TS033
Integrative studies on the role of Ccbe1 in cardiogenesis: from the embryo to ES cell derived cardiac tissue

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A central challenge for the future perspective of cardiac regenerative medicine is the generation of large numbers of patient-specific cardiac myocytes. *Ccbe1* encodes a secreted molecule that was firstly identified using an Affymetrix GeneChip differential screen for chick heart precursor cells expressed genes (Bento et al., 2011). In mouse and chick, *Ccbe1* is expressed in major cardiac progenitor lineages that contribute to distinct heart structures during heart organogenesis (Facucho-Oliveira et al., 2011). Moreover, analysis of gain and loss of function performed in both mouse and chick embryos showed abnormal cardiac morphogenesis and aberrant chamber formation further elucidating the role of Ccbe1 for cardiac development. Similarly, in mouse and human ES cells, increased levels of *Ccbe1* expression were detected after cardiac lineage commitment demonstrating well-coordinated expression of various early and late cardiac specific markers and *Ccbe1*. Knock-Down in mouse and human ES cells demonstrated the requirement of Ccbe1 for proper cardiogenesis. Modulation of mCcbe1 activity in differentiating mES cells using media supplemented with mCcbe1 recombinant protein has demonstrated a remarkable inductive potential of mCcbe1 to enhance cardiogenesis. Taken together, this data strongly suggest that Ccbe1 has the ability to direct the expression of cardiac inducers and to control cardiac progenitor expansion *in vitro* and *in vivo,* allowing the generation of non-genetically manipulated cardiac cells from a renewable cell source for regenerative cardiovascular medicine.

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Development of a bilayered scaffold based on silk fibroin and silk fibroin/nano-calcium phosphate for osteochondral regeneration

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Objectives: Osteochondral defect is a common condtion in clinic. Satisfactory outcomes are rarely achieved by traditional methods. Tissue engineering might be a promising strategy for this hinder. The aim of this study is to mimick the stratified structure of osteochondral tissue, by developing a bilayered scaffold for osteochondral regeneration. The developed bilayered scaffold is composed of a porous silk fibroin scaffold as the cartilage-like layer and a porous silk fibroin/nano-calcium phosphate (CaP) scaffold as the bone-like layer.

Methods: The silk and silk/nano-CaP bilayered scaffolds were prepared by a combination of salt-leaching and freeze-drying approaches, as previously reported [1,2]. Briefly, the concentrated silk fibroion aqueous solution (16%) was mixed with calcium chloride and ammonium phosphate dibasic solution to generate the silk/nano-CaP suspension. The bottom layer was prepared by adding the sodium chloride particles (500–1000 μm) into the suspension. A 16% silk fibroin solution was then added onto the top of the silk/nano-CaP layer. Sodium chloride particles of the same size were added into the silk solution to produce the top layer. After 48 h, sodium chloride was leached out from the layered scaffold and the final bilayered scaffolds were lyophilized. The generated scaffolds were characterized by SEM, micro-CT, and EDX.

Results and Discussion: SEM images showed that a macro/micro porous structure was observed in both layers. These two layers integrate well, without the formation of a clear interface. Micro-CT analysis allowed observing that the layered scaffolds were of porous structure, with homogeneous porosity distribution in each layer. The CaP was homogeneously distributed in the bottom layer, while there was no CaP detected in the top layer. By EDX analysis, the amount of CaP from the bottom to the top layer was mapped, which presented a gradient decrease in the interface region, indicating a good integration between both layers.

Conclusions: Silk fibroin and silk fibroin/nano-CaP bilayered scaffolds were successfully generated. The porous structure was maintained in each layer. The CaP was homogeneously distributed only in the bottom layer, and presented a gradient decrease at the interface. The most important features of this bilayered construct is the good integration between the two layers at the interface region. This is important since when implanted this region can be particularly sensitive to mechanical stresses. Therefore, this bilayered scaffold may be useful for an osteochondral regeneration approach.

References:

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