Tubular composite scaffolds produced via Diffusion Induced Phase Separation (DIPS) as a shaping strategy for anterior cruciate ligaments reconstruction

Ilenia Vitrano*[a], Francesco Carfì Pavia[a], Valerio Brucato[a], Clemens Goegele[a], Aldo R. Boccaccini[c], Vincenzo La Carrubba[a], Gundula Schulze-Tanzil[b]

[a]*Department of Civil, Environmental, Aerospace, Materials Engineering Universita' di Palermo, Viale delle Scienze building 18 Palermo, Italia*

[b]*Institute of Anatomy, Paracelsus Medical University, Salzburg and Nuremberg, Nuremberg, Prof.-Ernst-Nathan Strasse 1, 90419 Nuremberg, Germany*

[c] *Institute of Biomaterials, Department of Materials Science and Engineering, University of Erlangen-Nuremberg, Cauerstraße 6 Erlangen, Germany*

Injuries of tendons and ligaments are common, especially among the young population. Anterior cruciate ligament (ACL) injuries do not heal due to its limited vascularization and hence, surgical intervention is usually required. The ideal scaffold for ligament tissue engineering (TE) should be biocompatible and possess mechanical and functional characteristics comparable to the native ACL. The Diffusion Induced Phase Separation (DIPS) technique allows the preparation of homogenous porous tubular scaffold with micro-pores using a rather simple procedure. Composites based on biodegradable polymers and bioglass have attracted much attention in tissue reconstruction and repair because of their biological and physicochemical advantages.

In this work a new approach in ACL TE will be proposed focussing on the development of a suitable technique for *in vitro* seeding of lapine ACL fibroblasts into tubular-shaped instructive Poly-lactic-acid (PLLA) scaffolds, supplemented or not with bioglass (BG) 1393, produced via DIPS. Tubular composite scaffold (diameters: 1.2 and 2 mm, +/- BG) were obtained through a dip coating around a cylindrical support followed by a DIPS. An 8%wt PLLA/dioxane solution was prepared with 5%wt of BG-1393 as filler. Preliminary *in vitro* cell culture trials were carried out by seeding lapine ACL fibroblasts inside the scaffolds (2 cm as length) employing different seeding strategies in order to find the best way that allows to obtain a homogeneous fibroblast distribution inside the tubes. (1) First trials consisted in the inoculating of the cell suspension inside the tubes and maintaining them in dynamical culture. (2) The second one was done by suspending the cells in a fibrin gel polymerized within the tubes by using of thrombin. (3) The third approach was carried out by using cell spheroids (three-dimensional self-assembled cell agglomerates). Cell attachment, viability and morphology were examined by live-death and Hematoxylin/Eosin stainings after 1, 7, 14 d and vimentin immunolabelings (7 d). Scanning electron microscopical analysis revealed that the internal surface of the tubes was homogeneously structured with micropores sized around 5 µm and a mean thickness of the wall of 60 µm. The results showed cell adhesion to the wall of the tubes with all seeding techniques applied even though with fibrin gel it was more homogenous. Furthermore, colonized areas expanded with culture time and the majority of cell survived irrespectively of seeding techniques. (1) In inoculation phase, many cells left the scaffold and attached on the plate. Even after the dynamic culture (rotating device) most cells covered only half the tube inner surface. (2) In the second trial, a fibrin gel was used to achieve a homogenous cell distribution during seeding. In the early stage (48 h) cells remained captured inside the fibrin, but after 7 d they become elongated and migrated from the fibrin to the inner tube surface forming a compact cell layer. So, the fibrin appears helpful to achieve an immediate high cell seeding efficiency and an almost homogeneous cell distribution inside the tubes. (3) Although using the spheroid technique the scaffold internal surface was not homogeneously colonized with cells, after 7 d cell migration to the inner scaffold surface from the attaching spheroids could be observed. In longitudinal sections cells were elongated like typical ligament fibroblasts parallel to the longitudinal tube axis. Therefore, it can be affirmed that employment of tubular scaffolds produced by DIPS could be a promising approach of ligament TE. In the future, it would be interesting to evaluate the effectiveness of seeding by combining the spheroids and the fibrin gel.