LAMPIRAN

Prosedur Pembuatan Larutan

DMEM Blank	Dalam 1 liter ddH20 (Sterile Water For Irrigation – WIDAWI ™ Unicap) 13,36gr DMEM powder + 3,7gr Sodium bicarbonate + 5,5mg Phenol red
Larutan L-ascorbic acid	L-ascorbic acid (mg) : PBS (ml) 3 : 1 Sebagai contoh: $\frac{3\ (mg)}{1\ (ml)} \times \frac{12\ (mg)}{PBS\ (ml)}$ PBS (ml)= $\frac{12\ (mg/ml))}{3\ (mg)}$ = 4 (ml)
Larutan Dextran sulfate	Dextran sulfate (mg) : DMEM blank (ml) 10 : 1 Sebagai contoh: $\frac{10\ (mg)}{1\ (ml)} \times \frac{40\ (mg)}{DMEM\ blank\ (ml)}$ DMEM blank (ml) = $\frac{40\ (mg/ml))}{3\ (mg)}$ = 4 (ml)
Larutan Sodium deoxycholate	0,5% didalam PBS 1× $\frac{0,5}{100} \times \frac{S.deox\ (gr)}{PBS\ (ml)}$ Jika ditimbang 27,7 maka akan dilarutkan dalam 5,5 ml PBS 1×
Larutan DNAse I (Untuk Deselulerisasi)	Dalam 10ml PBS 10ml × 6U = 5U × X 60mlU = 5U × X $\frac{60mlU}{5U}$ = X 12ml = X
TBE 10×	Dalam 300ml aquades 1M Tris (36,33 gr) + 1M Boric acid (18,549 gr) + 20mM EDTA (2,2335 gr)
APS 10%	Dalam 10ml Aquades dilarutkan 1gr Amonium persulfate

Acrylamide 30%	Dalam 100ml Aquades 29gr acrylamide + 1gr Bis
Agarose 1,5%	1,5gr Agarose + 100ml TAE 0,5×
TAE 50×	Dalam 200ml Aquades 48,4gr Tris base + 20ml 0,5M EDTA pH 8 + 11,42ml Glacia acetic acid

Spesifikasi Alat

Mikroskop CKX41 Olympus – Jepang

Biosafety cabinet class 2 tipe 2A Airtech – China

Tali-cytometry Invitrogen – Amerika Serikat

Mikropipette dan *tips*

ELISA Reader Glomax Multi Detection System (Promega) – Amerika Serikat

Mikroskop Konfokal Olympus - Jepang

T100 Thermal Cycler (BioRad PCR – Amerika Serikat)

BioRad Mini Protean Tetra (vertikal elektroforesis) dan komponen lengkap

Inkubator CO_2 - Memmert

Flask kultur, pipet serologi, cawan petri, dan botol media kultur - Corning

Tube sentrifugasi, micro-tube, dan rak

Gelas Kimia

Shaker LR *Invitroshaker* Taitec – Jepang

Microcentrifuge Maxpin C-12 mt – Daihan Brand

Tanki nitrogen cair 8 rak

Sentrifus Tomy AX-310

Bio Rad GelDoc XR – Amerika Serikat

Syringe 10cc –Terumo
dan
Millex-GP Filter 0.22 µm

Spesifikasi bahan

Nama Bahan	Diproduksi oleh:	Wujud Bahan
Dulbeccos modified eagle's medium (DMEM powder)	Sigma aldrich	Bubuk
DMEM / F12	Lonza	Cair (magenta)
Sodium bicarbonate	Gibco	Cair (tidak berwarna)
HEPES 1M	Gibco	Cair (tidak berwarna)
Penstrep		Cair (tidak berwarna)
Foetal bovine serum	Gibco	Cair (coklat keemasan)
Phenol red		Bubuk (magenta)
PBS		Cair (tidak berwarna)
Sterile water for irrigation (ddH2O)	WIDAWI^{TM} Unicap	Cair (tidak berwarna)
Dimethyl sulfoxide (DMSO)	Sigma	Cair (tidak berwarna)
L-ascorbic acid	Himedia	Bubuk
Dextran sulfate	Sigma	Bubuk
Sodium deoxycholate	Himedia	Bubuk
DNAse I		Cair
Kit Isolasi RNA	Zymo	Cair
Primer spesifik	Integrate DNA Technology (IDT)	bubuk
Nuclease free water	Sigma	Cair (Tidak berwarna)
Random primer	invitrogen	Cair
dNTP	promega	Cair
5× Cdna synthesis buffer	Invitrogen	Cair
0,1 M DTT	Invitrogen	Cair
RNAse out 40U/µl	Invitrogen	Cair
Thermoscript Reverse transcriptase	Invitrogen	Cair
Dream taq PCR master mix 2×	Invitrogen	Cair (hijau)
Acrylamide	Bio Basic Inc	Bubuk
Tris base	Bio Basic Inc	Bubuk
Boric acid		
Ammonium persulfate	Bio Basic Inc	Kristal
Topvision agarose powder	Thermoscientific	Bubuk
Tripan blue stain 0,4%	Gibco	Cair (biru tua)
Trypsin EDTA 0,25% 1×	Gibco	Cair (magenta)

EDTA		Bubuk
Hematoxylin and Eosin stain kit (Nucleus)	Abcam	Cair (multi warna)
Trichrome stain kit (Connective tissue)	Abcam	Cair (multi warna)
Sodium hipochloride	Bayclin	Cair

Optimasi Uji Tali-cytometer dengan Ekstrak Alami Kulit Manggis

File Name: SCAF-AROEM010421_1
Experiment Date : 2021-04-01 17-13-04
Assay : Green
Control Name : SCAF-AROEM010421_00
Sensitivity : 5
Circularity : 8

	Conc.	% cells	# cells
Green:	0.00X10e4 cells/mL	0%	0
No Green:	4.05X10e4 cells/mL	100%	85

Average cell size: 11 µm
# of cells counted: 85
Total cell conc.: 4.05X10e4 cells/mL

File Name: SCAF-AROEM010421_2
Experiment Date : 2021-04-01 17-18-02
Assay : Green
Control Name : SCAF-AROEM010421_00
Sensitivity : 5
Circularity : 8

	Conc.	% cells	# cells
Green:	0.05X10e4 cells/mL	1%	1
No Green:	3.57X10e4 cells/mL	99%	75

Average cell size: 8 µm
# of cells counted: 76
Total cell conc.: 3.62X10e4 cells/mL

File Name: SCAF-AROEM010421_3
Experiment Date : 2021-04-01 17-22-09
Assay : Green
Control Name : SCAF-AROEM010421_00
Sensitivity : 5
Circularity : 8

	Conc.	% cells	# cells
Green:	1.14X10e4 cells/mL	1%	24
No Green:	9.80X10e5 cells/mL	99%	2056

Average cell size: 6 µm
# of cells counted: 2080
Total cell conc.: 9.91X10e5 cells/mL

File Name: SCAF-AROEM010421_4
Experiment Date : 2021-04-01 17-24-27
Assay : Green
Control Name : SCAF-AROEM010421_00
Sensitivity : 5
Circularity : 8

	Conc.	% cells	# cells
Green:	0.14X10e4 cells/mL	1%	3
No Green:	1.22X10e5 cells/mL	99%	257

Average cell size: 7 µm
# of cells counted: 260
Total cell conc.: 1.24X10e5 cells/mL

File Name: SCAF-AROEM010421_5
Experiment Date : 2021-04-01 17-28-47
Assay : Green
Control Name : SCAF-AROEM010421_00
Sensitivity : 5
Circularity : 8

	Conc.	% cells	# cells
Green:	0.10X10e4 cells/mL	1%	2
No Green:	9.96X10e4 cells/mL	99%	209

Average cell size: 6 µm
# of cells counted: 211
Total cell conc.: 1.01X10e5 cells/mL

File Name: SCAF-AROEM010421_6
Experiment Date : 2021-04-01 17-30-50
Assay : Green
Control Name : SCAF-AROEM010421_00
Sensitivity : 5
Circularity : 8

	Conc.	% cells	# cells
Green:	7.58X10e4 cells/mL	2%	159
No Green:	3.42X10e6 cells/mL	98%	7184

Average cell size: 9 µm
# of cells counted: 7343
Total cell conc.: 3.50X10e6 cells/mL

File Name: SCAF-AROEM010421_7
Experiment Date : 2021-04-01 17-36-04
Assay : Green
Control Name : SCAF-AROEM010421_00
Sensitivity : 5
Circularity : 8

	Conc.	% cells	# cells
Green:	4.88X10e5 cells/mL	3%	1023
No Green:	1.36X10e7 cells/mL	97%	28457

Average cell size: 12 µm
# of cells counted: 29480
Total cell conc.: 1.40X10e7 cells/mL

File Name: SCAF-AROEM010421_8
Experiment Date : 2021-04-01 17-37-41
Assay : Green
Control Name : SCAF-AROEM010421_00
Sensitivity : 5
Circularity : 8

	Conc.	% cells	# cells
Green:	7.34X10e5 cells/mL	4%	1540
No Green:	> 1.5X10e7 cells/mL	96%	38224

Average cell size: 11 µm
# of cells counted: 39764
Total cell conc.: > 1.5X10e7 cells/mL

File Name: SCAF-AROEM010421_9
Experiment Date : 2021-04-01 17-41-12
Assay : Green
Control Name : SCAF-AROEM010421_00
Sensitivity : 5
Circularity : 8

	Conc.	% cells	# cells
Green:	6.74X10e5 cells/mL	3%	1415
No Green:	> 1.5X10e7 cells/mL	97%	39299

Average cell size: 11 µm
# of cells counted: 40714
Total cell conc.: > 1.5X10e7 cells/mL

File Name: SCAF-AROEM010421_10
Experiment Date : 2021-04-01 17-43-13
Assay : Green
Control Name : SCAF-AROEM010421_00
Sensitivity : 5
Circularity : 8

	Conc.	% cells	# cells
Green:	0.38X10e4 cells/mL	1%	8
No Green:	5.91X10e5 cells/mL	99%	1240

Average cell size: 6 µm
# of cells counted: 1248
Total cell conc.: 5.95X10e5 cells/mL

File Name: SCAF-AROEM010421_11
Experiment Date : 2021-04-01 17-48-19
Assay : Green
Control Name : SCAF-AROEM010421_00
Sensitivity : 5
Circularity : 8

	Conc.	% cells	# cells
Green:	0.43X10e4 cells/mL	1%	9
No Green:	3.62X10e5 cells/mL	99%	759

Average cell size: 6 µm
# of cells counted: 768
Total cell conc.: 3.66X10e5 cells/mL

File Name: SCAF-AROEM010421_12
Experiment Date : 2021-04-01 17-50-09
Assay : Green
Control Name : SCAF-AROEM010421_00
Sensitivity : 5
Circularity : 8

	Conc.	% cells	# cells
Green:	0.71X10e4 cells/mL	0%	15
No Green:	3.14X10e6 cells/mL	100%	6594

Average cell size: 12 µm
# of cells counted: 6609
Total cell conc.: 3.15X10e6 cells/mL

File Name: SCAF-AROEM010421_13
Experiment Date : 2021-04-01 17-52-08
Assay : Green
Control Name : SCAF-AROEM010421_00
Sensitivity : 5
Circularity : 8

	Conc.	% cells	# cells
Green:	1.10X10e4 cells/mL	0%	23
No Green:	2.36X10e6 cells/mL	100%	4962

Average cell size: 8 µm
# of cells counted: 4985
Total cell conc.: 2.38X10e6 cells/mL

File Name: SOXRF60421_1
Experiment Date : 2021-04-06 15-50-50
Assay : Green
Control Name : SOXRF60421_00
Sensitivity : 5
Circularity : 8

	Conc.	% cells	# cells
Green:	0.05X10e4 cells/mL	0%	1
No Green:	1.09X10e6 cells/mL	100%	2278

Average cell size: 11 µm
# of cells counted: 2279
Total cell conc.: 1.09X10e6 cells/mL

File Name: SOXRF60421_2
Experiment Date : 2021-04-06 15-53-25
Assay : Green
Control Name : SOXRF60421_00
Sensitivity : 5
Circularity : 8

	Conc.	% cells	# cells
Green:	0.29X10e4 cells/mL	6%	6
No Green:	4.86X10e4 cells/mL	94%	102

Average cell size: 9 µm
# of cells counted: 108
Total cell conc.: 5.15X10e4 cells/mL

File Name: SOXRF60421_3
Experiment Date : 2021-04-06 15-58-21
Assay : Green
Control Name : SOXRF60421_00
Sensitivity : 5
Circularity : 8

	Conc.	% cells	# cells
Green:	0.00X10e4 cells/mL	0%	0
No Green:	1.67X10e4 cells/mL	100%	35

Average cell size: 8 µm
# of cells counted: 35
Total cell conc.: 1.67X10e4 cells/mL

File Name: SOXRF60421_4
Experiment Date : 2021-04-06 16-00-41
Assay : Green
Control Name : SOXRF60421_00
Sensitivity : 5
Circularity : 8

	Conc.	% cells	# cells
Green:	0.05X10e4 cells/mL	1%	1
No Green:	3.86X10e4 cells/mL	99%	81

Average cell size: 9 µm
# of cells counted: 82
Total cell conc.: 3.91X10e4 cells/mL

File Name: SOXRF60421_5
Experiment Date : 2021-04-06 16-08-14
Assay : Green
Control Name : SOXRF60421_00
Sensitivity : 5
Circularity : 8

	Conc.	% cells	# cells
Green:	0.24X10e4 cells/mL	4%	5
No Green:	5.43X10e4 cells/mL	96%	114

Average cell size: 11 µm
# of cells counted: 119
Total cell conc.: 5.67X10e4 cells/mL

File Name: SOXRF60421_6
Experiment Date : 2021-04-06 16-10-12
Assay : Green
Control Name : SOXRF60421_00
Sensitivity : 5
Circularity : 8

	Conc.	% cells	# cells
Green:	2.67X10e5 cells/mL	4%	561
No Green:	7.00X10e6 cells/mL	96%	14691

Average cell size: 13 µm
# of cells counted: 15252
Total cell conc.: 7.27X10e6 cells/mL

File Name: SOXRF60421_7
Experiment Date : 2021-04-06 16-13-41
Assay : Green
Control Name : SOXRF60421_00
Sensitivity : 5
Circularity : 8

	Conc.	% cells	# cells
Green:	2.20X10e5 cells/mL	7%	461
No Green:	3.05X10e6 cells/mL	93%	6402

Average cell size: 13 µm
# of cells counted: 6863
Total cell conc.: 3.27X10e6 cells/mL

File Name: SOXRF60421_8
Experiment Date : 2021-04-06 16-15-15
Assay : Green
Control Name : SOXRF60421_00
Sensitivity : 5
Circularity : 8

	Conc.	% cells	# cells
Green:	4.57X10e5 cells/mL	3%	958
No Green:	> 1.5X10e7 cells/mL	97%	35565

Average cell size: 12 µm
# of cells counted: 36523
Total cell conc.: > 1.5X10e7 cells/mL

File Name: SOXRF60421_9
Experiment Date : 2021-04-06 16-18-23
Assay : Green
Control Name : SOXRF60421_00
Sensitivity : 5
Circularity : 8

	Conc.	% cells	# cells
Green:	3.76X10e5 cells/mL	2%	788
No Green:	> 1.5X10e7 cells/mL	98%	39600

Average cell size: 11 µm
# of cells counted: 40388
Total cell conc.: > 1.5X10e7 cells/mL

File Name: SOXRF60421_10
Experiment Date : 2021-04-06 16-20-31
Assay : Green
Control Name : SOXRF60421_00
Sensitivity : 5
Circularity : 8

	Conc.	% cells	# cells
Green:	0.05X10e4 cells/mL	2%	1
No Green:	2.43X10e4 cells/mL	98%	51

Average cell size: 8 µm
# of cells counted: 52
Total cell conc.: 2.48X10e4 cells/mL

File Name: SOXRF60421_11
Experiment Date : 2021-04-06 16-23-28
Assay : Green
Control Name : SOXRF60421_00
Sensitivity : 5
Circularity : 8

	Conc.	% cells	# cells
Green:	0.00X10e4 cells/mL	0%	0
No Green:	2.05X10e4 cells/mL	100%	43

Average cell size: 13 µm
# of cells counted: 43
Total cell conc.: 2.05X10e4 cells/mL

File Name: SOXRF60421_12
Experiment Date : 2021-04-06 16-25-08
Assay : Green
Control Name : SOXRF60421_00
Sensitivity : 5
Circularity : 8

	Conc.	% cells	# cells
Green:	0.14X10e4 cells/mL	0%	3
No Green:	1.15X10e6 cells/mL	100%	2417

Average cell size: 10 µm
# of cells counted: 2420
Total cell conc.: 1.15X10e6 cells/mL

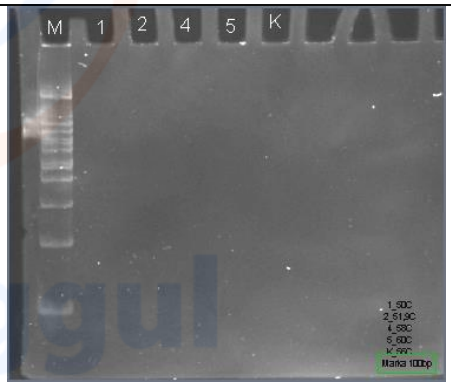
File Name: SOXRF60421_13
Experiment Date : 2021-04-06 16-26-20
Assay : Green
Control Name : SOXRF60421_00
Sensitivity : 5
Circularity : 8

	Conc.	% cells	# cells
Green:	0.14X10e4 cells/mL	0%	3
No Green:	1.17X10e6 cells/mL	100%	2454

Average cell size: 10 µm
# of cells counted: 2457
Total cell conc.: 1.17X10e6 cells/mL

Optimasi PCR melalui hasil pembacaan elektroforesis

<table>
<tr>
<td> 

RNA dengan primer penanda berupa Octmid</td>
<td> 

c-DNA dengan primer penanda berupa GAPDH</td>
</tr>
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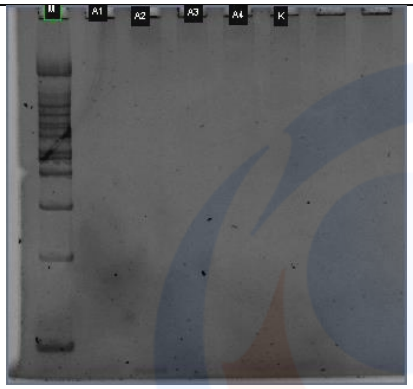
<td> 

c-DNA dengan penanda berupa GAPDH_1</td>
<td> 

RNA dengan primer penanda berupa Soxmid</td>
</tr>
</table>

Dokumen Publikasi

Bioscaffold from Mouse Embryonic Fibroblast maintains pluripotency of Mouse Embryonic stem cells

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Keyword: BioScaffold, Stem Cell, Mouse Embryonic Fibroblast, Decellularization, Oct4, Sox2

Abstract.

Culturing cell using 3D method provides a lifelike environment just like inside the body. Various method have been developed using synthetic, biological material and also combination of both. Bioscaffold is expected has more biocompability for stem cells growth. Bioscaffold from Mouse Embryonic Fibroblast (MEF) provides tissue integrity for cells attachment, interaction and growth factors. It was prepared from mouse fibroblast culture which had been crowded by Dextran sulphate supplemented with Ascorbic Acid to increase the production of extracellular matrix. Decellularization of cells was performed using Deoxycholate to remove parts of cells except their extracellular matrix. The extracellular matrix form 3D shape then called Bioscaffold. It served as culture media for Embryonic stem cells (ECS) but DMEM media was still added to support the growth. ECS culture using bioscaffold showed can maintain the Pluripotency of ECS marked by the presence of ECS marker (Oct 4 and Sox2). This biological scaffold can be developed as media for stem cell culture for propagation and differentiation. Furthermore, it will be useful for a model for tissue engineering and *in vitro* organ development.

Introduction

Stem cell are a special cell in multicellular organism that have the ability to developed to many different types of cells, and have potential to renew themselves. Stem cells are from embryo and adult cells. Stem cells are important because their ability to repair themselves, and in the future can be applied in medical world for repairing tissues and even organs [1] . Two properties are generally considered to define a stem cell are their capacity for long-term self renewal without senescence and pluripotency, their ability to differentiate into one or more specialized cell types. Protein marker which are crucial in pluripotent stem cell called *"Core Nuclear Transcription factors"* which consist of Oct3/4, Sox2, Klf and Nanog [2]. In 2006, Yamanaka et al. showed that that pluripotent stem cells can be obtained from mouse embryonic fibroblasts by combined expression of four factors, namely, Oct4, c-Myc, Sox2, and Klf4. It is called Induced-Pluripotent Stem Cell (iPS) [3]

Biomaterial of scaffold or natural scaffold, is supporting factor for stem cells, so physiologically safe for given as a medical cure to patients, and that is make it easy to blend with the area to be repaired. It provides tissue integrity for cells attachment and interaction and growth factors as well [4]. Naturally derived and synthetic polymers, bioresorbable inorganic materials, and respective hybrids have been developed. Decellularized tissue served as scaffold biomaterials which can boost structural, mechanical,

and biological properties. This method has potential to be developed for tissue engineering to be implanted directly to patient with defect cells [5].

Molecular crowding is a method to accelerate biochemical reaction by inserting inert macromolecules in solution to occupy a significant volume of medium [6]. Studies of crowding condition revealed that macromolecular crowding might affect protein structure, folding, shape, conformational stability, binding of small molecules, enzymatic activity, protein-protein interactions, protein-nucleic acid interactions, and pathological aggregation [7]. The intracellular environment condition is extremely crowded with volume occupancy of 5%–40% molecules [8] and creates a crowded medium, with considerably restricted amounts of free water [9]. It is very different with usual culture media used as cell culture media.

In this research, Bioscaffod formed from MEF were applied for embryonic stem cell culture. The pluripotency of stem cell were observed based on Sox2 and Oct 4, proteins marker for pluripotency, to know the effect of molecular crowding and bioscaffold in ESC culture. The application of Bioscaffold will be developed for ESC propagation and differentiation. Furthermore, it will be projected for tissue engineering in regenerative medicine.

Material and Methods

This research is start from January – May 2021 in Laboratory of Virology and Cancer Pathobiology Research Center, Faculty of Medicine, Universitas Indonesia. Stored MEF and ESC cells from liquid nitrogen were used for this research.

Mouse Embryonic Fibroblast Culture Under DMEM and Crowding Medium. MEF were seeded on 12-well plate at 50.000 cells per well in DMEM with 10% FBS, HEPES, Sodium bicarbonate, and 1% Penicilline-streptomycine. After 76 hours culture cells in 37°C 5% CO_2, crowd media (500kDa Dextran sulphate, 100µm L-ascorbic acid 2-phosphate) were added and incubated for 24 hour

Decellularization of cells to obtain bioscaffold . Sodium deoxycholate concentration of 0.5% in PBS were added in cell culture for 45 minute incubation, followed by 1 h incubation in DNAse I 6U/µL solution, then scaffold is ready for ESC culture or can be stored in 4°C until use.

Tali-cytometer for fluorescent cells count. ESC in extracellular matrix scaffold are cultured for 5 -7 days. After that, cells were harvested using Trypsin EDTA and collected into centrifuge tube then washed with flowcytometer buffer. Oct4 or Sox2 antibodies was added and followed by incubation for 1 hour. After washing, anti-mouse or rabbit anti-goat FITC antibodies as secondary antibodies were added and followed by 1 hour incubation and finally stored in 1% formaldehyde solution until reading in Tali-cytometer machine.

Results

Cells isolated from 10 days mouse embryos were cultured in DMEM media after being removed from Liquid nitrogen storage. After few days, the ECS will form a spheroid or grape-like shape, semi-floating in the culture (fig 1a) whereas the mouse embryonic fibroblast (MEF) adhere in the bottom of plate (fig 1b). After being separated, each type of cells were cultured in the different culture media. MEF were cultured in 12-well plate until 70-80% confluent and some of them crowded using Dextran and supplemented with Ascorbic acid to enhance extracellular protein production. ESC were cultured in DMEM-F12 media and stored in liquid nitrogen tank until use.

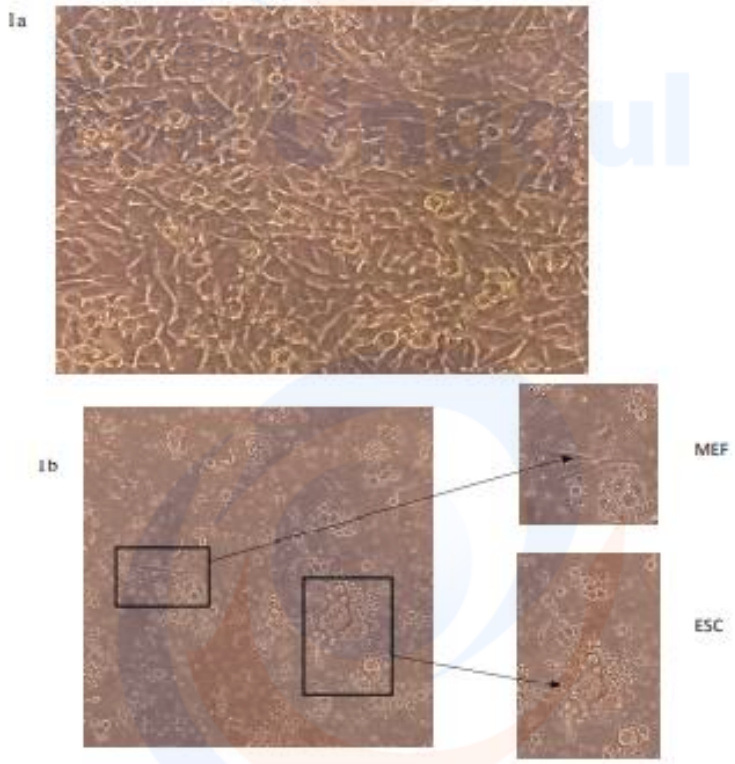


Figure 1. Mouse Embryonic Fibroblast (MEF) (1a) for preparing bioscaffold and Mouse Embryonic Stem cell (ESC) (1b) in DMEM culture media.

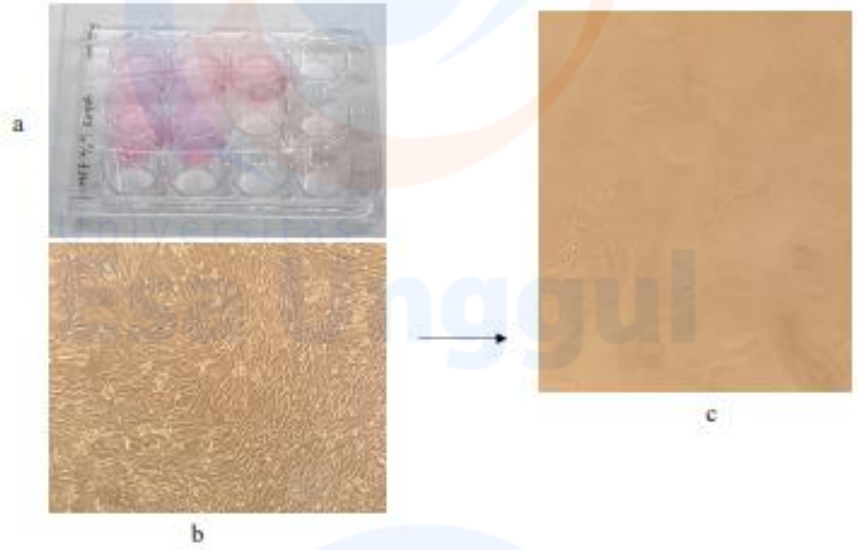
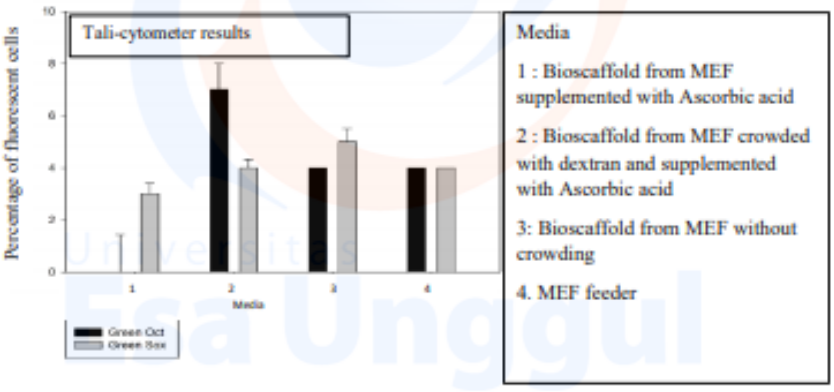


Figure 2. Bioscaffold formed by extracellular matrix after decellularization of confluent Mouse Embryonic Fibroblast (MEF) (b). MEF culture was crowded with Dextran sulphate and supplemented with Ascorbic acid to enhance the production of extracellular matrix. Cells then were decellularized using Sodium Deoxycholate resulting Bioscaffold for ESC culture (c).

MEF cells were cultured and propagated to obtain necessary number of cell for bioscaffold formation (fig. 1a). Cells then were seeded in 12-wells plate in DMEM (fig. 2a). After 1 day, crowder and supplement were added and incubated at 37°C for 48h (fig. 2b) Cells then were decellularized with Sodium Deoxycholate (fig. 1c). Extracellular matrix were obtained after decellularization and used as Bioscaffold for culturing ESC.



p-value				
Group	1	2	3	4
1		0,395	0,6	0,439
2	0,395		0,426	0,641
3	0,6	0,426		0,641
4	0,439	0,641	0,641	

p-value				
Group	1	2	3	4
1		0,6	0,439	N/A
2	0,6		0,293	N/A
3	0,439	0,293		N/A
4	N/A	N/A	N/A	

Figure 3. Tali-cytometer results for counting the fluorescent cells number which correspond to the presence of Oct4 and Sox2 in cells. After staining using Oct4 or Sox2 antibody as primary antibodies followed by the addition of secondary antibody conjugated with green fluorescent fluorophore (FITC), cells were analyzed using Tali-cytometer.

Discussion

Stem cells have great promise for cell therapy, tissue engineering, regenerative medicine and biotechnology. ESC are usually cultured as a monolayer in two-dimensional (2-D) culture plates and often xenogenic materials were added for attachment substrates, cytokines and growth factors, as well as serum. Xenogenic material from animal derived added in the media can potentially transmit pathogens and limit reproducibility between cultures due to lot-to-lot fluctuation of the material used [10]. Natural 3-D niches allow for complex spatial interactions between cells, ECM components, and gradients of nutrients, oxygen, and waste [11].Bioscaffold prepared from crowded MEF cells provide a better 3-D niches for ESC. So, they can propagate and maintain their pluripotency. However, nutrients, growth factors and other materials still need to be added and animal material need to be replaced for future application. The difference between media 1,2,3 and 4 still not statistically significant. Even though ESC cultured in bioscaffold from MEF crowded with dextran and supplemented with Ascorbic acid and ESC cultured in MEF feeder not statistically different, it tend to go higher in pluripotency showed by the increase number of protein Sox 2 and Oct 4 compared to ESC cultured in MEF feeder. As we know MEF feeder and conditioned medium now are very common to be used as media for embryonic stem cell and induced-pluripotent stem cell culture [12].

Bioscaffold 3-D will be applied in the next experiment for propagating, maintaining and also differentiating embryonic stem cells with the development of media and growth factors. As the characteristic of embryonic stem cells is pluripotent, this stem cell can be developed to various types of cells by adding growth factors, modifying media etc. In addition, natural products as herbal, active compounds and plant extract are being explored for propagation and differentiation of stem cells to various type of cells [13,14]

Conclusion

Bioscaffold made from extracellular matrix shows capability to maintain the pluripotency of ESC. It provides 3D culture which mimic ESC niche *in vivo*. Furthermore, it has more biocompatibility for implantation in the body and blend easily with area to be repaired. It is suitable for a model for tissue engineering and *in vitro* organ development.

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