Enzyme responsive acetaminophen hydrogels

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Abstract—Utilization of enzyme catalysis as a tool to disassemble self-assembled hydrogels to control the release encapsulated drug provides an opportunity to design a wide range of enzyme-specific low-molecular-weight hydrogelators (LMWGs). Herein, we report a novel approach for controlled delivery of multiple drugs by an enzyme triggered hydrogel degradation mechanism. In this proof-of-concept work, we report the synthesis of LMWGs (amphiphiles) from well-known drug acetaminophen (which is known as Tylenol®), and their ability to self-assemble into nanoscale structures in aqueous solutions to form hydrogels that subsequently encapsulate a second drug such as curcumin which is a known chemopreventive hydrophobic drug. Upon enzyme triggered degradation, hydrogels showed single and double drug delivery at physiological conditions in vitro. After treating with prodrug amphiphiles, mesenchymal stem cells (MSCs) retain their stem cell properties such as maintaining their adhesive and proliferation capacities with high viability. This new platform approach will have prospective effect on hydrogel based drug delivery research through developing drug delivery vehicles from a wide range of prodrug-based gelators.

I. INTRODUCTION

Self-assembly proved to be a powerful strategy to develop molecularly defined and functional material [1]. Hydrogels are one among them, and particularly they have been widely applied as intelligent delivery systems in controlled drug delivery [2]. A study of the literature reveals that there are only limited self-assembly based systems (low-molecular-weight gelators (LMWGs) are available as opposed to polymeric hydrogels [3]. One of the major rationales for this scenario is undesired toxicity of unknown metabolites that are generated from degradation of LMWGs hydrogelators. As a consequence, here we propose a novel conceptual route to surpass possible toxicity of in situ generated fragments of LMWGs (Fig. 1a). The key step in this approach is selecting either clinically practiced drugs or appropriate drugs that have safe metabolic pathway as precursor molecule to generate a library of amphiphiles which can undergo self-assembly to form hydrogels. Though covalent conjugation of known-drugs to the polymer backbone is well documented, however, polymer degradation can lead to polymer fragments with heterogeneous chain lengths that may generate potential toxicity or unwanted side effects. Hence, our approach, synthesizing prodrug-based amphiphiles which can degrade in presence of an enzyme to generate parent drug and another biocompatible fatty acid would surmount existing drawbacks.

Figure 1. a) Schematic representation of single and multiple drug delivery from prodrug-based hydrogels in response to enzyme catalysis. b) Synthesis of acetaminophen based amphiphiles. Scanning and transmission electron microscope images of curcumin encapsulated hydrogels (c and d, respectively).

Despite of having various stimuli such as temperature, pH and ionic strength etc to trigger drug delivery, an enzyme triggered drug delivery considered to be most safe method. In addition, often it allows the release of encapsulated therapeutics in desired locations. In this present approach we have overcome existing hurdles to develop biocompatible amphiphilic prodrugs as LMWGs to encapsulate hydrophobic drugs and subsequently release single and multiple drugs upon enzyme mediated gel degradation (Fig. 1a) in this investigation. To demonstrate the proof-of-concept, we choose the well-known drug TYLENOL, N-(4-hydroxyphenyl)acetamide, which is also known as acetaminophen (Apn), its drug activity is well studied [4,5]. Apn is a common analgesic and antipyretic drug that is used for the relief of fever, headaches, and other minor aches and pains. It is remarkably safe in recommended doses and importantly its route of metabolism in humans has extensively been investigated and found to be safe. A set of amphiphiles were synthesized in a single step from Apn as shown in Fig. 1b.

II. MATERIALS AND METHODS

Prodrug-based amphiphiles (Apn-7-COOH and Apn-13-COOH) were synthesized using a single step esterification
reaction from acetaminophen (Fig. 1b). Typically, in a round bottom flask, \( \alpha,\omega \)-dicarboxylic acid (12 mmol) was dissolved in dry THF, to that 1.2 equivalent of DCC and catalytic amounts of DMAP was added at room temperature. After stirring for 1 hr, 4-Hydroxyacetanilide (Apn, 1.36 g, 10 mmol) was added in one lot and stirring continued for 24 hrs. After completion of the reaction, mixture was filtered to remove DCU, washed with THF. After removal of THF, slightly acidic water was added, and thrice extracted with chloroform; organic layer was dried over anhydrous Na\( \text{SO}_4 \). After evaporating the solvent, the obtained crude products were purified by silica gel column chromatography using methanol:chloroform (1:9) as eluent, afforded pure products as white solid. Yields of the reactions were ~ 50%.

### III. RESULTS AND DISCUSSION

Gelation abilities of Apn based amphiphilic prodrugs have been investigated thoroughly in a wide range of solvents. The amphiphile Apn-7-COOH forms excellent gels in water and other aqueous buffer solutions even in the presence of salts. In addition, Apn-7-COOH is soluble in basic solutions (pH greater than 8), on contrary it forms hydrogel in acidic and neutral pH solutions. This clearly shows that terminal carboxylic acid groups play a vital role in self-assembly through formation of a hydrogen bonding network. However, due to the longer hydrophobic chain Apn-13-COOH was sparingly soluble in water and thus additional co-solvent (5-15 v/v% of alcohol such as methanol, ethanol or isopropanol) is necessary to make the hydrogel. Hydrogels of Apn-7-COOH or Apn-13-COOH were found to encapsulate high concentrations of curcumin (up to 0.5 mmol); this observed enhanced concentration of curcumin could be due to localization of hydrophobic curcumin within the hydrophobic pockets of the hydrogen's three-dimensional network. Morphology of curcumin encapsulated hydrogels were examined under electron microscope; SEM and TEM images (Fig. 1c and d, respectively) showed that prodrug gels form branched or entangled fibrous / sheet-like gel networks with fiber thickness of 50-400 nm, and fiber lengths of several microns.

To examine multiple (two) drug delivery (Fig. 2a), lipase was added to the curcumin encapsulated hydrogel of Apn-7-COOH (2 wt%) and kept it at 37 °C. Absorbance spectra were recorded on aliquots which were collected (after the addition of enzyme to the hydrogel) at regular intervals. Interestingly, initial aliquots did not show any absorbance peak, but aliquots collected after 4 hrs showed absorption maxima at 425 nm, which corresponds to the absorption peak of curcumin. To determine the role of the enzyme on hydrogel degradation, similar experiments were performed by adding only buffered solution without an enzyme. As we expected curcumin encapsulated gel was still intact over a month, there was no absorbance peaks corresponding to the curcumin were observed suggesting that the enzyme is required for gel degradation. Interestingly, increasing either the concentration of enzyme or incubation temperature to 45 °C doubled the rate of drug release.

![Figure 2](image-url)

**Figure 2.** a) Absorption spectra curcumin released from degraded hydrogel of Apn-7-COOH at different time points. Examining the effect of Apn-7-COOH prodrug on MSCs cellular properties such as b) viability, c) adhesion and d) proliferation.

To test the cytocompatibility of prodrug-based amphiphiles, and their effect on cell characteristics such as viability, proliferation, and adhesion; a series of experiments were performed with MSCs. In these experiments unmodified drug (Apn) and modified drug Apn-7-COOH were examined with trypsin blue assay. The presented results (Fig. 2b) suggest that there was no significant decrease in the viability of the MSCs after 48 hours incubation with prodrug-based amphiphile. These results clearly show that Apn-derived prodrug amphiphiles did not induce cell toxicity. In addition, data showed in Fig. 2c and d clearly suggesting that Apn-7-COOH amphiphile did not affect the inherent cell properties such as adhesion and ability to proliferate.

### IV. CONCLUSIONS

In conclusion, Apn-based amphiphilic prodrugs have showed excellent hydrogelation ability and could encapsulate curcumin. Delivery of single and multiple drugs has been achieved upon enzyme-triggered gel degradation at physiological condition. Newly synthesized prodrugs were highly cytocompatible in nature.

### REFERENCES