Long Term Mesenchymal Stem Cell Culture on a Defined Synthetic Substrate with Enzyme Free Passaging

Abstract

Mesenchymal stems cells (MSCs) are currently the focus of numerous therapeutic approaches in tissue engineering/repair because of their wide multi-lineage potential and their ability to modulate the immune system response following transplantation. Culturing these cells, while maintaining their multipotency in vitro, currently relies on biological substrates such as gelatin, collagen and fibronectin. In addition, harvesting cells from these substrates requires enzymatic or chemical treatment, a process that will remove a multitude of cellular surface proteins, clearly an undesirable process if cells are to be used therapeutically. Herein, we applied a high-throughput 'hydrogel microarray' screening approach to identify thermo-modulatable substrates which can support hES-MP and ADMSC growth, permit gentle reagent free passaging, whilst maintaining multi-lineage potential. In summary, the hydrogel substrate identified, poly(AEtMA-Cl-co-DEAA) cross-linked with MBA, permitted MSCs to be maintained over 10 passages (each time via thermo-modulation), with the cells retaining expression of MSC associated markers and lineage potency. This chemically defined system allowed the passaging and maintenance of cellular phenotype of this clinically important cell type, in the absence of harsh passaging and the need for biological substrates.