The use of four-colour immunofluorescence techniques to identify mesenchymal stem cells.

Abstract:
In stem-cell research a major difficulty is caused by the lack of distinctive features that allow the identification of human mesenchymal stem cells (hMSC). Until now, there has been no specific marker and the most common way to identify hMSC is by their characteristic stem-cell properties: self-replication and differentiation potential. However, these findings can only be revealed retrospectively, and, once differentiated, hMSC lose their stem-cell character. The aim of this study was to establish four-colour immunofluorescence of several markers simultaneously in order to address the problem of how to identify hMSC on the single-cell level. The four markers collagen-I, collagen-IV, fibronectin and CD44 are known to be expressed by hMSC. Antibody binding was detected using secondary antibodies conjugated to FITC, Alexa546, TexasRed and AMCA. Because the distinction between Alexa546 and TexasRed was not possible on conventional digital images using standard filter sets, we performed spectral image acquisition. The image was subsequently decomposed into its pure spectral components, which permitted linear unmixing. Using this procedure we were able to demonstrate four-colour immunofluorescence on hMSC. With the possibility of using more sophisticated marker profiles and/or additional markers, four-colour immunofluorescence offers the opportunity of identifying hMSC on the
single-cell level without performing differentiation assays.