# ABSTRACTS (MASTER THESIS)

## Development of cellulose nanofiber/collagen sponge for 3D cell culture device

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### Introduction

Recently, the field of regenerative medical science have been getting attention. Especially in cell research, “Three-dimensional culture (3D cell culture)” for research has been attracted because it is required to mimic the in-vivo situation in cell culture. At present, collagen sponge with high biocompatibility has been used as a scaffold of cell for therapeutic area, however, using collagen sponge for cell research or cell experiment as cell culture device have not progressed. That is because collagen sponges have low physical strength, and there is a problem that the space where cells can survive is getting narrow by the deformation of the sponge during cell culture. Then, we tried to reinforce collagen sponge with cellulose nanofiber which has high elasticity, and aimed at development of Cellulose nanofiber/Collagen sponge for 3D cell culture device which can be cultured for a long time.

### Experiment

A suspension of pH3 TEMPO-oxidized cellulose nanofiber (TOCN) (about 3 nm in width) was mixed with a collagen (COL) solution at 4 ℃, and then the mix solution was immediately frozen at -20 ℃ after pH-induced. Next, these are dried with a freeze dryer to produce a sponge. After that, this sponge was subjected to dehydrothermally cross-linked treatment by a vacuum oven, sterilized by EOG, and then subjected to scanning electron microscope observation, physical property test, and cell culture test.

### Results and discussion

In a 7-day shaking cell culture, the COL sponge broke in the medium on 4 day, while the TOCN/COL sponge retained its shape. Furthermore, the cell mitochondrial activity was significantly increased as the TOCN amount was increased in the MTT test for measuring the cell mitochondrial activity (Figure 1). And also, after 4 day of culture, cell cryopreservation was performed, and cell mitochondrial activity after cryopreservation was compared. As a result, cell mitochondrial activity of TOCN/COL sponge was significantly higher than that of COL sponge (Figure 2).

These results suggest that reinforcement by TOCN is effective on maintaining the space where cells can survive during culture and also after cryopreservation, and the TOCN/COL sponge is useful for 3D cell culture.

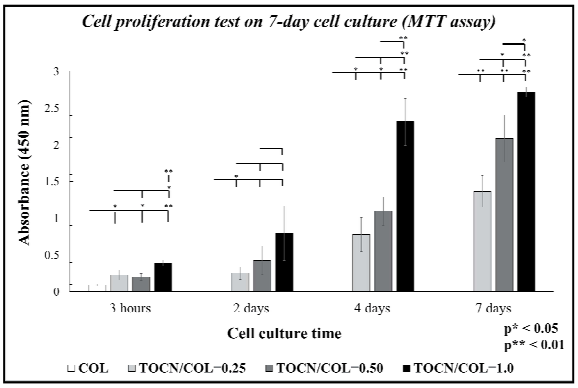


Figure 1. Cell proliferation on 7-day cell culture by MTT assay. (p*<0.05, p**<0.01)

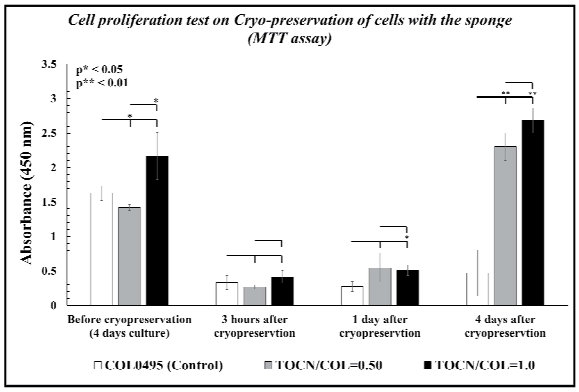


Figure 2. Cell proliferation on cryo-preservation of cells with the sponge by MTT assay. (p*<0.05, p**<0.01)