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On the application of ‘seeding’ techniques in the primary separation of plasmid DNA from neutralised E. coli lysates*

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Abstract

BACKGROUND: Initial extraction of plasmid DNA from *Escherichia coli* and its separation from host-derived contaminants is a difficult task to perform. Here, we examine the application of particle ‘seeding’ solid-liquid separation methods for primary recovery of plasmid DNA from neutralised alkaline cell lysates.

RESULTS: Planting magnetic particle ‘seeds’ during cell lysis resulted in enhanced phase separation, facile magnetic separation of the floc, slight improvements in plasmid purity, but diminished plasmid recoveries. When CaCO$_3$-coated low-density microspheres were seeded into flocs, phase separation was impaired, shear-induced floc damage and contamination of the plasmid liquor with genomic DNA and cell debris occurred, but plasmid DNA recovery was improved. Introduction of hydrophobic low-density microspheres into the floc dramatically improved floc stiffness, phase separation and flotation efficiency, and reduced the solids content in the plasmid liquor ten-fold. However, strong reinforcement of the cell debris lattice by these microspheres hindered plasmid release into the liquor beneath.

CONCLUSION: By incorporating magnetic or buoyant seeds during cell lysis we have identified new routes for separation of shear-sensitive cell debris solids from crude plasmid-containing liquors. Effective use of seeding approaches for difficult solid-liquid separation tasks will require evaluation of a wide range of seeds of varying architecture, size, shape, density and chemistry.

Keywords: alkaline lysis; fillers; flotation; gene therapy; genetic vaccination; magnetic particle seeds; low density gas-filled microspheres
INTRODUCTION

Gene therapy, one of the fastest growing fields in the New Medicine arena, promises prevention, treatment and cure of diseases against which many present day drugs are ineffective (http://www.wiley.com/wileychi/genmed/clinical). Currently vectors for gene delivery fall into one of two broad categories, namely ‘viral’ (disabled viruses) or ‘non-viral’ (i.e. plasmid based genes, in naked form or complexed with agents such as lipids or polypeptides). One of the principle advantages of plasmid DNA (pDNA) is its potential to be used for both medical therapy and genetic vaccination. Should non-viral gene therapy and/or genetic vaccination in particular, prove to be effective treatments, then both the ultimate scale of production of pDNA and number of applications, are expected to be large.

The initial isolation of plasmid DNA from *Escherichia coli* host cells and the primary separation of it away from major host derived contaminants presents a significant challenge to downstream process development. In most large-scale procedures for the purification of plasmid DNA for gene therapy and DNA vaccination, the plasmid is released from within the cells by the alkaline lysis method, which was first described by Birnboim and Doly. A brief, but necessary review of the mechanisms involved in this important chemical extraction protocol is presented below.

The addition of an alkali-surfactant solution to the cell suspension results in rapid cell lysis and release of the intracellular contents. In the strongly alkaline environment that results, the bulk of the macromolecular components denature irreversibly, whereas the supercoiled plasmid is reversibly denatured provided that the pH is kept below a critical value, which varies for different plasmids. The cell wall lysis and solubilisation reaction is fast, and the release, denaturation and subsequent structural changes of the chromosomal DNA molecules are responsible for extreme changes in viscosity of the solution. Following a brief incubation period, sufficient to ensure complete lysis and solubilisation of the cell walls, the resulting alkaline cell lysate is then neutralised through the addition of a high ionic strength acidic solution (typically 3 M potassium
acetate, pH 5.5) the changes in physical appearance, chemical composition and rheological properties of the lysate on neutralisation and subsequent aging are dramatic. On returning to neutral pH, supercoiled plasmid molecules renature and remain in solution. Simultaneously, potassium ion concentration causes denatured SDS/protein complexes to pull out of solution. The formation of this highly flocculating suspension entraps cell wall debris, denatured chromosomal DNA and the bulk of cellular RNA. Over the space of 0.5-1 h in a ‘low shear’ environment an off-white, highly-porous and fragile gelatinous mass of flocculated solids forms, and rises to the surface of the reaction vessel, leaving beneath it a crude liquor containing plasmid DNA, colloidal debris and fine solids.

The floating gel matrix possesses both viscous and elastic properties typical of viscoelastic substances, and is highly sensitive to shear. For example, ‘moderate mechanical shear forces inflict serious damage on the gelled solids, whereas ‘high’ shear fields liquefy it. Gentle processing of the neutralised lysate is crucial in order to minimise degradation of the floc and subsequent contamination of the plasmid containing liquor with large amounts of insoluble and soluble contaminants. Previously we reported that efficient and gentle ‘shear free’ separation of the plasmid from the cell debris floc formed following neutralisation and ageing could be achieved by simply draining the liquor from beneath the floating raft of solids and further that the efficiency of flotation increased with rise in scale of operation. However, despite the simplicity of the ‘flotation & draining’ approach, the acute shear sensitivity of the residual fine solids makes conventional elimination of this material from the drained liquor by centrifugation or filtration a difficult task. The challenging situation posed by such intractable biological solids encourages the evaluation of solids removal techniques that are not normally employed within the pharmaceutical industries.

Since their introduction during the 1970’s, ‘seeding’ methods, i.e. the deliberate physical entrapment of dense and/or magnetic particulate materials as ‘seeds’ within the insoluble colloidal network of a biological floc or precipitate, have found widespread application,
especially at large scale in water pollution control for depletion of an incredibly diverse range of contaminants (e.g. inorganic suspended solids; biological suspended solids; algae, bacteria, viruses, biopolymers, inorganic sulphur, phosphates, heavy metals, natural and synthetic dyes, and waterborne organics including mineral oils, phenol, polychlorinated biphenyls, alkanes, naphthene, polycyclic hydrocarbons) from many different aqueous liquors (e.g. various factory effluents, sewage, rivers, petroleum refinery process and ground waters). While the use of dense particles to seed biological precipitates enhances the settling velocity of the latter, the use of magnetic materials also makes the precipitate amenable to magnetic separation technology. Contrary to the popular belief held in biotechnology, magnetic separations are highly scaleable. Rotating drum permanent magnetic separators produce relatively low levels of fluid shear, and in light of this would appear to be the most suitable large magnetic apparatus for removing intractable biological solids from moving suspensions.

In this work we describe the application of seeding methods in the primary separation of plasmid DNA from crude neutralised E. coli lysates. We have investigated two different approaches to enhance/manipulate separation of the gelatinous solid flocs (formed during chemical extraction and ageing) away from the plasmid liquor. In the first of these, we rendered the flocs magnetic and amenable to magnetic separation by using magnetic seed particles, while in the second we attempted to enhance the floc’s natural tendency to float, by seeding it with ultra low density floating microspheres.

MATERIALS AND METHODS

Materials
Nickel powder Type 123 was received as a gift from INCO Speciality Powder Products (London, UK). Before use, the nickel power was placed in a 350°C oven for 1 h. The resulting product was then ground back to a fine powder in a pestle and mortar. Magnetic iron (II, III) oxide powder was purchased from Sigma Aldrich Company (St. Louis, MO, USA). Ultra-low density gas-filled
calcium carbonate coated (Dualite® M-6017AE-04, Pierce and Stevens Corp., Buffalo, NY, USA) and uncoated hollow acrylonitrile-based thermoplastic microspheres were kindly provided by UpFront Chromatography A/S (Copenhagen, Denmark). The uncoated microspheres had been prepared by treating the calcium carbonate coated material with 1 M hydrochloric acid to remove the inorganic surface coating and reveal the underlying non-porous thermoplastic polymer and were then washed thoroughly with water. The 8.5 kb runaway plasmid, pOU61, was received as a gift from S. Molin (Technical University of Denmark) and the non-mucoid ‘low endotoxin producing’ Escherichia coli host strain DH5α was obtained from Life Technologies (Roskilde, Denmark). BCA Protein Assay Reagent was purchased from Pierce Ltd. (Rockford, IL, USA). All other chemicals used in this study were supplied by Sigma-Aldrich Company Ltd (St. Louis, MO, USA).

Production of plasmid-containing cells, cell lysis and seeding experiments

Detailed procedures for the transformation, fermentative production, centrifugal recovery and storage of pOU61 containing E. coli DH5α cells have been presented elsewhere. Likewise detailed procedures for the chemical extraction of plasmid DNA from E. coli cells and separation of the plasmid containing liquors from bulk insoluble cellular contaminants by flotation have also been described previously, thus only a brief description of these methods is presented here.

Alkaline cell lysis, neutralisation and ageing were carried out at ambient temperature (20 – 22°C) at a variety of scales (13.5 – 300 ml final volume of neutralised lysate) in glass or transparent polycarbonate vessels (aspect ratios of ~1.5:1) fitted with taps for draining. Defrosted cells harbouring pOU61 were thoroughly resuspended in 10 mM Tris/HCl, 1 mM EDTA, pH 8 buffer to give cell suspensions containing ~120 g wet cells per litre. Plasmid was released from E. coli cells by lysis with NaOH and SDS. The resulting alkaline cell lysate was subsequently neutralised by addition of potassium acetate and left to age at 6°C for 1 h (low temperature enhances the
flocculating power of the SDS-protein complex by lowering its solubility\(^{15,35}\). The ratio of volumes of cell suspension (solution 1) to alkaline lysis solution (solution 2: 0.2 M NaOH, 1% SDS) to neutralising solution (solution 3: 3 M potassium acetate, pH 5.5) was 1:1:1 in all experiments. Alkaline lysis solutions were added to cell suspensions over \(\sim10\) s with gentle mixing provided by manual swirling of the flask. After 60 s the alkaline cell lysates were then neutralised by addition of the neutralising solution over a 10 s period, and swirling was continued for a further 5 s prior to ageing at 6\(^\circ\)C. After the ageing period, the cell debris formed an insoluble raft of floating gelatinous solids. Recovery of the plasmid-containing liquor from beneath the floc was then be achieved by opening the tap, and draining through a layer of gauze (to hold back any large solids).

The principles behind the floc-seeding experiments undertaken in this study are schematically illustrated in Fig. 1. In experiments with magnetic materials (Fig. 1a), 1 g portions of NiO-coated nickel powder or fine particle magnetite were added to 100 ml volumes of solutions 1, 2 or 3 contained in 500 ml carboys immediately prior to plasmid extraction. Following neutralisation and ageing the ‘magnetic’ solids were separated with the aid of a 0.15 Tesla flat plate permanent magnet (PerSeptive Diagnostics, Cambridge, MA, USA). The magnet was placed vertically up against the vessel wall and after separation of the gelatinous solids had been achieved, the tap was opened and the liquid phase was recovered by draining.

Preliminary floc-seeding experiments with the floating microspheres, employed at final concentrations in the lysates of 0 – 50 mg ml\(^{-1}\), were performed at 13.5 and 100 ml scales (added in solution 1). In subsequent studies 3 g quantities of the CaCO\(_3\) coated and uncoated low density plastic seed materials were added to 50 ml portions of cell suspension (solution 1) immediately prior to alkaline cell lysis (see Fig. 1b). Following neutralisation and ageing, separation of plasmid liquors from the floating gelatinous solids (containing entrapped hollow microsphere particles) was achieved by draining (as described earlier) and plasmid-containing liquor entrained in the solid flocs was recovered following centrifugation at 10,000 rpm at 4\(^\circ\)C in the SLA 3000
rotor of a Sorval RC5C refrigerated centrifuge. Drained and floc entrained liquors arising from all of the above experiments were subjected to analysis for plasmid (pDNA), chromosomal or genomic DNA (gDNA), RNA, protein and solids contents.

Analysis

Hollow microsphere seed materials were examined with a Nikon Optiphot 2 microscope (Nikon, Melville, NY, USA) fitted with a Kappa CF-8/L FMC monochrome video camera (Kappa Opto-electronics GmbH, Gleichen, Germany) and digitised micrographs and particle size distributions were produced with the Image-Pro® Plus software (version 4.1 for Windows™; Media Cybernetics, Silver Spring, MD, USA) program. Images during flotation experiments were recorded with an Olympus C-400L digital camera (Tokyo, Japan). The solid contents in drained liquors arising from lysis and seeding experiments were determined by turbidimetric measurements at 600 nm using a Lambda 20 UV/VIS Spectrophotometer (Perkin Elmer, Norwalk, Ct, USA). Prior to assaying soluble RNA, DNA and protein contents, samples of drained liquors were centrifugally clarified in a microfuge (Biofuge-Pico, Heraeus Instruments, Osterode, Germany) operated at 10,000 rpm for 600 s and the supernatants were separated from the pelleted solids. Soluble RNA, DNA and protein contents were routinely measured using modified orcinol\textsuperscript{36}, diphenylamine\textsuperscript{37} and bicinchoninic acid (BCA) colorimetric assays respectively, employing baker’s yeast RNA, highly polymerised calf thymus DNA and human serum albumin (HSA, fraction V) as standards. The BCA assay was adapted for use in a spectrophotometric robot (Cobas Mira, Roche Diagnostics, Switzerland). In order to determine the relative amounts of plasmid and genomic DNA, samples with known DNA content were electrophoresed in horizontal 0.8% (w/v) agarose gels at a constant voltage of 100 V for 2 h. Relative amounts of pDNA and gDNA were then estimated by scanning densitometry analysis of captured images of ethidium bromide-stained gels. A Gel Doc 2000 gel documentation system
RESULTS AND DISCUSSION

In the present study we have found that many of the properties that seeding bestows on insoluble biological precipitates, coagula or flocs can best be appreciated from studies on the use of fillers in plastics and building materials. The term ‘filler’ is most often applied to solid additives incorporated into a polymer to modify its physical (usually mechanical) properties. It is known that the average particle size and distribution, particle shape and porosity, chemical nature of the filler’s surface, and presence of impurities can all strongly influence the physico-mechanical properties of elastomeric systems. For example, it is commonly observed that the finer the particle size, the stronger the modified material will be. This enhancement phenomenon is known as ‘reinforcement’. In contrast, the use of coarser filler particles may often yield compounds that are less strong than those with filler absent. The particle shape and porosity of fillers can also influence structural properties, for example particles with rough or uneven surfaces can be difficult to wet with polymer, whereas porous fillers may adsorb selected components in their pores thereby compromising the effectiveness of the filler. The chemical nature of the filler’s surface is vitally important. For example, due to their highly polar surfaces, mineral fillers, such as calcium carbonate, are attractive to hydrophilic components, but not to hydrophobic species. Finally, the presence of impurities, such as coarse sharp-edged particles (i.e. grit), especially in the case of mineral fillers, create points of weakness in soft polymers causing them to fail under applied stresses below those which they might be expected to survive.
Magnetic seeding experiments

Nickel (3-7 μm; 8.9 g cm$^{-3}$; <0.4 m$^2$ g$^{-1}$) and magnetite (<<1-5 μm; 5.2 g cm$^{-3}$; ≈10 m$^2$ g$^{-1}$) powders were chosen as seed materials in preference to other magnetic particle materials primarily for reasons of low cost. Prior to use in magnetic seeding experiments, the nickel powder was heated in air for 1 h at 350°C so as to impart a high degree of corrosion resistance to the seed material. The results of floc–seeding experiments conducted at 300 ml scale using magnetite and NiO-coated nickel particles at a final concentration of 3.33 mg ml$^{-1}$ are summarised in Table 1. Although incorporation of either NiO-coated nickel powder or fine particle magnetite during the cell lysis step (i.e. by addition of particles in solutions 1 or 2) did not induce any visible reinforcement of the floc formed, phase separation was enhanced in all cases and separation of the flocculated solids from the plasmid liquor was significantly facilitated. In stark contrast, addition of particles during the neutralisation step (i.e. within solution 3) led to poor entrapment of the seeds within the gelled debris solids and consequently impaired phase separation. With ageing, magnetic particles (especially the very dense NiO-coated nickel powder) tended to fall out of the solids network. The finding that better particle entrapment was observed during lysis than during neutralisation can be understood by considering the extreme variations in viscosity that are observed during the alkaline lysis procedure. The initial cell suspension and the SDS-NaOH solution exhibit low Newtonian viscosities (<2 mPas), the lysed mixture is highly viscous (e.g. at fixed shear rates of 46 s$^{-1}$ and 367 s$^{-1}$ Ciccolini et al. recorded maximum viscosities of 160 mPas and 35 mPas respectively) and exhibits non-Newtonian properties, and on neutralising the liquid phase viscosity decreases dramatically to 5-10 mPas.

In addition to enhanced phase separation observed following magnetic seeding during cell lysis (i.e. seeds added to solutions 1 or 2; see Fig. 1a & Table 1), slight improvements in plasmid purity were observed albeit at the expense of reduced plasmid recoveries. In contrast, seeding of magnetic particles during neutralisation (i.e. seeds added to solution 3) significantly impaired plasmid purity, especially when fine particle magnetite was used. Furthermore, the levels of
contaminating protein were markedly increased. Evidently, the introduction of small (and sharp) particles during mixing of the neutralised solution with the viscous cell lysate caused additional shear damage to the formed solids liberating both soluble and insoluble contaminants (arising from the solids) into the plasmid liquor. The greater damage in experiments with the fine particle magnetite (reflected by the highest levels of contaminating soluble genomic DNA and protein in the recovered plasmid liquor) is presumably related to the much higher specific surface area (ca. 10 m² g⁻¹) of this magnetic seed material cf. the NiO-coated nickel powder.

**Seeding experiments with floating microspheres**

Rather than fight the natural tendency of the solids to float, attempts to assist it and improve the flotation procedure were made through the addition of ‘buoyancy aids’ planted as seeds within the flocs. Hollow low density particles and tubes of various composition (i.e. plastics, borosilicate glass, zeolite, ceramics) have been used in the recent past in a wide variety of applications such as: improving the texture of cosmetics; use as fillers to give structure, strength and elasticity to building, road transport, aerospace and nautical materials without the penalty of increased weight.⁴⁰⁻⁴²

The nearest related application to that described in this work is the use of hollow glass microspheres as core materials for the production of low density fluidised bed adsorbents intended for use in inverse fluidised bed separations.⁴³ In the work described herein we have employed two types of non-porous hollow microspheres (Fig. 2a & b) of low density (0.13 g cm⁻³) and surface area (<0.2 m² g⁻¹), which differ only in the nature of their exterior surface (see descriptions under Materials). Few differences in particle size distribution (Fig. 2c & d apparent density and physical appearance of the coated and uncoated microspheres were noted; however, on close inspection, the coated microspheres appeared slightly darker and lightly speckled (presumably reflecting surface deposited CaCO₃) relative to the uncoated material (compare Figs. 2a & b). Indirect evidence for the increased hydrophobicity of the uncoated microspheres relative
to that of the coated version was provided by observations that the uncoated microspheres floated much more rapidly in aqueous solution and formed a more cohesive particulate layer at the air-liquid interface, which according to Hórvölgyi et al.\textsuperscript{44,45} is indicative of a strongly hydrophobic material. The concerted effects of microsphere particle concentration and surface chemistry on reinforcement of the cell debris floc into which these fillers were seeded), and turbidity of the resulting plasmid containing liquor are summarised in Fig. 3, whereas Fig. 4 shows photographs taken from lysis/seeding experiments conducted at 150 ml scale. Increasing the content of both types of microspheres resulted in floc reinforcement (Fig. 3a), but whereas this effect was only slight when coated hydrophilic seeds were employed, quite dramatic floc reinforcement was observed with the uncoated and hydrophobic materials. At this small scale of operation flotation works very poorly,\textsuperscript{17,19} but when uncoated microspheres were incorporated into the lysis mixture (in solution 1) at a final concentration in excess of 20 mg ml\textsuperscript{-1} of neutralised lysate, flotation efficiency and solid–liquid phase separation were markedly improved and the turbidity of the drained plasmid liquor was very reduced (~10 fold) compared to the control (see Figs. 3b and 4). In contrast, the inclusion of even low amounts (<10 mg ml\textsuperscript{-1} lysate) of coated seeds exerted the opposite effect. Phase separation was impaired and the solids content approximately doubled.

As mentioned above, the only significant difference between the coated and uncoated microspheres lies in the physicochemical nature of their surfaces. According to Brydson\textsuperscript{38}, the reinforcement phenomenon appears to depend on three main factors: (i) ‘extensivity’, i.e. the total surface area of filler per unit volume in contact with the material to be modified; (ii) ‘intensity’, i.e. the specific activity of the filler-polymer material interface, which results in chemical and/or physical bonding; and (iii) ‘geometry’, i.e. arising from the degree of aggregation and porosity of the filler particles. The most likely explanation for the marked difference in observed structural reinforcement of the cell debris floc with the two microsphere preparations is that the uncoated microsphere particles interact ‘intensely’ with components of the gelatinous cell debris lattice, whereas the coated microspheres do not. The strong reinforcement effect exhibited on seeding the
cell debris floc with uncoated beads is most probably caused by the formation of multiple hydrophobic interactions between the denatured cellular components and the seeds. As mentioned earlier, the addition of an alkaline/surfactant lysis solution to an E. coli suspension converts condensed double stranded nucleic acids into open and tangled single stranded forms, results in the formation of amphiphilic highly flocculating complexes of dodecyl sulphate denatured proteins, and releases lipids and structural polysaccharides from the cell envelope into solution. The resulting viscous environment is predominantly hydrophobic. The difference in intensity of interaction of the hydrophilic and hydrophobic microspheres with the gelatinous cell debris floc exerts fairly dramatic effects on the recovery of pDNA from E. coli. Table 2 summarises the results of seeding experiments conducted at 150 ml scale with both materials at a final concentration of 20 mg ml⁻¹ lysate. Seeding of the hydrophilic microspheres into the lysis mixture seems to enhance pDNA recovery in the drained liquor relative to the control lysis (see Table 2a), but evidently this is at the expense of some damage to the floc given the significant rises in soluble (especially gDNA and protein) and insoluble contaminants appearing in the plasmid liquor. It is possible that the lack of substantial interaction of these filler particles with floc components (i.e. poor reinforcement; see Fig. 3a) could result in a situation where the coated (the presence of a precipitated CaCO₃ coat could be envisaged to lend some sharp abrasive surface features to the filler) microsphere fillers are allowed the freedom to move through the gel lattice during its formation, thereby creating regions of weakness within it. Our observations of enhanced pDNA release from the floc into the liquor beneath, combined with the attritive damage to it, are consistent with the above possibility. In stark contrast, the inclusion of hydrophobic microspheres during lysis does not inflict damage on the resulting floc (no gDNA was detected in the plasmid liquor), but it is deleterious to pDNA recovery in the drained liquor (Table 2a). Following centrifugal ‘squeezing’ of the floc, much of the plasmid could be recovered (Table 2b). Clearly, the strong reinforcement observed between the uncoated hollow filler particles and components of the floc (Fig. 3a) is responsible, at least in part, for the hindered release of pDNA.
from the gel/particle lattice into the liquor below. Another possible explanation for the low overall recovery of pDNA (Table 2c) could be that some plasmid is lost during lysis when it becomes single stranded and able to participate in the formation of the ‘hydrophobic microsphere/cell debris’ gel structure.

CONCLUSIONS

One of the more challenging tasks in the large-scale purification of therapeutic plasmids is extraction from *E. coli* host cells and primary separation from the issuing cell debris solids. The laboratory based alkaline lysis protocol originally described by Birnboim and Doly is currently the method of choice for extracting pDNA at large-scale. In this work we have applied so called ‘particle seeding techniques’ that are rarely employed in the biopharmaceutical sector, but which have found widespread use in low grade biotech applications such as wastewater treatment, to enhance the removal of shear sensitive gelatinous solids from crude plasmid-containing lysates following alkaline extraction/neutralisation. Reeve has reported that facile clarification of plasmid extracts can be achieved at small scale by employing magnetic seeding during chemical extraction. This involves incorporating magnetic particles during cell lysis/neutralisation so as to entrap them within the floc, which can then be removed from the plasmid DNA liquor with the aid of a magnet. However, with rise in scale the floc formed exhibits an increased tendency to float. This property combined with a lack of physical or chemical interaction with the magnetic seed (i.e. reinforcement or, strictly speaking, intensity), and a propensity for the heavy seeds to rapidly fall through the loose and weak structure of the floc, make this approach problematic at scales much above 10 ml.

Given the floc’s affinity to float, in this study we attempted to enhance its flotation efficiency by entrapping low density, gas-filled hollow microspheres within the lattice of the cell debris solids. The surface chemistry of these seed materials clearly played an important role. While hydrophilic seeds exerted no beneficial effects, hydrophobic floating seed materials of
identical size interacted very strongly with floc components resulting in not only dramatically enhanced phase separation and floc flotation, but also significantly hindered release of pDNA from the ‘hollow microsphere/gel lattice’. Whether the seeding approach described herein offers any benefits for plasmid recovery (or indeed other tricky solid/liquid separation tasks) is difficult to predict from this study alone. Clearly, answering this would require more detailed investigations using a wider set of floating seeds (e.g. differing in size distribution, shape, density and chemistry) combined with appropriate rheological measurements (e.g. texture analysis). It is clear however, that seeding doesn’t offer an easy quick fix solution to the problem of large-scale primary isolation of pDNA.

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REFERENCES


Table 1. Summary of data and observations from magnetic seeding experiments conducted at 300 ml scale.

<table>
<thead>
<tr>
<th>Seed</th>
<th>Seed added to solution #</th>
<th>Phase separation&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Drained liquor recovered (ml)</th>
<th>Liquor contents (mg)</th>
<th>pDNA&lt;sup&gt;b&lt;/sup&gt;</th>
<th>gDNA&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Protein&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
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<tbody>
<tr>
<td>None</td>
<td>n.a.</td>
<td>Normal</td>
<td>153</td>
<td></td>
<td>6.7</td>
<td>0.2</td>
<td>48.2</td>
</tr>
<tr>
<td>NiO: Ni powder</td>
<td>1</td>
<td>Enhanced</td>
<td>148</td>
<td></td>
<td>5.9</td>
<td>n.d.</td>
<td>39.2</td>
</tr>
<tr>
<td>NiO: Ni powder</td>
<td>2</td>
<td>Enhanced</td>
<td>145</td>
<td></td>
<td>4.8</td>
<td>n.d.</td>
<td>45.7</td>
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<tr>
<td>NiO: Ni powder</td>
<td>3</td>
<td>Deteriorated</td>
<td>205</td>
<td></td>
<td>9.1</td>
<td>1.2</td>
<td>92.3</td>
</tr>
<tr>
<td>Magnetite</td>
<td>1</td>
<td>Enhanced</td>
<td>150</td>
<td></td>
<td>5.8</td>
<td>n.d.</td>
<td>51.0</td>
</tr>
<tr>
<td>Magnetite</td>
<td>2</td>
<td>Enhanced</td>
<td>125</td>
<td></td>
<td>4.2</td>
<td>n.d.</td>
<td>25.0</td>
</tr>
<tr>
<td>Magnetite</td>
<td>3</td>
<td>Deteriorated</td>
<td>200</td>
<td></td>
<td>8.7</td>
<td>4.3</td>
<td>94.6</td>
</tr>
</tbody>
</table>

Key: solution 1, cell suspension; solution 2, 0.2 M NaOH/1% SDS; solution 3, 3 M CH₃COO⁻.K⁺, pH 5.5; <sup>a</sup>relative to 'control' - lacking seed; <sup>b</sup>determined by diphenylamine assay and scanning densitometry; <sup>c</sup>determined by BCA assay; n.a. = not applicable; n.d. = not detected.
Table 2. Summary of data and observations from seeding experiments with floating hollow microspheres conducted at 150 ml scale.

<table>
<thead>
<tr>
<th>Seed</th>
<th>Phase separation</th>
<th>Floc volume (ml)</th>
<th>Drained liquor volume (ml)</th>
<th>turbidity (OD$_{600nm}$)</th>
<th>Drained liquor pDNA (mg)</th>
<th>gDNA (mg)</th>
<th>RNA (mg)</th>
<th>Protein (mg)</th>
</tr>
</thead>
<tbody>
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<td>Normal</td>
<td>93</td>
<td>58</td>
<td>1.2</td>
<td>2.8</td>
<td>n.d.</td>
<td>31.5</td>
<td>27.2</td>
</tr>
<tr>
<td>CHM</td>
<td>Deteriorated</td>
<td>106</td>
<td>49</td>
<td>2.2</td>
<td>3.4</td>
<td>1.3</td>
<td>32.5</td>
<td>33.6</td>
</tr>
<tr>
<td>UHM</td>
<td>Enhanced</td>
<td>107</td>
<td>56</td>
<td>&gt;0.1</td>
<td>0.5</td>
<td>n.d.</td>
<td>23.0</td>
<td>8.5</td>
</tr>
</tbody>
</table>

Key: CHM = coated hollow microspheres; UHM = uncoated hollow microspheres; *recovered following centrifugation of the floc at 10,000 rpm for 0.5 h in the SLA 3000 rotor of a Sorval RC5C refrigerated centrifuge; $^b$ i.e. ‘drained’ plus ‘floc-entrained’ liquors; n.d. = not detected.
Figure legends

Fig. 1. Schematic illustration of seeding experiments with (a) magnetic and (b) floating particles.

Fig. 2. Light micrographs and particle size distributions of calcium carbonate coated (a & c) and uncoated (b & d) hollow thermoplastic microspheres.

Fig. 3. Effect of seed type and concentration on (a) floc reinforcement and (b) solids content in the resulting plasmid containing liquor. The seeding experiments were conducted at scales of 13.5 (up-triangles), 100 (circles) and 150 ml (squares) using coated (open symbols) and uncoated (filled symbols) hollow plastic microspheres. The reinforcement index was scored on the basis of visual observation (0 = no reinforcement; 10 = maximum reinforcement). We observed complete entrapment of seeds within the flocs in all cases, thus the turbidity of the drained lysates is solely attributable to the presence of insoluble biological materials.

Fig. 4. Photographs taken during floc seeding experiments at 150 ml scale. The above images (a – control ‘no seeds added’; b – coated hollow microspheres; c – uncoated hollow microspheres) were taken after neutralisation at 6°C for 1 h.
Figure 1
Figure 2
Figure 3
Figure 4