Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B

Supplementary material

Growth and spontaneous differentiation of umbilical-cord stromal stem cells on activated carbon cloth

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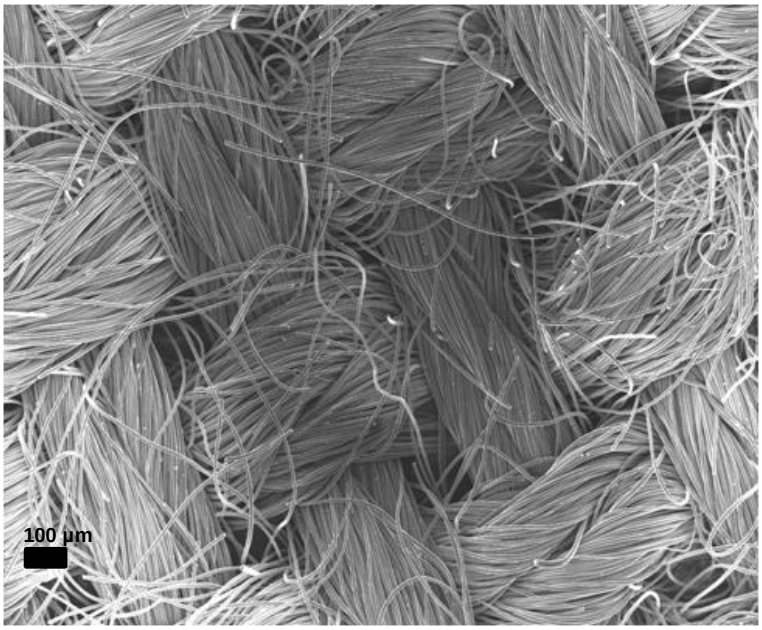
Fig. S1 A) SEM micrograph of ACC. **B)** N_2 adsorption-desorption isotherm at -196 ºC; adsorption (black triangles) and desorption (red triangles).

A)



B)

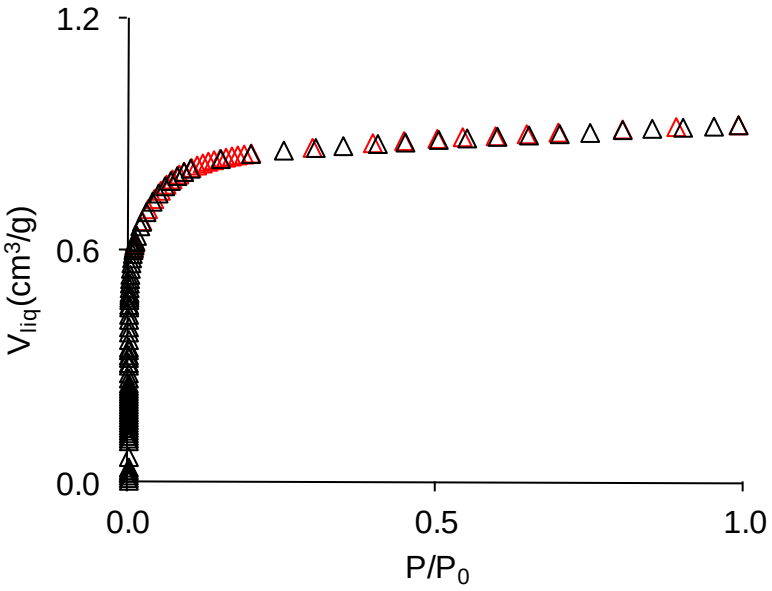


Fig. S2 UCSSCs growing on ACC under control conditions for 21 days. Nuclei are stained with DAPI (blue dots in **A** and **B** and 1, 2 and 3 in **C**), which proves the viability of the cells. **A**) Sirius Red staining reveals the presence of an abundant secretion of ECM proteins, among which collagen type I is the most important component. **B**) Some cells or some component of ECM are autofluorescent (green in the image in **B**, and 4, 5 and 6 in **C**, **AFL**) after excitation with white light; notice that nuclei and fluorescent images are separate (B, and 7, 8 and 9 in **C**) and that both DAPI and autofluorescent signals increase with time.

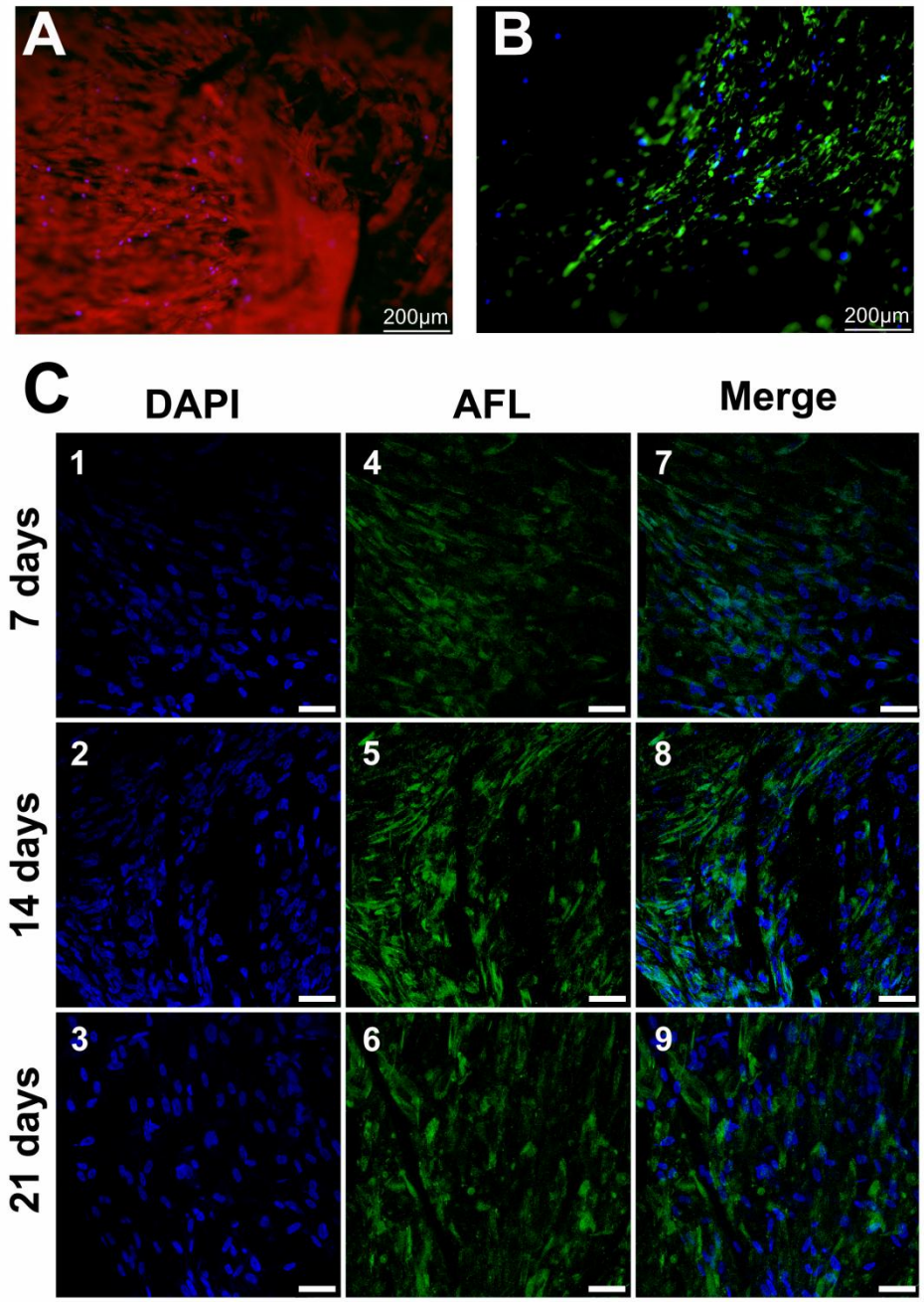


Fig. S3 UCSSCs growing on ACC under control conditions for 3, 10 and 21 days. Nuclei are stained with DAPI and EMC proteins with Sirius Red staining. Notice the relative variation between number of nuclei and expression of EMC proteins.

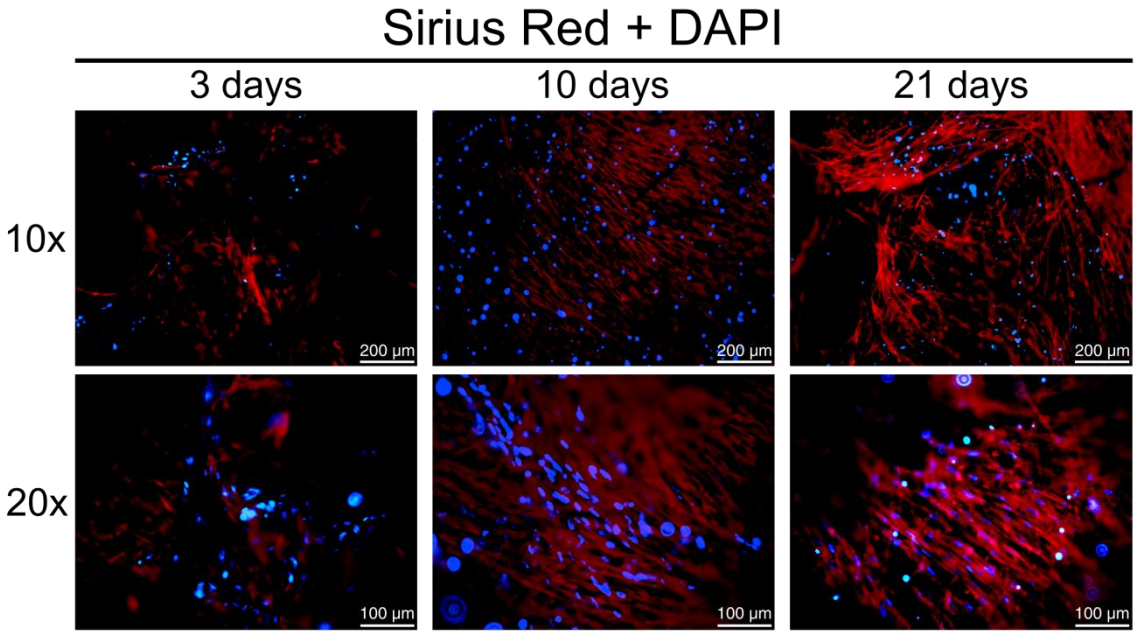


Fig. S4 Confocal images of the UCSSCs committed to osteocyte differentiation at 7, 14 and 21 days during the time-course experiment. Nuclei are stained with DAPI (A, B and C). In D, E and F we have summarised the autofluorescent signal observed after excitation with white light. G, H and I correspond to the specific osteoblast marker osteopontin (red signal). Finally, J, K and L show the merged images.

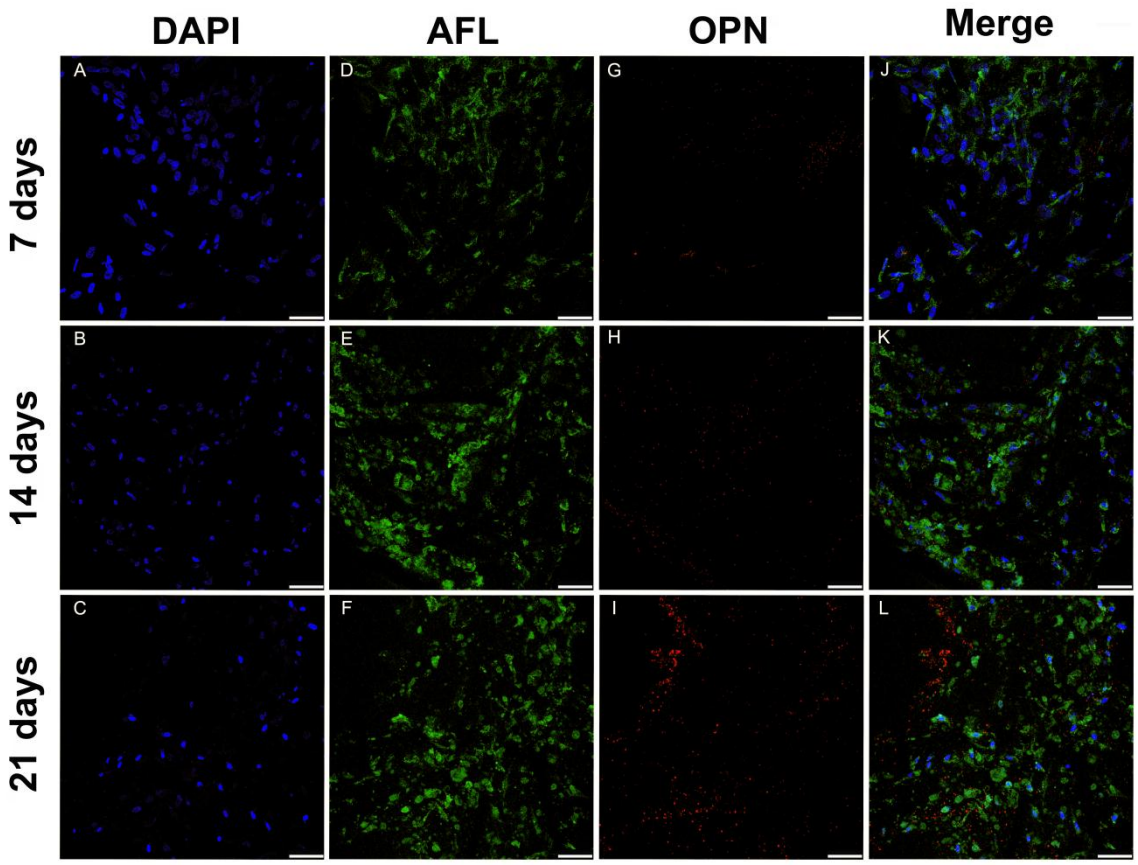


Fig. S5 Confocal images of the UCSSCs committed to chondrocyte differentiation after 7, 14 and 21 days during the time-course experiment. Nuclei are stained with DAPI (A, B and X); In D, E and F we have summarised the images corresponding to the specific chondroblast marker, collagen type II (green signal). Finally, G, H and I show the merged images. It should be noted that the green staining is concentrated around the nuclei, indicating the location of collagen II.

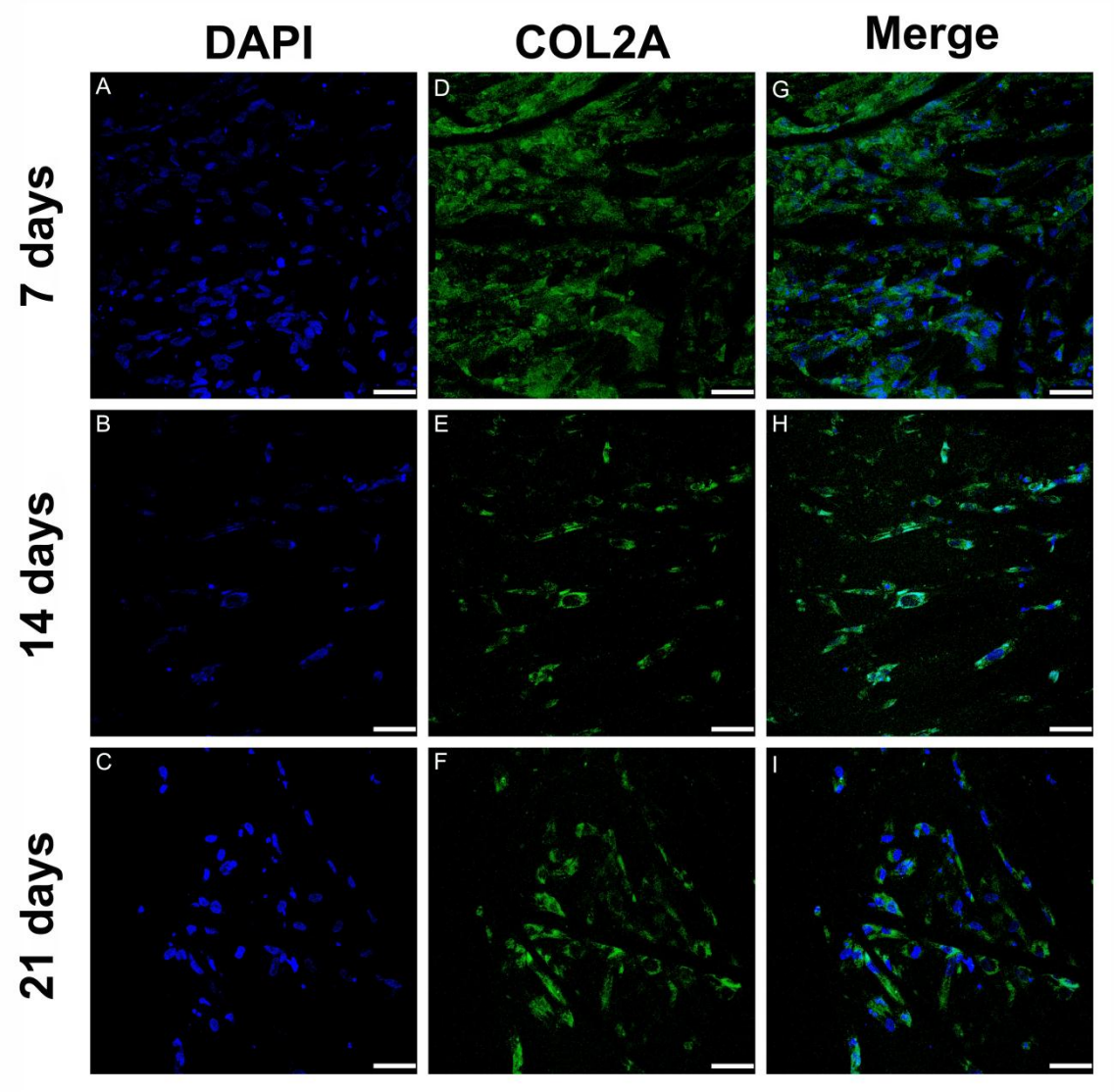


Fig. S6 Examples of the fibres measured in this work. **A**) On the structure of the ACC (marked with a red *) numerous microfilaments can be discerned attached to the pores on the carbon surface. The sample corresponds to an experiment of differentiation of UCSSCs to osteocytes examined at 14 days. We call these fibres type I. **B**) Sample corresponding to an experiment with UCSSC cultures under control conditions examined at 21 days. On the microphotograph there are numerous type II fibres, marked with **. Dendritic processes and materials formed by cellular secretion can also be made out. **C**) The type III fibres (marked with ***) are easily identifiable in cultures of more than 4 weeks. In this case the image corresponds to an experiment with UCSSCs cultured in a medium to stimulate differentiation towards osteoblast lineages. Noteworthy are the different thicknesses of the fibres and the presence of two spherulites, which are normally taken to be an initial deposit of hydroxyapatite. In **D**) we have plotted the histogram of the frequency distribution of the width of fibres measured in this work. We did not measure the type I fibres although they are known to be under 10-20 nm in size (an asterisk has been included). The histogram shows a bi-modal distribution with maxima approximately centred on 60 and 160 nm.