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Exocrine damage leads to a loss of beta cell function in a zebrafish model

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Abstract

Type 1 diabetes (T1D) is a chronic autoimmune disease affecting the beta cells in the endocrine pancreas, impairing insulin production. Recent T1D research has taken a turn towards the role of the exocrine pancreas in the disease. We focused on the effects of exocrine damage on beta cell function in a zebrafish model (*Danio rerio*). Previous research has found correlations between exocrine dysfunction and T1D, but a causal relationship of one leading to the other has yet to be illustrated. We visualised the beta cells by staining insulin using indirect immunofluorescence. Other antigens have also been tested, such as elastase, glucagon, amylase, trypsin and L-plastin. We optimised the existing protocol and found that methanol fixation is a necessary step in whole-mount immunostaining. An exocrine damage model was made by using a double transgenic zebrafish line expressing nitroreductase (NTR) in the exocrine pancreas, which reduces nifurpirinol (NFP) to cytotoxic products. The functional beta cell count was quantified and we found that exocrine damage affects the function of beta cells in this zebrafish model. This finding is significant for T1D research as it brings us one step closer to finding a cause related to the exocrine pancreas or developing a preventative treatment.

Introduction

In a world where the incidence of type 1 diabetes (T1D) is rising, researchers are searching for both its root causes and new treatment options. T1D is described as an autoimmune disease that affects the beta cells in the islet of Langerhans, the endocrine pancreas. As a result, insulin is not produced when needed. This leads to hyperglycemia, a hallmark symptom of the disease, along with many other complications. T1D incidence has over the years been rising by 3 to 4% per year, with Northern European countries such as Finland and Sweden at the top (Tuomilehto et al., 2020). Since the discovery of insulin in 1922, many analogues have been developed and insulin injections remain the prime therapy for T1D today. However, despite such effective treatments and the rising incidence, the root cause of the inflammation that triggers T1D is actually unknown. It is thought to be a complex phenomenon, including genetic and environmental factors.

Recent research has demonstrated that the beta cells, though the main actors in the story, are not the only type of cell that may be affected in T1D. The exocrine pancreas has shown to be involved in T1D, demonstrated to have a smaller volume and mass than in healthy individuals. Next to this disparity, a higher number of active immune cells can be found in the exocrine pancreas, and abnormalities such as pancreatic exocrine insufficiency (PEI) are more common among type 1 diabetics (Foster et al., 2020, Alexandre-Heymann et al., 2019). However, it is not known what effects exocrine damage may have on the beta cells directly. Lastly, the beta cells themselves may play a lead role in the development of T1D, instead of just being the victim of the autoimmune response. In the past few years, more attention has been drawn to the endoplasmic reticulum (ER), an essential organelle for proper beta cell functioning. When ER stress is significant and the unfolded protein response (UPR) seems out of control, this may contribute to the pathogenesis of T1D (Sahin et al., 2021). These responses could in part be attributed to oxidative stress (Eguchi et al., 2021, Leenders et al., 2021).

One model that allows for microscopic visualisation of the whole pancreas is the zebrafish. Transgenic zebrafish are often used to achieve ablation of specific organs, including the endocrine and exocrine pancreas. A known system for this is cell ablation using nitroreductase (NTR), which is expressed under a cell-specific promoter and reduces a prodrug such as metronidazole (MTZ), a nitroaromatic antibiotic, into cytotoxic products. A compound tested on the zebrafish pancreas that is more effective than MTZ due to a considerably lower toxic effect, is nifurpirinol (NFP), another nitroaromatic antibiotic. That the beta cells of the endocrine pancreas have the ability to regenerate after chemical or surgical ablation (Moss et al., 2009), which has also been confirmed after ablation by NFP (Bergemann et al., 2018).
Earlier studies have shown that zebrafish are also a fitting model for studying the exocrine pancreas (Wan et al., 2006). Not only are zebrafish cheap and relatively non time-invasive to work with, they are also transparent as embryos (Teame et al., 2019). When 1-phenyl 2-thiourea (PTU) is added to the growth medium between 1 and 4 days post-fertilisation (dpf), the zebrafish will stay transparent, making them excellent models for microscopy (Westerfield M, 2007). Due to the rapid development of zebrafish, it takes only a few days to use their larvae for imaging. At 5 dpf, already a fully developed pancreas can be observed with one islet of Langerhans (Tiso et al., 2009). Histologically, zebrafish larvae have a similar pancreas to humans, and are therefore excellent models for diabetes research and other kinds of research on the pancreas. Another advantage of zebrafish larvae is that until 120 hours post-fertilisation (hpf), they are excluded from the European Union's animal experimentation regulations (Strähle et al., 2012).
There are different microscopy techniques that can be used to image zebrafish. When working with fluorescent markers, confocal laser-scanning microscopy (CLSM) is a favourable technique. Due to the pinholes that block light outside of the area of focus, not only is the resolution better than in simpler types of microscopy, it also causes only minimal photodamage to the sample (Sanderson et al., 2014, Jafari et al., 2017, Faraj et al., 2022). While CLSM has proven to create good quality images of the zebrafish pancreas, microscopy technique is not everything in fluorescence imaging. There are several ways to study the zebrafish pancreas including whole mount immunostaining and transgenic fish lines with pancreas-specific fluorescent reporters. In our experiment, we will use different antibodies of the pancreas for immunostaining and use GFP as a control to study the beta cells of the pancreas.
The results you obtain during imaging - if through manual immunolabeling rather than transgenic expression of fluorescent proteins - are highly dependent on the protocol used and the antibodies used in the experiments. Therefore, it is important to look into the efficiency of immunohistochemistry protocols, including the use of fixation steps and the duration of exposure to the chemicals in the protocol. Procedures need to be optimised both for imaging of the endocrine and exocrine pancreas.

It has yet to be studied how damage of the exocrine pancreas can affect the beta cells, and optimised techniques should be tested, such as the NTR-mediated ablation of exocrine cells after NFP treatment. Next to the immunostaining of insulin, exocrine enzymes elastase, trypsin and amylase will be stained. Double stainings of insulin together with trypsin have failed in prior experiments, and the antibody has yet to be tested separately with the protocol currently in use. Next to these antibodies, glucagon will be stained separately to assess the alpha cells in the

sample and L-plastin will be stained separately - which has also failed in prior double staining experiments with insulin - for the purpose of observing macrophages around the pancreas, indicating inflammation. We provide a critical eye to a much-used protocol of whole mount immunostaining of zebrafish larvae at our department and several changes will be tested to increase efficiency, including the replacing of overnight methanol (MeOH) fixation with a more time-efficient 4% PFA fixation. The main aim of this study is to study the effect of exocrine damage on the beta cells of the zebrafish pancreas, while several other immunostainings are tested and the protocol used is optimised along the way.

Materials & Methods

Zebrafish lines and husbandry

For the first set of experiments, wild-type (AB) zebrafish (*Danio rerio*) were used. The fish were cultured in standard conditions: they were kept in a 14-10 hour light-dark cycle, and all procedures involving the fish were done according to the European and Dutch animal welfare legislation. The eggs were collected in a petri dish with E3 medium and kept at an optimal temperature of 28 ℃ (Urushibata H, et al. 2021). After 24 hours of incubation dead embryos, fish excrement and other impurities were removed from the medium. PTU was added to the medium and the embryos were kept at 28 ℃ for 72 hours. Transgenic fish were handled in the same manner. These fish are a double transgenic line, crossed between +Tg(ins:GFP) fish and +Tg(elaA:PM-DEVD-mScarlet-NTR) fish, both developed in the BSCS department of the University Medical Centre Groningen (UMCG), creating a double transgenic line. For the NFP treatment experiment, transgenic zebrafish were divided into two groups. NFP treated in which NFP (x1) was added to the medium at 4 dpf. The fish were kept for 12 hours at 28 ℃.

Fixing and immunostaining of zebrafish larvae

After 12 hours all zebrafish larvae were euthanized using tricaine (MS-222) (10x). They were separated into 1.5 mL Eppendorf tubes. The larvae were centrifuged at 11000 rpm briefly to collect them at the bottom after which the medium was removed. 4% PFA in PBS solution was added to the tubes after which they were inverted 5 times to expose all the larvae to the solution. With the PFA solution the larvae were incubated at 4 ℃ for 24 hours. After 24 hours in 4% PFA the larvae were washed using PBS + 0.1% Tween 20 (PBS-T) at least twice for 5 min. A stepwise dehydration using methanol (MeOH) series followed in 25%, 50%, 75% and 100% methanol (dilution in PBS-T) with 5 min per step. The larvae were stored at -20 ℃ for a period of time between 1 and 3 days. When proceeding, larvae were stepwise rehydrated in 75%, 50%, 25% and 0% (PBS-T) methanol, with 5 min per step. The PBS-T was removed from the larvae and they were incubated in 10 µg/mL proteinase K in PBS-T for 40 minutes or 1 hour. After an hour the proteinase K solution was removed and the larvae were fixed for 20 minutes in 4% PFA. The larvae were then washed using PBS-T twice for 5 minutes. The larvae were then incubated in a blocking solution (3% BSA in PBS-T, 0.1% donkey serum) for 2 hours. Afterwards, the larvae were incubated with primary antibody solution at 4℃ for at least 16 hours. Antibody dilutions are made with PBS-T/3% BSA. **Supplementary Table 1** shows the primary antibodies and the secondary antibodies that were used for each experiment. After incubation of the first antibody the larvae were washed 10 times with PBS-T for 3 minutes per

wash. All washes were done while gently rocking the tubes. The PBS-T was removed and the larvae were incubated with a secondary antibody solution at 4 ℃ for at least 16 hours. Dilutions were made with PBS-T/3%BSA mix. Furthermore, all samples were incubated with Dapi (Sigma, D8417-10 MG) in a dilution of 1:1000 (5 µg/ml).

Imaging

After washing the larvae at least 6 times with PBS-T for 10-15 min, they were pipetted into WillCo-dishes®. Excess medium was discarded and 1% low-melting agarose gel (diluted in PBS) was added. The larvae were put on their left side and pushed into the medium to visualise the pancreas on the right side. For microscopy a Leica Sp8 confocal microscope (using LasX software) and a Zeiss LCM 780 confocal microscope (using Zeiss ZEN lite software) were used. Objectives used: HC PL APO CS2 40x/1.30 OIL (Leica Sp8), HC PL APO CS2 63x/1.40 OIL (Leica Sp8), HC PL FLUOTAR 5x/0.15 DRY (Leica Sp8), EC Plan-Neofluar 40x/1.30 Oil DIC M27 (Zeiss LCM 780). Magnifications are mentioned in the respective figures. Images were analysed with ImageJ (Fiji) 2.14.0.

Quantification and statistical analysis

Images of the control (DMSO-incubated) vs. the exocrine damage (NFP-treated) model were compared in LasX Office. Insulin-producing cells were counted for each sample (control n=3, treated n=5). These numbers and the means were compared to each other using an unpaired two-sample t-test. The standard deviations were calculated and the p-value was considered significant at <0.05.

Results

Immunofluorescence was used to image areas of the pancreas in the 5 dpf zebrafish larvae. Some stainings had influence on other areas of the larvae, but the pancreas can be found by acquiring an overview of the larvae's anatomy (**Figure 1**). The aim was to first test the protocol provided on whole-mount immunostaining by removing the methanol fixation step and comparing the results. While insulin staining is well-established, we tested different antibodies with this protocol, namely elastase, glucagon, amylase, trypsin and L-plastin. These stainings could prove valuable in many kinds of pancreas research. We also attempted a double staining of insulin and elastase together, which has only succeeded once before. After optimising the protocol and testing antibodies, we used a transgenic model involving NFP-mediated exocrine damage induction through NTR. Here, we assessed the effect of exocrine damage on the beta cells through insulin staining.

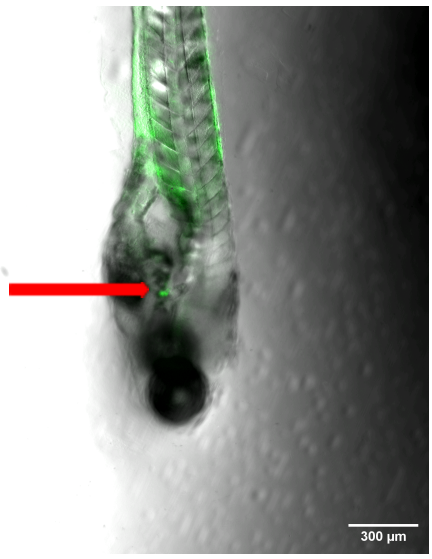


Figure 1. Brightfield image of a zebrafish larva (5x/0.15 DRY, Leica Sp8), 5 dpf, merged with GFP to show the beta cells of the pancreas. These are pointed out by the red arrow. The small black area stretching above the beta cells are the exocrine pancreas. The fish is pointed with its head to the bottom and lying on its left side.

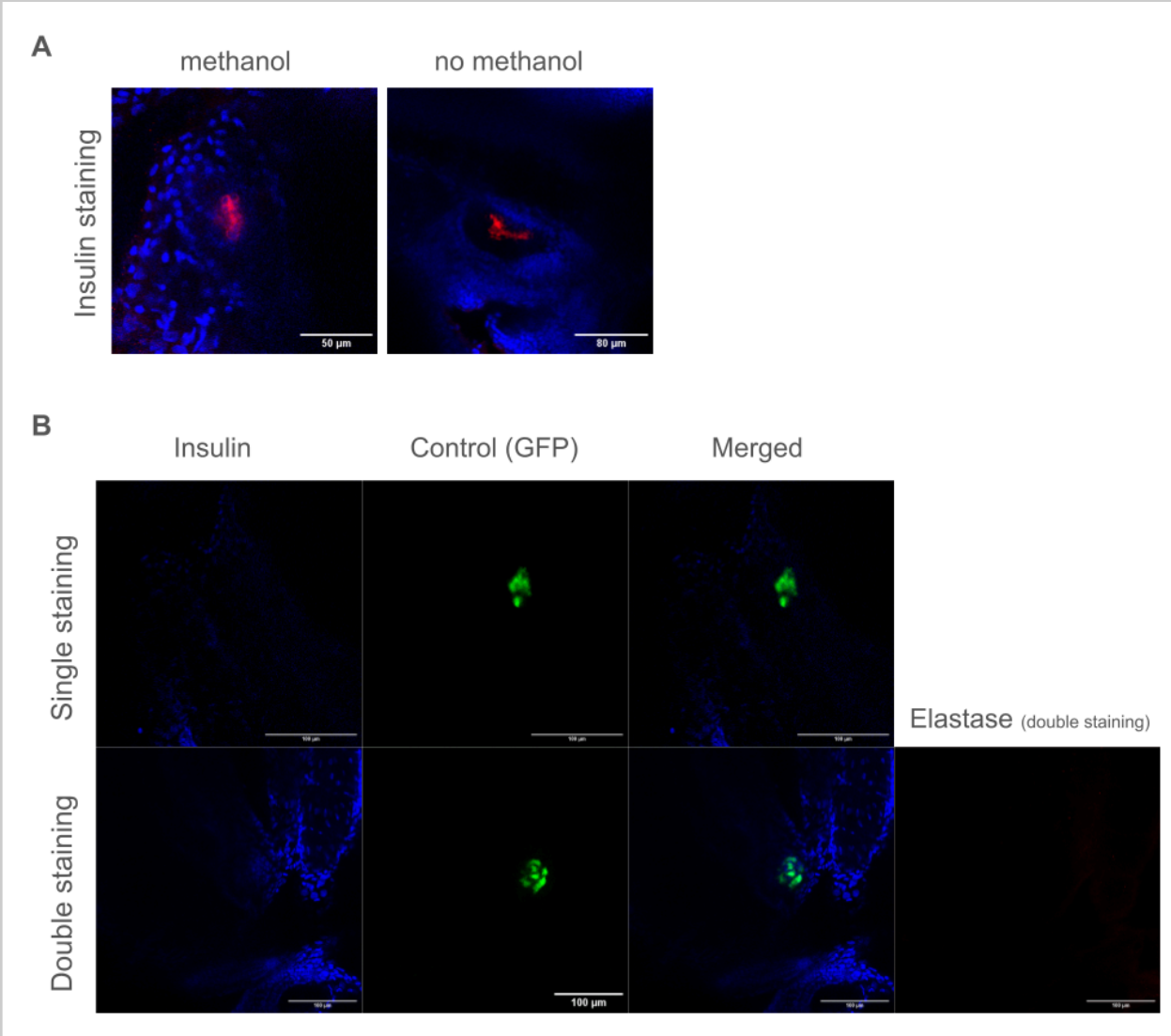
Methanol fixation of samples increases the quality of the staining

To assess whether time could be saved during immunostaining, we skipped the usual 24-96 hour -20 ℃ methanol incubation step and incubated the samples overnight in 4% PFA (4 ℃) instead. The samples were then stained with DAPI and anti-insulin or DAPI and anti-elastase (**Figure 2a**). When comparing the results, DAPI seems clear in both cases. An insulin staining shows up in both PFA-only and methanol incubated samples, but the overlap with DAPI seems to be missing in the PFA-only image. This is most likely a case of background staining of insulin, however it was not visible in the control. Other possibilities are that either DAPI did not appropriately stain all areas of the larvae, or an area of insulin has been stained other than the beta cells in the pancreas, for example in the ear where insulin tends to accumulate. Several stainings were performed to assess the effect of 4% PFA without the extra night of incubation, shortening the protocol by a day. Transgenic fish were used and GFP was chosen as a control for the beta cells, since due to the absence of methanol fixation in this experiment, GFP had no chance of being washed out of the sample. Insulin, glucagon and amylase were stained separately, and an insulin-elastase double staining was also performed (**Figure 2b**). Both the DAPI and antibody stainings did not show up clearly in the microscope images, while GFP controls were bright except in amylase. This indicates that while the lack of methanol fixation leaves GFP signalling intact, no antibody signals are picked up and DAPI is either more washed out than usual or does not penetrate the tissue enough due to the absence of methanol fixation, which also contributes to the permeability of the sample.

Elastase could not be detected in both single and double staining

While elastase did not show up in the non-methanol fixed stainings, its staining also failed in other experiments. As part of the methanol fixation versus 4% PFA-only fixation, elastase could not be imaged in both cases (**Figure 3a**). Insulin on the other hand was clearly stained,

indicating a defect in the staining of elastase. After establishing that methanol fixation was a necessary step, another elastase staining was performed along with an insulin-elastase double staining (**Figure 3b**). In the first experiments, the secondary antibody Alexa Fluor 488 (Invitrogen) was used for visualisation, but in this staining Dylight 649 (Jackson IR) was used to control for the secondary antibody as a confounding factor. While DAPI and insulin were clearly stained in both the single and double staining, there is no visible elastase in the area of the pancreas.



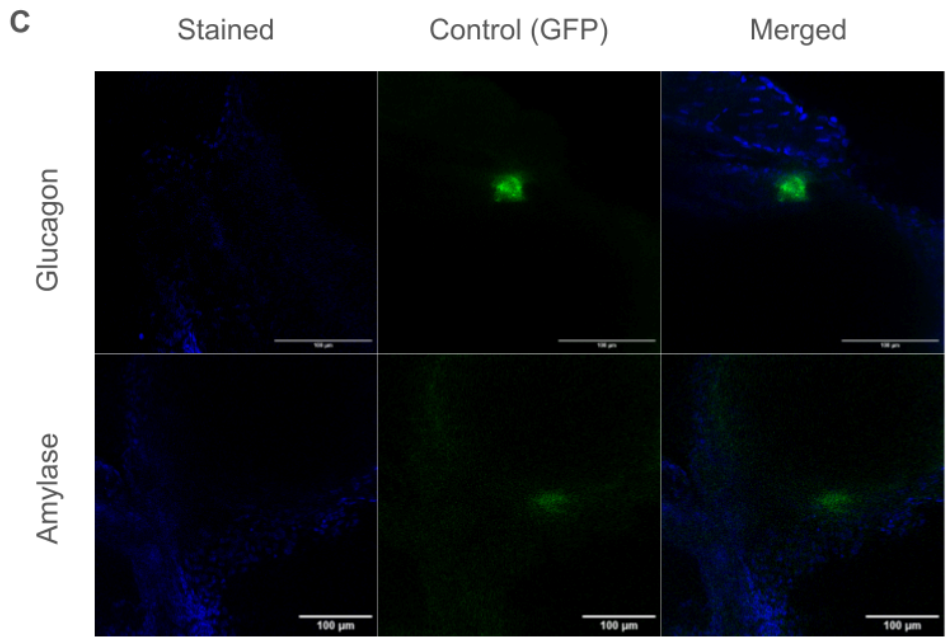


Figure 2. Methanol fixation was compared to 4% PFA only fixation (no methanol) in both wild-type and transgenic zebrafish for different antigen stainings. **A.** Insulin (red) and DAPI (blue) staining in wild-type (AB) zebrafish larvae. The methanol fixation showed normal insulin staining. No methanol fixation led to insulin staining that is either due to background staining or found in an area outside of the pancreas such as the ear, where insulin can accumulate in zebrafish larvae. Scale bars: methanol = 50 µm, no methanol = 80 µm. **B**. Insulin (red) was stained in transgenic zebrafish larvae (ins:GFP). Additionally, a double staining of insulin and elastase was performed. As control, GFP (green) is excited in the beta cells and DAPI (blue) stains the nuclei. There were no signals from the elastase and insulin stainings, possibly because the larvae were not fixated in methanol. Scale bars: 100 µm. **C**. Glucagon (red) and amylase (red) were stained in the same transgenic line as figure B. Again, only GFP expressed in the beta cells (green) and DAPI staining the nuclei (blue) are expressed. Glucagon and amylase stainings have failed. Scale bars: 100 µm. The conclusion from these images is that methanol fixation is a necessary step to get quality stainings in immunofluorescence.

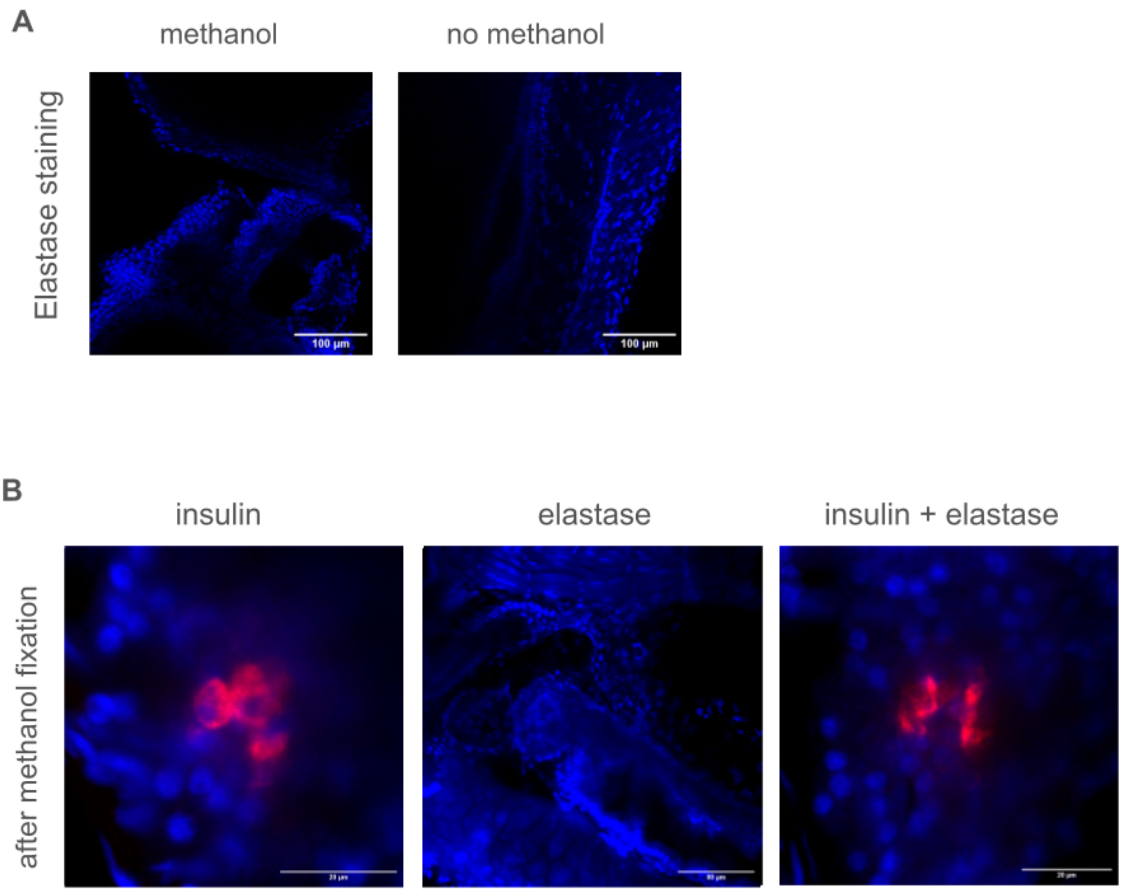


Figure 3. Several elastase stainings, with and without methanol fixation, with and without an insulin double staining in wildtype (AB) zebrafish larvae. Insulin in red, DAPI in blue, elastase in green. **A.** While comparing stainings fixated by methanol to stainings fixated only in 4% PFA, elastase did not show a signal in either case. Scale bars: 100 µm. **B**. Different fish were stained with only insulin, only elastase, or a double staining of insulin and elastase. In both cases insulin did show but elastase staining failed. Scale bars: insulin and double staining = 20 µm, elastase = 80 µm.

Single stainings of L-plastin and trypsin did not show a significant result

L-plastin and trypsin are two antigens which have not been successfully stained in double stainings together with insulin. We sought to confirm their capability to stain individually using the current protocol (**Figure 4**). Insulin staining was used as a positive control for this experiment, and the negative control consisted of an incubation with rabbit serum instead of the primary antibody (anti-insulin, rabbit origin). Both trypsin and L-plastin did show a signal during microscopy, however, it is not significant. The shapes and intensity identified in the images compared to the DAPI and insulin staining indicate that this is not a staining of real exocrine cells for trypsin or macrophages for L-plastin, but rather an amplified background.

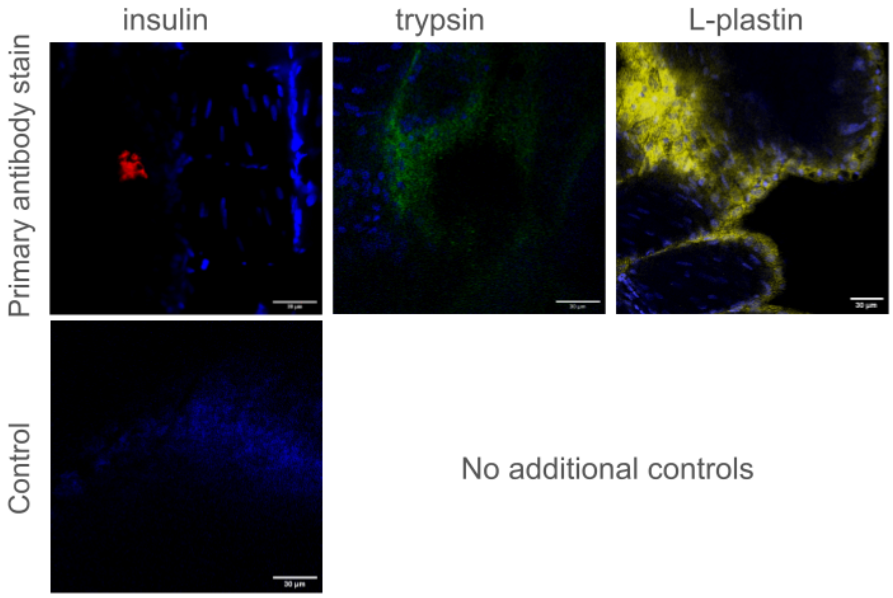


Figure 4. Single staining insulin (red), trypsin (green), L-plastin (yellow) and DAPI (blue) staining in wild-type (AB) zebrafish larvae. All stainings showed signals and the control (rabbit serum) only showed DAPI signals. However, the trypsin and L-plastin signals are not significant for exocrine cells and leukocytes respectively, and likely due to amplified background instead. The trypsin and insulin stainings were captured around the area of the pancreas while the L-plastin staining was captured more towards the posterior end of the yolk sac. Scale bars: 30 μm.

Insulin-producing cells are impacted by exocrine damage

In our final experiment, transgenic larvae were treated with NFP to induce exocrine damage, after which the effect on the beta cells was observed. Several images of the control (DMSO) group (n=3) were laid out against images of the treated (NFP) group (n=5) (**Figure 5a**). When comparing the z-stacks, the treated group showed less insulin staining than the control group in all images, both in 2D surface area and over a smaller amount of layers. Negative controls were incubated with rabbit serum instead of the primary antibody and indeed did not show an insulin signal. Insulin staining was quantified by counting the cells in ImageJ, after which statistical analysis (see Materials and Methods) showed that there was indeed a significant difference between the control group (DMSO) and the treated group with exocrine damage (NFP) (**Figure 5b**). The means of the beta cell, or insulin-producing cell counts are compared to each other with a more than three-fold difference (mean treated group = 6.4, mean control group = 19.7). The two-tailed p value equals 0.0122, which is considered statistically significant. The standard deviation of the control group is 6.944222219, for the treated group 1.624807681. Therefore, the beta cells' insulin production is affected by the exocrine damage caused by NFP treatment, suggesting that the beta cell count is significantly lower than normal when exocrine damage occurs.

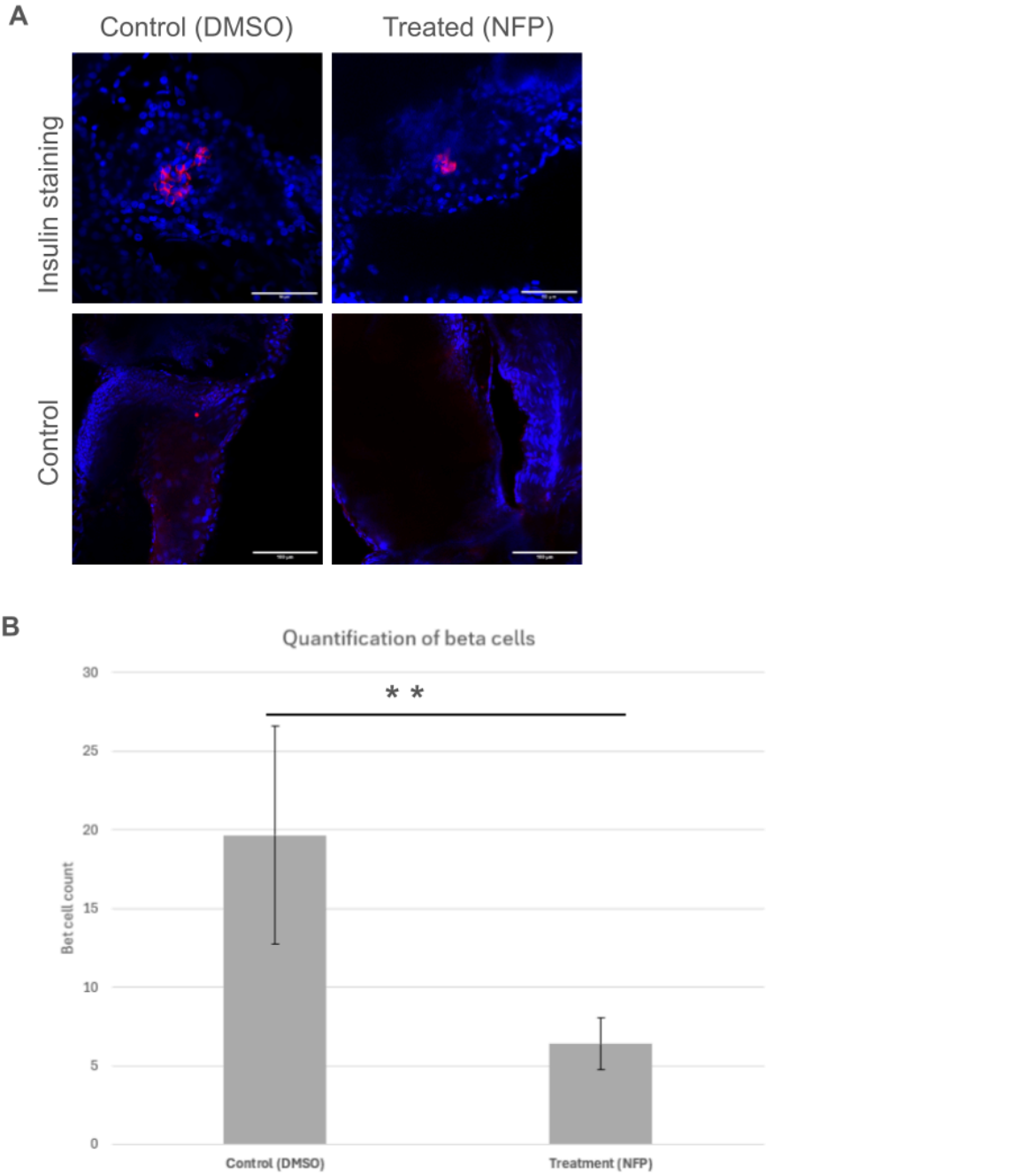


Figure 5. Insulin production of the beta cells is negatively impacted by exocrine damage. Figure **A**. Single staining of insulin was done, comparing larvae treated with NFP for 12 hours (exocrine damage model) to larvae held in DMSO. The treated and DMSO larvae both showed insulin (red), clearly marking the beta cells along with DAPI staining of nuclei (blue). The control group was incubated with rabbit serum. The amount and surface area of beta cells is larger in the control than in the exocrine damage model. Scale bars: insulin staining = 50 μm, control = 100 μm. **B**. Quantified means of the beta cell count of the treated group

(NFP for 12 hours, mean = 6.4) compared to the control group (DMSO, mean = 19.7). The standard deviations are shown: treatment group (NFP)= 1.624807681, control group (DMSO) = 6.944222219). P-value is significant (p = 0.0122), p < 0.01 = ** .

Discussion

We carried out this work to address the zebrafish larvae model used to study T1D. Several aspects of the protocol have been put to the test, by experimenting with methanol fixation and by staining antigens which have not worked before with this protocol. The direct effects of exocrine damage on the beta cells of the zebrafish pancreas have been measured for the first time. We used whole-mount immunofluorescence of zebrafish larvae for this research and quantified the exocrine damage measurements by counting the stained cells and performing a statistical analysis. Over the weeks, the same protocol has been used and updated to fit our experiments and improve our results. Methanol fixation seems to be necessary for immunofluorescence imaging, as samples fixated only with 4% PFA did not show staining. Elastase could not be stained, including when controlled for methanol fixation and the secondary antibody used. L-plastin and trypsin could also not be stained regardless of methanol fixation, so changes should be made to the protocol to bind and stain these antigens in the future. The same goes for glucagon and amylase, which were stained in PFA-only fixated samples, but also did not show any signal. The most important finding of this research is that exocrine damage affects the function of the beta cells in the endocrine pancreas of the zebrafish larvae. This is a new finding and undoubtedly important for the future of type 1 diabetes research, as researchers are looking beyond the beta cells to the exocrine pancreas and finding correlations between exocrine dysfunction and the onset of type 1 diabetes (Foster et al., 2020, Alexandre-Heymann et al., 2019).

Methanol fixation is a useful step in immunofluorescence imaging. While it may not be mandatory in all circumstances, it is a necessary step when doing a whole-mount staining of a zebrafish larva. Methanol dehydrates the sample, shrinking the cells in the process. Therefore, methanol also plays a role in the permeabilization of the sample (Jamur et al., 2010). This is why it may be so significant for the immunostaining of zebrafish larvae, as the sample needs to be permeable enough for the primary and secondary antibodies to enter the pancreas. Paraformaldehyde is also a necessary fixative as it cross-links proteins and lipids, preventing their leakage out of cells due to methanol fixation, and thus fixation with paraformaldehyde and methanol produces greater fluorescence signal than using one fixative alone (Pollice et al., 1992). It is also known from literature that using only methanol is not recommended and fixation with paraformaldehyde and methanol together is standard for immunofluorescence staining, which is in line with our findings (Hoetelmans et al., 2001, Hobro et al., 2017).

Immunofluorescence staining of elastase has not worked with the primary and secondary antibodies we used. In the first experiment we used the secondary antibody Alexa Fluor 488 (Invitrogen, donkey origin, anti-mouse), which did not work. This prompted us to try a different secondary antibody, Dylight 649 (Jackson IR, donkey origin, anti-rabbit), however, this staining also failed to work. These secondary antibodies are also well-tested in other samples and therefore we can conclude that the secondary antibody is likely not the reason the staining

failed. There is currently no literature available on elastase antibody staining in zebrafish, nor are results for other species accessible (not to be confused with publications on human neutrophil elastase and other related enzymes outside of the pancreas). Therefore, immunostaining of elastase requires more research, and another way of imaging elastase that can be used is the fluorescent reporter line elaA:gfp (Wan et al., 2006, Faraj et al., 2022). The same can be said about amylase and glucagon, though the lack of methanol fixation in our experiment likely played a role in their failure to be stained.

In our experiments, both trypsin and L-plastin stainings returned signals, but these were not significant. While literature on trypsin immunostaining in zebrafish is lacking, L-plastin has been imaged using antibodies before (Prajsnar et al., 2012, Fenaroli et al., 2014, Jim et al., 2016). In the future, literature should be consulted about the antibodies used for L-plastin in zebrafish. Staining L-plastin could provide more insight into the process of primary inflammation in the pancreas before the onset of T1D. Since there was a high amount of background staining in the images for trypsin and L-plastin, steps should be taken to prevent this in the future, such as increasing the blocking incubation period, reducing the concentration of primary or secondary antibody used, or checking and controlling for autofluorescence.

As mentioned earlier, the fact that exocrine damage leads to a loss of beta cell function is the most significant result of this study. Exocrine dysfunction has been linked to T1D before (Foster et al., 2020, Alexandre-Heymann et al., 2019), along with an overall decrease of acinar cells (Wright et al., 2020). The exocrine pancreas is a recent field of interest for researchers in relation to T1D and our findings are in line with clinical evidence that exocrine disease can lead to a loss of beta cell function, and even diabetes, such as in chronic pancreatitis-induced or type 3c diabetes (Hart et al., 2016, Rickels et al., 2020). Studies also reveal that the level of enzymes of the exocrine pancreas are decreased in individuals with T1D, suggesting the onset of exocrine dysfunction (Lankisch et al., 1982, Li et al., 2017, Foster et al., 2020).

While our finding of beta cell loss is statistically significant, it should be taken into account that the number of zebrafish counted is low, with n=3 and n=5 for the control and exocrine damage group respectively. Therefore, repeat experiments should be performed to increase the confidence of the data. Due to the lack of immunofluorescence images of L-plastin and glucagon, we cannot conclude more about the role of leukocytes or alpha cells in our exocrine damage model. In the future, L-plastin should be stained with this model to assess the role of the primary immune system in exocrine damage. Glucagon should also be stained to observe the alpha cells, since the involvement of alpha cells in T1D is another upcoming field of research (Yosten, Gina L. C., 2018, Takahashi et al., 2019, Brawerman et al., 2022). Since a staining of the exocrine cells in the exocrine damage model could not be performed due to time constraints and the current lack of a working protocol for elastase, trypsin and amylase, there was no way to incorporate a control group for the exocrine damage via immunofluorescence. Therefore, we cannot know the exact extent of the exocrine damage, as we could not visualise the pancreas while the damage was induced. Further experiments should control for this either by first optimising the immunofluorescence stainings for exocrine markers or by including an elaA:gfp or similar reporter line in the transgenic zebrafish. Despite these obstacles, we were able to attain results with translational value for T1D research by observing the loss of beta cell function in the exocrine damage model. This is due to the zebrafish pancreas having a good genetic and physiological comparison to the human pancreas with a fully developed islet of

Langerhans at just 5 dpf (Tiso et al., 2009). This short development time is a significant advantage over mammalian models. The zebrafish model being transparent as an embryo and easily kept transparent with PTU makes it a valuable asset for whole-mount microscopy, making imaging of the whole pancreas possible (Teame et al., 2019).
In this study, a point of focus was the exocrine damage model, one that should be used to further study the effects of exocrine pancreas pathology and the impact they could have on T1D. We conclude that exocrine damage leads to a loss in beta cell function. However, it cannot be concluded with current literature if T1D causes exocrine dysfunction/damage or vice versa. We suggest that future studies look into this, which is not only valuable for direct exocrine dysfunction-induced diabetes, but also to eventually find the cause of T1D or develop new preventative treatments. Targeting alpha cells and macrophages using fluorescence microscopy could also enlighten this area of research.

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Supplementary data

Supplementary table 1. Antibodies used for immunofluorescence stainings.

Antigen	Target	Primary antibody	Secondary antibody
Insulin	Beta cells	Anti-insulin (rabbit) 2:200 Abcam - AB210560	Alexa Fluor 594 1:250 (donkey, anti-rabbit) Invitrogen - A21207
Elastase	Exocrine cells	Anti-elastase (mouse) 1:200 Novus biologicals - lot 23436-2p170313	*Wild-type (AB) zebrafish experiments* Alexa Fluor 488 1:250 (donkey, anti-mouse) Invitrogen - A21202 *Transgenic zebrafish experiments* Dylight 649 1:250 (donkey, anti-rabbit) JacksonIR 711-495-152
L-plastin	Leukocytes	Anti-L-plastin (mouse) 4:200 Santa Cruz - sc-133218	Alexa Fluor 488 1:250 (donkey, anti-mouse)
Trypsin	Exocrine cells	Anti-trypsin (sheep) 15:200 ThermoFisher - PA5-47544	Alexa Fluor 647 1:250 (donkey, anti-sheep)
Glucagon	Alpha cells	Anti-glucagon (mouse) 1:200 Santa Cruz sc-57171	Alexa Fluor 488 1:250 (donkey, anti-mouse) Invitrogen A21202
Amylase	Exocrine cells	Anti-α-amylase (rabbit) 2:200 Sigma A8273-IVL	Dylight 649 1:250 (donkey, anti-rabbit) JacksonIR 711-495-152