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Abstract

Strategies that enable hydrogel substrates to support cell attachment typically incorporate either entire extracellular matrix proteins or synthetic peptide fragments such as the RGD (arginine–glycine–aspartic acid) motif. Previous studies have carefully analysed how material characteristics can affect single cell morphologies. However, the influence of substrate stiffness and ligand presentation on the spatial organisation of human mesenchymal stem cells (hMSCs) have not yet been examined. In this study, we assessed how hMSCs organise themselves on soft ($E = 7.4 - 11.2$ kPa) and stiff ($E = 27.3 - 36.8$ kPa) poly(ethylene glycol) (PEG) hydrogels with varying concentrations of RGD (0.05 – 2.5 mM). Our results indicate that hMSCs seeded on soft hydrogels clustered with reduced cell attachment and spreading area, irrespective of RGD concentration and isoform. On stiff hydrogels, in contrast, cells spread with high spatial coverage for RGD concentrations of 0.5 mM or higher. In conclusion, we identified that an interplay of hydrogel stiffness and the availability of cell attachment motifs are important factors in regulating hMSC organisation on PEG hydrogels. Understanding how cells initially interact and colonize the surface of this material is a fundamental prerequisite for the design of controlled platforms for tissue engineering and mechanobiology studies.

Keywords: cell attachment, RGD, PEG, hydrogel, human mesenchymal stem cells, tissue engineering

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