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 O_2 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% H_2O_2 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% CO_2, 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% CO_2 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% CO_2 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 Tryphan 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% CO_2 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 µL culture medium into each well, then after 1 hour add another 700 µL culture medium into each well
- Incubate it in the incubator with 5% CO_2 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

Validation	Prepared by:	Checked by:	Authorized by:
Name	Jennifer Lheman	Dr. dr. Siufui Hendrawan, M.Biomed	Prof. Dr. Med. Hans U Baer
Signature			
Date	16 September 2019	16 September 2019	

REPUBLIK INDONESIA
KEMENTERIAN HUKUM DAN HAK ASASI MANUSIA

SURAT PENCATATAN
CIPTAAN

Dalam rangka pelindungan ciptaan di bidang ilmu pengetahuan, seni dan sastra berdasarkan Undang-Undang Nomor 28 Tahun 2014 tentang Hak Cipta, dengan ini menerangkan:

Nomor dan tanggal permohonan	:	EC00201972907, 26 September 2019
Pencipta		
Nama	:	**Dr. dr. Siufui Hendrawan, M.Biomed**
Alamat	:	Jl. Letjen S. Parman No.1, Universitas Tarumanagara, Fakultas Kedokteran, Jakarta Barat, Dki Jakarta, 11440
Kewarganegaraan	:	Indonesia
Pemegang Hak Cipta		
Nama	:	**Dr. dr. Siufui Hendrawan, M.Biomed**
Alamat	:	Jl. Letjen S. Parman No.1, Universitas Tarumanagara, Fakultas Kedokteran, Jakarta Barat, Dki Jakarta, 11440
Kewarganegaraan	:	Indonesia
Jenis Ciptaan	:	**Buku Panduan/Petunjuk**
Judul Ciptaan	:	**Fibroblast Matrix Implant (FMI) For Hernia Repair**
Tanggal dan tempat diumumkan untuk pertama kali di wilayah Indonesia atau di luar wilayah Indonesia	:	16 September 2019, di DKI Jakarta
Jangka waktu pelindungan	:	Berlaku selama hidup Pencipta dan terus berlangsung selama 70 (tujuh puluh) tahun setelah Pencipta meninggal dunia, terhitung mulai tanggal 1 Januari tahun berikutnya.
Nomor pencatatan	:	000155958

adalah benar berdasarkan keterangan yang diberikan oleh Pemohon.
Surat Pencatatan Hak Cipta atau produk Hak terkait ini sesuai dengan Pasal 72 Undang-Undang Nomor 28 Tahun 2014 tentang Hak Cipta.

a.n. MENTERI HUKUM DAN HAK ASASI MANUSIA
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