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	Fibroblast Matrix Implant (FMI) for Hernia Repair	Release date: 16 September 2019
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Scaffold fabrication

- PLLA pellets (#B6002-2) are purchased from Durect Lactel (Cupertino, California, USA)
- Grind and Sieves sodium chloride (NaCl) particulates ranging in diameters from 355 to 425 μm
- Put 9 gr of the NaCl particulates into an aluminum pan (diameter of 40 mm)
- Dissolve 1 gr of PLLA pellets in 5.3 mL of chloroform & vortex the PLLA solutions
- Pour the PLLA solutions into the aluminum pans and mix with NaCl particulates
- Air-dry the mixtures in a fume hood at room temperature for more than 24 hours to evaporate the chloroform
- Detach the PLLA/NaCl composite from the aluminum pans
- Place the composite in a vacuum drying service (Yamato, Tokyo, Japan) at -0.1 mPa for another 3 days
- Wash the PLLA/NaCl composites in deionized water to leach out the NaCl particulates and the water is changed every hour for 20 times
- The PLLA scaffold (matrix) is formed after drying
- Cut the scaffold into discs with 1 cm diameter and 2 mm thickness for further experiments.

Surface coating with collagen

- Immerse the PLLA scaffold discs in the 1% (w/v) type I porcine collagen solution (Wako, Osaka, Japan) and deaerate under a reduced pressure (20 Pa) with a vacuum freeze dryer FDU-2200 (EYELA, Tokyo, Japan)
- Place the collagen/PLLA constructs onto 100 μm cell-strainer membranes (Falcon, Durham, USA)
- Centrifuge at 2000 x g for 10 minutes at 4°C to remove collagen solution excess from the pores
- Freeze the collagen solution-coated scaffold discs at -80°C for a minimum of 4 hours
- Then, freeze-dry the scaffold discs under vacuum (<5 Pa for more than 24 hours)

Plasma surface treatment and sterilization

- Treat the PLLA collagen-coated discs with plasma for surface cleaning and activation with O₂ for 10 min (custom-made plasma treatment system, Diener electronic GmbH, Ebhausen, Germany)
- Seal the matrices in Tyvek® bag, and sterilize in the Diener machine with 30% H₂O₂ solution (Merck, Darmstadt, Germany) at 30°C for 12 hours
- Then transfer the matrices into 24-well plates and store under vacuum at 4°C until use.

Isolation of fibroblast cells from rats

- The primary rat fibroblasts are isolated from abdominal skin specimens of approximately 4x3 cm Sprague Dawley rats (obtained from National Agency of Drug and Food Control, Jakarta)
- Under aseptic conditions, dissect and remove the abdominal skin
- Wash the tissue 5 times with PBS (Gibco, Grand Island, NY) containing 2% antibiotic-antimycotic (Sigma)
- Wash 5 times with EGTA solution (Sigma) containing 2% antibiotic-antimycotic
- Digest it for 5 hours with 30 mL of 200 U/mL collagenase type I (Gibco) solution at 37°C, with 5% CO₂, and 95% relative humidity
- Gently scrap the dermis side of the skin with cell scraper
- Sieve the cells (1 mm and 100 µm mesh size)
- Wash twice and resuspend with Dulbecco's Modified Eagles Medium (Sigma, St.Louis, Missouri, USA) buffered with N-(2-hydroxyethyl)piperazine-N0-2-ethanesulfonic acid (HEPES) (Sigma), supplemented with 20% fetal bovine serum (Gibco, Grand Island, New York, USA) and 1% of antibiotic/antimycotic (Sigma)
- Culture the cells in a T75 cell culture flask
- Incubate the cells in incubator equilibrated with 5% CO₂ at 37°C
- When the cells have reached confluence, dissociate the cells with 2 ml 0.05% Trypsin-EDTA (Gibco) for 5 min in incubator equilibrated with 5% CO₂ at 37°C
- Add 5 ml of complete culture medium to stop the dissociation
- Centrifuge at 130 G for 5 min to pellet the cells
- Aspirate the medium and resuspend it with fresh culture medium
- Count the cells using the Trypan blue exclusion method.

Cytocompatibility tests

- In order to test the scaffolds cytocompatibility, the PLLA collagen-coated matrices are seeded with primary rat dermal fibroblasts and cells viability is analyzed
- Seed the primary rat dermal fibroblasts onto the PLLA collagen coated matrices (500,000 fibroblasts/matrix)
- As an internal control, seed a same number of cells (500,000 fibroblasts) directly into tissue culture wells-plate
- Incubate at 37°C, with 5% CO₂ and 95% relative humidity for 24 hours
- Analyze the cell viability using Cell Counting Kit-8® viability assay (CCK-8) (Sigma)
- Measure the absorbance using the Multiskan reader at λ450 nm (Multiskan Ex, Thermo Scientific, USA)
- Results were expressed as fold change relative to control percent adhesion (cells adhered on the wells, 100%).

Cells labelling and seeding

- Label the fibroblasts with CellVue® NIR815 dye (Li-Cor #929-90020, Lincoln, Nebraska, USA) according to the kit manual before seeded onto the PLLA matrices
- Seed 100 µL of the fibroblast cell suspensions onto the PLLA scaffolds (500,000 fibroblasts/matrix)

- Add 300 μ L culture medium into each well, then after 1 hour add another 700 μ L culture medium into each well
- Incubate it in the incubator with 5% CO₂ at 37°C for overnight before implantation in order to allow the cells adhere to the scaffolds

FMI in hernia animal model

- Male Sprague-Dawley (SD) rats are used as hernia model by making 2 cm incision at the midline abdomen
- Separate peritoneum from the muscle to create space or pouch for the matrices implantation (both side of the midline)
- Close the peritoneum using 6-0 suture
- Implant or place the engineered fibroblast-matrices (2 pcs) at the abdominal area, between the abdominal muscle and peritoneum (sublay hernia repair method), one on each side.
- Close the muscle and the abdominal skin using 4-0 suture
- Close the surgical wound with tegaderm and hypafix
- Scan the implantation sites using Imaging machine (Li-Cor Pearl Trilogy Digital Imaging System) to observe the viability and distribution of fibroblast in the matrix at the implantation site
- After the end-point, anesthetize and euthanized the rats. Then, necropsy is performed to collect the tissue at implantation sites; store it inside the formalin solution for histopathological analysis

Histopathology analysis

- Fixate the specimens in 10% buffered formalin and prepare the histological slides
- Stain the histological slides with Hematoxylin and Eosin, Masson Trichome to observe the collagen formation, and Immunohistochemistry for fibroblast

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