Development of 3D combined bioactive tissue matrices and evaluation of myogenic cell activities on these matrices under electrical stimulation

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Introduction

Skeletal muscle tissue has a high regenerative capacity, unlike many tissues, due to the satellite cells in its structure which become active as a result of injury and differentiate into myoblasts. However, in case of large damage, or in chronic muscle diseases such as muscular dystrophy, in which muscle cells are irreversibly damaged, the natural biological environment of the cells is also damaged. In this case, the self-renewal capacity of the muscle tissue remains insufficient. Cell-based therapies and different methods including natural or synthetic grafts have been proposed for the repair of damage. However, these treatments with a single functional component are often inadequate to repair complex tissue damage. In this study, a spiral form 3-dimensional (3D) matrices with electrical conductivity were developed for fast and effective treatment of major injuries to skeletal muscles. These structures were supposed to act as a carrier for muscle cells and at the same time stimulate the growth and differentiation of these cells and accelerate the formation of 3-dimensional muscle tissue.

Experimental Methods

The abdominal rectus muscle was decellularized using enzymatic and chemical methods. The amounts of DNA and GAGs in the samples were analyzed to determine the efficiency of the process and whether the protein structure is preserved or not. To produce a conductive oriented nanofiber membrane, a mixture of polyaniline (PANI) and polycaprolactone (PCL) polymer solutions was electrospun onto a rotating drum collector. The morphology of the nanofibers was determined using scanning electron microscopy (SEM). The electrical conductivity of the membranes was measured with a multimeter and to determine the chemical structure, FTIR-ATR analysis was performed. For 3D spiral matrices, a viscous solution of DCM was poured on the nanofiber membrane and rolled into a spiral form, and lyophilized. A tensile test and in vitro degradation studies were also conducted in PBS under physiological conditions.

To evaluate the interactions of 3D matrices with skeletal muscle cells, myoblast cells (C2C12) were seeded on both surfaces of plane DCM/PANI/PCL matrices and matrices/cell constructs were analyzed using the Alamar Blue assay and Phalloidin/DAPI staining at 1, 7, and 14th days of the culture. To further assess the effect of electrical properties of 3D nanofiber matrices on cell viability, morphology and myotube formation, electrical stimulation was applied during cell culture studies. Immunofluorescence staining with anti-troponin (TNNT) and anti-desmin was performed to determine the muscle-specific marker expression of cells in tissue matrices after electrical stimulation.

Results and Discussion

The results revealed that more than 75% of DNA reduction and 90% of GAG and protein protection were

obtained after decellularization. Nanofiber membranes have been successfully produced from a PCL/PANI polymer blend and the spiral form structures were also formed with good integration between the components as presented in SEM micrographs. The electrical conductivity of the PCL/PANI nanofiber membrane was calculated as between $3x10^{-9}$-$2,5x10^{-9}$ S/cm. The presence of both components, PANI and PCL, on the surface of nanofiber membranes were confirmed by FTIR-ATR spectra. The results from degradation studies revealed that the weight of 3D combined matrices was higher than PCL/PANI nanofibrous mats after 90 days.

Regarding cell culture with myoblast cells, it was observed that the cells on the nanofibrous surface continued to survive and proliferate until the 14^{th} day, while the cell viability on the DCM surface decreased between the 7^{th} and 14^{th} days. Supporting the results of the Alamar Blue cell viability test, it was determined with DAPI/Phalloidin staining that the nanofibrous surfaces of the matrices showed well spread on the surfaces. Tube formation and alignment in the direction of electrical stimulation were observed in the cells (E+). Tube formations were observed in patches in samples without electrical stimulation (E0), but the orientation did not occur (Fig1). According to the immunofluorescent staining, TNNT and Desmin markers were observed in both groups (E0, E+). More intense anti-TNNT and anti-Desmin were observed in samples with electrical stimulation (E+).

Conclusion

The results obtained from the present work indicate that spiral shape combined structures possess favorable properties to serve as a matrix for the regeneration of damaged skeletal muscle tissue. In vitro cell, culture tests, and cell behavior under electrical stimulation have also successfully demonstrated that these 3D combined tissue matrices are suitable for the application of muscle tissue defects.

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Figure1
DAPI/Phalloidin staining of myoblast cells (C2C12) on the nanofiber surface of the matrix with (E+) and without (E0) electrical stimulation.