Three-dimensional Construction of Micrometer Level in Rat Stomach by Synchrotron Radiation

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Research article

Keywords:  Synchrotron radiation phase-contrast imaging, 3-dimensional gastric structure images, different stages

DOI: https://doi.org/10.21203/rs.2.23423/v1

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Abstract

Objectives

This article shows an imaging method of the stomach that does not use imaging agents. X-ray phase-contrast images of different stages of gastric development were taken using X-ray in-line phase-contrast imaging (XILPCI). The aim of the study was to demonstrate that XILPCI is a micron imaging method for gastric structures.

Methods

The stomachs of 4-, 6- and 12-week-old rats were removed and cleaned. XILPCI has 1000 times greater soft tissue contrast than that of X-ray traditional absorption radiography. The projection images of the rats’ stomachs were recorded by an XILPCI charge coupled device (CCD) at 9 μm image resolution.

Results

The X-ray in-line phase-contrast images of the different stages of rat gastric specimens clearly showed the gastric architectures and the details of the gastroduodenal region. 3-dimensional stomach anatomical structure images were reconstruction.

Conclusion

The reconstructed gastric 3D images can clearly display the internal structure of the stomach. XILPCI may be a useful method for medical research in the future. Keywords: Synchrotron radiation phase-contrast imaging, 3-dimensional gastric structure images

Background

The stomach is an important organ in the alimentary track. The observation of the development of the stomach by imaging has not been previously realized. General clinical imaging of the stomach mainly employs gastroscopy. The gastroscopy image resolution is millimetre level. There are high-resolution images of X-ray absorption imaging of the human skeleton, but the poor images of the human abdominal organs. X-ray in-line phase-contrast imaging (XILPCI), has emerged as an imaging method. The imaging principle of XILPCI is the X-ray phase-change after an X-ray passes through objects. It is micrometre level image resolution of XILPCI of soft tissues.

XILPCI can be combined with computed tomography (CT). Phase-contrast CT is also diffraction CT. It will be a possible imaging method for soft tissues without the need for imaging agents. The micrometre level image resolution of XILPCI of soft tissues can reach 0.37 μm. XILPCI can obtain the more accuracy gastric structure. This type of XILPCI method could be a good research method in the future of medicine.
A growing number of studies have shown that intestinal development has obvious temporal characteristics and is regulated by many factors[1]. The rapid growth and functional maturation of the stomach and small intestine in newborns may reflect an adaptation process, which are exposed to an open environment and to process nutrient soon after birth the newborns. Morphometrical analyses revealed that the growth rates are greater in the gastric body region than in the cardiac and pyloric regions, and greater in the mucosal layer than in other layers. It has been shown in rats that gastric mucosal cell proliferation was elevated during early postnatal development[2]. There is evidence that growth of gut mucosal tissues is associated with an increased DNA synthesis rate[3] and a decreased cell turn over rate in neonatal animals[1, 4]. As the DNA synthesis in cells of the gut was investigated previously[5-7]. However, experimental studies of characteristic morphological in stomach and intestine of the rats, especially in XILPCI. So we choose the young and adults rats as the object of study.

**Methods**

**Setup and specimens**

Twenty-one Sprague-Dawley healthy male rats were purchased from the Animal Centre of Capital Medical University in China. The rats were randomly divided into three groups, seven rats per group. The rats, housed in cages under a controlled temperature of 22.0±1.0°C and 12 hours light–dark cycles, were fed on standard laboratory chow and water and allowed to acclimate for more than 7 days. The rats were starved for 12 hours and sacrificed under anaesthesia with an intraperitoneal injection with 5mg/100g of the concentration of 1% pentobarbital sodium solution at the three time point 4, 6 and 12 weeks of age. The stomachs of the rats were removed and the stomach cavity is filled with 10% formalin to maintain the morphological structure of the stomach for X-ray image scanning as shown in Fig. 1. These stomach tissues were cleaned with normal saline, fixed in 10% neutral phosphate-buffered formalin solution for 24 hours and embedded in paraffin. 5–6 μm thick of the specimens were obtained from the stomach and stained with HE staining. This animal study was in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal welfare Committee on the Ethics of Animal Experiments of Capital Medical University (Protocol NO. AEEI-2016-079) approved the experiment protocol. All surgery was performed under pentobarbital sodium anaesthesia, and all efforts were made to minimize suffering.

**Imaging principle of XILPCI**

XILPCI experiments were done at synchrotron radiation[8] facility. Synchrotron radiation is electromagnetic radiation which is emitted by relativistic charged particles traveling along a turning orbit under the action of electromagnetic field. Synchrotron radiation, as a light source, has an obvious high-brilliance and flux, wide energy spectrum and very short pulses.

The gastric XILPCI experiments were performed using the BL13W1 beamline of the Shanghai Synchrotron Radiation Facility (SSRF). The BL13W1 beamline partial facility of SSRF was depicted as shown in Fig. 2. A. Snigirev[9, 10] obtained phase contrast images by using a synchrotron radiation light
in 1995. The XILPCI method can use multi-colour light sources, therefore eliminating the need for the burdensome complexity of a monochrome system.

The complex refractive index $n$ can be used to describe XILPCI characteristics. The refractive index $n$ is smaller than the number 1 and $n$ formula is shown as:

$$n = 1 - \delta - i\beta$$  \hspace{1cm} (1)

After X-rays go through an object, their phase and amplitude change. Real component $d$ presents the phase changes, and imaginary part $b$ presents the amplitude attenuation. In XILPCI of lighter elements (C, H, O, etc.) of the object, $\delta$ is 1000 times greater than $\beta$, so the phase change quantity is much larger than the change quantity of X-ray absorption attenuation. There is a phase-contrast imaging of micron scale image resolution, so XILPCI images can show microstructures of objects.

**Steps of XILPCI**

Specific experimental methods: Firstly, in order to reduce the XILPCI artifacts caused by specimens deformation, the specimens are placed in the air for a period of time, and the specimens are dried. Secondly, the rat gastric dry specimens were wrapped with insulating materials and placed on the sample table.

After repeatedly evaluating different levels of X-ray energy, the X-ray energy for this experiment is 17.5 keV. The images were whiter if the energy was higher than 17.5 keV, and the imaging exposure time increased when the energy was lower than 17.5 keV. Images will be dark if the exposure time is not appropriate. It will take a longer time to shoot the more than 1000 images required for CT if the exposure time increases and gastric specimens will undergo serious deformation. Therefore, 17.5 keV is an optimal parameter of comprehensive factors. The distance was 59.3 m from the light source to the specimen. The detector was 60 cm from the specimen to CCD, with 9 $\mu$m image resolution and an exposure time of 8 ms. It took more 20 minutes to obtain XILPCI projection images of a gastric specimen by using 0.1 degree steps from 0 to 180 degrees over the gastric specimen.

**Histology and pathology scoring**

Stomach tissue specimens were dewaxed and made HE stain. Sections were fixed on microscope slides and observed with an Olympus DP72 MacroView (Japan). Histological scoring was based on a previously adapted scoring systems.

**Statistical analysis**

The data were analyzed by using GraphPad Prism 5.0 software package (GraphPad Software Inc., San Diego, CA, USA) of variance of the gastric wall thickness and all values were expressed as mean and standard error of mean (S.E.M.); $n$ was the number of animals in each experiment. The differences
among groups were analyzed using a one-way analysis of variance followed by Dunnett’s multiple comparison. A p value less than 0.05 was considered statistically significant. The power of the results was 86.5% by the software power analysis and specimen size software.

Results

Rats’ gastric specimens characteristics in XILPCI projection images

In order to be identification of the purpose of the experiment, the XILPCI projection images of gastric specimens are shown in Fig. 3. Changes were observed at different weeks of age on the gastric images. Fig. 3A demonstrates the characteristics of a 4-week-old young gastric normal specimen XILPCI image. The XILPCI image show that the gastric tissues are ordered and regular, and the gastric walls are smooth without any hyperplasia. The XILPCI image shows more detailed than the X-ray traditional image of a normal gastric specimen\[10-13\]. The absorption images of the stomach are very fuzzy and unclearly show the internal texture, and we only observe the overlapping walls of the stomach.

On XILPCI images, a 4-week-old image shows a uniform grey level, which indicates that the body of the 4-week-old gastric wall is as thick as the gastric fundus. It is obvious that the wrinkles of the fundus are abundant, and the fundus of the stomach is much thinner. In the middle of Fig. 3B and C, there is a demarcation line between the gastric body and the gastric fundus. Fig. 3B demonstrates the characteristics of a 6-week-old adult gastric normal specimen XILPCI image. The gastric wrinkles are more extensive, but the wrinkles of the 6-week-old gastric fundus are fewer than the wrinkles of the 4-week-old gastric fundus. The XILPCI images show the grey of a 6-week-old gastric fundus is lighter than that of a 4-week-old fundus. The body of the 6-week-old stomach is thicker than the body of the 4-week-old stomach. The performance of XILPCI images is that the grey of a 6-week-old gastric body image is deeper than that of 4-week-old image. Fig. 3C presents a 12-week-old normal gastric specimen XILPCI image, demonstrating the characteristics of mature gastric normal tissues. The wrinkles in the walls of the gastric body and duodenum are the most abundant.

CT images of the same rats’ gastric specimens

The XILPCI 3-dimensional slices were rebuilt by means of a filter back projective algorithm. The details can be visualized inside the gastric tissue from Fig. 4.

The results are shown in Fig. 4 observed under the gross anatomy. The gastric inner surfaces clearly presented longitudinal branching wrinkle and gastric pits. The d, e and f parts of the gastric specimens in Fig. 4 were taken out and fixing in formalin solution. The d, e and f part of Fig. 4 could display the same shape as Fig. 5, but XILPCI images could not show the clear internal structure the same as Fig. 5. At present, the gold standard for diagnosis is still biopsy. Hematoxylin-eosin(HE) staining process was as follows: these gastric specimens were dehydrated and dried, and then the d, e and f part of specimens were embedded in paraffin. Paraffin-embedded sections were made into pathological section and stained with HE to evaluate general morphology. In the body portion of the stomach after HE staining, the
micrograph and macrograph showed a cross section of the gastric wall (Fig. 5). Similar to the other parts of the gastrointestinal tract, there are four layers of structure in the gastric wall, which are an outer mucosa, inner submucosa, muscular external layer, and serosa. During the growth and development of the rat, the fundus, formed by the upper curvature of the organ from the muscular external layer, is the thickest part at all three ages (Fig. 5d, e, f). There is an obvious line between the fundus and body of the stomach. There is a palpable mucosa layer, but there are also overt differences seen on HE staining, and we can see that the mucosa layer in the 4-week-old rats show more alkalinity than that in the 6- and 12-week-old rats, especially at 12 weeks (Fig. 5f). Fig. 5d e and f can also observe line between the fundus and body of the stomach.

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Three-dimensional reconstruction images of the same rats’ gastric specimens

In order to further reconfirm the advantage of the the XILPCI 3, were reconstructed 3-dimensional images of the different stages of the stomach as shown in Fig. 6. The XILPCI 3-dimensional images show a clear structure of the interior of the stomach (showed as video 1), and we can clearly see the villus of the fundus of the young rat stomach. There is a clear demarcation line between the fundus and the body of the stomach. The walls of the 12-week-old rat’s stomachs are thicker than those of the 4-week-old rat’s stomachs. The conclusion here shows that XILPCI has high anatomical accuracy to image stomachs. In addition, the thickness of the gastric wall at various stages was measured in Fig. 6 to compare the thickness of gastric specimens. As Fig. 7 shown, the results indicate that the average values of gastric wall at same stages are very similar, and the measurement of gastric wall by 3-dimensional images in Fig. 6 has certain reference significance. In Fig. 7, the thickness of 4-week-old gastric wall for the right panel is lower than that of the left panel, but the thickness of 6-week-old gastric wall is higher for the right
panel because of the randomness of specimen selection and the existence of measurement error. The above results are appeared. But these results are also in line with the requirements of statistics and within a reasonable range.

Discussion

Limitations of XILPCI must be overcome if XILPCI is to have wider applications. The one limitation is that takes around one hour to shoot a larger specimen because the larger specimen must shoot images in several segments. The experiment is only good image effect for static specimens, so the device must to be improved to shorten the imaging time required for living specimens. In addition, XILPCI will occur to movement artifacts when the specimens being taken are moving. There is common imaging defect to CT imaging equipment. In this experiment, because of the high resolution of the imaging, only a little movement can produce imaging artifacts. Specimens shrinkage occurs when the specimens are taken on the sample table. The shrinkage of the specimens under X-ray irradiation also produces movement artefacts. The current solution is to make the specimen as dry as possible and shorten the experiment time. Movement artefacts are also the factor that affect the image quality, and are also the problem that needs to be further solved in the later research.

The CCD camera of BL13W1 of SSRF can obtain a resolution of 0.37 μm, and XILPCI is helpful for angiography. At present, Applications on humans in the field of phase contrast mammography have been reported by using both synchrotron radiation and conventional sources[14] With further development, XILPCI would be a valuable imaging method for medical research.

Conclusion

In summary, we have applied the XILPCI method to imaging of the rat's stomach without the need for imaging agents. The XILPCI projection images showed that the development of the normal gastric structure may cause thickening of the gastric wall and coarseness of the gastric texture.

XILPCI is an X-ray phase variation imaging method that differs from X-ray traditional absorption imaging methods. The XILPCI method can achieve micron-scale image resolution of biological tissue. The ordinary micro-focus X-ray sources can be instead of synchrotron radiation sources from XILPCI. It is possible that the XILPCI method will be widely applied in the future at a low cost because of this advantage.

Electronic Supplementary Material

Video 1 3D video of a 4-week-old rat's stomach

Declarations
Availability of data and materials

The datasets generated during the current study are available from the corresponding author on reasonable request.

Abbreviations

XILPCI: X-ray in-line phase-contrast imaging

CCD: charge coupled device

BL13W1: Imaging and Biomedical Application Beamline

SSRF: Shanghai Synchrotron Radiation Facility

3D: three dimensional

Acknowledgement

Our deeply gratitude goes the assistance of all the staff in BL13W1 from Shanghai Synchrotron Radiation Facility.

Role of funding

This study was partly supported by the National Natural Science Foundation of China (Grant No. 81673671 and 81274173) for the rat research in XILPCI method to imaging; the Natural Science Foundation of Beijing (Grant No. 7144189 and 7122017) for the research assistant fee of the students in this experiment and the transportation fee to and from Beijing and Shanghai; and the Science Foundation of Capital Medical University (Grant No. 17ZR24) for SZY learning computer simulation reconstruction technology includes learning and training.

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Contributions
QT completed the experimental data, worked on the algorithm design, measured the thickness of gastric wall and drafted the manuscript. JDX conceived the study, contributed to specimen preparation and conclusions and manuscript modification. XHT and CCG contributed to the animal handling. SZY and TTW reconstructed the 3-dimensional reconstruction images. All authors read and approved the manuscript.

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Ethics declarations

Ethics approval and consent to participate
Informed consent was sought and granted (AEEI-2016-079) in accordance with the ethical recommendations of Guide for the Care and Use of Laboratory Animals of the National Institutes of Health on animal research.

Consent for publication
Consent for publication is inherent in the ethics granted. More information available on request.

Competing interests
The authors declare that they have no competing interests.

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Provenance and peer review: Not commissioned; externally peer reviewed.

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**Figures**

*Figure 1*

Rat gastric specimens. (a) A 4-week-old gastric specimen. (b) A 6-week-old gastric specimen. (c) A 12-week-old gastric specimen. 1 Cardia·2 Gastric corpus·3 Pylorus. These are the positions noted on the gross anatomy.
**Figure 2**

Schematic diagram of BL13W1 beam line of SSRF. The light source is used for the calibration location of the light, specimen and the CCD. A multidimensional sample table. The specimens are placed on the sample table to rotate and the specimens’ images are then obtained at different angles. An X-ray CCD. It obtains specimens’ projective images with high-resolution. Data processor. It can calculate optical density from the CCD to the specimens and image conversion.
Figure 3

XILPCI projection images of rat gastric specimens. (A) A 4-week-old specimen. It shows that the gastric walls are smooth. (B) A 6-week-old specimen. It shows that the gastric walls inside the stomach are uneven. (C) A 12-week-old specimen. It shows thick gastric body walls and more gastric body wrinkles. 1 Cardia, 2 Gastric body, 3 Gastric fundus, 4 Pylorus.
The XILPCI transverse CT image of the rats’ gastric specimens in Fig. 3. (a) The transverse image along the black line a as shown in Fig. 3. It is a transverse CT image of a 4-week-old rat stomach. It can clearly show that there are grey changes in different gastric structures. There is the same thickness for the body and the fundus of the stomach because the XILPCI image shows the same width of gastric wall, and it is obvious that there are multiple bulges in the fundus of the 4-week-old rat stomach. (b) The transverse image along the black line b as shown in Fig. 3. The XILPCI image shows the wider the body of stomach than the fundus. There is a thicker the wall of gastric body than that of the fundus. This obviously shows that there are grey changes in the different gastric structures and the duodenum. The bulges have decrease in the fundus of the 6-week-old rat stomach. (c) The transverse image along the black line c as shown in Fig. 3. It can obviously be seen that there are many gastric body wrinkles in the 12-week-old gastric specimen. 1 Cardia, 2 Gastric body, 3 Gastric fundus, 4 Pylorus.
Figure 5

Rats gastric HE staining images. (d) A 4-week-old gastric specimen; (e) A 6-week-old specimen; (f) A 12-week-old gastric specimen.

Figure 6

3D anatomical structure images of the coronal plane of different stages of the stomach. (A) 3D internal structure image of the 4-week-old rat stomach. The stomach has obvious wrinkles in the fundus part, and there is a clear demarcation line between the fundus and the gastric body. The shape of the stomach is round. (B) 3-dimensional internal structure image of the 6-week-old rat stomach. The wrinkles of the fundus part are decreasing, and the gastric body is becoming rugged. (C) 3-dimensional internal structure image of the 12-week-old rat stomach. The fundus part of the stomach has become smooth. The shape of the stomach is an ellipse. ▲ represents the wrinkles of the fundus of the stomach. ▲ is the same size in the A B and C part of Fig. 6 and represents the thickness of the wall of the gastric body. 1 Cardia, 2 Gastric body, 3 Gastric fundus, 4 Pylorus.
Figure 7

The comparison results of the thickness of the gastric wall at various stages in Fig. 6 with gastric specimens. The thickness of the gastric wall of the specimens is significant increased from 0.71±0.04mm to 0.99±0.05mm, 1.33±0.32mm about 28.28%(p<0.001, n=7), 46.61%(p<0.001, n=7) in the 4 weeks, 6 weeks and 12 weeks postnatal respectively. While the thickness of gastric walls calculated as shown in Fig. 6, were marked enhancement from 0.62±0.024mm to 1.13±0.02mm, 1.34±0.04mm about 45.13% (p<0.001, n=7) and 53.73% (p<0.001, n=7). All the data at the same stage has no difference. Data represents mean ± S.E.M. (***P<0.001, △△△P<0.001, ▲▲▲P<0.001). ***4-week-old vs 6-week-old, △△△4-week-old vs 12-week-old, ▲▲▲6-week-old vs 12-week-old.

Supplementary Files

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