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Technical note

Real-time monitoring of amyloid growth in a rigid gel matrix

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We demonstrate the real-time monitoring of the growth of amyloid-protein aggregates in a semi-rigid gel environment constructed from a 5% w/v gelatin solution. The kinetics of amyloid fibril growth from reduced and carboxy-methylated κ-casein occurring in the gel medium was contrasted against that obtained in a regular solution assay. Aggregation kinetics were recorded using Thioflavin T fluorescence. Transmission electron microscopy was used to confirm the aggregates' existence and morphology. The current demonstration of controlled amyloid growth in a gel environment represents the first step towards development of an experimental model for investigating the role of spatial and medium factors in the kinetics of aggregation-based proteopathies.

The production of aberrant protein aggregates is believed to play a causal role in a large number of disease states, collectively termed the aggregation based proteopathies [1–3]. Due to their cytotoxic nature [4–6], the time course and kinetic mechanism of aggregate growth has been intensively studied [7–9] along with factors that accelerate or retard aggregate production [10,11]. Although protein aggregation kinetics is a much researched topic, the near total majority of investigations have studied the growth of aggregates in shaken or stirred aqueous solutions [12,13]. Under these conditions, the well-mixed approximation is valid and the kinetic rate equations accounting for aggregate growth take the form of ordinary differential equations, with the concentration of aggregate defined in terms of the total volume of the reaction vessel [1,7,9,13]. From PET [1] and MRI imaging studies of the dispersion of disease-related aggregate within the body, it is clear that such spatial uniformity does not always comport to the reality of disease progression [14,15]. Indeed, a more realistic paradigm of aggregation based disease development may involve slow aggregate movement from one spatial center to another occurring via a diffusion and/or convection based mechanism [1,16] (Fig. 1). When spatial aspects have been previously considered, either compartment modelling approaches [17,18] or reaction/diffusion/convection models [16,19,20], have been used. To the best of our knowledge, no *in vitro* experimental model has been developed to accompany these theoretical approaches. To experimentally interrogate this spatial aspect, a suitable model system, that is capable of spatially resolving amyloid growth, is required. Due to its ability to largely eliminate complications arising from convective processes, an aqueous-based gel environment, doped with soluble (non-aggregated) protein, represents an attractive model candidate. Here, we take the first steps towards realizing the inherent promise of such a model system by demonstrating conditions for formation of amyloid aggregates within a semi-rigid gel formed from a 5% w/v gelatin solution.

Fig. 2 describes the kinetics of amyloid formation from six concentrations of reduced and carboxy-methylated κ-casein protein, detected by use of the specific amyloidogenic dye marker Thioflavin T [21,22]. Fig. 2A and B describes the baseline corrected Thioflavin T fluorescence signal indicative of amyloid growth, occurring in either a quiescent gel matrix or a stirred solution environment. Fig. 2C and D respectively shows transmission electron microscope (TEM) images of amyloid fibers, either recovered from the gel, or via direct plating onto the TEM grid from solution. Supplementary Fig. 1A and B describes the corresponding non-baseline corrected kinetic traces along with baseline reading. Supplementary Fig. 1C and D describes higher magnification versions of the corresponding TEM images in Fig. 2C and D.

Understanding how the spatial dependent migration of amyloid occurs has been recognized as the next major challenge in amyloidosis research [1,2]. The gaining of such an understanding will help to delineate the causal effectors of the systemic and localized natures displayed by many aggregation-based

Abbreviations: PET, Positron Emission Tomography; MRI, Magnetic Resonance Imaging; ThT, Thioflavin T; TEM, Transmission Electron Microscope.

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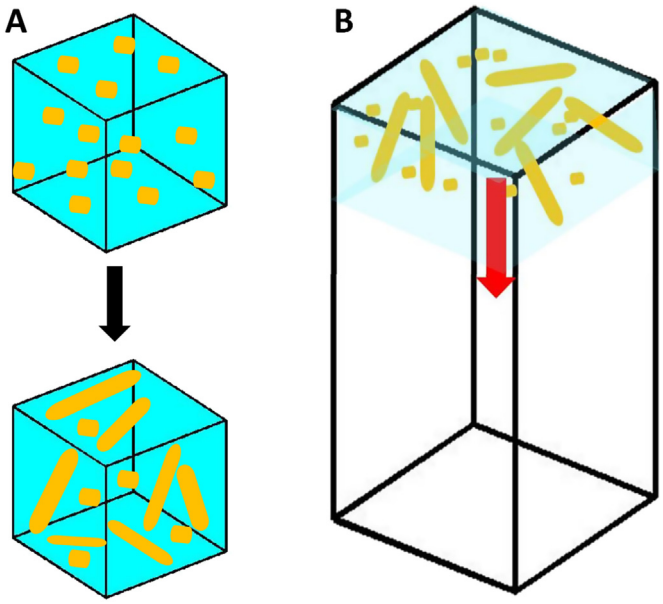

Fig. 1. (A) Schematic demonstrating uniform growth of aggregate within a gel-like medium. (B) Schematic showing spatial-dependent migration of aggregate through a tissue-like space.

proteopathies [23,24]. Such information will also provide valuable insight into the efficacy of drug-based patient treatment approaches [25,26]. To date, limited progress has been made in understanding how spatial and medium effects influence the progression of amyloidosis and other aggregation-based proteopathic diseases. This dearth of knowledge stems from a lack of experimental systems capable of providing temporally and spatially resolved data. In the current experiments, gelatin was trialed as a supporting gel medium in which to grow aggregates. Gel formation for a 5% gelatin solution occurs at 40 °C and produces a semi-rigid gel with a range of pore sizes between 370 and 660 μm [27,28]. We have used this gelatin system to demonstrate the capability to grow amyloid aggregates within a semi-rigid medium. The RCM-κ-casein reaction is a relatively well-characterized amyloid forming model system [21,29]. As it aggregates at relatively gentle pH and temperature conditions it is suited for use with the 5% w/v gelatin supporting matrix. This model system, along with others compatible with the gel setting and existence conditions, are well poised to further interrogate spatial dependent aspects of aggregate growth. This will be achieved by injection of aggregate particles within the gel, with subsequent monitoring of their movement using spatially variable detection systems based on both fluorescence and turbidimetric [30] principles. Such spatially-resolved kinetic measurements of aggregate growth in supported gel systems hold potential for the

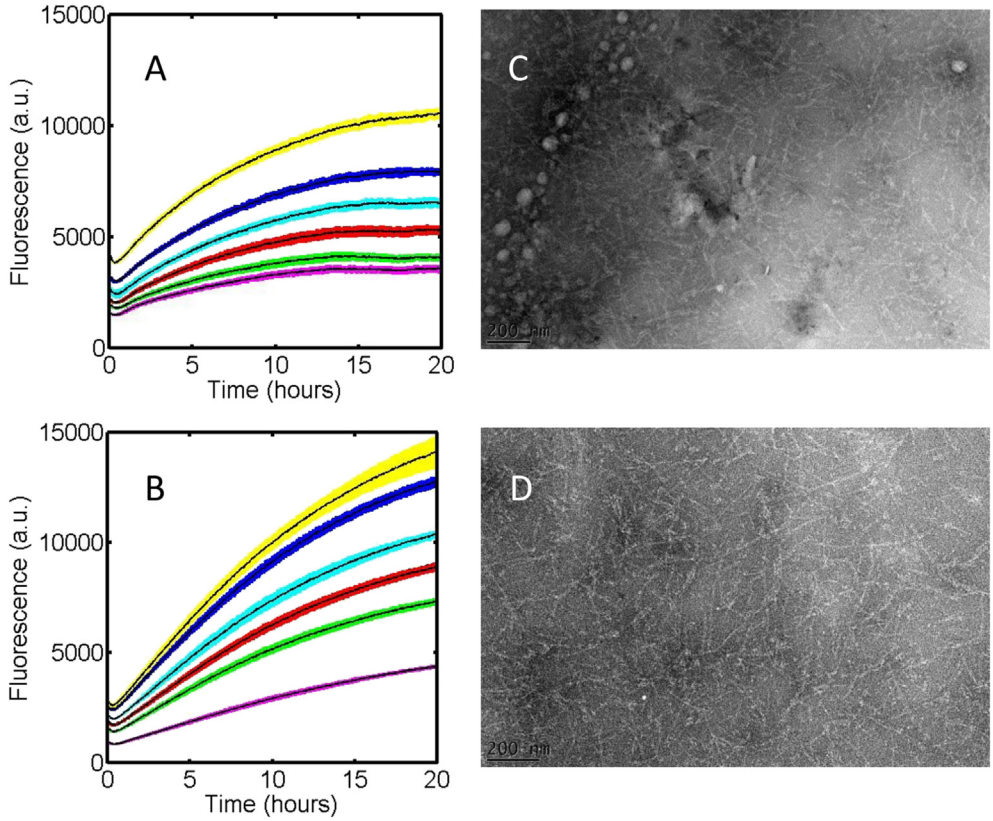

Fig. 2. RCM κ-casein aggregation in gel and solution conditions measured fluorometrically using 10 μM ThT fluorescence (30 °C incubation over 20 h) and visualized using TEM. (A) ThT fluorescence assay of RCM κ-casein aggregation in 5% gelatin shows a concentration-dependent increase in aggregation between 50 μM and 150 μM [Black lines denote average values for seven replicates with the associated colored error envelope describing the average value ± 1s.d. The six different concentrations are indicated by the following colors – pink-50, green-70, red-90, cyan-110, blue-130, yellow-150 μM, {a.u. arbitrary units}]. (B) Solution-based aggregation of RCM κ-casein aggregation in 50 mM sodium phosphate solution (pH 7.4) – average value and colored error envelope as per A, {a.u. arbitrary units}. (C) TEM image of RCM κ-casein fibrils in 5% gelatin (w/v) at 80,000 × magnification. (D) TEM image of RCM κ-casein fibrils in 50 mM sodium phosphate solution at 80,000× magnification. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)