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Evaluating full-thickness skin grafts in intraperitoneal onlay mesh position versus onlay position in mice

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Background: Importance: Hernia surgery requires reinforcement material with few side effects when used in the intraperitoneal position. Autologous skin grafting may meet this requirement, but animal experiments are obligatory before being applied in humans.

Objective: To compare survival and effects of isogeneic full-thickness skin grafts in the intraperitoneal onlay mesh (IPOM) position in mice, with a control group using the onlay position. Primary end point was graft survival and secondary end point adhesion formation and inflammation through NF-κB activity.

Methods: Design: Intervention study with 8-week follow-up in accordance with ARRIVE criteria, performed between 2015 and 2016.

Setting: Animal laboratory.

Participants: Transgenic C57BL/6 mice with isogeneic background were used. Recipients were female wild-type phenotype mice >3 mo (n = 24). Donors were male or female mice >7 mo, with phenotype-positive for the luciferase gene (n = 20) or positive for NF-κB-luciferase gene (n = 4).

Intervention: Full-thickness skin was grafted in the IPOM position and compared with grafts in the onlay position as controls. Survival was evaluated by regular longitudinal postoperative luminescence imaging over 8 wk. Adherence formation was evaluated macroscopically after sacrifice. Inflammation of full-thickness skin grafts in IPOM position of NF-κB mice was evaluated in four additional mice.

Main outcome and measure: Survival of grafts, evaluated by luminescence.

Results: Ten animals received grafts in the IPOM position, and 10 in the onlay position as controls. Graft survival after 8 wk was 100% (20/20). Average luminescence at the end of the
Introduction

The treatment of complex hernias requires improvement. It is estimated that 15% of abdominal procedures give rise to incisional hernias, with 300,000 repairs performed each year in Europe. Sauerland (2011, laparoscopic versus open surgical techniques for ventral or incisional hernia repair). Large and complex ventral hernias have few treatment options, and there is no best practice consensus.

Intraperitoneal onlay mesh (IPOM) is the mesh position with the highest risk for adverse effects due to direct contact with the intestine, thereby increasing the risk for enterocutaneous fistulae. On the other hand, IPOM positioning is mechanically favorable in the repair of several abdominal wall defects such as lateral and parastomal hernias. Synthetic mesh reinforcement, used in small to moderate ventral hernia repair, is not always suitable for large abdominal hernia repair due to the complexity of associated conditions such as obesity, skin problems, multiple abdominal surgical procedures, and need for concomitant intestinal surgery. Synthetic mesh decreases hernia recurrence rate but has side effects with aggregate long-term complication rates approaching 27%-50%, including postoperative pain, infection, encapsulation, and fistula formation.

An alternative to synthetic mesh is biologic mesh, or bioprothetic acellular porcine scaffold. This material showed encouraging results in terms of vascular ingrowth and resistance to infection when introduced about 10 y ago. However, long-term follow-up has shown high recurrence rates due to problems with ingrowth, degradation, and cross-linking of materials giving rise to encapsulation. Furthermore, biologic mesh comes at a substantial price. The European consensus conference in Berlin, January 2016, recommended restricted use of biologic material in hernia repair.

Many of the severe complications associated with both synthetic and biologic mesh are attributed to immune reactions and immune-mediated degradation. The risk of an immune reaction within the abdominal wall is no longer relevant with autologous grafts. In a proof-of-concept study in humans, no sign of rejection was seen with autologous grafts in the onlay position nor was rejection seen in a randomized study comparing synthetic mesh with autologous full-thickness skin graft in giant ventral hernia repair. Hence, this study uses the onlay position as controls, to evaluate autologous full-thickness skin grafts in IPOM position.

The aim of this intervention study was to evaluate the effect of positioning of isogeneic full-thickness skin grafts, as surgical implant, on viability and adhesion formation. This study is part of a long-term process aiming toward improved treatment of high-risk patients with complicated or giant hernias using graft reinforcement, but without the complications often associated with traditional mesh materials.

Key Points

Question

- Will a full-thickness skin graft survive when transplanted in the intraperitoneal onlay mesh position compared to onlay, and if so, without adhesions or negative clinical side effects?

Findings

- Graft survival was 100% (20/20) and adhesions were sparse. The mice showed no adverse signs.

Meaning

- Full-thickness skin grafts in the intraperitoneal position have the potential to be used as reinforcement material in the repair of abdominal wall hernia.

Materials and methods

Animal background information

The use of transgenic mice with C57BL/6 isogeneic background, expressing luciferase driven by either a constitutively active promoter or NF-κB activation has been described previously. In short, the firefly-derived luciferase gene was incorporated into either a plasmid behind NF-κB-binding sites or a CMV-based promoter, then injected into the pronucleus of fertilized C57BL/6 mouse eggs, thus producing mouse strains expressing either luciferase constitutively or through coexpression with NF-κB activation during inflammatory processes.

All animal experiments were conducted at a single center in Oslo during 2015-2016 in accordance with the ARRIVE criteria and EU Directive 2010/63/EU for animal experiments. Animal protocols were approved by the Animal Care
Institutional Review Panel in agreement with FOR-1996-01-15-23 (Oslo University, Oslo University Hospital) with ethics approval id FDU 6476 (Mattilsynet) in Norway. Animals were housed in cages at regulated room temperature (20 ± 2°C) with 40%-60% relative humidity and exposed to 12-h light/dark cycles (30–60 lux). Regular laboratory feed was always available. Playthings were provided in larger cages. Weights recording and overall well-being, including additional clinical inspection for infection or failure of repair, were all performed at the time of luminescence imaging.

**Luminescence model and imaging**

Schematic picture of follow-up is seen in Figure 1. The technique of bioluminescence has previously been described in detail.¹⁹,²⁰ Luminescence derived from firefly luciferase is used to monitor local cell viability in grafts postoperatively. In short, luminescence refers to photons emitted in proportion to enzymatic oxidation activity—the higher the luminescence value, the larger the number of viable cells. The unit of luminescence is flux (photons per second). The technique has the advantage that it permits noninvasive longitudinal postoperative follow-up in live animals. The luminescence measurement procedure was identical throughout the follow-up period and performed as follows. Animals were anesthetized to obviate mobility, and an intraperitoneal injection of substrate D-luciferin (120 mg/kg; BIOSYNTH AG, Staad, Switzerland, dissolved in 200 μL phosphate-buffered saline, pH 7.8) was administrated. Ten minutes later, pictures were taken using a photon camera, IVIS Spectrum μCT instrument (PerkinElmer, Waltham, MA, USA). Photons emitted from an identically sized region of interest encompassing the graft were counted using Living Image software v. 4.4 (PerkinElmer, Waltham, MA, USA). Background radiation was subtracted from each region of interest before calculations were performed.

**Preoperative survival test of grafts in vitro**

In vitro graft survival studies were conducted to ensure graft ability to survive the bridging period between implantation and ingrowth of an adequate blood supply. To assess survival of explants divested of their blood supply, luciferase-positive skin flaps 1 × 1 cm were kept in a basal culture of Dulbecco’s Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F-12) for 2 wk, during which survival was regularly evaluated by luminescence imaging.

**Anesthesia**

Anesthesia was obtained through the intra-abdominal injection of ZRF (Zolazepam, Tiletamine, Xylazine, Fentanyl). Animals were kept in a dark and quiet environment during induction. Buprenorphine was used for analgesia. Homeostasis was maintained with eye gel, automatic heat pads with rectal temperature probes, and a bolus of normal saline.

When measuring graft luminescence during the postoperative follow-up period, the mice were anesthetized using 2% isoflurane in medical oxygen.

**Graft preparation**

The selection of animals for surgery was conducted through consecutive sampling from the research lab population. Eligibility criteria for recipients were female age >3 mo and wild-type phenotype. Eligibility criteria for donors were male or female age >7 mo, and phenotype-positive for the luciferase gene. Exclusion criteria for both groups were prior experimentation and signs of disease. Dorsal skin 1 × 1 cm was excised macroscopically in the adventitia tissue layer, thus being naturally free from fat and of approximately 1-mm thickness. The fur was shaved down to the skin, and the areas of incision were disinfected with alcohol before incision. To optimize quality, grafts were harvested from live anesthetized donors as close as possible to the time of implantation and kept hydrated in phosphate-buffered saline in the interim period.

**Surgical procedure**

To be able to compare grafts in the IPOM position with control grafts in the onlay position (Fig. 2), luciferase-positive grafts were sutured on to the rectus abdominis muscle with dermis facing the muscle layer in both groups, 10 mice in the onlay...
position, and 10 mice in the IPOM position. Surgical access was achieved through a 1-2 cm incision on the lateral abdominal wall so that wound healing would not interfere with luminescence monitoring. Grafts were attached to the rectus muscle with a single suture at each corner of the graft, approximately 1 mm from the edge. Then, 7-0 coated vicryl absorbable sutures V1028 were used for IPOM placement, whereas 6-0 coated vicryl absorbable sutures were used for onlay placement (both Ethicon, US). The wound was closed with single sutures in one or two layers depending on the graft position: rectus muscle (if applicable) and skin. Each suture length was 2-3 mm with bites taken about 1-2 mm from the edges. Graft coverage corresponded to approximately 25% of the abdominal wall area.

**Primary end point**

Assessment of graft survival was performed by longitudinal luminescence imaging; daily during the first week and then every 2 to 3 d until 8 wk after implantation.

**Secondary end point**

After 8 wk, the mice were sacrificed, and the transplants were examined macroscopically for graft adhesion tenacity and graded using a modified Jenkins’ scale for intra-abdominal adhesions. The Jenkins’ scale grades adhesion tenacity from 0 to 4 (Table 1). The tenacity scale was applied without the usual adjustment for percentage of mesh surface affected by adhesion since the grafts were too small to obtain accurate figures.

**Graft inflammation**

Four wild-type mice (not included in the cohort as previously described) received an IPOM graft from NF-κB-luciferase-positive mice, with the dermis facing the rectus muscle. These were subsequently followed up with luminescence over 33 d and analyzed for inflammation. Surgical, anesthesia, and luminescence follow-up procedures were identical to those previously described.

**Table 1 – Jenkins’ scale of adhesion tenacity.**

<table>
<thead>
<tr>
<th>Adhesion characteristics</th>
<th>Score</th>
<th>Onlay (n = 10)</th>
<th>IPOM (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No adhesion</td>
<td>0</td>
<td>10/10</td>
<td>7/10</td>
</tr>
<tr>
<td>Firm adhesion: viscera/omentum not attached to mesh, disrupted manually</td>
<td>1</td>
<td>-</td>
<td>2/10</td>
</tr>
<tr>
<td>Dense adhesion: viscera/omentum attached to mesh requiring blunt dissection to separate viscera/omentum from mesh</td>
<td>2</td>
<td>-</td>
<td>1/10</td>
</tr>
<tr>
<td>Dense adhesion: viscera/omentum attached to mesh requiring sharp dissection to separate viscera/omentum from mesh</td>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dense adhesion: viscera/omentum entwined in the mesh requiring sharp dissection to separate mesh from abdominal wall, leaving mesh attached to viscera/omentum</td>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Adhesion tenacity of onlay and IPOM grafts graded by Jenkins’ scale. Graft adhesion to the intestines was judged macroscopically, post mortem, as the difficulty in prizing the tissues apart at dissection. The greatest difficulty encountered at any point during dissection was recorded as the adhesion score. In the onlay group, adhesions between the abdominal wall and intestines were assessed. In the IPOM group, adhesions between the abdominal wall and intestines, and the graft and intestines were assessed.
Results

The in vitro study, performed prior to in vivo grafting, measuring luminescence from full-thickness skin explants, showed that luminescence was maintained (i.e., graft survival) throughout the 2-wk follow-up period.

Graft implantation was performed on 20 mice; 10 in the IPOM position and 10 in the onlay position. Recipient median age at intervention was 4 (4-11) mo in the IPOM group and 5 (4-12) mo in the onlay group. Median age of donors was 11 (7-14) mo. Median overall weight loss postoperatively was 7%, returning to baseline after a median of 8 d.

Primary end point

Hundred percent (20/20) grafts survived the postoperative follow-up period. As seen in Figure 3, the average luminescence value at the end of the 8-wk follow-up period was 928,068 flux (min 61,783, max 2,521,530) in the IPOM group n = 10 and 769,708 flux (min 76,590 max 2,164,080) in the onlay control group n = 10. A subset of female to female mice (n = 4) from the IPOM group showed an average luminescence of 1,663,622 flux (min 1,066,320 max 2,521,530). A corresponding subset of female to female mice in the onlay control group (n = 4) showed an average of 1,559,010 flux (min 1,164,080 max 2,164,080) (Fig. 3). Grafts in the onlay position showed a steady increase in luminescence over the 8-week period (Fig. 4). Grafts in the IPOM group showed an increase in luminescence over the first 5 d after transplantation followed by a decrease to a stable high level similar to baseline (Figs. 3 and 5). Background luminescence (6000-30,000 flux throughout the experiment) was subtracted from all results presented.

Secondary end point

On macroscopic examination, transplants were smooth and even in all but one animal. Adhesion tenacity on Jenkins’ tenacity scale showed no adhesion in the onlay position and three adhesions grades 0-2 in the IPOM group (Table 1).

The only complication observed in these experiments was a faulty heat pad that resulted in a small burn on the back of one animal. This was considered not to have influenced the graft. Throughout the follow-up period, there were no clinical signs of infection, abscess, fistula, bleeding, cannibalism, wound dehiscence, necrosis, or rejection in any of the implanted animals. No signs of pain or discomfort were detected nor was there any visible compromise in mobility.

No systematic evaluation of size or tensile strength of the grafts was performed.

Graft inflammation

A further four animals received an IPOM graft for assessment of inflammation. No complications or adverse effects were seen during the 33-day follow-up period. Luminescence from NF-κB activation was highest by day 2 returning to a stable low level by day 5, where it remained for the duration of the experiment (Fig. 6).

Fig. 3 – Quantification of luminescence from luciferase-positive skin flaps IPOM grafted to wild-type animals. Blue line shows all IPOM graft (n = 10) values. Red line shows a subset, consisting of n = 4 animals where both donor and recipient were female. Quantification of luminescence (flux) was performed by imaging IPOM skin grafts expressing luciferase (values shown with standard deviation). Background flux (6000-30,000 photons/s) was subtracted from each reading.
Discussion

The results of this in vivo study show that full-thickness skin graft survives when placed in the IPOM position as well as in the control onlay group, with few adhesions. The course of the inflammatory reaction to the graft corresponded to that expected after surgical trauma with subsequent healing. As the isogeneic female donors and recipients shared identical genetic background down to sex chromosomes, autologous skin implantation was mimicked, and the highest luminescence values were seen in this group, indicating better survival. In plastic surgery, autologous grafts have a well-documented ingrowth and survival rates. The safety of isogeneic IPOM graft placement shown in this study suggests that autologous full-thickness skin could be used as IPOM reinforcement in future hernia repairs.

Before considering autologous skin grafting in hernia repair in the human setting, we must be confident that the thickness of the grafted skin will enable sufficient uptake of nutrients and oxygen for long-term survival. Initial simple diffusion of oxygen is later superseded by neovascularization. Knife-meshed skin is preferred to avoid seroma formation, and this in turn provides a larger surface area for initial oxygen exchange. Grafted skin is presumably transformed into stable connective tissue/fascia-like structure as seen in biopsies from onlay grafts,\(^13,14\) providing extra strength to the abdominal wall.

A drawback with the luminescence model is that it is impossible to distinguish between low luminescence because of fewer living cells or whether there is a compromised vascular supply with substrate deficiency. Furthermore, the number of photons emitted shows interindividual and intra-individual variation, although standardized methods and longitudinal follow-up are used. Some photons from IPOM position do get blocked by the rectus muscle but still produce values about equal or higher than the control group in onlay position. Taken together, the reader is recommended to look
at the comparison between the groups rather than the exact flux values.

Successive improvement in vascularization during the healing process could explain the steady increase in luminescence values in the onlay group. Macroscopic findings at the end of the study could neither confirm nor reject this; onlay grafts were imbedded in the abdominal wall, and IPOM grafts were somewhat healed and attached to the abdominal wall.

Inflammation increases NF-κB activity, so that NF-κB luminescence gives a direct indication of the level of inflammation present. In this study, the NF-κB luminescence recorded suggests a natural transient postoperative course reflecting trauma. A low level of inflammation during the healing process is considered beneficial for repair. The fur of the mice could potentially have influenced the inflammation, although hair follicle stem cells have been suggested to modulate inflammation. Human skin has far lower density of hair follicles, theoretically making the transition of adnexa structures to stable connective tissue/fascia easier.

As proposed by Dumanian and Reith, tension in the biologic or skin scaffold seems to counteract encapsulation, shrinkage, and extrusion. Tension may also facilitate vessel ingrowth and minimize the risk for encapsulation and formation of seroma under the graft. However, the exact degree of tension on grafts in these studies is unclear. We did not measure the tensile strength of the grafts, but tensile measurements are a present study area within the research group. Also, cost-effectiveness evaluations in future clinical applications are important. With positive results come the possibility to study the widening of the techniques application, but that is beyond the scope of this article and a subject for further research.

A potential drawback of using autologous skin in the human setting is that this often involves performing surgery on patients with complex comorbidities, often ASA 3 or 4. Important issues specific for hernia patients is skin quality, long-term steroid use, and the possibility of genetic alterations in collagen composition. Skin for harvest is always available from various parts of the body with minimal donor site morbidity, and meshing increase the graft size. Taken together, this technique is not limited significantly in clinical practice by the theoretical upper limit of harvested graft size. In human studies, autologous skin in the onlay position has been evaluated with promising results. In view of the adverse side effects associated with the use of conventional mesh, and the complexity of, for example, giant ventral hernia repair, the IPOM full-thickness skin graft may be a more suitable reinforcement material.
Conclusion

In conclusion, we have demonstrated that skin isografts can be grafted to the IPOM position in mice with no detriment to health and with little adherence formation. Transplanted skin grafts, both onlay and IPOM, showed long-term survival. We thus believe that autologous full-thickness skin can be a suitable alternative to synthetic and biologic material in further studies on abdominal wall reinforcement in complex hernia repair in humans. Vascular supply and cell survival are important factors if skin grafts are to attain their full potential as successful implantation material, and these require further studies at the histologic level.

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Disclosure

The authors reported no proprietary or commercial interest in any product mentioned or concept discussed in this article.

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