Cell-cell communication mimicry with PEG hydrogels for enhancing β-cell function

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Statement of Purpose: Cell-cell communication is important in many cellular activities, including survival, cellular function, proliferation, and differentiation.[1,2] Surprisingly, very few strategies have been implemented in hydrogels to recapitulate critical cell-cell interactions. While increasing cell packing density in hydrogels may provide sufficient cell-cell interactions, this simple strategy may not be practical due to the lack of an ample donor cell supply. We hypothesize that restoring critical cell-cell communication signals in synthetic hydrogels will enhance the survival and function of pancreatic β-cells. To demonstrate this, we utilized a natural cell-cell interaction found in pancreatic β-cells, namely EphA5-ephrinA5 binding,[3] to design biomimicry hydrogels. Thiolated EphA5-Fc receptor and ephrinA5-Fc ligand were immobilized in poly(ethylene glycol) (PEG) hydrogels via a thiol-acrylate photopolymerization. In addition to their crucial role in regulating insulin secretion, we also found that this unique binding enhances the survival of dispersedly encapsulated β-cells. The effects of cell adhesive peptides (RGDS) were also studied to reveal their synergistic effects on cell survival. This unique gel design employs a new strategy to tailor biomimetic extracellular microenvironments and may find broad applications in tissue engineering.

Methods: Cell encapsulation was achieved using photopolymerization in the presence of a cytocompatible photoinitiator (0.05wt% LAP,[4] UV: 365nm, 4mW/cm^2, 2 min). MIN6 β cells were encapsulated in 10wt% PEG diacrylate (mw: 10kDa) hydrogels at different packing density (5×10^6~2×10^7 cells/mL). At pre-determined time, survival of MIN6 cells was measured quantitatively by CellTiter Glo reagent, as well as qualitatively by live/dead staining and confocal microscopy imaging. Insulin secretion following static glucose challenge (2 and 25mM glucose) was quantified by insulin ELISA kits. Insulin secretion index was defined as insulin secretion at high glucose divided by insulin secretion at low glucose. To synthesize biomimetic hydrogels, EphA5 and ephrinA5 fusion proteins were first thiolated using Traut's reagent and conjugated in PEG hydrogels (in the presence of cells) via a thiol-acrylate reaction.[5] Viability and insulin secretion were measured as described above.

Results: We found that the survival of pancreatic MIN6 β-cells in un-modified PEG hydrogels increased when encapsulated at high cell packing density. A minimum packing density of 10^7 cells/mL was necessary to maintain the survival of encapsulated β-cells without the addition of cell adhesion ligands (e.g., RGDS). After 10-day culture, most cells did not survive when encapsulated at lower cell density (<6.7×10^6 cells/mL) (Fig. 1a). Higher cell packing density resulted in higher viability and better glucose responsive insulin secretion (Fig. 1b). When PEG gels were functionalized with fusion proteins, the survival of encapsulated MIN6 β-cells at low packing density (6.7×10^6 cells/mL) increased dose-dependently as a function of fusion protein concentrations (data not shown). The encapsulated β-cells, even at low cell density, not only survive in biomimetic hydrogels, but formed islet-like cell clusters after 21 days (Fig. 2).

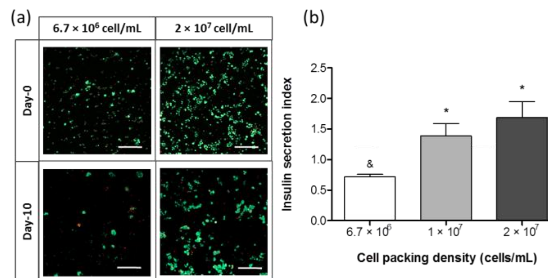

Fig. 1. (a) Images of live(green)/dead(red) staining of MIN6 β-cells encapsulated in PEG hydrogels (different cell packing density; Scale: 200μm) (b) Glucose responsive insulin secretion as a function of cell packing density in PEG hydrogels. (&: <1; *: >1; p<0.05)

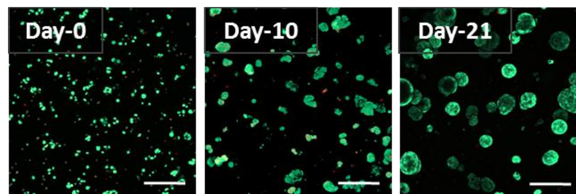

Fig. 2. Images of live/dead staining of MIN6 β-cells (packing density: 6.7×10^6 cells/mL) encapsulated in biomimetic PEG hydrogels immobilized with 200nM of EphA5 and ephrinA5 (1:1). (Scale: 200μm)

Conclusions: We found that increasing cell packing density provides important cell-cell contact cues and is sufficient to maintain cell survival and function in unmodified synthetic PEG hydrogels. Furthermore, a biomaterial strategy mimicking natural cell-cell interaction was used in PEG hydrogels to significantly enhance β-cell fate. This strategy may be useful in other applications in which cell-cell interactions are critical in directing cell fate processes.

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