Definable surface chemistry is essential for many applications of microfluidic polymer systems. However, small cross-section channels with a high surface to volume ratio enhance passive adsorption of molecules that depletes active molecules in solution and contaminates the channel surface. Here, we present a one-step photochemical process to coat the inner surfaces of closed microfluidic channels with a nanometer thick layer of poly(ethylene glycol) (PEG), well known to strongly reduce non-specific adsorption, using only commercially available reagents in an aqueous environment. The coating consists of PEG diacrylate (PEGDA) covalently grafted to polymer surfaces via UV light activation of the water soluble photoinitiator benzoyl benzylamine, a benzophenone derivative. The PEGDA coating was shown to efficiently limit the adsorption of antibodies and other proteins to <5% of the adsorbed amount on uncoated polymer surfaces. The coating could also efficiently suppress the adhesion of mammalian cells as demonstrated using the HT-29 cancer cell line. In a subsequent equivalent process step, protein in aqueous solution could be anchored onto the PEGDA coating in spatially defined patterns with a resolution of <15 μm using an inverted microscope as a projection lithography system. Surface patterns of the cell binding protein fibronectin were photochemically defined inside a closed microfluidic device that was initially homogeneously coated by PEGDA. The resulting fibronectin patterns were shown to greatly improve cell adhesion compared to unexposed areas. This method opens for easy surface modification of closed microfluidic systems through combining a low protein binding PEG-based coating with spatially defined protein patterns of interest.