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

This study is designed to compare the cell 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 skin tissue.

Materials and Methods

Tissues used

Skin tissue of 3 ICR mice aged 8 weeks

Tissue sampling

Removing the skin	For the skin tissue sampling, three mice were anesthetized with 1g/kg urethane. NairTM, hair remover, was applied with a soft brush on ICR mice to remove the fur before taking skin samples from the flank. Then the hair remover was wiped out after three minutes along with the hair removed. After applying povidone to the area with fur removed, the skin was sterilized by applying alcohol three times. After sterilization, the mice were euthanized by cervical dislocation, and then the skin was kept free from alcohol. The skin on both sides of the flank was taken after euthanasia.

Skin tissue was collected in uniform size using the 4 mm punch biopsy. Tissue biopsy slices at a maximum of 60 were taken from one mouse, generating 180 slices from the three mice. The obtained skin tissue slices were sterilized twice using povidone (5 min) and sterile phosphate-buffered saline (PBS, with antibiotics, 5 min). After that, povidone was washed off from the skin using PBS (with antibiotics, 10 min). The skin tissue was tenderized through trypsin pretreatment (30 min, 37°C, shaking). The adipose tissue was then trimmed off from the skin tissue. Each skin tissue slice was chopped into small sizes (<1 mm^3) using sterilized scissors.Ten skin tissue slices were chopped and placed in a 2mL tube. 180 skin slices were randomly divided and inserted into 18 units of 2 mL tubes. The tubes were divided into three groups with six tubes per group, and each group was treated with different collagenase (n=6).

Preparation of each enzyme solution

Tissue type	TDzyme® C, T (Connext)	Liberase™ TM (Roche)	Collagenase Type IV-S (Sigma-Aldrich)
Skin tissue	Diluent: Ice-cold Milli-Q water Concentration: 200 µg/mL Incubation: 90 min, 37°C	Diluent: Injection quality-sterile water or tissue-dissociation buffer Concentration: 200 µg/mL Incubation: 90 min, 37°C	Diluent: Krebs Ringer buffer with calcium and BSA Concentration: 200 µg/mL Incubation: 90 min, 37°C

The collagenases (TDzyme®, Liberase™ TM, and Collagenase type IV-S, n=6) with a concentration of 200 µg/mL were applied. In order to apply the enzyme, 1 mL of collagenase solution was added to the prepared tube and the tube was kept on the tube roller at 37°C for 90 minutes. The solution was then filtered using a 70 µm cell strainer. Centrifugation (20°C, for 5 min at 1200 rpm, 190 mm (rotor)) was performed after putting the filtered solution into a 15 mL tube and adding the 4 mL of sterile DMEM. The supernatant liquid was then discarded. Red blood cells were removed from the remaining solution by using an RBC lysis buffer for a minute. Centrifugation (20°C, for 5 min at 1200 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 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
Skin tissue	Advanced DMEM, 10mM HEPES, 10% FBS 37°C CO2 incubator

Results

Tissue dissociation efficiency

The number of cells dissociated from skin tissue was counted after treating skin tissues with the three different collagenase products, and the dissociation rate for each collagenase was evaluated. The total number of cells dissociated from skin tissues was 0.28 x 10^6 on average for TDzyme® and 0.24 x 10^6 for Liberase™ TM, but the difference was not statistically significant ($p > 0.05$). For Collagenase type IV-S, it was 0.11 x 10^6, significantly lower than those for TDzyme® and Liberase™ TM ($p < 0.05$). The number of living cells and their viability were examined in the total number of cells obtained from skin tissue. The average number of living cells for TDzyme®, Liberase™ TM, and Collagenase type IV-S was 0.14 x 10^6, 0.11 x 10^6, and 0.01 x 10^6, respectively. Compared to TDzyme®, the results of the other two enzymes were significantly lower ($P < 0.05$).

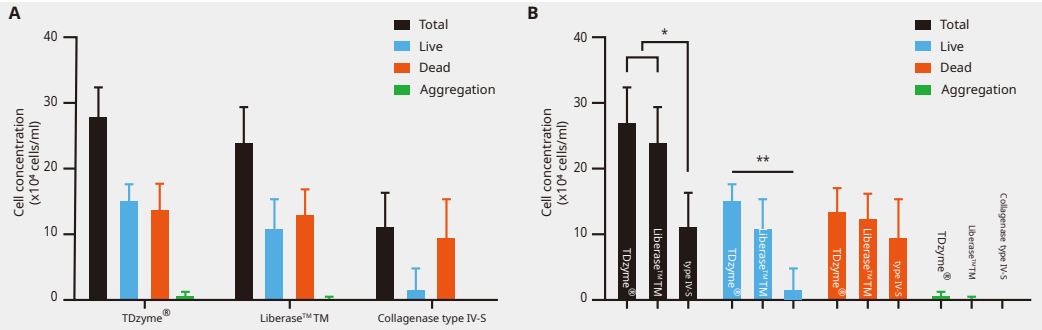

Fig 1. Comparison of dissociation efficiency among enzymes

The cell viability of dissociated cells from skin tissues for TDzyme®, Liberase™ TM, and Collagenase type IV-S was 51.40%, 45.50%, and 10.21%, respectively. There was no statistically significant difference between Liberase™ TM and TDzyme®, However, the cell viability was significantly lower for Collagenase type IV-S than the other two ($p < 0.05$).

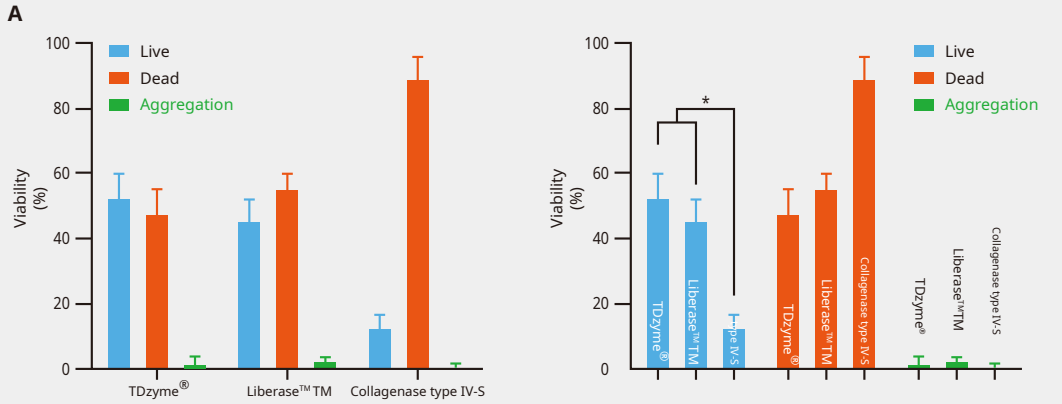

Fig 2. Comparison of cell viability

Cell viability (Cultured primary cells)

In order to observe the cultivation of cells obtained from skin tissue, the cultured cells were examined under the microscope 72 hours after cultivation. A large number of growing cells attached to the cell culture plate could be noticed clearly for TDzyme® and Liberase™ TM (Red arrow).However, a significantly lower number of cultured cells was detected for Collagenase type IV-S, compared to TDzyme® and Liberase™ TM. 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 90.17%, and that for Collagenase type IV-S was 7.22%. Compared to TDzyme® and Liberase™ TM, Collagenase type IV-S showed a statistically significant lower value ($p < 0.05$).

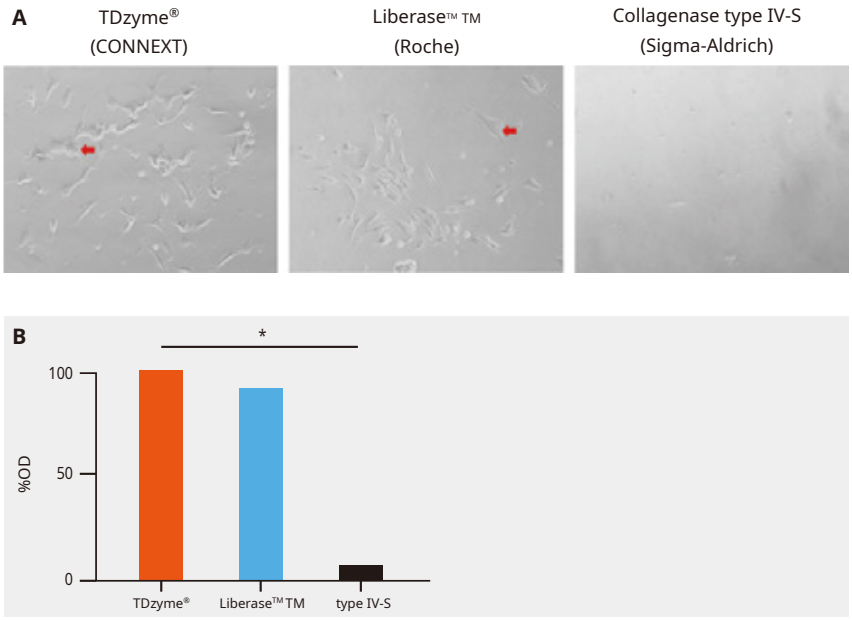

Fig 3. The patterns of cultured cells and the assessment of cell viability

A. The cells from skin 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 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 (* $p < 0.05$).

Discussion

When it comes to the cell dissociation rate for each enzyme, the number of cells from the skin tissues 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. For the patterns of cells dissociated from the tissues, the number of living cells for TDzyme® was significantly larger than Liberase™ TM, but both had similar patterns in terms of viability. Compared with Collagenase type IV-S, TDzyme® showed significantly higher results.In summary, based on the comparison of the number of cells dissociated from skin tissue and their viability by different enzymes, TDzyme® and Liberase™ TM showed similar results, but had higher dissociation efficiency than 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® had similar patterns of cells proliferating on the cell plate compared to those of Liberase™ TM. According to the MTT assay results, 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. The MTT assay results showed a statistically significant drop for Collagenase type IV-S compared to the other two enzymes.

The number of dissociated cells from the skin tissue and their viability for TDzyme® were similar to those for Liberase™ TM. Since there was no statistically significant difference in the viability of cultured cells and the patterns of cell culture, the study results of TDzyme® are similar to those of Liberase™ TM in cell dissociation from the skin tissue. Collagenase type IV-S showed a significant decrease in the dissociated cell number and viability compared to the two enzymes, and also a drop in the dissociated cell viability and cultured cell viability.

Conclusion

In the comparative experiment, skin 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 that of other commercial collagenases, Liberase™ TM and Collagenase type IV-S. TDzyme® was similar to Liberase™ TM in terms of cell dissociation efficiency for skin tissue, dissociated cell viability, and cultured cell viability for skin 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.