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Short Communication

Peptide-based spherulitic films—formation and properties

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ABSTRACT

Peptide nanotube-based spherulitic films are a recently discovered phenomenon, which was demonstrated in the case of the self-assembled diphenylalanine peptide nanotubes. Here we show that the film-formation method can be implemented with other peptides. We also demonstrate that a critical physical parameter, an elevated level of environmental hydration, is required for film growth. A possible formation mechanism is suggested. The optical, morphological and mechanical properties of these films are characterized and are found to be substantially different from those of non-spherulitic deposits.

Spherulites are poly-crystalline entities comprised of a nucleation center from which multiple lamellas span out, maintaining a space-filling shape [1,2]. Although spherulitic growth has been recognized since the early days of crystal growth research, its mechanism is not yet completely understood [1,2]. Nevertheless, spherulitic moieties can be found in various types of materials—inorganic [3–5], organic [6–10] biologic [11–20], and even in the element selenium [21]. In particular, spherulites are encountered in polymers [1]. The significance of their existence in soft matter stems from the fact that spherulitic composition has been shown to alter the polymers' physical properties, such as optical and mechanical [6,22–25].

It was previously shown [26] that the peptide diphenylalanine (FF) can self-assemble to form peptide nanotubes (PNTs). The formation of PNTs has been previously discussed in view of its self assembly properties [26]. Self assembly [27,28] is an important phenomenon with great importance in nature and in nano-sciences since it offers a way to construct complex nanostructures. Interestingly, self assembly can be seen in various scales, ranging from molecular and up to micro-sized objects [28].

We have previously shown [29] that self-assembly capability of FF can be utilized to form spherulitic films on solid surfaces. These films are an excellent example of multi scale self assembly, that is ranging from the molecular self assembly, and up to the spherulitic structure. Here we demonstrate that the spherulitic film growth phenomenon is governed by several physical factors, and not by the chemical composition of the peptide molecules. We show that environmental humidity level is a critical factor in the peptide's spherulitic growth. This is demonstrated by the formation of spherulitic films from another PNT-forming peptide—dileucine (LL). We also illustrate that the mechanical properties of the peptide spherulitic films differ from those of their non-spherulitic counterparts.

Our method of forming peptide spherulitic films [29] consists of depositing an aqueous peptide solution, diluted with the additive N-methyl pyrrolidone (NMP), onto a heated flat solid substrate. The solution is subsequently allowed to completely evaporate. The conditions for such spherulitic growth are not as harsh as in the case of some polymer spherulites: similarly shaped and packed spherulites can be found in bulk polymers, which are crystallized from the melt at temperatures exceeding 100 °C, such as nylon [7] and *n*-alkanes [10]. The mild conditions required for peptide spherulitic film formation suggest that their formation from other peptides should not interfere with peptides' functions; thus, various peptide films with enhanced mechanical properties can be formed which still retain their function. Moreover, although spherulites have been previously shown to be formed by peptides [13–15], such structures were three-dimensional in nature and were spread sporadically in the matrix in which they were formed. Our films are comprised of two-dimensional closely arranged spherulites, similar to those found in bulk polymers [2,6], and therefore have potential uses in biologic coating technology.

We have recently shown that the beta-amyloid derived diphenylalanine [26] (FF, Fig. 1a) can be induced to form films on flat solid surfaces [29]. These films are comprised of multiple closely arranged two-dimensional spherulites, each exhibiting a nucleation center and multiple lamellas. These lamellas grow from the nucleation center until they encounter the growth fronts of neighboring spherulites, thus forming a grain boundary. Optical, atomic

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force (AFM) and scanning electron (SEM) microscopy of the films are shown in Fig. 1.

We found that FF spherulitic films form only when a specific physical parameter exists. Specifically, a high level of environmental relative humidity (RH) (RT, RH > 75%). The high humidity was found to be a critical factor: without it, no spherulites formed.

To test whether spherulitic films can also form from other peptides, and whether our film-formation procedure can be applied to various peptides or proteins, the spherulitic film-formation procedure found suitable for FF was applied to a different dipeptide—dileucine (L-Leu–L-Leu; LL, Fig. 2a). It was also of interest to note whether the dependency on humidity levels would still exist.

LL, like FF, is a dipeptide with hydrophobic side chains, but it differs from FF by its lack of aromatic moieties. LL has been previously found to be a PNT-forming peptide [30], as it is comprised of channels that form due to its crystalline structure, like FF. The channels in LL are smaller than the 10 Å channels found in FF, and are rectangular, with dimensions of 2.5 Å by 6.0 Å [30].

When the FF-based film-formation procedure was applied to LL, spherulitic films were also successfully formed. The LL films were similarly comprised of closely arranged spherulites, exhibiting distinct nucleation centers and grain boundaries. Optical, AFM and SEM analyses of the film are shown in Fig. 2. As in the case of the FF films, the same humidity dependency was found with the LL spherulitic films, which formed only at elevated levels of environmental humidity.

For both FF and LL, the dependence on humidity was found to outlive the film-deposition process itself. At RH > ~75% (RT), films were observed immediately after deposition. In contrast, film-deposition attempts carried out at lower environmental humidity (e.g. RT, RH ~ 60%) did not initially yield spherulitic films. However, when the samples were exposed to elevated levels of humidity, spherulitic films appeared on the substrate. An example of this effect can be seen in Fig. 3.

Fig. 3a shows the result of attempted FF film deposition at a humidity level below 70% (RT). No spherulitic film is formed, and the only observable feature is a small fibrous branched shape. Similar behavior was observed for LL, as can be seen in Fig. 3c, which shows only small fibrous features appearing shortly after deposition. Exposure of the samples to elevated levels of environmental humidity resulted in the formation of a spherulitic film (Fig. 3b and d, FF and LL, respectively). The fiber seen in Fig. 3a became the nucleation center of the spherulites, and is an example of a nucleation "sheaf", which represents a stage of homogeneous nucleation [1,31].

It should be noted that spherulites comprised of small organic molecules are rarely encountered [2]. Nevertheless, the few small organic molecules that have been previously described [1] have a common property—they are all aromatic hydrocarbons. LL is therefore unique in the sense that it is a small non-aromatic organic molecule that forms spherulites.

Based on our observations, and on what is known about spherulitic composition in polymers, we determined the effect of

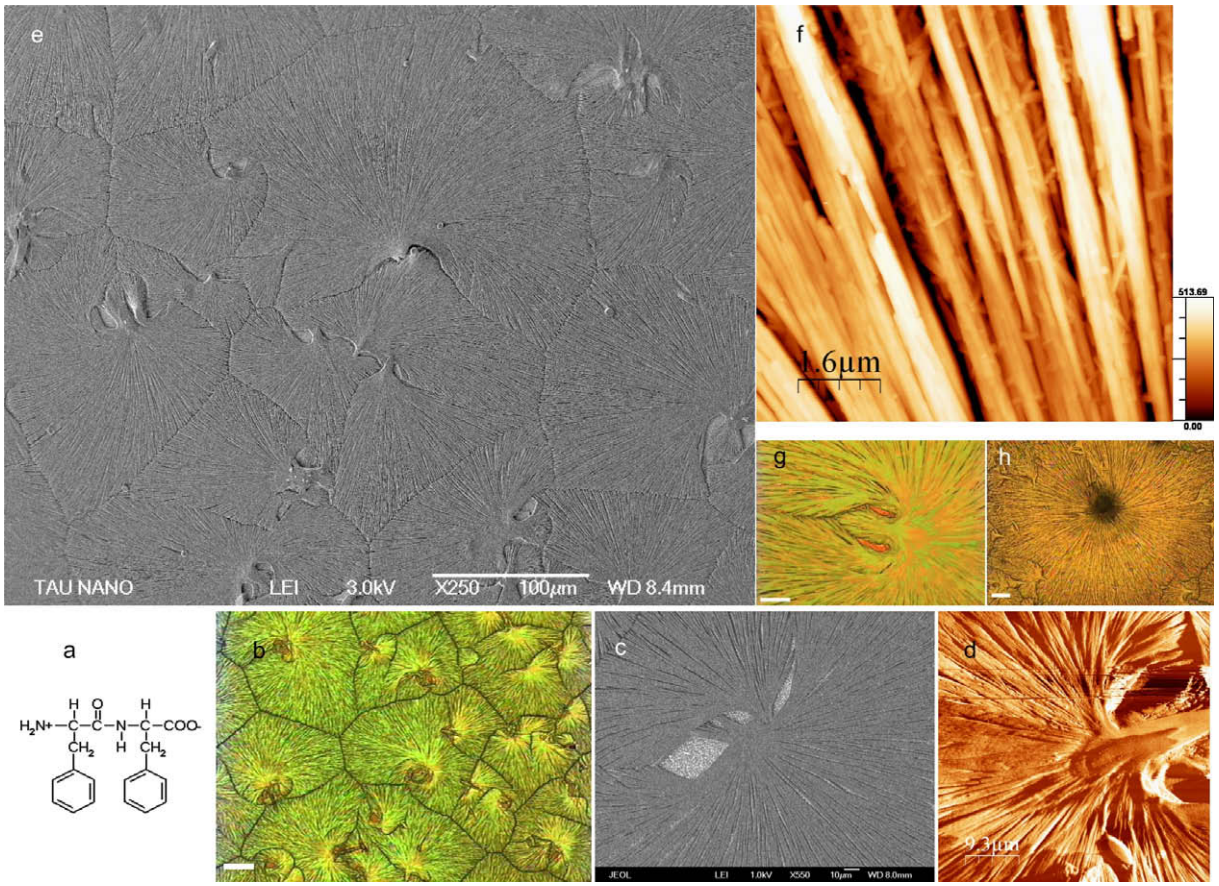

Fig. 1. (a) Scheme of L-Phe–L-Phe (FF). (b) An optical image of an FF spherulitic film. Bar = 50 μm. (c) An SEM image of a homogenous nucleation site of a FF spherulite, bar = 10 μm. (d) An AFM phase image of a spherulite's homogenous nucleation site. Bar = 9.3 μm. (e) An SEM image of an FF spherulitic film. Multiple closely arranged spherulites can be seen. Bar = 100 μm. (f) An AFM image showing the lamellas comprising an FF spherulite. Bar = 1.6 μm. (g) and (h), Optical images of a homogenous nucleation site of FF spherulite (bar = 20 μm) and a heterogeneous one (bar = 50 μm). Films were prepared as follows: the peptide was dissolved in water, at a conc. of 2 mg/ml. N-methyl-2-pyrrolidone was added at a ratio of 10% v/v and the solution was drop-casted and dried on the substrate at 60 °C. The resulting films are 700–1500 nm thick.