Gradual conversion of cellular stress patterns into pre-stressed matrix architecture during in vitro tissue growth

Abstract

The complex arrangement of the extracellular matrix (ECM) produced by cells during tissue growth, healing and remodelling is fundamental to tissue function. In connective tissues, it is still unclear how both cells and the ECM become and remain organized over length scales much larger than the distance between neighbouring cells. While cytoskeletal forces are essential for assembly and organization of the early ECM, how these processes lead to a highly organized ECM in tissues such as osteoid is not clear. To clarify the role of cellular tension for the development of these ordered fibril architectures, we used an in vitro model system, where pre-osteoblastic cells produced ECM-rich tissue inside channels with millimetre-sized triangular cross sections in ceramic scaffolds. Our results suggest a mechanical handshake between actively contracting cells and ECM fibrils: the build-up of a long-range organization of cells and the ECM enables a gradual conversion of cell-generated tension to pre-straining the ECM fibrils, which reduces the work cells have to generate to keep mature tissue under tension.

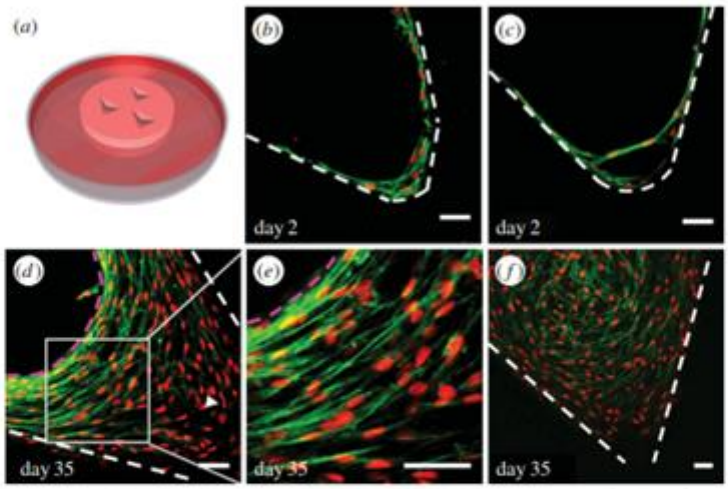

Figure 1. Cell organization during tissue growth in triangular millimetre-sized pores. (*a*) ECM-rich tissue was grown in triangular pores of HA scaffolds incubated in culture medium containing MC3T3-E1 cells. After fixation, the tissue was stained for actin (green) and nuclei (red) for fluorescent confocal imaging. Samples were fixed after 2 days (*b*) to reveal the elongated shape of the cells, which occasionally pulled out of the surface of the scaffold (white dashes) by the associated forces (*c*). Scaffolds fixed after 35 days of culture reveal the organization of the cells in the tissue at a later stage of growth and the apparition of an actin ring at the tissue–medium interface (pink dashes) (*d*). Throughout the culture period, cells at the tissue–medium interface have an elongated morphology (*e*), whereas cells embedded in the bulk spread in three dimensions (*d*, arrow). This transition in cell morphology as they become embedded in a three-dimensional environment also appears at the centre of a pore filled with tissue after 35 days of growth (*f*). Scale bar, 50 μm.