Endothelial Progenitor Cell Differentiation and Paracrine Activity on Self-Assembling Peptide Amphiphile Nanofibers

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Introduction
The in situ release and maintaining of endothelial progenitor cells (EPCs) in cardiovascular tissue engineering offers new possibilities for achieving an effective vascularisation of ischemic or injured tissue. For this reason it is important to use biodegradable scaffolds mimicking the structure and biological function of native extracellular matrix (ECM). ECM does not only provides a physical support for cells, but also a substrate with specific ligands for cell adhesion and migration and regulates cellular proliferation and function by providing various growth factors. Peptide-amphiphiles (PA) are self-assembling biocompatible molecules with the potential of forming nanofibers when mixed with opposite charged solution. In a previous work we have demonstrated that this PA does not affect cell viability, independently by the PA concentration and by the culture method used (2D or 3D). The objective of this study was to investigate if our PA is a good alternative to fibronectin for EPC differentiation and functional activity.

Materials and Methods
The PA was prepared by a fully automated peptide synthesizer. The final chemical structure contained RGD, eight aminoacids and an alkyl tail of 16 carbons. The PA was dissolved in water and the solution was maintained at 4°C until use. PA gel was formed by mixing the aqueous PA solution at different concentrations (1% - 2%) with an endothelial medium with 5% FBS, growth factors and containing CaCl2. The scaffold morphology was investigated by scanning electron microscopy (SEM), atomic force microscopy (AFM) and cryogenic SEM (CRYO-SEM). Mononuclear cells obtained from the peripheral blood of healthy donors were seeded either on the surface (2-D) or inside the gel (3-D) and cultured for 1 week to obtain EPC. EPC obtained on fibronectin were used as a control. The expression of endothelial markers (CD31, KDR, vWF, Ve-Cadherin) was assessed by confocal microscopy. Finally, the paracrine release of cytokines involved in inflammatory process by EPCs was also investigated by using a multiplexable bead assay.

Discussion and Conclusions
The three-dimensional complexity of PA containing RGD sequence, characterised by both a nanometric fibrous structure, important to mimick the ECM and an interconnected porous network that guarantees an excellent nutrition supply, suggests that it can be a suitable scaffold for EPC differentiation and function. The development of a class of self-assembling PA created through molecular design and able to selectively release mediators involved in cell recruitment at sites of neo-vessels offers new possibilities in ischemic tissue regeneration.

Disclosures: Not commercial conflict of interest