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Innovative encapsulation platform based on pancreatic extracellular matrix achieve substantial insulin delivery

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ABSTRACT

Cell-based therapies for the treatment of diabetes, generally aim to provide long-term glucose regulated-insulin delivery using insulin producing cells. The delivery platform is crucial for the therapeutic outcome as well as for immunoisolation of the entrapped cells. We have developed a novel artificial pancreas encapsulation platform for the treatment of diabetes that is based on solubilized whole porcine pancreatic extracellular matrix (ECM). These unique capsules were used to entrap human liver cells and mesenchymal stem cells that were induced to differentiate into glucose-regulated insulin-producing cells. We demonstrate that the ECM-microcapsule platform provides a natural fibrous 3D niche, supporting cell viability and differentiation, while significantly improving insulin delivery. *In vivo*, ECM-encapsulated cells were shown to be non-immunogenic, and most importantly, to significantly improve the glycemic control in diabetic mouse preclinical model, thus establishing a proof-of-concept for this new cell-based insulin delivery platform.

1. Introduction

The major advantage of cell-based therapies, such as artificial pancreas, for the treatment of diabetes lies in the potential of the system to deliver long-term glucose regulated-insulin. This can be achieved using glucose-responsive insulin producing cells [1–3]. Microencapsulation platforms, in which cells are isolated from the host immune system by a polymeric semipermeable membrane, have long been studied for the treatment of diabetes and other diseases, gaining substantial success in pre-clinical studies [4–6]. The most important considerations for designing cell microencapsulation platforms are the choice of biomaterial and the choice of cells. The biomaterial has to provide the physical structure and mechanical support that will allow cellular attachment. In addition, the biomaterial should provide biological cues so that the cell environment mimics the natural tissue niche and can thus support cell survival, differentiation and function. In natural tissues, cells synthesize and deposit macromolecules that provide the framework, the extracellular matrix (ECM), on which they grow and remodel. The ECM is primarily comprised of polysaccharides and proteins, mainly collagen and elastin, that provide a physical niche for cellular attachment [7]. It is also responsible for transmitting a wealth of chemical and mechanical cues, which affect cellular differentiation, maintenance, and biological function [8–11]. The implementation of ECM-based biomaterials for biomedical platforms is, therefore, highly beneficial for the creation of a tissue-specific microenvironment which can promote specific biological functions [12,13]. In diabetes cell-based therapies the use of ECM is of particular interest as ECM components, such as collagen, were shown to improve pancreatic islet survival and culture as well as insulin secretion [14–17].

On the cell aspect, the cells chosen for microencapsulation need to produce and secrete insulin in a glucose-regulated manner and in clinically relevant doses. Additional considerations are cell availability, long-term survival and function *ex-vivo* and *in vivo*, as well as their immunogenic potential. Though the vast majority of the efforts to develop cell-based therapies for the treatment of diabetes make use of pancreatic islets, an increased research effort has been recently directed at the differentiation of cells from various sources into insulin producing cells (IPCs) [18–20]. Reprogramming of adult human liver cells (AHLC) toward IPCs by ectopic expression of pancreatic transcription factors (pTFs) was suggested as an unlimited source of beta cell replenishment [21,22]. Transdifferentiated AHLCs produce, process, and secrete insulin in a glucose-regulated manner, ameliorating hyperglycemia by *in vivo* implantation in diabetic SCID mice [18,23]. Human mesenchymal stem cells (hMSC), on the other hand, are known for their immune-evasiveness, ease of isolation and culture, and controlled

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differentiation [24–27]. Moreover, these cells are already approved for different clinical applications. To achieve insulin secretion, the cells are transduced with pTFs to induce differentiation into glucose regulated insulin-producing cells [22,23]. We have therefore, developed a new microencapsulation platform for diabetes cell-based therapy which is based on the soluble form of a whole porcine pancreatic native ECM (Fig. 1). We hypothesized that microcapsules made from pancreatic decellularized ECM hydrogel could provide the optimal conditions for cell differentiation and maturation into glucose-regulated insulin producing cells, and maintain their differentiated state and survival. Such a platform could provide stronger and longer lasting therapeutic effects than when using traditional inert polymers. For these studies we entrapped AHLC and hMSC that were differentiated into IPC using ectopic expression of pTFs. The ECM-based platform was designed and comprehensively characterized in terms of structure, physical properties and interaction with the transduced cells. Most significantly, ECM-microcapsule biocompatibility was confirmed, and the platform's therapeutic potential was validated and demonstrated *in vitro* and in pre-clinical diabetic model.

2. Materials and methods

2.1. Study design

The overall objective of this study was to develop and evaluate the pre-clinical feasibility of a novel cell microencapsulation platform, which is based on the natural porcine pancreatic ECM, for the treatment of diabetes. First, we developed methods for isolation, solubilization and gelation of the ECM, and characterized the obtained biomaterial. Concurrently, in order to provide an available, reliable cell source that secretes insulin in a glucose regulated manner and in clinically relevant doses, the differentiation of hMSC using ectopic expression of pTFs was addressed and compared to the transdifferentiation of AHLC using the same method. Following the above, we investigated the potential of the ECM-gel to support cell viability and function. Once this potential was validated, an ECM-microencapsulation technology was developed, using alginate for the initial structural support and for the formation of an alginate-PLL semipermeable membrane. Following the ECM gelation, the alginate was removed, leaving intact and stable ECM microcapsules. The developed ECM-microencapsulation platform was then evaluated *in vitro* in terms of its ability to support the encapsulated cell viability and function in the long term, and in terms of biocompatibility. Finally, *in vivo* studies were conducted to assess the system immunogenicity (using an immunocompetent mouse model) and the system therapeutic efficacy (using an immunodeficient diabetic mouse model). In all the *in vitro* studies and in the *in vivo* biocompatibility studies, the ECM microcapsules were compared to alginate-PLL microcapsules, the most investigated system for cell encapsulation. For all the experiments, the number of replicates or the number of mice used is outlined in each figure legend.

2.2. Cell culture and genetic modification

Adult human liver cells (AHLC) were isolated from human liver and cultured as previously described [18,28]. The human mesenchymal stem cells (hMSC) were from Lonza™, Basel, Switzerland. The hMSC were cultured in Alpha MEM containing 5 ng/ml of basic fibroblast growth factor (bFGF, Biological Industries, Israel), and supplemented with 10% Fetal Calf Serum (FCS), 1% Pen-Strep and 0.4% amphotericin B (Fungizone, Biological Industries). The RAW 264.7 murine macrophage cell line (TIB-71™; ATCC) was cultured in DMEM (25 mM glucose, Sigma) and supplemented with 10% FCS and 5 ml glutamine. hMSC and AHLC were modified to express insulin by transduction with recombinant adenoviruses Ad-CMV-PDX-1, Ad-PAX4-CMV-GFP and Ad-CMV-MAFA at a multiplicity of infection (MOI) of 1000, 100 and 10, respectively, for 48 h [29]. The following growth factors and soluble factors were added directly to the culture medium: Nicotinamide 10 mM (Sigma), human Epidermal Growth Factor 20 ng/ml (PeproTech) and Exendin-4 5 nM (Sigma).

2.3. Analyses of transduced cells

The percentiles of GFP positive cells after viral transduction of *Ad-PAX4-CMV-GFP* (100 MOI) were analyzed using flow cytometry (FACSCalibur™, BD Biosciences). In addition, transduced and non-transduced hMSC and AHLC were immuno-fluorescently stained for insulin

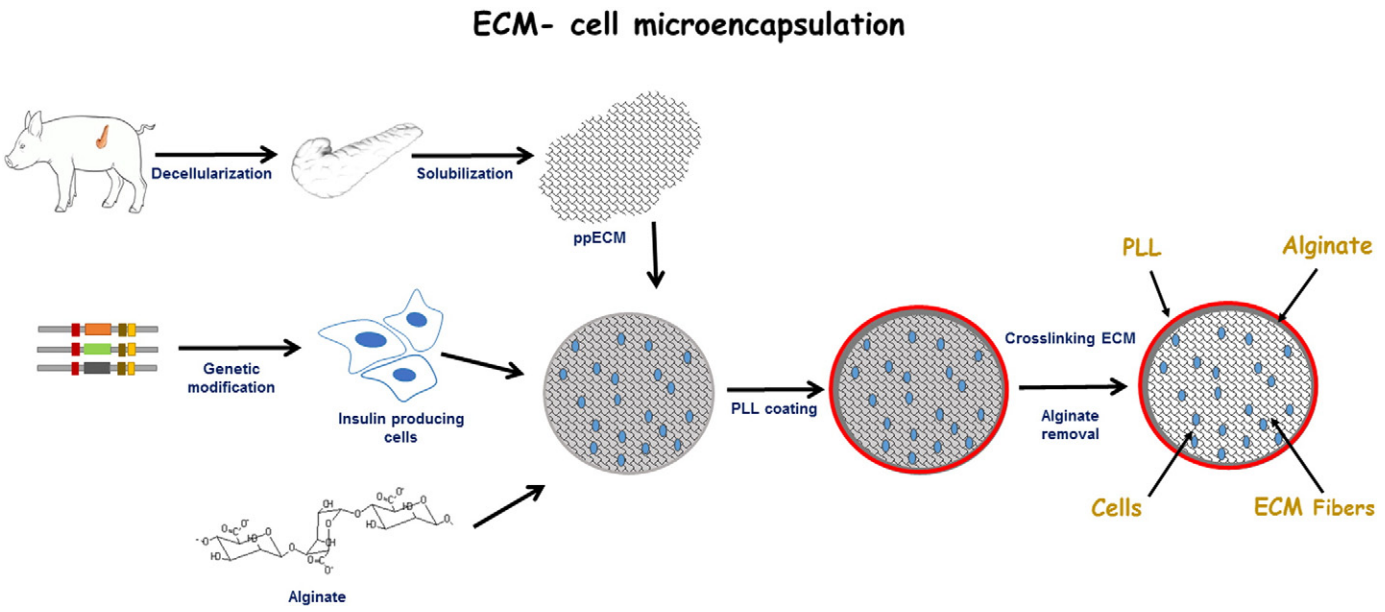

Fig. 1. Schematic illustration of the ECM microencapsulation system. Porcine pancreatic ECM is isolated and solubilized. The porcine pancreatic ECM is then used together with alginate to encapsulate insulin producing cells. Microcapsules are coated with poly-L-lysine (PLL) and the ECM is crosslinked. Finally, the alginate is removed by chelation to produce ECM-based microcapsules.