



# Novel NGR anchored pullulan micelles for controlled and targeted delivery of doxorubicin to HeLa cancerous cells

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## Abstract

Doxorubicin (DOX) is used to treat different kinds of cancers, including cervix carcinoma. However, it has various side effects such as cardiotoxicity. Nano-sized controlled releasing carriers such as polymeric micelles are of interesting approaches to overcome these side effects of doxorubicin in cancer chemotherapy. Regarding the up-regulation of CD13/APN receptors on the cervix carcinoma cells, which can bind to peptide sequences specially NGR (asparagine–glycine–arginine) with high affinity, peptide sequence (NGR) targeted micelles would lead to effective treatment of this carcinoma. In this study, the NGR peptide sequence was synthesized using the solution-phase strategy from asparagine, glycine, and arginine residues. The pullulan–retinoic acid conjugate and pullulan–retinoic acid–NGR conjugate were prepared by the amide and ester bond formation between the hydroxyl groups of pullulan and carboxylic acid groups of retinoic acid and peptide sequence. Pullulan–retinoic acid–NGR micelles were prepared by the direct dissolution method. The optimized micelles, according to their particle size (124.5 nm), zeta potential (− 3.65 mV), entrapment efficiency (85%), and release of DOX (70%, within 72 h) were assessed for their cytotoxicity on HeLa cells using MTT assay. NGR-targeted pullulan/retinoic acid micelles had higher cytotoxicity than the free DOX in cell culture studies on the HeLa cell line, and this can be a promising result in the treatment of cervix carcinoma.

**Keywords** Aminopeptidase N · NGR · Pullulan · Polymeric micelle · Doxorubicin (DOX) · Cervix cancer

## Introduction

Cervical cancer is originated from the certain types of human *papillomavirus* (HPV). Cervical cancer is the second most common cancer amongst women in less developed regions with an estimated 445,000 new cases in 2012 (84% of the new cases worldwide). In 2012, about 270,000 women died from cervical cancer; more than 85% of these deaths occurred in low- and middle-income countries [1].

Doxorubicin (DOX) as an anthracycline antibiotic is an effective chemotherapeutic agent for a wide range of cancers comprising the cervix, ovaries, etc., but it causes different side effects like cardiotoxicity that occurs as chronic congestive heart failure [2]. Targeted drug delivery methods were developed to enhance the selective delivery of chemotherapeutic drugs to the tumor site by minimizing the drug resistance along with the reduced side effects [3, 4].

Aminopeptidase N (APN/CD13) is a transmembrane glycoprotein of 150 kDa, which is included into the cell membrane through a hydrophobic N-terminal part. The

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