# Dermal nipple-areola complex perfusion through full thickness circumareolar scars : a porcine model for safe delay in two-stage nipple sparing mastectomy

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# Dermal Nipple-Areola Complex perfusion through full thickness circumareolar scars: a porcine model for safe delay in two-stage nipple sparing mastectomy.

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*Short title: Delayed nipple-areola complex scar perfusion*

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Products and devices list:

1. Sterile silicone sheet (Groupe Sebbin, Paris, FRANCE)
2. VS3 Iridium-Visionsense Infrared Fluorescence Vision System (Elevision IR Platform-Medtronic, Fridley, Minnesota, USA)
3. Indocyanine green dye (Infracyanine – SERB, Paris, FRANCE)

ABSTRACT

Introduction

Nipple sparing mastectomy (NSM) has evolved to a standard surgical option. NSM complication rate remains high in large breasts. To reduce the risk of necrosis several authors propose delayed procedures to enhance blood supply to nipple-areola complex (NAC). The purpose of this study in a porcine model is to show adequate redirection of NAC perfusion by neoangiogenesis through circumareolar scars.

Methods

Delayed two-staged NSM is simulated in 52 nipples (6 pigs) with a 60-days interval. The nipples undergo a full thickness circumareolar incision onto the muscular fascia with preservation of underlying glandular perforators. After 60 days NSM is performed through a radial incision. A silicone sheet is introduced in the mastectomy plane to prevent NAC revascularization by wound bed imbibition. Digital colour imaging is used to assess necrosis. Near-infrared fluorescence with indocyanine green (ICG) is used to assess perfusion patterns as well as perfusion in real time.

Results

No NAC necrosis is seen after 60 days delay in all nipples. In all nipples ICG-angiography shows complete alteration of NAC vascular perfusion pattern from subjacent gland to a capillary fill following devascularization exhibiting a predominant arteriolar capillary blush without distinct larger vessels.

## Conclusion

NAC delay reverses glandular perfusion to adequate dermal neovascularization. Neovascularization through full thickness scars provides sufficient dermal perfusion after 60 days delay. Identical staged delay in humans may be a surgically safe NSM option and could broaden therapeutic NSM indications in difficult breasts. Large clinical trials are necessary to provide identical results in human breasts.

## INTRODUCTION

Nipple sparing mastectomy (NSM) with immediate or delayed breast reconstruction has evolved to a standard surgical option. NSM can be performed for early breast cancer, for ductal carcinoma in situ (DCIS) and in the risk reducing setting (1). In large breasts, many surgeons remain reluctant to perform this procedure due to higher risk of nipple and skin necrosis (2). In NSM blood supply to the nipple-areola complex (NAC) is reduced from dermoglandular to dermal perfusion.

In immediate NSM skin-and-nipple necrosis rate of 14.95 % remains high (3). To reduce the necrosis rate several authors propose delayed procedures, allowing enhanced blood supply to nipple-areola complex in multiple stages, resulting in a total necrosis rate of 4.06 % (4,5). In our review on NSM in large breasts the overall complication rate is as high as 29,08 percent (5). The incidence of necrosis is in favor of the delayed procedures. The incidence in one-stage versus delayed reconstructions was 5,36 versus 2,15 percent for partial NAC necrosis, 5,08 versus 0,48 percent for complete NAC necrosis and 4,8 versus 1,43 percent for skin flap necrosis (5).

NSM in larger breasts also has the surgical difficulty of nipple positioning and skin excess. Therefore, NAC delaying has the advantage of simultaneous nipple repositioning and preshaping of skin and breast mound. In 2012 Spear finds evidence that a preshaped larger breast with circumareolar scars after reduction has an acceptable necrosis rate when an NSM is performed in this preexisting status (6). Alperovich (7) and Vaughn (8) target the reliability of reperfusion through existing circumareolar scars in 2013 by performing NSM in patients with a history of breast reduction with Wise pattern incisions.

Despite the above-mentioned necrosis rates, no consensus exists in literature on the advantage of delayed versus immediate NSM for nipple necrosis. Nipple blood supply after NSM is influenced by many factors (breast size, age, smoking, diabetes, incision, reconstruction type, direct or staged approach, radiotherapy…) (5,9). This makes assessment of the correlation between NSM timing

(direct or staged approach) and perfusion (necrosis) difficult in clinical series without large experimental numbers. These factors are eliminated in this animal model, focusing on the presence of perfusion.

The purpose of this study in a porcine model is to show adequate redirection of NAC perfusion by staged NAC delay in NSM, resulting in neoangiogenesis through circumareolar scars. The delayed redirection of glandular to complete dermal NAC perfusion may result in less necrosis. To date this is the first study that correlates the anatomical findings with qualitative dynamic angiography in a delayed NSM porcine model. The dermal formation of new blood vessels to NAC is evaluated clinically and dynamically. Near infrared fluorescence (NIR) is used to assess qualitative differences in skin perfusion and to evaluate differences in perfusion patterns after the first and second stage, 60 days later, each time during a 10-days period.

The article is written and checked according to STROBE-Vet Statement checklist.

## MATERIALS AND METHODS

### Species model

The presence of NAC is only found in humans. The areola is of ectodermal origin: the presence of an areola or the presence of normal skin around the nipple makes no difference. The blood supply of the pig's teat resembles that of human NAC (10). The human nipple is supplied through the gland by internal mammary and intercostal perforators (11). The pig has up to sixteen teats in two lateral rows. The pig's nipples are supplied through the gland by large cutaneous deep superior epigastric artery perforators from proximal to caudal of the umbilicus. The distal nipples are supplied by superficial inferior epigastric artery (12).

### Animals, Anesthesia and Analgesia

All animal procedures were performed under good laboratory practice, with the presence of an independent observer. The study protocol was approved by the Ethics Committee for Animal

Experiments of the University of Antwerp, Belgium (Protocol ECD 2020-42), and conformed to European Union Directive 2010/63/EU on the protection of animals used for scientific purposes. The pigs acclimatized 3 days after arrival. Twenty-four hours prior to surgery a 50-µg fentanyl patch was placed on the back of each pig. Six female Aachener mini pigs (Carfil, Belgium), averaging 45.6 kg, were induced with an intramuscular injection of atropine 0,04 mg/kg, ketamine 30 mg/kg and midazolam 0,5 mg/kg, intubated with an intravenous bolus of midazolam 0,5 mg/kg and alfentanil 30 µg/kg. General anesthesia was maintained with 2,5% sevoflurane and oxygen. Electrocardiography, heart rate, oxygen saturation, and body temperature were monitored. An 18-gauge venous catheter was inserted into the auricular vein prior to surgery. Antibiotics were given: cefazoline 1g IV with induction, continued with 500 mg/2 hours of surgical time. After the procedure buprenorphine 0,01 mg/kg was administered subcutaneously at the end of the procedure surgery and repeated every 6 to 8 h when indicated.

**Porcine Staged Nipple-sparing Mastectomy Model ~~(Table 1)~~**

The model's time schedule is shown in Figure 1. Four nipples could be visualized in the same field of view at any given time. The pig has no areola. Therefore, the circumareolar incisions were designed with a 6-cm diameter cookie cutter, centered around the nipple to simulate areolar skin. One out of four nipples was designated as control. The other three nipples underwent a circumareolar full thickness incision onto the muscular fascia with preservation of the underlying glandular vasculature as in a breast reduction dermoglandular pedicle (Figure 2). To eliminate nipple position as a possible confounder, the position of each model was rotated in every subsequent pig and for every four nipples within the same pig (13). At the second stage (60 days) NSM was performed through a 3 cm radial incision perpendicular to the circumareolar scar (Figure 3). The interval of 60 days is also used in our clinical practice. The underlying glandular vasculature in the pedicle was sacrificed by wide subdermal undermining and gland removal.

Wide undermining was performed at least two centimeters away from the circumareolar scar to mimic breast skin flap delay. Limitation of this model was the absence of the inverted T incision pattern because of pig's anatomy. A sterile silicone sheet (Groupe Sebbin, Paris, FRANCE) was introduced in the undermined mastectomy plane to mimic NSM (14). This sheet prevented NAC vascularization by wound bed imbibition: blood supply was redirected to strictly dermal, depending on the neovascularization through the circumareolar scar.

**Clinical evaluation**

Nipple viability was evaluated at postoperative day (POD) 0, 10, 60 and 70 by digital color imaging to assess color, epidermolysis, necrosis, wound dehiscence, and infection. Digital color videography showed presence or absence as well as speed of capillary refill.

**Dynamic Indocyanine Green Fluorescence Dermal Angiography and Perfusion Patterns**

Assessment of nipple perfusion was performed by NIR imaging by VS3 Iridium-Visionsense Infrared Fluorescence Vision System (Elevision IR Platform-Medtronic, Fridley, Minnesota, USA), to visualize perforator perfusion in the first two stages (POD 0 and 10) as well as circumareolar neovascularization in the last stages (POD 60 and 70). Elevision IR (IR) is working with Indocyanine green (ICG) fluorescence dye which has an excitation band between 800-815nm and emission band between 825-850nm. The imaging system was positioned at 50 centimetres from the surgical field.

ICG (Infracyanine) was purchased from SERB (Paris, FRANCE) and dissolved in distilled water at 2.5 mg/ml prior to injection. 0.1 mg/kg of ICG was injected intravenously.

Images were obtained from the time of the injection to 2 minutes post injection, every 2 sec for the first 30 sec, every 5 sec for the next 30 sec and every 10 sec until 2 min. At POD 60 video was prolonged to 5 minutes. When breathing spontaneously, animals were allowed to recover from anaesthesia in a separate cage for the first 24 h and housed in group afterwards. ICG angiography

was taken preoperatively and at the end of surgery on POD 0 and 60, as well as during clinical evaluation on POD 10 and 70. The difference in relative fluorescence intensity (FI) percentages was quantified using custom software (Visionsense). Relative FI (pixel FI compared with autobase which is highest FI measured on the screen) is automatically calculated. Skin perfusion is displayed as a blue green to red colour on the combined image (white colour on the IR camera screen). Pink skin colour (absence of ICG dye filling) represents absence of arterial skin perfusion (black colour on IR camera screen).

ICG nipple perfusion patterns were qualitatively classified in four categories according to Bertoni (15): V1 (from subjacent breast), V2 (surrounding skin), V3 (combination of V1 + V2) and V4 (capillary fill following devascularization exhibiting a predominant arteriolar capillary blush without distinct larger vessels).

## RESULTS

### Clinical evaluation

A total amount of 69 nipples was divided in 17 control nipples and 52 study nipples. At POD 0 and 10 all nipples remained pink-colored without necrosis and with normal refill after 3 seconds. At POD 60 transection of the glandular pedicle resulted almost immediately in white nipples without any refill (see Figure, Supplemental Digital Content 1, shows two hypoperfused white NAC after NSM and a well perfused pink NAC before NSM at POD 60). This effect faded gradually away over an interval of 5 minutes in all nipples. Eight nipples presented a blue blush with a fast refill (less than 3 seconds) (see Figure, Supplemental Digital Content 2, demonstrates a congested NAC after NSM at POD 60). At POD 70 all nipples were pink colored without necrosis and with normal refill (Figure 4). No wound dehiscence occurred during all stages of the experiment.

Infection did not occur in the first stage. After insertion of the silicone sheet in the second stage infection was seen in four nipples. Wound swab cultures remained sterile. Ten days after insertion, no extrusion of the silicone sheet was seen.



**Dynamic Indocyanine green Fluorescence Dermal Angiography and Perfusion Patterns**

At POD 0 and 10 NIR fluorescence was seen at the control and treated nipples within 15 seconds after intravenous injection. Preoperatively the nipple fluorescence intensity was higher than the surrounding skin. FI peak at the nipples disappeared quickly within 30 sec: we confirm the suggestion of nipple perfusion by a perforator (13,16) (see Figure, Supplemental Digital Content 3, where the deep superior epigastric artery with large perforators and glandular perfusion by a large perforator are shown).

At POD 0 all nipples showed a V1 perfusion pattern. Four study nipples in the same pig showed a V3 pattern at POD 10; all other study nipples maintained a V1 pattern.

In the second stage (POD 60) Indocyanine green (ICG) arrived later in treated NAC compared to control NAC. Treated NAC was gradually filled with ICG which took longer than in control NAC (Figure 5). FI peak in treated NAC was also later and less intense.

All treated NAC express a V4 perfusion pattern (see Figure, Supplemental Digital Content 4, shows three consecutive fused near-infrared images each with a 2-seconds interval on POD 10, 60 and 70). At POD 70 all treated NAC filled at the same speed as the surrounding skin (see Video, Supplemental Digital Content 5, demonstrates V1 perfusion pattern in all nipples on POD 0 and POD 10. On POD 60 all treated nipples show adequate but in time delayed dermal V4 perfusion. On POD 70 the video demonstrates adequate V4 perfusion that is not delayed in time in the treated nipples as well as V1 perfusion in control nipples. The video also shows identical FI levels in treated and control nipples which demonstrates complete recuperation of perfusion by neoangiogenesis in the circumareolar NAC scar).

## DISCUSSION

The similarity in cutaneous and deep territories of the angiosomes between humans and pigs lies at the basis of the suggested porcine model. The pig has large, long vessels which are spaced further apart, where the skin is mobile as in its anterior torso. The deep superior epigastric artery (DSEA) is large, and its cutaneous perforators extend caudal to the umbilicus. NIR fluorescence shows a 1 to 2 seconds delay on POD 0 filling of the nipples proximal versus distal of the umbilicus: difference in anatomical length and vessel position regarding the porcine aorta supports these findings (Figure 6).

Perforator preservation is more important to nipple perfusion than the dermal/subdermal plexus (13). Since all pig's nipples have a V1 perfusion, the circumareolar incision gives a higher perfusion at 0h in the first stage compared to control. This higher perfusion equilibrates over time in 72h (13). The ideal scenario to ensure blood supply to NAC is a staged approach that creates a V1 pattern in the first stage (hyperperfusion to NAC).

Flap delay stimulates reduced-caliber choke vessels to supply adjacent vascular territories (17). The porcine model shows adequate redirection of NAC perfusion by staged delay, resulting from neoangiogenesis through circumareolar scars. The delayed redirection of glandular to dermal NAC perfusion results in the absence of necrosis: all treated nipples survived completely.

Due to its robust V1 perfusion pattern the pig's nipple is an ideal model that mimics (dermo)glandular pedicled perfusion. NAC is delayed at the distal end of the pedicle within its angiosome (12). Delay will induce hypoxia-driven vasodilatation in the pedicle as well as neo-angiogenesis at the distal end (18,19). After the circumareolar incision in the first stage no signs of ischemia or necrosis occur. The perfusion of the first-stage-created pedicle remains strictly glandular through musculoglandular perforators. Due to the presence of direct perforators no necrosis is seen in the first stage: NAC is preserved on a pedicled flap. Random flaps have no

fixed vascular pattern to rely upon (20). This explains the distal formation of adequate neoangiogenesis throughout the circumareolar scar. Delay not only improves vascularity within the flap but is strong enough to create perfusion through newly formed vessels in the scar (18).

Wide undermining on POD 60 shows adequate redirection by delay from perforator perfusion to dermal plexus neoperfusion through the scar. All challenged nipples show pale color due to hypoperfusion immediately after undermining. The induced ischemia-hypoperfusion triggers the newly formed dermal microvasculature (see Figure, Supplemental Digital Content 6, is a near-infrared image showing microvessel ingrowth on POD 60 in a V4 capillary perfusion pattern immediately after transection of the glandular pedicle). The dermal neoangiogenesis is adequate since necrosis does not occur. Compared to the staged technique in human breasts, NAC experiences a double delay: first at the distal end of the glandular pedicled flap, secondly within the challenged random skin flaps. Dynamically the new dermal vessels will act as choke vessels: NAC is hooked to the adjacent dermal skin flaps as an extra territory in which the acute induced hypoxia of NAC results in vasodilatation and vessel hypertrophy (21). Appearance of V3 perfusion in some treated NAC on POD 10 suggests early presence of adequate transcicatriccial perfusion. The delay V1 perfusion of NAC in the first stage will allow the dermal/subdermal plexus to adapt to relative ischemia through neoangiogenesis in the circumareolar scar.

As described in Bertoni's series we also observe a shift to V4 patterns in all treated nipples on POD 60 suggesting the development of new microvessels because of the surgically induced ischemia (15). Ischemia is the driving force behind angiogenesis or sprouting of new microvessels from the existing capillary network. Dermoglandular staged delay allows NAC to recover sufficiently in the first stage, to withstand the second ischemic challenge. Staged ischemic challenging of NAC allows these nipples to tolerate further ischemic insult.

Evaluation of skin flap viability is a safe option. This can be achieved intraoperatively using ICG angiography. ICG angiography qualitatively shows the presence of dermal perfusion in real-time. Gorai et al. showed a significant decrease in full thickness necrosis from 17.8% to 4.8% with the use of ICG angiography-guided skin trimming (22). In pedicled perforator flaps or for large skin paddles, intraoperative ICG angiography is strongly correlated with postoperative outcomes (23). No necrosis is seen in our porcine model: on POD 70 all nipples showed levels of ICG fluorescence like the adjacent skin. However, on POD 60 a clear delay is noted in ICG appearance as well as in the level of ICG fluorescence.

Anatomical differences between pigs and humans contribute to the limitations of our porcine model. All pig's nipples have a direct perforator perfusion pattern, but the perfusion of the proximal nipples depends on large cutaneous DSEA perforators whereas fewer distal nipples are SIEA dependent. The pig's nipple lacks an areola which we simulated by the nipple's surrounding skin: both areola and nipple surrounding skin are of ectodermal origin. We noticed that ICG appearance in the nipple is faster than in the surrounding skin; fluorescence intensity however was the same. We did not measure this time difference since it was beyond the scope of this experiment. Other variables we could not exclude are peroperative blood pressure, injection rate, dispersion by the venous and pulmonary circuit, and recirculation time (cardiac output or venous return) (24).

In pigs the delay mechanism occurs within 2-3 days (21). In this experiment the length of delay is 60 days, like our clinical protocol in preventive surgery. We reported this technique to be safe for preventive surgery in high-risk patients (25). Further research must show whether the second stage can be scheduled earlier: a shorter delay would possibly broaden therapeutic NSM indications in difficult breasts without significant postponement of adjuvant therapy.

## CONCLUSION

NAC delay reverses glandular perfusion to adequate dermal neovascularization through the newly formed circumareolar scar. Neo-angiogenesis in the scar acts as choke vessels during flap delay. ICG angiography qualitatively shows the presence of dermal perfusion in a real-time manner. Neovascularization through full thickness scars provides sufficient dermal perfusion after 60 days delay. Identical staged delay in humans may be a surgically safe NSM option and could broaden therapeutic NSM indications in difficult breasts. Large clinical trials are necessary to provide identical results in human breasts.

Note: online supplementary material

1. Figure, Supplemental Digital Content 1, shows two hypoperfused white NAC after NSM and a well perfused pink NAC before NSM at POD 60.
2. Figure, Supplemental Digital Content 2, demonstrates a congested NAC after NSM at POD 60.
3. Figure, Supplemental Digital Content 3, shows deep superior epigastric artery with large perforators and glandular perfusion by a large perforator.
4. Figure, Supplemental Digital Content 4, shows three consecutive fused near-infrared images each with a 2-seconds interval on POD 10, 60 and 70.
5. Video, Supplemental Digital Content 5, demonstrates glandular perfusion patterns on POD 0 and POD 10 as well as redirected dermal perfusion patterns at POD 60 and POD 70.
6. Figure, Supplemental Digital Content 6, is a near-infrared image showing microvessel ingrowth on POD 60 in a V4 capillary perfusion pattern immediately after transection of the glandular pedicle.

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**Legends**

Figure 1: Study design porcine model on POD 0, 10, 60 and 70.

Figure 2: (left) Schematic drawings of the surgical model. (right) Overview of four-nipple-model in pig 4 at POD 10 with nipple 3 as a control nipple and nipples 1,2 and 4 as study nipples. Circumareolar full thickness skin incisions (mimicing NAC) are shown in study nipples (S) (red arrow). Surgery was not performed on the control nipple (C). The underlying pedicle and perforators were preserved.

Figure 3: Schematic drawing of surgical model at second stage (POD 60). NSM was performed through a 3 cm radial incision perpendicular to the circumareolar scar. The underlying pedicle vasculature was sacrificed and a silicone sheet was introduced to prevent NAC vascularization by wound bed imbibition.

Figure 4: real-time image/fused near infrared image/near-infrared image are shown from left to right at different stages in pig 5 (C= control nipple).

From top to bottom:

First row: preoperative POD 0 with V1 perfusion pattern (green ICG filling)

Second row: POD 10 after circumareolar incision with fast nipple filling in all nipples with V1 pattern (red higher FI intensity than blue)

Third row: postoperative POD 60 showing hypoperfused treated NAC after NSM through a perpendicular incision and insertion of silicone sheet – absence of ICG filling in near-infrared (black NAC) and fused near infrared (pink NAC surrounded by blue skin) image at 20 seconds after ICG-injection.

Fourth row: POD 70 with normal ICG filling in a V4 perfusion pattern (capillary fill following devascularization exhibiting a predominant arteriolar capillary blush without distinct larger vessels) in all treated nipples

Figure 5: Relative fluorescence intensity over time. Steep ICG arrival curve in 3 control nipples versus delayed ICG slope in 9 treated nipples in the same pig (red arrows show control ICG peak, green arrow shows study nipples with delayed slope)

Figure 6: NIR fluorescence images are shown with a 2 seconds interval. The nipples proximal from the umbilicus are earlier perfused than the distal ones. This delay in ICG filling is due to the difference in anatomical length and vessel position with regard to the porcine aorta. This delay is even more obvious in the most distal nipples because of their perfusion by SIEA instead of DSEA.

Figure, Supplemental Digital Content 1 POD 60 hypoperfusion photo shows (left) two hypoperfused white NAC (white arrows) after NSM through a perpendicular incision and insertion of silicone sheet and well perfused pink NAC (white dotted arrow) before NSM in pig 6. Perfusion by wound bed neovascularization is prevented by the silicone sheet in the subcutaneous mastectomy plane (right).

Figure, Supplemental Digital Content 2: congested NAC at POD 60 after NSM through a perpendicular incision and insertion of silicone sheet (white arrows)

Figure, Supplemental Digital Content 3: Glandular perfusion by a large perforator (white arrow-left image) and (right image) deep superior epigastric artery (DSEA- dotted white arrows) supplies gland and nipple with large perforators (white arrow-right image)

Figure, Supplemental Digital Content 4 :

Left to right : all fused near-infrared images are taken with a 2-seconds interval

First row: POD 10 all nipples are similarly perfused but treated nipples show hyperperfusion in NAC

Second row: POD 60 after NSM; treated NAC filling is delayed

Third row: POD 70 all NAC show adequate ICG filling. Control NAC has V1 perfusion, but treated NAC show V4 perfusion patterns

Figure, Supplemental Digital Content 6: Near-infrared image of microvessel ingrowth (red arrows) on POD 60 (T=treated nipples and C=control nipple) in a V4 capillary perfusion pattern through the circumareolar scar (blue arrows) immediately after transection of the glandular pedicle.

Figure 1

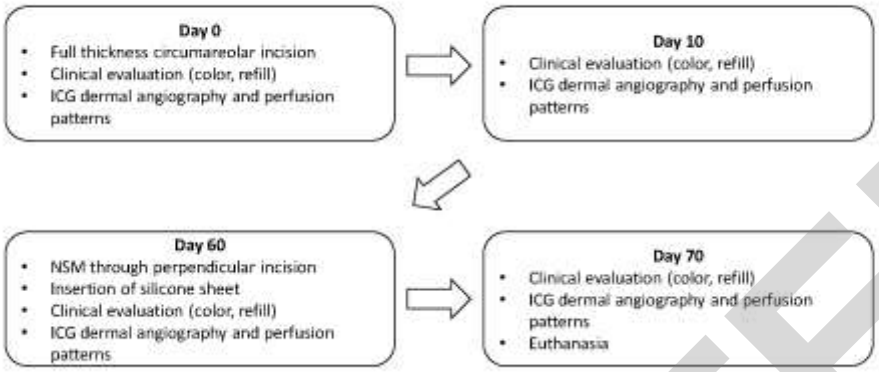


Figure 2

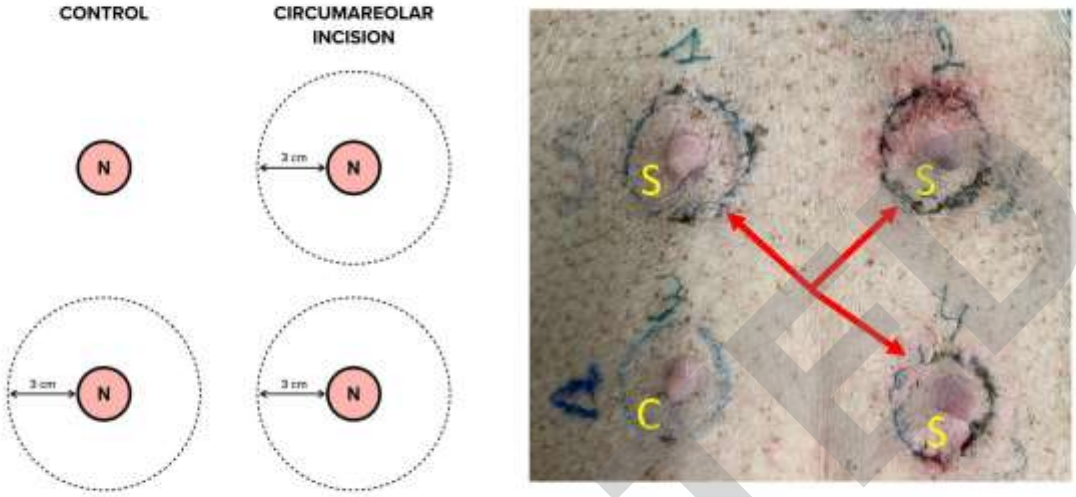


Figure 3

Figure 4

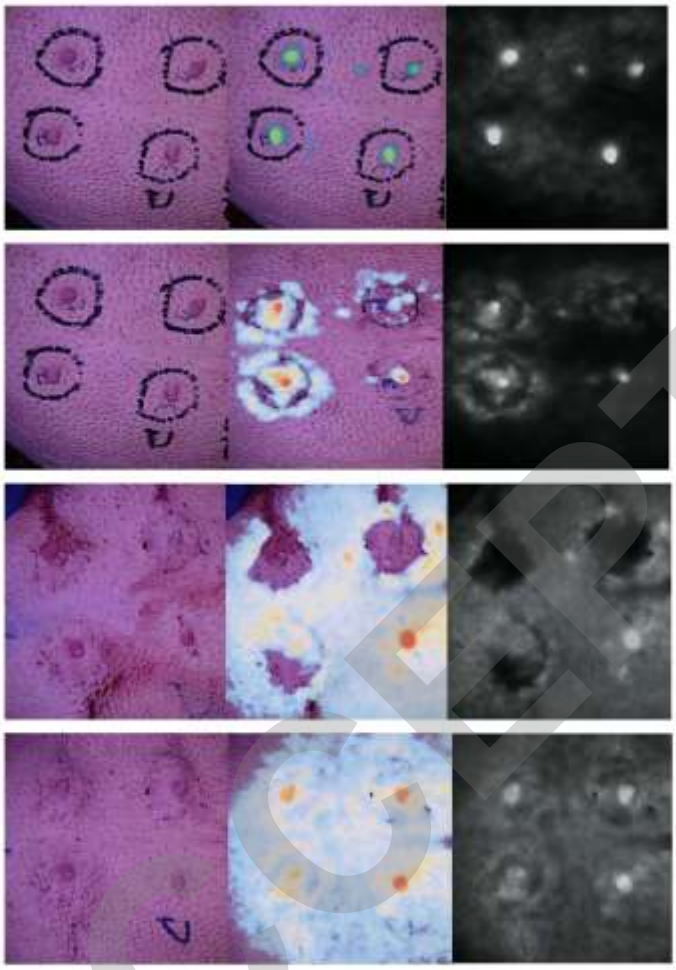


Figure 5

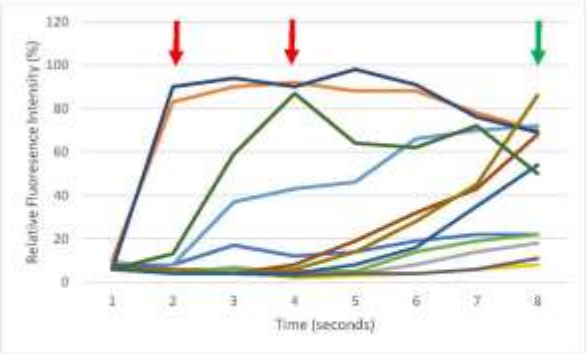
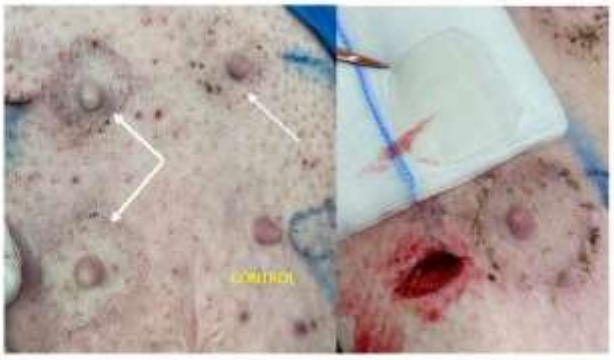
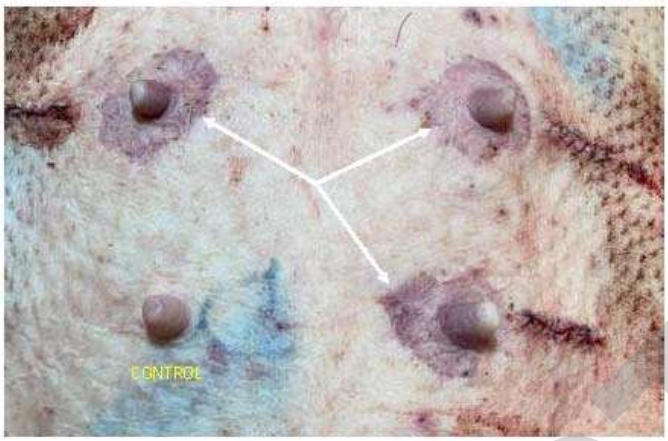


Figure 6

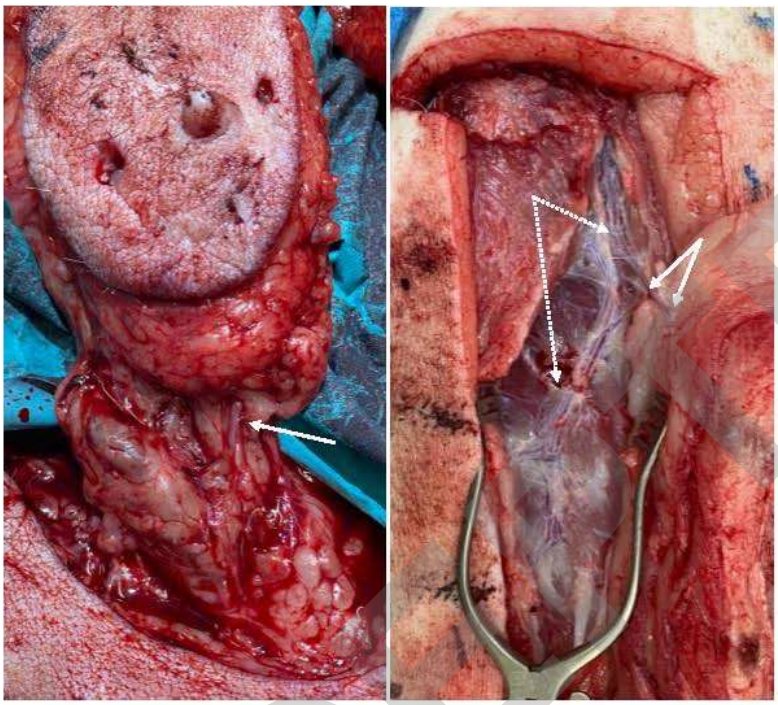
SDC 1



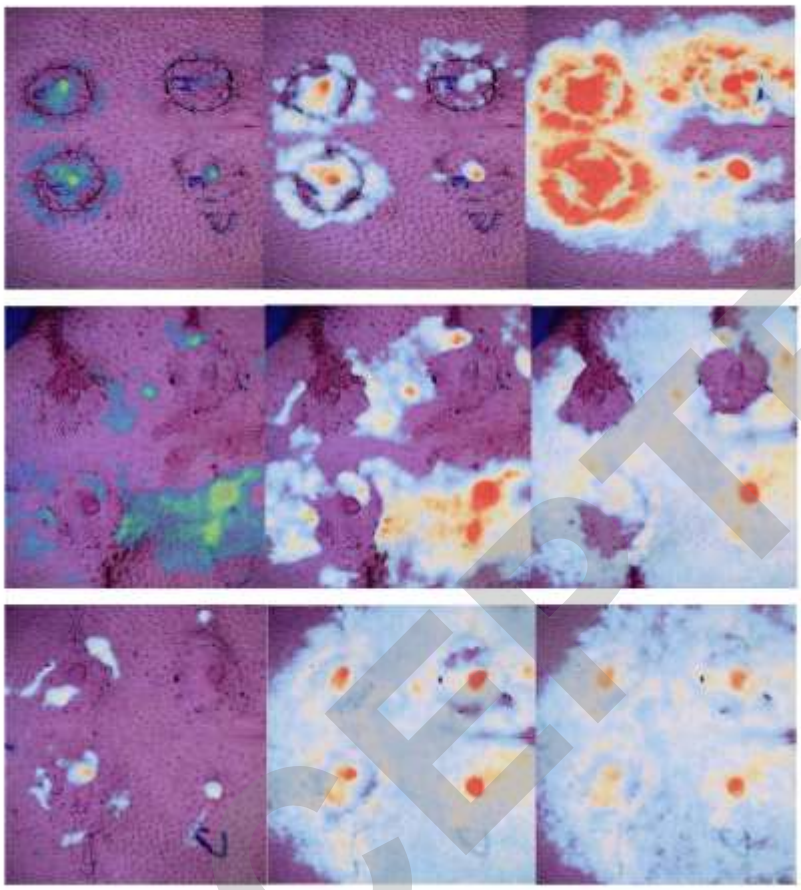
SDC 2



SDC 3



SDC 4



SDC 6