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

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Introduction

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 tubular scaffolds based on biodegradable polymers and bioglass (BG) might support cell alignment and could be integrated in larger scaffold.

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 1393, produced via DIPS.

Materials and Methods

Scaffold production 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.

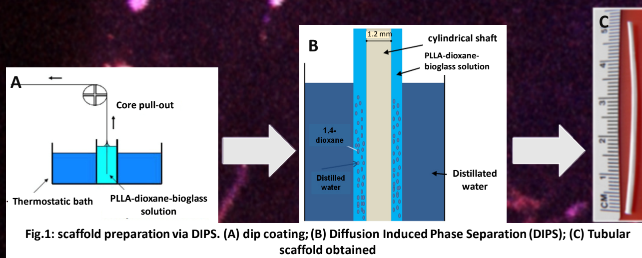

Fig.1: scaffold preparation via DIPS. (A) dip coating; (B) Diffusion Induced Phase Separation (DIPS); (C) Tubular scaffold obtained

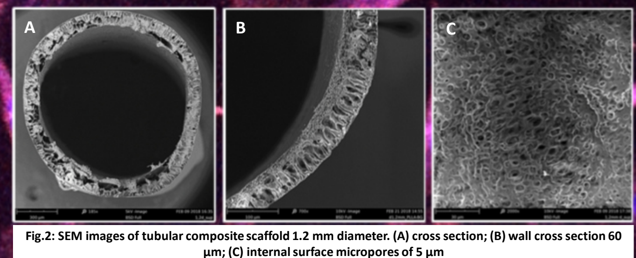

Fig.2: SEM images of tubular composite scaffold 1.2 mm diameter. (A) cross section; (B) wall cross section 60 µm; (C) internal surface micropores of 5 µm

Biological tests with Lapine ACL fibroblasts

***In vitro* different seeding strategies inside scaffolds of 2 cm as length:**

1. **Inoculating of the cell suspension inside the tubes – dynamic culture**
2. **Suspending the cells in a fibrin gel**
3. **Cell spheroids (3D self-assembled cell agglomerates)**

Cell attachment, viability and morphology were examined after 1, 7, 14 days.

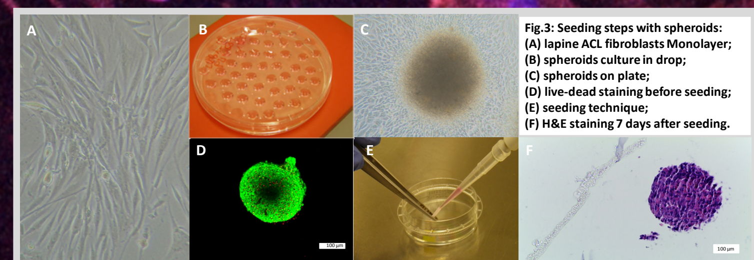

Fig.3: Seeding steps with spheroids: (A) lapine ACL fibroblasts Monolayer; (B) spheroids culture in drop; (C) spheroids on plate; (D) live-dead staining before seeding; (E) seeding technique; (F) H&E staining 7 days after seeding.

Results and Discussion

(1) Many cells left the scaffold and attached on the plate but most cells covered only half the tube inner surface. (2) At 1 day cells remained captured inside the fibrin (Fig.7.2). After 7 days, the cells become elongated and migrated from the fibrin to the inner tube surface forming a compact cell layer. (3) Scaffold internal surface was not homogeneously colonized but after 7 days cell migration to the inner scaffold surface (Fig.7.3). Fibrin appears helpful to achieve an immediate high cell seeding efficiency and an almost homogeneous cell distribution inside the tubes. Colonized areas expanded with culture time and the majority of cell survived irrespectively of seeding techniques. Cells were elongated like typical ligament fibroblasts mostly parallel to the longitudinal tube axis (Fig.6.B1, 6.B2)

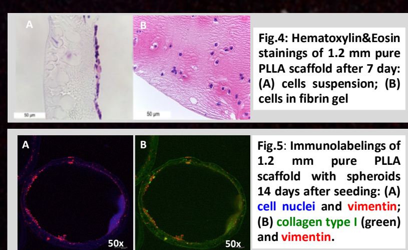

Fig.4: Hematoxylin&Eosin stainings of 1.2 mm pure PLLA scaffold after 7 day: (A) cells suspension; (B) cells in fibrin gel

Fig.5: Immunolabelings of 1.2 mm pure PLLA scaffold with spheroids 14 days after seeding: (A) cell nuclei and vimentin; (B) collagen type I (green) and vimentin.

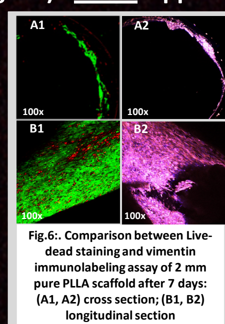

Fig.6:. Comparison between Live-dead staining and vimentin immunolabeling assay of 2 mm pure PLLA scaffold after 7 days: (A1, A2) cross section; (B1, B2) longitudinal section

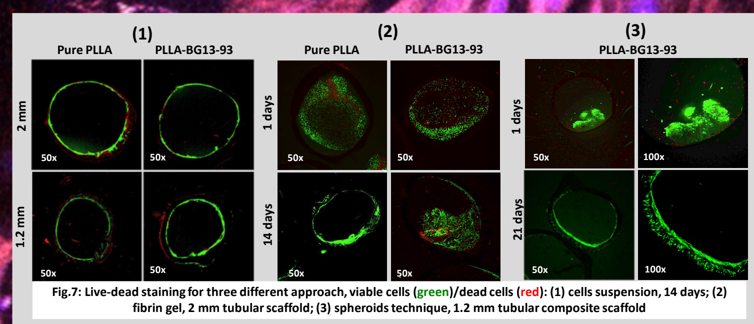

Fig.7: Live-dead staining for three different approach, viable cells (green)/dead cells (red): (1) cells suspension, 14 days; (2) fibrin gel, 2 mm tubular scaffold; (3) spheroids technique, 1.2 mm tubular composite scaffold

Conclusion

The results show that employment of tubular scaffolds produced by DIPS could be a promising approach of ligament TE.