Scanning Electron Microscopy

Volume 1985
Number 1 *1985*

Article 33

8-30-1984

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SCANNING ELECTRON MICROSCOPY/1985/I (Pages 339-344)
SEM Inc., AMF O'Hare (Chicago), IL 60666 USA

0586-5581/85$1.00+.05

THE ROLE OF THE VESSELS IN THE GROWTH PLATE : MORPHOLOGICAL EXAMINATION

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(Paper received March 6 1984, Completed manuscript received August 30 1984)

Abstract

Combined methods of light microscopy to present ossification zones by means of fluorochrome dyes make it possible to explain the contradicting presentations of the vascular system of the growth plate in the literature. The vascular system was casted with methacrylates which can be presented in the scanning electron microscope 3-dimensionally together with the trabeculae as a result of their resistance in the electron beam.

The 3-dimensional presentation in the electron microscope allows a clear distinction between the various vascular sections in the arterial flow system. In the microcorrosion casts the vessels of the epiphyseal side of the growth plate can be clearly distinguished from those of the metaphyseal side. The combination of both methods: labelling with fluorochromes investigated in the incident fluorescent light and casting of the vessels studied in the SEM shows close connection between the arterial vascularization and osteogenesis. These findings also explain the reactions on the part of growth behavior following traumatic injuries to the growth plate - reactions which could not be clearly explained up until now. Our findings do not contradict results of studies in the literature. They permit a uniform interpretation of these findings,however. Presentations of the venous drainage system on the epiphyseal side of the growth zone have not been made to date.

KEY WORDS: Vascularization of the growth plate, epiphyseal blood supply, metaphyseal blood supply, injection replicas, scanning electron microscopy.

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Introduction

McLean and Bloom (1940) divided the growth plate into 4 microscopic-anatomic zones: 1. the zone of resting cartilage, 2. the zone of proliferation, 3. the zone of mature or even hypertrophic cartilage, 4. the zone of mineralized cartilage.

Trueta and Morgan (1960) added the base plate of bone as the first zone and the zone of cell degeneration as the fifth zone. This was followed by the zone of bone formation. Trueta and Morgan also divided the growth plate into an epiphyseal and a metaphyseal section. According to them the growth plate was supplied with arteries primarily from the epiphyseal side.

Dividing the growth plate into an epiphyseal and a metaphyseal part does not necessarily make sense. More recent studies have shown that the growth plate is part of the metaphysis. It is only the anchoring zone for the plate's cartilage in the base plate of bone in the epiphysis which can be considered epiphyseal.

Combined studies with the fluorescence and scanning electron microscopes were performed to determine the relationship between the arterial metaphyseal blood supply and the rates of apposition in the bone growth. The role of the vessels in osteogenesis was described by Trueta (1963).

Materials and Methods

Six Sprague-Dawley rats weighing 200g were perfused via the abdominal aorta after having been anaesthetized with Nembutal.Ringer's solution with an admixture of 5000 I.U.heparin and 0.1 g/l papaverine was used for perfusion. The injection was made by hand via a 1.3 mm catheter. The inferior vena cava was drained with a 1.9 mm catheter. After sacrificing the animals with an overdose of Nembutal, fixation was performed with Karnovsky's solution at body temperature (36°C). 120 ml fixative was injected by hand until there was a pronounced extension

contracture of the lower extremities from strong muscle fibrillations. The fixative in the vasculature was washed out with 40 ml Ringer's solution. A 20 ml disposable syringe was used to inject a mixture of 10 ml Mercox® and 10 ml MMA into the vascular system. After the resin had hardened in a 45°C water bath, both were carefully prepared. The bones were cut in frontal sections with a diamond saw and then corroded in a 3% H_2O_2 solution. The myeloid and cartilage tissue had been removed after 1.5 to 2 hours at temperatures just under 60° C. The bone and its vascular system were seen. The specimens were then dehydrated and air-dried and sputtered with gold. They were then studied in the PSEM 500 X scanning electron microscope.

In order to study the relationship between arterial circulation and bone apposition, six 6-month old rabbits which had been labelled continuously with fluorochromes in the 6th, 7th, 8th and 9th weeks as well as in the 18th, 19th, 20th and 21st weeks were prepared to be studied in the fluorescence microscope. Oxytetracycline, calcein blue, alzarine complexon and calcein were the fluorochromes used. The animals were perfused as described above 11 days after the last labelling. The bones were then prepared with a diamond saw and the specimens were studied in the low-power incident fluorescent light.

Results

The vascular system in the diaphysis

At times, the corrosion preparations of the vasculature together with the rat's tibial bone presented complete vascular casts of the medullary cavity (Fig. 1). Four different vascular morphologies could be distinguished in the SEM. The large nutrient vessels with their ascending and descending branches were particularly striking due to their spiral course. Branching off from these acute angles, we could see smaller arterial vessels with a straight course which passed through the medullary cavity and parallel to one another towards the metaphysis. Branching off from these vessels we found smaller arteries which passed through the medullary spaces. These arteries were straight as they flowed back and branched off further to form a wide-meshed capillary network. In addition to the spiral-shaped vessels and those with a straight course and branchings at acute angles, there were also thick, coarse venous vessels running through the medullary space.The thickest trunk, the central vein, was found in the center of the medullary cavity. A coarse network of veins was formed around the straight main arterial vessels by arched vessels flowing into the central vein nearly at right angles, as well as by 3-dimensional branches joining together at obtuse angles. The wide meshes of the larger venous and arterial vessels were filled in by a narrow-meshed 3-dimensional network of medullary sinusoids.

The vessels in the metaphysis

In the metaphysis of rat's tibiae, strong vessels which were straight and parallel to one another ascended towards the growth plate (Fig. 2). Rami with horizontal course branched off from the stronger arterial vessels at the level of the primary spongiosa trabeculae. These rami ascended with a regular and vertical course into the intertrabecular medullary spaces beneath the plate's cartilage with arterial capillaries branching off at nearly right angles.

The arterial vessels with a horizontal course were connected to the metaphyseal centripetal arterial influxes; they were found at the level of the vertical spongiosa trabeculae of the metaphysis (Fig. 3). These trabeculae ended in the free medullary cavity. The arterial capillaries- without branching off further - could be followed into the cartilage's zone of mineralization. The terminal capillaries formed various sprouts which forged ahead towards the cartilage. The transition to a widely branching parietal vascular system of venous capillaries was located beneath the end buds (Fig 4). Several spindle-shaped impressions could be seen on the capillaries. The end buds of these arterial vascular networks presented a totally irregular outline. Coarse, usually thicker sprouts were found most frequently. These clearly stood out against the 3-dimensional network of the delicate capillaries.

The epiphyseal vessels in the growth plate

The epiphysis with its base plate concave to the cartilage's cell columns, formed a large anchoring surface in the cartilaginous growth plate (Fig. 5) due to its greatly projecting bony protuberances. The corrosion specimens of vascular casts present a widely branching, regular vascular network which rests on the entire plate and is protected by the bony anchoring protuberances (Fig. 6a, 6b and 6 c). These vessels are wide, thin-walled venous capillaries with a varying morphology which best corresponds to the sinusoids in the medullary space. Unlike the network of the medullary sinusoids, these venous vessels presented a 2-dimensional, coarse network. The vessels either flowed through the epiphyseal base plate into the medullary spaces of the epiphysis, or were directly connected to the perichondral veins by the thin metaphyseal cortex layer. With respect to their course to the trabeculae and their morphology, the arrangement of the venous sinusoids corresponded to that of the sinusoids in the trabeculae in bone marrow. Their distance to the bone was nearly constant. The view upon the metaphyseal side in incident light corresponded to an arterial flow system, while the base plate in the epiphysis presented

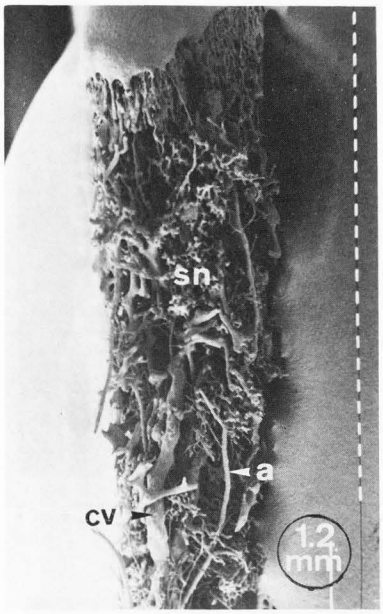

Fig. 1. Vasculature of a rat's tibial bone revealing the central vein(cv), smaller arteries (a) with their straight course and the sinusoid network(sn).

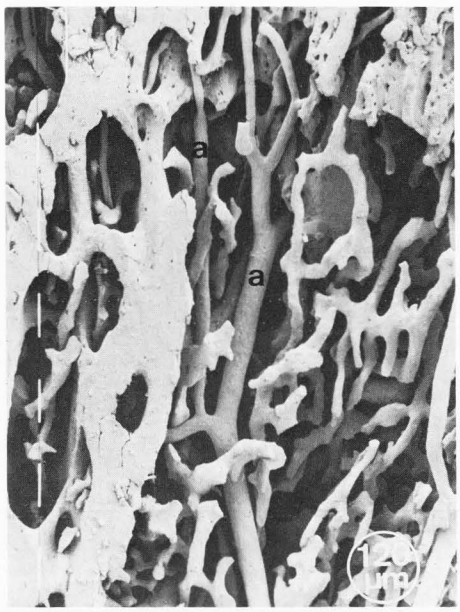

Fig. 2. Strong arterial vessels (a) ascending toward the growth plate.

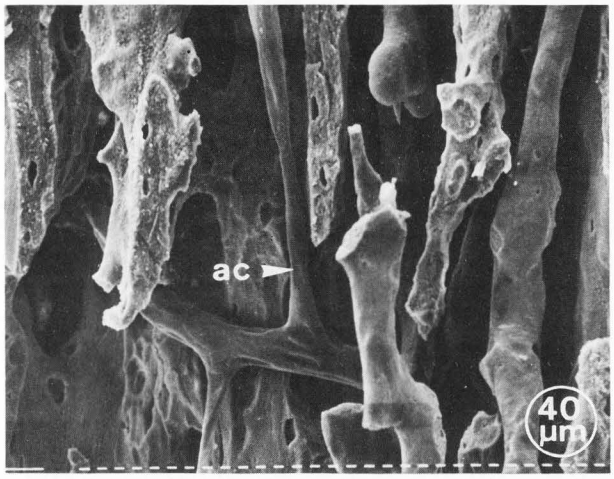

Fig. 3. Arterial capillaries (ac)- without branching off further - can be followed towards the zone of mineralization.

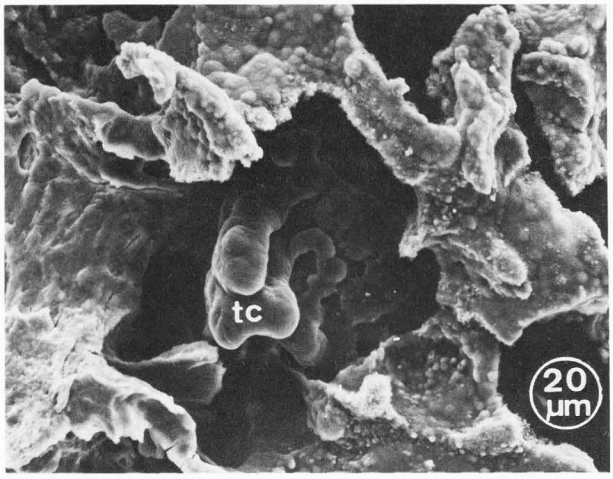

Fig. 4. The terminal capillaries(tc)form various sprouts which forged ahead towards the cartilage. View upon the end buds.

a venous, 2-dimensional network. Only rarely were small-caliber arterial capillaries found in the epiphyseal base plate. These also had a straight course and passed through the intertrabecular spaces, i.e. the medullary spaces.

The role of the vessels in the growth of long bones

The stiffening of the long tubular bones during ontogenesis begins with a mantle-like ossification in the middle of the shaft. Continuous sequential labelling with fluorochrome dyes using the rabbit's tibia as an example have shown that the cuff of bone expands like a funnel towards the adjacent joints. The metaphyses are taken up into the funnel in the shape of a "V" (Fig. 7).

Using continuous sequential labellings we were able to show that the bone growth was added on only in that part of the metaphysis where the extensive arterial vascularization could be shown in the microcorrosion casts.

The joint-supporting epiphyses grew as a result of apposition of their entire circumference. At the same time, the spongiosa trabeculae were reinforced and the medullary spaces drifted, resulting in an excentric apposition pattern (Fig. 7). The epiphyses surpassed the metaphyses, making it look as if hats had been placed on the growth plate. The fibers in the articular cartilage continued as perichondrium in the plate and periosteum in the metaphyses. They formed the longest fibers in the intercolumnar septae of the

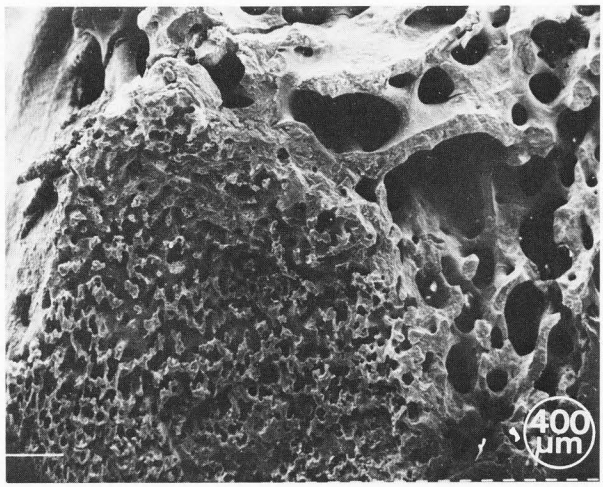

Fig. 5. Large anchoring surface of the epiphysis' base plate.

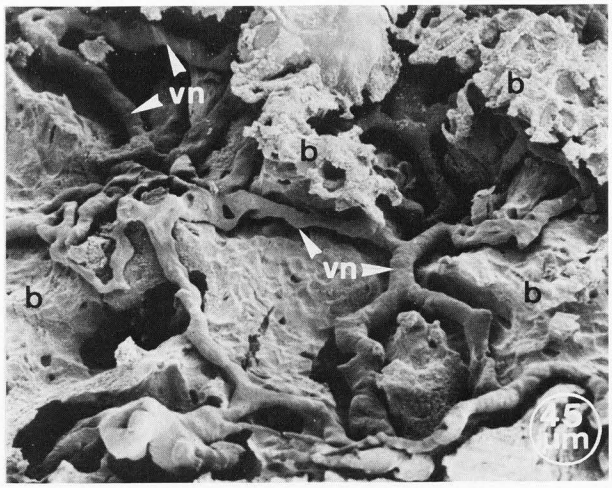

Fig. 6a. Regular venous network (vn) of the epiphysis'base plate protected by the bony protuberances (b).

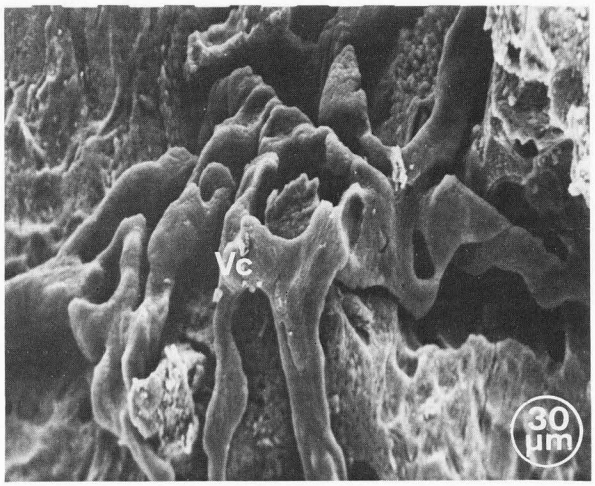

Fig. 6b. Thin-walled venous capillaries (Vc) similar to the sinusoids of the bone marrow.

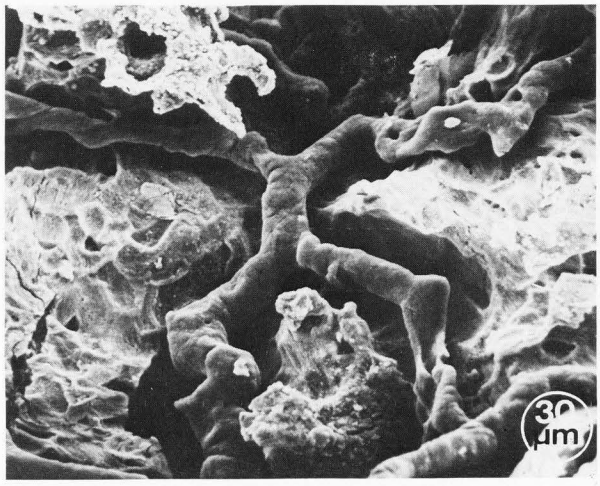

Fig. 6c. Venous vessels on the epiphysis' base plate presenting a 2-dimensional network of thin-walled capillaries.

growth plate's cell columns. They are also part of the primary spongiosa trabeculae and the compact substance of the cortical bone. Using polychrome fluorescent labellings in incident fluorescent light, we could very impressively show that all bone appositions occur in the metaphysical region (Fig. 7). At this point in the time only a fraction of deposition activities could be seen on the epiphyseal side of the growth cartilage. The formative character of these bone appositions corresponded to the morphology of the epiphysis and were completely different from the growth in length of the metaphysis.

Discussion

Trueta and Morgan (1960) were the first to break the growth zone into an epiphyseal and metaphyseal portion. They concluded that the plate was supplied with arteries primarily from the epiphyseal side. These results do not agree with the morphology which can be found in corrosion specimens of the bone's vascular system. The morphology of the vessels on the metaphyseal side reflects an arterial flow system, while the epiphyseal side of the growth plate represents a venous drainage system. These findings are in good agreement with the fact that the osteogenetic activities are very closely related to the arterial side of the vascularization. It could be clearly demonstrated that the arteries were part of the metaphysis.

Trueta and Harrison (1953) distinguished between an epiphyseal and a metaphyseal blood supply in all of their casts. In their presentation performed under the light microscope, Trueta and Harrison(1953)

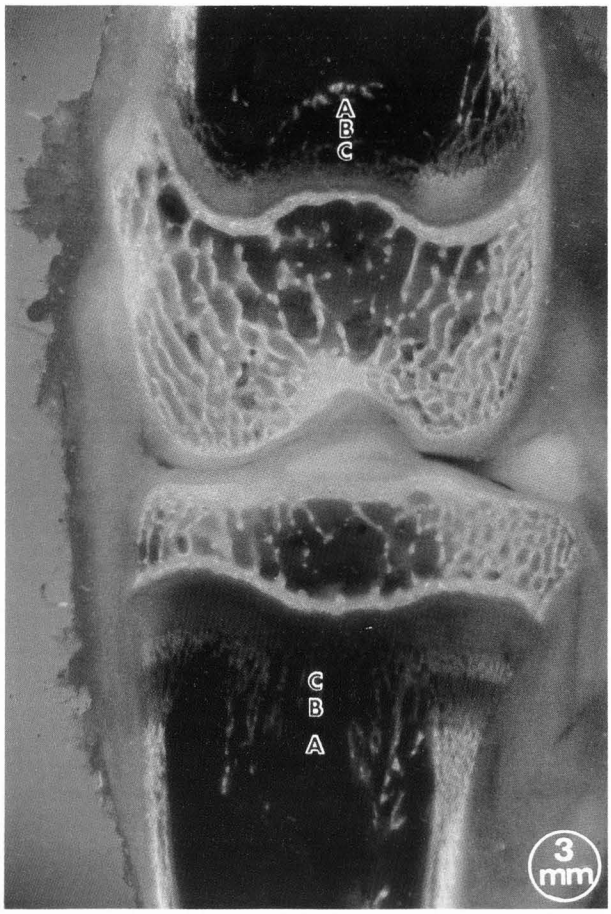

Fig. 7. Growth of a 5 month old rabbit's knee joint labelled sequentially with fluorochromes in the 18th, 19th, 20th and 21st week of growth. (A) Continuous label of oxytetracycline during the 18th week of growth. (B) Label of alizarine complexon during the 20th week of growth. (C) Label of calcein green during the 21st week of growth. The weekly continuous labellings reveal impressively that all bone apposition of the growth in length occur in the metaphyseal region. The calcium blue label of the 19th week is not revealed on this micrograph.

could only show the large vascular arcades in the metaphyseal arteries and the vessels in the epiphysis itself. The fine vascular networks at the bottom of the epiphyseal base plate could not be seen in any of their photographs. Trueta and Morgan (1960) presented the terminal capillaries of the metaphyseal vascular loops somewhat more precisely. They found that they all ended at the same level at the 2 lowest rows of maturing cartilage cells in the hyaline cartilage. They also described the close relationship between the invasion of the vessels and the mineralization of the intercolumnar septae. Morgan (1959) gave a very impressive description of the course taken by the metaphyseal vessels. He discovered the branching of the arteries and their further branchings off into numerous thin-walled vessels which all ended in capillary vascular loops. Morgan's presentation under the light microscope is the equivalent of the photograph of vascular casts which can be obtained in the SEM.

Trueta (1963) described the great significance of arterial vascularization for osteogenesis. The fact that Morgan (1959),Trueta and Morgan (1960) and Trueta (1963) came to the conclusion in joint studies that the growth plate is supplied almost exclusively by the epiphyseal side can only be explained by the fact that the venous network of the anchoring protuberance in the epiphyseal base plate could not be presented in their casts. As a result, it was only the thick arterial vascular arcades in the epiphysis which attracted one's attention and gave one the impression that they were responsible for the arterial vascularization of the epiphyseal plate due to their close connection to it.

From the fluorescent labellings it can be concluded that the first osteoblast layers are formed on the cartilage protuberances with the vessels sprouting up along the longitudinal septae, and as a result, cartilage-bone protuberances are formed.

According to Kember (1960), the proliferation layer comes after the uppermost zone of the cartilaginous growth cells, the zone of reserve cells. Based on his findings, all of the cells in the reserve cell zone and in the proliferation layers can undergo division. Kember labelled thymidine with tritium, a beta-emitting hydrogen isotope. He determined the cell rates on 5 µm thick sections of the growth plate. He found the generation time for cells in the proliferation zone to be approx. 2 days in rats. The generation time was longer in the uppermost cell layers, which is why he called this zone the reserve cell zone. The cells in the zone of maturing cartilage were no longer divisible. According to Lacroix (1951), the fibers in the intercolumnar septae were subjected to longitudinal traction as a result of the cartilaginous cell's increase in volume. This caused the bundles of fibrillae in this zone of "hypertrophic cartilage" to become longer and thinner. McLean and Bloom (1940) and then Matthieu (1951) and Trueta and Little (1960) described the calcification of the septae. Dodds (1932) had already found that the sidepieces of the ladders mineralized at the level of the 3 lower rows of maturing cartilage cells. Dodds had also determined the close relationship between the "mineralization zone" and the vessels. It was only later, however, that Trueta and Little (1960) described this finding in more detail. According to Schenk, Spiro and Wiener (1967), mineralization of the matrix of cartilage is a precondition for

invasion by metaphyseal vessels. All of these descriptions and studies show the close relationship between oxygen partial pressure and ossification , i.e. mineralization of the opening zone. Studies in which the growth plate was damaged experimentally have also clearly shown the close relationship between oxygen supply and osteogenesis.

Jansen (1928) and Bragard (1932) found that the sensivity of the growth cartilage was in the following order: first the ossification zone, then the zone of maturing cartilage and finally the proliferation zone show disturbed development. More recent studies by Neddelblad (1984), in which plate transplants were performed with micro-surgical vascular anastomosis of the nutrient vessels of a dog's proximal fibula, have shown that if the vascular anastomosis failed, the result was not a shortening of the transplant, but a broadening of the growth plate. As a result, the transplant increased in length. Thus growth in length appears to be controlled by the braking effect of ossification.

The contradiction in Trueta and Morgan's (1960) morphological findings and their conclusion with respect to the vascularization of the growth plate can be eliminated by the high resolution of vascular casts in the scanning electron microscope. The morphology of the vascular system corresponds to an arterial flow system with capillary terminal loops on the metaphyseal side and a venous drainage system with a coarse network of venous sinusoid vessels on the epiphyseal side of the growth zone. These sinusoid vessels drain both into the epiphysis and through the thin metaphyseal compact substance in perichondral veins.

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Discussion with Reviewers

Reviewer I: What is fluorescent resin?
Authors: In the incident fluorescent light methacrylates show a blue luminescence in the UV-spectrum.

Reviewer I: What is the proof that the vessels in the anchoring plate are veins?
Authors: The vessels are wide and thin-walled and correspond to the marrow sinusoids. Endothelial replicas and branchings off can clearly be distinguished from the arterial system.