Plasmid DNA Hydrogels as a Scaffold for 3D Cell Culture

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Introduction

Three-dimensional (3D) cell cultures mimic the heterogeneity, structure, and function of tissues, bridging classical two-dimensional (2D) cultures and living animal models (**Fig. 1)**. Utilizing plasmid DNA (pDNA) as the generic polymer in 3D cell culture may provide significant control over the topological tunability, biocompatibility, and biodegradability of the hydrogel which are major challenges with synthetic scaffolds. However, large quantities of pDNA are needed to generate 3D cell culture.

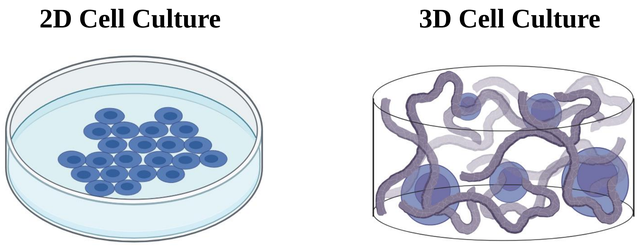

Figure 1. *Comparison of the structure of a 2D and 3D cell culture.*

Experimental Approach

To address this need, we worked to develop a simple, scalable and unified method for pDNA cultivation and purification that is easily implemented in an academic setting (**Fig. 2**).

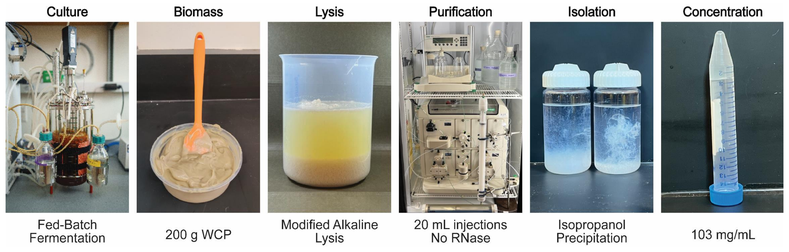

Figure 2. *Process overview for bulk pDNA generation and purification. Cells containing target plasmid are grown in a 7 L bioreactor vessel via fed-batch fermentation, producing wet cell paste (WCP). pDNA is purified using a modified alkaline lysis protocol. Lysate is purified with anion exchange chromatography to separate RNA from pDNA. pDNA is precipitated out of solution using isopropanol, then concentrated. Target concentration is 100 mg/mL.*

This work will focus on key advancements in large-scale purification as culture optimization is the objective of other projects within our group. Purification of pDNA with alkaline lysis and anion exchange chromatography (AEX) is a promising route, however many industrial protocols rely on digests or additional chromatographic steps, as well as specialized equipment to facilitate, homogenous mixing and sterile filtration. In this work, we present solutions for major obstacles of large-scale purification suitable for academic labs.

pDNA Purification

Large-scale purification was accomplished by applying industrial techniques to academic protocols. Cells are lysed using a modified scalable alkaline lysis (**Fig. 3**) and purified using anion exchange chromatography (**Fig. 4**)

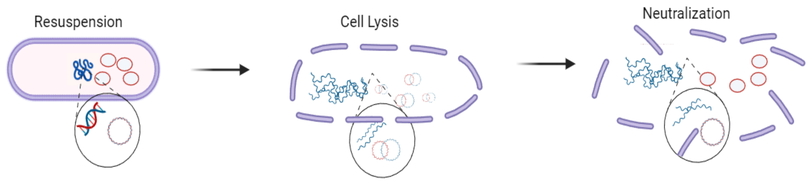

Figure 3. *Cellular view of general mechanism of the alkaline lysis process. Cells containing target plasmid are resuspended, then lysed with a highly alkaline solution. Upon neutralization, cellular debris "crashes out" of solution, leaving pDNA and RNA in solution for separation using anion exchange chromatography.*

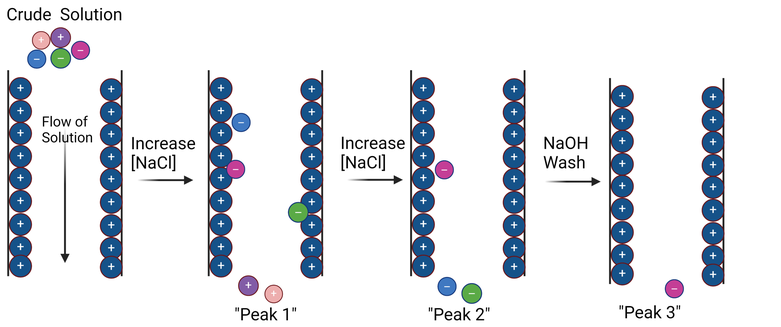

Figure 4. *General mechanism of anion exchange chromatography.*

Results

Integration of ammonium acetate precipitation into the protocol replaced laborious sterile filtration. 5L beakers and stir plates were found adequate for large-scale homogenous mixing. An optimized NaCl gradient was developed to produce pure pDNA samples without the need for RNase digests or additional chromatographic steps.

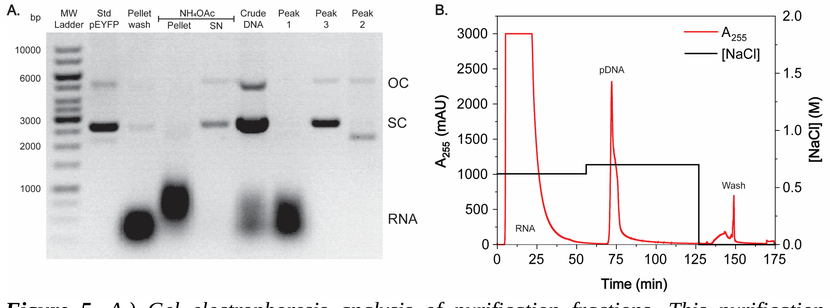

Figure 5. *A.) Gel electrophoresis analysis of purification fractions. This purification process results in ~70% yields and has been used to purify pUC19 (2.868 kbp), pUCP20T-eYFP (pEYFP, 5.045 kbp), and PIC11 (11 kbp) without modification. B.) Chromatogram of pDNA purification using anion exchange chromatography in a 193.9mL column packed with Q-Sepharose™ FF using our optimized NaCl gradient.*

Future Work

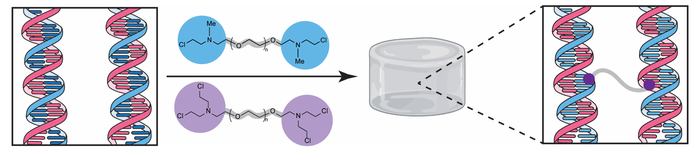

Figure 6. *Formation of chemically cross-linked pDNA hydrogels using bisfunctionalized PEG cross-linkers.*

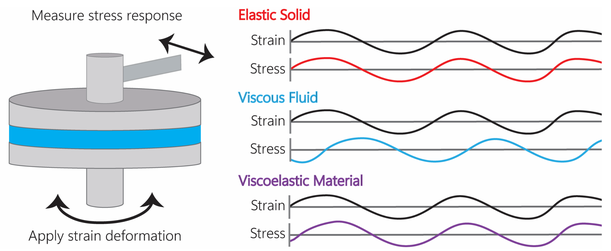

Figure 7. *Rheological analysis will provide insight on the stiffness of the created pDNA hydrogels, as well as other mechanical properties that impact cell growth and proliferation.*

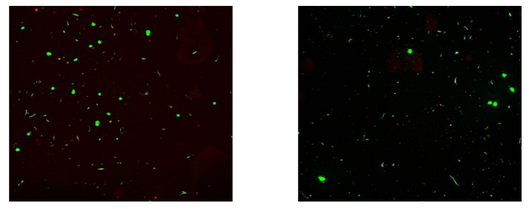

Figure 8. *Live/Dead cytotoxicity assay on 3D cell culture with pDNA scaffolding. Green indicates live cells while red represents dead cells.*

Acknowledgments

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