Histological fate of abdominal dermis–fat grafts implanted in the temporomandibular joint of the rabbit following condylectomy


Abstract. The histological fate of abdominal dermis–fat grafts implanted into the temporomandibular joint (TMJ) following condylectomy was studied. 21 rabbits underwent left TMJ discectomies and condylectomies; 6 were controls (Group A; no graft used); 15 (Group B) had autogenous abdominal grafts transplanted into the left TMJ. Animals were killed after 4, 12 and 20 weeks. Specimens of the TMJ were histologically and histomorphometrically evaluated. At 4 weeks, fat necrosis was clear in all specimens. The dermis component survived and formed cysts with no necrosis. By 12 weeks, viable fat deposits appeared with no evidence of necrotic fat. At 20 weeks, large amounts of viable fat were present in Group B specimens. Group A had no fat, although the missing condyles regenerated. In the presence of viable fat, Group B showed little condyle regeneration 20 weeks after condylectomy. Non-vascularised fat grafts do not survive transplantation, but stimulate neoadipogenesis. The fate of the dermis component of the graft is independent of the fat component. Fat in the joint space disrupts the regeneration of a new condylar head. Neoadipogenesis inhibits growth of new bone and cartilage. This has clinical implications for TMJ ankylosis management and preventing heterotopic bone formation around prosthetic joints.

Keywords: temporomandibular joint; condylectomy; abdominal dermis–fat graft; neoadipogenesis; rabbit.

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Autologous fat grafts have been used in reconstructive surgery for over a century. Despite the abundance of adipose tissue that can be easily harvested from multiple sites with minimal morbidity, the results of free fat grafting have been generally disappointing. While the fate of free fat grafts in soft tissue augmentation and contour repair for soft tissue defects has been unpredictable, the same degree of unpredictability is also encountered when fat is used to obliterate bony cavities such as the frontal sinus.

A recent radiological study using magnetic resonance imaging (MRI) showed that non-vascularised dermis–fat grafts not only appear to survive, but the fat component also thrived in significant quantities when transplanted to the human temporomandibular joint (TMJ). This raises interesting questions as to whether the survival and growth of a non-vascularised fat graft is dependent on unique factors found only in certain recipient sites.
in the body, or whether the addition of
dermis to the non-vascularised fat graft
facilitates its survival.

Fat grafts alone are difficult to handle
and difficult to sculpture to suitable sizes.
They also fragment easily when placed in
confined spaces. The addition of dermis to
the fat greatly facilitates the harvesting of
finite quantities of fat tissue and simplifies
the sculpting of the fat, which is bound to
the dermis. Placement into various cav-
ities is also expedited by the dermis, which
acts as a convenient carrier for the fat graft
that can be easily orientated and anchored
to the surrounding recipient bed when
attached to dermis.

The dermis–fat graft was introduced to
TMJ surgery by Dimitroulis in 2004 when
it was first described as an interpositional
material for use in gap arthroplasties for
the management of TMJ ankylosis. Since
2000, Dimitroulis has also used autoge-
 nous dermis–fat as an interpositional graft
in joint cavities following TMJ disect-
omy. The graft, which is harvested from the
periumbilical region of the lower
abdomen, was never expected to replace the
missing disc but was intended as a soft
tissue plug to fill the joint cavity when the
disc was removed. In the absence of a
disc, the intention was for the dermis–fat
graft to provide a physical barrier between
the condyle and glenoid fossa to prevent
heterogenous bone formation and perhaps
also to prevent direct contact between the
joint surfaces, to minimise wear and tear
on the articular cartilage.

The clinical outcomes of TMJ disect-
omy with dermis–fat grafting have been
favourable, but little is known about the
histological fate of the dermis–fat graft
within the TMJ, and whether the dermis
is essential for the growth and mainte-
nance of the fat graft as suggested in a
previous MRI study. Using a rabbit
model, this study aims to investigate three
issues. First, the survival mechanism of
the dermis–fat graft when implanted into
the TMJ will be assessed at three time
points under light and virtual microscopy.
Second, the role, if any, the dermis com-
ponent of the dermis–fat graft plays in the
survival of the fat when implanted into the
TMJ will be assessed. Third, what influ-
ence the presence of dermis–fat graft
material has on the regeneration of man-
dibular condyles in young adult (3 month
old) rabbits will be determined.

Materials and methods

This study was approved by the Animal
Ethics Committee at St. Vincent’s Hospi-
tal Melbourne in accordance with guide-

| Table 1. Summary of the 21 rabbits divided into 2 groups (A and B). |
|-------------------|-------------------|-------------------|-------------------|
| 4 weeks           | 12 weeks          | 20 weeks          |
| Rabbits 1A, 2A    | Control – left TMJ condylectomy, no graft | Experimental – left TMJ condylectomy with dermis–fat graft |
| Rabbits 1B–5B     | Control – left TMJ condylectomy, no graft | Experimental – left TMJ condylectomy with dermis–fat graft |
| Rabbits 3A, 4A    | Control – left TMJ condylectomy, no graft | Control – left TMJ condylectomy, no graft |
| Rabbits 6B–10B    | Control – left TMJ condylectomy with dermis–fat graft | Control – left TMJ condylectomy, no graft |
| Rabbits 5A, 6A    | Control – left TMJ condylectomy, no graft | Control – left TMJ condylectomy, no graft |
| Rabbits 11B–15B   | Experimental – left TMJ condylectomy with dermis–fat graft | Control – left TMJ condylectomy, no graft |

All rabbits were 3 month old females and weighed a minimum of 2.0 kg at the time of surgery. An average of 0.5 cm³ of abdominal dermis–fat graft was implanted into the left TMJ of each of the 15 experimental (Group B) rabbits. Five experimental (Group B) and 2 control (Group A) animals were killed at each of the 3 time intervals (4, 12 and 20 weeks following surgery).

Surgical technique for TMJ

A horizontal skin incision was made from
just posterior to the lateral canthus of the
eye to just anterior to the external acoustic
meatus. The zygomatico-squamosal
suture line was exposed and a section of the
zygomatic process overlying the TMJ
capsule was carefully removed. In the 6
control rabbits (Group A) a left side dis-
ectomy using sharp dissection and 5 mm
condylectomy using fine bone ronguers
was performed and the wound was imme-
diately repaired without any graft. In the
15 Group B rabbits, a left side discectomy
using sharp dissection and 5 mm con-
dylectomy using fine bone ronguers
was performed and an autogenous piece of
dermis–fat graft (0.5 cm³) was passively
inserted into the left TMJ and the joint
capsule securely closed. The greatest
cross-sectional area of each graft was
50 mm².

Fig. 1. Dermis–fat graft being harvested from the lower abdomen. The skin remains attached to
the fat tissue bed until the epidermal layer is removed to leave the underlying dermis before the
graft is raised.
capsule was closed and the surgical wounds were repaired in layers with 4/0 vicryl sutures.

The animals were killed using IV sodium pentobarbitone (2 mg/kg) 4, 12 and 20 weeks following surgery (Table 1). The animals were decapitated and conveyed to the histopathology laboratory where the left TMJs were dissected out and placed in formalin. The specimens were decalcified prior to histological sectioning. Coronal sections of each TMJ specimen were prepared for histological evaluation under light microscopy. At least 3 sections, 3 mm apart, from each joint specimen were prepared and stained with haematoxylin–eosin for histological examination under light microscopy. Pertinent findings were recorded using digital photography and histomorphometric analysis was performed with virtual microscopy.

Virtual microscopy (quantitative) analysis

The haematoxylin–eosin stained histological slides were digitally scanned using the ScanScope T3 virtual microscopy slide scanner (Aperio, Vista, CA, USA) and ScanScope Console software v7.00.08.1020 provided the user interface. After all the slides were scanned, the digital images were analysed using the ImageScope(r) software package. The fat was selected using the ‘pen tool’ and the ‘positive pixel count’ algorithm was run on the selected tissue. The colour saturation threshold was calibrated for each group, based on the intensity of the stain of the positive control slide, containing adipose tissue alone, to achieve uniformity in measuring the stain for all sections of that group. The same procedure was repeated for the (non-fat) fibrous and epithelial elements to determine the background stain. The number of positive pixels was divided over the surface area to obtain the number of positive pixels per mm² for each slide. This value was subtracted from that of the negative control slides, containing non-fat, fibrous and epithelial elements, to exclude background stain and provide an absolute value of the area of fat present for each slide.

Results

Each TMJ specimen had 3 slices taken 3 mm apart in the coronal plane with the middle section sliced at the centre of the specimen. The results are summarised in Tables 2–4.

<table>
<thead>
<tr>
<th>Percentage proportion of necrotic fat</th>
<th>Percentage proportion of viable fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 weeks</td>
<td>12 weeks</td>
</tr>
<tr>
<td>Rabbit 1A 0%</td>
<td>Rabbit 2A 0%</td>
</tr>
<tr>
<td>Rabbit 1B 86.5%</td>
<td>Rabbit 12B 0%</td>
</tr>
<tr>
<td>Rabbit 2B 94.9%</td>
<td>Rabbit 13B 0%</td>
</tr>
<tr>
<td>Rabbit 3B 92.3%</td>
<td>Rabbit 14B 0%</td>
</tr>
<tr>
<td>Rabbit 4B 88.7%</td>
<td>Rabbit 15B 0%</td>
</tr>
<tr>
<td>Rabbit 5B 97.1%</td>
<td>Rabbit 16B 0%</td>
</tr>
<tr>
<td>Rabbit 6B 100%</td>
<td>Rabbit 13B 0%</td>
</tr>
<tr>
<td>Rabbit 7B 100%</td>
<td>Rabbit 14B 0%</td>
</tr>
<tr>
<td>Rabbit 8B 100%</td>
<td>Rabbit 15B 0%</td>
</tr>
<tr>
<td>Rabbit 9B 100%</td>
<td>Rabbit 16B 0%</td>
</tr>
<tr>
<td>Rabbit 10B 100%</td>
<td>Rabbit 17B 0%</td>
</tr>
</tbody>
</table>

Summary of mean values of necrotic fat (percentage proportion)

<table>
<thead>
<tr>
<th>4 weeks</th>
<th>12 weeks</th>
<th>20 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>91.9 ± 3.5%</td>
<td>0%</td>
</tr>
<tr>
<td>Group B</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

Table 3. Evidence of regenerating condyle and epidermoid cyst in the TMJ in Group B and Group A rabbits.

<table>
<thead>
<tr>
<th>Evidence of regenerating condyle</th>
<th>Presence of epidermoid cyst in the TMJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 weeks</td>
<td></td>
</tr>
<tr>
<td>Rabbit 1A Yes</td>
<td>No</td>
</tr>
<tr>
<td>Rabbit 2A Yes</td>
<td>No</td>
</tr>
<tr>
<td>Rabbit 1B No</td>
<td>Yes</td>
</tr>
<tr>
<td>Rabbit 2B No</td>
<td>Yes</td>
</tr>
<tr>
<td>Rabbit 3B Yes</td>
<td>No</td>
</tr>
<tr>
<td>Rabbit 4B Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Rabbit 5B Yes</td>
<td>No</td>
</tr>
<tr>
<td>Rabbit 6A Yes</td>
<td>No</td>
</tr>
<tr>
<td>Rabbit 6B Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Rabbit 7B Yes</td>
<td>No</td>
</tr>
<tr>
<td>Rabbit 8B (poor) Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Rabbit 9B No</td>
<td>Yes</td>
</tr>
<tr>
<td>Rabbit 10B Yes (poor)</td>
<td>Yes</td>
</tr>
<tr>
<td>Rabbit 11B Yes (poor)</td>
<td>No</td>
</tr>
<tr>
<td>Rabbit 12B No</td>
<td>No</td>
</tr>
<tr>
<td>Rabbit 13B No</td>
<td>Yes</td>
</tr>
<tr>
<td>Rabbit 14B No</td>
<td>No</td>
</tr>
<tr>
<td>Rabbit 15B No</td>
<td>No</td>
</tr>
</tbody>
</table>

4 weeks

In the 2 rabbits in Group A, the joint showed extensive bone remodelling of the condylar stump with evidence of cartilaginous islands forming in areas above the amputated stump. There was irregular regeneration of the condylar head (Table 3) in both specimens, which was composed predominately of a cap of fibrous...
tissue with early cartilaginous formation below the irregular fibrous outline. No fat tissue was seen.

In the 5 rabbits in Group B, extensive areas of fat necrosis were found in all rabbit tissue specimens examined (Figs. 2 and 3). There was very little viable fat seen (mean 8%) (Table 2). The condylar stump surrounded by thick band of fibrous tissue was identified in 2 rabbits while the remaining 3 rabbits demonstrated evidence of early regeneration of a new condyle (Table 3). The appearance of the new condyle resembled a bulbous expansion of the condylar stump with active growth signified by the presence of osteoblasts within the condylar process. Immature cartilage was seen covering the new condylar process but was irregular in appearance (Fig. 2). Small dermoid cysts were found in 3 of the 5 rabbits (Rabbits 1B, 2B and 4B; Table 3) with dermal elements such as hair follicles and sweat glands also visible within the cyst lining (Fig. 2). The mean size of the viable fat graft (Fig. 4) was 39.5 mm² which was 79% the size of the original fat graft (50 mm²) implanted into the TMJ (Table 4).

20 weeks

In the 2 rabbits in Group A, both joint specimens showed an almost fully regen-

Fig. 2. Extensive fat necrosis in the TMJ of the 4 week Group B rabbit with evidence of early regeneration of condyle. A small epidermoid cyst is visible in the lower right part of the Photomicrograph, Haematoxylin–eosin 10×.

<table>
<thead>
<tr>
<th>0 weeks</th>
<th>Group A</th>
<th>0 mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group B</td>
<td>31.2 ± 6.3 mm² (necrotic fat)</td>
<td></td>
</tr>
<tr>
<td>12 weeks</td>
<td>31.2 ± 6.3 mm² (necrotic fat)</td>
<td></td>
</tr>
<tr>
<td>Group A</td>
<td>0 mm²</td>
<td></td>
</tr>
<tr>
<td>Group B</td>
<td>39.5 ± 10.1 mm² (viable fat)</td>
<td></td>
</tr>
<tr>
<td>20 weeks</td>
<td>39.5 ± 10.1 mm² (viable fat)</td>
<td></td>
</tr>
<tr>
<td>Group A</td>
<td>0 mm²</td>
<td></td>
</tr>
<tr>
<td>Group B</td>
<td>98.7 ± 23.6 mm² (viable fat)</td>
<td></td>
</tr>
<tr>
<td>Fat graft at time of implantation</td>
<td>50.0 mm²</td>
<td></td>
</tr>
</tbody>
</table>

Volumetric analysis could not be done because specimens were sectioned 3 mm apart. So measurements of each specimen were taken in 2 dimensions (height and width) only. The mean cross-sectional area of the fat graft at its widest point when sliced down the middle was 10 mm × 5 mm = 50 mm² which was implanted in the TMJ. Measurements recorded from the TMJ specimens were through the histological sections that showed the greatest area of fat graft present for each animal using the virtual microscope.
generated condyle, which was the size of a normal condyle but with some irregularity in the outline (Fig. 6). One of the two regenerated condyles demonstrated a bifid head. Some of the regenerated cartilaginous cap was composed of areas of immature hyaline cartilage surrounded by larger areas of fibrocartilage. The regenerated condylar stump was filled with osteoclasts compared with the normal condyle (Table 3). There was no fat present.

In the 5 rabbits in Group B, only 1 rabbit showed evidence of a poorly regenerated condyle (Table 3). The other 4 rabbit specimens only showed what appeared to be insignificant remnants of condylar stumps with the joint space completely filled with adipose tissue (Fig. 7). Of the 5 rabbits in this group, only 1 rabbit showed evidence of a large dermoid cyst with dermal elements within the lining such as hair follicles and sweat glands (Table 3). No other specimens showed evidence of dermoid cysts in any of the sections. Viable fat was found in all specimens (Fig. 7) with only 1 rabbit showing some residual synovial lining. The mean size of the viable fat graft (Fig. 4) was 98.7 mm² which was about twice (197%) the size of the original fat graft (50 mm²) implanted into the TMJ (Table 4).

Discussion

This study showed that non-vascularised fat grafts do not survive transplantation. Fat necrosis (Fig. 3) was clearly demonstrated in all tissue specimens from the TMJ of the Group B animals at 4 weeks (Table 2). The dermis component of the graft seemed to survive and form cysts with no evidence of necrosis at any stage in the TMJ (Table 3). By 12 weeks, early signs of viable fat deposits appeared in the Group B animals in the TMJ with a notable absence of necrotic fat (Fig. 5). By the 20 week stage, large amounts of viable fat were present (Fig. 7) in the Group B specimens (Table 4). The large quantities of fat seen in the TMJ for Group B rabbits at 20 weeks (Table 4) confirms the findings of Dimitroulis et al. who found MRI evidence of significant fat deposits surrounding the mandibular condyles in all 15 humans who had undergone TMJ disectomy with dermis-fat graft replacement.

The findings of this study suggest that the fate of the fat and the dermis appears to be distinctly separate, with the dermis surviving to form cysts, while the fat becomes necrotic and is eventually replaced by new fat in the TMJ. This is in contrast to the suggestion that the dermal layer is vasoinductive for the underlying fat tissue graft, which was not found in this study. The propensity of the dermis component of the dermis-fat graft to form dermoid/epidermoid cysts has been shown in a previous study using full thickness skin. That study showed that when full thickness skin was implanted into the TMJ, all the rabbits (100%)
demonstrated cyst formation. The fact that cysts were not found in all specimens in the present study points to the possibility that the cysts were the result of epidermal remnants that were still present when the dermis–fat graft was implanted. It is likely that cysts failed to develop in those animals in which all the epidermal elements were thoroughly removed before transplantation. It appears the dermis has no influence on the fate of the fat graft at the light microscopy level (Fig. 2). From a clinical standpoint the dermis serves as a useful carrier for the fat graft, which makes it easier to handle.

Since no fat tissue was seen in the control animals (Fig. 6), it can be safely assumed that the viable fat deposits seen in the TMJ of the 12 and 20 week Group B rabbits (Figs. 5, 7) were derived from the original fat graft placed in the TMJ. This finding is a revelation as the results of the study appear to support the host replacement theory, because the original fat graft was shown not to survive transplantation. While the host replacement mechanism is unknown, perhaps the inflammatory reaction that surrounded the necrotic fat deposits might have been the trigger that resulted in the process of neoadipogenesis. That is, new fat tissue was created by recruitment of stem cells or pre-adipocytes from the tissues surrounding the TMJ, which was facilitated by the inflammatory process around the necrotic fat. This is in keeping with the results of a recent study, which demonstrated that a state of chronic, low-grade inflammation promoted neoadipogenesis in vivo through the mobilisation and recruitment of a circulating population of adipose precursor cells.

Neoadipogenesis was seen in the TMJ of all Group B rabbit specimens (Table 2) at the 12 and 20 week stages following condylectomy and graft implantation. New fat formation did not rely on the presence of an intact TMJ complex so the surrounding tissues appear to be the determinant for new fat growth. In the Group B specimens the fat in the TMJ continued to grow and take up additional space in the 20 week specimens (98.7 mm²) that was beyond the size of the graft (50 mm²) that was originally implanted (Fig. 4).

A significant finding derived from this study is that the presence of the fat graft within the TMJ following condylectomy appears to have inhibited the growth of new bone (Fig. 7). Compared with the Group A control rabbits, in which the condylar stump was seen to regenerate slowly over the 3 time periods (Fig. 6), the 15 Group B experimental rabbits showed initial attempts at regeneration at the 4 and 12 week stage (Figs. 2 and 5) but little sign of condylar regeneration at the 20 week stage (Fig. 7) (Table 3). It seems the presence of the fat graft following condylectomy appears to retard the regeneration of the condylar stump after viable fat begins to replace the necrotic fat in the 12 and 20 week rabbits (Tables 2 and 3). This has significant clinical implications for the management of TMJ ankylosis and the prevention of heterotopic bone formation following prosthetic joint replacements.

In conclusion, non-vascularised fat grafts do not survive transplantation, but appear to stimulate neoadipogenesis, which is abundantly evident when implanted in the TMJ. The fate of the dermis component of the graft appears to be completely independent and has no influence on the fate of the fat. Dermis has the propensity to form epidermoid cysts if the epidermal layer is not completely removed at the time of harvesting the graft. The long-term presence of viable fat within the joint space appears to disrupt the regeneration of a new condylar head. It seems that the process of neoadipogenesis inhibits the growth of new bone and cartilage within the joint space. This has clinical implications when it comes to the management of TMJ ankylosis and the prevention of heterotopic bone formation around prosthetic joints.

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Competing interests
This paper forms part of the PhD thesis of the first author.

Ethical approval
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