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Engineering of synthetic skeletal muscle tissue for cultivated meat

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Cultivated meat is a recent and promising technology that aims to develop actual meat without the need of animal slaughter, using tissue engineering techniques instead (1). In order to create a synthetic tissue that resembles the skeletal muscle *in situ*, a three dimensional substrate, called scaffold, that mimics the extracellular matrix of the native tissue is required (2,3). The scaffold's architecture is an important concern on the field because it modulates muscle cells adhesion, alignment and proliferation (2,3), but also may influence organoleptic properties of the final product, such as taste and texture. Cellulose acetate nanofibers (CAN) are good candidates as scaffolds for cultivated meat because they are biocompatible and edible biomaterials that have shown great affinity for myoblasts in culture (4). Besides, it is obtained by electrospinning, a versatile and affordable technique that is adaptable to different demands. In this work, we used aligned and random CAN, and investigated their interaction with C2C12 mouse myoblasts, a cell line model for cultivated meat (5). Myoblasts proliferation rates were much lower in the scaffolds, aligned or random, than in the monolayer. Cell cycle arrest in the scaffold was not triggered by high cell density, since cells were a lot more sparse and distributed along the *z* axis of the 3D substrate, when compared to cell distribution in the monolayer culture. To investigate whether this brake in proliferation could be due to an incipient triggering of myogenesis, cell morphology was first assessed. Scanning electron microscopy (SEM) and actin fluorescence images revealed that myoblasts in the CAN were more elongated through culture time as compared to the monolayers, even though cell density was not noteworthy. Also, as expected, cells were more aligned on the anisotropic scaffold. SEM images also showed that cells cultured under differentiation medium (DM - DMEM, 2% of horse serum) for 5 days (DM) presented the same morphology of cells cultured for the same amount of time on growth medium (GM). Accordingly, qPCR data of the expression profile of myosin heavy chain 3, a myogenesis marker, was similar for cells under GM or DM on the scaffold. These data suggest that CAN consist in an excellent substrate for skeletal muscle tissue engineering, not only allowing cell adhesion and population, but also its differentiation without the need of any external stimuli.

Key-words: Cultivated meat, Skeletal muscle, Nanotechlogy

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