Comparative analysis of reconstructed architectures from mice and human islets

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ABSTRACT

Intra-islet communication via electrical, paracrine and autocrine signals, is highly dependent on the organization of cells within the islets and is key for an adequate response to changes in blood glucose and other stimuli. In spite of the fact that relevant structural differences between mouse and human islet architectures have been described, the functional implications of these differences remain only partially understood. In this work, aiming to contribute to a better understanding of the relationship between structural and functional properties of pancreatic islets, we reconstructed human and mice islets in order to perform a structural comparison based on both morphologic and network-derived metrics. According to our results, human islets constitute a more efficient network from a connectivity viewpoint, mainly due to the higher proportion of heterotypic contacts between islet cells in comparison to mice islets.

Introduction

The optimal function of pancreatic islets relies on several processes at different levels of organization: from the mechanisms at the single cell level involved in the secretion of insulin, glucagon and somatostatin from β, α and δ cells, respectively, to intra-islet communication signals that, in conjunction with external regulation signals (i.e. endocrine, nutritional, neural, etc.), shape the release of islet hormones, key for the control of blood glucose homeostasis. Specifically, within the islets, α, β and δ cells regulate each other via direct electrical communication (between β cells and β and δ cells), as well as paracrine and autocrine signals, which are highly dependent on the composition and organization of islet cells (i.e. architecture). Given the fact that islet architecture is altered in type 2 diabetes, it is highly likely that intra-islet communication is consequently disturbed as a result of the disease.

Multiple studies have demonstrated that mouse and human islets differ in the composition and organization of α, β and δ cells. For instance, it has been shown that mice islets have more β cells than human islets (~75 vs 60%, respectively), and that human islets contain a higher proportion of α and δ cells (~30 and 10%, respectively), in comparison with mice islets (~20 and 5%, respectively). In addition, functional interspecies differences have also been described, mainly related to β cells, such as the different electrical behavior observed experimentally, the glucose threshold for the secretion of insulin, and the ionic channels expressed.

In spite of these advances, linking the structural and functional properties of pancreatic islets has not been an easy task, as several complex mechanisms are involved at different levels of organization. Moreover, the possibility that rodent and human islets are structurally and functionally different, limits the extrapolation of experimental observations between species.

Recently, a methodology based on computational optimization was proposed to reconstruct islet architectures from experimental data, giving as a result islets composed of non-overlapping cells, also allowing to quantify cell-to-cell contacts within the islets. Based on this methodology, in this article we reconstruct architectures of both mouse and human islets in order to perform a structural comparison between species based on common structural characteristics such as cell-to-cell contacts and islet volumes, but also on connectivity related...
metrics derived from the analysis of reconstructed architectures using a network-based approach. In addition, we evaluate the impact of β-cell loss in the islet connectivity and network properties.

**Material and methods**

**Reconstruction of islet architectures**

Human and mouse islets were reconstructed using the iterative optimization algorithm described in detail in a previous work. In short, the reconstruction algorithm consists of proposing an initial islet using the experimental nuclei coordinates as center coordinates of spherical cells with radii assigned randomly from reported experimental distributions. At each step of the iterative optimization algorithm, a cell is randomly selected and new center coordinates and radius are proposed for the selected cell. At each iteration the number of overlapped cells is calculated and compared to the minimum value obtained during the whole process. If the number of overlapped cells calculated is lower, the change in the cell radius and center coordinates is accepted; otherwise, it could be either accepted or rejected based on a monotonically decreasing probability as a way of preventing the algorithm from reaching a local minimum. This process is repeated until either convergence or a stop criterium is reached.

The reconstructed islets were provided in previous works by Hoang et al. (available under the terms of the Creative Commons Attribution License). In total, 56 mouse and human islets were reconstructed (n = 28 in both cases). Given that only the position and identity of α and β cells were reported, δ cells were not considered for the reconstruction process. Human islets were reconstructed using the radii distributions reported by Camunas-Soler et al. (β: 6.49 ± 1.6 μm, α: 5.04 ± 0.9 μm). On the other hand, the radii distributions of mouse islet cell given by Briant et al. were used to reconstruct the mouse islets (β: 6.9 ± 4.5 μm, α: 5.84 ± 3.7 μm).

A selection of the reconstructed human and mouse islets is shown in Figure 1. As described in Table 1, most of the reconstructed islets (52 of 56) included > 99% of the cells identified experimentally, with a considerable percentage (~41%, i.e. 23 islets) even reaching 100%. Only 4 mouse islets included a slightly lower percentage of the experimentally identified cells (~96%). These results demonstrate that the reconstruction algorithm is capable of reconstructing islets from different species in spite of the differences in cell size, composition and distribution of cells.

![Figure 1. Selection of reconstructed human (top) and mouse (bottom) islets.](image-url)
The computing time of the reconstruction of mouse and human islets heavily depended on the number of cells in the islet (see Table 1), ranging from ~32 minutes for an islet composed of 340 cells to more than 9 days, 9 hours and 15 minutes for a considerably larger islet (4159 cells). In terms of the number of iterations, at least $6.92 \times 10^6$ iterations were needed to fully reconstruct an islet, while the maximum number of iterations performed were $7.51 \times 10^7$. Details of the reconstruction process including the computing time and number of iterations needed for all the islets reconstructed can be consulted in Table 1. Loss of β-cell mass was simulated by randomly removing 50% of β-cells from the reconstructed islets.

**Construction and analysis of islet structural networks**

Undirected and unweighted networks were constructed using the cell-to-cell contacts identified during the reconstruction process, based on the assumption that cells are the network nodes and that cell-to-cell contacts are the network links. Two different structural networks were constructed for each islet analyzed: a network composed only of β cells (β-β network), and a network composed by both α and β cells (α-β network). An example of the β-β and α-β networks are shown in Figure 5(a). The resulting networks were therefore composed of $N$ nodes and $L$ links joining the nodes, with a maximum number of links given by $L_{\text{max}} = N(N-1)/2$. All the networks were characterized by the following network metrics:

(a) Average degree. Denoted by $\langle k \rangle$, it is the average number of links per node in the network. Given that $k_i$ is the degree of node $i$, defined as the number of neighbors or links of node $i$, the average degree of the network was calculated as:

$$\langle k \rangle = \frac{\sum k_i}{N} \quad (1)$$

(b) Density. Denoted by $d$, it is a measure of connectedness of the network, given by the ratio of actual cell-to-cell contacts in the network to all possible contacts.
\[ d = \frac{L}{L_{\text{max}}} = \frac{2L}{N(N-1)} = \langle k \rangle \quad \text{(2)} \]

(c) Average clustering coefficient. Interpreted as a measure of interconnection of the neighborhood of each node in the network, it was calculated as:

\[ C = \frac{\sum_{i\{k_i>1\}} C_i}{N_{k_i>1}}, \quad \text{(3)} \]

where \( C_i \) is the clustering coefficient of node \( i \), defined as the fraction of neighbors of node \( i \) that are connected to each other. Mathematically, \( C_i \) can be calculated as:

\[ C_i = \frac{\tau_i}{\tau_{\text{max}}} = \frac{2\tau_i}{k_i(k_i-1)}, \quad \text{(4)} \]

where \( \tau_i \) and \( \tau_{\text{max}} \) are the actual number of triangles including node \( i \) and the maximum number of possible triangles that could include node \( i \), respectively. Note that nodes with less than two neighbors (i.e. \( k_i < 2 \)) were excluded from the calculation.

(d) Global efficiency. Defining \( d_{ij} \) as the distance between any two nodes \( i \) and \( j \) (i.e. the number of edges in the shortest path between them), the efficiency between \( i \) and \( j \) can be defined as \( e_{ij} = 1/d_{ij} \) (for \( i \neq j \)). The global efficiency of a network is then given by the average of efficiencies over all the pairs of nodes:

\[ e_g = \frac{1}{n(n-1)} \sum_{i \neq j} e_{ij} \quad \text{(5)} \]

Note that when nodes \( i \) and \( j \) are not connected, \( d_{ij} = \infty \) and \( e_{ij} = 0 \). The global efficiency can be interpreted as a measure of integration of the network.

(e) Diameter. Given by the longest short path between all nodes in the network, it is used as a measure of size of the network.

(f) Largest component. When a network can be described in terms of disconnected subnetworks, the largest component of the network is simply the subnetwork containing the greatest number of nodes.

**Statistical analysis**

Statistical differences between human and mouse islets were assessed using the Student’s t-test for the interval/ratio variables that showed a normal distribution according to the results of the Kolmogorov-Smirnov normality test and visual inspection of the data and QQ plots. Differences between species in interval/ratio variables not showing a normal distribution were assessed using the Mann-Whitney test. For comparisons of categorical variables, the \( \chi^2 \) test with the Yates’ correction was used. In all cases, a \( P \)-value < .05 was considered to be statistically significant (* \( P < .05 \), ** \( P < .01 \), *** \( P < .001 \), **** \( P < .0001 \)). Results are reported as mean ± SD (standard deviation) and/or median ± IQR (interquartile range). Throughout the article, mean and median values are denoted as \( \bar{x} \) and \( x \), respectively, where \( x \) represent the variable of interest.

**Computational aspects**

Islet reconstruction was performed in the Yoltla cluster of the Laboratorio Nacional de Cómputo de Alto Desempeño (LANCAD) at the Universidad Autónoma Metropolitana, Iztapalapa, México City, México. Calculations were performed in Intel Xeon E5-2670 nodes (20 physical processors and 20 threads) with 64 GB DDR3 RAM memory. Code was written in C using the OpenMP library. Postprocessing and visualization of the optimized islets were performed in Wolfram Mathematica 12.0 (Champaign, IL). Statistical analysis was performed in Prism version 9.0.0 for Mac OS (GraphPad Software, San Diego, California USA). Network analysis was performed in Python 3.7 using NetworkX.\textsuperscript{35}

**Results**

**Populations and volumes of \( \alpha \) and \( \beta \) cells in reconstructed islets**

In agreement with a previous report by Hoang et al.,\textsuperscript{22} mouse islets were composed of a greater number of cells than human islets (Figure 2(a)). Human islets had more \( \alpha \) cells than mouse islets, with minimum and maximum number of \( \alpha \) cells of 63 and 1162 in human islets and 19 and 469 in mouse
islets (Figure 2(b)). In contrast, mouse islets had more β cells than human islets, with the number of β cells ranging from 257 to 1931 in human islets and from 498 to 3876 in mouse islets (Figure 2(c)).

Table 2. Basic characteristics of human and mice islets.

|               | Number of cells | Radii | Volume |
|---------------|-----------------|-------|--------|
|               | N₀ (%) | N₁ (%) | Total (%) | r₀ (µm) | r₁ (µm) | r₀ (µm) | r₁ (µm) | n = 10879 | n = 27609 | n = 38488 |
| Human         | 10879 (28.3) | 27609 (71.7) | 38488 (100) | Mean (SD) | 4.8 (0.4) | 5.3 (0.7) | Mean (SD) | 2.5 (1.4) | 9.3 (5.1) | 11.7 (6.5) |
| Mouse         | 3637 (6.7) | 50798 (93.3) | 54435 (100) | Median (IQR) | 4.7 (0.6) | 5.1 (0.9) | Median (IQR) | 2.5 (2.1) | 9.3 (3.5) | 11.8 (11.6) |
|               | Mean (SD) | 5.2 (0.9) | 5.2 (0.7) | Median (IQR) | 1.2 (0.6) | 16.5 (10.6) | Median (IQR) | 1.0 (1.1) | 14.5 (20.2) | 15.6 (21.3) |
|               | 4.9 (1.0) | 5.0 (1.0) | t = 4.45 (df = 37.36) | t = 3.24 | t = 2.42 |
|               | U = 1.77 | U = 6.68 | (df = 38.73) | (df = 43.11) |
The percentages of α and β cells differed between species. In human islets, 28.3% of islet cells were α cells and 71.7% were β cells. In contrast, in mouse islets, only 6.7% were α cells while 93.3% were β cells (see Table 2 and Figure 2(d)).

The median values of the radii of α cells in the reconstructed human and mouse islets were $\bar{r}_{\alpha, h} = 4.74$ μm and $\bar{r}_{\alpha, m} = 4.87$ μm, respectively (Figure 2(e)). Similarly, the median radii of β cells were $\bar{r}_{\beta, h} = 5.09$ μm and $\bar{r}_{\beta, m} = 4.97$ μm in human and mouse islets, respectively (Figure 2(f)). It should be noted that, although statistically significant, the radii of α and β cells in human and mouse islets (as well as the mean values presented in Table 2) were extremely similar, even though the experimentally derived distributions used in the reconstruction process were different. In terms of cell volumes, mouse islets had a higher average volume when compared to human islets (Figure 2(g)). The mean volume of α cells was higher in human islets (Figure 2(h)). In contrast, mouse islets had a higher β cell volume than human islets (Figure 2(i)). These results suggest that volume differences between human and mouse islets are most likely due to the differences in the number of cells and not because of differences in cell size. Statistical details about the number, proportions, cell’s radii and islet volumes can be consulted in Table 2.

### Table 3. Cell-to-cell contact information obtained from the reconstruction process of human and mice islets

|        | N$_{\alpha\alpha}$ (%) | N$_{\alpha\beta}$ (%) | N$_{\beta\beta}$ (%) | Homotypic (%) | Heterotypic (%) | Total (%) |
|--------|------------------------|-----------------------|----------------------|---------------|----------------|-----------|
| Human  | 10388 (18.2)           | 13733 (24.1)          | 32819 (57.6)         | 43207 (75.88) | 13733 (24.12) | 56940 (100) |
| Mouse  | 1228 (1.9)             | 5436 (8.6)            | 56672 (89.5)         | 57900 (91.42) | 5436 (8.58)   | 63336 (100) |
| Chi-square (df = 1) |                        |                       |                      |               | 5400          |           |
| P value| <0.0001                |                       |                      |               | <0.0001       |           |

N$_{\alpha\alpha}$: α-α contacts, N$_{\beta\beta}$: β-β contacts, N$_{\alpha\beta}$: α-β contacts.
**Cell-to-cell contacts**

As mentioned before, given that the analyzed islets were only composed of α and β cells, only α-α, β-β and α-β contacts were considered (Table 3). No differences in the average total number of cell-to-cell contacts were found between human and mouse islets (Figure 3(a)). Human islets had significantly more α-α contacts than mouse islets (Figure 3(d)). In contrast, mouse islets had more β-β contacts than human islets (Figure 3(e)). On the other hand, human islets had a higher number of α-β contacts in comparison to mouse islets (Figure 3(c)). Overall, as a percentage of total contacts, the percentages of α-α, β-β and α-β contacts varied between human and mouse islets (Figure 3(f)). In human islets, 18.24% of total were α-α contacts, 57.6% β-β contacts and 24.1% α-β contacts. In contrast, in mouse islets only 1.9% were α-α contacts, 89.5% were β-β contacts and 8.6% were α-β contacts. As illustrated in Figures 3(b) and (c) (see also Table 3), the number of heterotypic contacts differed between species, being greater in human islets, while the number of homotypic contacts did not differ significantly. On the other hand, there were differences in the percentages of homotypic (i.e. α-α and β-β) and heterotypic (α-β) contacts between human and mouse islets, being homotypic 91.4% of contacts in mouse islets and 75.9% in human islets, while 8.6% and 24.1% were heterotypic, respectively (see Table 3 and Figure 3(f)). According to our results, the great majority of homotypic contacts in mouse islets are β-β contacts, which is reasonable if we consider that, as described above, α cells only represent 6.7% of cells in the reconstructed mice islets. In contrast, in human islets both α-α and β-β contacts contributed considerably to the count of homotypic contacts, reflecting the much higher percentage of α cells present in human islets.

**Structural networks**

**Networks of β cells (β-β)**

As described in Table 4, where the network metrics are summarized, there were differences between human and mouse islets in both the average degree and clustering coefficient of the β-β network (Figure 4(a and c)), although in both cases, the mean and median values were barely different (see Table 4). In contrast, there were not differences between species in the density, diameter and global efficiency of the β-β network (Figure 4(b, d, e)). This results suggest that the connectivity between β-cells is marginally higher in mouse islets as a result of the higher proportion of β-cells and β-β contacts (see Figures 2(c and e)). However, given the fact that the density, diameter and global efficiency was similar in both species, it is reasonable to conclude that networks of β-cells in mouse and human islets are similar from a topological viewpoint.

**Table 4. Network metrics calculated from β – β and α – β networks in human and mice islets.**

| Network | Degree | Density | Clustering | Diameter | Efficiency |
|---------|--------|---------|------------|----------|------------|
|         | α – β  |         | α – β      | β – β    | α – β      | β – β    | α – β      | β – β    | α – β      |
| Human   | 1.7 (1.6) | 3.0 (1.6) | 1.7E-3 (1.2E-3) | 2.8E-3 (1.6E-3) | 0.08 (0.2) | 0.1 (0.2) | 49.1 (15.9) | 34.3 (5.3) | 0.03 (0.02) | 0.08 (0.02) |
| Mouse   | 2.0 (2.0) | 2.0 (2.0) | 1.3E-3 (1.6E-3) | 2.1E-3 (2.4E-3) | 0.0 (1.0) | 0.0 (0.2) | 46.5 (17.5) | 35.0 (7.75) | 0.02 (0.03) | 0.08 (0.02) |
|         |         |         |           | Mean (SD) | Median (IQR) | Mean (SD) | Median (IQR) |        |           |
| n       | 38488  | 28      | 38488      | 28       |             |           |             |        |           |
| U       | 968    | 868     | 329        | 175      | 169         | 968      | 349         | 147.5   | 390        | 130        |
| P-value | <0.0001 | <0.0001 | ns         | <0.0001 | <0.0001     | ns       | <0.0001     | ns      | <0.0001    |
Networks of α and β cells (α-β)

As also described in Table 4, when α cells were added to the networks of β cells, both the average degree and density increased in both species as can be appreciated when Figure 4(a, b, f and g) are compared. It is worth noting that the α-β network of mouse islets showed a lower density when compared to that of human islets, which can be attributed to the fact that, on average, mouse islets had a higher number of cells, while the average number of total contacts between species showed no significant differences. On the other hand, the average clustering coefficient showed only a slight increment in both species (~25% in humans and ~10% in mice). Interestingly, the diameter of the networks in human islets decreased ~30%, while in mice, on the contrary, the diameter increased slightly (~10%). Moreover, the global efficiency of the α-β networks in human islets increased threefold, while in mice islets there was only a modest increase of 33%. These results indicate that the greater proportion of α cells in human islets generates more efficient networks by increasing

Figure 4. Figure 4. Network metrics calculated from the corresponding β–β (A–E) and α–β (F–J) networks in mice and human islets.

Figure 5. Componentes-02-02.tif.
the average number of connections in the network and reducing the path lengths between the network’s nodes.

The islet networks were composed of several subnetworks or components disconnected from each other (see Figure 5(a)). As depicted in Figure 5(b), there were not differences between the percentage of cells in the largest component of β-β networks from mice and human islets. Notably, when considering the α-β networks, the percentage of cells in the largest component increased considerably only in human islets (Figure 5(b)) while, according to our results, there were not differences between the largest components of the β-β and α-β networks in mouse islets. As shown in Figure 5(b), it is clear that the largest component in the α-β network in human islets is composed by a much larger percentage of cells than in mouse islets due to the considerable higher number of α-β contacts in human islets.

Effects of the loss of β-cells on cell-to-cell contacts and network metrics

When 50% of β-cells was removed from the reconstructed islets (see the top panels of Figure 6(a) and (b)), the number of β-β and α-β contacts, as well as the total contacts decreased considerably in both species (Figure 7(a) and (b)). Specifically, the number of β-β contacts dropped by ~75% both in human and mouse islets. In contrast, the number of α-β contacts decreased ~50 and 19% in mouse and human islets, respectively. Similarly, the total contacts decreased ~55 and 71% in human and mouse islets, respectively.

The loss of β-cells, and as a consequence, of cell-to-cell contacts led to a corresponding decrease in all the network metrics (average degree, density, average clustering coefficient and global efficiency), as shown in Figure 7(c) and (d). In particular, the average degree dropped from 3 ± 1.6 to 1 ± 1 in human islets, and from 2.3 ± 1.5 to 0.7 ± 0.8 in mouse islets. Similarly, the density of the networks decreased from 2.8E-3 ± 1.6E-3 to 1.5E-3 ± 0.9E-3, and from 1.5E-3 ± 0.9E-3 to 0.8E-3 ± 0.5E-3 in human and mouse islets, respectively. As expected, the global efficiency of the networks was severely affected, decreasing from 0.08 ± 0.02 to 3.8E-3 ± 3E-3 in human islets and from 0.04 ± 0.03 to 1.3E-3 ± 0.9E-3 in mouse islets. Likewise, the diameters were also affected, changing from 34.3 ± 6–3 to 17.71 ± 10.57 in human islets and from 54.7 ± 20.2 to 8 ± 4.7 in mouse islets. The impact of the loss β-cells can be clearly observed.

Figure 6. Example visualizations of the simulated loss of 50% β-cells in reconstructed human (A) and mouse (B) islets. The corresponding islet networks are shown below each islet.
in the graphical representation of typical networks of normal and perturbed islets shown in the bottom panels of Figure 6(a) and (b).

Discussion

In this article, we reconstructed human and mouse islets and performed a structural comparison using typical metrics such as volumes and proportion of cells, but also metrics obtained from the analysis of islet-derived networks.

We showed that the reconstruction algorithm used (for details see reference 31) is capable of satisfactorily reconstructing islets from different species despite the differences in radii distributions and proportions of cells. Notably, in most cases, the reconstructed islets included >99% experimentally identified cells.

According to our results, mouse islets are generally larger than human islets in terms of both the number of cells (as previously described by Hoang et al.22) and cell volume. In addition, the proportion of α and β cells differed considerably between the two species, being the proportion of β cells larger in mouse islets and the proportion of α cells larger in human islets, as it has been previously reported.14,19–24 Interestingly, despite the differences in islet size, the number of total cell-to-cell contacts was not statistically different, although there were in fact important differences in the
and corroborated in human islets in order to determine the functional implications of the structural differences between species.

While it is still difficult to determine a definitive relationship between the network metrics and the functional properties of the islets, it is reasonable to hypothesize that the network metrics could serve as indicators of the islet capability to exhibit an organized response, as they provide us with a quantitative measure of local (e.g. degree, clustering coefficient) and global properties (e.g. density, global efficiency and diameter) of the islet connectivity network. For instance, a decrease in the average degree as a consequence of the loss of β-cells could be indicating that regulatory inputs from neighbor cells might be missing as a result, and therefore local coordination and/or regulation could be impaired. Similarly, a decrease in the density, diameter and global efficiency of the islet network could indicate that the transmission of stimulatory and/or inhibitory signals from one islet region to another could be also impaired as a consequence of a reduced intrasilet connectivity.

In this work, we have used network analysis to characterize the structural networks in mouse and human islets, and therefore, functional aspects were not explicitly considered. In spite of this, we believe that the use of network-derived metrics in conjunction with morphological indicators will contribute to elucidate the relationship between the structural and functional properties of pancreatic islets, and eventually, to contribute to gain a better understanding of islet dysfunction in pathological conditions such as type 1 or 2 diabetes.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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