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Poly lactic-co-glycolic acid controlled delivery of disulfiram to target liver cancer stem-like cells

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Abstract

Disulfiram (DS), an anti-alcoholism drug, shows very strong cytotoxicity in many cancer types. However its clinical application in cancer treatment is limited by the very short half-life in the bloodstream. In this study, we developed a poly lactic-co-glycolic acid (PLGA)-encapsulated DS protecting DS from the degradation in the bloodstream. The newly developed DS-PLGA was characterized. The DS-PLGA has very satisfactory encapsulation efficiency, drug-loading content and controlled release rate *in vitro*. PLGA encapsulation extended the half-life of DS from shorter than 2 minutes to 7 hours in serum. In combination with copper, DS-PLGA significantly inhibited the liver cancer stem cell population. CI-isobologram showed a remarkable synergistic cytotoxicity between DS-PLGA and 5-FU or sorafenib. It also demonstrated very promising anticancer efficacy and antimetastatic effect in liver cancer mouse model. Both DS and PLGA are FDA approved products for clinical application. Our study may lead to repositioning of DS into liver cancer treatment.

Key words: Disulfiram; PLGA; Liver cancer; Cancer stem cells; Drug repositioning; Nano-technology; Drug delivery

Primary liver cancer is the fifth most common neoplasm and the third most common cause of cancer-related death worldwide.[1] Hepatocellular carcinoma (HCC) accounts for 70%-80% of cases of primary liver cancer.[2] The incidence of HCC is high in Asia and increasing in the Western world in the past decade. Although the therapeutic outcomes in many cancers have been improved significantly, the prognosis of advanced HCC remains very dismal.[3] Chemoresistance and metastasis are the major hindrances for the treatment of advanced HCC. HCC cells are resistant to all currently available anticancer drugs. Sorafenib (Nexavar) is the sole drug showing marginal efficacy in HCC with only approximately 3 month improvement for overall survival.[4] Therefore, development of efficacious chemotherapeutic drugs is of significant clinical importance for treatment of the advanced HCC.

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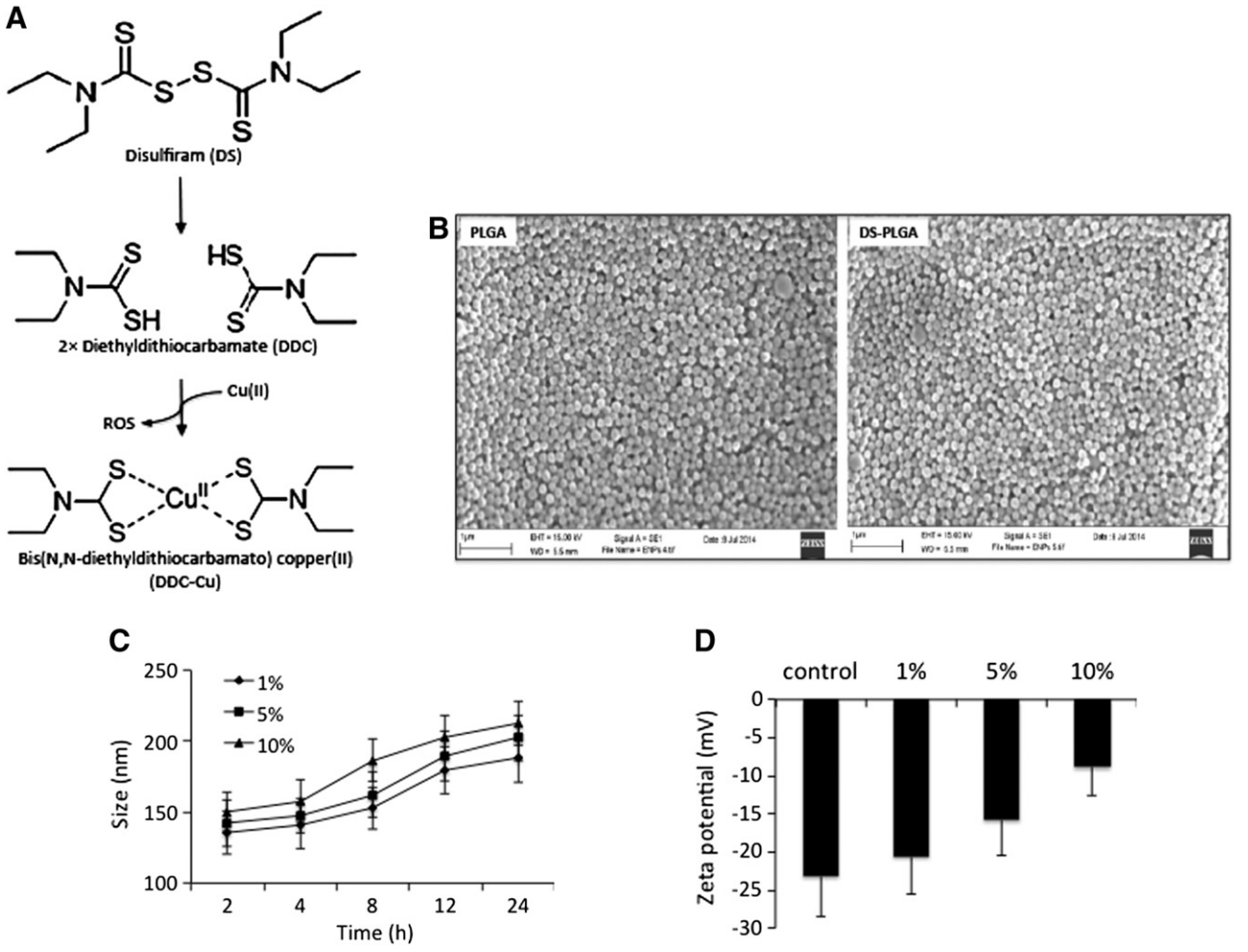

Figure 1. *In vitro* characterization of DS-PLGA. **(A)** The scheme of DS metabolism and reaction with copper(II). **(B)** Scanning electron micrographs of PLGA and DS-encapsulated PLGA (DS-PLGA). **(C)** The influence of serum concentration on the size of DS-PLGA. **(D)** The influence of serum concentration on zeta potential. **(E)** *In vitro* release profiles of DS. **(F)** *In vitro* half-life of DS in horse serum. **(G)** The *in vitro* half-life of free DS and DS-PLGA in horse serum. $^{**}P < 0.01$.

HCC is a highly heterogenic disease containing a small population of cancer stem-like cells (CSCs). HCC CSCs can be identified by detection of the expression of stem cell markers *e.g.* ALDH, CD133, CD90, CD44, EpCAM, and CD13.[5] Previous studies suggest that CSCs are maintained by the hypoxic milieu,[6,7] quiescent and highly resistant to currently available chemotherapeutic agents. Conventional anticancer drugs eliminate bulk of cancer cells but could not eradicate CSCs, which become the source of the cancer recurrence and metastasis. Therefore, development of efficacious CSC-targeting drugs may improve the therapeutic outcomes of HCC.

New drug development is a time and cash consuming procedure. This leads to the current interest in drug repositioning.[8] Previous studies demonstrate that disulfiram (DS), a commercially available anti-alcoholism drug,[9] is highly cytotoxic to a wide range of cancer cell lines[10–13] and enhances conventional anticancer drug-induced apoptosis in colon, breast and brain cancer cell lines.[10,14–16] Importantly, DS is highly cancer specific and specifically inhibits the activity of aldehyde dehydrogenase (ALDH), a functional marker of CSCs and reactive oxygen species (ROS) scavenger[17,18] to eliminate CSCs.

Although the anticancer activity of DS has been reported for almost 3 decades, its translation into clinical cancer treatment is severely limited by its very short half-life in the bloodstream.[19] The anticancer activity of DS is copper (Cu), zinc and some other divalent transitional metal elements dependent.[11,20–22] DS is quickly reduced to diethyldithiocarbamate (DDC), a strong chelator of copper(II). The reaction between DDC and Cu(II) generates ROS which are highly toxic to cancer cells. Due to the extremely short lifespan of ROS in human tissues,[23] the extracellular ROS can target cancer cells only when the reaction takes place within the cancer tissues.[22,24] DDC-Cu, the final product of the DS and Cu reaction, can also penetrate into cancer cells and induce apoptosis.[22] The sulfhydryl group of DDC is essential (Figure 1, *A*) for chelation of Cu(II) by DDC. The currently available oral version of DS is quickly reduced to DDC and the sulfhydryl group of DDC is promptly destroyed by glucuronidation, methylation and degradation in the bloodstream of the portal vein.[25] This may introduce the discrepancy between the anticancer activity of DS *in vitro, in vivo* and in patients. In order to translate DS into cancer therapeutics, intact sulfhydryl group of DS or DDC must be protected in the bloodstream and