Data in Brief 48 (2023) 109213

Contents lists available at ScienceDirect

Data in Brief

journal homepage: www.elsevier.com/locate/dib

Data Article

Experimental datasets on the immunohistological assessment of δ-cells in the islet organs of the endocrine pancreas of Japanese medaka (*Oryzias latipes*) fish exposed to graphene oxide

Asok K. Dasmahapatra[a,b], Paul B. Tchounwou[a,*]

[a] *RCMI Center for Environmental Health, Jackson State University, Jackson, MS 39217, USA*
[b] *Department of Bio Molecular Sciences, Environmental Toxicology Division, University of Mississippi, Oxford, MS 38677, USA*

ARTICLE INFO

Article history:
Received 24 March 2023
Revised 2 May 2023
Accepted 2 May 2023
Available online 19 May 2023

Dataset link: Experimental Datasets on the Immunohistological Assessment of δ-Cells in the Islet Organs of the Endocrine Pancreas of Japanese Medaka (Oryzias latipes) Fish Exposed to Graphene Oxide (Original data)

Keywords:
Graphene oxide
Endocrine disruption
Japanese medaka
Endocrine pancreas
Delta cells
Immersion
Intraperitoneal injection
Cell sorting

ABSTRACT

The datasets of this article present the experimental parameters resulting from the assessment of δ-cells in the islet organs of the endocrine pancreas as a potential biomarker of endocrine disruption (ED) mediated by graphene oxide (GO), using Japanese medaka fish as the model. These datasets support the article "Evaluation of pancreatic δ-cells as a potential target site of graphene oxide toxicity in Japanese medaka (*Oryzias latipes*) fish". GO used in the experiments was either obtained from a commercial source or synthesized in the laboratory by us. GO was sonicated for 5 min in ice temperature before application. The experiments were conducted on reproductively active adult fish maintained as a breeding pair (one male and one female) in 500 ml balanced salt solution (BSS) either by immersion (IMR) in GO (20 mg/L) continuously for 96 h with the refreshing of media once in every 24 h, or by a single intraperitoneal (IP) administration of GO (100 μg/g) to both male and female partners. Control fish were maintained in BSS only (IMR experiment), or nanopure water (vehicle) was injected into the

DOI of original article: 10.1016/j.ecoenv.2023.114649
* Corresponding author.
E-mail address: paul.b.tchounwou@jsums.edu (P.B. Tchounwou).

https://doi.org/10.1016/j.dib.2023.109213

peritoneal cavity (IP experiment). The IP experimental fish were anesthetized in MS-222 (100 mg/L in BSS); the injected volume (0.5 µL/10 mg fish) never exceeds 50 µl/fish. After injection, the injected fish were allowed for recovery in clean BSS and after recovery both partners were transferred to 1 L glass jars with 500 mL BSS. During depuration, the media of the breeders refreshed once every 24 h and the eggs were collected. After 21 days, the survived fish were anaesthetized, and the trunk region was preserved in 4% paraformaldehyde in PBS (20 mM) containing 0.05% Tween 20. The phenotypic sex of adult fish was assessed externally by secondary sex characters (fin features) and internally by gonad (testis and ovary) histology. Once the location of pancreas was determined after HE stains, immunohistochemical technique was applied on next few slides using rabbit derived polyclonal antisomatostatin antibody as primary antibody and a commercial kit for colorimetric determination of δ-cells in the islet organs was used. Images were captured using an Olympus CKX53 inverted microscope with DP22 camera and CellSens software. Using imagej software, a minimum 3 images of principal islets and one image of secondary islets were assessed. The immunoreactivity of δ-cells, due to neuron-like appearance and filopodia like processes, enabled us to separate them from other cell types found in the pancreatic islets of medaka. Based on immunoreactivity, we have classified islet cells into three categories; noncommunicating delta cells (NCDC), communicating cells (CC), and non-delta cells (NDC), and expressed as number of cells (NCDC/CC/NDC)/mm^2 of islet organs. The nuclear area (µm^2) and the linear length of filopodia of NCDCs were also considered for evaluation. Numerical data were analysed by Kruskal-Wallis test followed by Mann-Whitney's test as post hoc test and presented as means $\pm$ SEM. Statistically significant differences were considered for $p \leq 0.05$.

Specifications Table

Subject	Biological Sciences
Specific subject area	Zoology
Type of data	Table Image Graph Figure
How data were acquired	Microtome (Olympus cut 4055) Transmission Electron microscope (TEM) Barnstead Nanopure, model D11901, Thermo Fisher Scientific, Waltham, MA, USA Microsyringe (Hamilton 1700 Series Gastight Syringes, 22-gauge, 2 in. Needle: Hamilton company, Reno, Nevada, USA) Haematoxylin Eosin Staining (HE) (Sigma-Aldrich, St. Louis, MO, USA) Immunohistochemistry using polyclonal rabbit antisomatostatin antibody as primary antibody (Abcam, Waltham, MA, USA) ABC kit (Vector lab, Burlingame, CA, USA) Vector Dab (Vector lab, Burlingame, CA, USA) Bloxall (Vector lab, Burlingame, CA, USA)

(*continued on next page*)

	Olympus CKX53 inverted microscope with DP22 camera and CellSens software (Hunt optics & Imaging, Pittsburgh, PA, USA) GraphPad Prism 7 (San Diego, CA, USA) imagej (imagej.nih.gov)
Data format	Raw Analyzed
Description of data collection	Medaka breeders were exposed to GO (20 mg/L) either by continuous immersion for 96 h or by a single IP injection (100 µg/g). The endocrine pancreas was assessed after 21 days post-treatment. Immunohistochemical detection of δ-cells was made by using rabbit derived polyclonal antisomatostatin antibody. Distribution of islet cells (number/ mm^2) as NCDC, CC, or NDC and nuclear area (μm^2) and linear length of filopodia (µm) of NCDC were determined using imagej software.
Data source location	Jackson State University 1400 JR Lynch Street Jackson, MS 39217 USA
Data accessibility	Repository Name: Figshare Direct ULT to data https://doi.org/10.6084/m9.figshare.21153139
Related research article	Author' s names: Asok K Dasmahapatra, Paul B. Tchounwou Title: Evaluation of Pancreatic δ- Cells as a Potential Target Site of Graphene Oxide Toxicity in Japanese Medaka (*Oryzias latipes*) Fish Journal: Ecotoxicology and Environmental Safety, 253 (2023) 114,649. https: doi.org/10.1016/jecoenv.2023.114649

Value of the Data

- Due to neuron-like morphology and dynamic filopodia-like processes, δ-cells in endocrine pancreas of medaka secret somatostatin which can activate the functions of nearby or distant cells distributed within the islet organs and have potential impact on glucose metabolism. Our innovative data indicate that GO-induced impairments of endocrine pancreas in medaka are nonspecific and could be affected by factors such as exposure routes, sex, and age of the fish.
- Data will be used by the organizations worldwide who are developing strategies/tools for the control of diabetes. Such organizations include the American Diabetic Association, European Association for the Study of Diabetes (EASD), Asian Association for the Study of Diabetes (AASD), and others. Also, these data can be used to other agencies such as United States Environmental Protection Agency, and the European Union, who are evaluating the potential of GO in ED.
- Evaluation of pancreas as a target site of EDCs is challenging because they can act on multiple cells including α and β cells found in endocrine pancreas and produce an adverse effect on carbohydrate metabolism. However, histology/histochemistry is a sensitive tool for identification of target cells in a specific organ. The medaka pancreas is unique, and this study highlights the value of pancreatic islet organs as an alternative tool to be used during EDC evaluation.
- Due to the extensive use of engineered nanomaterials in biomedicine, it is necessary to evaluate the potential health hazards of GO by using unique model organisms. The cutting-edge data we generated in this study are valuable in sharing knowledge on the potential EDC effects of GO targeting the endocrine pancreas of a model fish species, Japanese medaka, which has unique δ-cells with dynamic filopodia-like features, common to humans and mice.

1. Objectives

The specific aims of this paper were to provide the experimental data generated from our toxicology research and to fully describe these data to strengthen our recently published article entitled "Evaluation of pancreatic δ-cells as a potential target site of graphene oxide toxicity in Japanese medaka (*Oryzias latipes*) fish".

2. Data Description

Fig. 1 represents the flowchart of the experimental plan we designed to study of the effect of GO on medaka pancreas. We have used both IMR and IP exposure techniques during GO treatment. The breeders (one healthy mature male and one healthy mature female /jar which were 16 $\pm$ 2 weeks old) were transferred to 1 L glass jars containing 500 mL of BSS for acclimatization and allowed normal breeding in the laboratory conditions (25 $\pm$ 1 °C; light cycle 16 h light: 8 h dark). The media were changed once every day and the eggs laid by the female were collected either from the bottom of the tank or from the abdomen of the female for evaluation of breeding activities. The number of fertilized eggs indicated the breeding activity of males, and the total eggs (both fertilized and unfertilized) were indicated the breeding activity of females. Once they are acclimatized and laid eggs continuously for consecutive 7 days, the breeders are ready for GO exposure. GO was sonicated for 5 min in ice temperature before experimental use. In IMR experiment, GO (20 mg/L) was sonicated for 5 mins in ice temperature and the media was refreshed once every 24 h. After 96 h of treatment, the fish were maintained in a GO-free environment until sacrifice (21 days). Controls were maintained in identical conditions in clean BSS (only BSS, no GO). For IP experiments, GO (100 µg/g) was also sonicated for 5 min in ice temperature, and was injected (0.5 µL/ 10 mg body weight) into the intraperitoneal cavity of the fish by a microsyringe (Hamilton 1700 Series Gastight Syringes, 22-gauge, 2 in. Needle: Hamilton company, Reno, Nevada, USA). Both male and female fish of the same breeding pair was injected with GO; moreover, the volume of the injected materials never exceed 50 µL/fish. Controls (both partners) were injected with nanopure water (0.5 µL/10 g body weight). The surviving fish (IP experiment) were maintained in BSS for 21 days in a GO-free environment. During depuration, the fish were fed with TetraMin Flakes and hatched nauplii of brine shrimp, twice daily. The eggs were collected, and the media refreshed once every 24 h. On the 21st day of recovery, the fish were sacrificed, and the trunk tissues were fixed in 4% paraformaldehyde in 20 mM PBS

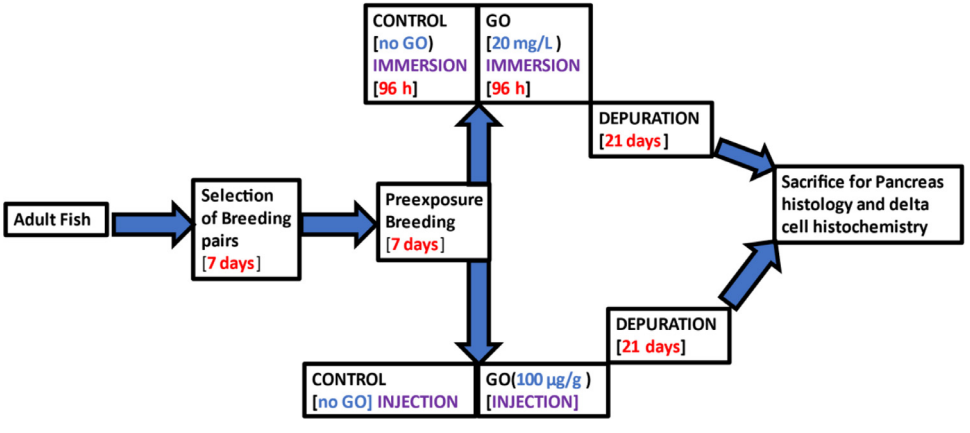

Fig. 1. The flowchart illustrating the GO exposure paradigm to adults of Japanese medaka fish during experiments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

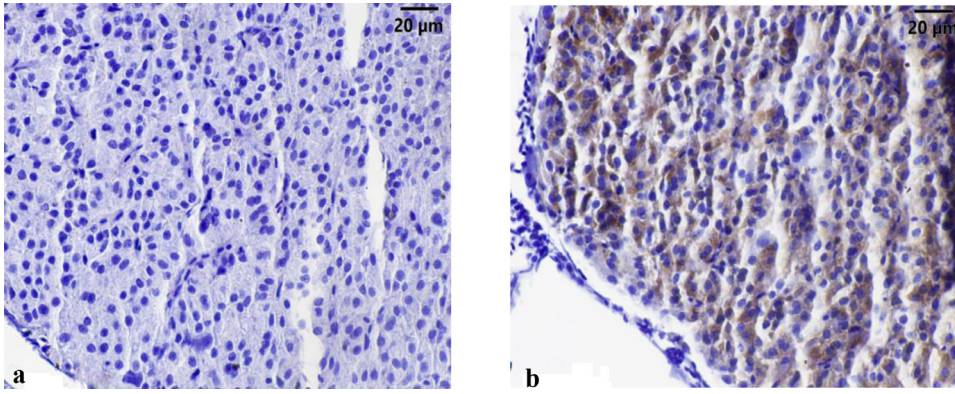

Fig. 2. Representative photomicrographs showing immunohistochemical staining of the pancreatic islets of Japanese medaka adults, counterstained with hematoxylin. a: principal islet without primary antibody; b: principal islet with primary antibody. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

for histological and histochemical evaluation of the gonads, pancreas, and the pancreatic islet organs (primary and secondary). The sections were evaluated and photographed under microscope (Olympus CKX53 inverted microscope). Antibody positive δ-cells were brown in color with long filopodia-like processes (Fig. 2b and Figs 3a-j; SF1, SF2), whereas unreactive cells and the cells in the sections devoid of primary antibody, stained blue (Fig. 2a).

Fig. 2 and the supplementary Figures (SF) SF1 and SF2 show the detection of the δ-cells in the islet organs of the medaka pancreas by immunohistochemical techniques using rabbit-derived polyclonal antisomatostatin antibody. Only the filopodia of δ-cells were found to be positive to stomatostatin antibody (Figs 2a-2 h; SF1 and SF2). In both IMR (Figs 2a-2d; SF1) and IP (Figs. 2e-2 h; SF2) experiments, the GO- exposed male (Figs. 2b, 2f; SF1e, SF1g, Sf1i, SF2e, SF2g, SF2i) and female fish (Figs.2d, 2 h; SF1f,SF1h, SF1j; SF2f, SF 2 h, Sf 2j) showed apparently more immunoreactivity than the corresponding control males (Figs 2a, and 2e; SF1a, SF1c, SF2a, SF2c) and female fish (Figs 2c and 2 g; SF1b, SF1d,SF2b, SF2d).

The δ-cells of medaka found in the islet organs were detected immunohistochemically by using rabbit-derived polyclonal antisomatostatin antibody (Abcam, Waltham, MA, USA) as primary antibody. Fig 3a = control male fish in immersion (IMR); b: male fish exposed to GO by IMR (20 mg/L); Fig. 3c = control female fish in IMR; d: female fish exposed to GO (20 mg/L) by IMR. e: control male fish intraperitoneally (IP) injected with vehicle (nanopure water); f: male fish exposed to GO (100 µg/g) IP; g: control female fish, IP-injected with vehicle (nanopure water); h: female fish exposed to GO (100 µg/g) IP. Cells in green circles represent noncommunicating δ-cells (NCDC); cells in red circle represent communicating cells (CC), and cells in black circle represent non-δ-cells (NDC).

The nuclear area (μm^2) and the linear length of filopodia (µm) in the noncommunating δ-cells (NCDC) are presented in Table 1 and Tables ST1 and ST2. Distribution of islet cells including immunoreactive δ-cells in an islet organ as NCDC, communicating cells (CC) and non-δ-cells are presented in Table 2 and in ST3, ST4, and ST5.

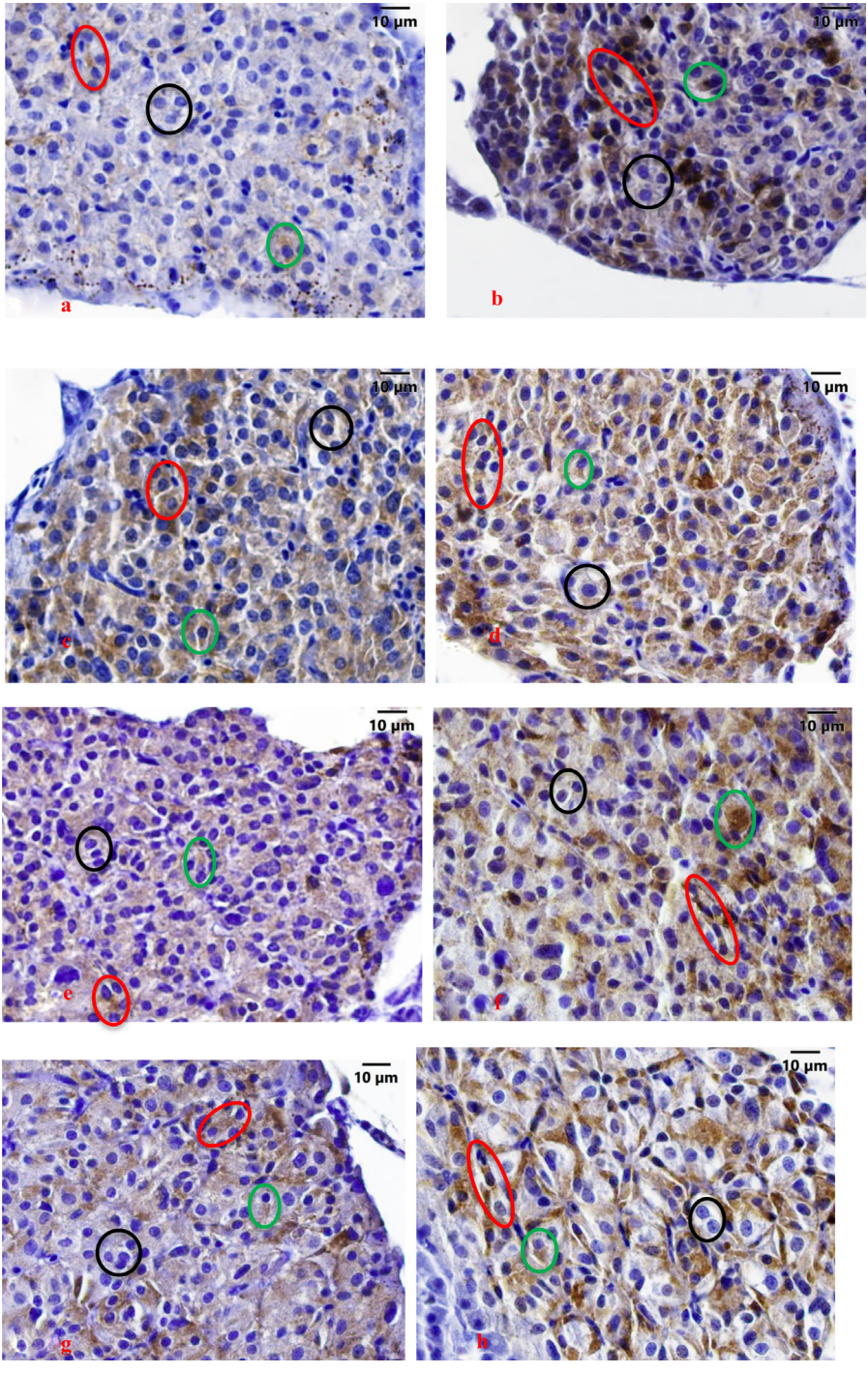

Fig. 3. Effects of GO on the pancreatic islet organs of Japanese medaka. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Effect of GO in the nuclear area and filopodial length of NCDC in the islet organ of Japanese medaka.

	IMR				IP			
	Control		GO (20 mg/L)		Control		GO (100 μg/g)	
Parameters	Male	Female	Male	Female	Male	Female	Male	Female
Nuclear area (μm^2)	14.43 ± 0.39 (N = 79)	14.31 ± 0.27 (N = 177)	12.63 ± 0.24 (N = 218) *	13.00 ± 0.41 (N = 74) *	12.37 ± 0.34 (N = 96) #	11.33 ± 0.32 (N = 98) #, $	13.13 ± 0.41 (N = 69)	11.87 ± 0.32 (N = 84) #, $
Filopodia length (μm)	4.99 ± 0.26 (N = 81)	4.17 ± 0.81 (N = 177) $	4.36 ± 0.16 (N = 112)	4.58 ± 0.19 (N = 96) *	4.49 ± 0.19 (N = 96)	4.70 ± 0.18 (N = 98) #	5.67 ± 0.30 (N = 69) #,*	5.01 ± 0.22 (N = 85)

Data are presented as means ± SEM; N = number of nucleus or filopodia; NCDC = noncommunicating delta cell; IMR = immersion; IP = intraperitoneal injection. Asterisks (*) represents significant difference ($p < 0.05$) between control fish (males or females) with corresponding males or female fish exposed to GO either by IMR or by IP; pound symbol (#) indicates significant difference ($p < 0.05$) between the route of exposures (IMR vs IP) of the male or female fish exposed as control or in GO. Dollar ($) symbol indicates significant difference ($p < 0.05$) between the sex of the fish (male vs. female) exposed either as control or in GO.

Table 2
Effect of GO exposure on number of NCDC, CC and NDC in an islet organ of Japanese medaka.

	IMR				IP			
	Male		Female		Male		Female	
Cell types	Control	GO (20 mg/L)	Control	GO (20 mg/L)	Control	GO (100 μg/g)	Control	GO (100 μg/g)
NCDC (number/mm^2)	1953 ± 240 (n = 13)	1601 ± 103 (n = 85)	1880 ± 103 (n = 29)	2047 ± 166 (n = 18) $	1941 ± 191 (n = 16)	2431 ± 307 (n = 11) #	1849 ± 258 (n = 16)	2355 ± 249 (n = 15) #
CC (number/mm^2)	5254 ± 898 (n = 13)	7792 ± 719 (n = 20) *	4779 ± 447 (n = 29)	5133 ± 709 (n = 12) $	7555 ± 773 (n = 15)	3184 ± 569 (n = 9) #,*	3867 ± 531 (n = 15) $	4205 ± 406 (n = 16)
NDC (number/mm^2)	6755 ± 832 (n = 13)	6606 ± 796 (n = 19)	8676 ± 546 (n = 29) $	8093 ± 805 (n = 18)	9498 ± 1182 (n = 16)	8641 ± 690 (n = 11)	12,062 ± 817 (n = 16) $, #	6889 ± 537 (n = 16) $, *

NCDC = noncommunicating δ-cells; CC = communicating cells; NDC = non-δ-cells. The data are expressed as number of cells/mm^2 of an islet organ and presented as mean ± SEM. n = number of fields examined. IMR = immersion; IP = intraperitoneal injection. The data are presented as means ± SEM; Asterisks (*) represents significant difference ($p < 0.05$) between control fish (males or females) with corresponding males or female fish exposed to GO either by IMR or by IP; pound symbol (#) indicates significant difference ($p < 0.05$) between the route of exposures (IMR vs IP) of the male or female fish exposed as control or in GO. Dollar ($) symbol indicate significant difference ($p < 0.05$) between the sex of the fish (male vs. female) exposed either as control or in GO.

3. Experimental Design, Materials and Methods

The Institutional Animal Care and Use Committee (IACUC) of the Jackson State University, Jackson, MS, USA approved all the experimental protocols after critical evaluation as the Protocol number (#IBC 08–01–17 and IBC 09–01–17) followed the guidelines of National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

3.1. Maintenance and exposure of the fish to GO

In our previous publications, we have described the source and the detailed maintenance procedure of the Japanese medaka fish colony at the Jackson State University (JSU) campus, Jackson, MS, USA, [1–9]. Briefly, in September 2017, a protocol transfer agreement was signed by the official/authorized representatives of the JSU and the University of Mississippi (UM) for the official

transfer of 50 adult fish (both males and females) from the medaka fish colony maintained at the University of Mississippi, Oxford, MS 38,677. Accordingly, 50 adult breeding pairs (20 males and 30 females) were transferred to JSU, and the breeding colony was set up at the Plant Science building of JSU. In our laboratory at JSU, the breeders were maintained in 35 L tanks (6 females and 4 males) containing 25 L of BSS at 25 ± 1 °C temperature with light cycle 16 h L: 8 h D; the media was recirculated and passed through disposable bio and carbon filters (That Fish Place-That Pet Place, Lancaster, PA, USA). The fish were fed twice a day with brine shrimp nauplii (reared in the laboratory) and Tetramin flakes (That Fish Place-That Pet Place, Lancaster, PA, USA); the morning feeding was scheduled between 9 and 10 a.m. and the afternoon feeding was made between 4 and 5 p.m. Circulating media was changed every two/three week, depending on the pH (7–8.5) and the ammonia concentration (1–3 ppm). With all these conditions, the breeders bred successfully and laid excellent quality/number of eggs. The laid eggs were generally collected either from the lower abdominal part of the female fish or from the bottom of the tanks within 1–3 h after the light was turned on.

3.2. GO preparation

The source and the procedure of GO synthesis, dilution, sonication, application to the animal was described previously [1,2,7–9]. Briefly, the GO we used in our experiments was either synthesized in the laboratory by us or purchased from Sigma-Aldrich, St. Louis, MO, USA. The concentration of GO we received from commercial source was 2 mg/mL which was dispersed in water. We, therefore, dissolved the laboratory synthesized GO in nanopure water (Barnstead Nanopure, model D11901, Thermo Fisher Scientific, Waltham, MA, USA) and made the concentration of GO as equivalent to the concentration we received from the commercial source (2 mg/mL). Moreover, in IMR experiment, commercial GO was further diluted to desired concentration (20 mg/L; 100-fold dilution) in BSS. For IP experiments, no further dilution of GO was made (2 mg/mL). The GO either in aqueous solution (2000 mg/L) or in BSS (20 mg/L) was sonicated in ice temperature for 5 min (2 s on-1 s off pulse, 225 w) using a probe sonicator (ultrasonicator LPX 750, Cole Palmer, Chicago, IL, USA). The sonicated nanomaterial was checked by TEM at the Electron Microscopy Core laboratory at JSU [1,2]. The commercial GO was used in both IMR and IP experiments, however, the synthesized GO was used only in IP experiment.

3.3. Exposure of adult fish to GO

The experimental adult medaka fish we used for immunohistochemical evaluation of the pancreatic islets, were obtained from our previous experiments on GO exposure by IMR [7–9] and IP [1,2] Briefly, before exposure to GO (either by IMR or IP), the fish were under paired breeding (one male and one female) in the laboratory conditions (25 ± 1 °C and 16 h light: 8 h dark Light cycle) in 1 L glass jars containing 500 mL BSS. The reproductive activity of the breeding pairs was checked carefully for 1 week (7 days), by collecting the eggs from the breeding tanks within 1–3 h after the light turned on. The breeding activity of the males was considered by counting the number of fertilized eggs produced by a breeding pair every day; for females, both fertilized and unfertilized eggs (total number of eggs) were considered. For evaluation of pancreatic islet cells, we have used only one concentration (20 mg/L) of GO for IMR and one dose of GO (100 µg/g) for IP experiments (Fig. 1). For IMR experiment, the fish as a breeding pair (one male and one female; the same partner acclimatized as a breeding pair before GO exposure) were exposed to 20 mg/L GO in BSS continuously for 96 h with refreshing of media every 24 h and then depurated for 21 days in a GO-free environment; IMR controls were exposed to BSS only (no GO) and run parallelly to the GO-exposed group. For IP experiment, GO (2 mg/mL; maximum injected volume was 0.5µL/ 10 mg body weight) or vehicle (nanopure water) were injected (0.5 µL/10 mg body weight) into the intraperitoneal cavity of the fish by a

micro-syringe (Hamilton 1700 Series Gastight Syringes, 22-gauge, 2 in. Needle; Hamilton Company, Reno, Nevada, USA). The injections were given to both partners (one male and one female, which were acclimatized as breeding pair before injection), and the volume of the injected materials never exceed 50 µL/fish. To avoid movement during IP injection, the fish were anesthetized in MS 222 (100 mg/L) and after successful injection returned to BSS until they recovered completely from the stress we imposed during injection (judged from the normal swimming activity of the injected fish) (the IMR fish, either exposed to GO or served as control were not anesthetized prior to GO exposure or served as controls). After IP injection the surviving fish (the same breeding pair) were maintained in 1 L glass jars in 500 ml BSS for 21 days in a GO-free environment in the laboratory. Both IMR and IP fish were allowed breeding during depuration period with refreshing of media every day and the collected eggs were checked for fertility. On 21st day, for histopathological observations, the fish were anesthetized in MS 222 (100 mg/L), sacrificed and the trunk tissue was preserved in 4% PFA in 20 mM PBS. The histopathology of pancreatic islets of the fish exposed to GO by IMR [7–9] was also compared with the fish administered GO (100 µg/g) by IP-injection [1,2]. The phenotypic sex of the breeding pairs was confirmed by checking the histology of the gonads (testis/ovary) following OECD guidelines [10]. No ovotestis or testis-ova was observed in any of the control or experimental fish exposed to GO either by IMR or IP.

3.4. Somatostatin immunohistochemistry

In pancreatic islets, the hormone somatostatin is secreted by the δ-cells. We intend to use immunohistochemical technique for the detection of somatostatin in the δ-cells of the experimental fish which are different from other islet organ cells (such as α and β cells) in shapes and with the presence of filopodia like processes. We used rabbit-derived polyclonal antisomatostatin antibody as primary antibody (catalog no. ab108456, Abcam, Waltham, MA, USA) and a kit (Vector laboratory, Burlingame, CA, USA) for colorimetric estimation of somatostatin in the δ-cells of pancreatic islet organs (Figs 2a-2 h; SF1a-SF1j). The polyclonal antibody (catalog number ab108456 Abcam, Waltham, MA) we used, showed significant amino acid sequence identity with the protein sequences of somatostatin-1(Genbank Accession XP_004084505), somatostatin-2 (GenBank Accession XP_004084506), and somatostatin-1b (GenBank Accession_004070427 reported in GenBank (ncbi.nlm.nih.gov); the BLAST search was made by the Abcam technical support, technical@abcam.com) [9]. During standardization of the technique, we followed the guidelines provided by the commercial companies (Abcam, Waltham, MA, USA; Vector laboratory, Burlingame, CA, USA)[7–9]. In brief, once the position of the islet organs in tissue sections (5 µm thickness) were identified after hematoxylin-eosin (HE) staining, the sections on nearby slides (probably two consecutive glass slides, containing 3–5 sections) were deparaffinized in xylene, rehydrated in graded alcohols (100%−30%) and brought to tap water. Antigen retrieval (unmasking) of pancreatic islets was done for 40 min in citrate buffer (pH 6.0) at 85–90 °C in a water bath. After washing the slides in 0.1 M phosphate buffered saline-Tween 20 (Phosphate buffered saline with 0.3% Tween 20, PBST; Sigma-Aldrich, St. Louis, MO), for blocking of endogenous peroxidase and alkaline phosphatases, the sections were treated for 10 min at room temperature with bloxall (Vector laboratories, Burlingame, CA). Next, the sections, after a brief wash in PBST, were incubated with 3% normal goat serum in PBST for 1 h at room temperature and finally with (Fig 3B) and without (Fig 3A) primary antibody (polyclonal rabbit-derived antisomatostatin antibody with a final dilution of 1:600 in 3% goat serum in PBST) overnight at 4 °C (Figs 2a-2j; SF-1, SF2). Next day (after 16–24 h), sections were washed several times in PBST and incubated for 1 h at room temperature with biotinylated goat antirabbit secondary antibody (Catalog number PI-1000; lot number: ZH0823; Vector laboratories, Burlingame, CA) diluted (1:300) with 3% goat serum in PBST. Sections were washed again in PBS and incubated with avidin-biotin complex in PBS for 1 h at room temperature as per recommendation made by the manufacturer (Vector laboratory, Burlingame, CA, USA). The sections were washed several times in PBS and incubated again for 1 h at room temperature with vectastain elite ABC reagent (Vector laboratory,

Burlingame, CA, USA). After incubation, sections were washed several times in PBS and incubated again at room temperature for 10 min with freshly prepared peroxidase substrate (vector DAB) as recommended by the manufacturer (Vector laboratory, Burlingame, CA, USA). After incubation, the sections were washed in tap water and counter stained with hematoxylin, dehydrated in graded alcohols (30–100%), cleared in xylene and mounted in permount (Fisher Scientific, St. Louis, MO). The sections were evaluated and photographed under microscope (Olympus CKX53 inverted microscope). Antibody positive δ-cells were brown in color with long filopodia-like processes (Figs 2b; Fig 3a-j; SF1, SF2), whereas unreactive cells and the cells in the sections devoid of primary antibody, stained blue (Fig. 2a).

3.5. Distribution of islet cells in the endocrine pancreas of medaka

We have used 20 experimental fish for identification of pancreatic δ-cells by immunostaining. In IMR experiments, 2 breeding pairs (2 males and 2 females) were used as controls (no GO) and 3 breeding pairs (3 males and 3 females) were used as GO-treated fish (exposed to GO for 96 h continuously); for IP experiments, like IMR, 2 breeding pairs (2 males and 2 females) were used as control (no GO; vehicle) and 3 breeding pairs (3 males and 3 females) were injected with GO (100 µg/g). After identification of the pancreas by HE staining, we generally immunostained 3–5 slides and evaluated 3–5 sections/slide/ fish. Due to the small sample size the counting of cells were not blinded to treatment, however, we are very careful and cautious about that. Based on somatostatin immunoreactivity, the cells in an islet organ of medaka pancreas were classified into three categories (blood cells are excluded from counting) [9]. The filopodia of immunoreactive δ-cells (neuron-like morphology) when remained free (not in contact with other neighboring or distant cells as judged from low microscopic observations) were identified and counted as noncommunicating δ-cells (NCDC); those cells which were in communication or in contact with other neighboring or distant cells with immunoreactive filopodia, we classified and count them as communicating cells (CC); and the third type of cells remained negative to somatostatin antibody, we classified and count them as non-delta cells (NDC). After counting all these cells in an islet organ (counting all cells in a principal islet organ requires three fields and for secondary islets requires only one field), the cells were classified as NCDC, CC, and NDC and the area under cell counting was determined by imagej software (http://www.imagej.nih.gov/ij); data (cell count) were expressed as number of cells (NCDC/CC/NDC)/mm$^{2.}$ (Table 2; ST3, ST4, ST5). Moreover, the nuclear area (µm^2) and the linear length of filopodia (µm) were also determined by imagej software (Table 1, ST1 and ST2).

3.6. Statistical analysis

We were able to track the pancreatic islets of 20 experimental fish; 2 males and 2 females as controls and 3 males and 3 females as GO-exposed (20 mg/L) fish from the IMR experiment. Similarly, in IP experiment, we were also able to track 2 males and 2 females as control fish and 3 males and 3 females as GO-exposed fish (100 µg/g). Data analyses were made by following the statistical programs we used previously [1–9]. In brief, all the data were analyzed by using GraphPad prism version 7.04 (GraphPad Prism, San Diego, CA) software. We used descriptive analysis to evaluate nuclear area (µm^2), linear length of filopodia in NCDCs and the distribution of NCDC, CC and NDC within an islet organ (number/mm^2). D'Agostino-Pearson (DP) or Shapiro-Wilks (SK) tests were used to determine the normality of the data. Because our data were not distributed normally and did not meet the criteria of using a parametric test, we performed the Kruskal-Wallis test followed by Mann-Whitney's test (nonparametric test) as a post-hoc test and $p < 0.05$ was considered as the level of significance. All the numerical data were expressed as means $\pm$ SEM.

Ethics Statement

No human subjects were involved in this research.

The experiments conducted in this study were approved by the Institutional Animal Care and Use Committee (IACUC Protocol number IBC 08–01–17 and IBC 09–01–17) of the Jackson State University, Jackson, MS following the guidelines of National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships which have or could be perceived to have influenced the work reported in this article.

Data Availability

Experimental Datasets on the Immunohistological Assessment of δ-Cells in the Islet Organs of the Endocrine Pancreas of Japanese Medaka (Oryzias latipes) Fish Exposed to Graphene Oxide (Original data) (Figshare).

CRediT Author Statement

Asok K. Dasmahapatra: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Validation, Visualization, Writing – original draft, Writing – review & editing; **Paul B. Tchounwou:** Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Writing – review & editing.

Acknowledgments

The research was supported by NIH/NIMHD grant # G12MD07581 (RCMI Center for Environmental Health), NIH/NIMHD grant #1U54MD015929 (RCMI Center for Health Disparities Research) and NSF grant #HRD 1547754 (CREST Center for Nanotoxicity Studies) at Jackson State University, Jackson, Mississippi, USA

Supplementary Materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.dib.2023.109213.

References

[1] A.K. Dasmahapatra, D.K. Powe, T.P.S. Dasari, P.B. Tchounwou, Assessment of reproductive and developmental effects of graphene oxide on Japanese medaka (Oryzias latipes), Chemosphere 259 (2020) 127221, doi:10.1016/chemosphere.2020.127221.

[2] A.K. Dasmahapatra, D.K. Powe, T.P.S. Dasari, P.B. Tchounwou, Experimental data sets on the characterization of graphene oxide and its reproductive and developmental effects on Japanese medaka (Oryzias latipes) fish, Data Brief 32 (2020) 106218, doi:10.1016/j.dib.2020.10628.

[3] A. Myla, A.K. Dasmahapatra, P.B. Tchounwou, Sex-reversal and histopathological assessment of potential endocrine disrupting effects of graphene oxide on Japanese medaka (*Oryzias latipes*) larvae, Chemosphere 279 (2021) 130768, doi:10.1016/j.chemosphere. 2021.130768.

[4] A. Myla, A.K. Dasmahapatra, P.B. Tchounwou, Experimental data sets on sex-reversal and histopathological assessment of potential endocrine disrupting effects of graphene oxide on Japanese medaka (*Oryzias latipes*) larvae at the onset of maturity, Data Brief 38 (2021) 107330, doi:10.1016/j dib.2021.107330.

[5] T.E. Asala, A.K. Dasmahapatra, M. Myla, P.B. Tchounwou, Experimental data sets on the evaluation of graphene oxide as a thyroid endocrine disruptor and a modulator of gas gland cells in Japanese medaka (Oryzias latipes) larvae at the onset of maturity, Data Brief 39 (2021) 107625, doi:10.1016/j dib.2021.107625.
[6] T.E. Asala, A.K. Dasmahapatra, M. Myla, P.B. Tchounwou, Histological and histochemical evaluation of graphene oxide on thyroid follicles and gas gland of Japanese medaka larvae (Oryzias latipes), Chemosphere 286 (2021) 131719 2021. 131719, doi:10.1016/j.chemosphere.
[7] A.K. Dasmahapatra, P.B. Tchounwou, Histopathological evaluation of the interrenal gland (adrenal homolog) of Japanese medaka (*Oryzias latipes*) exposed to graphene oxide, J. Enviro. Toxicol. 37 (2022) 2460–2482 PMID 35809259, doi:10.1002/tox.23610.
[8] A.K. Dasmahapatra, P.B. Tchounwou, Experimental data-sets on the histopathological and immunohistological assessment of the interrenal gland (adrenal homolog) of Japanese medaka (*Oryzias latipes*) fish exposed to graphene oxide, Data Brief 45 (2022) 108693, doi:10.1016/j.dib 2022.108693.
[9] A.K. Dasmahapatra, P.B. Tchounwou, Evaluation of pancreatic δ-cells cells as a potential target site of graphene oxide toxicity in Japanese medaka (*Oryzias latipes*) fish, Ecotoxicol. Environ. Saf. 253 (2023) 114649, doi:10.1016/j.ecoenv.2023.114649.
[10] Guidance document of the diagnosis of endocrine-related histopathology in fish gonadsOECD Series on Testing and Assessment. No 123, OECD, Paris, 2010 https://www.oecd.org/chemicalsafety/testing/42140701.pdf.