Equipment:

1. Digital Balance
2. Orbital Shaker Incubator (Biosan, ES-20)
3. Fume Hood (ESCO, Ascent Max)
4. Analytical Sieve Shaker (Retsch, AS200 basic), test sieve ⌀=8" x t=2" (203x50mm), 355µmx425µm
5. Vacuum Drying Oven (Yamato, DP200)
6. Vacuum Freeze Dryer (EYELA, FDU-2200)
7. Ultra Low Temperature Freezer/Deep Freezer -80°C (New Brunswick, Innova U101)
8. Linear Shaking Bath (Grant, GLS Aqua Plus Series)
9. Mortar & Pestle
10. Spatula, spoon
11. Blunt forceps
12. Test tube & rack
13. Measuring pipette (glass)
14. Rubber Pipet Filler
15. Desiccator
16. Beaker plastic 300 ml
17. Aluminum Tray
18. Scissors
19. Vortex
20. Plastic Container
21. Incubator oven
22. Duran bottle
23. Waste Disposal Bottle
24. Plastic Sealer
25. Diener Plasma Machine

Materials / Solutions:

1. PLGA (Sigma, Saint Louis, USA, #P1941-5G) or;
   PLGA 75:25, Ester Terminated (Durect Lactel, Birmingham, USA, B6007-1P)
2. Chloroform (Wako, Osaka, Japan, #038-18495)
3. Sodium Chloride (Wako, Osaka, Japan, #191-01665)
4. Ethanol 70%
5. Double-distilled water (ddH_2O)

6. H_2O_2 30% (Merck, Darmsatdt, Germany, #108597)

Consumables:

1. Nitrile gloves
2. Surgery mask
3. Weighing boat
4. Centrifuge tube 50 ml
5. Aluminum pan (12 ml) or Aluminum cup 5 cm x 5 cm
6. Aluminum foil
7. Rubber bands
8. Petri dish Ø 100 mm
9. Petri dish Ø 55 mm
10. Autoclavable bags
11. Plastic bag
12. Plastic wrap
13. Parafilm

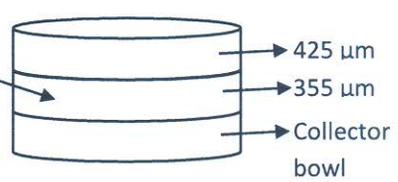
Work sheet: **Protocol for preparation PLGA Sponges**

Date : _____
Batch : _____
Start at : _____

sticker

Researcher:

- ☐ Take out the polymer materials (PLGA) from the freezer (-20°C) and place it inside a desiccator (vacuum condition) at room temperature for 1 hour
- ☐ After 1 hour at room temperature, weigh _____ (target: 1 gram/matrix) the PLGA with weighing boat and put it in a glass test tube then close the lid with aluminum foil and bring the tube into fume hood
  Note: If there is remaining PLGA, seal the leftover and store it in freezer (-20°C)
- ☐ Add **5 ml** of chloroform into each tube, then seal it with aluminum foil and rubber band (double seal)
- ☐ Dissolve the PLGA pellets in chloroform to prepare 20 (w/v) % solutions, place the tube inside orbital shaker incubator (bioshaker) at 37°C, and shake (150 rpm) for _____ (target: 3 hours)
  (!) Close any window to avoid light exposure during incubation (light sensitive)
- ☐ Grind Sodium Chloride (NaCl) particulates using mortar and pestle; then sieve the particulates with analytical sieve shaker (or sieve manually) to obtain NaCl particulates ranging from 355 to 425 µm
  Note: Grind gently, do not grind excessively
- ☐ Collect NaCl particulates that trapped on 355 µm siever
  Repeat the process until obtain enough NaCl particulates
  Rinse and soak the siever in ddH_2O after used



- ☐ Weigh NaCl particulates _____ (target: 9 gram/matrix/tube), pour it in a centrifuge tube then seal it and put inside desiccator
- ☐ After 3 hours incubation, check whether the PLGA pellets are already completely dissolved or not.
  If the pellets are already completely dissolved, stop the incubation and vortex the PLGA solutions;
  If the pellets are not yet completely dissolved, continue the incubation
- ☐ Pour the NaCl particulates into an aluminum pan, then pour the PLGA solutions gently and mixed them quickly and thoroughly using spatula inside the fume hood **(!)**
  (!) Immediately rinse the test tube with ethanol absolute and towel paper after pouring the solutions into aluminum pan to prevent the solutions stick on the glass tube. Rinse twice with ethanol 70%. Discard the excess solutions into a designated waste disposal bottle.
- ☐ After mixing, tap the aluminum pan repeatedly to release air bubble
- ☐ Allow chloroform to evaporate by air-drying for > 24 h in a fume hood (Fan ON)

☐ Detach the PLGA composites from the aluminum pan, scissors edge of aluminum pan with a distance of 1 cm

☐ Then gently peel off the aluminum pan; turn over the sponge and place it in a new aluminum pan and let it dry for the rest of the 24 h

☐ Place the sponges inside an aluminum tray without lid into the vacuum drying oven YAMATO (vacuum chamber). Vacuum it under -0.1 MPa for 3-4 days

☐ After 3-4 days inside the vacuum chamber, stop the vacuum drying process

☐ Fill 100 ml ddH_2O into a plastic beaker 300 ml; Put the sponge inside the beaker (1 beaker → 1 sponge)

☐ Degass the sponge using vacuum freeze dryer EYELA 2-3 times each sponge (gently turn the knob into vacuum)

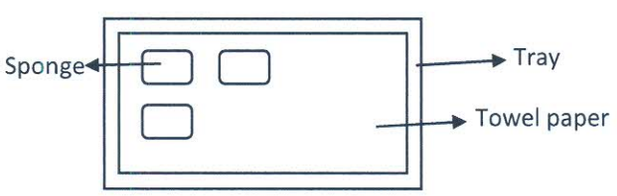
Note:

1. **Use the 600 ml EYELA bottle and put the beaker contains matrix and ddH_2O into EYELA bottle**
2. **Spray a little amount of ethanol 70% into the bottle cap to close the bottle easily**

☐ After degassing, add ddH_2O until 200 ml of volume into each beaker; then put inside linear shaking bath at 25°C (room temperature), shake (60 rpm) to leach/wash out the NaCl particulates

☐ Change the water in the beaker every 1-2 hours for the next 48 hours (for more than 20 times)

☐ After 48 hours, put the sponges on towel paper for drying process



☐ Keep moving the sponges on towel paper until no water is seen on the paper

☐ Then move the sponges inside the fume hood; leave them for overnight to dry completely

☐ The PLGA sponge was formed after drying, weigh the sponge (target: <1 g); If more than 1 gram wash the sponge again

☐ Cut the sponges into the desire size

Protocol for Plasma treatment and Sterilization Matrices

- ☐ Turn to the right (clockwise) "main switch" located at the back of the Diener machine, to start the machine.
- ☐ Wait for the computer to *booting* and screen lights up (manual desktop).
- ☐ Click the "Programs" to show the *main picture (F1)* "Plasma Reaktor Steuerung".
- ☐ Wait, until the *warm up MFCs* icon changes from the red color to green color.
- ☐ Click "on" the "controller" to turn on the vacuum pump O_2.
- ☐ Open the oxygen flow, by turning the knob (cylinder valve) located above the tank counter clockwise, then rotate the screw handle (pressure adjusting knob) below to adjust the oxygen pressure: 1 mbar.
- ☐ Select the *automatic program 1*, which is *warm up and cleaning chamber*.
- ☐ Click the button "start" and fill in the comment (wait until the program runs completed).
- ☐ Put the samples/matrices on the glass plate openly and then insert the glass into the chamber.
- ☐ After the program completed, close the oxygen flow by turning the knob (cylinder valve) and the screw handle (pressure adjusting knob) clockwise.
- ☐ Prepare 2 ml H_2O_2 30% solution at a glass bottle (working in fume hood).
- ☐ Remove the sample from the chamber, wrap the sample with tyvex bag and sealed it, place on top the glass plate then place it inside the chamber.
- ☐ Put the H_2O_2 glass bottle, under the sample glass plate (the tip of the bottle facing outward).
- ☐ Choose the *automatic program 3,* which is *H_2O_2 Sterilization*.
- ☐ Click the button "start" (leave it overnight).
- ☐ After the running programs completed, click the controller off, close the program, remove the H_2O_2 glass bottle **(Note: Immediately close/wrap the bottle lid with aluminum foil and put inside the fume hood!)**, take out the sample and the glass plate, shut down the computer and turn off the machine by turning the "main switch" counterclockwise (to the left).

Note:

No. 1. *Warming up chamber and cleaning*:

- 15 min plasma / 50 % Power / O2 plasma / 0,4 mbar
- Vacuum pump and plasma chamber are getting warm so that process conditions are always the same.

No. 3. *H2O2 for 12 hours*

- Sub-program 1: "STEP 1: H2O2 steam for 12 hours"
- Sub-program 2: "STEP 2: purging and pumping"
- Starting program no. 3 both sub-programs will run automatically.
- Samples in Tyvek bags are treated here.
- No plasma, just H2O2 steam

Validation	Prepared by:	Checked by:	Authorized by:
Name	Jennifer, Nuraeni	dr. Siufui Hendrawan	Prof. Dr. Med. Hans U Baer
Signature			
Date	14 October 2020	14 October 2020	

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	:	EC00202047007, 6 November 2020
Pencipta		
Nama	:	**Dr. dr. Siufui Hendrawan, M.Biomed**
Alamat	:	Jl. Letjen S. Parman No.1, Universitas Tarumanagara, Fakultas Kedokteran, Jakarta, 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, Dki Jakarta, 11440
Kewarganegaraan	:	Indonesia
Jenis Ciptaan	:	**Buku Panduan/Petunjuk**
Judul Ciptaan	:	**PLGA Matrix Preparation For Hernia Repair (Anti-adhesion)**
Tanggal dan tempat diumumkan untuk pertama kali di wilayah Indonesia atau di luar wilayah Indonesia	:	28 Oktober 2019, 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	:	000214312

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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