JANUS: Bilayer Decellularized Collagen Matrix Supports Pancreatic β cells

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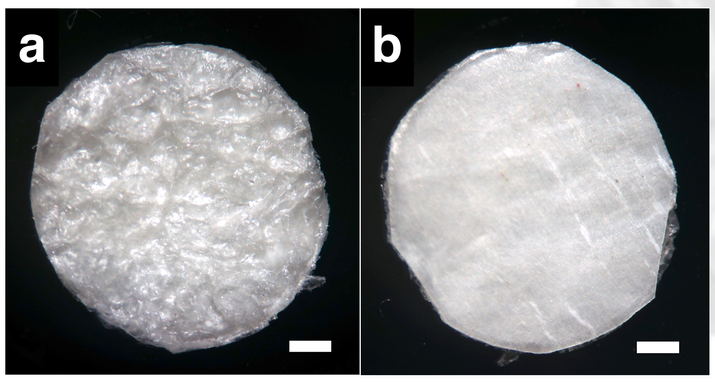
Abstract

Decellularized collagen matrix (DCM) refers to acellular tissue that retains collagen IV, the major components of islet microenvironment. DCM have been proved functional in islet transplantation into diabetes animals according to previous studies, but few passed long-term assay[1].

Here, we designed a new matrix, JANUS. With two differently oriented layers, JANUS is supposed to provide both porous environment for cluster forming and mechanical strength to support in vivo cultivation. This work explored physiological properties of the matrix, marked by the cell cluster morphology, cell metabolism level and cell's responsiveness to high glucose concentration. Results turned out that the matrix supported pancreas β cells's growth, giving license to future in vivo studies.

Introduction

Islet transplantation has been considered as a potential substitution for daily insulin injection. However, most direct transplantation trials in animal model failed, possibly due to immunological rejection, lack of nutritions and/or disruption of extracellular matrix of pancreas β cells which resulted in unstable islet clusters that cannot survive for long.



c Tensile elastic modulus (MPa)	Suture retention strength (N)	Indentation elastic modulus (MPa)	
		Upper layer	Basal layer
47.1±8.2	5.56±0.29	0.087±0.04	6.2±0.6

Figure 1 Macroscopic structure and mechanical properties of JANUS. Upper layer (a) derived from bovine tendon is porous and basal layer (b) derived from bovine pericardium is more compact. bar=10mm. c, mechanical properties (reproduced with permission, from Xi WANG)

Extracellular matrix (ECM) are major component of islets' microenvironment which provide both mechanical support and physiological cues. Since ECM is disrupted during islet isolation, we made use of decellularized tissue to mimic and restore the native environment of islet clusters.

We named this bilayer decellularized collagen matrix 'JANUS', since it couples hardness with softness as the god in ancient Roman myth.

Materials and Methods

Cell lines Rat insulinoma (INS-1)-cell line

Insulin secretion assay After pre-incubation, cells were stimulated sequentially with FMS plus 2.8 mM glucose and 16.7 mM glucose. Obtained buffers were sent to Beijing Northern company for insulin concentration through radioimmunoassay. Glucose stimulation index (SI) was calculated as[2]

$$SI = \frac{\text{insulin concentration at high glucose level}}{\text{insulin concentration at low glucose level}}$$

Results and Discussion

INS-1 cells have tendency to form islet-like clusters both in the matrix and on ultra-low attachment plates. These clusters gradually aggregated into a malformed 'supercluster' when cultured on ultra low attachment plates, which is possibly caused by the self-clustering properties of INS-1 and mechanical disturbance during culture changing. However, INS-1 clusters can maintain stable appearance and size for at least a week in the matrix (fig 2), suggesting that JANUS provide reliable protection to cell clusters and would probably function similarly in vivo, sheltering transplanted islet clusters from toss and turns.

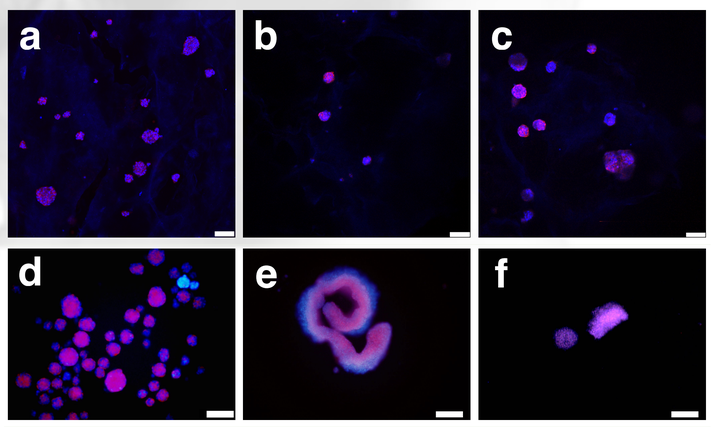


Figure 2 Morphology of pancreatic β cell clusters in the matrix (a-c) and on ultra-low attachment plate without matrix (d-f), observed 1 day (a,d), 4 days (b,e) and 7days (c,f) after seeding.blue: DAPI. red: Rhodamine-3. bar=100μm (a-d). bar=200μm(e-f)

To evaluate the function — the responsiveness to high glucose concentration of INS-1 clusters, we launched the glucose challenge assay after 2 days or 10 days after seeding. Although cells in the matrix did not show significant difference compared to those on the plate,

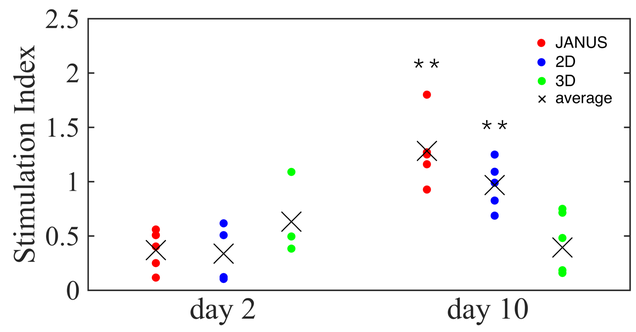


Figure 3 Glucose-induced acute insulin release from ins-1 clusters cultured on JANUS matrix, control dishes and ultra-low attach dishes. Stimulations were carried out after 2 days and 10 days of incubation. 2D: on common 96-well plates. 3D: on ultra-low attachment 96-well plates. **: $p < 0.01$, compared to day 2.

they subtly outperformed both control groups in the assay carried out 10 days after seeding, indicating that the 20,000 INS-1 cells in the matrix as a whole held increased responsiveness to high glucose concentration than those without matrix. This is possibly due to increased cell clusters retained in the matrix, or improved insulin expression and secretion in high-glucose environments. The exact reason will be further analyzed by normalizing to the total DNA content of cells retained in the matrix or on the plate.

Significance and Prospects

Although INS-1 cells are not really good candidates to replace islets in terms of transplantation, the research into INS-1 provide insight into islet transplantation, since INS-1 cell line share many similarities with pancreatic β cells (e.g., the responsiveness to physiologically high glucose concentration and a relatively high insulin content)[3]. The positive preliminary result obtained from INS-1 cells thus give license to further experiments using isolated islets and in turn, transplantation. The ultimate goal of this project is to see that JANUS provides support for islets in vivo and regulates the blood glucose level of Diabetes Mellitus animals in a long time range.

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Reference

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