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Function Assessment of a Fabricated Artificial Vascular Graft in Sheep Carotid Artery

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ABSTRACT

Background & Objective: In the field of vascular surgery, the use of tissue-engineered vascular grafts is advancing and new synthetic tissues are being utilized to replace damaged blood vessels. These synthetic vessels, made through tissue engineering techniques, must mimic the shape and mechanical properties of native vessels. This study was performed to assess the function of an artificial vascular graft in an animal model.

Materials & Methods: The evaluation of artificial vessels was carried out on rat and sheep models. The artificial vascular scaffolds were made of Polyethylene terephthalate (PET), Polyurethane (PU), and Polycaprolactone (PCL) polymers. In the first phase, the fabricated scaffolds were implanted in rats and after 45 days, the grafts were removed and evaluated pathologically. In the second phase, the structures were implanted into the carotid arteries of sheep. Doppler ultrasound and angiography imaging were done to assess changes in carotid blood flow. Eleven months later, the artificial grafts and surrounding tissues were removed and evaluated pathologically.

Results: In the rat samples, no hypodermic infections, systemic inflammation, or fibrosis of adjacent tissues were observed. In the sheep samples, no local or systemic complications were reported one week after surgery. No complications were seen after 11 months in the two sheep that received PCL/PU grafts. In contrast, ultrasound evaluation showed thrombosis in the two other sheep that received PET/PU/PCL grafts.

Conclusion: This study shows that the implanted artificial vessel used in sheep carotid arteries has a favorable patency rate and satisfactory clinical results, and in terms of mechanical properties, it may be a good candidate for vascular replacement.

Keywords: Artificial Vessel, Vascular Graft, Carotid, Sheep, Polyurethane (PU), Polycaprolactone (PCL), Polyethylene Terephthalate (PET)

Introduction

Coronary and peripheral artery diseases cause numerous deaths in developed countries, particularly in the United States (1). Additionally, congenital heart anomalies affect about 1% of live births, with about 25% requiring surgical treatment over their lifetimes (2). Risk factors for heart disease include age, gender, ethnicity, genetics, family history, diabetes, obesity, hypertension, hyperlipidemia, unhealthy dietary habits, smoking, physical inactivity, and psychological disorders such as stress, grief, and depression (3). Treatment options include lifestyle and dietary modifications, drug therapy, and surgery in severe cases (4).

Autologous transplantation is the standard treatment for surgical candidates, but its use is restricted due to limited donor sites (5). Bypass grafting is the last resort in coronary and peripheral artery diseases to restore blood flow to underserved tissues (6). Synthetic grafts have been widely used in cardiovascular surgery since the mid-20th century, and advancements in tissue engineering have led to the development of tissue-engineered vascular grafts (TEVGs) that mimic the structure and biochemistry of native vessels (7).

Ideal characteristics of a vascular prosthesis include long-term patency, durability, low porosity, ease of handling, kink resistance, desirable biological properties, and low production costs (8). Polyethylene terephthalate was the first prosthesis used in aortic surgery and was introduced by DeBakey in 1957 (9). However, studies have shown that commercial synthetic vascular grafts are not a suitable alternative to large blood vessels, as they lack the ability to adapt to surface forces and plasma protein absorption and activation, leading to inflammation, thrombogenicity, anastomotic intimal hyperplasia, aneurysms, and atherosclerosis (10, 11).

In some patients with conditions such as aperture, varicosity, anatomic variation and previous removal, the autologous vessels were not applied as vascular grafts (12). On the other hand, patients with disorders in the saphenous veins, the internal mammary or radial arteries and those with peripheral arterial occlusive disease (PAOD) are excellent candidates for vascular substitutes (13).

Successful TEVGs must meet two major principles in vascular surgery: adaptation to hemodynamics and tissue physical changes and non-thrombogenic luminal surfaces to prevent platelet adhesion. The durability of vascular grafts is directly related to blood flow rate (14). In a previous study, we reported the fabrication of vascular scaffolds with different PCL/PU weight ratios and evaluated their mechanical properties (15). In the present study, we assessed the in-vivo characteristics of our fabricated vascular scaffolds, including immunological reactions in rats and biomechanical properties in sheep.

Sheep are appropriate animal models for evaluating TEVGs in the carotid region as the site for carotid surgery is readily accessible, allowing for the implantation of vessels up to 15 cm long. We expect that the implanted artificial vessels will be able to adapt mechanically to different blood pressures (6).

Materials and Methods

A) Graft Fabrication and Characterization: The vascular scaffolds used in this study were fabricated using electrospinning techniques with high porosity, as previously described (15). To achieve maximum mechanical performance, two different compositions of nano-fibrous scaffolds were used. The first composition was a triad-hybrid composite of polyethylene terephthalate (PET), polyurethane (PU), and polycaprolactone (PCL) with a blending ratio of 33% each (PET33/PU33/PCL33). The second composition consisted of two polymers, PCL and PU, with a blending ratio of 75% and 25% respectively (PCL75/PU25). PCL and PU were chosen for their relatively high strength and appropriate elastic properties, respectively.

B) Assessment of Local Immunological Response:

The local possible immunological reactions were evaluated by subcutaneous implantation of the fabricated scaffolds in rats.

Animal Characteristics: The rats were provided by the Mashhad University of Medical Sciences (MUMS) medical faculty animal lab and had an average age of 45 days and weight of 170 grams.

Preoperative Considerations: Before the procedure, the artificial vessels were sterilized for 14 hours using ethylene oxide in the CSR section of the open-heart surgery ward.

Anesthesia Management and Implantation: The rats were anesthetized with a mixture of ketamine (1.5 cc) and Xylazine (0.7 cc) via intraperitoneal injection. After shaving and sterilizing the skin with Povidone-iodine, an 8 mm incision was made on the right side of the abdomen. The subcutaneous tissues were explored using sterile scissors, and pieces of the fabricated scaffold with 8mm2 diameter were implanted in the subcutaneous area using forceps (Fig. 1). The skin was then closed with 5-0 nylon sutures. A total of 69 rats underwent this procedure

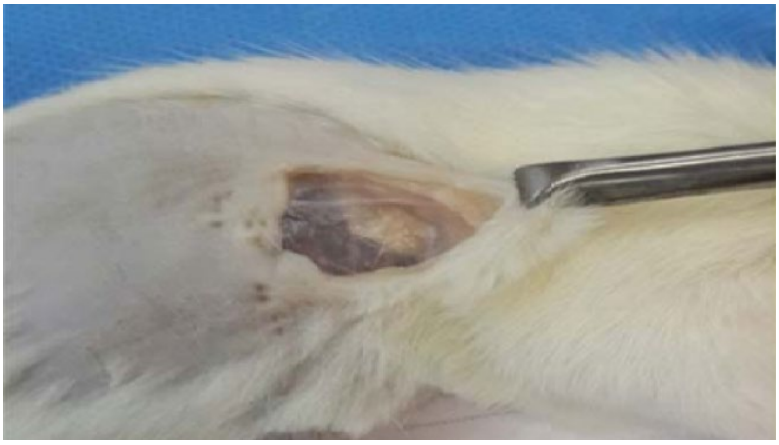

Figure 1. Implantation of Tissue Engineering Scaffolds in Rats through an 8mm Incision on the Skin

After 45 days, the rat grafts were removed followed by pathological tests and the evaluation of the implantation site.

C) Evaluation of graft performance: To be able to conduct research on Sheep, an agreement was signed between the Mashhad University of Medical Sciences and the Animal Research Center of Agriculture faculty in Ferdowsi University in Mashhad. Four young healthy female Sheep weighing 23 kilograms on average were provided. All the surgical procedures were performed in the Faculty of Veterinary Medicine at Ferdowsi University.

Implantation in Sheep: To evaluate the mechanical properties of the fabricated vascular scaffolds, this structure was grafted into the common carotid artery in Sheep. The advantages of using the Sheep model are the suitable anatomical location for surgery and the possibility of using the same surgical set and suture material used in humans **(16)**. Vascular surgeons and veterinary surgeons along with veterinary anesthetists collaborated in the procedure and grafted one artificial vessel on the carotid artery. Sheep were not fed for 24 h before surgery. For each animal the weight and age were recorded and they were numbered. Four Sheep categorized in two groups (group A labeled as 416, 491 and group B labeled as 420, 477) were used. One type of artificial vessel (PET33/PU33/PCL33) was implanted in group A and another type (PCL75/PU25) in group B (Fig. 2). Carotid flow and its size were determined by Doppler ultrasound prior to surgery.

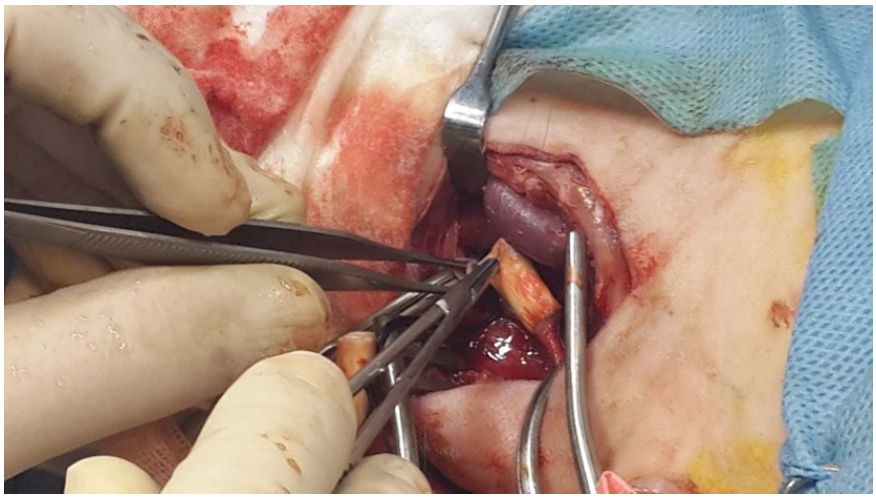

Figure 2. Implantation of artificial vascular PCL/PU (75:25) scaffold in sheep carotid

Anesthesia was applied by intramuscular injection of xylazine and topical lidocaine in the surgery site. Xylazine injection was repeated one time during the operation. The left carotid artery of Sheep was selected for vascular implantation. To achieve the best surgery site, the sheep was rested on the right lateral position on the surgery table. The Sheep neck was prepared for the operation with Chlorhexidine and Povidone-iodine and then draped sterilely The Internal jugular vein and carotid artery were exposed and surrounding tissues were dissected from the artery. Cefazolin (1gr) and heparin (100 IU/kg) were injected intravenously during surgery. The proximal and distal portions of the carotid artery were clamped. A 50 mm length of the left carotid artery was dissected. The implanted vascular scaffolds were 3cm long and had a 5mm diameter. The vessel was transplanted with the vascular grafts using a 7-0 Prolene suture. The artificial vessel (3-cm long) was sutured end-to-end as an interposition graft using running suture. Incisions were closed in two layers with Nylon suture. The duration of surgery was 1.5 hours. Serum therapy was performed to maintain fluid and electrical balance by the injection of 500 ml of normal saline (NS) and 500 ml of Ringer's solution during the surgery (Fig. 3).

Finally, using polypropylene sutures, an end-to-end anastomosis was performed using the beveled technique (Johnson & Johnson, New Jersey, USA). A blood sample was taken, and the surgical site was treated with Oxytetracycline spray. The surgery lasted 1.5 hours and the animal had one urination during the procedure. The total amount of bleeding was 70 cc. After the surgery, the animal was transferred to the recovery room and was able to stand up after half an hour.

Postoperative Considerations: The sheep were monitored for surgical complications, such as blindness, coma, and paralysis. One hour after surgery, the carotid flow rate was measured using Doppler ultrasound imaging, and the carotid artery pulse was checked daily. Enoxaparin 40 was injected subcutaneously into the sheep three hours after surgery, and the sheep were allowed to begin feeding three hours after surgery. They were then transferred to the livestock and poultry research center and were given a daily dose of Aspirin 80 for seven days. The surgical site was checked daily for wound healing and carotid pulse. Doppler ultrasound imaging was conducted before and after surgery to monitor changes in blood volume and carotid diameter. Additionally, to assess the degree of patency of the implanted prosthetic vessel, Doppler ultrasound was used one, three, and six months

after surgery, and angiography (SIEMENS) was performed six months after implantation.

Eleven months after implantation, to evaluate tissue responses in sheep, the grafted vessel and surrounding tissue were surgically removed and examined pathologically. Local anesthesia was applied to the sheep's neck using 5 cc of lidocaine. An incision was made at the site of the artificial vessel, and after separating the vessel from the surrounding tissues, the sheep were sacrificed. The tissues were then transferred to the pathology department for further examination. They were assessed using a microscope after being properly stained (Fig. 4). The study protocol was approved by the ethics committee of Mashhad University of Medical Sciences (approval code: IR.MUMS.REC.1393.964).

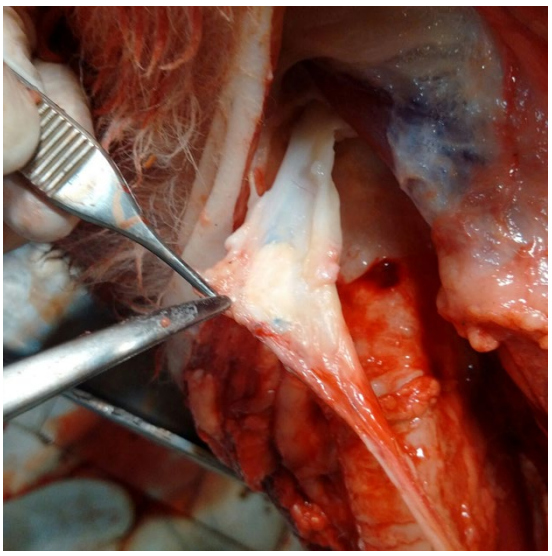

Figure 3. Exposure of previously implanted artificial vessel in the sheep carotid

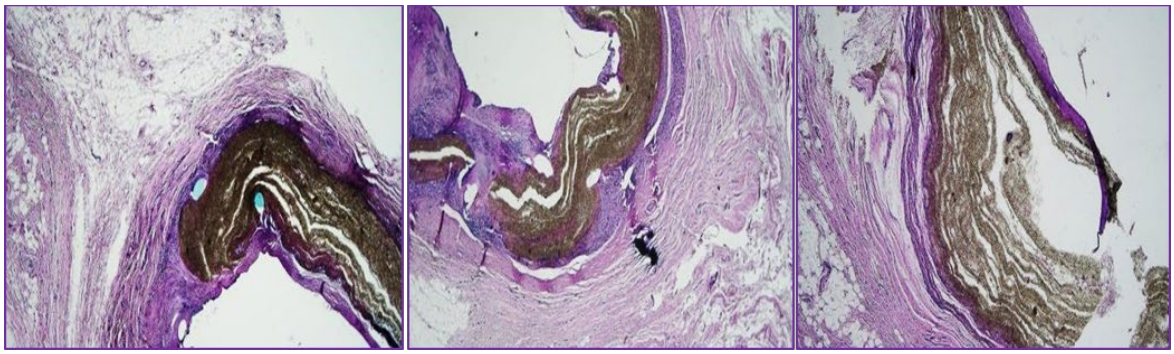

Figure 4. Hematoxylin and eosin (H&E) sections of the implanted vessel and the surrounding tissue for pathological evaluation..

Results

To evaluate the immune system's response to the polymers used in artificial vessel scaffolds, all specimens were subjected to pathological and histological examinations after being removed from the bodies of sheep and rats. The samples were placed in 4% formalin for 24 hours, then fixed with paraffin, and examined for signs of edema, fibrosis, and granulomatous foreign body reaction.

Results in Rat:

During the study, we observed no significant local or systemic inflammatory responses in rats. The pathologist evaluated the rats' reaction, and no infection was seen at the surgical site. The polymers used in the composite structures, either the PCL/PU or the PET/PU/PCL type, did not elicit any harmful or inflammatory responses in rats. Therefore, composite polymer structures are non-toxic in terms of degradability and do not lead to adverse responses or tissue death in adjacent tissues. The degree of reactions, including edema, fibrosis, and granulomatous foreign body reaction, was moderate.

Results in Sheep:

One day after surgery, cardiac examinations showed that the heart sounds were normal and that the carotid pulse was palpable (mean 105 beats/min) in all four sheep. There was no evidence of hematoma.

Blood samples were analyzed, and the mean values were as follows: Red Blood Cell count (11 × 10^9/L), Hematocrit (36%), Platelet count (× 10^3/µL), White Blood Cell count (× 10^3/µL), Neutrophil count (30%), and Lymphocyte count (60%).

Two sheep that underwent vascular graft transplantation using PCL/PU grafts were monitored for over eleven months. Doppler ultrasound and angiography imaging were performed at different time intervals to assess changes in the blood flow through the implanted vascular scaffold in vivo and compare it with the native vessel. This technique was used to investigate the blood flow volume and carotid diameter of the sheep before surgery, with values of 400 mL and 4.8 mm, respectively. Ultrasound was performed pre- and postoperatively on these two sheep. Ultrasound was also performed on these sheep one, three, five, seven, and eleven months after surgery, and no evidence of thrombosis was seen. Eight months after surgery, angiography was performed on the sheep, and the results showed that the grafting process was successful.

Ultrasound evaluation of the two other sheep that underwent graft surgery using the PET/PU/PCL graft showed thrombosis.

At the end of the study, the average weight of the sheep was 47 kg.

Histological investigation of the implanted artificial vessel composite in sheep showed satisfactory results in terms of pathology assessments. Mild edema, moderate fibrosis, and foreign body granulomatosis were observed in reaction to body tissues, which is remarkably favorable given the one-year time frame in the animal (Table 1).

Complications:

No sheep mortality was observed during the study, and duplex imaging after synthetic graft implantation in sheep did not reveal significant stenosis, hematoma, or torsion. However, thrombosis was seen in two out of the four sheep.

Table 1. Results of pathological reaction evaluation in sheep

Edema	Fibrosis	Foreign body Granulomatosis	Necrosis
Mild	Moderate	Moderate	Absent

Discussion

The limited availability of autologous tissue necessitates the use of synthetic grafts in cardiovascular surgeries. When the patient's own blood vessels are unsuitable for grafting, synthetic or prosthetic vessels are needed (17). Thrombi and intimal hyperplasia are the two major complications that occur following synthetic vascular graft implantation. These consequences are a result of the structural differences between the composition of synthetic vascular grafts and autologous tissue. Biocompatibility and mechanical durability are two important objectives in achieving successful tissue engineering technology (18).

There are several types of vascular grafts that can be created through tissue engineering techniques. It is important to design vascular grafting studies that evaluate the physiological functions of prosthetic vessels under different conditions such as hypertension in experimental animal models (6). The selection of the appropriate structural material and suitable surgery design will lead to successful vascular implantation. In the past three decades, there have been significant advancements in the TEVG field for producing high-quality vascular conduits in vitro conditions. These developments have played a critical role in accelerating the treatment process for arterial atherosclerosis patients who require bypass surgery. Lack of immune response against the graft and infection resistance are two important requirements for the commercial use of these artificially made tissues.

In this study, evaluation of the surgery site in rats and analysis of pathological samples demonstrated that the artificial vessel used does not pose any immunological threat.

Despite the potential of these tissues, there are still limitations to their use such as commercial availability and ambiguous costs (19). PCL/PU and PCL/PU/PET scaffolds are electrospinning structures that can act as a replacement for damaged tissue. They are nanofiber scaffolds containing porous substrates with a structure similar to the extracellular matrix, and have a wide range of applications in tissue engineering. It has been shown that cellular interactions, such as adhesion, migration, proliferation, and differentiation, are optimal on these structures (20).

A key principle in achieving patency is maintaining a single-layer cell with a non-thrombogenic surface that reduces the risk of activation of the coagulation cascade and platelet adhesion. Furthermore, before vascular implantation, these structures must have suitable integrity and a stable endothelium (21).

This study was designed to evaluate the in vivo function of artificial vessels made using tissue engineering techniques. Sheep are an appropriate animal model for evaluating vascular grafts in the carotid region, as their neck is a suitable anatomical site for carotid surgery. The carotid artery is accessible and, after passing through tissue layers, a vessel of up to 15 cm can be implanted (6). To interpret the results of preclinical animal studies that were conducted in vivo, it is essential to know their hematological and

hemodynamic characteristics. Since the hemodynamic properties of sheep are similar to humans, they are a suitable choice for experimental vascular graft studies. The mean cardiac index of sheep is 115 (mL/min/kg), heart rate is 95 (beats/min), and arterial and diastolic blood pressure are 140 and 90 mmHg, respectively (22).

Another advantage of using sheep in pre-clinical trials is their relatively large size, which allows for the use of similar surgical techniques and equipment as those used in human surgeries. Additionally, sheep have a lower cost compared to other large animal models such as pigs and non-human primates (23).

Despite the potential benefits of TEVGs, there are still challenges to overcome, such as the development of complications like stenosis and aneurysm (24). In most cardiovascular patients who require valve and artery replacement, autologous prostheses, bioprosthetic, and synthetic prostheses are used. Dilation, infection, and calcification are common complications which can lead to further surgeries and patient morbidity (25). To address these issues, researchers continue to explore new materials and technologies for the development of ideal prosthetic grafts.

Inoguchi et al used thermoplastic polyurethane for the production of artificial vessels and assessed its thermal stability in the body. They demonstrated that this polymer is irresolvable and biocompatible (26).

In the present study, PCL/PU (75:25) composite was prepared via co-electrospinning of PCL and PU nanofibers separately, retaining unique properties of both polymers. The obtained composite structures showed appropriate strength, Young's modulus, and compliance, comparable with that of native grafts, making them suitable for vascular tissue engineering. Synthetic vascular scaffold has shown successful clinical applications such as biocompatibility and biodegradability with no trace of toxicity, chronic inflammation, or tissue necrosis after implantation. Observations performed after Doppler ultrasound and angiography showed that blood flow in the implanted artificial vessel was normal and did not show significant changes.

T. Matsuda et al used polyurethane for the matrix and assessed the rotational speed of electrospinning and type of solvent on mechanical properties of the matrix (27). Browning et al mixed collagen and PEG hydrogel to prevent overgrowth of cells and blood coagulation. This product was reinforced with electrospinning polyurethane (28). Grsal et al made a scaffold with electrospinning polyurethane and assessed parameters such as homogeneity, continuity, proliferation, and tenacity in vivo (29). Ma et al published several articles about nanofiber as an artificial vessel scaffold and analyzed the impact of diameter, direction, and other parameters on the proliferation of cells. They used nanofiber that was copolymerized with Caprolactan and PET (30).

In this study, the mechanical properties of two different types of artificial vessels were examined. Follow-up investigations using Doppler ultrasound and angiography imaging showed normal carotid flow and no evidence of thrombosis in the two sheep in which the PCL/PU structure was implanted. Excellent patency was observed with no complications. In the other two sheep in which the PCL/PU/PET structure was implanted, thrombosis occurred as evaluated by ultrasonography; however, no other complications such as blindness or paralysis emerged, and the sheep were alive after 11 months.

We therefore suggest that the biochemical and mechanical properties of the first type of artificial vessels were appropriate for implantation in vivo.

Since fibro-intimal hyperplasia has been reported to be an important cause of late prosthetic vascular graft failure, many studies have shown that the use of heparin can prevent this complication (31, 32). In this study, heparin was also administered, and thus no evidence of hyperplasia was found.

We demonstrated that the implanted vessels remained in the sheep's body for 11 months until the animals were euthanized. In a study by Fukinishi et al, the degradation extent of nanofiber vascular grafts was compared between sheep and rats, and it was found that grafts lasted longer in rats than in sheep (33). Although we did not perform this comparison in our current study, the duration of the graft remaining in the sheep's body was longer in our study, 11 months, compared to the 6 months reported in the study by Fukinishi et al (33).

However, in a study by Wang et al, the implantation of vascular grafts in sheep was evaluated, and the animals were followed for 12 months. They reported the occurrence of aneurysms in the grafts (34).

In vivo studies are valuable because they not only support the positive results of in vitro studies but also reveal their limitations and provide insights for future studies.

Conclusion

In conclusion, our study demonstrated that the PCL/PU and PCL/PU/PET composite vascular scaffolds had suitable mechanical and biological properties for implantation in sheep. The implanted vessels remained patent with normal carotid blood flow for up to 11 months without any evidence of hyperplasia or chronic inflammation. However, thrombosis was observed in the PCL/PU/PET group. Further preclinical animal studies are necessary to evaluate the long-term performance of these scaffolds before clinical translation.

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Conflict of Interest

The authors declare no conflict of interest.

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