Flap Prefabrication – The Bridge Between Conventional Flaps and Tissue-engineered Flaps

BK Tan,[1] *FAMS, MBBS, FRCS (Edin)*, HC Chen,[2] *MD, FACS*, TM He,[3] IC Song[4]

Abstact

Flap prefabrication is one of the most exciting areas in Plastic Surgery because of its bridging role between conventional reconstructive surgery and tissue engineering. Using this technique, tissues such as bone, cartilage, skin and muscle can be preassembled to form precise composites that will fit any defect. In pre-lamination, for example, an ear may be created by burying cartilage underneath forearm skin and later harvested as a skin-cartilage composite free flap to replace the missing part. Vascular induction is yet another means of customising flaps where new blood supply is introduced to create transplantable tissue. For example, bone chips wrapped in a vascular carrier such as muscle can become vascularised grafts. Our experiment describes jejunal prefabrication in a rat model using the same technique. Intestinal segments wrapped in muscle flaps become independent of their mesenteric blood supply by "parasitising" on the muscle's blood supply. This idea arose from our initial observations that intestinal segments transferred to the neck to reconstruct the oesophagus could survive accidental disruption of the pedicle if sufficient time had elapsed. Clearly, the bowel had picked up new blood supply from its bed. Subsequently, jejunal prefabrication was used to reconstruct the oesophagus in a patient in whom there were no recipient vessels for free jejunal transfer. The pedicled latissimus dorsi muscle flap was used as a carrier for the jejunum. Another application of this idea could be in the area of allogeneic trachea or pancreatic transplantation, since present-day techniques have yet to overcome problems such as insufficient vascularity and unpredictable transplant survival. Future applications incorporating biomaterials and cultured cells will usher in the era of tissue-engineered flaps.

Ann Acad Med Singapore 2004;33:662-6

Key words: Transplantation, Vascular induction

Introduction

Master of the Academy of Medicine, Singapore; Mr Chairman; Chairman, Chapter of Surgeons; Ladies and Gentlemen:

I would like to express my gratitude to the Chapter of Surgeons and the Academy of Medicine, Singapore for the honour of delivering this 8th Yahya Cohen Lecture.

Flap prefabrication is one of the most exciting areas in Plastic Surgery because of its bridging role between conventional reconstructive surgery and tissue engineering. Using this technique, tissues such as bone, cartilage, skin and muscle can be pre-assembled to form precise composites that will fit any defect.[1-6] In its simplest form, pre-lamination, for example, an ear may be created by burying cartilage underneath the forearm skin and later harvested as a skin-cartilage composite free flap to replace the missing part.

Another method, vascular induction, is one in which new blood supply is introduced to create new transplantable tissue. It has been reported that bone chips wrapped in a vascular carrier such as muscle become vascularised grafts.[7] Instead of bone, our experiment describes bowel prefabrication using the same technique.[8] Intestinal segments wrapped in muscle flaps become independent of their mesenteric blood supply by "parasitising" on the muscle's

[1] Department of Plastic Surgery
Singapore General Hospital, Singapore
[2] Chang Gung Memorial Hospital, Taiwan
[3] Animal Research Laboratory
Chang Gung Memorial Hospital, Taiwan
[4] Department of Experimental Surgery
Singapore General Hospital, Singapore
Address for Reprints: Dr Bien-Keem Tan, Department of Plastic Surgery, Singapore General Hospital, Outram Road, Singapore 169608.
Email: bienkeem@singnet.com.sg

blood supply.

This idea arose from our initial observations that intestinal segments transferred to the neck to reconstruct the oesophagus could survive accidental disruption of the pedicle if sufficient time had elapsed.[9] This is because the bowel had picked up new blood supply from its bed. With this observation, could we devise a technique to deal with difficult cases of oesophageal reconstruction, in which recipient vessels for free jejunal transfer are often unavailable because of previous surgery or irradiation? In such instances, jejunal transfer employing a muscle flap as a "vascular carrier" may provide a solution to the problem. Could jejunum be induced to acquire a new blood supply from a muscle flap wrapped around it? If so, the bowel could be carried by the pedicled muscle flap up to the neck for oesophageal reconstruction.

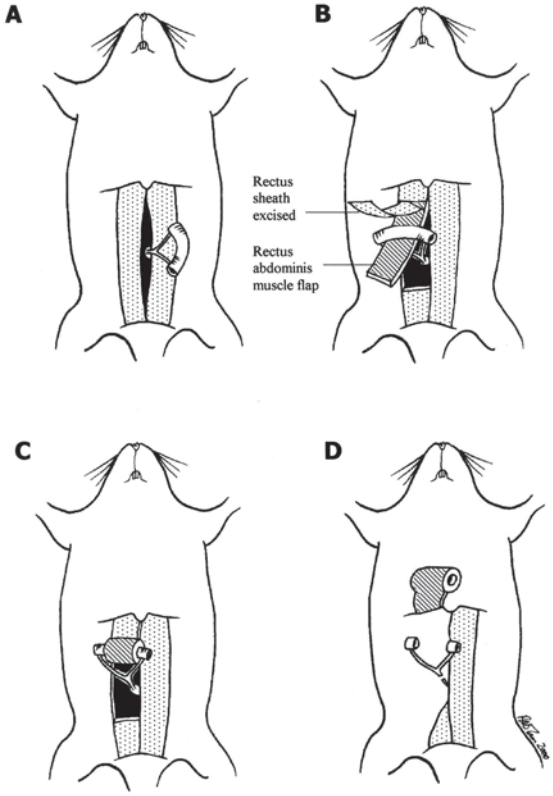

Fig. 1. Schematic representation of jejunal prefabrication using the rectus abdominis muscle flap.
(A) A 1.5 cm proximal jejunal segment based on 2 jejunal pedicles was isolated.
(B) The anterior rectus sheath over the right rectus abdominis muscle was excised.
(C) A superior-pedicled rectus abdominis muscle flap was elevated and folded around the bowel segment. Bowel ends were exteriorised as stomas.
(D) At the second stage, the mesenteric pedicle was ligated and divided. The bowel ends were cut flush with the muscle flap and the composite flap transposed to a new subcutaneous location.
Published with permission from: Tan BK, Chen HC, Wei FC, Ma SF, Lan CT, See LC, et al. Formation of independently revascularized bowel segments using the rectus abdominis muscle flap: A rat model for jejunal prefabrication. Plast Reconstr Surg 2002; 109:655-63.

Animal Study

The present study was designed to evaluate whether small bowel could be vascularised by a muscle flap for survival without mesenteric blood supply.[8] Muscle was selected as the vascular carrier because it is commonly used in head and neck reconstruction. The time required for successful revascularisation was evaluated by mesenteric ligation at predetermined time intervals. The mechanism and quality of revascularisation was assessed by histology and microangiography.

Twenty-four mature (500 to 700 g) rats were divided into 6 experimental groups of 4 animals each. In each animal, a 1.5 cm segment of proximal jejunum was isolated on 2 jejunal arteries and wrapped around with a superior pedicled rectus abdominis muscle flap (Fig. 1). To determine the time of neovascular takeover, the mesenteric pedicles were ligated on postoperative day 2 (group I), day 3 (group

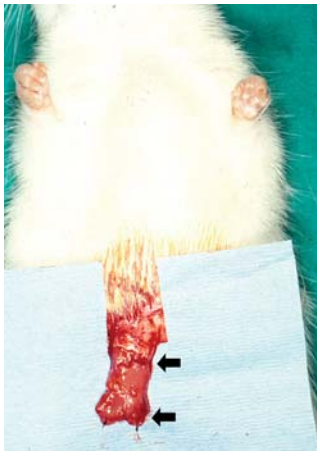

Fig. 2.

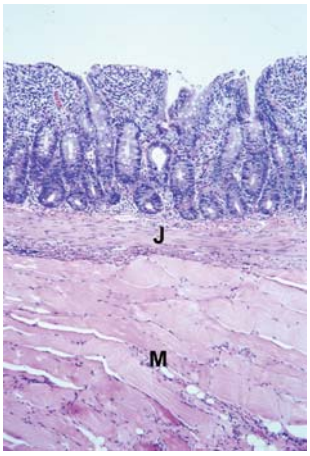

Fig. 3.

Fig. 2. Prefabricated jejunal segment opened longitudinally to expose the mucosal surface. The jejunal segment demonstrated patency of lumen and mucus production. It was lined with pink velvety mucosa coated with clear/cream-coloured viscous fluid characteristic of mucus. There was peristaltic activity which could be elicited by stroking the mucosal surface with a cotton tip. The bowel was tightly adhered to muscle and did not separate easily with manipulation.

Fig. 3. Histologic section of group V prefabricated jejunum (mesenteric ligation on postoperative day 6). Notice the intact jejunal mucosa characterised by tall villi and deep intestinal glands (crypts of Lieberkühn), features typical of normal jejunum. These were lined by intact columnar epithelium interspersed with numerous plump goblet cells. At the muscle-bowel interphase, union of the skeletal muscle fibres with serosa was observed. Acellular gaps between the 2 surfaces were few.
Note muscle (M)-jejunum (J) integration. Villous height and glandular distribution are compatible with normal jejunum; original magnification x25.

II), day 4 (group III), day 5 (group IV), day 6 (group V) and day 7 (group VI). At the time of pedicle ligation, the composite flap was transposed to a new subcutaneous location. Viability of bowel was assessed according to gross appearance and histology 48 hours after transfer.

Complete survival of revascularised jejunum in 11 of 12 animals was obtained after pedicle ligation on postoperative day 5 and beyond (P <0.0001, Fisher's exact test). These bowel segments demonstrated luminal patency, intact pink mucosa, mucous production and visible peristalsis (Fig. 2). Histology showed healthy intestinal epithelium and tissue integration along the serosa-muscle interphase (Fig. 3). Goblet cells which secrete an acid mucous were located along the crypts and villi. These stained pink with mucicarmine and had a normal distribution pattern. Alkaline phosphatase activity was demonstrated by red deposits along the brush borders.

In contrast, pedicle ligation on day 4 and earlier resulted in varying degrees of bowel necrosis characterised by flattening or ulceration of mucosa (day 4), mucosal sloughing and necrosis of mural musculature (day 3), and complete loss of bowel architecture with lumen obliteration (day 2).

This study demonstrated the feasibility of vascularising small bowel with muscle flaps to create bowel segments that are independent of their native mesenteric blood supply. Complete survival and normal function were evidenced by structural integrity, mucous production, enzyme secretion and peristalsis. The observations in this study indicate that the process of jejunal revascularisation is time-dependent. In the present rat model, a minimal period of 5 days was required. This was statistically significant (P <0.0001) as no viable bowel segment was found in animals that underwent mesenteric ligation 4 days and earlier. To explain the all or none survival of bowel, we postulate that day 4 represented a critically ischaemic

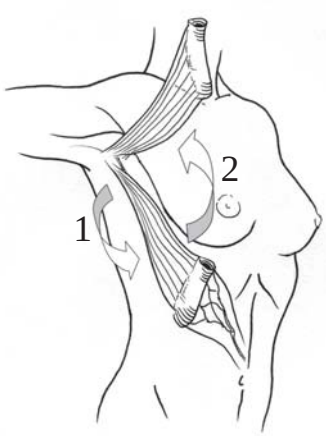

Fig. 4. Oesophageal reconstruction in a patient by jejunal prefabrication using the latissimus dorsi muscle as a vascular carrier. Reconstruction was achieved in two stages eight weeks apart — first, to vascularise the intestinal segment with muscle and second, to transfer the composite flap to the neck to bypass the strictured oesophagus.

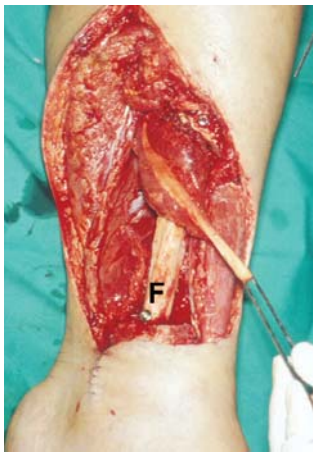

Fig. 5.

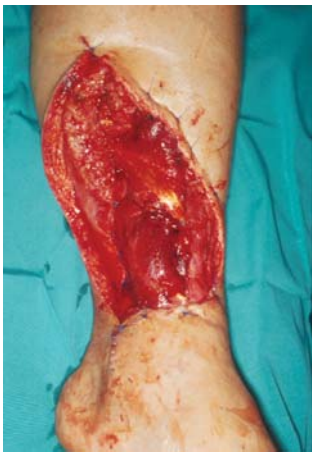

Fig. 6.

Fig. 5. A non-vascularised fibula (F) was used to replace the tibia in a patient after tumour resection.

Fig.6. To ensure vascularity, the bone was completely wrapped with the tibialis posterior and flexor hallucis longus muscles.

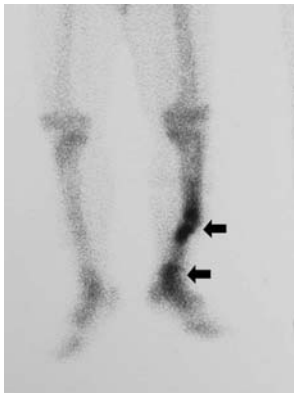

Fig. 7.

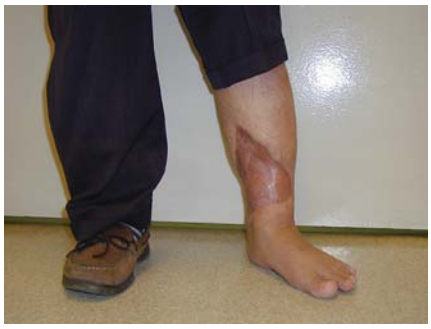

Fig. 8.

Fig. 7. Bone scan of the same patient showing tracer activity in the reconstructed segment.

Fig. 8. One-year follow-up assessment demonstrated the patient's ability to weight-bear and walk with a brace.

phase beyond which survival was assured if neovascularisation remained uninterrupted. Short of this, all bowel segments underwent progressive autolysis resulting in necrosis.

Interestingly, it was observed that the quality of prefabricated jejunum improved with time. Microscopically, day 7 and day 6 specimens had taller villi and more numerous goblet cells than day 5 specimens, although no discernible difference was seen on gross examination. One could postulate that with time, better neovascularisation had promoted epithelial regeneration and growth.

Contact between muscle and bowel appeared an important factor in jejunal prefabrication. Microscopically, areas with good contact showed direct muscle to serosa healing, whereas areas with poor contact showed granulation tissue composed of fibroblasts, new blood vessels, loose extracellular matrix, and a variable collection of inflammatory infiltrate. Although the presence of granulations in the muscle-bowel interphase did not affect survival, we feel that these might predispose to scarring and consequently strictures in the long term. Hence, efforts should be made to maximise contact by using stitches or tissue adhesives such as fibrin glue.

Clinical Application

From a clinical standpoint, jejunal prefabrication may offer some problem-solving alternatives in difficult cases of oesophageal reconstruction. In instances where neck vessels are absent, jejunum transfer could be accomplished using a muscle carrier with an adequate reach. The potential for such an application was realised recently in a patient in whom neck vessels were unavailable because of irradiation.[10] An exteriorised segment of jejunum was wrapped with a latissimus dorsi muscle flap for 8 weeks before transfer. At maturity, the mesenteric pedicle was divided and the jejunum successfully transposed to the neck based on the muscle flap (Fig. 4). With further experience, we should be able to speed up the process, using indicators such as goblet cell density and villous height as determinants of vascular sufficiency.

Second, in patients with diffuse intraperitoneal adhesions precluding dissection of the jejunal vessels, microsurgical jejunum transfer could still be attempted employing the muscle flap as a vascular carrier and pedicle.

Third, in patients undergoing long segment oesophageal reconstruction, flap prefabrication could be employed for lengthening the jejunal flap. Since bowel curvature is determined by its mesenteric attachment, it is conceivable that bowel can be straightened by substituting the native curved mesentery with a straight muscle flap neomesentery.

The uses of muscle flap vascularisation need not be confined to the gastrointestinal tract. Other organs, aside from bowel, may be revascularised in like manner. Recently, we salvaged a thrombosed live fibula graft used for tibia reconstruction by stripping away the periosteum and completely enveloping it with the tibialis posterior and flexor hallucis longus muscles (Figs. 5 and 6). One year later, bone scans showed tracer activity in the graft with enhancement over the ends where healing was taking place (Fig. 7). The patient was able to walk with a brace (Fig. 8).

Another application could be in the area of allogeneic trachea or pancreatic transplantation, since present-day techniques have yet to overcome problems such as insufficient vascularity and unpredictable transplant survival.

To transplant a portion of the pancreas, say its tail, the pancreas is first wrapped with a muscle flap, and then transplanted at a later stage as a prefabricated pancreatic flap. This would obviate the need for whole organ transplant, reducing donor sacrifice and morbidity. The technique could also be employed to create an axial blood supply where there is none. The trachea, for example, has fine segmental blood supply which makes microvascular transplantation difficult, if not impossible. If, however, its native blood supply is substituted with a single extrinsic source from a free muscle flap, then transplantation becomes feasible with a single set of vessels supplying the entire composite flap for anastomosis to suitable recipient vessels.

Why is muscle such an effective vascularising agent? We postulate the following reasons: 1) Muscle has stores of growth factors and angiogenic factors, which are liberated to promote tissue healing and adhesion.[11] 2) Its extracellular matrix regulates angiogenesis and tissue repair in an orderly fashion. How this is achieved is unclear but the existence of regulation is evident. In the rat model, for example, we observed that viable prefabricated flaps did not exhibit florid neovascularisation nor excessive scar proliferation in the bowel-muscle interphase as one would have expected. Instead, the junctions seemed quiescent, suggesting the presence of few but precisely-connected vessels in a background of "minimal-scar" type tissue repair. 3) Muscle has a dense capillary network of 2000 capillaries per square mm. By contrast, skin has 150 capillaries for the same unit area.[12,13] 4) Muscle has high blood flow – its resting blood flow is a fifth of cardiac output and 4 times that of skin. We postulate that this driving force contributes to angiogensis.[13]

The Future: Tissue-engineered Flaps

The ultimate goal of flap prefabrication is to generate vascularised tissues in desired compositions that are normally non-existent. Imagine this scenario – large quantities of rib cartilage could be assembled to form an ear construct. This could in turn be transformed into a flap by inducing a suitable vascular carrier (e.g. axial artery and

vein, fascial flap) to perfuse it. One step further would be to incorporate cultured cells and stem cells for new tissue generation and specialised function. Findlay et al[14] and Dolderer et al[15] recently reported transforming adipocytes into mammary cells grown around arteriovenous bundles in tissue chambers. If these transformed cells remain differentiated and organised around the axial vessels, these tissues would qualify as tissue engineered flaps. By introducing alloplastic or bioengineered materials, flap composition could be further altered to create specialised tissue. Tham and Song[16] recently reported vascularising acellular dermal matrix (Integra®) with rat superficial inferior epigastric vessels and microsurgically transferring them as bioengineered dermal flaps. These flaps were viable up to the time of inspection 72 hours after transfer.

In conclusion, tissue engineering and bioengineered flaps are key areas of rapid development in plastic surgery and surgery in general, and we would do well to channel our efforts in these directions. The ultimate goal is to produce like-for-like body parts to replace what's diseased or missing, without the expense of donor tissue. Thank you.

Acknowledgements

We thank Dr Tan Mann-Hong of the Department of Orthopaedic Surgery, Singapore General Hospital for his collaborative efforts; and Ms Magdalene Foo for assistance in manuscript preparation.

REFERENCES

1. Khouri RK, Upton J, Shaw WW. Prefabrication of composite free flaps through staged microvascular transfer: an experimental and clinical study. Plast Reconstr Surg 1991;87:108-15.
2. Shintomi Y, Ohura T. The use of muscle vascularized pedicle flaps. Plast Reconstr Surg 1982;70:725-35.
3. Erol OO, Spira M. Secondary musculocutaneous flap: An experimental study. Plast Reconstr Surg 1980;65:277-82.
4. Erol OO. The transformation of a free skin graft into a vascularized pedicled flap. Plast Reconstr Surg 1976;58:470-7.
5. Morrison WA, Dvir E, Doi K, Hurley JV, Hickey MJ, O'Brien BM. Prefabrication of thin transferable axial-pattern skin flaps: an experimental study in rabbits. Br J Plast Surg 1990;43:645-54.
6. Erol OO, Spira M. New capillary bed formation with a surgically constructed arteriovenous fistula. Plast Reconstr Surg 1980;66:109-15.
7. Fisher J, Wood M. Experimental comparison of bone revascularisation by musculocutaneous and cutaneous flaps. Plast Reconstr Surg 1987;79:81-90.
8. Tan BK, Chen HC, Wei FC, Ma SF, Lan CT, See LC, et al. Formation of independently revascularized bowel segments using the rectus abdominis muscle flap: A rat model for jejunal prefabrication. Plast Reconstr Surg 2002;109:655-63.
9. Chen HC, Tan BK, Cheng MH, Chang CH, Tang YB. Behavior of free jejunal flaps after early disruption of blood supply. Ann Thorac Surg 2002;73:987-9.
10. Chang SY, Chen HC, Tang YB, Tan BK, Wei FC. Prefabrication of jejunum for challenging reconstruction of cervical esophagus. Plast Reconstr Surg 1999;104:2112-5.
11. Frank JM. Angiogenesis. Clinically Applied Microcirculation Research. In: Barker JH, Anderson GL, Menger MD, editors. New York: CRC Press, 1995:375-89.
12. Barker JH, Ryan TJ. Skin microcirculation. In: Barker JH, Anderson GL, Menger MD, editors. Clinically Applied Microcirculation Research. New York: CRC Press, 1995:315-37.
13. Hill MA, Meininger GA. Skeletal muscle microcirculation. In: Barker JH, Anderson GL, Menger MD, editors. Clinically Applied Microcirculation Research. New York: CRC Press, 1995:297-314.
14. Findlay M, Dolderer J, Cooper-White J, Thompson E, Penington A, Morrison WA. Creating large amounts of tissue for reconstructive surgery – a porcine model. Aust N Z J Surg 2003;73:240.
15. Dolderer JH, Findlay M, Cooper-White J, Thompson E, Penington A, Morrison WA. In-vivo generation of 3-dimensional vascularized tissue constructs. Aust N Z J Surg 2003;73:250.
16. Tham CH, Song C, Ng R, et al. The vascularisation of bioengineered acellular dermal replacement (Integra®) in a rat model. Reconstructive Microsurgery: Proceedings of the Inaugural Congress of the World Society for Reconstructive Microsurgery 2001:579-81.