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Functional chitosan microcarriers for selective cell attachment and expansion

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Introduction: The success of many stem cell applications in the biomedical field is highly dependent on the development of reliable techniques either for isolation or selection of specific cell populations with a very high yield and purity.[1] In this work we propose the use of chitosan microparticles (μPs) to capture a specific cell type based in the concept of antibody-antigen binding. Our goal was to create new biomaterials capable of selecting within a heterotypic cell suspension, a specific sub-population, and supporting subsequent cell expansion. Such system simultaneously allows the selection and acts as a microcarrier for a specific target, thus reducing cell manipulation and time-consumption.
Materials and methods: μPs were fabricated by spraying a chitosan (HMC) solution (2% w/v) into a NaOH solution. Free amines on the μPs were used to tether biotin. Biotinylated antibodies were then immobilized through the modification of the μPs with streptavidin. The functionalization was performed with anti-CD90 and anti-CD31 (eBioscience) to attach and expand human adipose stem cells (hASCs) and human umbilical vein endothelial cells (HUVECs), respectively. For assessing the yield of the selection process, cells were pre-stained with Dio (green) and Dil (red). Additionally the cells recovered from the μPs were evaluated for cell-surface marker expression (CD90, CD73 and CD31). Differences between the experimental results were analyzed according to a Student t-test ($p < 0.05$).
Results: The μPs that were functionalized with the defined antibodies were selectively recognized by the respective cell type. In addition, both the attached cells were able to proliferate on the μPs. (Fig. 1A) The capacity of the μPs to select a specific cell type from a heterogeneous cell population was then assessed. The results confirmed the ability of the developed microparticles to separate hASCs and endothelial cells (Fig. 1B) from a complex cell mixture with approximately 30% of $CD31^+$ and 65% $CD90^+$ cells. After selection with anti-CD90 μPs, 97.31% of the obtained cells was positive for CD90. Likewise, 98% of the population isolated with the anti-CD31 μPs expressed the endothelial marker.

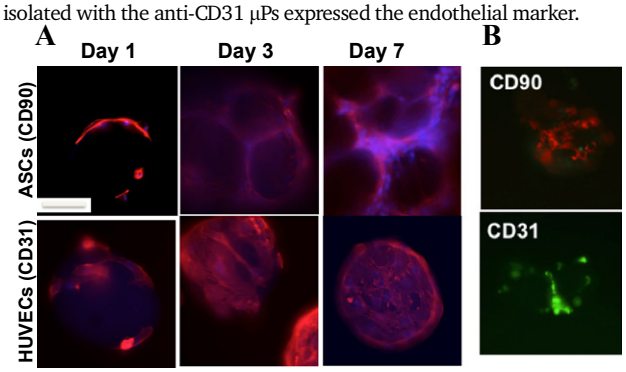

Figure 1 (A) Fluorescence microscopy images of (A) adhered hASCs on anti-CD90 and HUVECs on anti-CD31 μPs, along the time of culture, and (B) the sub-populations 24 hours after selection from the mixture.
Discussion and conclusions: Chitosan μPs were successfully conjugated with biotin and streptavidin. The versatility of this method allows the combination of the functional particles with any biotinylated molecule. It was shown that μPs functionalized with different antibodies presented selective affinity to cells, making them potentially suitable for separating subpopulations of cells from complex mixtures. Besides the ability for cell separation, the particles proved to be also suitable for cell growth.
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