Influence of Silicone Sheets on Microvascular Anastomosis

The Hoang Nguyen MD, PhD, Marcus Kloeppel MD, PhD,
Christoph Hoehnke MD, PhD, Rainer Staudenmaier MD, PhD

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Abstract The use of silicone products combined with free flap transfer is well established in reconstructive surgery. We determined the risk of thrombosis as a result of direct contact between the silicone sheet and the point of microanastomosis. We performed microvascular surgery in 24 female Chinchilla Bastard rabbits weighing 3500 to 4000 g using two groups: Group 1 (n = 12), microanastomosis directly in contact with silicone sheets; and Group 2 (n = 12), microanastomosis protected by a 2 × 3 × 1-cm muscle cuff before being placed in contact with the silicone. We assessed flow-through of the microanastomosis by selective microangiography and histology at 1 and 3 weeks. All microanastomoses in Group 1 were occluded by postoperative thromboses, whereas all microanastomoses in Group 2 had adequate flow-through. Histologic analysis revealed thromboses in Group 1 formed

from collagenous bundles of fiber securely attached to the intraluminal wall of the vessel. Three weeks after the procedure, these thromboses were canalized by varying small vessels. In Group 2, a slight luminal stenosis with evidence of infiltration of inflammatory cells at the microanastomosis line was observed histologically in all cases. Prefabricated flaps using silicone sheets and muscular cuffs placed around the anastomoses appear to reduce the risk of thrombosis and enhance neovascularization.

Introduction

Silicone implants are widely used for breast augmentation [3, 7, 9, 29, 31], restoration of contour deficiencies [18], scar management [20], and intensifying neovascularization in prefabricated flaps [15, 21, 28]. They also have been used experimentally and clinically in association with free vascularized flaps to reconstruct tissue defects in the ear and nose [4, 12, 16], and the trachea [6]. For mandibular defect reconstruction, Bhathena and Kavarana [1] described the procedure of microvascularized free flap transfer combined with a primary silastic mandibular prosthesis in 69 patients. In Poland's syndrome, Shipkov et al. [25] reported one-stage surgery with a prefabricated vascularized latissimus dorsi muscle flap and configured silicone prosthesis.

In orthopaedic surgery, vascularized flap and silicone implants have been used to enhance clinical outcomes. In 1983, Stern et al. [26] reported favorable postoperative results of primary implantation of silicone prostheses in an acute open degloving hand injury to reconstruct the metacarpophalangeal (MCP) joint of the index, middle, and ring fingers combined with primary insertion of silicone spacers and rods to preserve extensor tendon function. In this case,

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T. Hoang Nguyen (✉)

Department of Hand Surgery and Microsurgery, Institute of Trauma and Orthopaedics, Central University Hospital 108, Khoa B1-2, Benh vien 108, So 1 Tran Hung Dao, Hanoi, Vietnam
e-mail: hoangkolpinghaus1@yahoo.com

T. Hoang Nguyen, M. Kloeppel, C. Hoehnke

Department of Plastic and Reconstructive Surgery, University Hospital "rechts der Isar", Technical University of Munich, Munich, Germany

T. Hoang Nguyen, R. Staudenmaier

ENT Department, University Hospital "rechts der Isar", Technical University of Munich, Munich, Germany

the entire wound and silicone implants were covered primarily with a well-vascularized skin flap. For hand reconstruction of congenital anomalies, Nishijima et al. [22] described silicone rod insertion to prepare for a flexor pollicis longus transfer combined with a free microvascularized transfer of the proximal interphalangeal joint of the second toe for carpometacarpal joint replacement. For finger microsurgical replantation at the level of the MCP joint, primary silicone implantation to preserve joint function may be a valuable and practical solution in selected cases [2].

In 1992, Itoh and Arai [17] described prefabricating a thin rectus abdominis flap by inserting a silicone sheet before transferring the flap to cover deep burns of the foot in obese patients. Hong et al. [13] reported prefabrication of a sartorius muscle flap in two stages to cover chronic knee wounds. In the first stage the sartorius muscle was lifted with the vascular supply intact and silicone sheets were used under the flap to increase its vascularity and size. After 3 weeks the muscle was transposed to the knee wound with a distal pedicle. The flap was used in six patients to close dead space and cover moderate-sized wounds.

Pribaz et al. [23, 24] described successful prefabricated flaps for facial defects, but suggested the failures either were attributable to insufficient neovascularization in the prefabricated flaps or to pedicle damage. We have experienced flap failure after simultaneous use of silastic implants for wrist and MCP joint reconstruction and free vascularized cutaneous flap transfer owing to skin defects after severe hand and finger contractile burn scars when we performed the vascular microanastomosis at the level of the silicone wrist-spacer implantation. Furthermore, based on previous experimental observations of prefabricated flaps using silicone and pedicles with arteriovenous anastomosis [10, 11, 28], we found flap transfer was unreliable when the microanastomosis was placed in direct contact with silicone sheets. However, how these sheets influence the microanastomoses is not known nor are the most effective ways to reduce or avoid this undesirable risk.

We therefore asked: (1) whether direct contact of silicone sheets with a microanastomosis influenced thrombosis, (2) whether prefabrication with silicone sheets enhance neovascularization, and (3) if a muscle cuff placed around the microanastomosis reduced thrombosis.

Materials and Methods

We used two experimental groups of 12 animals each to investigate the risk of thrombosis as a result of direct contact between the silicone and the microanastomosis. In Group 1 the microanastomosis was placed directly in

contact with the silicone; and in Group 2 the microanastomosis was protected using a muscle cuff before being placed in contact with the silicone. For each group, we randomly subdivided the surgically treated animals into two groups of six animals each for evaluation at either 1 week or 3 weeks. Twenty-four adult female Chinchilla Bastard rabbits weighing 3500 g to 4000 g were used for the two experimental groups. To determine the original vasculature and morphologic features in the random pattern abdominal skin flap, we operated on an additional three rabbits (six flaps) as controls. All the incisions were made in the same manner as in the experimental groups, but there was no vascular loop pedicle and no silicone sheet implantation. All animals were treated and housed in accordance with the European Directive for the Care and Use of Laboratory Animals (Reg Obb AZ 211-2531-38/95).

Anesthesia was induced via an intramuscular injection of a mixture of 40 mg/kg ketamine and 4 mg/kg xylazine. The right abdominal region and lower limb were shaved, washed with soap, and sterilized with iodine and isopropyl alcohol. We administered intravenous infusions through an ear vein during the surgical procedures, and all operations were performed under sterile conditions.

Medial and craniocaudal incisions were made in the rabbit's right abdomen to create an 8 × 15-cm skin flap (Fig. 1A). This abdominal flap was dissected together with the panniculus carnosus and lifted laterally. After controlling hemostasis in this area, we placed a silicone sheet (LPI, Perouse, France) measuring 8 × 15 cm × 0.25 mm on the abdominal wall anchored with Ethilon 3-0 (Ethicon,

Fig. 1A–C In this procedure, (A) an 8 × 15-cm flap is created, and (B) end-to-end microanastomosis of the artery and vein stump is achieved using 10-0 nylon suture to establish a vascular loop with maximal blood flow in the pedicle. (C) Selective microangiography shows the implanted vascular loop.

Norderstedt, Germany). We then made an incision extending from the inguinal ligament to the heel along the medial axis of the femoral and large saphenous vascular bundles. The femoral and saphena magna bundle with a minimum of perivascular tissue were dissected to create a 10-cm long pedicle. All branching vessels were ligatured or coagulated. Under $\times 5$ magnification, we identified and freely dissected the artery and vein of the vascular bundle. Next, the artery and vein stump were anastomosed end to end (interrupted) under a $\times 25$ magnification microscope using 10-0 nylon suture (Ethicon) to establish a vascular loop with maximal blood flow in the pedicle (Fig. 1B–C). Subsequently, the study animals were randomly assigned to one of two different groups. In Group 1 this vascular loop with the microanastomosis was turned in the cranial position, fixed beneath the abdominal skin flap using Vicryl 8-0 (Ethicon), and placed directly in contact with silicone sheets. In Group 2 before turning in the cranial position, the microanastomosis was carefully protected by a $2 \times 3 \times 1$ -cm avascular muscle cuff harvested from the gracilis muscle of the affected thigh, which was placed around the point of microanastomosis. This muscle cuff functions as a muscle barrier to protect the microanastomosis from direct contact with the implanted silicone sheet. We then closed the incisions.

All study animals were injected intraoperatively with an antibiotic (5 mg/kg Baytril 2.5% subcutaneously; Provet, Lyssach, Switzerland), an analgesic (0.05 mg/kg Temgesic subcutaneously; Essex, Munich, Germany), and 500 IU heparin (25,000 IU Heparin-Natrium intravenously; Ratiopharm®; Ratiopharm GmbH, Ulm, Germany). Postoperatively, heparin 1000 IU/daily $\times$ 5 days, Baytril 5 mg/kg/daily $\times$ 5 days, and Temgesic 0.05 mg/kg $\times$ 2 days were given subcutaneously.

At 1 or 3 weeks, we ascertained the flow-through of the microanastomosis. The animals were given heparin (5000 IU Heparin-Natrium) intravenously, and the implanted vascular loop was freely dissected. The input of the vascular loop was cannulated with polyethylene tubes (0.9 mm $\times$ 25 cm), and the prefabricated flap was irrigated through this tube with warmed heparinized saline (heated to 37°C) for 20 minutes. After irrigation, we immediately injected the loop with a suspension of Micropaque® 30% (Guerbet, Sulzbach/Taunus, Germany) with Rheomacrodex 10% (Pharmalink, Upplands Väsby, Sweden) in a ratio of 2:1 at a constant injection pressure of 110 mm Hg for 45 minutes. After giving a lethal injection of Nacoren (160 mg/kg pentobarbital; Merial, Hallbergmoos, Germany), the flaps were excised and pinned on cardboard holders for microangiography using a Radiofluor machine (120; TORR Philips Electronic Instruments, Munich, Germany) and Kodak radiographic film (Kodak-Pathe, France). We (MW, AF, KH, OP) then

determined, by visual observation, the flow-through (or occlusion) of microanastomosis by the way the microanastomosis was filled (or occluded) by Micropaque. We quantified neovascularization development using angiograms. We used a standard integral line plate including 25 evenly spaced lines corresponding to a 15-cm flap length. The vessel quantity was determined by counting the total number of times vessels perfused by Micropaque intersected the integral lines over the entire surface of the flap [11]. All selective angiograms were evaluated in blinded fashion by the four observers, and the final results were determined by the average value of the four.

After microangiography, we immediately fixed all flaps in 10% formalin for 5 days. Slices were cut perpendicular to the long axis of the implanted vascular loop at the point of microanastomosis, embedded in paraffin, and stained with hematoxylin and eosin, elastica van Gieson, and elastica Ladewig. We (MW, AF, KH, OP) then recorded the morphologic structures of the vessel loop, including the position of the vessel loop and muscle cuff in the flap, development of new connective capsule, inflammatory reaction, and changes around the microanastomosis.

For microangiography in the control animals, we gave heparin intravenously and used general anesthesia. We inserted plastic intravenous cannulae (0.5 $\times$ 0.9 mm) in the carotid artery and jugular vein. Two thousand milliliters of heparinized saline heated to 38°C was infused into the carotid artery at a standard pressure of 110 mm Hg and then allowed to drain through the jugular vein. After infusion, a suspension of Micropaque 30% (Guerbet) and Rheomacrodex 10% (Pharmalink) in a ratio of 2:1 was infused into the carotid artery at a constant injection pressure of 110 mm Hg for 5 hours. After the flaps were excised, all evaluation procedures were performed following the same steps as used for the two experimental groups.

Statistical differences in vessel numbers from angiograms between the 1-week and 3-week groups and between Group 1 and Group 2 were determined by a two-tailed t-test based on the sample size (six flap values). The vessel value of each flap was a mean of four measurements from four different observers.

Results

All microanastomoses of the 1-week and 3-week groups in Group 1 showed visible occlusion angiographically as a result of postoperative thrombosis formation, whereas all microanastomoses of both groups in Group 2 exhibited flow-through. The thromboses appearing on the angiograms in Group 1 were confirmed histologically. At 1 week, we observed collagenous bundles securely

Fig. 2 Histologic analysis of the 1-week group in Group 1 showed the thrombosis formation at the point of microanastomosis. The thrombosis consisted of collagenous bundles of fiber attached securely to the intraluminal wall of the vessel (Stain, hematoxylin and eosin; original magnification, $\times 20$).

attached to the intraluminal wall of the vessel loop (Fig. 2). At 3 weeks, these thromboses were canalized by small vessels of various calibers (Fig. 3). All skin flaps from both groups appeared viable and similar to normal epidermis with hair follicles, connective tissue, and blood vessels like those seen in the control group. As early as 1 week after surgery, a newly developed fibrous capsule was visible macroscopically beneath the skin flaps in both groups. This capsule was smooth with a glossy gray surface. The implanted vascular loop and the skin flap were closely attached such that the pedicle lay within the skin flap. This new fibrous capsule contained thin parallel layers of connective tissue and became thicker based on the length of retention time of the silicone implant. Microscopically, a local inflammatory reaction after silicone implantation was observed. Around the microanastomosis line, there was evidence of infiltration of inflammatory cells such as macrophages, lymphocytes, granular leukocytes, and fibroblasts. Inflammatory cells, however, varied depending on the length of retention time of the vascular loop implant.

In the 1-week group, it consisted predominantly of macrophages and granular leukocytes, whereas in the 3-week group, it was predominantly fibroblasts.

There were differences in the numbers of vessels and percentages of neovascularization in prefabricated flaps quantified from the angiograms between controls and Groups 1 and 2 and between Week 1 and Week 3 in the two experimental Groups (Table 1). Neovascularization development of 1-week and 3-week intervals in Group 2 was angiographically intensified compared with the development in Group 1 based on the vessel quantity. Corresponding to the angiographically quantifying value, the reliability of the prefabricated flap transfers of Group 2 was confirmed. In Group 1, the 1-week group (Fig. 4A) had a very small number of newly formed vessels and vascular connections. In the 3-week group (Fig. 4B), newly formed vessels sprouting from the implanted vascular loops were more dense and there were various communications with the available flap vasculature; however, the vessel quantity of this group was only 69.4% compared with 100% in the control group. In contrast, neovascularization in the 1-week group of Group 2 (Fig. 4C) showed numerous newly formed vessels, some of which were connected to the original vessel system of the flap. The 3-week group (Fig. 4D) had innumerable newly formed vessels and vascular-rich communications. The vessel quantity of the 3-week group in Group 2 had increased to 103% compared with the control group. At this time the entire flap was well perfused by the blood flow supplied from the newly implanted vascular loop.

A muscle cuff placed around the anastomosis line reduced thrombosis formation. All implanted vascular loops in Group 2 revealed no thrombosis formation in the vessel lumen (Fig. 5A); however, a slight luminal stenosis at the microanastomosis line was observed in all cases resulting from local proliferation and thickening of the intima. In Group 2, all anastomoses in the 3-week group were stably protected in the surrounding muscle cuff (Fig. 5B).

Fig. 3A–B (A) Low- (Stain, hematoxylin and eosin; original magnification, $\times 10$) and (B) high-power (Stain, hematoxylin and eosin; original magnification, $\times 40$) views of the 3-week group in Group 1 showed canalization of newly formed thrombosis by varying small vessels of different calibers.

Table 1. Comparison of vessels counted on angiograms

Group	Control group			Group 1			Group 2			p value**
	Average number of vessels	Percent	SD	Average number of vessels	Percent	SD	Average number of vessels	Percent	SD	
Control	238.8	100	±15.8							
1-Week				12.4	5.2	±5.6	74.6	31.2	±11.4	1.4×10^{-4}
3-Week				165.8	69.4	±22.3	246.2	103.1	±31.6	8.6×10^{-4}
p value*				1.5×10^{-5}			4.2×10^{-5}			

SD = standard deviation; Percent = percentage of counted vessels compared with the control group. * = difference between 1-week and 3-week values; ** = difference between Group 1 and Group 2.

Fig. 4A–D Selective microangiography of Group 1 after (A) 1 week and (B) 3 weeks shows the visible vascular occlusion at the point of anastomosis. In contrast, there was no vessel occlusion observed in Group 2 after (C) 1 week and (D) 3 weeks. Neovascularization development in the prefabricated flaps of Group 2 was intensified in comparison to Group 1.

Fig. 5A–B The implanted vascular loop in Group 2 revealed no thrombosis formation in (A) the vessel lumen (Stain, elastica van Gieson; original magnification, $\times 10$), and (B) the anastomoses in the 3-week group were stably protected in the surrounding muscle cuff (Stain, hematoxylin and eosin; original magnification, $\times 40$). M.C. = muscle cuff.

Discussion

Microvascular free flaps are reliable and effective for reconstruction of large soft tissue defects. In orthopaedic surgery, free vascularized flaps can be combined with silastic constructs to enhance the final functional and aesthetic results [2, 13, 17, 22, 23, 26, 27]. However, we have experienced flap failures resulting from vessel occlusions using free tissue transfers combined with silastic implants. Wishing to identify an easy, practical, and effective way to reduce or avoid thrombosis we therefore asked whether: (1) direct contact of silicone sheets with a microanastomosis influenced thrombosis, (2) whether prefabrication with silicone sheets enhance neovascularization, and (3) whether a muscle cuff placed around the microanastomosis reduced thrombosis.

Because silicone sheets were applied at only 1-week and 3-weeks, we cannot describe all changes of microanastomoses in direct contact with silicone implants. Additional studies with more detailed observations would be necessary to identify all important changes. With *in vivo* silicone implantation, postoperative formation of a fibrous connective tissue capsule around the silicone implant is inevitable [3, 13, 30, 32]. Essentially, the formation of this capsule is one of the body's defense mechanisms to completely isolate foreign bodies [7, 9, 30–32]. Vistnes et al. [30] reported that in the acute stage of the initial inflammatory response, if phagocytes failed to remove large implants as a silicone sheet, collagenous fibers would infiltrate and be arranged directly around the implant to form a fibrous tissue capsule isolating the foreign body. A capsule eventually developed with fibrous layers and vascularity. During the entire chronic stage, acid mucopolysaccharides were associated predominantly with the collagenous fibers. The foreign body reaction process that produces the fibrous capsule continues as long as the implant is present but disappears when the silicone sheet is completely removed [30–32]. Formation and development of this fibrous capsule are influenced by several factors such as properties of the silicone implant [7, 30], local surgical trauma [9, 14, 30], postoperative formation of seroma or hematoma [30], and bleeding from local small vessels [29–31].

Owing to the lack of available explanations in the literature, we presume the local foreign body reaction that occurs after *in vivo* silicone implantation is likely the cause of postoperative thrombosis formation in our study animals (Group 1). We suspect, based on the acute inflammatory reaction, the anastomosis is infiltrated by granulocytes and collagenous fibers directly through the weakest points between vessel sutures. Combined with initial intraluminal injuries caused by sutures and inevitable vessel stenosis after microanastomosis, it is likely

thrombosis will form. Furthermore, owing to the lack of related explanations in the literature and histologic absence of collagenous fiber infiltration observed in the muscle cuff, we presumed the thick muscle cuff placed around the anastomosis in Group 2 would function as a barrier to prevent infiltration of collagenous fibers into the vessel lumen during the acute foreign body reaction.

Flap prefabrication based on the concept of neovascularization by means of implanting a vascular pedicle into a random pattern tissue area presents a new trend in flap reconstruction. The procedure provides the option of creating thin, large, and suitable flaps with minimal morbidity regardless of natural vascular anatomy. This type of flap has been experimentally investigated [5, 8, 10, 11, 15, 19, 21, 23, 27, 28]. The most important requirement for determining successful transfer of prefabricated flaps is maturation of neovascularization. In principle, neovascularization is considered mature when the newly vascularized system is identical in number and form to the original physiologic flap perfusion [11].

An important point related to clinical use of prefabricated flaps is to intensify neovascularization development. Experimental investigations indicated increasing blood flow in the implanted vascular pedicle combined with silicone sheet implantation played important roles [10, 11, 15, 21]. Using a silicone sheet and arteriovenous pedicle with microanastomosis, numerous authors have reported 20 days is the minimal time required for neovascularization maturation [10, 11, 16, 15, 27, 28]. As a result of thrombosis we found delayed neovascularization development in Group 1 compared with Group 2. The vessel quantity of the 3-week flaps in Group 1 was only 69.4%, whereas in Group 2, it was 103% compared with the control group. Our observations suggest a negative influence of silicone sheets placed directly in contact with microanastomoses. We question the transfer of a free prefabricated flap in Group 1 because of the unreliable neovascularization and blood output of the flap resulting from anastomosis occlusion.

Based on the similarity between animals and humans, and the fact the local foreign body reaction is essentially the same in all mammalian hosts [30], it is important to carefully consider the surgical designs and operative techniques involved in simultaneously using silicone implants and microvascular procedures to avoid direct contact between silicone implants and the microanastomosis.

Our data suggest implanted silicone sheets placed in direct contact with the microanastomosis exert increased postoperative thromboses at the point of the anastomosis. A muscle cuff placed around the anastomosis may be the easiest way to reduce this complication. Prefabricated flaps combined with silicone sheets and muscle cuffs appear to provide flaps with enhanced vascularization.

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