Characterization of novel autologous leukocyte fibrin platelet membranes for tissue engineering applications

<u>Silvia Barbon</u>¹ - Elena Stocco¹ - Francesca Grandi² - Alessio Borean³ - Stefano Capelli³ - Martina Contran⁴ - Veronica Macchi⁴ - Pier Paolo Parnigotto⁵ - Claudio Grandi¹ - Raffaele De Caro⁴ - Andrea Porzionato⁴

¹Dipartimento di Scienze del Farmaco, Università degli Studi di Padova, Padova, Italia – ²Dipartimento della Salute della Donna e del Bambino, Università degli Studi di Padova, Padova, Italia - ³Dipartimento d'Immunoematologia e Medicina Trasfusionale, Ospedale San Martino, Belluno, Italia- ⁴Istituto di Anatomia Umana, Università degli Studi di Padova, Padova, Italia - ⁵Fondazione per la Biologia e la Medicina della Rigenerazione - Tissue Engineering and Signaling - TES, Onlus, Padova, Italia

Autologous hemocomponents have recently emerged as potential biologic tools for regenerative purpose, consisting mainly of platelet concentrates which locally release growth factors (GFs) to enhance the tissue healing process. Despite two decades of clinical studies, the therapeutic efficacy of platelet concentrates is still controversial. This work represents a first characterization of a novel autologous leukocyte fibrin platelet membrane (LFPm), which is prepared by the Department of Immunohematology of Belluno Hospital according to a well standardized protocol. The quantification of their specific content showed that LFPms are enriched not only with platelets, but also with monocytes/macrophages, fibrinogen and CD34+ cells. Mechanical properties of LFPms were investigated by tensile tests, revealing that the specific elasticity of membranes was maintained over time. Furthermore, the release kinetics of Platelet Derived Growth Factor, Vascular Endothelial Growth Factor, Tumor Necrosis Factor alpha and Interleukin-10 was assessed by ELISA, demonstrating that LFPms act as GF delivery systems which sustain the local release of bioactive molecules. For in vitro biodegradation analysis, LFPm samples were incubated into PBS solution for 4, 7, 14, 21 days. SEM micrographs showed a progressive loss in cellular elements associated to a simultaneous exposure of the fibrin scaffold, also confirmed by histological and immunohistochemical investigations. In parallel, LFPm disks were implanted into a subcutaneous dorsal pouch of healthy nude rats and explanted after 4, 7, 14, 21 days for in vivo biodegradation study. SEM, histological and immunohistochemical analysis revealed that the typical LFPm fibrin structure was maintained until day 7, with a contemporary loss of cellular elements. From day 14, the morphology and texture of samples became less and less recognizable, confirming that a progressive biodegradation occurred. Overall, collected evidences could support the rationale for the clinical use of LFPms, shading some light on the regenerative effect they may exert after the autologous implant on a defect site.

References

[1] Caloprisco et al. (2010). New method to produce hemocomponents for regenerative use from peripheral blood: integration among platelet growth factors monocytes and stem cells. Transfus Apher Sci 42, 117-24;doi: 10.1016.

Keywords

Autologous hemocomponents; platelets; tissue healing.