

ABSTRACT

In the field of regenerative medicine, regeneration of cartilage defects (caused either by injury or age-related degeneration) has become a widely discussed topic. Nanofibrous scaffolds provide a suitable environment for cell adhesion, proliferation, differentiation, and also for the local involvement of bioactive substances. Nanofibrous scaffolds mimic the extracellular matrix (ECM) of hyaline cartilage. These scaffolds are seeded with autologous chondrocytes. After having been isolated from the patient, the cells must be cultivated *in vitro* in order to obtain a sufficient amount of chondrocytes. Scaffolds with cultivated chondrocytes are later implanted back into the patient. Chondrocytes, however, when grown on a 2D tissue culture plastic rapidly de-differentiate and thus lose the ability to synthesize ECM molecules. The aim of the work was modulation of chondrogenic differentiation medium through finding the ideal concentration of chondrogenic supplements, composed of L-ascorbate-2-phosphate (A2P) and dexamethasone (DEX), in the culture of primary chondrocytes seeded on a nanofibrous polycaprolactone (PCL) scaffold. The effect of different concentrations of the chondrogenic supplements on chondrocyte adhesion to the scaffold and their proliferation and differentiation was studied. The influence of each of the supplements alone in the medium was studied, then their synergistic effect together with other chondrogenic supplements, and finally A2P and DEX were incorporated into the PCL fibers themselves. It has been demonstrated that A2P has a positive effect on chondrocyte metabolic activity. DEX has been proved to induce gene expression for chondrogenic markers. If the supplements were added into the medium at the same time, their effects became even more potent. The supplements were studied in 21-day experiments. If the supplements were incorporated in the fibers, the 5PLM group containing double concentrations of chondrogenic supplements than those present in the standard differentiation medium was best for maintaining chondrogenic differentiation. The advantage of incorporating the supplements inside the nanofiber scaffolds is the fact that it enables their gradual release at the defect site.

KEY WORDS: chondrocytes, chondrogenic differentiation, centrifugal spinning, L-ascorbate-2-phosphate, dexamethasone