**Protein Patterning: Investigating the use of different protein deposition techniques to transfer proteins onto various surfaces**

Kate Clancy*, Sebastien Dery, Véronique Laforte, David Juncker, Dan Nicolau

Micro and NanoEngineering Lab, Biomedical Engineering Department, McGill University, Montreal, Canada

Bioengineering Department, McGill University, Montreal, Canada

kathryn.clancy@mail.mcgill.ca

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**1. Introduction**

Protein microarrays are used in various research areas including drug discovery, diagnosis, and analysis of protein-ligand interactions. Their efficacy depends on a well-defined pattern of immobilized proteins that also have retained their bioactivity otherwise termed spot uniformity.

Numerous studies have formulated different printing solutions to control the drying rate of spots and decrease inhomogeneity within their drying pattern. Other approaches have looked at modifying the surface parameters to increase uniformity of deposited biomaterials.

Pin printing is a contact serial protein deposition method that utilizes a liquid transfer/deposition pin that loads a controlled volume of solution then dispenses the solution as a droplet onto the substrate. This is compared to inkjet printing, a non-contact serial deposition method that ejects a droplet of protein solution onto the substrate.

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**2. Purpose**

In this work we quantitatively compare the distribution of fluorescently labeled proteins deposited using inkjet and pin printing onto various functionalized glass surfaces of different contact angles through fluorescent microscopy.

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**3. Datasets**

**Inkjet** / Non-contact deposition

- 8x8 single nozzle of 64 arrays

**Pin-printing / Contact deposition**

- 4 pins of 5x5 arrays

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**4. Methods**

- **Surface Preparation**
  1. Cleaning by sonication of the glass slides in 100% ethanol
  2. Plasma treatment (1 min) creates surface hydroxyl groups
  3. Silanization of the glass surface
    a. GPS and OTS slides prepared by liquid deposition
    b. APTES and PFS were prepared by vapor deposition
  4. Water contact angle of the surface were obtained through a goniometer
  5. Slides were cleaned using N₂ gas between each step

- **Printing of Protein Solution**
  1. Eight protein solutions were created (0, 1.0, 2.5, 5.0, 6.25, 12.5, 25, 50) in either 1X PBS or 1X PBS with 25%, 2.5%, or 2% solvent
  2. Protein solutions were transferred to the source plate for either pin or inkjet printing then printed in their respective aforementioned pattern
  3. After printing washed with 1X PBS and ddH₂O

- **Pseudo-automatic extraction**
  1. Pre-defined search areas isolating each set by its functional relevance
  2. Automatic thresholding using Otsu method

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**5. Results**

- **Fluorescence intensity changes across surfaces and method**
  As the concentration of the protein solution decreases there is a decrease in deposition proteins on all surfaces. The mean intensity of deposited protein varies across all surfaces for both methods and proteins with the greatest intensity on APTES, followed by OTS and PF respectfully. The exception being IgG deposited by inkjet, where the opposite is observed. IgG is found to adhere more to all surfaces when compared to BSA.

- **Area changes across surfaces**
  Area of the deposited protein solution varied across all surfaces for both proteins. The pin printing method produced a smaller area on all surfaces when compared to inkjet printing. This is property inherent to the machines used. The one outlier is IgG printed by inkjet at a 50 µg/mL illustrating the wealth of patterns observed.

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**6. Conclusion**

The results found in this work, where proteins were deposited with a greater mean intensity on APTES, then OTS and PF, agrees with previous findings. Droplet size was observed to decrease as the hydrophobicity of the surface increased. This is consistent with the idea that a droplet of predetermined volume will have a larger contact area with the surface on a hydrophilic surface as compared to a droplet of the same size on a hydrophobic surface. The PSD metric for measuring uniformity is not adequate for the complexity of the data and additional texture measures need to be pursued.

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**References**