

Article

# Dissolution of Chitin in Deep Eutectic Solvents Composed of Imidazolium Ionic Liquids and Thiourea

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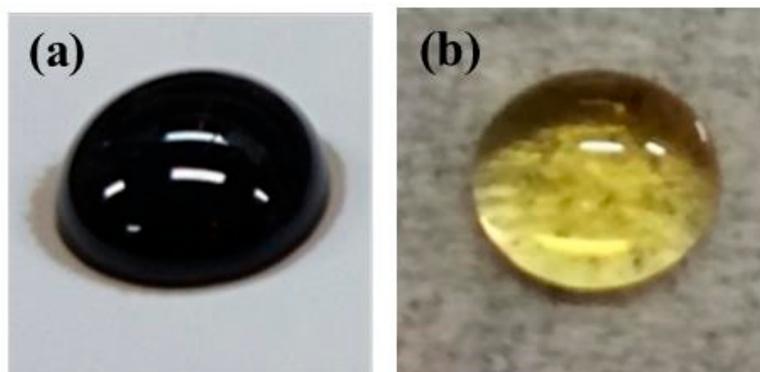
**Abstract:** Chitin is an abundant organic resource but shows poor solubility, leading to difficulty in utilization as materials. We have already reported that an ionic liquid (IL), 1-allyl-3-methylimidazolium bromide, dissolves chitin at concentrations up to ca. 5 wt %. However, the color of the resulting solution is blackened, mainly owing to the presence of bromide. On the other hand, some deep eutectic solvents (DESs) have been already reported to dissolve chitin. In this study, we found that DESs composed of imidazolium ILs and thiourea dissolved chitin without obvious coloring. DESs are systems formed from eutectic mixtures of hydrogen bond accepters and donors. We first prepared DESs by heating mixtures of imidazolium ILs with thiourea at 100 °C for 30 min with stirring. Predetermined amounts of chitin were then added to the DESs, and for the dissolution, the mixtures were left standing at room temperature for 24 h, followed by heating at 100 °C for 24 h with stirring. The dissolution processes were evaluated by CCD camera views, which revealed in most cases the dissolution of chitin at 2–5 wt % concentrations with the present DESs.

**Keywords:** chitin; deep eutectic solvent; dissolution; ionic liquid; thiourea

## 1. Introduction

Ionic liquids (ILs) are low-melting-point (low-mp) salts that form liquids at temperatures below the boiling point of water. ILs have been identified as good solvents for natural polysaccharides [1,2], since an IL, 1-butyl-3-methylimidazolium chloride (BMIMCl), was found to dissolve cellulose in 2002 [3]. For more than a decade, various ILs have been reported to dissolve cellulose and to be used as media for cellulose derivatization, modification, and functionalization [4–10]. On the other hand, there are not many ILs to dissolve chitin [2,11–15], because chitin shows quite poorer solubility even compared with cellulose. Such a solubility problem is principally caused by the presence of acetamido groups at the C-2 position in  $\beta(1\rightarrow4)$ -linked *N*-acetyl-D-glucosamine repeating units, which form very strong intermolecular hydrogen bonds [16]. Even nowadays, only several types of ILs, which dissolve chitin, are known [17], including 1-alkyl-3-methylimidazolium acetates, 1-ethyl-3-methylimidazolium alkanoates, 1-alkyl-3-methylimidazolium dimethylphosphate, tetrabutylphosphonium amino acid salts, and 1-allyl-3-methylimidazolium bromide (AMIMBr) [11,18–21]. For example, we found that AMIMBr dissolved chitin at concentrations up to ca. 5 wt % and formed ion gels with higher contents of chitin [21]. AMIMBr is more stable and could be easily prepared, owing to the fact that it consists of a bromide counter anion, compared with the two kinds of ILs mentioned above. In the following studies, accordingly, we used AMIMBr solutions as media for facile acylation and self-assembled regeneration of chitin to successfully produce chitin esters and nanochitins [22–26]. The presence of

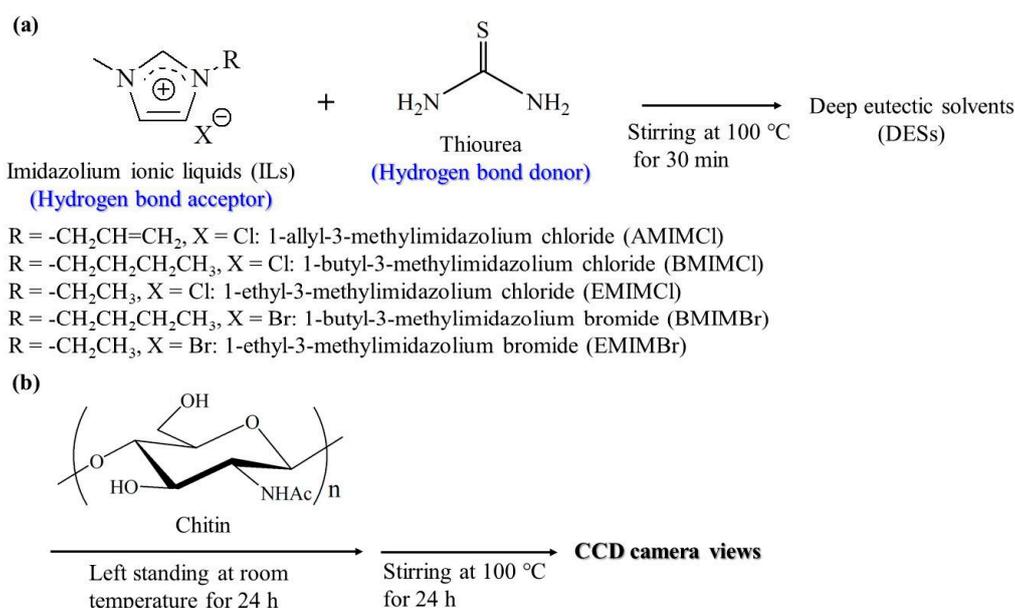
bromide in AMIMBr, however, causes the solution and gel to be blackened, as shown in Figure 1a, resulting in the production of colored chitin materials. Based on the above viewpoints, new solvent systems for chitin have still attracted a great deal of attention, which can be used as media to construct new chitin-based functional materials.



**Figure 1.** Photographs of solutions of chitin in 1-allyl-3-methylimidazolium bromide (AMIMBr) (a) and a deep eutectic solvent (DES) composed of 1-allyl-3-methylimidazolium chloride (AMIMCl) and thiourea (ca. 5 wt %) (b).

Recently, some deep eutectic solvents (DESs) as analogs of ILs, mainly composed of choline chloride, have also been found to dissolve chitin [27–35]. DESs are fluids formed by adequate mixtures of hydrogen bond acceptors (HBAs) and hydrogen bond donors (HBDs), which are capable of self-association through hydrogen bonding interactions to form eutectics with lower melting points (mps) in comparison to each individual component [36]. For example, a DES composed of choline chloride and thiourea was reported to dissolve chitin [27,28]. Furthermore, DESs have been used for extraction and purification of chitin from raw native resources such as lobster shells [29]. However, mps of DESs obtained from choline chloride are relatively high, e.g., higher than room temperature, which are disadvantage to be employed as media for further applications of chitin [36]. Therefore, considerable efforts should be devoted to developing new DESs for solvents of chitin, which have potential to be used as media in efficient materialization of chitin, such as derivatization and modification.

On the other hand, DESs composed of ILs as HBAs have been found to dissolve biopolymers, such as starch [37]. In this study, we prepared DESs from imidazolium ILs having different substituents and thiourea, which were employed for the dissolution experiments of chitin. The imidazolium ILs, used in this study, were 1-allyl-3-methylimidazolium chlorides (AMIMCl), BMIMCl, 1-ethyl-3-methylimidazolium chlorides (EMIMCl), and 1-butyl- and 1-ethyl-3-methylimidazolium bromides (BMIMBr and EMIMBr) (Figure 2a). It should be noted that the pure ILs do not dissolve certain amounts of chitin. Consequently, in most cases, the addition of thiourea to the ILs enhanced the dissolution ability of chitin in the resulting DESs.



**Figure 2.** (a) Preparation of DESs and (b) dissolution experiment procedure of chitin in DESs.

## 2. Materials and Methods

### 2.1. Materials

The  $\alpha$ -chitin powder from crab shells was purchased from Wako Pure Chemicals, Tokyo, Japan. BMIMCl (purity  $\geq 98.0\%$ ) and EMIMCl (purity  $> 97.0\%$ ) were purchased from Sigma-Aldrich, Darmstadt, Germany and Tokyo Chemical Industry Co., LTD., Tokyo, Japan, respectively. The other reagents and solvents were available commercially and used without further purification.

### 2.2. Preparation of Ionic Liquids

A typical procedure for preparation of ILs was as follows. 1-Chloro-1-propene (14.0 mL; 0.183 mol), 1-bromobutane (20.0 mL; 0.185 mol), or bromoethane (20.0 mL; 0.257 mol) was slowly mixed with 1-methylimidazole (10.0 g; 0.122 mol) at 80 °C, and the resulting mixture was then maintained at this temperature for 15 h with stirring. After the reaction mixture was washed with diethyl ether several times, the residue was dried under reduced pressure at 100 °C for 24 h to give the ILs, that is, AMIMCl, BMIMBr, and EMIMBr (ca. 17–22 g; 73%–87% yields). The structures of the ILs were confirmed by <sup>1</sup>H NMR spectra (CDCl<sub>3</sub>, Supplementary Figure S1) according to the data previously reported in the literature [38,39].

### 2.3. Preparation of DESs

A typical procedure for preparation of DESs was as follows. A mixture of an IL with thiourea at a predetermined molar ratio was heated at 100 °C for 30 min with stirring. After cooling the mixture to room temperature, the formation of a clear solution was evaluated by a charge-coupled device (CCD) camera view.

### 2.4. Dissolution Experiments of Chitin with DESs

A typical procedure for a dissolution experiment of chitin with a DES was as follows. A predetermined amount of chitin was mixed with a DES at an IL/thiourea molar ratio of 1:0.5. The mixture was then left standing at room temperature for 24 h and subsequently heated at 100 °C for 24 h with stirring. After cooling the mixture to room temperature, the dissolution was evaluated by a CCD camera view.

### 2.5. Regeneration of Chitin from a Solution with a DES

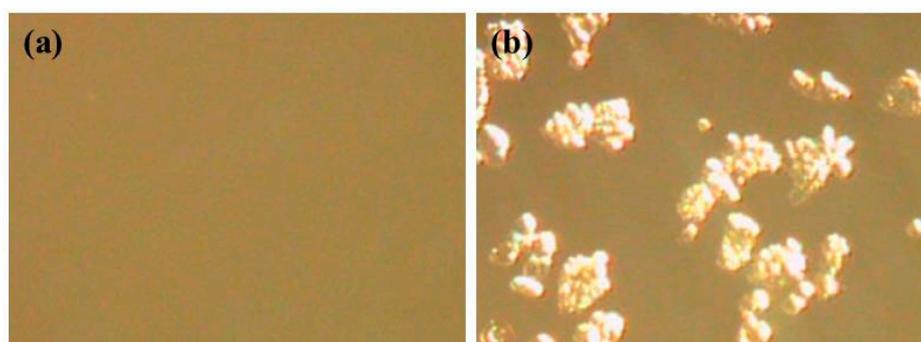
A mixture of chitin (0.0526 g; 0.259 mmol) with the AMIMCl/thiourea DES (1:0.5 molar ratio; 1.00 g) was left standing at room temperature for 24 h and subsequently heated at 100 °C for 24 h to give a 5 wt % solution. After the addition of methanol (6.0 mL) to the resulting solution, the mixture was centrifuged, and the supernatant was decanted. The processes for the addition of methanol to the residue, centrifugation, and decantation were additionally performed twice. The resulting residue was isolated by filtration, washed with methanol, and dried under reduced pressure at room temperature for 2 h to give the regenerated chitin (0.0508 g).

### 2.6. Measurements

The  $^1\text{H}$  NMR spectra were measured on the JEOL ECA600 spectrometer (JEOL, Akishima, Tokyo, Japan). CCD camera observation was conducted using the Dino-Lite Digital Microscope AM-311 (AnMo Electronics Corporation, Hsinchu, Taiwan). Differential scanning calorimetry (DSC) was performed on the DSC 6220 (Seiko Instruments Ins. Chiba, Japan). Samples (ca. 5–8 mg) were hermetically sealed in an aluminum pan and heated from room temperature to 100 °C at a heating rate of 20 °C  $\text{min}^{-1}$  under nitrogen atmosphere as the first scans for removal of moisture. After the samples were cooled to 0 °C, the second scans were then conducted by heating the samples from 0 to 400 °C at the same heating rate to obtain the DSC profiles. Powder X-ray diffraction (XRD) measurements were conducted using the PANalytical X'Pert PRO MPD (PANalytical B.V., Almelo, The Netherlands) with Ni-filtered Cu K $\alpha$  radiation ( $\lambda = 0.15418$  nm). Infrared (IR) spectra were recorded on the PerkinElmer Spectrum Two spectrometer (PerkinElmer Japan Co., Ltd., Yokohama, Japan).

## 3. Results and Discussion

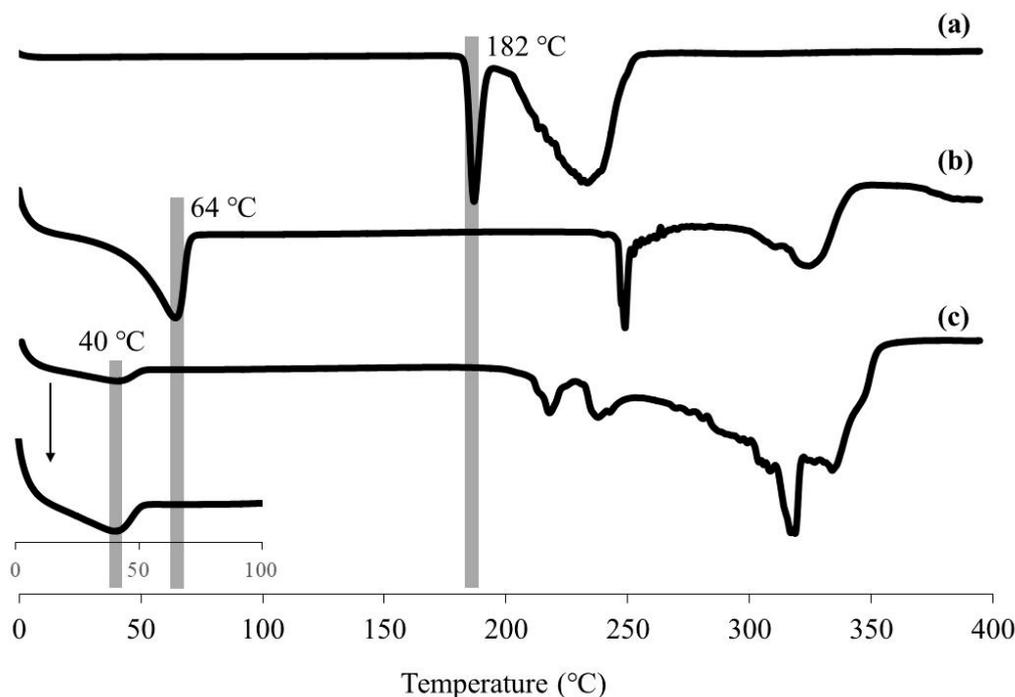
We first performed the preparation of DESs by heating the mixtures of imidazolium ILs with thiourea at different molar ratios at 100 °C for 30 min with stirring (Figure 2a). The CCD camera views of the resulting liquids with a 200 times magnification indicated that clear solutions were obtained from the mixtures at IL/thiourea molar ratios up to 1:0.5 (Figure 3a), except the mixtures with EMIMCl, while at higher IL/thiourea molar ratios such as 1:0.6, thiourea was not completely dissolved in the ILs in all the cases (Figure 3b). Hereafter, we employed a 1:0.5 IL/thiourea molar ratio for DESs. In the case of EMIMCl, a maximum molar ratio of EMIMCl/thiourea for the formation of a clear solution was 1:0.3, which was used for the subsequent dissolution experiment.



**Figure 3.** CCD camera views of the mixtures of AMIMCl with thiourea at molar ratios of 1:0.5 (a) and 1:0.6 (b) after heating at 100 °C for 30 min with stirring for preparation of DESs. The magnification is 200 times.

The formation of the DESs was confirmed by DSC measurements. Figure 4 shows the DSC profiles of thiourea, EMIMBr, and the DES prepared from the mixture of EMIMBr and thiourea (molar ratio of 1:0.5). The DSC profile of the DES exhibits an endothermic peak at 40 °C, whereas those of the pure thiourea and EMIMBr show endothermic peaks at higher temperatures, i.e., 182 and 64 °C,

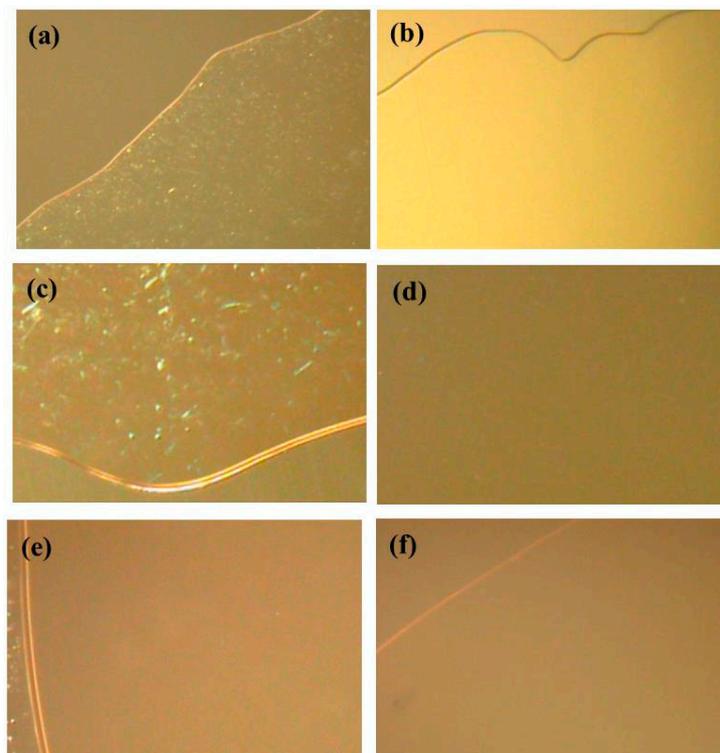
respectively, corresponding to their mps; the mp values of EMIMBr in the literature are 65–79 °C [40]. These data support the occurrence of mp depression in the mixture of EMIMBr with thiourea, strongly indicating the formation of the DES. Based on the DSC results of the prepared DESs, the mp value of the EMIMBr/thiourea DES was reasonably evaluated to be around 40 °C as shown in Figure 4c, while the mp values of the other DESs were estimated to be lower than 0 °C. Indeed, the former DES was frozen at 10 °C, whereas the latter DESs were kept to be liquids even in a refrigerator (at –20 °C). The as-prepared EMIMBr/thiourea DES was liquid at room temperature below the mp, owing to the undercooling state, which could be employed for the dissolution experiments. However, after the DES was frozen once, it was kept to be solid even at room temperature.



**Figure 4.** Differential scanning calorimetry (DSC) profiles of thiourea (a), EMIMBr (b), and DES (c) (molar ratio of EMIMCl to thiourea = 1:0.5).

The dissolution experiments of chitin using the prepared DESs were then carried out as follows. Predetermined amounts of chitin were mixed with DESs. The mixtures were then left standing at room temperature for 24 h for pretreatment and subsequently heated at 100 °C for 24 h with stirring. The dissolution processes were followed by CCD camera views with a 200 times magnification, as shown in Figure 5. The maximum concentrations of chitin dissolved were evaluated by whether solids of chitin were seen or not in the CCD camera views. For example, the CCD camera view of the 5 wt % mixture of chitin with the AMIMCl/thiourea DES after heating at 100 °C for 24 h does not show any solids of chitin (Figure 5b), unlike the CCD camera view of this mixture before heating (Figure 5a). This result suggested that the DES dissolved chitin at a 5 wt % concentration (Entry 1, Table 1). Similarly, Table 1 lists the maximum concentrations of chitin for dissolution in the prepared DESs to obtain clear solutions. Besides the system from BMIMCl, the addition of thiourea contributed to the enhancement of the dissolution ability of chitin in the DESs. Indeed, the CCD camera views in Figure 5d–f show the formation of clear solutions, by the dissolution experiments, in the mixtures of 5 wt % chitin with the BMIMBr/thiourea DES (Entry 4, Table 1) and 2 wt % chitin with the EMIMCl/thiourea and EMIMBr/thiourea DESs (Entries 3 and 5, Table 1). On the other hand, insoluble solids of chitin appeared in the mixture of a lower content (1 wt %) of chitin with the BMIMCl/thiourea DES after heating at 100 °C, as shown in Figure 5c (Entry 2, Table 1), indicating that the addition of thiourea was not significantly effective in the enhancement of the dissolution ability of the DES. These results indicated

that both the substituents on the imidazolium rings and the counter anions of the ILs strongly affected the dissolution ability of the DESs, but roles of them for the dissolution in the DESs are not yet clear. Furthermore, it is notable that the solutions of chitin with these DESs did not exhibit obvious coloring, as shown in Figure 1b.



**Figure 5.** (a) CCD camera view of the mixture of chitin with AMIMCl/thiourea DES. (b–f) CCD camera views of products of chitin in the AMIMCl-, BMIMCl-, EMIMCl-, BMIMBr-, and EMIMBr-thiourea DESs in the dissolution experiments. Contents of chitin are as listed in Table 1, except the content for the BMIMCl/thiourea DES, which was 1 wt %. The magnification is 200 times.

**Table 1.** Dissolution of chitin in the DESs <sup>a</sup>.

Entry	IL in a DES (Molar ratio of IL to Thiourea)	Maximum Concentration of Chitin for Dissolved (wt %) <sup>b</sup>
1	AMIMCl (1:0.5)	5
2	BMIMCl (1:0.5)	~0
3	EMIMCl (1:0.3)	2
4	BMIMBr (1:0.5)	5
5	EMIMBr (1:0.5)	2

<sup>a</sup> The dissolution experiments were conducted by standing mixtures at room temperature for 24 h and subsequently by heating at 100 °C for 24 h with stirring. <sup>b</sup> Estimated by the CCD camera views of the mixtures after heating.

Finally, regeneration of chitin from the AMIMCl/thiourea DES solution was carried out by addition of methanol as a poor solvent for chitin. The XRD profile and the IR spectrum of the regenerated chitin were totally identical with those of the original chitin, as shown in Supplementary Figures S2 and S3. These results strongly suggested that degradation and change in the chemical structure of chitin did not obviously occur during the dissolution experiments.

#### 4. Conclusions

In this study, we found that the addition of thiourea to imidazolium ILs provided the dissolution ability of certain amounts of chitin in the produced DESs in most cases. The preparation of DESs

was conducted by heating the mixtures of imidazolium ILs with thiourea at 100 °C for 30 min with stirring. The formation of the DESs was confirmed by the DSC measurements of the produced solutions. The dissolution experiments were conducted by standing mixtures of chitin with the DESs at room temperature for 24 h for pretreatment and subsequently by heating at 100 °C for 24 h with stirring. The dissolution processes were evaluated by CCD camera views, which indicated that the AMIMCl- and BMIMBr-thiourea DESs and the EMIMCl- and EMIMBr-thiourea DESs at the molar ratios listed in Table 1 dissolved chitin with 5 wt % and 2 wt % concentrations, respectively. On the other hand, the BMIMCl/thiourea DES did not dissolve chitin. Accordingly, the results of the investigation suggested that both the substituents on the imidazolium rings and the counter anions of the ILs strongly affected the dissolution ability of the DESs. The XRD and IR results of the regenerated chitin from the AMIMCl/thiourea DES solution suggested no obvious occurrence of degradation and change in the chemical structure of chitin during the dissolution experiments. Furthermore, clear solutions without obvious coloring were obtained using the present DESs, unlike the phenomenon of chitin dissolved in an AMIMBr solution, as previously reported. The present DESs, facily prepared from imidazolium ILs and thiourea, can be considered as useful solvents, which will be employed as media for derivatization and functionalization of chitin in the future.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/2305-7084/3/4/90/s1>, Figure S1: <sup>1</sup>H NMR spectra of AMIMCl (a), BMIMBr (b), and EMIMBr (c) in CDCl<sub>3</sub>, Figure S2: XRD profiles of the original chitin (a) and the regenerated chitin (b), Figure S3: IR spectra of the original chitin (a) and the regenerated chitin (b).

**Author Contributions:** K.Y. and J.-i.K. conceived the project, designed the experiments, directed the research, and wrote the manuscript. S.I. performed the experiments. All the authors discussed the results and edited the manuscript.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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