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Clinical observation, imaging, and histopathology of 3D polypropylene mesh for abdominal hernia in rabbits

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ABSTRACT

Polypropylene 3D hernia mesh for abdominal hernia repair may trigger foreign body reactions. In this research, we evaluated 3D hernia mesh developed for animal hernia through clinical and hematological, blood biochemistry, imaging through ultrasound, X-ray, and tissue histopathology in rabbits. This research enrolled 10 male New Zealand white rabbits aged 6-9 months weighing 1-2 kg divided into 2 groups, which were sham (without implant) and implant group (with 3D hernia mesh). The 1st surgical created hernia defect by incision on the abdominal muscle 4 cm long which was left for 2nd weeks. The 2nd surgical closed the hernia defect by auto-tissue (sham) and by 3D hernia mesh from polypropylene. Evaluation on hematology, blood biochemistry, and imaging through ultrasound and X-ray of the 3D hernia mesh were performed on day 6th, 12th and 24th after implant. Macroscopic and microscopic evaluation on the tissue around 3D mesh were performed on day 24th, 48th, and 96th after implant. Examination of hematology and blood chemistry profile showed that 3D mesh trigger minimum response from the body. The result of tissue imaging around 3D mesh showed radiopaque density with hernia defect completely closed without severe exudative inflammation and hyperechoic echogenicity. The microscopic examination of peritoneum muscle layer around the 3D mesh did not show severe pathological reaction. Histopathological examination on day 96th after implant still found inflammatory cell around the surgical stitches. The use of 3D hernia mesh is safe and viable to close abdominal hernia based on comparative study in rabbit.

Keywords: Polypropylene, 3D mesh hernia, Imaging, Histopathology

Hernia is an abnormal protrusion of an organ through the abdominal wall or the diaphragm (Sangwan *et al*. 2013, Pardesi *et al.* 2020). In several cases of big hernia rupture, treatment attempt to close the rupture might be hindered by tension around the closing stitches and thus requires a closing tissue in hernia defect formed in 3D structure (Brescia *et al*. 2017). The main concept of hernia treatment using hernia mesh has been used since 50 years ago (Shah and Shah 2019). Therefore, he introduced a three-dimensional mesh prosthesis (3D) that was anatomically designed for easy positioning, fixation free and reducing pain (Koch *et al.* 2006).

Polypropylene is often used in medical field as implant material in 3D mesh structure. Polypropylene mesh is categorized as a classic heavy weight type with small pores and high tensile strength. Other types are the middle weight and the light weight meshes. The light weight meshes have comparatively greater pore sizes and an increased flexibility (Pardesi *et al.* 2020). Attachment of foreign materials into the body triggers inflammatory response. Mesh-induced inflammatory responses may lead to postoperative complications and inflammation (Conze *et al.* 2004). We synthesized polypropylene polymer as 3D hernia mesh utilizing 3D printing for use in pet animals. Therefore, the present study was undertaken to evaluate the efficacy of 3D mesh hernia graft polypropylene for implant of hernia *in vivo* examination in rabbits.

MATERIALS AND METHODS

This research was approved by the Veterinary Ethics Committee of Faculty Veterinary Medicine, Universitas Syiah Kuala with certificate number: 62/KEPH/V/2020. The 3D hernia mesh implant was developed from polypropylene polymer. The design for 3D hernial mesh was created by CAD software, which then printed by laser sintering 3D printing process with polypropylene polymer with filament size being 1.75 mm. The size and temperature of the nozzle used to produce 3D hernial mesh were 0.4 mm and 230°C respectively. Meanwhile, the bed temperature was set on 100°C. The 3D hernia meshes were tested for material tensile strength according to ASTM D638 Type 5 test standard.

In vivo study utilized 10 New Zealand White rabbits aged 6-9 months weighing 1-2 kg. Defect was created on all rabbits by incising the abdomen 4 cm in length (abdominal

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hernia). Abdominal hernia defect included incisions on the skin, musculature, and peritoneum. Two weeks after, the defect was closed by surrounding tissue (sham) and by 3D hernia mesh from polypropylene. Post-surgery implant was performed in individual cages and each rabbit was given several drugs two times a day for 5 days which included antibiotic and anti-inflammatory PO.

Clinical observation comprised of wound healing, skin colour, organ location and protrusion (Erwin *et al*. 2023). Clinical examination and biocompatibility tests on 3D hernia mesh membrane which includes hematology and serum biochemistry of liver and kidney on day 0 before implant and day 6th, 12th, and 24th after implant. Tissue imaging examination for tissue condition around 3D hernia mesh was performed by ultrasound (USG) Vet DP-50® (Mindray, Shenzhen Mindray Bio-Medical Electronics Co, China) and Direct-Radiography (DR-Xray) Multix-Fusion® (Siemens, Jerman) on day 0, 6th, 12th and 24th after implant. X-ray was performed to observe hernia defect and organ location in the abdominal cavity, while ultrasound was used to observe the structure of the abdominal wall. Tissue around the 3D hernia mesh implant was biopsied on day 24th, 48th, and 96th after implant to examine the tissue response against the implant material. Lesions observed were hyperaemia, hemorrhage, inflammatory cells, neovascularization, and fibroblast or collagen whose severity categorized as few (1-5 lesions), moderate (6-10 lesions), and a lot (11-10).

Data from haematology and serum biochemistry were analysed by ANOVA (*Analysis of Varian*) with confidence level of 95% utilizing SPSS 24 software. Imaging and histopathology data were analysed descriptively.

RESULTS AND DISCUSSION

The choice of material for mesh hernia is often left to the surgeon's preference and cost factor. Repairing of large abdominal hernias using polypropylene mesh and polyester mesh (Sagar *et al*. 2010). However, the use of polypropylene mesh for abdominal hernia repair in small animals is an alternative to prevent recurrent hernias (Singh *et al.* 2012). Polypropylene 3D hernia mesh produced by 3D printing was 100x100 mm in size. Material tensile strength test result according to ASTM D638 Type 5 test standard. The pulling test obtained were tensile strength of 321.67 kgf per mm^2, yield strength of 261.67 kgf per mm^2, and elongation of 55.35 % (Fig. 1). The clinical observation on day 12th after surgery, both wound edges have closed well. The skin around the wound has the same colur as the surrounding skin, and no swelling is detected. The wound has healed completely, the stiches have begun to be removed, and the hernia defect has been well closed. The goals of hernia repair include minimizing intraoperative and postoperative complications, achieving effective repair, lowest possible recurrence, and early return to normal life, as well as cost effectiveness and better cosmetic results (Rashid *et al.* 2018).

Implant polypropylene material for abdominal hernia is a foreign object to the body and thus affect hematology and serum biochemistry as well as triggering inflammatory symptoms due to the body defence mechanism (Obongo *et al.* 2006). The meshes were made of polypropylene fibers arranged into a net with pores of differing sizes. PPM is classified on the basis of density of the material and its surface area as heavyweight (90 gm per sq meter to 100 gm per sq meter); middle weight (45 gm per sq meter to 50 gm per sq meter) and light weight (less than 45 gm per sq meter) (Shah and Shah 2019).

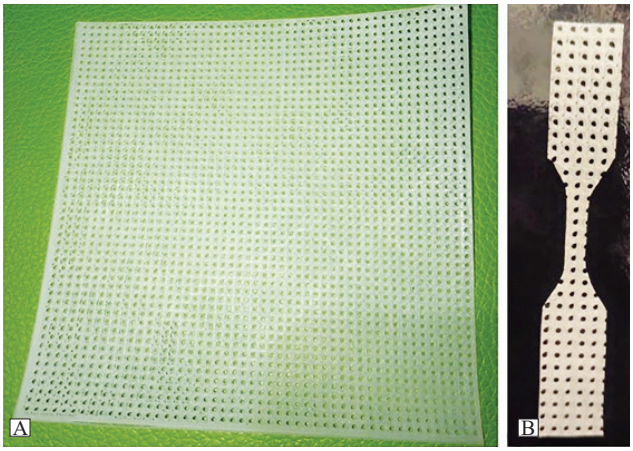

Fig. 1. (a) Polypropylene 3D hernia mesh produced by 3D printing method. (b) The pulling test result of 3D hernia mesh in accordance to ASTM D638 Type 5.

The number of erythrocyte, leucosyte, hemoglobin, hematocrit, and trombocyte did not show significant difference between sham group and 3D hernia mesh group ($p>0.05$) (Table 1). The number of erythrocyte, leucosyte, hemoglobin, hematocrit, and trombocyte did not show signfiicant difference within observation period ($p>0.05$). Increase in erythrocyte and hemoglobin are the physiological attempt from the body towards healing. Lowered number of erythrocyte and hemoglobin in a long period of time can cause hypoxia which may be followed by tissue death. The bond between oxygen and haemoglobin protein molecule not only play a role in biological oxidation but also tissue oxygenation (Siallagan *et al.* 2019).

The vascular system is key to the inflammatory response since most components of the inflammatory response transit through the blood and vessels (Zanoli *et al.* 2020). During acute inflammation, blood vessels react by vasodilatation induced by activation of endothelial cells, mediated by inflammatory cytokines. This vasodilatation is the cause of erythema or redness and static blood flow (Pober and Sessa 2015). Hemoglobin value goes in line with erythrocyte which makes an increase in hemoglobin value indicates an increase of erythrocyte value. The primary function of erythrocytes is the transport of oxygen to tissues. This increases the capacity of the blood to carry oxygen, reduces the hypoxic stimulus and provides a negative feedback of stopping EPO production (Panjeta *et al.* 2015).

Severe oedema and leakage of fluid from blood vessel into the tissue cause haematocrit value to increase (Casey

Table 1. Hematology profile of 3D hernia mesh on abdominal defect from day 0 until day 24th after implantation in rabbits

Day of observation	Group	Eritrosit ($\times10^6$/µL)	Leucocyte ($\times10^3$/µL)	Hemoglobin (g/dL)	Hematocrit (%)	Thrombocyte (/µL)
	Douglas, 2010	4.99-7.94	5.5-12.5	10.4-17.4	32-48	200-600
0	Sham	5.90±0.66	9.46±2.34	12.43±0.45	38.26±4.22	433.33±8.22
	3D mesh	5.12±0.47	10.66±2.75	12.60±0.40	33.63±1.57	436.66±4.42
6	Sham	5.42±0.45	10.90±3.10	11.43±0.94	35.43±4.70	736.22±7.44
	3D mesh	4.96±0.52	6.63±1.55	10.40±0.70	32.80±2.86	728.32±5.64
12	Sham	5.46±0.22	9.70±3.14	11.66±0.35	36.10±2.52	565.33±6.80
	3D mesh	5.88±0.34	6.96±1.71	12.00±0.72	38.93±3.05	463.66±6.48
24	Sham	5.31±0.60	9.20±1.90	11.10±0.82	34.90±4.45	401.23±5.68
	3D mesh	5.01±0.33	9.90±1.83	10.43±0.55	33.26±2.31	536.34±7.24

2000). Thrombocyte located in the damaged tissue will release thromboplastin and reacts with prothrombin and calcium to form thrombin, which will react with fibrinogen to form fibrin and cover the wound. Lowering thrombocyte value may increase the period needed for blood clotting, and thrombocyte value in normal blood circulation is always dynamic. The increase of leucocyte value after 3D hernia mesh implantation was due to stress and the formation of granulation on the surface of the wound which was still not ready to accept the implant. Inflammation phase will be shorted if there is no infection (Casey 2000). Leucocytosis in research animal occurs is the number of leucocyte increases 35% above its normal standard (Landén *et al.* 2017).

Serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), blood urea nitrogen (BUN), and creatinine (Table 2). SGPT, SGOT, BUN and creatinine level appeared to have increased in 3D hernia mesh group with no significant difference with sham ($p>0.05$). The BUN level on both groups increased in day 6th, 12th and 24th after implant with significant difference ($p>0.05$) against day 0 before surgery. Meanwhile, the SGPT, SGOT and creatinine levels did not show significant difference between observation period ($p>0.05$). An increase in BUN level indicated high protein metabolism in the body. The increase and decrease of SGPT, SGOT, BUN and creatinine levels during the research were within normal range. The increase of SGPT level is indicated by hepatocyte damage with nucleus and cytoplasm swelling, which causes the content of the cell to spill to the extracellular region of hepatocyte. Light liver damage is followed by an increase of SGPT in the blood, while severe liver damage will cause a drop of SGPT level (Ganai *et al.* 2014).

Intoxication and infection are among the causes of liver damage. The level of SGOT and SGPT may reach 20-100 times the normal range. Excessive presence of foreign object may trigger inflammatory process with macrophage presence, causing SGOT enzyme to be released in large number into the blood vessel which changes the permeability of cell membrane. Lowering BUN level is caused by feed with low protein, liver disease, severe malnutrition, liver function, the ability of intestine in absorbing protein, urease enzyme activity in the caecum and dehydration status. Protein intake may influence BUN level before and after implant (Siallagan 2019). Polypropylene compound contained in the implant ingredients is assumed to not be toxic to the kidney. Most of the compounds circulating in kidney is bonded with transferrin receptor. Thus, no nephrotoxicity occurring in the kidney caused by the implant. Gender, hunger, and muscle tissue sizes are factors that influence creatinine level. Creatinine level can be influenced by bone muscle mass, toxic compounds, and high protein consumption; creatinine level tends to fluctuate during several days observation post treatment (Pauletto 1999).

The results of DR-X Ray and ultrasound imaging of the tissue surrounding 3D hernia mesh (Fig. 2). Radiography is a non-invasive procedure to understand possible initial changes after implant, such as inflammation, bleeding, and exudate (Gu *et al*. 2006). It gives information about the density or radiodensity of an implant material.

Table 2. Blood biochemistry for liver and kidney of 3D hernia mesh on abdominal defect from day 0 until day 24th after implantation in rabbits

Day of observation	Group	SGPT (µL)	SGOT (µL)	Creatinine (mg per dL)	Urea (mg per dL)
0	Sham	47.00±6.56	60.33±4.24	1.10±0.17	16.00±4.00
	3D mesh	59.33±1.53	72.66±2.62	1.40±0.43	23.00±5.10
6	Sham	45.66±7.11	37.00±5.81	1.30±0.20	32.66±2.10
	3D mesh	39.00±3.00	31.33±4.10	1.26±0.05	38.33±4.23
12	Sham	45.33±6.52	41.66±6.22	1.20±0.17	38.00±2.64
	3D mesh	75.66±5.42	118.00±5.41	1.40±0.36	44.66±4.60
24	Sham	39.66±8.62	27.66±7.40	1.30±0.20	41.66±4.82
	3D mesh	39.00±5.21	25.33±4.64	1.06±0.32	41.33±4.90

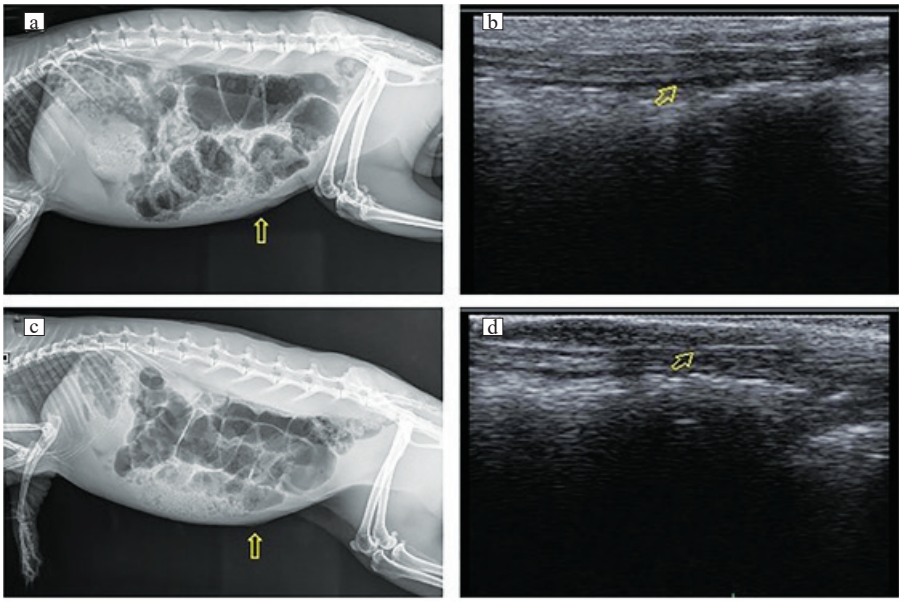

Fig. 2. DR-X Ray and ultrasound imaging (yellow arrow) of tissue structure around 3D hernia mesh on day 24th post-surgery. (a) DR X Ray imaging, (b) ultrasound imaging on sham group, (c) DR X Ray imaging and (d) ultrasound imaging on 3D mesh hernia.

The ultrasound and X ray on day 6th and 12th showed hyperechoic echogenicity around 3D hernia mesh due to inflammation. The accumulation of hyperechoic fluid is no longer visible on day 24th after implant and indicated that 3D mesh hernia accepted by the body. A stable density (echogenicity) of implant material means the material is biocompatible (Erwin *et al*. 2021). The main responses against the presence of mesh graft material are the formation of palmitic protein layers such as albumin, IgG and fibrinogen by the area around the implant soon after implantation (Elango *et al.* 2017). The gross anatomy of abdominal muscles around 3D hernia mesh did not show significant difference between sham group and 3D hernia mesh group.

Hernia defect was closed well; the muscles around 3D hernial mesh did not show pathological changes and the tissue had anasthomosed into the 3D mesh hernia's pores. Connective tissue proliferation and neovascularization into 3D hernia mesh accelerates anastomoses that would close the hernia defect. General result of histopathology structure examination by hematoxylin-eosin staining is provided in Table 3.

The histopathology of the muscle surrounding the implant sites did not appear to be significantly different between the sham and 3D mesh hernia (Fig. 3). A week after implantation, the phagocytic mononuclear cell will differentiate into macrophage. These cells release messenger which helps modulating inflammatory response. Inflammatory cells marginate into injured tissue and an efflux of leukocytes and plasma proteins enter the wound site. Neutrophils arrive initially and function to phagocytose and debride the wound. Macrophages phagocytose injured tissue and debris as well as secrete multiple growth factors. The macrophage orchestrates tissue repair and appears to be the only inflammatory cell type absolutely required (Franz 2008). This sell also plays a role in the synthesis of connective tissue especially collagen as a form of wound healing. After implant surgery, implant material will continuously react with the tissue or body fluid and cells will respond the recovery process with acute inflammation. Incompatible implant material will cause pathological reaction around the implant. Inflammatory cell invasion pathological reaction may require months to years for foreign material to be phagocyted until it is completely destroyed (Knapik *et al.* 2013).

Histopathology showed that proliferation of fibroblast into implant is more prevalent in 3D mesh hernia group compared to sham. Fibroblasts migrate into acute wounds

Table 3. Histopathology of the muscles around 3D hernia mesh on abdominal defect from day 24th until day 96th after implantation in rabbits

Microscopic lesion	Lesion severity on day					
	24		48		96	
	sham	3D mesh	sham	3D mesh	sham	3D mesh
Hyperemia	Moderate	Moderate	Few	Few	Few	Few
Hemorrhage	Few	A lot	Few	Few	Few	Few
Inflamation	A lot	A lot	Moderate	Moderate	Few	Few
Neovascularization	Moderate	A lot	Few	Moderate	Few	Few
Fibroblast or collagen	A lot	A lot	A lot	A lot	A lot	A lot

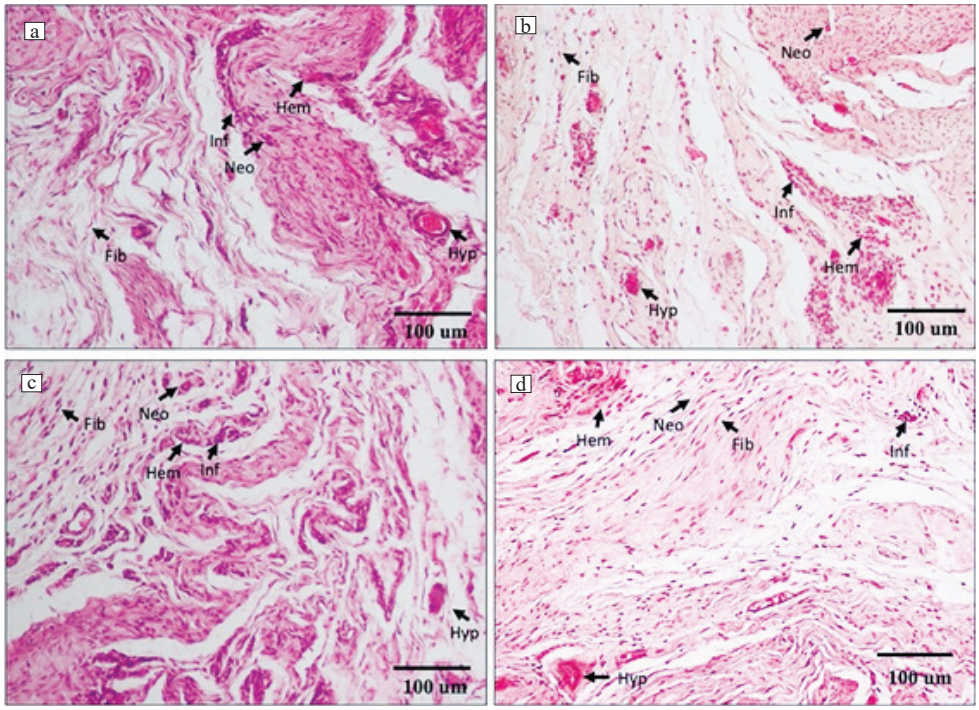

Fig. 3. Histopathology muscle around of 3D mesh hernia in abdominal defect in day observation. (a) sham group on day 24th post-surgery, (b) 3D mesh group on day 24, (c) 48th and (d) 96th post-surgery. Hyperemia (Hyp), hemorrhage (Hem), Inflamation (Inf), Neovascularization (Neo), Fibroblast or collagen (Fib). Hematoxylin-eosin staining.

within two days and are the major cell type in granulation tissue by post-injury day four. Receptor mediated interactions are increasingly described between the wound extracellular matrix and activated repair fibroblasts (Franz 2008). Fibroblast synthesize extracellular matrix and collagen which are very important in wound healing (Erwin *et al.* 2017). Collagen will provide strength to the wound area and promote the development toward normal skin (Tracy *et al.* 2016). Mesh graft from polymer is an option in hernia case treatment due to low biological response and side effects as well as lowering the risk of post-surgery infection in hernia case (Greisler *et al.* 1991).

The study has successfully determined the strength of 3D hernia mesh as well as observe its clinical condition, haematology, serum biochemistry, imaging and muscle integration histopathology around 3D mesh hernia. 3D mesh hernia made of polypropylene material had good biocompatibility and covered hernia defect in experimental rabbits. The next step is clinical trial of 3D mesh graft in pets with hernia.

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