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# Role of Tissue Dissociation Enzymes on Human Islet Yield Intended for Clinical Islet Cell Transplantation

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## Introduction

Clinical islet transplantation is a well-established and a relatively safe procedure for diabetic patients, whereas autologous islet transplantation is performed as an adjunctive therapy following a total or partial pancreatectomy from patients with chronic pancreatitis. Successful clinical human allo or auto-islet transplantation requires recovery of a sufficient number of functional islets from cadaveric or chronic pancreatitis pancreata. During the last two decades, significant progress has been made in the islet isolation procedures and in the use of tissue dissociation enzymes. However, it is still challenging to recover all available islets from pancreas. In many centers, about 50% of the islet isolations do not generate a sufficient number of islets for single donor transplants. Poor islet recovery remains, a critical issue which must be addressed so to achieve widespread adoption of islet transplantation. This review will present information focusing on role of tissue dissociation enzymes and their properties during islet isolation, particularly emphasizing on the current developments within the field.

## Bacterial Collagenase from *Clostridium histolyticum*

Historically, it has been demonstrated that utilizing a precise, collagenase containing enzyme blend for pancreatic islet isolation is a critical parameter for successful islet transplantation.

Collagenase is an endopeptidase enzyme produced from the fermentation broth of *Clostridium histolyticum*. By definition, collagenase has a unique specificity since it cuts the triple helical structure of native collagen. Proteases are ineffective at degrading collagen's triple helix. Collagens are the major component of the connective tissue extracellular matrix (ECM). The biological and enzymatic properties of *C. histolyticum* collagenase are well documented [1]. Collagenase is produced by two distinct genes, *colG* and *colH*, coding for Class I (C1) and Class II (C2) collagenases having molecular weights of 116 kDa and 114 kDa, respectively [2]. C1 and C2 collagenases appear to digest all forms of collagen since the triple helical structure is a key component of these molecules. The C1 and C2 enzymes work synergistically to hydrolyze these helical structures into smaller peptides [3].

Collagenase was first commercially available from Worthington Biochemical Corporation in 1959. By 1970's many other suppliers manufactured a "crude collagenase" produced from *C. histolyticum*, which contained many other enzymes such as collagenase, phospholipase, clostripain, elastase, aminopeptidase, galactosidase, and other proteases [4]. This crude product was widely used to isolate specific cell types from different tissue sources such as pancreas [5], liver [6], cartilage [7], heart [8], adipose [9], and bone [10].

Until the 1990's, pancreatic islet isolation was processed using the crude or enriched collagenase [11]. The major hurdles of using these products for islet isolation were lot to lot variability of enzyme activity and high endotoxin content. To overcome these issues, a purified enzyme blend from Boehringer Mannheim Biochemicals (now sold by Roche), "Liberase-HI Purified Enzyme Blend" was introduced in 1994. This enzyme was provided as one vial with a fixed enzyme dose. It contained C1/C2 collagenase from *C. histolyticum* and thermolysin, a thermostable neutral protease from *Bacillus thermolyticus* rokko, and low endotoxin contamination. Introducing this enzyme in the market immediately improved islet yields from clinical islet isolation procedures [12]. As a result, crude enzyme usage declined for human islet isolation. However, purification of collagenase did not eliminate batch variability because many laboratories obtained inconsistent islet isolation results.

## Class I and Class II Collagenases

The purified enzymes blend currently used for islet isolation comprise of class I and class II collagenases. The C1 and C2 collagenases work synergistically to degrade native collagen [13]. The ratio between C1 and C2 collagenases is important for optimal islet isolation in rat and human pancreas [14,15]. C1 is more stable and has a greater activity towards native collagen, whereas C2 has the ability to digest a broader range of peptide substrates when compared to C1. Among the three well-known enzyme suppliers (VitaCyte, Serva, and Roche), only the VitaCyte and Roche C1/C2 collagenase ratio is known (60:40) [16]. Our previous studies have demonstrated that different C1/C2 ratios and doses of purified collagenase, when tested for human islet isolation, had no significant effect on digestion and islet yield [17].

## Role of Proteases in Islet Isolation

Collagenase and proteases are required for successful islet isolation. Purified collagenase lack proteases which necessitates the addition of proteases that complement the collagenase activity, leading to successful islet recovery. Neutral protease from *C. histolyticum* and thermolysin have been extensively used in clinical islet isolations. In addition, BP protease ('dispase' equivalent enzyme) from *Paneibacillus polymyxa* has also successfully been used for human islet isolation [17].

## Collagenase Enzyme Activity Assessment

Currently, clinical islet isolation enzyme suppliers (VitaCyte, Serva/Nordmark, and Roche), express their enzyme (collagenase) activity in 'Wunsch units'. The Wunsch and FALGPA assays use a peptide substrate that preferentially detects collagenase activity, these peptidase assays primarily detect C2 activity and assess only the function of the catalytic domain. In contrast, the true functional collagenase activity is detected by using a native collagen substrate. VitaCyte's fluorescent microplate collagen degradation activity (CDA) assay measures enzyme that contain a catalytic domain and a collagen binding domain. The collagenase must first securely bind to native collagen before the catalytic domain in the same molecule can cut collagen's triple helical structure. Treatment of collagenase with chymotrypsin leads to removal of collagen binding domains and loss of nearly all the CDA activity with minimal change in the Wunsch activity. Studies at VitaCyte has shown the specific CDA activity of C1116 kDa is about 7-8 times higher than the C1100 kDa or C2114 kDa forms of collagenase [28].

## Clinical Islet Isolation Enzymes

In 2007, the purified enzyme Liberase-HI, was withdrawn from the market because of safety concerns associated with the use of bovine brain-derived materials during the fermentation process. Identifying a replacement enzyme blend became imperative for successful continuation of clinical islet transplant programs across the globe [18]. During that time, the only available enzyme for clinical isolation was Serva/Nordmark collagenase NB1 [19]. The purified clinical grade collagenase and neutral protease enzymes were supplied in independent vials. Left with no alternatives, many centers took time to standardize and establish isolation protocols with these enzymes, which gave lower islet yields that led to fewer islet transplantation procedures [18,20].

Eventually, Szot et al. [2009] in used the Serva/Nordmark enzyme to isolate islets and use a high percentage of these preparations for clinical allo-transplantation [21]. As part of the islet group at the University of Minnesota, we also reported that the same enzyme could be successfully utilized for autologous islet isolation from chronic pancreatitis pancreata. The results from autologous islet isolation using the Collagenase NB1 were comparable with traditional Liberase-HI in terms of islet yield and clinical outcome [22].

Purified tissue dissociation enzymes (TDEs) are critical to successful human islet isolation, but little was known about the key enzymes-class I (C1) and class II (C2) collagenase characteristics. We were the first group to demonstrate differences between the C1 collagenase found in purified collagenase products manufactured by three main suppliers and evaluated the impact of C1 differences between two suppliers on human islet yield. Our results indicated the importance of intact C1 collagenase, necessary for successful human islet isolation and transplantation [23].

Kin et al. [2009] improved the islet isolation outcome by non-simultaneous administration of collagenase and protease [24]. Furthermore, Brandhorst et al. [2009] performed a detailed enzyme activity analyses for the current enzyme lots [25] and they determined the high tryptic-like activity as a key parameter for the functioning of pancreatic dissociation enzymes. In order to identify suitable enzyme combinations, we also evaluated many different enzyme products containing different levels of intact or truncated collagenases, used in combination with thermolysin or neutral protease. We incorporated biochemical analysis of the collagenase enzymes as part of our evaluation criteria and identified a new blend containing intact class I (C1)/class II (C2) collagenase and neutral protease, both from *C. histolyticum*, and called it the new enzyme mixture (NEM). Isolations with our NEM were a lot more effective than any other enzyme combination, recovering consistently high yields of quality islets from human pancreata [23]. Additionally, clinical autologous and allo-transplantations with islets isolated from the NEM achieved greater success [23].

During this course of enzyme standardization, Roche introduced Liberase MTF, a new preparation of, purified collagenase and purified thermolysin. HPLC analysis confirmed that their product had intact C1 collagenase. This enzyme was successfully utilized for clinical islet isolations [26,27].

Over the years, the trend has been to utilize collagenase with a protease as an enzyme combination to improve islet yield. In the last decade (2007-2016) particularly, three different enzyme combinations (from Serva, Vitacyte, and Roche) have been implemented for clinical preparations. Though all these enzymes were successfully utilized for processing clinical isolations, only an average of 50% have yielded sufficient islet numbers for transplantation [20].

Despite successful utilization of enzyme combinations from different providers, one of the persistent challenges which requires immediate attention is the variability in islet yield. Several factors contribute to this variability. Total islet number within whole pancreas has not yet been determined. In this context, further optimization of the enzyme type or dose and better understanding of the extracellular matrix of human pancreas, is required for improving islet yield.

## Conclusion

Collagen is the most abundant protein within human pancreas. Human donor's differ tissue characteristics due to genetic and environmental factors. Critical analysis of how collagenase and protease enzymes may act on known and novel tissue substrates, present within the ECM, for better tissue dissociation is subject to further investigation. In this context, we recently introduced a recombinant collagenase enzyme which demonstrated better outcomes when compared to previously used enzyme combinations [17]. Further evaluation is necessary to optimize enzyme doses and combinations to reach a degree of consensus. An enzyme blend which core islet facilities across the world can utilize as a consensus mixture would be ideal, this becoming one of the ultimate goals within clinical islet research requiring collaboration between islet centers and core facilities, enzyme companies and clinicians.

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