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	:	EC00202323784, 28 Maret 2023
Pencipta		
Nama	:	**Dr. dr. Siufui Hendrawan, M.Biomed**
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Kewarganegaraan	:	Indonesia
Pemegang Hak Cipta		
Nama	:	**Dr. dr. Siufui Hendrawan, M.Biomed**
Alamat	:	Universitas Tarumanagara, Fakultas Kedokteran, Gedung J, Lt. Dasar Jl. Letjen S. Parman No. 1, Jakarta Barat, DKI JAKARTA, 11440
Kewarganegaraan	:	Indonesia
Jenis Ciptaan	:	**Buku Panduan/Petunjuk**
Judul Ciptaan	:	**Secretome Matrix Implant (SMI) For Liver Cirrhosis**
Tanggal dan tempat diumumkan untuk pertama kali di wilayah Indonesia atau di luar wilayah Indonesia	:	13 Maret 2023, di 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	:	000456707

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
Direktur Jenderal Kekayaan Intelektual
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Disclaimer:
Dalam hal pemohon memberikan keterangan tidak sesuai dengan surat pernyataan, Menteri berwenang untuk mencabut surat pencatatan permohonan.

3D PLLA collagen-coated matrix fabrication

- 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 Poly-L-lactic acid (PLLA) (Durect Lactel, Cupertino, California, USA) 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 3-4 days
- Put the PLLA/NaCl composites in a plastic beaker filled with deionized water to leach out the NaCl particulates using shaking water bath and the water is changed every hour for 20 times
- The PLLA sponge (matrix) is formed after drying
- Cut the sponge into round discs with 1 cm diameter and 2 mm thickness
- Immerse the PLLA matrix discs in the 0.5% (w/v) type I bovine collagen solution (Koken, 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 a 100 µm cell-strainer (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 matrix discs at -80°C for a minimum of 4 hours
- Then, freeze-dry the matrix discs under vacuum (<5 Pa for more than 24 hours) using vacuum freeze dryer FDU-2200

Surface treatment and sterilization of 3D PLLA collagen-coated matrix

- 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, sealed with parafilm, and store under vacuum at 4°C until use.

Preparation of hepatocytes

Donor: the primary rat hepatocytes are isolated from liver specimens of Sprague Dawley rats (obtained from National Agency of Drug and Food Control, Jakarta)

Method: this procedure was done according to Standard Operating Procedure (SOP) S-007 (Rat Liver Perfusion and Hepatocytes Isolation) of THCT Lab which already registered as intellectual property (registration number: 000107024) at Directorate General of Intellectual Property, Ministry of Law and Human Rights Republic of Indonesia.

Cytocompatibility tests

- In order to test the matrices cytocompatibility, the PLLA collagen-coated matrices were seeded with primary rat hepatocytes (500,000) and cells number and viability is counted manually
- As an internal control, seed a same number of cells (500,000 hepatocytes) directly into tissue culture wells-plate (2D culture)
- Incubate at 37^{o}C, with 5% CO_2 and 95% relative humidity for overnight
- Change the medium with fresh pre-warmed William E medium completed with 10% FBS and 1% Ab/Am
- Incubate at 37^{o}C, with 5% CO_2 and 95% relative humidity for another 48 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%).

Cirrhosis induction (modelling liver cirrhosis in rat)

Method: this procedure was done according to SOP S-026 (Thioacetamide-induced Cirrhosis Animal Model in Rat) of THCT Lab which already registered as intellectual property (registration number: 000380018) at Directorate General of Intellectual Property, Ministry of Law and Human Rights Republic of Indonesia.

- After 11 weeks of cirrhosis induction, the rats are used as animal model for liver cirrhosis and are implanted with engineered cell-matrix (matrix seeded with cells)

Engineered cell-matrix for Secretome Matrix Implant (SMI)

- Seed 100 µL of the hepatocytes cell suspensions onto the PLLA matrixs (750,000 hepatocytes/matrix)
- Add 300 µL complete culture medium into each well, then after 1 hour add another 700 µL complete culture medium into each well
- Incubate it in the incubator with 5% CO_2 at 37°C for overnight in order to allow the cells adhere to the matrices
- Change the medium with 1 ml of pre-warmed fresh complete culture medium supplemented with conditioned medium (CM) derived from human umbilical cord mesenchymal stem cells (hUC-MSC), then continue incubation

    - **Notes:** CM production was done according to Standard Operating Procedure (SOP) S-022 (Conditioned Medium Umbilical Cord Mesenchymal Stem Cell Preparation) of THCT Lab which already registered as intellectual property (registration number: 000156983) at Directorate General of Intellectual Property, Ministry of Law and Human Rights Republic of Indonesia.
- Incubate it in the incubator with 5% CO_2 at 37°C for another 48 hours before implantation

SMI procedure

- Cirrhosis-induced male Sprague Dawley (SD) rats is used as animal model for liver cirrhosis
- After the liver cirrhosis is confirmed (liver function parameter analyses)
- Implantation surgery is started by making 2 cm incision (laparotomy) at the midline abdomen
- Place the matrices between the serosal surface of two adjacent mesenteric loops of the small bowel intestine. Each matrix is fixed by using 6-0 suture (non-absorbable).
- Each rat is received four pieces of engineered hepatocytes-matrices.
- Close the muscle and the abdominal skin using 4-0 running sutures (absorbable).
- Close the surgical wound with Tegaderm™ and Hypafix®
- At the end-point, anesthetize the rats, then collect the blood via intracardiac for liver parameter analysis, then euthanized the rats. After that the necropsy is performed to collect the liver and implantation sites at the small bowel mesentery; store it inside the paraformaldehyde for histopathological analysis

Histopathology analysis

- Fixate the specimens in paraformaldehyde solution and prepare the histological slides
- Stain the histological slides with Hematoxylin and Eosin, Sirius Red (Abcam, UK) to observe the cirrhosis stage, and Immunohistochemistry for albumin (Invitrogen, USA)