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

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

Materials and Methods

Tissues used

Liver tissue of 3 ICR mice aged 5-7 weeks

Tissue sampling

For the liver tissue sampling, three mice were anesthetized with 1g/kg urethane. The abdominal cavity of the anesthetized mouse was then exposed, and the small intestine was moved to the right to reveal the liver, kidney, caudal vena cava, and portal vein. Blood perfusion was performed using PBS (sterilized solution with antibiotics) injected into the portal vein with a 27-gauge needle. After the liver sufficiently swelled, an incision was made on the portal vein to check the changes in the color of the liver while draining the blood. After the liver tissue got collected, it was moved into the sterilized PBS (with antibiotics, 4°C). Connective tissue, gallbladder, and cystic duct were removed from the liver tissue except for liver parenchyma. Liver tissue was minced using a sterilized razor blade, and then it was put into a 15 mL tube where it contained 9 mL of the sterilized DMEM. After suspension using the electric pipette, it was divided into 1 mL and placed in nine 5 mL tubes (n=3). Through the same process, 27 tissues (n=9) were obtained from three liver tissues of three ICR mice.

Preparation of each enzyme solution

Tissue type	TDzyme®C, T (Connext)	Liberase™ TM (Roche)	Collagenase Type IV-S (Sigma-Aldrich)
Liver tissue	Diluent: Ice-cold Milli-Q water Concentration: 40 µg/mL Incubation: 30 min, 37°C	Diluent: Injection quality-sterile water or tissue-dissociation buffer Concentration: 40 µg/mL Incubation: 30 min, 37°C	Diluent: Krebs Ringer buffer with calcium and BSA Concentration: 40 µg/mL Incubation: 30 min, 37°C

The collagenases (TDzyme®, Liberase™ TM, and Collagenase type IV-S, n=9) with a concentration of 40 µg/mL were used to make a 5 mL tissue solution. The 27 tubes of the tissue solution containing enzyme were placed on the tube roller for 30 minutes at 37°C for mixing. The solution was filtered using a size 70 µm cell strainer. The supernatant liquid was discarded after centrifugation (4°C, for 2 min at 50 G (485 rpm, 190 mm (rotor)) by adding the filtered solution to the 15 mL tube. Red blood cells were removed from the remaining solution by using an RBC lysis buffer for a minute. Centrifugation (4°C, for 2 min at 50 G (485 rpm, 190 mm (rotor)) was performed again after neutralizing the RBC lysis buffer by adding 5 mL of DMEM to the remaining solution. After removing the supernatant liquid from the centrifuged solution, 10 µl of the remaining solution was taken and stained with 10µl 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).

Tissue type	Culture Conditions
Liver tissue	Advanced DMEM, 10mM HEPES, 10% FBS, 37°C CO2 incubator

Results

Tissue dissociation efficiency

The number of cells dissociated from liver tissue was counted after treating liver tissue with three different collagenases, and then the dissociation rate for each collagenase was evaluated. The average number of cells dissociated from the liver parenchyma was 0.29 x 10^6 cells for TDzyme® and 0.33 x 10^6 cells for Liberase™ TM. The total number of cells from Liberase™ TM did appear to increase compared to that of TDzyme®, but the difference was not statistically significant ($p > 0.05$). Collagenase type IV-S had 0.51 x 10^6 cells, which is significantly larger compared to TDzyme® and Liberase™ TM ($p < 0.05$).

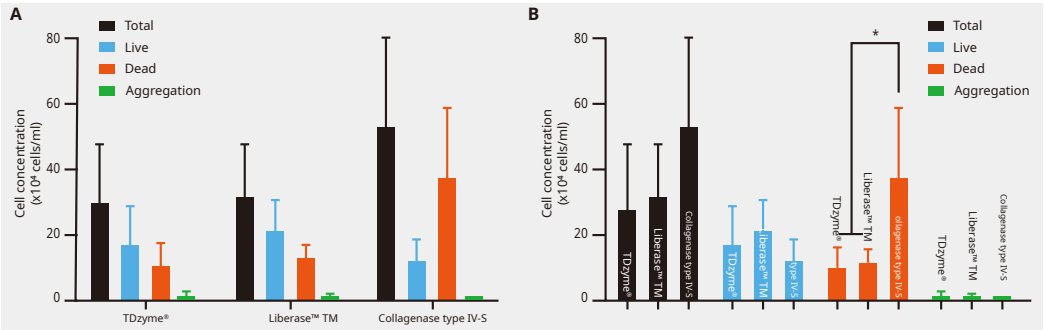

Fig 1. Comparison of dissociation efficiency among enzymes

The number of living cells and their viability in the total number of cells from liver tissues were examined. The average number of living cells was 0.17 x 10^6 for TDzyme® and 0.20 x 10^6 for Liberase™ TM, and their viability was 58.08% and 59.35%, respectively. The number of living cells and their viability in the liver obtained using TDzyme® were not significantly different from the results obtained using Liberase™ TM ($p > 0.05$). The number of surviving cells and their viability in the liver obtained using Collagenase type IV-S were 0.13 x 10^6 and 25.76%, respectively, and these numbers were significantly lower compared to those of TDzyme® and Liberase™ TM ($p < 0.05$).

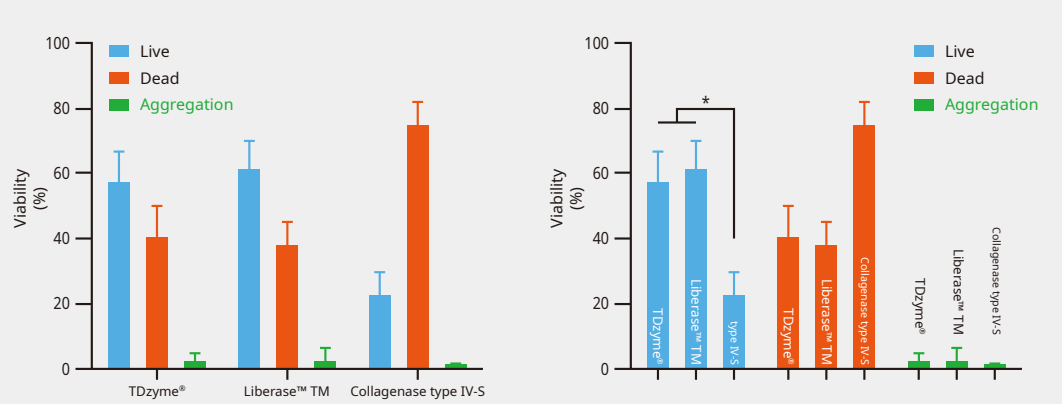

Fig 2. Comparison of cell viability

Cell viability (Cultured primary cells)

In order to observe the cultivation of cells obtained from liver tissue, the cultured cells were examined under the microscope after cultivating for 72 hours and removing culture media. A large number of dissociated cells attached to the cell culture plate could be noticed clearly for TDzyme® and Liberase™ TM (Red arrow). However, the number was significantly lower 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 154.78%, and that for Collagenase type IV-S was 22.05%. Compared to TDzyme® and Liberase™ TM, Collagenase type IV-S showed a statistically significant lower value ($p < 0.05$).

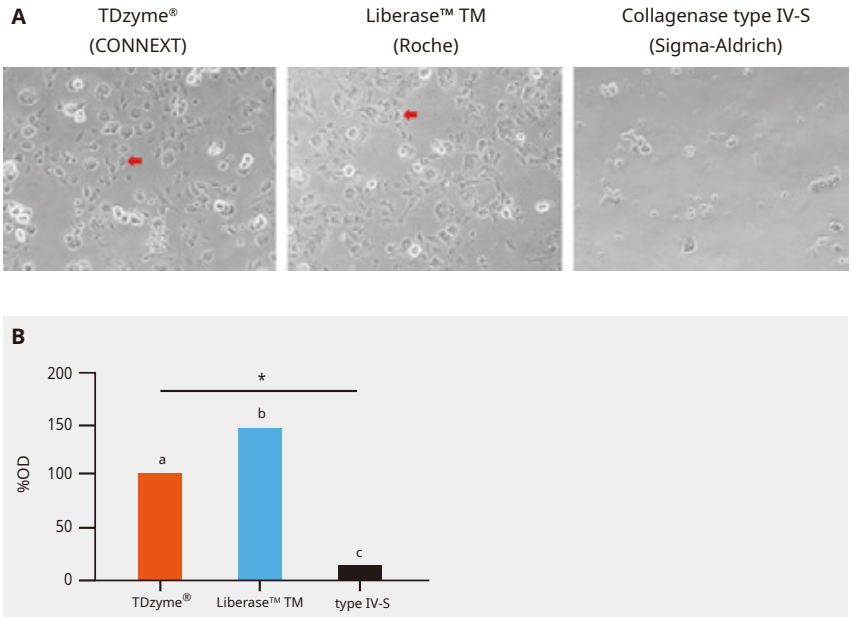

Fig 3. The patterns of cultured cells and the assessment of cell viability

A. The cells from liver tissue were cultured on a 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 are shown relative to TDzyme® calculated as 100%. C is statistically significant compared to others (* $p = 0.0441$).

Discussion

When it comes to the cell dissociation rate for each enzyme in the liver tissues, the total number of cells dissociated from the liver tissues for TDzyme® and Liberase™ TM was similar. In contrast, Collagenase type IV-S showed a significantly larger number of dissociated cells than the other enzymes. In the case of TDzyme® and Liberase™ TM, the percentage of live cells was similar at 57.08% and 59.35%, respectively. However, Collagenase type IV-S showed a statistically significant lower percentage of 25.76%. In other words, the percentage of viable cells is very small despite a large number of cells obtained through Collagenase type IV-S. In the case of TDzyme®, albeit fewer than Collagenase type IV-S the obtained number of cells and the viability were similar to that of Liberase™ TM.
The patterns of cultured cells and viability of cells attached to the cell plate were examined through the MTT assay. TDzyme® showed patterns of cells proliferating on the cell plate similar to those for Liberase™ TM; even the result of the MTT assay showed no statistically significant difference. However, there was a significantly reduced number of proliferating cells attached to the cell plate for Collagenase type IV-S and the MTT assay result also showed a statistically significant drop compared to that of the other two enzymes. The viability of cells dissociated from the liver tissue for TDzyme® was similar to that of Liberase™ TM. Since there was no statistically significant difference in the viability of cultured cells and the patterns of cultured cells, the study results of TDzyme® are similar to those of Liberase™ TM in cell dissociation from liver tissue. Moreover, the two enzymes had significantly higher dissociated cell viability and cultured cell viability, compared to Collagenase type IV-S.

Conclusion

In the comparative experiment, liver tissue was collected from ICR mice. 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 cell dissociation efficiency, dissociated cell viability, and cultured cell viability for liver 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.