

## Sulfation degree of glycosaminoglycans triggers distinct cytoskeleton organisation in mesenchymal stem cells

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Glycosaminoglycans (GAGs) comprise the closest cellular environment: they are building elements of the ECM and can be also found on cells surface. Their biological activity depends on several parameters among which the negative charge is of prime importance[1]. This charge is generally associated with the presence of sulfate groups (-OSO<sub>3</sub>H). Sulfation is a dynamic modification: it can occur at various positions within the glycan and different sulfation patterns have been identified for the same organs and cells during their development. However, the mechanisms of coding and transferring information by these functionalities are not yet complete understood, mainly because of (i)the complex physiological microenvironment in which GAGs interactions occur and (ii)the inability to access homogeneous GAGs[2].

In this work, we propose model surfaces bearing GAGs with different sulfation degree as platform to investigate the pathways by which mesenchymal stem cells (MSCs) sense and respond to this peculiar functionality: the -OSO<sub>3</sub>H. We have selected two natural GAGs for this study: hyaluronic acid (HA) because it is the only non-sulfated glycan and heparin (HEP) as it is the GAG with the highest degree of sulfation. To obtain a larger range of sulfation degrees, we have also prepared a synthetic analogue of HA with a sulfation degree of 1.4 (sHA). All these GAGs were covalently bonded to aminothiols deposited on gold surfaces. MSCs, both from bone marrow and adipose tissue, adhered well to all surfaces. Formation of focal adhesions was observed after only 1h of culture for bone marrow derived MSCs regardless the used substrate. The presence of -OSO<sub>3</sub>H groups induced different morphology and cytoskeleton organisation: formation of longer filopodia and well pronounced actin fibers were visible for the MSCs from both sources. Moreover, cells were more spread after 24h in contact with -OSO<sub>3</sub>H containing surfaces. Cells behaved similarly on both sulfated surfaces (sHA and HEP) and differences in cell morphology were less obvious: higher sulfation degree induced less lamellipodia formation while filopodia number and length increased.

In summary, the present study provides evidence that sulfation degree of GAGs triggers distinct cytoskeleton organisation in mesenchymal stem cells that may be related with the differentiation of those cells. However, further studies at the molecular level about the exact mechanism of these processes need to be carried out.

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2. Soares da Costa D. et al., *Journal of Materials Chemistry*, 2012.**22**(15):7172-7178.

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