Two stages in three-dimensional in vitro growth of tissue generated by osteoblastlike cells

Abstract

Bone regeneration is controlled by a variety of biochemical, biomechanical, cellular, and hormonal mechanisms. In particular, physical properties of the substrate such as stiffness and architecture highly influence the proliferation and differentiation of cells. The aim of this work is to understand the influence of scaffold stiffness and cell seeding densities on the formation of tissue by osteoblast cells within polyether urethane scaffolds containing pores of different sizes. MC3T3-E1 preosteoblast cells were seeded on the scaffold, and the amount of tissue formed within the pores was analyzed for culture times up to 49 days by phase contrast microscopy. The authors show that the kinetics of three-dimensional tissue growth in these scaffolds follows two stages and can be described by a universal growth law. The first stage is dominated by cell-material interactions with cell adherence and differentiation being strongly dependent on the polymer material. After a delay time of a few weeks, cells begin to grow within their own matrix, the delay being strongly dependent on substrate stiffness and seeding protocols. In this later stage of growth, three-dimensional tissue amplification is controlled rather by the pore geometry than the scaffold material properties. This emphasizes how geometric constraints may guide tissue formation in vitro and shows that optimizing scaffold architectures may improve tissue formation independent of the scaffold material used.

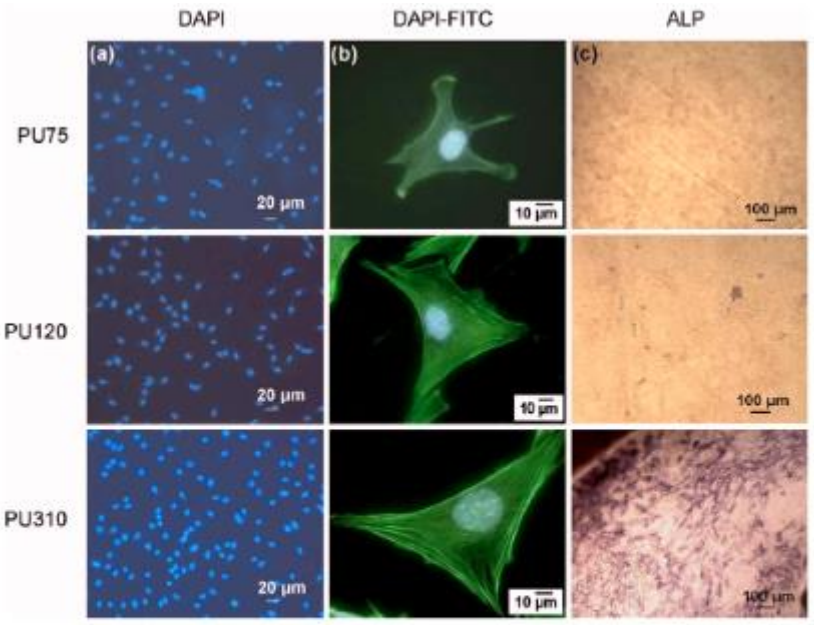

FIG. 2. (Color online) (a) DAPI staining of cells (nuclei) seeded at 10^5 cells/cm^2, 6 h after cell seeding (scale 20 μm). (b) FITC-DAPI staining of MC3T3-E1 cells showing the cytoskeleton and nucleus on PU75, PU120, and PU310, respectively (scale 10 μm). (c) Alkaline phosphatase enzyme staining of PU75, PU120, and PU310, respectively, after 21 days of culture (scale 100 μm).