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Biocompatible nanoparticles sensing the matrix metallo-proteinase 2 for the on-demand release of anticancer drugs in 3D tumor spheroids

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ABSTRACT

The balance between dose-dependent tolerability, effectiveness and toxicity of systemically administered antitumor drugs is extremely delicate. This issue highlights the striking need for targeted release of chemotherapeutic drugs within tumors. In this work, a smart strategy of drug targeting to tumors relying upon biodegradable/biocompatible nanoparticles releasing cytotoxic drugs after sensing physiological variations intrinsic to the very nature of tumor tissues is exploited. Here, the well-known over-expression of matrix metallo-proteinase 2 (MMP2) enzyme in tumors has been chosen as a trigger for the release of a cytotoxic drug. Nanoparticles made up of a biodegradable poly(D,L-lactic-co-glycolic acid) (PLGA) – block – polyethylene glycol (PEG) copolymer (namely PELGA), blended with a tumor-activated prodrug (TAP) composed of a MMP2-sensitive peptide bound to doxorubicin (Dox) and to PLGA chain have been produced. The obtained devices are able to release Dox specifically upon MMP2 cleavage of the TAP. More interestingly, they can sense the differences in the expression levels of endogenous MMP2 protein, thus modulating drug penetration within a three-dimensional (3D) tumor spheroid matrix, accordingly. Therefore, the proposed nanoparticles hold promise as a useful tool for *in vivo* investigations aimed at an improved therapeutic efficacy of the conjugated drug payload.

1. Introduction

The performance of systemic chemotherapy, accomplished with both traditional and innovative drugs, is restricted by a series of biological barriers hindering an effective drug delivery after intravenous administration. Actually, solid tumors have inherently aberrant features, such as a highly fibrous matrix and an abnormal blood flow in their inner regions and frontier, which limit the delivery of drugs to the target tissue because of arterial-venous shunting and a strong interstitial pressure gradient [1–3]. Furthermore, only a tiny fraction of chemotherapeutic agent(s) can reach the tumor site because of their non-specific distribution and uptake by the reticuloendothelial system (RES) [4]. Therefore, the administered drug(s) accumulate within both target tissues and healthy organs and, owing to their low therapeutic index, often entail severe side effects, such as irreversible cardiotoxicity and nephrotoxicity [5–7]. These characteristics, together with the susceptibility toward drug resistance, reduce the healing potential of anticancer drugs [8–10], thus highlighting the striking need for more effective strategies to release chemotherapeutic drugs within tumor sites.

In this context, drug-loaded nanoparticles (NPs) are recognized to be cardinal platforms [11]; indeed, their nanometric size, coupled with superficial poly(ethylene glycol) (PEG) segments, help to circumvent RES and, therefore, to release the drug payload preferentially to tumor tissues, taking advantage of the enhanced permeability and retention (EPR) effect, which results from the leakiness of the immature and non-organized vasculature of solid tumors [12]. Even though EPR effect does actually enable a preferential accumulation of carriers/drugs to tumor sites and some reduction of side effects, the actual benefit is unpredictable because of individual variations in tumor microenvironment [12,13]. Actually, EPR allows only a few percent of intravenously administered NPs to accumulate at the target site [14] and, during their circulation in the bloodstream, NPs accumulate within the liver and spleen. This reduces the contact time between the NPs and the tumor site and, therefore, therapy effectiveness [15], thus resulting

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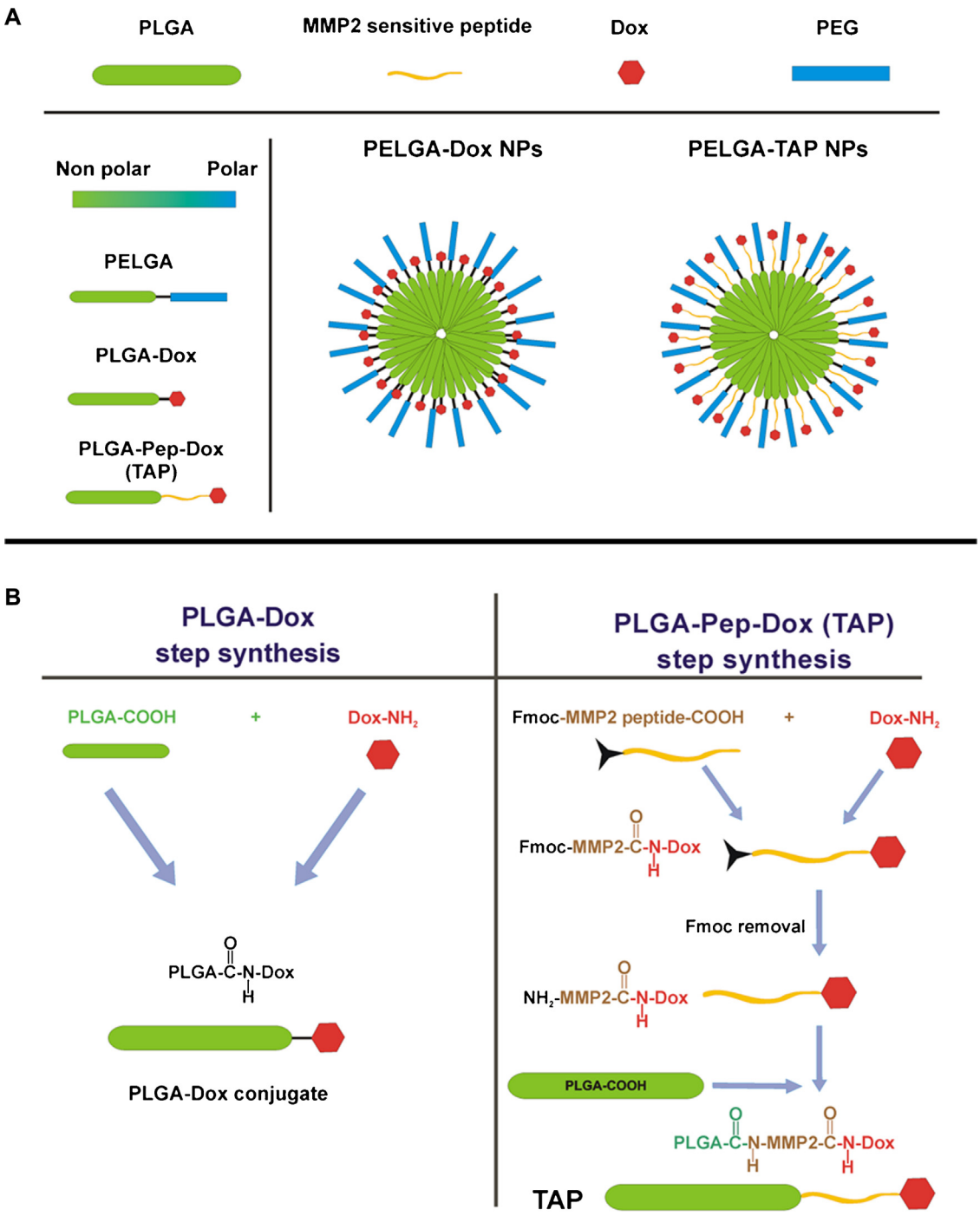

Fig. 1. Schematic representation of PELGA-TAP and PELGA-Dox NP formulations (A); Step synthesis of PLGA-Dox and PLGA-TAP copolymers (B).

into a heterogeneous NP accumulation in the tumor, and an unsatisfactory increase in overall patient survival [16–18]. The specificity of chemotherapy action can be increased if NPs are endowed with functional moieties to provide an active targeting, which can in principle improve NP performance in terms of targetability, cellular penetration and sensitivity to specific internal stimuli, such as the acidic pH in tumor microenvironment [19], altered redox potential [20], and up-regulation of specific proteins [21].

In this context, one strategy of drug targeting to tumors relies upon NPs releasing cytotoxic drugs by exploiting physiological variations that are intrinsic to the very nature of the tumor tissues. Indeed, compared to normal tissues, tumors secrete higher amounts of matrix metallo-proteinases (MMPs), which are proteolytic enzymes cleaving the natural extracellular matrix (ECM) of tumors and push tumor progression and metastasis [22,23]. In particular, MMP2 (also known as gelatinase A) plays a key role in tumor invasion and angiogenesis by hydrolyzing type IV collagen, which is a major constituent of tumor ECM [24,25]. Therefore, NPs delivering chemotherapeutic agents in response to MMP2 action offer the chance to exert their cytotoxic action toward target tumor sites with a high specificity, in order to prevent, or significantly reduce, the insurgence of toxic side effects against non-target tissues and organs.

In a recent study [26], we have synthesized two tumor-activated prodrugs (TAPs), composed of MMP2-sensitive peptides bound to doxorubicin (Dox) and PEG, tethered to model polystyrene NPs.