Simplifying Progress

Grafting Chromatographic Monoliths with Charged Linear Polymers for Highly Productive and Selective Plasmid DNA Purification

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Introduction

Increased requirements for plasmid DNA (pDNA) in gene therapy and vaccination efforts brings the need for efficient large-scale production processes with high purity and productivity. Chromatographic purification of pDNA is often a bottleneck due to low dynamic binding capacities (DBC), pDNA recovery and/or low selectivity of columns. Ion exchangers (IEX) with charged groups on surface extenders (grafted and surface modified polymer chains formed by grafting reaction) have attracted attention due to their greatly enhanced productivity compared with traditional IEX.

The goal of our investigation was to increase the DBC for pDNA on a monolith stationary phase by grafting the surface with linear polymethacrylate brushes, while retaining high pDNA recovery and chromatographic selectivity between pDNA and RNA impurities. The effect of graft length and density on DBC and recovery was investigated. The optimal grafted column (with 1 mL bed volume) was evaluated for pDNA purification from neutralized *E. coli* lysate for two pDNA sizes, 4.7 and 11.6 kbp. Performance and possible scale-up of the grafted monolithic column for pDNA purification was presented with productivity calculation for AEX capture step.

1. Experimental approach

Preparation of anion-exchanging (AEX) grafted monoliths: Grafting experiments were conducted on polymethacrylate-based, 1 mL CIMmultus monoliths (6 µm channel diameter). GMA was polymerized by atom transfer radical polymerization with an activator regenerated by electron transfer (SI-ATRP-ARGET) [1]. The amount of grafted GMA (chain length – grafted layer thickness) was controlled by the reaction time or initiator concentration. Reaction steps are presented in Figure 1.

DBC and recovery determination for pure pDNA: Prepared grafted AEX monolithic columns were characterized with purified pDNA sample (7.3 kbp, pAAV2/8) according to the protocol published in [2]. Loading and elution flow rates were 5 and 2 column volume (CV) per minute.

pDNA isolation from *E. coli* lysate: Biomasses containing 4.7 kbp (pUCBS4.7) and 11.6 kbp pDNA (pHelper) were lysed and clarified according to [3, 4]. 5 mg of pDNA material was loaded onto a column at 10 CV/min for both plasmids. AEX capture was performed according to [4]. All collected samples (loading, flow-through, elution of RNA and pDNA and CIP with 1 M NaOH) were analysed by PATfix® HPLC on CIMac pDNA 1.4 or 6 µm columns [5, 6].

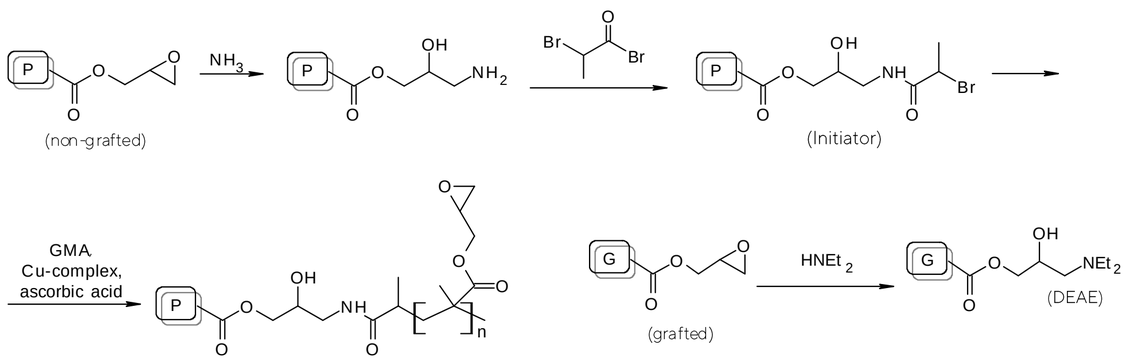

Figure 1: Preparation of grafted monolith and subsequent functionalization of grafted monolith with diethylamine (DEA) to finish with weak AEX stationary phase. P – base monolith, G – grafted monolith.

2. Results - Characterization of different graft varieties with pure pDNA (7.3 kbp)

- Compared to non-grafted CIMmultus DEAE with the same channel size (6 µm) grafted monoliths exhibited from 2 to 7.7-times higher DBC.
- Different variations of grafting conditions resulted in the DBC increase either with increased chain surface density or length - DBC varies between 5.2 and 17.6 mg/mL (Figure 3).
- pAAV2/8 elution recovery increases with decreasing density of graft polymers distributed over the monolith surface and decreasing the graft lengths (Figure 3).
- Over 80% recovery was achieved in all cases except where the highest relative initiator density was used (8-times relative initiator density, Figure 2).
- The compromise between high pDNA DBC and high recovery suggests the use of lower density graft with higher polymerization times.

3. Purification of 4.7 and 11.6 kbp pDNA from *E. coli* lysate with grafted column

- Optimal grafted DEAE column with respect to DBC and recovery (Figure 3) was evaluated for purification of 4.7 and 11.6 kbp large pDNA from clarified bacterial lysate.
- PATfix HPLC analysis of loading, flow-through, elution and CIP fractions revealed 95% pDNA elution recovery for 4.7 kbp pDNA and 78% for 11.6 kbp pDNA (Table 1).
- No bacterial RNA contaminants were detected in the main elution fraction (Figure 4), proving good selectivity between RNA and pDNA.

4. Theoretical productivity for downstream capture of 4.7 kbp pDNA with CIMmultus grafted DEAE 400 mL

- Theoretical productivity was calculated using optimal grafted DEAE (6 µm) column (Figure 5).
- Input parameters for lysis and pDNA capture were taken from real batch of biomass containing pUCBS4.7, purified on 1 mL monolith scale. These numbers were extrapolated to the amount of pDNA produced in 50 L bioreactor (1.1 mg pDNA/g of wet cell weight).
- 2650 L of clarified and diluted lysate would be theoretically processed in a single cycle using CIMmultus grafted DEAE 400 mL (DBC of 13.5 mg/mL - determined in previous experiments).
- At loading flow rate of 37 CV/min (maximal possible calculated flow rate for grafted DEAE (6 µm) 400 mL column – determined by taking into account the geometry and permeability of the monolith as well as pressure limitations of the 400 mL housing), one batch of bacterial lysate would be processed in 3.6 h.

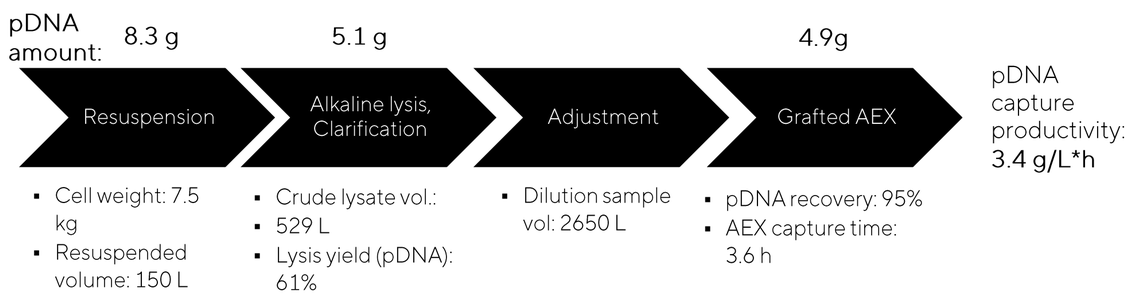

Figure 2: Purification process of 4.7 kbp pDNA from 50 L bioreactor with CIMmultus grafted DEAE 400 mL .

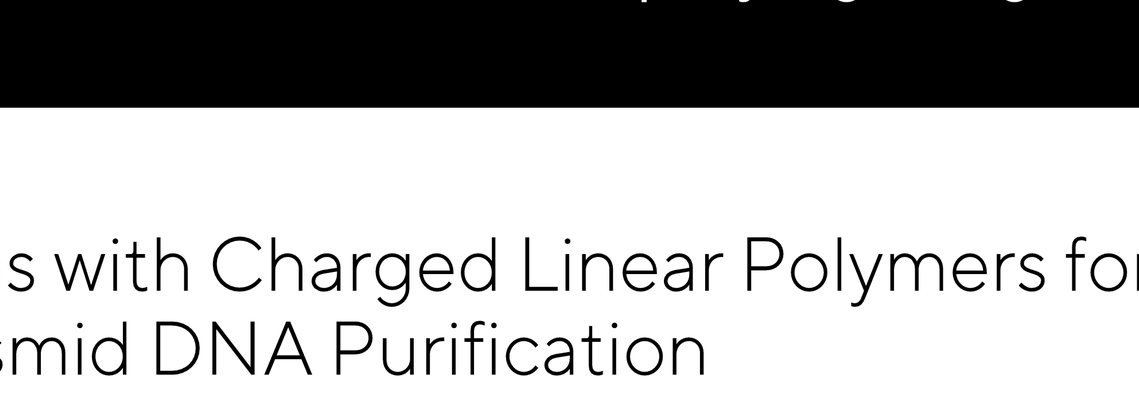

Figure 3: SEM images of a non-grafted epoxy (A) and heavily grafted monolith (B). A cross-section of grafted monolith globule visualizes 20-40 nm thick layer of grafted polymer deposited on the surface.

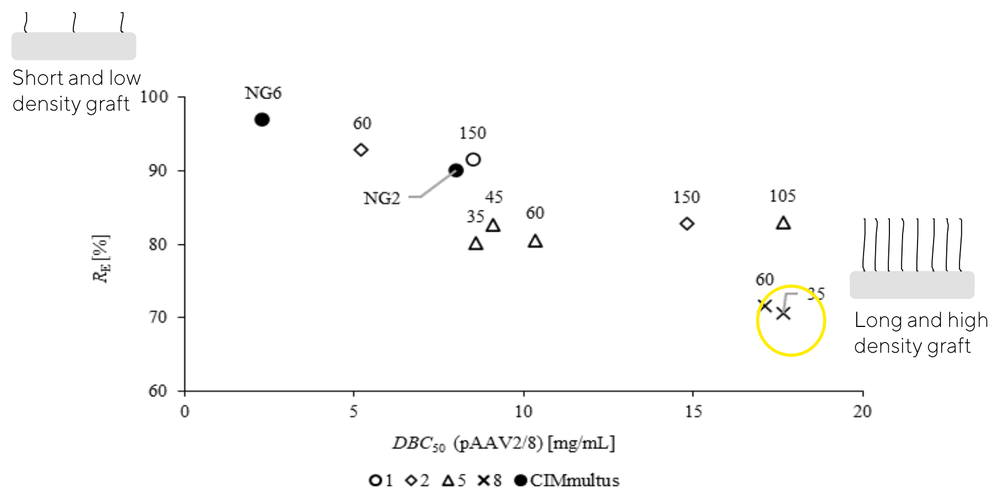

Figure 4: Correlation between DBC_{50} and elution recovery for pAAV2/8 (7.3 kbp). CIMmultus DEAE (6 µm) and CIMmultus DEAE (2 µm) labeled as NG2 or NG6. Shape determines the relative initiator (2-BPB) density, number signifies the grafting (polymerization) time. The circled number signifies the optimal grafted monolith.

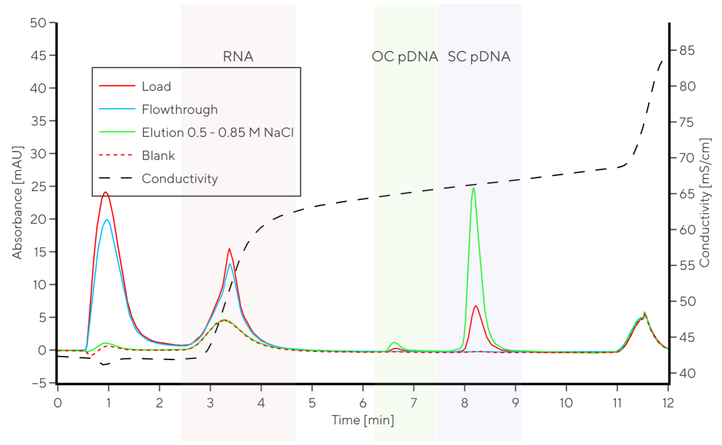

Figure 5: CIMac pDNA analysis of collected fractions from preparative pUCBS4.7 purification with CIMmultus grafted DEAE 1 mL (6 µm) : red - load, blue - flowthrough and green - elution. Analytical column used: CIMac pDNA (1.4 µm).

	Recovery in %	
pDNA size	NaCl gradient (elution)	Total (Including CIP)
4.7 kpp	95	96
11.6 kbp	78	88

Table 1: Elution recoveries for pUCBS4.7 (4.7 kbp) and pHelper (11.6 kbp) isolation.

5. Conclusions

- Dynamic binding capacities for 7.3 kbp pDNA were up to 8-times larger compared to non-grafted CIMmultus 1 mL columns.
- High permeability (in 10^{-13} m^2 range) enables flow rates on large column scale in range of 10-20 CV/min at pressure around 5 bar
- A compromise between high DBC and high recovery was obtained on columns with long and lower density graft.
- pDNA was successfully separated from other *E. Coli* impurities.
- High recovery (≥80%) of pDNA purification from bacterial lysate was achieved for up to 11.6 kbp large pDNA.
- High performance (flow rate, low pressure drop, high DBC) of grafted column resulted in high productivity for downstream capture of pDNA.

6. References

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