Seeded-Decellularized Scaffold Vascular Tissue Engineering for Small-vessels Vascular Tissue Engineering in Indonesia: a Systematic Review

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1. INTRODUCTION:

Cardiovascular disease is the leading cause of death globally and comprises one of the most lethal groups of non-communicable disorders worldwide. The World Health Organization (World Health Organization) estimates that 17.9 million people die from cardiovascular disease (2018). Bypass procedures and blood vessel replacement using a graft are options that have high therapeutic value for coronary disorders.Small diameter (<6 mm) vascular grafts are used primarily in revascularization and reconstructive vascular surgical procedures. The most commonly used graft is an autologous vein or artery.This graft was used among 519,000 heart bypass operations performed annually in the United States, with an estimate of about 800,000 in the world (Baim, 2003). However, venous grafts in coronary bypass grafts become blocked over time due to accelerated atherosclerotic changes with a 10-year patency of 50%, postoperatively. There are several reasons for this, such as mismatch of adherence, thrombogenicity and poor hemodynamics. Differences in radius adjustment between the graft and native vessels at the site of the anastomosis compressed by suture inelasticity have been shown to cause luminal narrowing due to intimal hyperplasia. The ideal vascular graft should be non-thrombogenic, compatible with high blood flow rates, and have the same viscoelasticity as native vessels (Kannan et al., 2005). Therefore, the increased demands of small-diameter vascular grafts (SDVGs) globally has forced the scientific society to explore alternative strategies utilizing the tissue engineering approaches. Therefore, the exploration of alternative vessel sources in this regards must be performed. The aim of this investigation is to developed a small diameter (<6 mm) vascular graft from bovine mesenteric artery in combination with Endothelial Progenitor Cell (EPC) and Mesenchymal Stem Cell (MSC) as a basis for the development of small diameter vascular grafts.This first preliminary report deals with how Scaffold which is derived from bovine mesenteric blood vessels (decellularized with sodium dodecyl sulfate (SDS) in distilled, deionized water) after tested its tensile strength could be seeded with Endothelial Progenitor Cell (EPC) combined with Mesenchymal Stem Cell (MSC).

AUTOLOGOUS VESSEL GRAFT, SYNTHETIC VESSEL GRAFT VERSUS BIOENGINEERED SMALL VESSEL CONDUIT

Autologous grafts such as the great saphenous vein and the internal mammary artery represent the gold standard for small diameter vascular grafts. However, these vessels are limited, require invasive harvesting, and are sometimes inappropriate (Pashneh-Tala Samand et al., 2015). Synthetic materials such as polyethylene terephthalate (PET) and expanded polytetrafluoroethylene (ePTFE) have shown success when used instead of medium-large (> 6 mm diameter) grafts . Unfortunately, small diameter vessel grafts (<6 mm) , synthetic vascular grafts are limited due to their low patency rate (XU et al., 2017) The smaller the diameter, the lower the blood flow and the higher the intraluminal resistance. This condition increases the risk of several complications such as aneurysm, intimal hyperplasia, calcification, thrombosis and infection. These complications are also associated with regeneration of the non-functional endothelial layer and mismatch between the mechanical properties of the graft and the host vessels. When compared with autologous grafts, synthetic grafts tend to have lower compliance , or about one-fifth of autologous grafts . The ideal vascular graft is expected to have long-term strength and mechanical resistance to hemodynamic loads, be non-toxic, non-immunogenic, and biocompatible . These characteristics can be assessed by carrying out several tests, such as toxicity tests, mechanical tests and most importantly, the success of EC lining formation on the intima surface of the blood vessel graft before it will be applied in vivo.

The first tissue engineering as a blood vessel substitution was carried out by Weinberg and Bell in 1986, they cultured bovine endothelial cells, smooth muscle cells, and fibroblasts, on the collagen layer supported by Dacron's mesh. Currently, the seeding of autologous blood vessel cells is being developed on biodegradable scaffolds, which can then be cultured in vitro or can be directly implanted. Increasing understanding of tissue bio-engineering and the relevance of vascular extracellular matrix (ECM) to vascular tissue regeneration and its success in in vivo studies have led to an alternative source of scaffold material. Decellularized vessels represent also an alternative valuable source for the development of SDVGs. Decellularized allogeneic or xenogeneic tissue was able to maintain ECM integrity and minimal inflammatory response (Thottappillil, 2015). The bovine mesenteric artery graft is reported to have the advantage of good anastomotic compliance due to the elasticity of the blood vessels so that the risk of low intimal hyperplasia and a reported patency rate of 100% on day 35 is proven through the tensile strength test (Lin et al, 2018).This condition is the basis for implanting stem cells in grafts cultured on in vitro media before being applied in vivo. Stem cells that are combined in the graft are expected to be able to differentiate into endothelial cells. The decellularized bovine mesenteric artery will act as a mold for stem cells that will differentiate into the intraluminal endothelial layer or hereinafter known as the tissue-engineered vascular graft (TEVG) (Pashneh-Tala S et al, 2016).

Stem cells have the characteristics of self-renewability, clonogenicity, and plasticity (differentiated capacity). Endothelial progenitor cells (EPCs) in the TEVG study as an angiogenic source have been extensively studied. Mesenchymal stem cells (MSC) are also the most studied stem cells in the TEVG study. When compared to EPC, MSCs have a lower potential for differentiation into Endothelial Cells (EC), but can differentiate into Smooth Muscle Cells (SMC). MSC is thought to have antithrombogenic qualities, which could potentially be used in TEVG in vivo without any requirement for EC (Melero-Martin et al., 2008; Pashneh-Tala Samand et al., 2015).

TISSUE ENGINEERING FOR SMALL VESSEL CONDUIT

Tissue engineering (TE) represents an emerging research field where the production of vascular grafts utilizing state-of-the-art manufacturing methods has gained great attention from the scientific society . In contrast to large (d > 8 mm) and medium (d = 6–8 mm) diameter vascular grafts, which have currently been applied in a wide variety of vascular applications, such as carotid and aorta replacement, the production of SDVGs (d < 6 mm) requires further improvement. The requirements for an ideal tissue engineered vascular graft (TEVG), whether large or small, was related to the function of endothelial regeneration and the similarity of the mechanical properties of TEVG to natural blood vessels. TEVG must have the proper biological behavior and mechanical properties to achieve clinical use. The biological performance of TEVG should support the complete integration of the graft in the human body to avoid induction of a chronic inflammatory response during the process of material degradation. In addition, the mechanical properties of TEVG must match those of the native blood vessels, particularly in terms of deformability, compliance and strength. Vascular TE strategies are increasingly moving from a classical to an *in situ* approach also known as directed endogenous regeneration. Here, cell-free scaffolds are implanted to be colonized and remodeled endogenously, resulting in autologous vessel substitutes. One of the important principles of TEVG is the choice of scaffold with a suitable level of degradation during the in vivo remodeling process. The speed of scaffold degradation must be in accordance with the rate of tissue regeneration so that it can be used as a substitute for functional blood vessels. A number of different cell types have been used in the in vitro tissue-engineered vascular graft (TEVG) preparation. The type of cell used can directly influence the structure of the graft and how it performs in vivo, and simultaneously influence the grafting process. Mature autologous vascular cells, such as smooth muscle cells (SMC), endothelial cells (EC), and fibroblasts, have been used in many cases in making TEVG. These cells can be cultured for long periods in a bioreactor or seeded into a graft prior to implantation. Despite their popularity, the use of these cells has several drawbacks. Extraction of these cells requires an invasive vascular biopsy, causes donor morbidity, and in some cases may not be possible due to vessel quality or availability (Cho et al., 2005).

DECELLULARIZED SCAFFOLD

To date, despite numerous scaffolds that have been manufactured through varied forms of tissue engineering techniques, the construction of entirely biomimetic blood vessels is still underway. The biological decellularization structures derived from vascular tissue from either allogeneic or xenogenic sources have potential as a substitute for artificial vascular conduits, which will help solve the problem of donor vessel deficiency and recipient immune reactions. Decellularized vascular grafts currently on the market include products such as Artegraft (Bovine carotid artery), Solcograft (Bovine carotid artery), ProCol (Bovine mesenteric vein), and SynerGraft (Bovine ureter); This product is available for clinical use. Most of these grafts are used for vascular access during hemodialysis or peripheral artery bypass when a relatively large diameter is required. However, the clinical results were unsatisfactory due to thrombosis, infection, and aneurysm on this graft (Kumar et al., 2011; Pashneh-Tala S. et al., 2016). At present, decellularized xenogenic grafts only offer lower yields compared to other alternative synthetic channels. For example, the results of studies using synergraft have revealed a risk of aneurysm formation, poor long-term patency, infection, and inflammation. The limited performance of commercially available decellularized vascular grafts is due to a lack of cellularity at implantation. Another disadvantage of these decellularized xenogeneic grafts is that they cost more than synthetic grafts. Thus, this type of graft has not been widely used in clinics (Kumar et al., 2011). Therefore, various decellularized matrices derived from

the human umbilical artery, human umbilical vein, porcine carotid artery, porcine radial artery, porcine saphenous artery, porcine iliac artery, small intestine submucosa (SIS), and bovine ureter are investigated for application in tissue engineering. vascular diameter is small (Lin CH et al., 2018). We intend to develop the decellularized scaffold bovine mesenteric artery by sodium dodecyl sulfate (SDS) in distilled, deionized water as scaffold to develop TEVG and seeding it with mesenchymal stem cells (MSC).

CELL SOURCE FOR SEEDING

Bone marrow-derived mononuclear cells (BM-MNC) can be extracted from the bone marrow and include mesenchymal stem cells (MSC) and hematopoietic stem cells. BM-MNC extracts have the potential to produce various cell types, including vascular EC, SMC, and fibroblasts (Matsumura et al., 2003). These cells also lack major histocompatibility complexes, along with other important immunostimulatory molecules that offer potential as allogeneic cells for TEVG production. Shinoka et al. Used autologous BM-MNCs extracted from the superior iliac bone, and had great success in their initial clinical trial. In addition, these cells can be used to produce SMC and EC for seeding to TEVGs in vitro (Pashneh-Tala Samand et al., 2015).Mesenchymal stem cells can be separated from BM-MNC extracts or other tissues, including blood, adipose tissue, muscle, and liver. These cells can differentiate into SMCs, with in vitro studies of TEVG highlighting the effects of mechanical stimulation and growth factors that can occur in this process. Mesenchymal stem cells show little potential to produce EC, but results in animal models have shown that MSCs have a role in assisting EC colonization of TEVG. In addition, MSCs are thought to have antithrombogenic qualities, which could potentially be used in TEVG in vivo without any requirement for EC (Melero-Martin et al., 2008; Pashneh-Tala Samand et al., 2015).Adipose tissue stem cells also has its own stem cells which have been shown to differentiate into SMC and EC and have been used as a source of cells for vascular tissue engineering. These cells can be extracted in high numbers from aspirated adipose tissue which is often available and easily retrieved. Since most revascularization procedures are performed in elderly patients, the use of adipose tissue stem cells has certain advantages. It has been shown that these cells maintain high patency, with their ability to form ECs that are not affected by age (Zhang et al., 2011). In addition, the number of these cells does not appear to decrease with age with some evidence suggesting that the number of adipose tissue cells is higher in older subjects (Pashneh-Tala Samand et al., 2015).

VASCULAR TISSUE ENGINEERING

Cardiovascular grafts should have a good prognostic patency prior to in vivo implantation. The graft of the bovine mesenteric artery that has been decellularized and becomes a scaffold will then be performed seeding progenitor cells, namely EPC and MSC. Seeding with EPC was proven successful when positive results were obtained on CD34, VEGFR-2, CD31, VE-Cadherin, and vWF, while seeding with MSC proved successful when positive results were obtained on CD73, CD90, CD105. Both of these will be proven through the toxicity test of the MTT assay to calculate the percentage of the number of living cells. These living cells will then be subjected to a histopathological examination to see the endothelial surface. Good viscoelasticity is obtained from mechanical tests (tensile strength) and positive results on Elastin, Fibronectin, Fibulin, Collagen type I, III, IV, V, VI, Proteoglycan. The findings of endothelial cells, elastin, and collagen on histopathological examination, and tensile strength test will be prognostic for the patency of the blood vessel graft.

This study was designed as an experimental study, in accordance with the problem to be investigated and aims to analyze the efficacy of blood vessel implants using a scaffold from

bovine blood vessels given 2 types of stem cells, namely Endothelial Progenitor Cell (EPC) and Mesenchymal Stem Cell (MSC).
1. Stage I: The process of preparing a blood vessel scaffold taken from a bovine vein
2. Phase II: The seeding process uses 2 kinds of stem cells, namely Endothelial Progenitor Cell (EPC) and Mesenchymal Stem Cell (MSC)
3. Stage III: in vitro efficacy analysis of vascular implants

STAGE I : THE PROCESS OF PREPARING A BLOOD VESSEL SCAFFOLD TAKEN FROM A BOVINE

Sampling Mesenteric Artery. Bovine mesenteric arteries were taken from Surabaya District Slaughterhouse. The mesenteric artery was taken *in toto* with bovine ileus and bovine colon. The mesenteric artery was skeletonized dissection from the surrounding tissue. The mesenteric artery was then fixed using NaCl solution into the urine sample tube which was then put in a box that has been prepared with an ice pack in it. Samples were taken to the "Bank Jaringan" within 2 hours of sampling. After arriving at the "Bank Jaringan", the artery was dissected and the additional tissues surrounding such as adipose tissue and connective tissue were removed. They were washedwith Natrium chloride 0.9% saline then stored in a sterile container with a streptomycin-penicillinsolutionat -20^{0}C. They were cut intosmallpieces (about 0.5 cm) and medium-sized (about 5-6cm).

DecellularizationMethods

The samples were dividedintosevengroupsdividedinto 1 groupofcontroland 6 ofexperimentalgroups were employedusing (a) SDS 0.5% in 1 week, (2) SDS 0.5% in 2 weeks, (3) SDS 0.5% in 4 weeks, (4) SDS 1% in 1 week, (5) SDS 1% in 2 weeks, (6) SDS 1% in 4 weeks. The arterywaswashedwithdistilledwater, continueonan orbital shaker (TS-100, StemCellsandFunctionalTissueLaboratoryResearchat Dr. SoetomoAcademic General Hospital Instrument Equipment Manufacturing, Surabaya Indonesia) (100r/min) forabout 1 hrandincubated in a sterilecontainerandsoaked in distilledwaterfor 8 hrs at room temperature with gentle agitation to demineralize the art eryforde cellularizing groups. After washing in distilled water for an additional 1 hr, they were treated with one of the following six different decellularization protocols and the results were compared with the control group. The decellularization protocol was based on detergents as the following steps. After onan orbital shaker, thesearteries were immersedintoeither 0.5 or 1 % SDS solution and continuously oscillated foreach time in subgroups (1week, 2 weeks, and 4 weeks). The decellularized arteries were washed by using the protocol toremove the residual detergents. All thesteps were performed in a sterileenvironmentat room temperature. At last, all samples from each treatment group were fixedwith 4% buffered formalde hydeforimmuno histochemistry (IHC) or 10% buffered for maldehyde for standardhematoxylin and eosin (H&E) histology.

Decellularization method. Blood vessels were cut as needed, washed using 0.9% NaCl solution, until there was no remaining blood. The samples were divided into seven groups. The blood vessels that had the appropriate size were immersed using SDS solution 0.1% and 0.5% with different duration. During the immersion process, the samples were stored in a closed container at room temperature 22-23 ºC. After 1-week, 2-weeks, and 4-weeks, the soaked blood vessels were washed with 0.9% NaCl, until there was no bubble.

Storage methods (frozen dried). Conducted at the "Bank Jaringan" at Dr Soetomo General Hospital, the specimens were frozen using a freezer with a temperature of -80 ºC for at least

1x24 hours. Freeze dried for 24 hours, then the specimens were stored in polyethylene plastic and vacuum packed to prevent interaction with outside air.

HistologyExamination
The histologyofthescaffoldswasexaminedtodeterminethe formalin-fixedspecimens were preparedforimagingbydehydration, embedding in paraffinsectioninginto 5 μmsections, andsubsequentlystainedwithhematoxylinand eosin. We alsouseMasson'sandVerhoeff-vamGieson'strichromestainingtoevaluatethevisualizationofthecollagenofthescaffold. The stainedtissuesections were analyzedunder a lightmicroscopeusing a digital image analysis system. Sections were visually evaluated bytheprofessional histopathology clinician in Dr. SoetomoAcademic General Hospital.

ScanningElectronMicroscopy
Decellularizedarteries were lyophilized for 48 hr to remove all moisture followed by sectioning using a scalpelandmountingonscanningelectronmicroscopystubs. The samples were coated in 18nm osmium metal using osmium plasma beforeimagingwith HITACHI FLEXSEM 100 scanningelectronmicroscope. Representativeimages were takenofcross-section sof the sample interior andexterior.

CellViability Study onScaffolds
Decellularizedscaffolds were sterilizedallthescaffoldsby 25 kGy gamma rays. The vascularscaffoldwasplaced in eachwellof a 24-well cellcultureplateand MG63 cellline (Pasteur Institute) was cultured one achdecellularized scaffold at a concentrationof 2 x 103 cells per scaffoldfor 1, 3, and 5 days. The MTT assay was performed to measure the metabolic activity of cells and estimated through color-change phenomenon from yellow-colored tetrazolium salt, MTT{3-(4,5-dimethyl thiazol-2yl)2,5-diphenyltetrazolium bromide} topurple-coloredformazan. The extractwaspreparedafter 24-26 hrincubationat 37 ± 10C in a 1 mLculture medium containing serum protein. The extractsolutionwasfurtherdilutedto 50%, 25%, and 12.5% in thesameculture medium. An equal volume (100 μL) ofextract, as obtainedfrom HGPR05, negativecontrol (high-densitypolyethylene), positivecontrol (dilutephenol), andcell were placedonthesubconfluentmonolayerof L929 cellsandincubatedfor 24 ± 2 h at 37 ± 1 °C. The culturedcells were treatedwith 50 μLof MTT andfurtherincubatedat 37 ± 1 °C for 4 h in humidifiedand 5% CO2atmosphere. The excess amount of MTT was removed by a spiration and 100 μL of is opropanol was added to dissolve the formazan crystals. Cytotoxicitytests were performed in triplicate. The color exchange was quantified by measuring absorbanceat 570 nmusing a spectrophotometer.

BiomechanicalTest: TensileStrengthTest
Samples in 5-6 cm length were preincubated in 0.9% NaCl in 15 mins to restore the grant texture. The measurements were performedby using an autograph micro computer control universal testingwith a 10N loadcelland a crossheadspeedof 1mm/min. The distance between clamps was 10 mm. The valley and peakdistance were 12 mm. The measurements were repeated atleast 4 times for each scaffold. Measurement was valid when a sample broke in the middle. The maximum strength was recorded. The thickness of the specimen depends oneach scaffoldproduced. The thicknesswasmeasuredusing a digital caliperwithanaccuracyof ± 0.1mm.

Biomechanical Test (Tensile Strength Test). This test was carried out using the *autograph microcomputer control universal testing* owned by the Faculty of Science and Technology,

Universitas Airlangga. Specimens that have been freeze-dried, with a minimum length of 4 cm were sent in polyethylene plastic packaging, before testing, the samples were immersed in 0.9% NaCl solution for 15 minutes to restore the graft texture. Tensile strength testing was done by one technician.

Histopathology Test. The histopathology test performed at the Anatomical Pathology Laboratory of Dr.Soetomo General Hospital, Surabaya. The aim of this test was to assess the effectiveness of decellularization in eliminating native cells while maintaining the extracellular matrix of blood vessels which was necessary for the growth of stem cells later. The test used two types of staining, namely Hematoxylin and Eosin (H&E) staining aimed at identifying the nucleus and Masson Trichrome (MT) staining to identifying collagen and elastin. The results of this coloring will be read by a PA specialist.

Scanning Electron Microscope (SEM). Decellularized scaffolds were sent to SEM laboratorium in Mechanical Engineering Faculty of InstitutTeknologiSepuluh November (ITS) Surabaya to be examined. The sample was prepared by putting on carbon double tape and then coated in Coxem Spt-20 ion sputtering machine with 5mA electric force parameter and holding time of 150 s. The examination was done by using Hitachi FlexSEM 1000 with two magnifications for each sample, 500x and 1000x. From this examination we evaluated the difference of porosity of the external and luminal surface area between six types of decellularization and one control. The result was presented in SEM images and read by a microbiologist.

Immunohistochemistry. Paraffinized scaffolds' samples were deparaffinized using xylene solution and rehydrated using ethanol solution. Samples were rinsed with running aquadest. Epitope retrieval was done by using a microwave and citrate buffer. The samples were left on room air temperature for 40-60 minutes after epitope retrieval. We used Novopen to limit the tissue and blocked the tissue with peroxidase block reagent. The samples were rinsed and given protein blocks. Primary antibody 150 microliter was given and left incubated overnight in the humidity chamber. The tissues samples were rinsed again and added post primary antibody. Compact polymer was given for 30 minutes each. Immunohistochemical staining and immunoblotting were done using chromogen (DAB). After Immunohistochemical staining the tissues were processed with haematoxylin staining. We used collagen type I antibody for the immunohistochemistry test. Collagen type I is the most abundant type of collagen in the body as it is one of the components in the smooth muscle and the extracellular matrix (ECM). This examination is done to evaluate whether there is any collagen type I left after decellularization.

CELL ISOLATION

Cell Sources

Mesenchymal Stem Cell from Human Adipose Tissue. All activities that involve exposed tissue were performed in a biosafety cabinet. We maintained sterility of the preparation throughout the procedure. Handle all human tissues and cells using Universal Precautions and disposed of all biohazardous waste as per institutional requirements. Sample for MSCs were resected fat tissue from open heart patients. We used 15-ml tubes for the preparation, and dispensed 4–5 ml adipose tissue into each tube together with 4 ml of 0.075% type I collagenase solution prepared. We minced tissue and lipoaspirate that can be drawn up into a 10-ml sterile plastic pipet with the tip broken off. We incubated adipose tissue-collagenase suspension on a rotisserie-style rotator at 37 C for 30 min. After 30-min incubation, we add an equal volume (4 ml or more) of hMSC complete growth medium (with serum) to the

digest to neutralize the collagenase. Total volume in a 15-ml conical tube will be ~ 12–14 ml. Then we centrifuged at 5000 x g for 10 min to pellet the MSC-rich dense cell fraction. Adipocytes and fat will be apparent as a yellow oily layer at the top of the tube. Decant supernatant (the oily layer and collagenase solution). A transfer pipet or other sterile tool may be used to dislodge the oily layer. We resuspended each pellet in 2 ml of 160 mMNH4Cl and incubated at room temperature (RT) for 10 min to lyse red blood cells. Samples may be pooled for convenient handling. We add more NH4Cl if necessary to achieve lysis of most of the blood cells. Unlysed red blood cells appear refractive and ‘‘donut’’ shaped by phase microscopy. Then we transferred samples to new centrifuge tubes and centrifuge at 1200 x g for 10 min to pellet the MSC-rich dense cell fraction. We removed supernatant and resuspended pelleted cells in hMSC complete growth medium. Then we filtered cell suspension through a 100-mm cell strainer. Seeded suspension into culture flasks containing the appropriate amount of medium. We has found that cells isolated from ~ 2.5 g of fat will become 75–80% confluent in ~ 7–10 days if seeded in a 75-cm^2flask (e.g., a 50-g tissue sample would be processed in eight 15-ml tubes, and the resulting cells seeded into sixteen 75-cm^2lasks). We incubated flasks at 37 C in a humidified incubator with 5% CO2 for 24 h and washed flasks twice with PBS to remove non adherent cells and add fresh growth media. We feed cells every 3–4 days. When cells reach 70–80% confluency (about 7–10 days), we harvested using the standard trypsinization method. We harvested cells may be cryopreserved or seeded at 1500 cells per cm^2 for continued expansion. At this point, cultures may also be established for surface marker characterization, and to test differentiation capacity. Before we did a marker characterization, the cells were washed with PBS containing 2 % FBS. FITC conjugated anti human CD45, and anti CD14 and PE conjugated anti human CD 34, CD29, CD73, CD90 and PerCP-Cy 5.5 conjugated anti human CD105 antibodies were used for staining the cells. All antibodies were purchased from BD Biosciences. Analysis was done on Flow Cytometer (BD FACS ARIA II) using FACS DIVA software (Version 6.1.2). Propidium iodide (PI) stains dead cells alone. Hence the cells unstained by PI during flow cytometric analysis represent the viable cell population (Hao et al. 1998; Mckenzie et al. 2007).

Table 1. Stem cells sources for tissue engineering of blood vessel :

Cell	Advantages	Disadvantages
Mesenchymal Stem Cells (MSCs)	(i) self-renewal capacity (ii) long-term viability (iii) pluripotent	(i) low frequencies of existence (ii) time-consuming expansion (iii) harvesting complications
adipose-derived stem cells (ADSC)	(i) superior multi-differentiation potential (ii) easily acquired with minimally invasive technique (iii) have lower donor-site morbidity (iv) abundant and practical	(i) susceptible to apoptosis during isolation (ii) cell expansion requires growth factors
Endothelial progenitor cells (EPCs)	(i) have exponential proliferation rate (ii) involved in hemostasis, angiogenesis, and arterial injury and endothelium repair (iii) can be evaluated *in vivo* in	(i) unknown *in vivo* EPC differentiation and migration signals and homing to the sites of injured endothelium or extravascular area (ii) EPCs from high risk

	Baboon model (iv) promote neovascularization in ischemic tissue, coating of vascular grafts, seeding hybrid grafts (v) can be harvested prenatally and noninvasively	cardiovascular patients have higher rates of *in vitro* senescence
Bone marrow cells (BMCs)	(i) readily accessible autologous cell source (ii) BMC aspiration is less invasive and associated with much lower morbidity at the donor sites (iii) have the potential to regenerate vascular tissues (iv) improve patency in tissue-engineered small-diameter vascular grafts	(i) may induce calcification and thrombus formation
Human artery-derived fibroblast (HAFs)	(i) promotes enhanced ECM formation and maturation ini	
Human umbilical cord vein endothelial cells (HUVEC)	(i) important in endothelialization after transplantation (ii) prevent platelet adhesion (iii) largely and routinely cultured from a readily available supply of discarded tissue (iv) have reproducible and enhanced angiogenesis capacity (*in vitro*)	(i) time-consuming isolation (ii) cell culture includes risk of infection and requires exogenous growth factor (iii) low proliferative capacity
Embryonic stem cells (ESCs)	(i) pluripotent (ii) may differentiate to SMC	(i) low induction efficiency (ii) low smooth muscle cell (SMC) purity

Endothelial Progenitor Cell from Human Adipose Tissue. **Adipose tissue collection.** Adipose tissue was collected from human adipose tissue. All procedures used in the study were approved by the ethical committee of the hospital. Approximately 50–100 mL of lipoaspirate was collected from each patient in two 50 mL sterile syringes. **Isolation of SVFs from adipose tissue.** SVFs from the adipose tissue (lipoaspirate) in syringes were collected using the SVF Rapid Kit (RegenMedLab, HCM, Vietnam). The extraction

procedure was performed according to the manufacturer's instructions. Briefly, two syringes of aspirated fat were connected with 2 syringes of the Rapid kit. The syringes were put into an extraction machine with the following parameters: time: 30 min, speed: 1 cycle/min. The lysed fat was then centrifuged at 1000 g for 5 min at room temperature to collect SVFs. Finally, SVFs were washed twice with phosphate buffered saline (PBS). The pellet was resuspended in PBS for determination of cell quantity and viability using an automatic cell counter (NucleoCounter; Chemometec, Denmark). **Primary culture.** SVF samples were used to isolate EPCs by a 2step procedure. In the first step, SVF samples were cultured in MSCCult medium (RegenMed Lab, HCM, Vietnam) containing DMEM/F12 supplemented with antibiotic-antimycotic, epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), with 10% FBS (Thermo-Scientific, MA). The cells were plated at 5 × 104 cells/mL in T-75 flasks (SPL, Korea) and incubated at 37°C with 5% CO2 to collect rapidly adherent cells. After 48 h, the supernatant was collected. In the second step, the supernatant was centrifuged at 1000 g for 5 min to harvest the cell pellet. The pellet was resuspended in EGM-2 solution from the EGM-2 BulletKit (Lonza, Basel, Switzerland); 3 mL of EGM-2 was used per 3 mL of original supernatant. The suspension was then incubated at 37°C with 5% CO2 to collect slowly adherent cells, which were considered to be adipose tissue derived endothelial progenitor cells (ADEPCs). After 7 d, the cell medium was replaced with fresh medium, and continually replaced every subsequent 3 d until the cells reached 70–80% confluence. At that point, the cells were subcultured by TrypLe Express (Thermo-Scientific, MA). **Flowcytometry.** Cell markers were analyzed following a previously published protocol. Briefly, cells were washed twice in PBS containing 1% bovine serum albumin (BSA). The cells were then stained with anti-CD31-FITC and antivascular endothelial growth factor receptor 2 (antiVEGFR2)-PE (both purchased from Santa Cruz Biotechnology, Canada) for ADEPCs, and anti-CD44PE, anti-CD73-PE, and anti-CD90-FITC (all purchased from Santa Cruz Biotechnology) for MSCs. Stained cells were analyzed by a FACSCalibur flow cytometer (BD Biosciences). Isotype controls were used in all analyses.

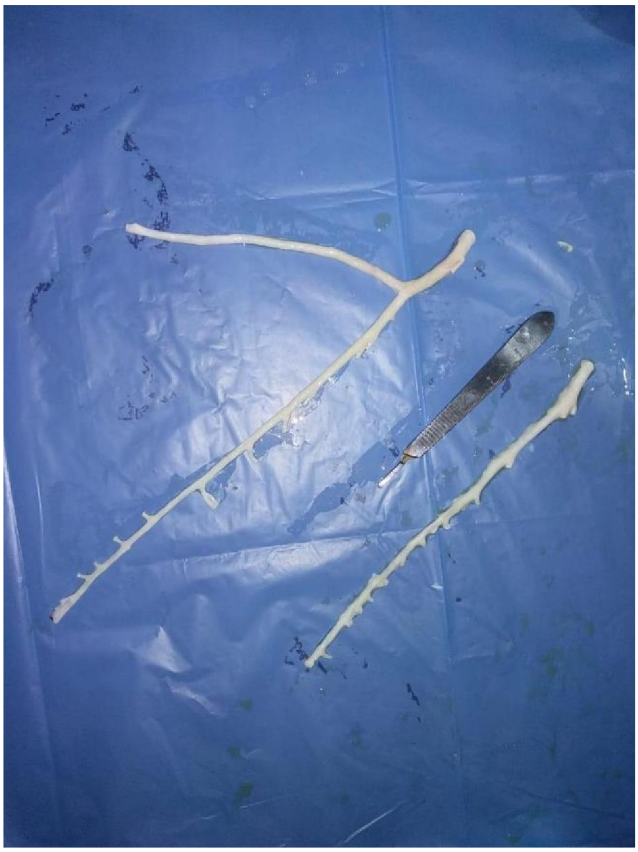

Figure 1. Skeletonized bovine mesenteric artery

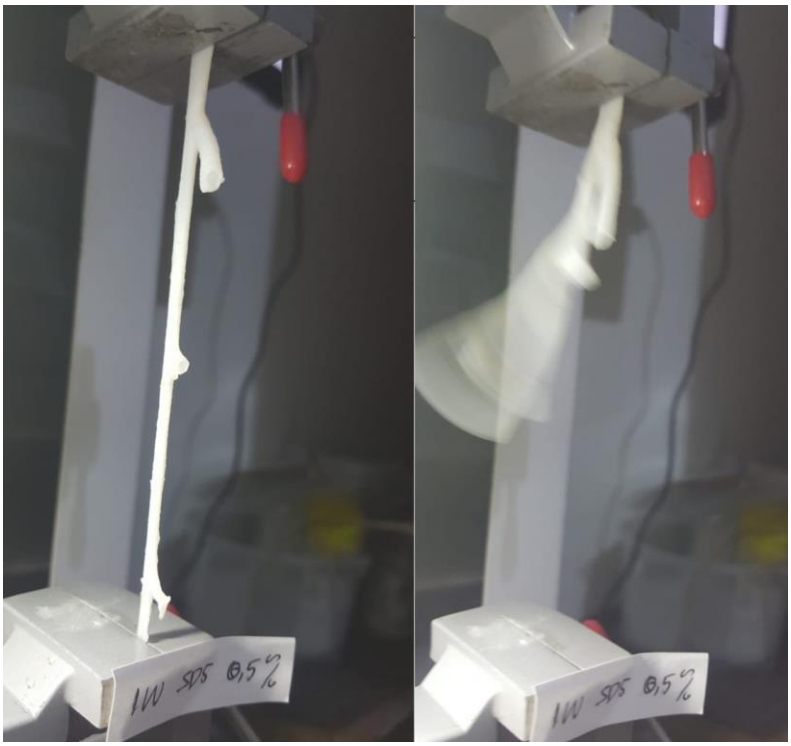

Figure 2. Tensile strength

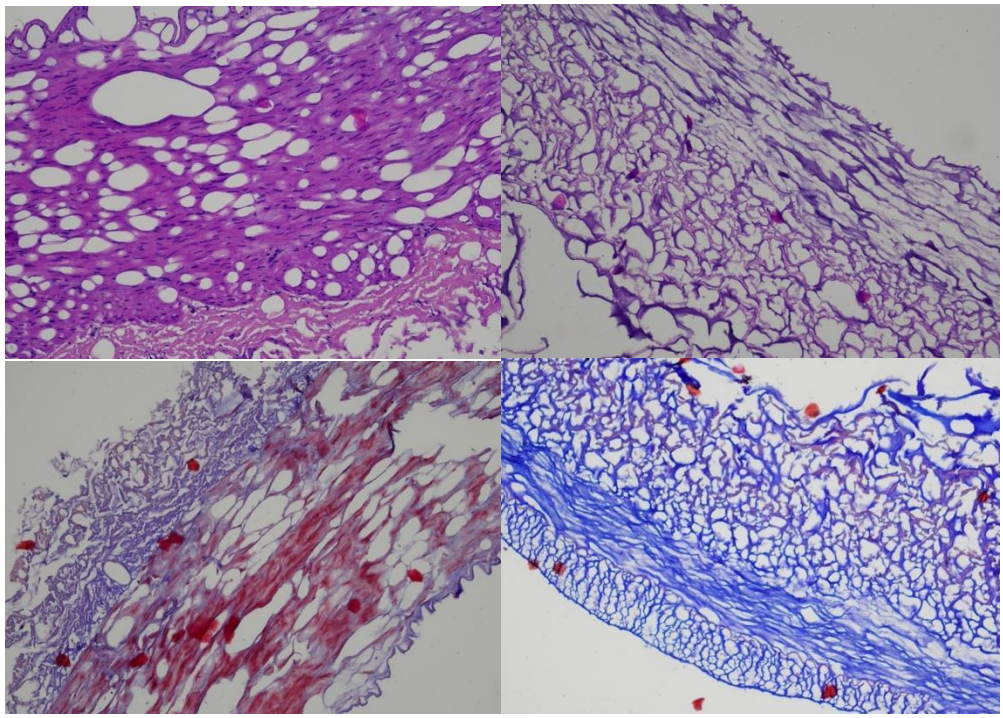

Figure 3. Mesenteric artery before decellularization on HE- staining (left-above; 40x) and MT-staining (left-below; 40x). Mesenteric artery after decellularization on HE-staining (right-above; 40x) and MT-staining (right-below; 40x)

CONCLUSIONS

The development of vascular bioengineering has led to a variety of novel treatment strategies for patients with cardiovascular disease. Notably, combining biodegradable scaffolds with autologous cell seeding to create tissue-engineered vascular grafts (TEVG) allows for in situ formation of organized neovascular tissue and we have demonstrated the clinical viability of this technique in patients with congenital heart defects. The role of the scaffold is to provide a temporary 3-dimensional structure for cells, but applying TEVG strategy to the arterial system requires scaffolds that can also endure arterial pressure. Both biodegradable synthetic polymers and extracellular matrix-based natural materials can be used to generate arterial scaffolds that satisfy these requirements. Furthermore, the role of specific cell types in tissue remodeling is crucial and as a result many different cell sources, from matured somatic cells to stem cells, are now used in a variety of arterial TEVG techniques. However, despite great progress in the field over the past decade, clinical effectiveness of small-diameter arterial TEVG (<6mm) has remained elusive. To achieve successful translation of this complex multidisciplinary technology to the clinic, active participation of biologists, engineers, and clinicians is required.

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