# Photocrosslinked silk fibroin microgels and microgel scaffolds for tissue engineering

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## Abstract

Hydrogels made from synthetic and natural polymers have been extensively explored for tissue engineering as an artificial extracellular matrix that supports tissue regeneration. However,

their nanometer-scale pore size hampers effective cell, tissue, and blood vessel integration when implanted. A novel category of microporous biomaterials, known as microgel scaffolds, is created by annealing microgels, which are hydrogels at microscale. This study introduces the creation of silk fibroin microgels using a microfluidic device for the first time, enabling control over the microgel diameter (100-400 μm) and shapes (rod and sphere) and stabilizing via visible light-initiated photo-crosslinking of the native tyrosine residues in silk. The photocrosslinking reaction grants the formation of microgels in the absence of silk modification, and the covalent encapsulation of biological molecules in the microgels. Microgels were then covalently annealed using silk solution as a glue, making this into a cytocompatible cell encapsulation platform. Unlike the nano-porosity of bulk photo-crosslinked silk hydrogels, the microgel scaffolds had an average pore diameter of 29±17 μm or 192±81 μm depending on the microgel size, with enhanced mechanical properties compared to bulk hydrogels. The tunable nature of this platform allows the formation of heterogeneous materials biofunctionalized with different molecules at different ratios and with spatial control. The microporosity supported enhanced cell spreading and proliferation *in vitro* with evidence for increased scaffold remodeling *in vivo*. Analysis of the immune reaction to these implants suggested that large microgels support pro-healing macrophages (CD206+) and material remodeling relative to small microgels. Finally, annealing of the microgels into microgels scaffolds supported enhanced immune response relative to lose microgels with fewer macrophages and fewer pro-inflammatory (MHCII+) macrophages compared to microgels in suspension. This research proves the potential of silk microgel scaffolds as viable options for tissue engineering and regenerative medicine purposes.