A comparative analysis of tissue dissociation efficiency and cell viability (Adipose tissue)

This study is designed to compare the tissue dissociation efficiency and viability of dissociated cells between TDzyme® (CONNEXT) and other two commercial collagenases, Liberase™ TM (Roche), Collagenase type IV-S (Sigma-Aldrich) in the primary cell culture process of adipose tissue.

Materials and Methods

Tissues used

Adipose tissue: Gonadal & retro-peritoneal adipose tissue of SD rats (2 rats aged 8 weeks)

Tissue sampling

Cardiac perfusion	Blood was removed from the adipose tissue using the rat's blood circulation. For the adipose tissue sampling, two rats were anesthetized with 1g/kg urethane. The chest of the anesthetized rat was exposed to have a needle with an extension line inserted into the apex of the left ventricle, and then the right auricle was incised. Sterile saline (0.9%) was added into the extension line using a perfusion pump at a rate of 10-12 mL/min to drain the blood from the rat.

The colors of liver and kidney tissues were observed, and the blood was confirmed to have drained completely from the rat. The extension line and needle connected to the heart were removed upon confirmation of blood removal from most organs, and the gonadal adipose tissue and retro-peritoneal adipose tissue that lies on the caudal surface of the kidney were collected using sterilized scissors and forceps. The collected adipose tissue was moved to sterile phosphate-buffered saline (PBS; with antibiotics, 4°C) and washed three times for 20 minutes at a time using sterile PBS. And then, the blood residue, connective tissues, and any other tissues were cut out from the washed adipose tissue to obtain only the adipose tissue. Trimmed adipose tissue was broken into small pieces by the homogenizer (12,000 rpm, 60 seconds) with the addition of 12 mL of sterile DMEM. The electronic pipette was then used for suspension. This suspended tissue solution was separated into 12 of 5 mL tubes (n=4). Through the same process, 24 samples (n=8) were obtained from two adipose tissue pieces of two SD rats.

Preparation of solution for each enzyme

Tissue type	TDzyme® C, T (Connext)	Liberase™ TM (Roche)	Collagenase Type IV-S (Sigma-Aldrich)
Adipose tissues	Diluent: Ice-cold Milli-Q water Concentration: 100 μg/mL Incubation: 30 min, 37°C	Diluent: Injection quality-sterile water or tissue-dissociation buffer Concentration: 100 μg/mL Incubation: 30 min, 37°C	Diluent: Krebs Ringer buffer with calcium and BSA Concentration: 100 μg/mL Incubation: 30 min, 37°C

The collagenases (TDzyme®, Liberase™ TM, and Collagenase type IV-S, n=8) with a concentration of 100 μg/mL were used to dissociate tissue into cells in a 5 mL tissue solution. The 24 tubes of the tissue solution containing enzyme were placed on the tube roller for 30 minutes at 37°C for mixing. Then each solution was filtered using a 70 μm cell strainer and collected into a 50 mL tube. Centrifugation (20°C, for 5 min at 250 G (1084 rpm, 190 mm (rotor)) was performed after adding 15 mL of sterile DMEM to the filtered solution. The supernatant liquid was then discarded. Red blood cells were removed from the remaining

solution. After removing the supernatant liquid from the centrifuged solution, 10 µl of the remaining solution was taken and stained with 10 µl of trypan blue. It was then assessed for cell viability with equipment, and the remaining solution was dispensed onto a cell culture plate and cultured (5% CO2, 37.6°C).

Assessment of cell dissociation efficiency

The trypan blue staining assay was used to count the number of cells dissociated after treating with collagenase using the cell counter. Quantitative comparison was made between living and dead cells per mL.

Primary culture and assessment of cell viability

The dissociated cells were cultured for 24 and 72 hours in the culture media to conduct the cell proliferation assay (MTT assay).

Tissues	Culture Conditions
Adipose tissues	Advanced DMEM, 10mM HEPES, 10% FBS, 37°C CO2 incubator

Results

Tissue dissociation efficiency

The number of cells dissociated from adipose tissue was counted after treating adipose tissue with three different collagenases, and the dissociation rate for each collagenase was evaluated. The total number of cells dissociated from adipose tissue on average was 0.13 x 10^6 for TDzyme® and 0.11 x 10^6 for Liberase™ TM, and there was no statistically significant difference ($p > 0.05$). Collagenase type IV-S was 0.07 x 10^6 cells, significantly lower than those for TDzyme® and Liberase™ TM ($p < 0.05$).

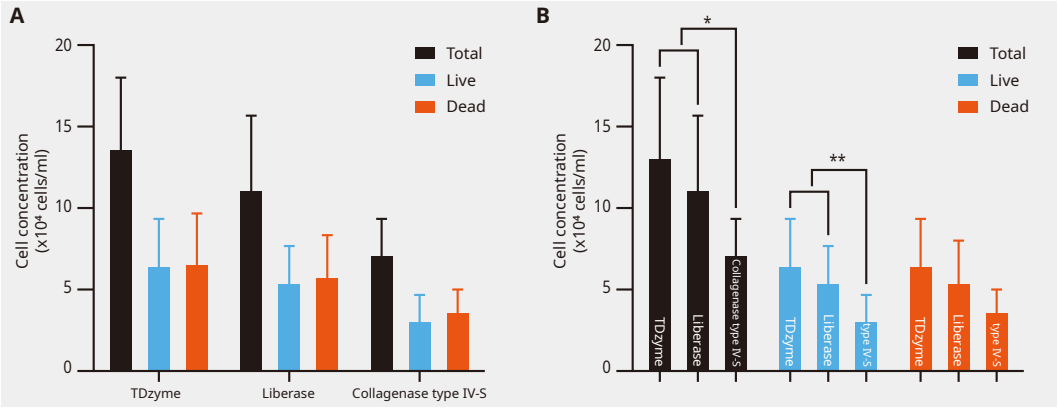

Fig 1. Comparison of dissociation efficiency among enzymes

The number of live cells and their viability were examined in the total number of cells from adipose The number of living cells and their viability were examined in the total number of cells from adipose tissue. The average number of living cells was 0.065 x 10^6 for TDzyme® and 0.053 x 10^6 for Liberase™ TM, and there was no statistically significant difference ($P > 0.05$). There were 0.03 x 10^6 cells when using Collagenase type IV-S, which significantly decreased compared to TDzyme® and Liberase™ TM ($p < 0.05$). There was no statistically significant difference found when comparing the cell viability for TDzyme®, Liberase™ TM, and Collagenase type IV-S as their viability was 48.83%, 49.32%, and 43.27%, respectively ($P > 0.05$).

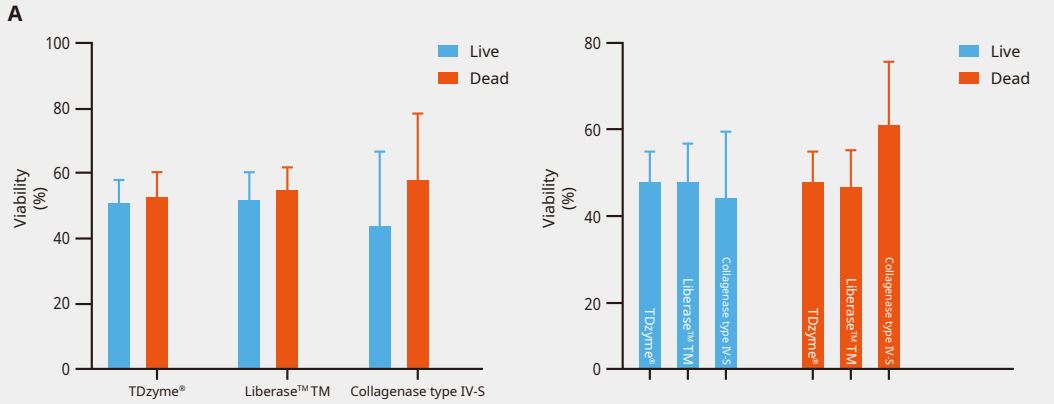

Fig 2. Comparison of cell viability

Cell viability (Cultured primary cells)

In order to observe the cultivation of cells obtained from adipose tissue, the cultured cells were examined under the microscope after cultivating for 72 hours and removing culture media. A large number of growing cells attached to the cell culture plate could be noticed clearly for TDzyme® and Liberase™ TM. (Red arrow). However, cells were rarely observed for Collagenase type IV-S. The MTT assay was performed to assess the viability of cells attached to the cell plate. In calculating the MTT assay results with TDzyme® as 100%, the average viability for Liberase™ TM was 70.37%, and that for Collagenase type IV-S was 5.22%. Compared to TDzyme® and Liberase™ TM, Collagenase type IV-S showed a statistically significant lower value ($p < 0.0001$).

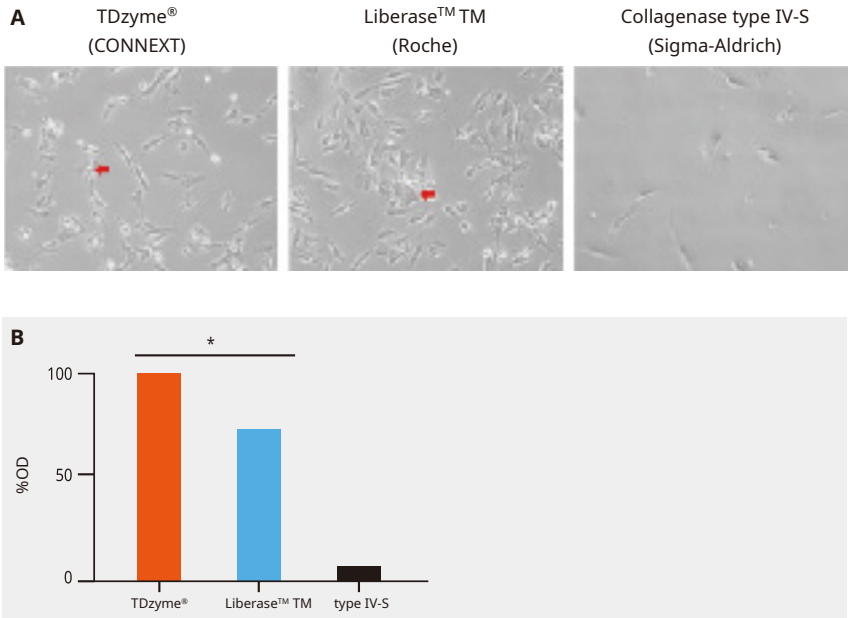

Fig 3. The patterns of cultured cells and the assessment of cell viability

A. The cells from adipose tissues were cultured on the 12-well cell culture plate; after 72 hours, the upper layer of culture media was discarded to film the patterns of cultured cells. Attached to the surface of the plate, a lot of differentiated cells were detected for TDzyme® and Liberase™ TM; for Collagenase type IV-S, however, a noticeably smaller number of cells were detected with the naked eye (Red arrow).

B. A graph assessing the viability of cells attached to the cell plate via MTT assay. The values of Liberase™ TM and Collagenase type IV-S (C1889) are shown relative to TDzyme® calculated as 100%. In the case of Collagenase type IV-S, a statistically reduced value was obtained compared to TDzyme® and Liberase™ TM (* $p < 0.0001$).

Discussion

When it comes to the tissue dissociation rate for each enzyme, the total number of cells dissociated from the adipose tissue for TDzyme® and Liberase™ TM was similar. In contrast, Collagenase type IV-S showed a significantly lower number of dissociated cells than the other enzymes. All three enzymes showed similar patterns in the cell viability of the cells dissociated from tissues. However, the number of living cells for TDzyme® and Liberase™ TM was found to be significantly bigger than that for Collagenase type IV-S.
The patterns of cultured cells and viability of cells attached to the cell plate were examined through the MTT assay. TDzyme® showed similar patterns of cells proliferating on the cell plate compared to those for Liberase™ TM. According to the MTT assay, TDzyme® showed better results than Liberase™ TM although the difference was not statistically significant. However, there was a significantly reduced number of proliferating cells attached to the cell plate for Collagenase type IV-S compared to the other two enzymes; even the MTT assay result showed a significant decrease, compared to that of the other two enzymes and a statistical significance was observed.
In the experiment on the adipose tissue dissociation, TDzyme® showed a similar pattern in the number and viability of cells detached from the tissue, compared to Liberase™ TM. However, Collagenase type IV-S showed a significantly low dissociated cell number and cultured cell viability in the same experiment, compared to the other two enzymes.

Conclusion

In the comparative experiment, adipose tissue was collected from SD rats. The tissue samples were then treated under the same conditions with different collagenases to compare the efficiency of TDzyme® with two commercial collagenases, Liberase™ TM and Collagenase type IV-S. TDzyme® was similar to Liberase™ TM in terms of tissue dissociation efficiency, dissociated cell viability, and cultured cell viability of the adipose tissue, and better than Collagenase type IV-S based on the obtained result. In conclusion, TDzyme® is shown to have similar efficiency to Liberase™ TM in the in vivo study while having significantly higher efficiency than the Collagenase type IV-S.