Nanocellulose hydrogels for biomedical applications: using atomic force microscopy techniques to understand and control cell adhesion and spheroid formation

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Ethical considerations and legislative initiatives have further encouraged the development of *in vitro* , three-dimensional (3D) cell culture models to replace or minimize animal experimentation in biomedical applications like drug testing or disease modelling. Nanocellulose hydrogels from plant biomass have proven to be a promising material in this field because, in addition to abundance and biocompatibility, their fibrillar network can structurally mimic the extracellular matrix of tissues. Nanocellulose hydrogels have been successfully used to grow 3D cell cultures *in vitro* , facilitating the formation of 3D cell aggregates called spheroids [1,2]. Nevertheless, to further exploit this biomaterial in biomedical applications and tissue engineering, a fundamental understanding on how cells interact with nanocellulose is needed. Using the versatility of atomic force microscopy (AFM) and the high sensitivity AFM-based force spectroscopy techniques, we carried out a detailed quantitative analysis of the adhesion and single binding forces between cellulose nanofibrils (CNFs) and human hepatocarcinoma cells (HepG2) and pluripotent stem cells (WA07 and iPS (IMR90)-4). For comparison, the interaction of cells with protein materials like laminin-521 (LN-521) and Matrigel was also analyzed. Our results indicate that the adhesion of cells to CNFs is governed by non-specific interactions, that is, interactions not mediated by binding proteins on cell membranes [3]. This is in contrast with cell adhesion to LN-521 substrates, where cell membrane integrins play a very important role in cell adhesion as specific receptors for LN-521 molecules. By analysing each of the multiple single bonds contributing to the total adhesion, we observed that the binding force of single bonds between cells and CNFs was similar to that of single integrin-protein bonds, within the range 20 - 60 pN [4,5]. Still, the presence of a higher number of single bonds made the adhesion of cells to LN-521 significantly stronger than to CNFs. In this regard, we proved that coating CNF hydrogels with LN-521 enhanced cell adhesion and proliferation, opening new routes for the development of nanocellulose-based hydrogels with tunable cell adhesion properties [5]. Another interesting conclusion was drawn when correlating the AFM results for cell-material and cell-cell interactions with gene expression tests: culturing cells in CNF hydrogels enhanced the expression of cadherins, cell membrane proteins involved in cell-cell adhesion. On the contrary, cadherin expression was down-regulated in 3D cell cultures using Matrigel as a matrix [4]. These results were in line with the experimental observation that spheroids formed in CNF hydrogels were more compact than the cell aggregates obtained in Matrigel. In summary, our work provides important insights into the molecular mechanisms involved in cell adhesion to CNFs and spheroid formation in nanocellulose hydrogels, supporting a rational understanding, tunability and application of this biomaterial in the biomedical field.

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