DNA-coated microcrystals†

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Coprecipitation leads to self-assembly of bioactive DNA on the surface of salt, sugar or amino-acid crystals and provides a rapid inexpensive immobilization method suitable for preparing dry-powder formulations of nucleic acids, useful for storage, imaging and drug delivery.

Processing of biomolecules into dry powders is carried out on a substantial scale in both the academic and commercial environment, and serves as a powerful stabilisation and storage method.[1] Of particular current interest is the production of well defined particles as they can form the starting point for making new functional biomaterials with applications in a number of areas including drug delivery, diagnostics, biocatalysis and screening. Hitherto, mature but complex technologies such as freeze-drying and spray-drying have been used for drying biomolecules, but these are time, energy and capital intensive and often provide particles with a limited range of physiochemical characteristics.[1,2] By comparison it is noteworthy that Nature assembles a wide variety of functional materials rapidly and spontaneously under ambient conditions. Therefore, alternative biomimetic approaches are being increasingly explored.[3]

Here we describe a coprecipitation process that leads to spontaneous immobilisation of dehydrated DNA onto the surface of water-soluble microcrystals. A critical component is the co-precipitant which must crystallise rapidly under high super-saturation conditions. It should also be aqueous soluble with negligible solubility in the particular water-miscible organic solvent used. Suitable crystalline carriers are amino-acids, salts and certain sugars and these can be used in combination with water-miscible solvents such as alcohols and acetone. The preparation of DNA-coated microcrystals (DCMC) is straightforward and applicable in any laboratory. An aqueous mixture is formed containing the DNA and a near saturated solution of the co-precipitant. This is then added drop-wise to a large excess of the solvent while mixing vigorously. Immediately a fine white co-precipitate can be observed that is made up entirely of DCMC. These microcrystals may be stored as a suspension or alternatively filtered/centrifuged, rinsed with solvent and air-dried to form a fine free-flowing powder. Significantly, coating of microcrystals during coprecipitation has proven to be an example of a generic bottom-up self-organisation process that we have termed **c**rystal **la**ttice **m**ediated **s**elf-assembly (CLAMS). As well as nucleic acids it can be applied to peptides, proteins[4] and nanoparticles.[5]

In this work the coprecipitant used was DL-valine. This is non-hygroscopic, has a convenient saturated aqueous concentration of around 80 mg/ml and tends to crystallise in the form of flat plates. For the initial experiments crude oligonucleotides prepared from herring sperm were used. DCMC were produced by adding an aqueous DNA/valine mixture to an excess of 2-propanol‡. The morphology of particles produced was assessed using Scanning Electron Microscopy (SEM). A control sample of valine precipitated in 2-propanol in the absence of DNA was found to contain thin plate-like crystals with dimensions of between 5–15 μm (Fig. 1a). No major surface features are visible (Fig. 1b).

The sample obtained upon co-precipitation of the oligonucleotides (< 50 bp) with the valine (10 wt% DNA loading) also contained crystals with similar morphology to the control (Fig. 1c). Significantly, when these were imaged at higher magnification (Fig. 1d) the surface structure was found to be different and was characterised by the presence of round clusters (< 100 nm). These features are attributed to clusters of DNA on the surface. Employing larger DNA (~2000 bp) produced the same crystal morphology and surface features. Importantly a second control sample obtained by precipitation of pure DNA into 2-propanol contained no crystals and only large oligonucleotide aggregates were observed†. To provide a better statistical analysis of particle

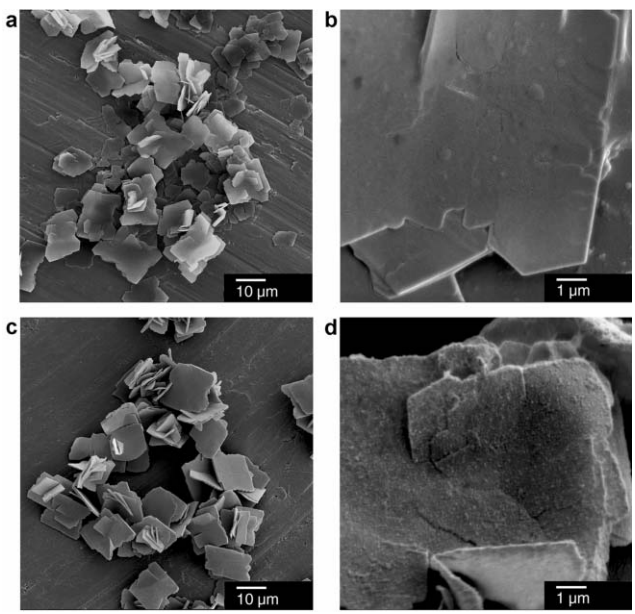

Fig. 1 SEM images of valine crystals, produced in the absence of DNA (a) and (b) and DNA-coated microcrystals produced with valine and crude oligonucleotides (< 50 bp) (c) and (d). Higher magnification images of a single valine crystal (b) and a DNA-coated microcrystal (d). Precipitation was into 2-propanol with a DNA loading of 10 wt%.

† Electronic supplementary information (ESI) available: SEM of DNA, redissolution experiment, PCR data and experimental details. See http://www.rsc.org/suppdata/cc/b5/b501045d/

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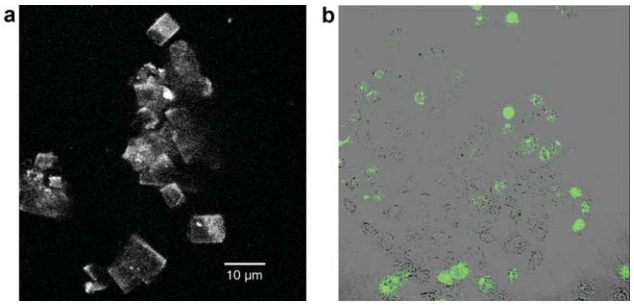

Fig. 2 (a) Fluorescence emitted by microcrystals of valine coated with fluorescently labelled oligonucleotide DQA-HEX. (b) Green fluorescence of HeLa cells 24 h after transfection with GFP-ARF plasmid/valine microcrystals (0.1 wt% DNA loading).

size, light-scattering measurements were carried out on a suspension of microcrystals in 2-propanol. The mean particle size of DCMC, produced with valine and DNA (~2000 bp, 10 wt% DNA loading), was found to be 13 μm. This is in good agreement with DCMC sizes obtained from SEM. Furthermore, light-scattering particle sizing indicated a narrow size distribution showing the SEM images obtained are representative (Span = 1.6).

The presence of the clusters in the SEM images suggests that DNA is present at the crystal surface. To investigate this further Confocal Laser Scanning Fluorescence Microscopy was carried out using a synthetic fluorescently-labelled oligonucleotide, DQA-HEX§ coated onto the valine core (0.3 wt% loading). The fluorescence emitted by DQA-HEX oligonucleotide was found to correspond faithfully to the position of the crystals, outlining geometric shapes with dimensions of approximately 5–15 μm (Fig. 2a). This is in good agreement with particle sizes determined by SEM. Additionally, the highest fluorescence intensity is found on the crystal edges as expected if the oligonucleotides are located on the carrier crystal surface. Further strong evidence that the majority of oligonucleotides are immobilised on the surface was obtained on suspension of the crystals in saturated aqueous valine solution as detailed in the ESI†. The DNA was found to immediately dissolve while the crystals remained intact.

In order for the DNA-coated microcrystals to be useful it is critical that bioactivity is retained. Therefore, we assessed if the oligonucleotides were compromised by the process. The oligonucleotide DQA-HEX§ was used to coat valine crystals and following redissolution tested as one of the primers in a PCR reaction. Processed DQA-HEX and unprocessed DQA-HEX (control) showed the same efficiency for DNA amplification (See ESI†). Sequencing data of the PCR products also confirmed that the right gene product was amplified in the PCR. Furthermore, it was found that the end sequence of the PCR product, which is dictated by the DQA-HEX primer, was identical and that no bases were altered or truncated. This demonstrates that the process for preparing DCMC is benign.

Having shown that short oligonucleotides retained bioactivity we were interested in looking at longer DNA sequences, in particular possible candidates for drug delivery, such as plasmids. A green fluorescence protein (GFP) construct was used for these studies because transfection efficiency can be easily followed by GFP expression. DNA-coated microcrystals were prepared using GFP-ARF plasmid and valine and following reconstitution used to transfect HeLa cells. As shown in Fig. 2b approximately 20% of the cells were successfully transfected. This is very similar to the result obtained with untreated plasmid and demonstrates that a complete plasmid can be passed through the DCMC process with good retention of bioactivity.

In conclusion we have shown for the first time that a straightforward coprecipitation process can be used to simultaneously dehydrate and immobilise DNA onto the surface of water-soluble microcrystals. Importantly there is no discernable loss of bioactivity, as judged both by PCR and transfection. The coated valine crystals are free-flowing, non-hygroscopic and easy to handle and provide a convenient carrier to store, handle and image DNA, RNA and their conjugates. Improvements to the process are expected to yield crystals with a size and morphology suitable for pulmonary drug-delivery applications including non-viral gene therapy.

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Notes and references

‡ In a typical preparation 0.1 ml of an aqueous solution of the DNA (10 mg/ml) was boiled for 1 min and upon cooling to RT mixed with 0.9 ml of an aqueous solution of DL-valine (60 mg/ml). (Alternatively solid DNA may be dissolved into the valine solution.) This mixture was added dropwise to 15 ml of 2-propanol (saturated with DL-valine) while stirring vigorously with a magnetic stirrer bar. The resulting DCMC were separated by filtration, thoroughly washed with 2-propanol (saturated with DL-valine) and either air-dried or resuspended in 2-propanol (saturated with DL-valine). This produces a theoretical loading of DNA of 18.2 wt%. See also ESI.

§ DQA-HEX = 5′-HEX (T*C)$_6$ GTG CTG CAG GTG TAA ACT TGT ACC AG, HEX = 2,5,2′,4′,5′,7′-hexachloro-6-carboxyfluorescein, T* = 5-(3-aminopropynyl)-2′-deoxyuridine.

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