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MICROCIRCULATION IN GRAFTED BONE

A Chamber Technique for Vital Microscopy of Rabbit Bone Transplants

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A method enabling *in vivo* observations of the microcirculation in orthotopically transplated bone in the rabbit is presented. The technique permits repeated study of a well-defined bone tissue compartment both before and after grafting to an autogenous or allogenous recipient site, respectively. It yields information about the reorganization of the microvascular system and the resorptive and formative processes of bone remodelling during incorporation of grafted bone. In the autologous experimental series, new capillaries were seen in the bone grafts 4 to 5 days after transplantation. No re-utilization of pre-existing graft vessels was observed.

Key words: animal experiments; bone transplantation; *in vivo* studies; microcirculation

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Biomicroscopy of the microcirculation in bone

Vital microscopy is the only technique that admits a *direct* and *simultaneous* registration of microvascular structure and function in a defined tissue compartment. Today, refinements of the technique allow analysis of the intimate relationship between capillary structure, capillary topography and flow pattern at a resolving level of less than 0.5 microns (Brånemark 1966). A method for intravital microscopy of bone tissue *in situ* was first described by Brånemark (1958, 1959). By grinding down the rabbit fibula to transparency Brånemark was able to study the microcirculation in the endosteal cortical layers and in the bone marrow. Earlier direct observations of the microcirculation within postnatal bone tissue had been limited to heterotropical bone grafts placed in the rabbit ear chamber (Sandison 1928, Kirby-Smith 1933, Clark & Clark 1942), in the rat skin chamber (Kiehn et al. 1952) or in the camera anterior of the guinea pig eye (Mosiman 1950). Brånemark's original method permitted a repeated study of the same bone tissue compartment but only over the first 7 days after the primary grinding procedure. Later on the bone window gradually lost its transparency due to appositional layers of regenerating bone. In order to enable long-term *in vivo* study of intraosseous vessels without repeated operations, Brånemark et al. (1964) developed the bone chamber technique. This technique was designed for analysis of bone marrow regeneration and it was based on the same principles as earlier types of transparent chambers used for biomicroscopy of regenerating tissue layers (Sandison 1924, Algire 1943).

The originally described bone marrow

chamber consisted of a titanium framework enclosing a light-conducting quartz glass rod, separated by a small gap from a cover glass. Regenerating bone marrow filled the gap during the 3-week period following installation of the chamber in the metaphysis of the rabbit tibia. Brånemark et al. (1964) presented a report on their observations of marrow regeneration and microvascular behaviour during this time interval. Using a modified Brånemark chamber, McCuscey et al. (1971) and McClugage et al. (1971, 1973) presented a detailed analysis of the microcirculation in regenerating bone marrow with special reference to the haemopoietic tissue elements.

Apart from the earlier studies of heterotropically grafted bone in the rabbit ear chamber mentioned above, there are also more recent ones (Ezra-Cohn et al. 1969, Sudmann 1975).

Biomicroscopy of the microcirculation in orthotopically grafted bone

No intravital study of microvascular reactions in bone grafted to skeletal sites could be found in the literature. The studies on the microvascular anatomy of orthotopically grafted bone published to date have been carried out with histological examination of fixed specimens (Stringa 1957, Ferguson et al. 1959, Kingma & Hampke 1964, Albrektsson 1971).

Of course, neither the histological studies nor the heterotropical chamber graft observations mentioned above permit any conclusions about the revascularization dynamics in grafted bone during different stages of "graft take" at a skeletal host site.

The aim of the present report is to describe a bone chamber technique enabling *in vivo* observations of orthotopically transplanted bone.

Figure 1. A. Chamber type A for implantation in the rabbit tibia. Chamber length 28 mm, outer diameter 4 mm. B. Chamber type B for implantation in the rabbit tibia. Chamber length 25 mm, outer diameter of the lower part 4 mm. This chamber type has a cover glass in a metal frame that is inserted into the chamber. The cover glass instead of the glass rod in type A permits the use of high resolution objectives.

MATERIAL AND METHODS

Modifications to the Brånemark chamber were needed to cope with the special problems associated with the transplantation procedure, which demands limited dimension and maximal stability of the graft-chamber-graft bed complex. Thus the bone transplantation chamber type A* (Figure 1A) consists of a hollow, externally threaded titanium cylinder with an outer diameter of 4 mm, containing two quartz glass rods (Vireosil®) separated by a 70–140 micron wide space. The width of the space is defined by interposing a suture thread (Ethilon monofil 7–0 70 microns, 6–0 100 microns, 5–0 140 microns, respectively) and the glass rods are glued in position with Araldit®. The type B chamber (Figure 1B) has, instead of the top glass rod, a cover glass placed in a removable metal frame. This chamber type makes it possible to work with high magnification (×23, ×55) water immersion objectives.

The chambers were inserted, in different experiments, into the femur diaphysis, the ulna diaphysis or the tibia metaphysis of male and female Belgian hares or Half-lop-eared rabbits at least 8 months of age.

* The chambers were skilfully manufactured by V. Kuikka, Department of Anatomy, University of Göteborg.

Installation of the chamber

The operations were carried out with the animals under general anaesthesia, maintained by i.v. injections of Nembutal Abbot at a dose of about 20 mg/kg body weight. Before the operation the animals were also given intraperitoneal injections of Valium, 1 mg/kg body weight.

Access to the chamber implantation site was gained by curved incisions through skin and fascia and by denudation of a circumscribed cortical bone area with the aid of a punch to cut the periosteum. The periosteum was removed only from the precise bone area to be perforated by the drill. A 3.0 mm well-sharpened spiral drill was used to make a hole through the proximal cortex, the marrow space and the distal cortex of the bone. A 3.8 tap was used for threading the hole. Great care was taken to produce minimal trauma to the tissues. During drilling, tapping and chamber installation sufficient cooling by saline irrigation was done to minimize the generated heat. Drilling was done with a speed of about 1,000 rev/min. The chamber and all drills and screws are of titanium. When the chamber was screwed into place, attention was paid to having the space in the proximal cortex oriented in the longitudinal direction of the bone, in the hope of achieving easier ingrowth of vascular tissue and bone into the observation area between the glass rods.

Registrations before grafting

Between 6 and 24 weeks after the implantation procedure the chamber was exposed via incisions in skin and fascia. The leg of the rabbit was supported on a stand mounted on a cross stage enabling careful adjustment of the position of the leg without undue compression of the soft tissues. The chamber was immobilized in a special holder securing horizontal positioning of the chamber space. Longitudinal movements of the leg with the chamber could be performed under controlled conditions by means of a micromanipulator. The bone and vessel architecture of the "future graft" was registered on video tape and film before carrying out the grafting procedure (Figure 2).

The biomicroscopical registrations were performed using a Leitz intravital microscope modified for the purpose. Objectives ×4–×55 together with eye-piece ×8 were used. The microscope is provided with a magnification changer enabling zooming up to ×4.5. To this set up a TV-camera with video tape recorder, a Vinten scientific film camera and a Nikon ESR camera were connected.

A Leitz stereomicroscope with binocular tubes ×25 and objectives ×4 and ×10 was also used.

Figure 2. Low power view (drawing from photograph) of chamber space tissue. The bone trabeculae (white) are seen between "islands" of connective tissue (dark). Vessels of varying width are crossing the microscope field. Picture prior to transplantation, 7 weeks after chamber installation. ×4.

Transplantation of the incorporated chamber

All transplantations were performed a minimum of 6 weeks after the installation of the chamber at the donor site. The procedure was always initiated by preparing the host site. A surgical procedure similar to that performed when the chamber was installed was now carried out on the contralateral extremity. The hole in the bone was cut with a trephine drill with an outer diameter of 6.3 mm to make room for the excised cylinder containing the titanium chamber and an enveloping sheath of bone. The chamber and its outer shell of bone were gently cut out *en bloc* by means of a sharp trephine with an inner diameter of 6.3 mm under continuous irrigation with saline (Figure 3). The excised bone cylinder with the chamber was transferred directly to the prepared host site. The technique described guaranteed an exact fit between graft and graft bed. To ensure stability admitting vital microscopy at early intervals after grafting, the transplant was splinted to the host tibia using an osteosynthesis plate threaded on to the top of the chamber. This plate was constructed in such a manner as to minimize the contact with the periosteum as can be seen in Figure 4.

Registrations after grafting

At regular periods, beginning immediately after the grafting procedure, repeated intravital

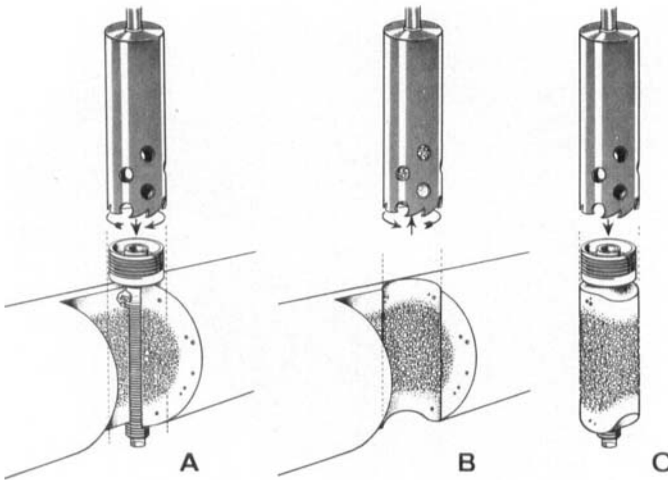

Figure 3. Schematic representation of the transplantation procedure: A. Under saline cooling a trephine is used to cut out the chamber and surrounding bone and marrow tissue. B. The trephine with chamber and graft is lifted out of the host bed. (A bone window has been opened on drawings A and B to get better visibility). C. Chamber and graft ready for implantation in a prefabricated hole in the contralateral extremity.

observations were performed under light general anaesthesia, the only limiting factor being the risk of anaesthetical complications. Microvascular and remodelling dynamics in the bone layer interpositioned between the glass rods have been registered up to 1 year after grafting. The sequences have been documented on film and video tape permitting graphical measurements and morphometrical analysis of bone remodelling at different stages during the experimental period. It was also possible to measure flow velocity. At the end of the observation period contrast-filling of vessels allowed for *simultaneous* microangiography and biomicroscopy. After sacrificing the animals the tissues were prepared for histology (Figure 5) and a comparison between intravital and histological findings was made.

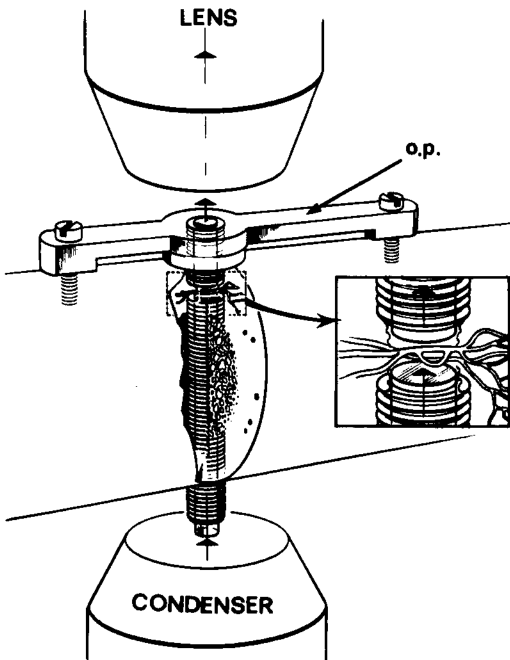

Figure 4. The chamber and graft in situ. *Enlarged section shows the chamber gap with bone tissue and vessels. This space is between 70 and 140 microns wide. o.p. = osteosynthesis plate.*

OBSERVATIONS AND DISCUSSION

Implantation sites

Different implantation sites were tested. The rabbit *femur* diaphysis was found to be less suitable because of the risk of fractures. When the chambers were transferred from an *ulnar* diaphysary donor to an ulnar host site in the contralateral extremity incorporation of the implant occurred without fractures. The radius, via the ossified intraosseous membrane, acted in the same way as a biological splint (Albrektsson 1971) stabilizing the transsected ulna. However, the foreleg of the rabbit was found to be less suitable for the purpose of vital microscopy because of the problem of respiratory movements disturbing the registrations. The proximal *tibia* was found to be the most suitable site. The medial facet of the tibial metaphysis is close to the skin surface, it is flat and its cortex is of

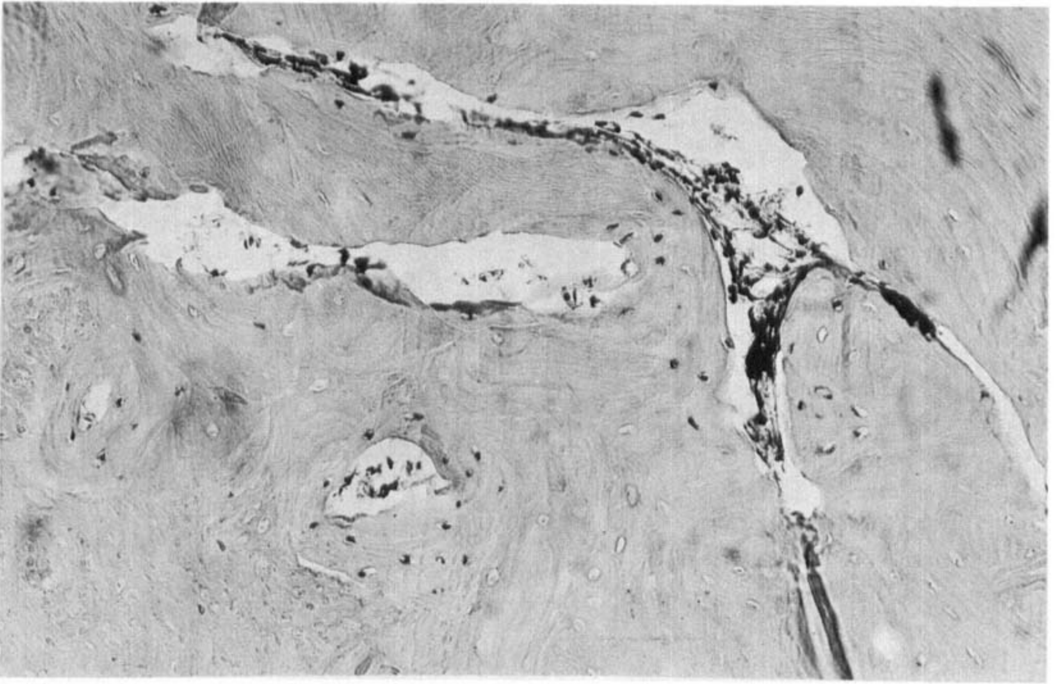

Figure 5. Histological section of the chamber space tissue 7 weeks after transplantation to a recipient site in the rabbit tibia. The section is taken from a part of the space where the bone post-grafting by vital microscopy was proved to undergo resorption. The lacunae in the vicinity of the vessels contain osteocyte nuclei indicating that the tissue is vital. Cement lines separating these vital osteocytes from the non-vital parts indicate that the vital bone probably has developed after grafting. ×180.

homogenous thickness facilitating a standardization of the implantation and registration procedures.

The bone graft

The graft consists of a cortical bone collar with the incorporated chamber. The part of the graft that could be studied with the vital microscope is found in the chamber space. This bone is a corticalized callus that has developed after chamber insertion.

In vivo *observations on bone tissue*

In about 90 per cent of the cases the chambers were firmly fixed to the bone 4 to 6 weeks after the operation and the glass space contained bone tissue and blood vessels. Contrary to Brånemark et al. (1964), who investigated the marrow circulation, the chamber space in our experiments was placed within the cortex to ensure better conditions for the study of the different events in the hard tissue components. With the space within the cortex one avoided fat cells in the test tissue. This gave better observation possibilities. The ingrowing tissue appeared in the form of bone ridges and a large amount of vessels of varying width (6–7 to 50 microns). Occasionally larger vessels (diameter 70–100 microns) were seen crossing the chamber gap, but these vessels did not give off any visible branches within the microscope field. Changes in flow direction and vessel pattern from the early stages of "graft take" to animal sacrifice were registered. The corpuscular flow rate could be determined by the flying spot technique (Brånemark 1959).

Scattered "islands" of connective tissue in the bone made the identification of the bone ridges and their subsequent rebuilding easier

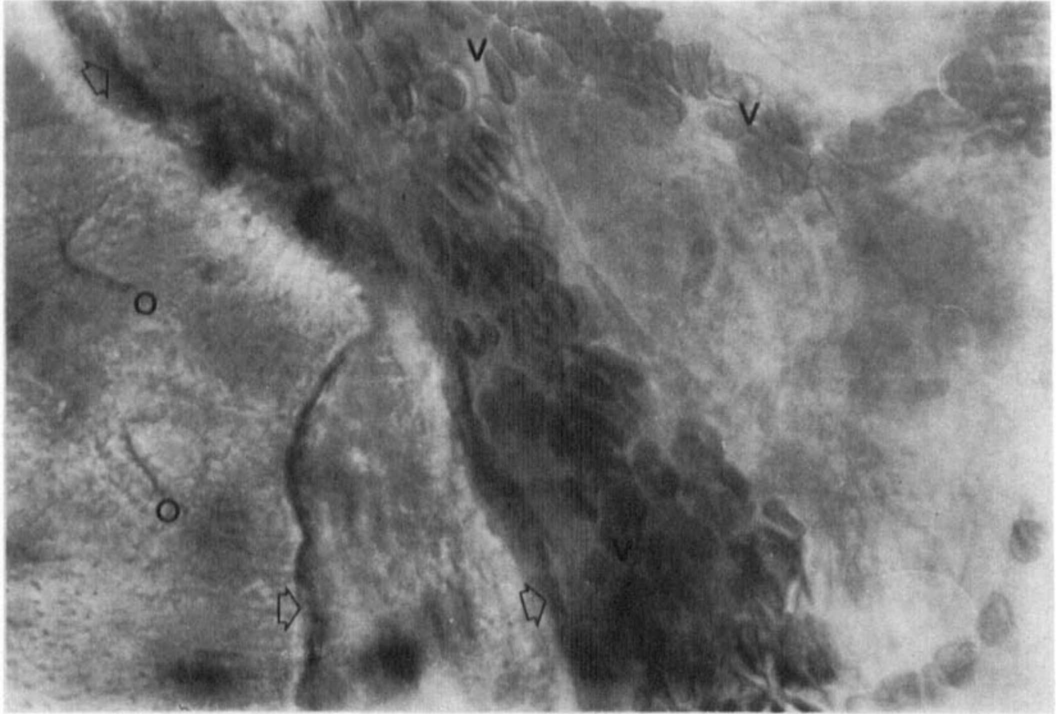

Figure 6. In vivo *microscopic view of the bone and vessels in the chamber space 6 months after installation, before transplantation. The arrows indicate trabecular bone edges. Arteriolar or venular vessels are often seen close to bone trabeculae. V = Venule with erythrocytes, O = Osteocyte lacuna with canaliculae. Photograph in Leitz intravital microscope, objective. Leitz ×55, N.A. 0.65.*

to register. Bone apposition and resorption was analysed morphometrically. The resolution made it possible to identify osteocytes with canaliculae and to study individual red and white blood cells (Figure 6).

During the first week after transplantation it was possible to observe to and fro movements of blood corpuscles in pre-existing graft vessels. True circulation however was not observed in these vascular channels. Four to five days after the transplantation procedure, circulation was observed in newly developed vessels in the chamber space. These capillaries of 6–7 micron diameter were primarily confined to the connective tissue "islands" (see Figure 2) in the chamber space. During the following weeks there appeared a network of regenerating vessels adjoining the bone trabeculae. These vessels were of a calibre which amounted to between 6 and 40 microns. From the second week postoperatively, a few vessels were observed to pierce the bone trabeculae. After revascularization, parts of the chamber bone tissue underwent resorption whereas other, also revascularized, bone areas remained structurally unchanged for follow-up periods of several months.

Further details of the revascularization and bone remodelling processes will be described in a later report.

Chambers without bone tissue

In about 10 per cent of the chambers no bone ingrowth had taken place 6 weeks after installation. In these cases either the chamber lacked primary stability or the implant was stable but had a glass space width of 70 microns. With this method 70 microns seemed to be the critical width to permit bone ingrowth. Undue movements of the chamber led to the production of an extensive callus

that stabilized the chamber after a prolonged healing period of another 4 to 6 weeks. Bone ingrowth however did not take place in spaces of implants lacking primary stability (4 cases). Instead of bone the glass space contained connective tissue with a limited amount of uniformly sized vessels (around 10 microns) arranged in a rather parallel fashion.

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