Reactive organization of particles into macroscopic length scales by myelin growth

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Investigations of precipitation reactions in equilibrium self-assembled structures like vesicles, micelles, microemulsions, etc. is an active area of research, but growing biological systems are not equilibrium systems. Here we propose the use of a well-characterized non-equilibrium system of myelins in AOT/water to carry out precipitation reaction of $CaCO_3$. It is found that the micro-flow patterns generated by myelin growth organize the particles formed into a chain of length of over 300 microns and whose form and structure are responsive to concentration of reactants and stresses in the system. It is suggested that this type of study of reactions in dynamic, structure-forming systems can be used to mimic some aspects of embryonic stage bone growth.

FORMATION and arrangement of particles into patterns on various length scales, from microns to centimetres, is of extreme importance in biological systems. One example, relevant in the present case, is that of bone growth in an embryo[1]. This is a stage in the growth of embryo where a large number of new cells and organs are being formed along with precipitation of calcium phosphate, leading to formation of a bone under these dynamic conditions[2].

Use of equilibrium structures like micelles, microemulsions, liquid crystals, vesicles, etc. for preparation of particulates of controlled shape and size to mimic biomineralization has been currently active and has been reviewed[3]. However, organizing these particles into various macroscopic structures is less developed. It is important to realize that equilibrium structures do not represent the dynamic situation existing in biological systems such as the one mentioned above. The present communication is an attempt to carry out reaction in a fairly well-characterized non-equilibrium condition of myelin structure formation and growth[4]. The benefit of such an attempt is immediate. We see that along with myelin growth, there is organization of $CaCO_3$ particles into chains over a length scale of several hundred microns (Figure 1).

The approach used in this paper is fairly general and flexible enough to enable one to vary the conditions, as mentioned below, to innovate in an attempt to mimic nature.

The surfactant AOT (sodium *bis* (2-ethylhexyl) sulfosuccinate) was purchased from Sigma, with 99% purity, chloroform AR grade from Thomas Baker (India), and calcium chloride (fused) from Merck India Ltd. Sodium carbonate AR grade was from S.D. Fine, India. All these chemicals are used as such without further purification. Doubly distilled water was used throughout the experiment.

A stock solution of 0.2 M AOT in chloroform was prepared. Microemulsions were prepared by mixing the aqueous solution of calcium chloride of different concentrations (0.1–0.5) with the 0.2 M AOT/$CHCl_3$ stock solutions. Water-to-AOT molar ratio R was kept equal to 10 in all cases. A drop of the above microemulsion containing the $CaCl_2$ solution was placed on a glass slide and the solvents, chloroform and water, were allowed to evaporate leaving a dry droplet of AOT containing $CaCl_2$. A cover slip was gently pressed onto the sample forming a film of the sample of about 50 μm on the glass slide. The surfactant phase was then contacted through capillary action with water containing 0.1 M Na_2CO_3 by introducing a drop of this solution at the edge of the cover slip close to the surfactant phase. After contact, calcium carbonate particles are formed through precipitation reaction. Similar procedure was used, but without $CaCl_2$ and Na_2CO_3 to measure the growth kinetics of myelins.

Olympus BX60 microscope in transmission mode was used for all experiments. The images were digitized using CCD camera XC77CE connected to a frame grabber and personal computer. The camera was alternatively connected to a video-television set-up for real-time video capture and analysis of the experiments at very short time intervals.

Image-Pro software for image analysis was used for timed-acquire of images at intervals of minimum two seconds, besides direct video capture. Magnifications of 160 ×, 64 × and 32 × were used.

This procedure is general enough and one can use other lipids and surfactants instead of AOT, which are known to form myelin structures[5]. One can also add polymers like gelatin and collagen into the surfactant phase and investigate the effect, both on myelin formation and on reactions. It is also possible to carry out precipitation reactions involving semiconductor and metallic particles[6]. These systems would be of importance in technology.

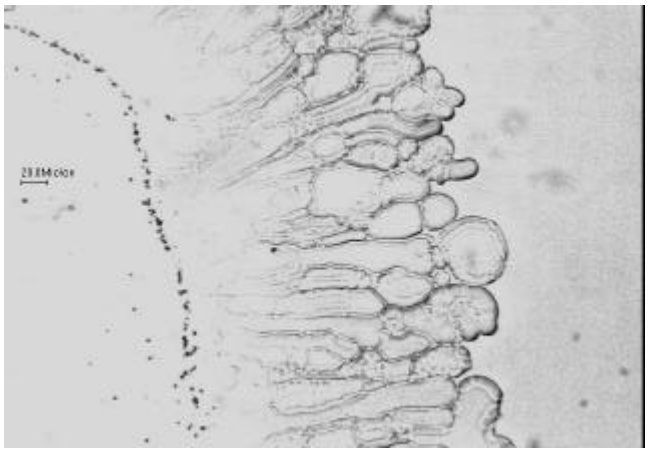

Figure 1. Optical micrograph of organization of $CaCO_3$ particles into a chain of over 300 μm in length (Bar 20 μm).

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Figure 1 shows the myelin formation. Na_2CO_3 was introduced from the right into the surfactant phase (on the left) containing $CaCl_2$. A lamellar phase is formed. The water phase penetrates the lamellar phase which swells, dissolving water into it. But the major amount of water gets reflected from the viscous lamellar phase inducing the myelin growth[4]. This is confirmed by investigations using polystyrene latex particles which are transported along with water. Myelin growth rate has been measured and the average length (L) shows the well-known 'diffusive' growth rate $L^2 \propto Dt$, with the apparent diffusion coefficient D ~ 80 micron^2/s and time (t). It is during this stage of evolution of several phases, that the precipitation of $CaCO_3$ takes place. The transport of particles generated is controlled by the phases formed, local changes in viscosity and microscopic flow patterns of water. These effects are absent in equilibrium studies. This results in the organization of particles into a chain of length ~ 300 μm shown in Figure 1.

The mechanism envisaged for chain formation is shown in Figure 2 and is consistent with the investigations of water flow in myelin-forming systems[4].

We attempted to see if we could form continuous structures instead of chains. This, we thought, would be important also for metallic particles where contact between particles is necessary for electrical conduction. We increased the concentration of $CaCl_2$ in the surfactant phase by preparing a microemulsion with 1 M $CaCl_2$ and drying it. All other conditions were kept the same. The result of such an effort is shown in Figure 3. It can be seen that the packing density has increased and there is a tendency to form a continuous structure (bottom of the figure). It is of interest to note that the interface has bent due to accidental mechanical stresses and the chain formation follows that. It is known[1] that each bone models its shape, structure and size according to the load-bearing stresses in the body. Surfactant structures are known to respond to stresses and shears. Now we have a case that reflects the action of stress/shear on reaction and organization of particles formed. One has to extend these methods to see if one can form continuous structures.

Experiments were performed using ordinary solutions of reactants without surfactant AOT. It was observed that as soon as particles were formed, they diffused away from the interface without forming narrow chains of $CaCO_3$ as in Figure 1. Similar experiments using glycerol as one of the liquids did not give organization of particles. This showed that the myelin formation and growth, which result in transport of particles, play an essential role in the formation of chains.

In summary, we have presented in this paper the following: (i) We propose the use of a well-characterized non-equilibrium system of myelins as a reaction medium. This is a more natural choice to mimic biological systems than equilibrium systems. (ii) Precipitation of $CaCO_3$ is used as a reaction to see if we can mimic some aspects of bone formation in embryonic stage. (iii) Due to formation of myelins and lamellar phases, micro-flow patterns are generated in the system which transport and localize the $CaCO_3$ particles into a chain of at least 300 μm in length. Organization of particles into these macroscopic lengths is one of the essential steps in formation of bones of well-defined shapes. These effects are absent in equilibrium systems. (iv) The number density and the size of the particulates seem to increase on increasing concentration of the reactants. (v) The chain formation responds to stresses, thus opening up investigations on the effect of stresses on organization of particulates.

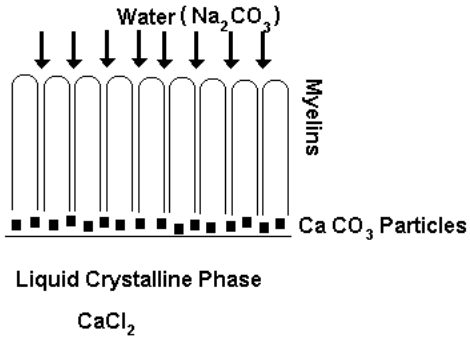

Figure 2. Mechanism for formation of $CaCO_3$ chains under dynamic non-equilibrium conditions of myelin growth.

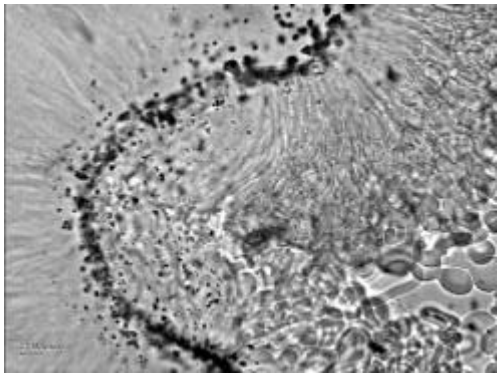

Figure 3. Optical micrograph showing increased packing density of particles with a tendency to form continuous chain. Note the effect of strain (Bar 20 μm).

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