Biological characterization of Poly-L-lactic acid (PLLA)/Hydroxyapatite (HA)/Bioglass (BG) composite scaffolds made by Thermally Induced Phase Separation (TIPS) hosting human Mesenchymal Stem Cells.

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In the last few years, Tissue Engineering has focused on the favourable effects that composite scaffolds have on cell adhesion, growth and differentiation. In fact, composite scaffolds, usually composed of a synthetic polymer supplemented with naturally occurring components, display superior mechanical properties and bioconductivity than scaffolds consisting of a single component. Hydroxyapatite (HA) is the major inorganic component of bones. Bioglass (BG) is known to exert stimulatory effects on cells by ion release and hence, could be also advantageous for Bone Tissue Engineering. Poly-L-lactic acid (PLLA) is a versatile synthetic polymer combinable with HA and BG.

The aim of this work was to assess the effectiveness of PLLA/HA and PLLA/HA/BG 1393 composite scaffolds as suitable artificial Extracellular Matrix (ECM) for human Mesenchymal Stromal Cells (h-MSCs). In order to check if composite scaffolds are actually superior, a comparison was made between the scaffolds above mentioned and PLLA and PLLA/BG scaffolds.

All four types of scaffolds (PLLA, PLLA/HA, PLLA/BG and PLLA/HA/BG) were manufactured in Palermo, at the University of Palermo, using the Thermally Induced Phase Separation (TIPS) technique, for which a PLLA/1,4-dioxane/water ternary solution was chosen. In composite scaffolds, HA and BG 1393 were added in the solution as powder phase. The temperature selected for promoting the phase separation was 30 °C and the residence time in the thermostatic bath was set to 80 minutes. The samples were then placed in a cooling bath at -20 °C for 15 minutes, in order to freeze the porous structure thus formed. With these process conditions, scaffolds with a porosity higher than 90% and a mean pore size of 100 μm were obtained. After subsequent washing and drying steps, the as-obtained cylindrical structures were cut in disk-shaped specimens with a diameter of 6 mm and a thickness of 1.5 mm.

Before the seeding stage, scaffolds were sterilized in 70% ethanol solution, stored in Phosphate Buffered Saline (PBS) and finally soaked in h-MSCs culture medium. The seeding of h-MSCs occurred in a 96-well plate and monitoring analyses were carried out at time points of 48 hours and 8 days. Before used for seeding experiments, undifferentiated MSCs were characterized for a set of markers (CD34, CD44, CD45, CD90, CD105, Vimentin) to show their typical expression pattern before seeding. Multilineage differentiation potential was proven by adipogenic, osteogenic and chondrogenic differentiation. Life/dead assays, Haematoxylin/Eosin, Alcian blue and Alizarin red stainings were employed to verify cells viability, ECM synthesis, adhesion to the polymeric structure and migration into the scaffold. Cell proliferation was calculated from DNA content using CyQuant assay.

h-MSCs expressed the typical markers, they were able to spread evenly on both upper and lower surfaces of all types of scaffold. The majority of the adhering cells survived on the scaffolds over the whole observation period. Scaffolds supplemented with HA revealed a higher seeded area compared

with PLLA and BG alone. Furthermore, h-MSCs penetrated in mean 260 μm into the porous polymeric structure of all scaffolds.
The next step will involve long time experiments with h-MSCs under osteogenic and non-osteogenic conditions in the same types of scaffolds. Successful osteogenic differentiation will be tested by monitoring osteogenic marker expression, e.g. type X collagen.