# Aerosol Jet Printing of Biomaterials for Creating Micropatterned Co-culture Systems

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Cellular micropatterning plays a crucial role in engineering in vitro microenvironments that closely mimic in vivo tissue architectures. This process enables the formation of predefined patterns of cell layers, facilitating studies on homotypic and heterotypic cell interactions within controlled microenvironments. Despite advances in single-cell patterning, there remains a significant gap in the development of co-culture systems where multiple cell types are patterned in proximity to one another. The aim of this project is to develop a novel platform for micropatterning multiple cell types in a co-culture system, utilizing Aerosol Jet Printing (AJP) technology which is a relatively new technique generally used for printing circuit boards for electronic devices. When compared with the other direct printing techniques, it offers a higher resolution and the ability to print bio-inks at higher concentrations. Additionally, this study focuses on the use of nanobodies—single-domain antibodies known for their small size, stability, and versatility—as cell adhesion molecules to pattern specific cell types. In this study, nanobodies targeting specific cell surface proteins are expressed in *E. coli* expression systems and purified using IMAC chromatography. Microscale patterns of specific nanobodies are printed onto glass substrates, functionalized with 3-glycidoxypropyltrimethoxysilane (GOPTMS) for surface crosslinking, followed by grafting of polyethylene glycol-bis amine (A-PEG) for blocking non-specific binding sites. These surface modifications are characterized through fluorescence microscopy, X-ray Photoelectron Spectroscopy (XPS), Atomic Force Microscopy (AFM), profilometry, and contact angle measurements. This project will provide a better understanding of the potential of using nanobodies as specific cell adhesion molecules for micropatterning which is an application of nanobodies that has not been explored. By integrating precise surface engineering with the specificity of nanobodies, this platform aims to overcome existing challenges in co-culture micropatterning, enabling advanced studies on cell-cell interactions and facilitating the development of functional tissue models for biomedical applications.