INTRODUCTION

Pancreatic islet transplantation is emerging as a therapeutic approach for patients affected by diabetes. This technique has been proven successful, but limitations have been identified. One of the major challenges of the process is the counting of the isolated pancreatic islets, which is currently performed by subluxation and macropatterson. Determination of the accurate islet number is a crucial factor in determining the correlation between the islet product and clinical outcome. In the presented study, we have developed software capable of objectively evaluating islet numbers and other viability variables by image analysis. This software is based on image processing and the image according to the objectives of the area of interest. This is the first step toward standardization of the isolation outcome and potential clinical success predictability.

BACKGROUND

Pancreatic islet transplantation has been proven successful but is still experimental due to its lack of standardization and cost representativeness. Major hurdles have been identified in the multiple steps of the procedure, which has re-opened investigative avenues that can benefit from intravascular technologies. Glucose-independent apoptosis is dependent upon the ability to achieve a high yield and purity of islets isolated from a human pancreas, obtained from cadaver donors. Pancreatic islets are isolated using a modification of the semi-automated method as previously described (1). Isolated islets are stained with DTZ and counted by an operator on a representative sample using an inverted microscope. The count that takes into consideration the different sizes with the use of a graduated microscope ocular, is then normalized using mathematical conversion to Islet Equivalents (IEq, units of an average diameter of 355 microns). The final preparation is then transplanted into the liver of diabetic patients by percutaneous cannulation of the portal vein. At the present time, counting is subjective and inaccurate and cannot be validated in vivo. Real image quality assessment (number, purity and preservation) is crucial to determine the relationship between isolation and clinical outcome and move forward in standardizing the procedure.

AIM

The proposed study aims to develop and test the effectiveness of digital image analysis to evaluate isolated islet samples of isolation and establish a correlation with clinical outcomes. In order to achieve this goal we have developed software capable of objectively evaluating the variables by image analysis.

MATERIALS & METHODS

Image processing consists of performing mathematical calculations on a digital input image to generate an output which can be an image with different characteristics suitable for subsequent feature extraction. The image analysis procedure was performed as follows:

1. Preprocessing: includes image framing, noise reduction, and background and region of interest extraction.
2. Feature extraction: involves the isolation of features of interest, such as size, shape, and texture.
3. Feature selection: involves the selection of the most relevant features for the analysis.
4. Feature classification: involves the classification of the selected features into different categories.

Our analysis began with a preliminary extraction of islets attained by DTZ staining (fig. a). The extraction of the regions with a specific color is then essential for the preliminary capture of the features. The color thresholding filter is our first tool to be processed with the image extraction. The filter receives the determination of a histogram range, desired by a low and high threshold, for color extraction. The color extraction is determined by predefining a range of colors on a palette. The colors are then extracted on the basis of their color rather than intensity (fig. b). According to the process, all thresholds must be properly established. However, the result obtained is typically affected by sparse residual pixels which do not match to features but have similar chromatic characteristics and must be removed. Statistical averaging schemes were adopted to remove noise while keeping the integrity of the image information obtained (fig. c). Light intensity thresholding was applied in order to obtain a binary image at a low and high light intensity level. By selecting image intensity as the thresholding parameter, color information is discarded and the image is translated into standard grey-scale format. In order to perform such segmentation, a proper intensity threshold was selected manually; however, automated methods may also be employed (2). As a result of the thresholding process, a Black and White (BW) image (fig. d) is finally obtained.

RESULTS

Feature extraction is the process to identify the regions of interest (ROIs) within an image. The proposed method requires the preliminary extraction of the region of interest (ROI). This is commonly achieved by selecting a predetermined region within which the feature is located. In such cases the border of the region of interest is not always very well defined. The region of interest (ROI) is defined as the set of pixels belonging to the rectangular and non-rectangular areas which are located above the top-left corner (x1,y1) and below the bottom-right corner (x2,y2) where the following conditions are satisfied:

\[
\begin{align*}
\text{ROI} &= \{(x,y) | x1 \leq x \leq x2, y1 \leq y \leq y2\} \\
\end{align*}
\]

Where (x,y) are the coordinates of the initial feature point found during the scanning process, and the ROI threshold, is the maximum number of feature pixels that can be found in a separating border.

Once the whole feature has been framed into the rectangular region (R), the rectangular area is then masked and the processed image surface is calculated by:

\[
\text{ROI} = \sum_{(x,y) \in R} \text{ROI}_i
\]

The scan process requires sequential repeating of this process until the whole image is processed.

This above discussed procedure aims to automatically determine the amount and the size of islets of Langerhans in a microscopic image. Effectiveness of the proposed method is confirmed by the preliminary results carried out on test images, although a deep investigation is necessary in order to measure the robustness and reliability of the approach on a substantial number of samples. The following figure (fig. e) gives the result on a complex image containing approx. 170 islets, obtained via an optimized microscope setup in the acquisition process. In more detail, fig. f is the acquired digitized image; fig. g is the output of the image processing phase, and fig. h is the final result of the feature extraction process. The image represents a highly fragmented feature distribution which is typically difficult to process for classical feature extraction algorithms, and consequently time consuming for professional experts. Time required for computation was approximately three seconds on a 2-3gtol core due to 2 g of RAM processing (RAM temps of 1300 X 900 with 70 dp) resolution. Computational times depend upon the number and density of features. With the approach proposed, a feature map of the image was judged satisfactory by the experts, thus confirming the helpfulness of the proposed system.

CONCLUSIONS

Digital image analysis has been proposed here as a new technology for assessing the amount of islets in a microscopic image of the sample of isolated pancreatic islets. Standardization of the information is fundamental to provide clinical outcome. The main goal achieved in our study is the establishment of a methodology for fully automated feature extraction of microscopic images. The methodology here proposed, however, involves several thresholds and operational parameters which ultimately determine the quality of the results. Proper optimization of such parameters should be carried out. In addition, the reliability and robustness of the proposed system should be validated on a proper number of samples and compared the results with the traditional manual process. Further investigations are required. The approach proposed bring significant innovation to the evaluation and prediction of clinical outcome precision, since it allows fast, accurate, and standardized evaluation of the isolated number and size. Additionally, the system is capable of providing a quick and accurate evaluation of the image, allowing the capability of measuring deviation from multiple components like fatigue, bias, interpretation and reproducibility. Finally, the automation and fast delivery of our method allows testing of multiple complex with better estimation of the success probability. The possibility of implementing additional indicators instead, for example in the shape and size of features, may also be analyzed with criteria to be employed within structured multi-criteria evaluation techniques for measuring the likelihood of transplant success. We believe this initiative opens a new area of research that can benefit the islet evaluation process and the entire islet transplant field.

REFERENCES