REPUBLIK INDONESIA
KEMENTERIAN HUKUM DAN HAK ASASI MANUSIA

SURAT PENCATATAN CIPTAAN

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Jenis Ciptaan	:	**Buku Panduan/Petunjuk**
Judul Ciptaan	:	**Preparation Of Collagen-Coated PLLA Matrix Seeded With Fibroblast And Supplemented With Secretome**
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1. Preparation of Culture Medium Supplemented with Secretome (DMEM+Sec)

- Dulbecco's Modified Eagle's Medium (DMEM; Sigma) complete medium is prepared by adding 10% Fetal Bovine Serum (FBS; Gibco), 1% sodium pyruvate (Sigma), 1% 4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES; Sigma), and 1% Antibiotic-antimycotic solution (Ab/Am; Sigma).
- For secretome supplementation (DMEM+Sec), DMEM complete medium is added with 1% secretome derived from human umbilical cord mesenchymal stem cells (hUC-MSCs).
  Note: hUC-MSCs secretome is obtained from the protocol of HKI No. 000284674.

2. Production of Ready to Use Matrix for Hernia Repair

- Collagen-coated poly-L-lactide (PLLA; Resomer L206S, Evonik) matrix is fabricated through salt-leaching method, following the procedure from Hendrawan *et al*. (2020)[1] (Fig 1).

  Note: The matrix is cut into a rectangular shape of 2 x 1 cm with blunt edges.

- One day before the hernia repair and implant surgery, each sterile matrix is seeded with 400 µL of fibroblast suspension, containing $2x10^6$ cells.
- After cell seeding, 800 uL of DMEM+Sec is added into the surrounding of matrix and further incubated for 1 hour at 37°C in a 5% CO_2 incubator.
- After 1 hour of incubation, another 1.2 mL of DMEM+Sec medium is added into each well and then incubated for 24 hours.
- Prior to hernia repair surgery, the matrix is transferred into a new 6 well-plate containing 800 µL of DMEM complete medium. The matrix is ready for use.

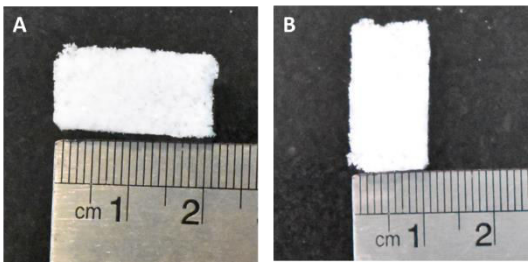

Figure 1 Rectangular collagen-coated PLLA matrix with 2 cm length (A) and 1 cm width (B).

3. Hernia Repair and Implant Treatment Procedure

Method is modified from Hendrawan et al. 2024[2].

- Male Wistar rat weighing 180-200 gr is anesthetized using 10% Ketamine (40-80 mg/kg BW) and 2% Xylazine (5-10 mg/kg BW).
- The hair on ventral abdominal side is removed using hair clipper and blade razor. The skin surface is disinfected with 70% ethanol and povidone-iodine.
- A midline laparotomy of ±2 cm in length is done along the *linea alba* to create hernia in the animal model (Fig 2A).
  Note: The abdominal muscle incision must be done gently without rupturing the peritoneum membrane.
- At the site of laparotomy, either side of the muscle is carefully separated from the peritoneum membrane to give a space for the matrix (Fig 2B).
- The implant is gently placed in sublay (in between of abdominal muscle and peritoneum membrane), without breaking the implant (Fig 2C).
- Abdominal muscle and skin are closed with a continuous suture using 4-0 absorbable vicryl (Ethicon W9386) (Fig 2D).
- Skin surgical site is applied with Gentamicin ointment and covered with Hypafix.
- Rats are kept until 2 months, for further tensile strength testing and histopathological staining of the abdominal muscle.

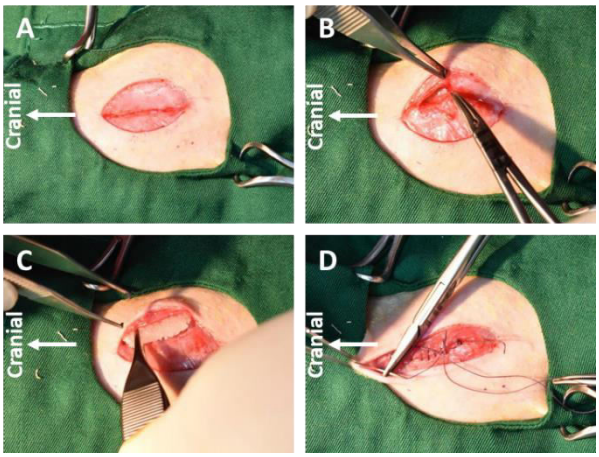

Figure 2 Procedure for hernia repair and implant treatment in rat model: midline laparotomy of 1.8 cm is created on the abdominal muscle (A), the peritoneum membrane is gently separated from the muscle (B), the implant is inserted sublay between the muscle and peritoneum (C), abdominal muscle and skin are sutured (D).

4. Euthanasia and Tissue Collection

- The rat is anesthetized using 10% Ketamine (80 mg/kg BW) and 2% Xylazine (10 mg/kg BW).
- Under deep anesthesia, rat is euthanized through intracardiac injection of sodium pentobarbital (200 mg/kg BW).
- Ventral abdominal side of the rat is shaved using a hair clipper.
- The ventral abdominal skin is separated from the muscle part and discarded (Fig 3A).
- The abdominal muscle on the area of midline laparotomy and implant is obtained according to the corresponding test:
    - <u>For tensile strength analysis:</u> a 6x4 cm of muscle is collected and submerged in a physiological salt solution for immediate tensile pull testing (Fig 3B).
    - <u>For histopathological staining:</u> a 3x2 cm of muscle is collected and submerged in a 4% paraformaldehyde solution to be made into a paraffin block (Fig 3C).

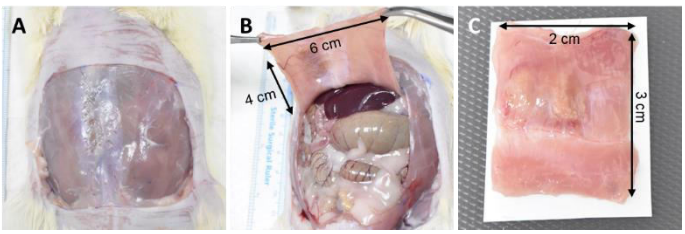

Figure 3 Sample collection and preparation of abdominal muscle: separation of abdominal skin from the muscle (A), collection of abdominal muscle for tensile strength analysis (B) and histopathological staining (C).

5. Tensile Strength Analysis of Abdominal Muscle at The Implanted Area

Method is modified from Melman et al. (2011)[3] and Holmdahl et al. (2019) [4].

- Tensile strength is measured using a universal testing machine with a dynamic force processor (Unipulse F381A).
- Obtained tissue is stretched and fixed into the upper and lower grips, with the direction of midline laparotomy is positioned laterally (Fig 4).
  <u>Note:</u> *The lower grip should hold the implant, while the upper grip holds the tissue near the midline laparotomy. Make sure that the tissue is all stretched without any parts folded.*
- A uniform speed of 400 rpm is applied and the pulling test is performed until the tissue ruptures.
- The maximum force borne by each specimen is recorded (in Newton).

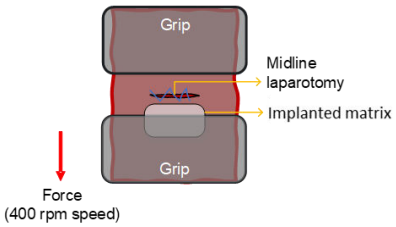

Figure 4 Tensile strength testing setup.

6. Collagen I:III Ratio Confirmation

To ascertain the effect of treatment for a stronger muscle development, collagen observation using Sirius Red solution is a widely-performed histology staining. Particularly through this staining, deposition of collagen type I and type III could be differentially observed using a polarized microscope. Collagen type I is formed as a thicker and stronger bundle, while collagen type III deposition is associated with a weaker muscle development. Hence, the ratio of collagen I:III could depict the strength of hernia repair[5].

- To distinguish the deposition of collagen type I from the type III, the stained specimen is observed under a polarized light using a light microscope (Olympus CX23) fixed with a custom-made analyzer and polarizer.
- Pictures are taken in 10x field views for further determination of collagen I:III ratio.
- The determination of ratio collagen I:III is quantified using ImageJ software (National Institute of Health)[6].

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