nanoparticulate drug carrier systems is polycaprolactone (PCL). PCL is synthetic, non-toxic, biocompatible and biodegradable polymer which is approved for therapeutic use in humans by FDA and several studies are reported with PCL nanoparticles to prepare controlled release drug carrier systems or implants in the scientific literature (Maria Ann and Dietmar Werner, 2010).

Personalized medicine is under growing interest in recent years. The basic idea of personalized medicine is to deliver the right drug and dose for the specific patient. This approach has an important place in the treatment of diseases such as cancer, which require different treatment approach according to the stage of the disease and the patient's individual differences. Especially the therapeutic dose of chemotherapy drugs is in a fairly narrow range and it can vary according to the biological condition of the patient and stage of the disease. This situation requires the necessity of patient-specific dose-adjustable drugs. It is expected to improve treatment efficacy and reduce side effects and cost of treatment by developing personalized medicine approach in cancer treatment (Mancinelli et al., 2000; Schilsky, 2010).

Printing technology has great potential in deposition drugs or drug-loaded nanoparticles on any surface including implantable systems, such as bioadhesive films. Inkjet printing technology works with an ink formulation, which contains the drug in free or carrier-bound form that can be printed on nearly any carrier substrate by using inkjet with a predefined dose and geometry. Furthermore, printing technology is a fast, economical and a relatively easy method and is suitable in industrial applications facilitating scale-up (Kolakovic et al., 2013).

In this study, an inkjet printed anticancer/antiviral combination delivery system was developed for local therapy of cervical cancer. With this approach, it may also be possible to benefit from the combined pharmacological effect of these drugs to improve the efficacy and safety of treatment by applying smaller doses of each drug to target site directly. The combination system comprises of PCX complexed to CD and cidofovir (CDV) encapsulated in PCL nanoparticles formulated into an ink that can be printed on a bioadhesive film depicted in our graphical abstract. The PCX:CD inclusion complex was developed to improve anticancer drug solubility. PCX was chosen as a model anticancer drug because it is known to be effective in cervical cancer and is associated with problems with solubility and rapid re-crystallization upon dissolution. On the other hand, CDV loaded PCL nanoparticle was specifically developed to control the drug release of the hydrophilic antiviral drug.

2. Materials and methods

2.1. Materials

Hydroxypropyl β-CD (HP-β-CD) was a kind gift from Barentz, Turkey. 6-O-Capro β Cyclodextrin (6-O-CaproβCD) and polycationic amphiphilic CD (PCβCDC6) were synthesized as described previously (Diaz-Moscoso et al., 2008; Ringard-Lefebvre et al., 2002). PCL (Mw = 65.000 Da) and PEG-PCL (PEG:PCL Mn: 5.000:5.000 Da) were obtained from Sigma&Aldrich, USA. Hydroxypropylcellulose (HPC) (Klucel™) was purchased from Ashland, USA. PCX (purity >99%) was purchased from LC Laboratories, USA and CDV (purity >95%) was obtained from Cayman Chemical, USA. Dialysis Tubing Cellulose Membrane (avg. flat width 25 mm, MWCO: 14,000 Da) was used in release studies and all other organic solvents and chemicals were purchased from Sigma&Aldrich, Germany. Ultrapure water was obtained from Millipore Simplicity 185 Ultrapure Water System Millipore, France and used without further purification.

2.2. Methods

Constituents of the ink were prepared and characterized separately as follows before incorporating them into the ink formulations;

2.2.1. Preparation and characterization of PCX:CDs inclusion complexes

Three different CD derivatives (hydrophilic HP-β-CD, non-ionic amphiphilic 6-O-CaproβCD and cationic amphiphilic PCβCDC6) were used as a polymer and 1:2 molar ratio inclusion complexes were prepared according to the co-lyophilization technique. Briefly, CD and PCX were dissolved in ethanol. This organic solution was then added to ultra-pure water (1:2 v/v) under magnetic stirring at room temperature. The solution was stirred for equilibrium for 7 days to obtain an inclusion complex and the organic solvent was evaporated under vacuum. The final dispersion was filtered through a 0.45 μm pore sized filter to eliminate free PCX and lyophilized to obtain a powder inclusion complex.

In vitro characterization of the inclusion complexes were carried out with several methods namely, Differential Scanning Calorimetry (DSC), Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), phase solubility and drug loading to select the optimal inclusion complex for further experiments.