In vivo tissues are rarely composed of a random and static arrangement of cells and matrix molecules. Instead, cells and matrix elements are intricately organized to form distinct tissue regions with specific biological functions, e.g. regions of the brain, lymph node, or tumor microenvironment, and are under constant interstitial fluid flow. However, many engineered models of these tissues lack these aspects of spatial organization and fluid flow.

To address this issue, we developed a unique system to generate micropatterned 3D cell cultures under physiological fluid flow rates. Photo-crosslinkable gelatin was selected as the backbone matrix material because it biomimetic cell adhesion and motility in a natural matrix, while enabling micropatterning. Using gelatin norbornene with a 4-arm PEG-thiol linker, lithium phenyl-2,4,6-trimethylbenzoylphosphinate, and a 405 nm collimated light source, we patterned features as small as 400 µm, at near-physiological density of $10^7$ cells/mL inside a 4 mm culture chamber housed on a PDMS/glass chip. Repeated patterning formed multiple freestanding or concentric structures, including ovoid shapes. Primary murine splenocytes, a fragile cell type, remained viable after patterning. Flow of media was controlled by syringe pump, for precise flow rates, or gravity-driven flow, for simplicity. This is the first demonstration of photopatterned 3D culture of primary cells inside of a microfluidic device, to our knowledge, and we demonstrated that it can mimic a variety of tissue organizations. Moving forward, we will use this method to generate models of organized immune responses on the chip, including rash-like reactions and a lymph node.