

Structural analysis of a micron-sized Cu⁰ in a person with diabetes's insulin ball

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Supplementary Information

Supplementary Methods

Detection of Cu deposits in the FFPE specimen

4 μm -sliced FFPE specimens were deparaffinized with xylene, washed with 100% methanol, 70% methanol, then distilled water, and dried completely. The FFPE specimen attached to the glass slide was fixed to the specimen holder of the SEM (JSM-IT510 (JEOL, Tokyo, Japan)) with conductive tape, and the specimens were observed at a voltage of 150 kV. The FFPE specimen of the case in which 100 μm metallic copper (Cu^0) had been found was used as a positive control. Many deposits were observed in the insulin ball and elemental analysis of metal deposits larger than 1 μm was confirmed by this simple SEM-EDS analysis. Four cases were randomly selected from 21 cases to perform SEM-EDS analysis; in three of the four cases, copper-containing deposits were identified in the FFPE specimens of insulin balls.

SEM-EDS analysis of the blade used for trimming the insulin ball tissue

To rule out the possibility of copper contamination from outside the body, the blade used to trim the extracted tissue (HISTO CUTTER PLASMA LH35, Muto Pure Chemicals Co., LTD., Tokyo, Japan) was analysed by SEM-EDS (JSM-IT510 (JEOL, Tokyo, Japan) at a voltage of 150 kV.

MALDI-TOF-MS analysis on the unfixed sample of the insulin ball

Unfixed insulin ball sample that had been frozen and stored at -80°C was fixed with methanol. After dissolving dimethyl sulfoxide (DMSO), the sample was mixed with DHB (2-hydroxy-5-methoxybenzoic acid). A JMS-S3000 SpiralTOF™ (JEOL, Tokyo, Japan) was used for measurements in spiral mode in high mass resolution mode.

Supplementary Table 1

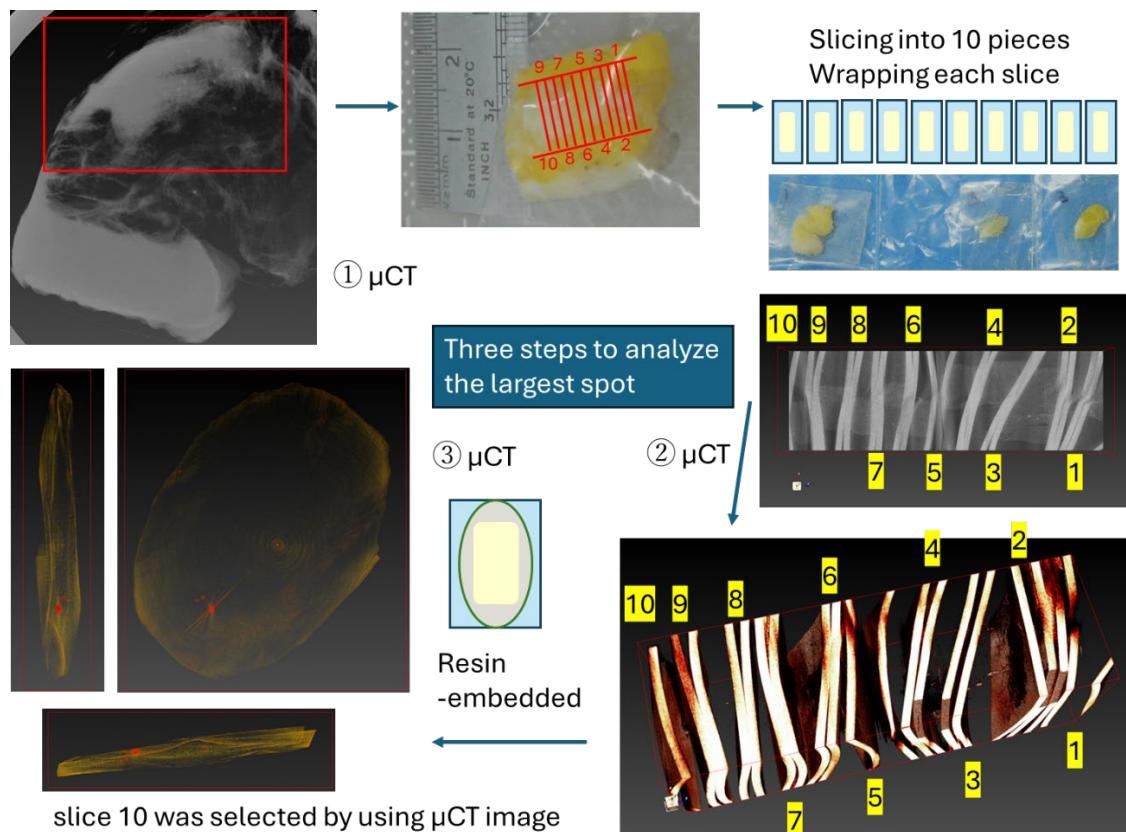
Table 1 Copper fragments detected in four FFPE specimens of insulin balls

	Copper presence	number	size (μm)	composition	distribution
PC	+	2	6 × 3	Cu, Zn	within IB
			6 × 5	Cu, Zn, Fe	within IB
1	+	1	11 × 7	Cu, Ni, Fe	within IB
2	+	1	1 × 1	Cu, Sn	within IB
3	—	—	—	Fe, Al	—
4	+	2	2 × 2	Cu, Zn	within IB
			7 × 1	Cu, Fe, Zn	within IB

PC; positive control

IB; insulin ball

Supplementary Figures (1 to 4)

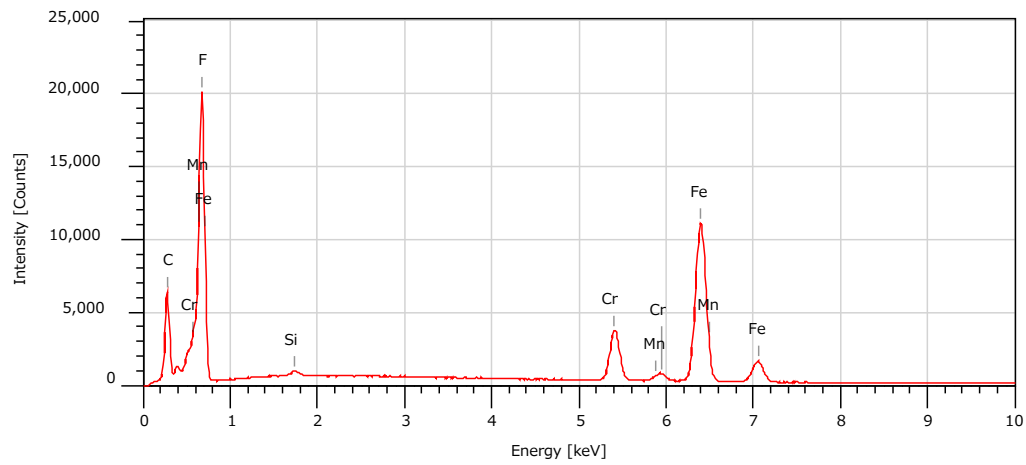


Supplementary Figure 1: Procedure to find out the largest bright spot

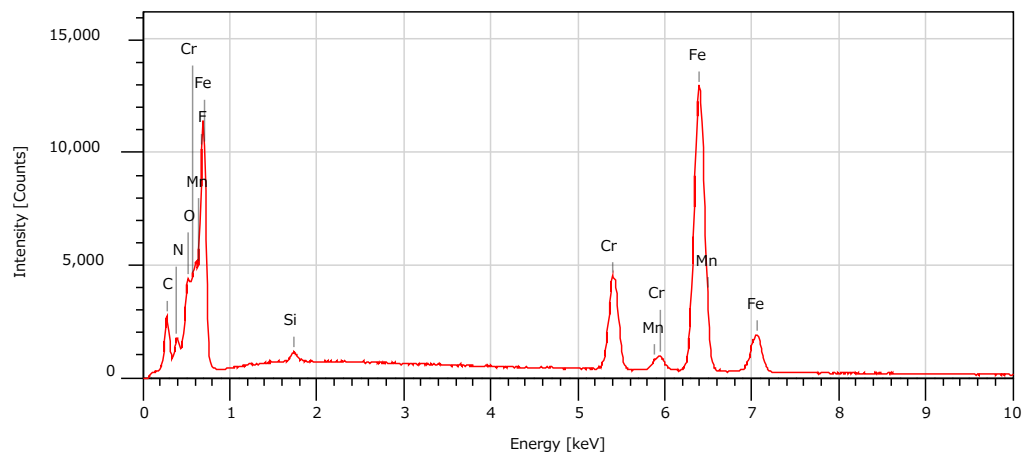
μ CT scans were performed three times to isolate the largest bright spot from the tissue.

- 1: μ CT of the entire insulin ball tissue was taken in the first step.
- 2: The insulin ball section was divided into 10 slices and μ CT image of all the divided slice was taken. The largest bright spot was included in the slice10.
- 3: After the slice 10 tissue was resin-embedded, μ CT image of slice 10 was taken. The largest bright spot was carefully cut out using the CT image as a guide.

a



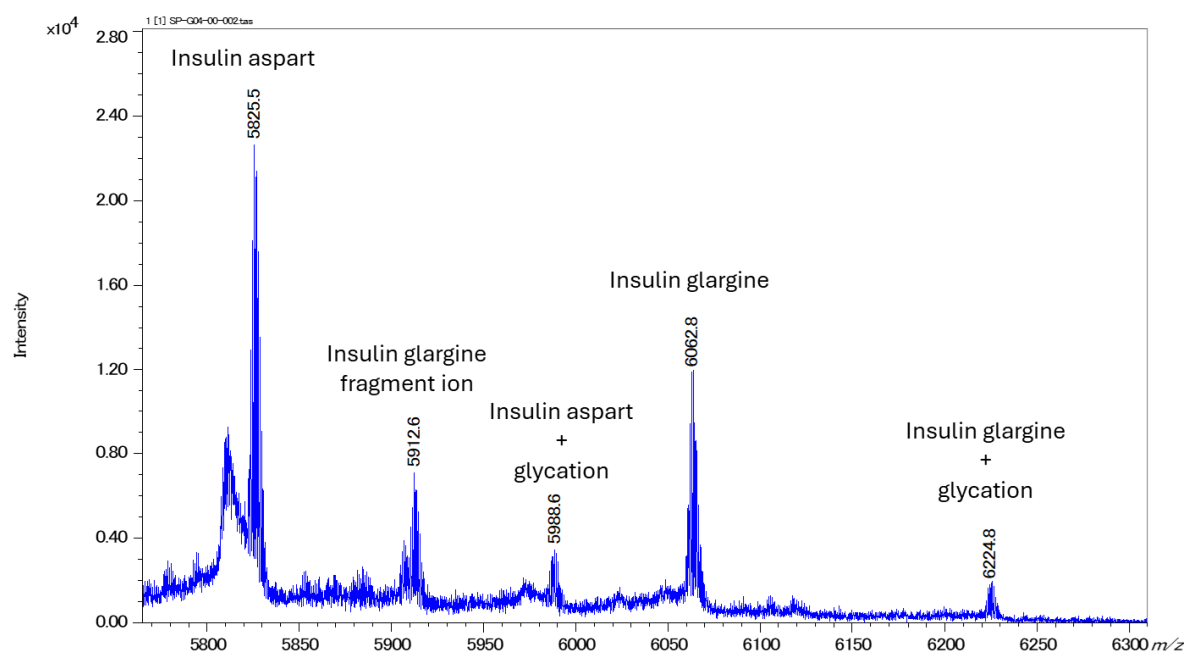
b



Supplementary Figure 2: SEM-EDS analysis of the blade used for trimming the insulin ball tissue

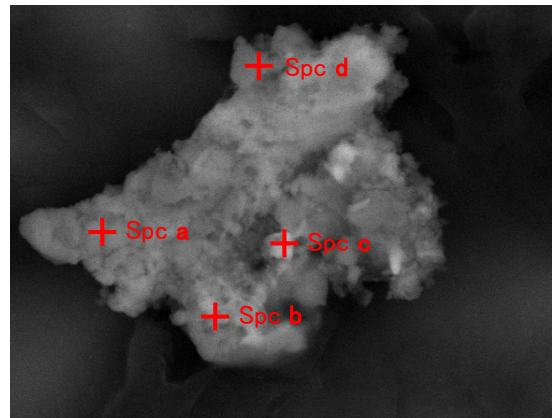
a: The cutting edge of the blade was made of stainless-steel (Fe (52.5%) and Cr (9.5%)). Fluorocarbon processing was applied to improve sharpness, and C (17.9%) and F (19.4%) were detected.

b: The main body of the blade was made of Fe (71.3%) and Cr (13.2%), confirming that it was a basic stainless-steel. The blade was plasma nitrogen treated, and N and F were detected. No Cu was detected.



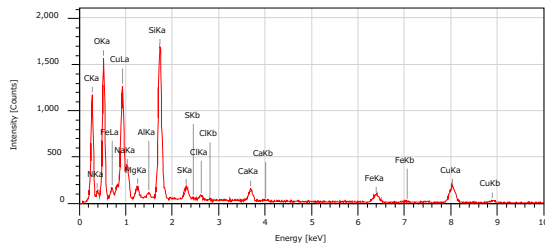
Supplementary Figure 3: MALDI-TOF-MS analysis on the unfixed sample of the insulin ball

Methanol-fixed sample showed glycation of both insulin aspart and insulin glargine, however, no apparent insulin oxidation, e.g. of the disulfide bonds, was observed.

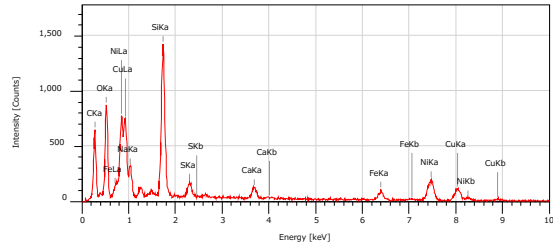


2 μ m

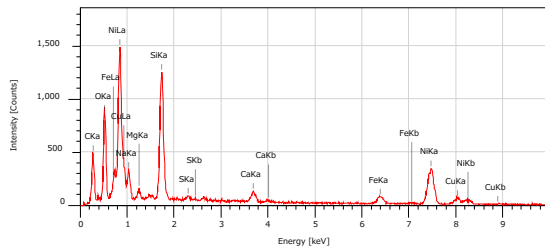
Spec a



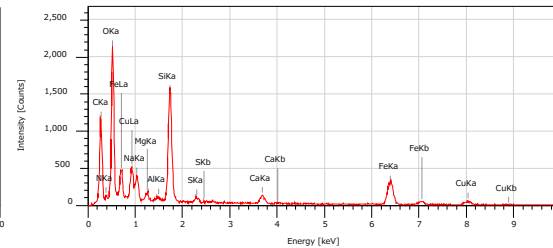
Spec b



Spec c



Spec d



Supplementary figure 4: Representative image of copper deposit of FFPE specimen by SEM-EDS analysis

The deposit was composed of mainly Cu mixed with Ni, and Fe.

Cu (18.3%), Fe (3.7%) was detected on Spec **a**.

Cu (15.3%), Ni (18.5%), Fe (4.4%) was detected on Spec **b**.

Cu (7.4%), Ni (34.2%), Fe (3.6%) was detected on Spec **c**.

Cu (4.5%), Fe (14.7%) was detected on Spec **d**.