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Substitution of natural coral by cortical bone and bone marrow in the rat femur (Part I)

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Natural coral consisting of calcium carbonate was investigated after the implantation into the cortex and marrow cavity of the rat femur in order to study the substitution by bone, soft tissue and bone marrow. The time intervals were 7, 14, 21 and 28 days and the tissue reaction was morphometrically determined. The absorption of coralline substance seemed to be locally enhanced by osteoclasts and otherwise due to dissolution by interstitial fluid. The development of bone was predominant in the marrow space after 7 and 14 days postoperatively. At the later stages, the development of bone was more pronounced in the cortex and reduced in the marrow cavity. Von Kossa staining for calcium-containing material revealed small seams of mineral at the coralline surface, suggesting that these structures exert some influence on the deposition of mineral. Soft tissue was replaced in the marrow cavity by a regenerate of hemopoietic tissue. Already after 28 days the tissues replacing the natural coral implant were highly organized in order to restore the original structures and functions.

1. Introduction

The first implants, made from the coral Porites, were prepared by hydrothermal conversion of skeletal calcium carbonate in the form of aragonite ($CaCO_3$) into hydroxyapatite by treatment in a fluid medium of $(NH_4)_2HPO_4$ and H_2O at 300 °C and high pressure [1]. It was thought that hydroxyapatite would be closer to bone mineral than the natural coral calcium carbonate. The surfaces of the interconnecting pores of this apatite or calcium carbonate provide a basis for bone deposition, and in the centre of the pores there is usually space for vessels and blood supply. The coralline material is used by the bone forming system and absorbing cells as a scaffold and can be remodelled over a long period of time [2].

At the surface of natural coral, which is deproteinized calcium carbonate (aragonite) treated with sodium hypochloride, bone deposition and absorption have been described [3–5]. The absorption rate of this coral seems to be higher than that of hydroxyapatite coral. Corals Porites and Acropora were studied in pig and sheep femur and tibia in cortical and trabecular bone sites at 1 and 2 months after implantation [4] for bone deposition and absorption. Despite these thorough morphometric investigations, questions still remain unanswered: what is the role of implantation bed? what is the role of cortical or trabecular bone or bone marrow in the dynamics of coral absorption and bone development? The role of osteoclasts, macrophages or soft tissue cells in the process of absorption is not clear and the chemical dissolution of the coralline substance is not separated from the activity of the absorbing cells. In order to better understand the mechanisms of calcium carbonate absorption and bone deposition, additional animal experiments were performed using quantitative histology (Part I of this report) and transmission and scanning electron microscopy (TEM, SEM) (contained in Part II).

2. Materials and methods

The natural coral implants (Biocoral^R) were obtained as cylinders of diameter 1.5 mm and length 6 mm from the species Porites. They were sterilized by gamma irradiation and contained 97% calcium carbonate in the form of aragonite and in the order of 0.07% amino acids and 0.5–1% oligo-elements. The macropores occupied approximately 45% of the volume, they were interconnected, and their diameter was around 150 μm. These data were supplied by the manufacturer. Using SEM (Fig. 1), the structure of the original surfaces is shown to be smooth and very different from the fractured surfaces, which have a multitude of gaps and clefts. Furthermore, the density of the surface structure is higher at the original inner surfaces than at the fractured surfaces.

Adult male Sprague–Dawley rats with a body weight between 450 and 650 g at the beginning of the experiment were housed in wire cages and given hard pellet diet Altromin standard (Lage, Lippe, FRG) and tap water *ad libitum*. Under general anaesthesia, the femur was exposed and a hole with a diameter of 1.5 mm was drilled midshaft and the implant cylinder inserted. The wound was closed in different layers with sutures and the animals given protective treatment with gentamycin 20 mg and 0.5 ml Belapharm Antiphlogisticum 30%. After 7, 14, 21 and 28 days postoperatively, the specimens were collected and prepared for light microscopy, including morphometry, SEM and

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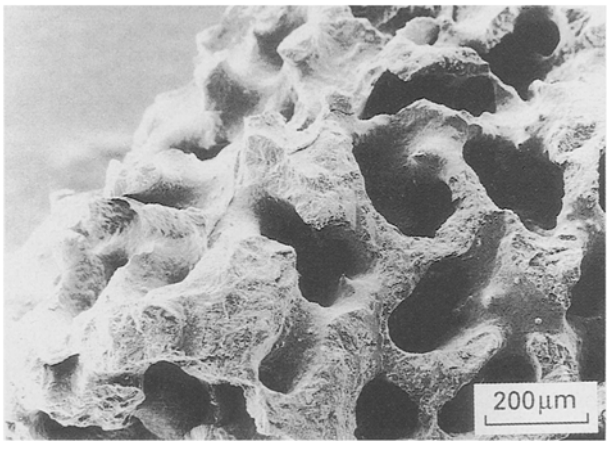

Figure 1 Natural, smooth surface and fractured rough surface of natural coral.

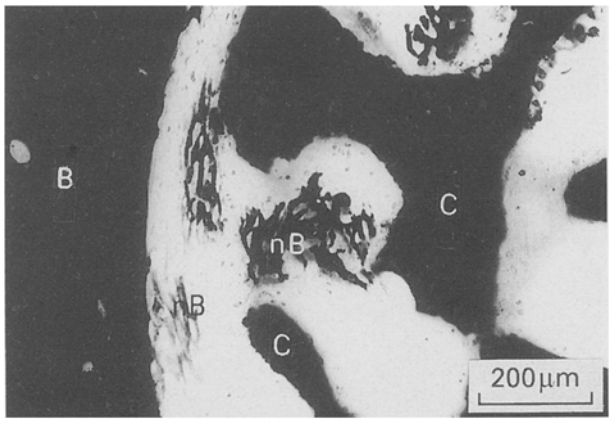

Figure 3 Mineralization of new bone (nB) between old bone (B) and coral (C) 7 days after implantation. Sawed section. Von Kossa and fuchsin staining.

TEM as described in [6]. The following parameters were determined in morphometry: area of coral, bone, osteoid and soft tissue for both cortical bone and the bone marrow sites. Four animals per time interval were used for light microscopy and morphometry, the others for SEM and TEM. At each time interval, seven animals were used for experimentation purposes and 14 implants were made.

3. Results

Seven days after implantation, the coral cylinders contain residues of blood clots, mainly of erythrocytes, in the centre of the specimens, whereas in the outer third of the cylinders there is already an organization of tissue comprising fibroblasts, capillaries, some macrophages and osteoblasts. Between the old bone, which provides one source of osteoblasts, and the coral implant there already exists bridging trabecular primary bone (Figs 2 and 3). The trabeculae sit on the pre-existing bone on one side and on the coralline material on the other side. They are already mineralized (Fig. 3) and display some osteoid and osteoblasts at the surface. Some osteoblasts are trapped in the trabeculae and are developing into osteocytes. After 7 days, there is only a small number of osteoclasts and other resorbing cells , whereas after 14 days multinuclear giant cells with features of osteoclasts and absorbing cells can be seen at the surface of the coral (Fig 4). At the sites of bone marrow, a small seam of fibrous tissue separates the blood cell precursors and the coral material.

After 14 days, bone development has already advanced to the centre of the implant cylinder (Fig. 5). There are some irregular surfaces due to areas of resorption, especially when located within the former bone marrow cavity. Pores are also filled up with soft tissue in which fibroblasts, round cells, very rarely polymorphonuclear leucocytes and some macrophages are encountered. In some areas, there is abundant resorption of coral by multinuclear giant cells (Figs 4 and 6). In sections which are closer to the marrow cavity, there is already a seam of bone at some surfaces of the coral pores (Fig. 5), the centre of the pores being made up of soft tissue with spacious capillaries. There are many osteoblasts lining the osteoid and surfaces of coral pores. In the marrow cavity, there is already advanced dissolution of the coral material on the one hand, and deposition of the bone at coral surfaces on the other hand. Osteoblasts produce elements of the extracellular matrix, govern

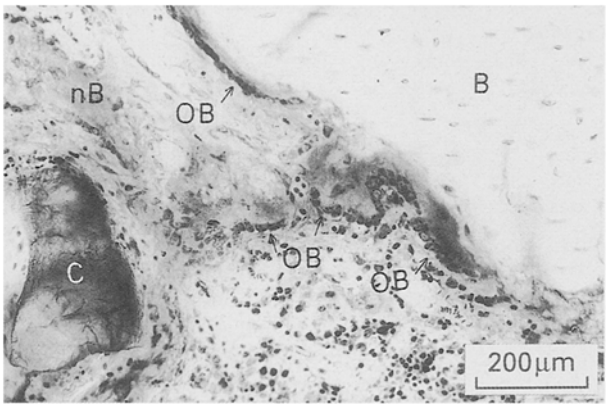

Figure 2 Development of new bone (nB) between old bone (B) and coral (C) 7 days after implantation. At the new bone surface seams of osteoblasts (OB). Sawed section. Giemsa staining.

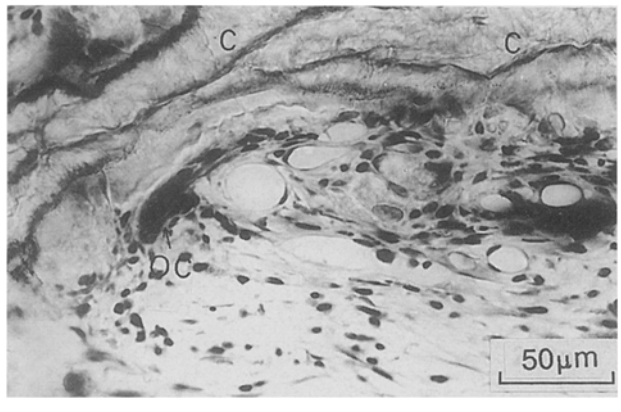

Figure 4 Lamellar structure of coral (C) indicating different stainability and large multinuclear absorbing cell (arrow), macrophages, fibroblasts and capillaries in the soft tissue within a pore 14 days after implantation. Sawed section. Giemsa staining.

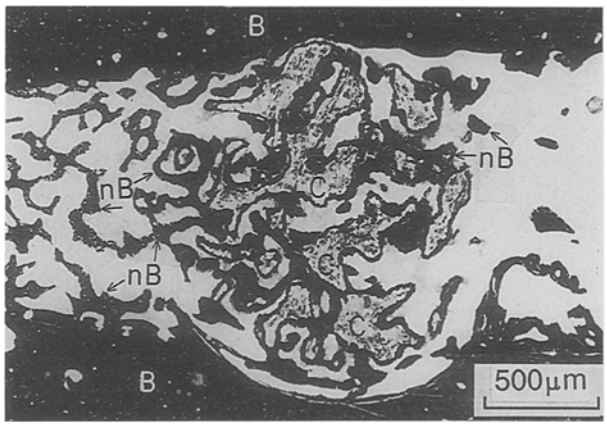

Figure 5 Porous coral cylinder (C) in the former drill hole with dense cortical bone (B). The coral surface partially covered by trabecular new bone (nB) 14 days after implantation. Sawed section. Von Kossa and fuchsin staining.

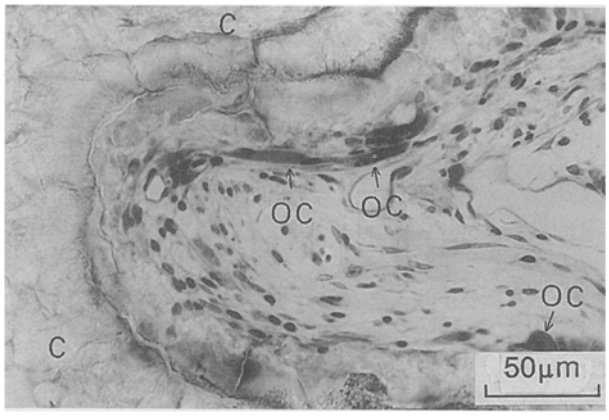

Figure 6 Osteoclastic (OC) resorption of coral (C) 14 days after implantation. Sawed section. Giemsa staining.

matrix vesicle mineralization and provide bone bonding to the coral substance.

At 21 and 28 days postoperatively, the absorption of coral advances slowly in cortical and marrow sites, whereas bone development is advancing in the cortex and decreasing in the marrow where the soft tissue and hemopoietic tissue increases. The localization of osteoclastic absorption and osteoblastic deposition of bone is very close (Fig. 7). This means a very precise

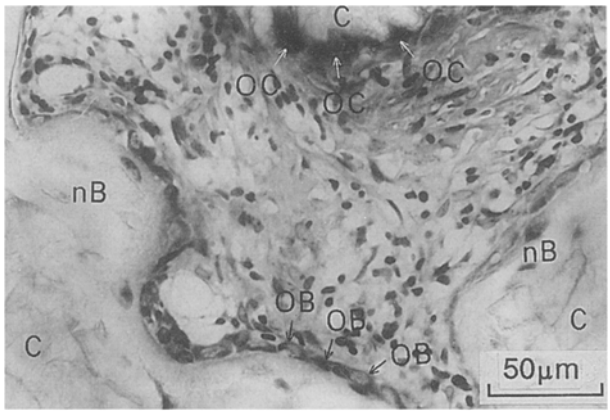

Figure 7 Seam of osteoclasts (OC) in resorption lacunae and new bone (nB) on coral (C) covered by a seam of osteoblasts (OB) 21 days after implantation. Sawed section. Giemsa staining.

local regulation of cellular activity in a small area. Already after 21 days, but more pronounced after 28 days, hemopoietic tissue can be found in the soft tissue spaces of the pores (Fig. 8). This observation indicates further regeneration of the physiological structures in the area of the former marrow cavity. In the cortical site, the density of bone increases which is consistent with the biomechanical necessities.

These processes are underlined by morphometric data (Table I). There is a decrease in the area covered by coral in the cortical and in the marrow part of the drill hole from 7 days to 28 days. The degree of coral loss seems to be higher in the marrow than in the cortex of the femur; this is, however, statistically not proven. On the other side, there is at 21 and 28 days more bone in the cortical part of the drill hole than in the marrow; again the statistical evaluation is on a level of $p = 0.07$. At the same time, the development of

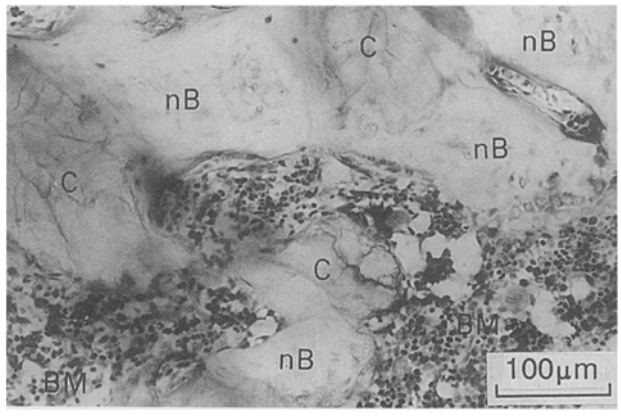

Figure 8 Coral (C) mainly covered by new bone (nB) and bone marrow regenerated in pores of the coral implant 28 days after implantation into the marrow part of the former drill hole. Sawed section. Giemsa staining.

TABLE I Percentage of coral, bone, soft tissue and osteoid covered by osteoblasts (OB) in sections from cortical bone and bone marrow sites 7, 14, 21 and 28 days after implantation into the femur diaphysis of adult male rats. Values represent the mean ± SEM (standard error of the mean) of up to 11 sections. Probability (p) for differences of values between cortical and marrow sites were calculated using the Wilcoxon test.

Days	7	14	21	28
Coral				
cort. bone	48.4 ± 0.5	40.8 ± 3.2	27.3 ± 3.9	32.3 ± 2.0
marrow	48.9 ± 0.5	44.0 ± 1.1	30.8 ± 3.7	28.2 ± 0.3
p	0.72	1.0	0.07	0.15
Bone				
cort. bone	0.6 ± 0.4	13.5 ± 0.6	23.0 ± 2.8	31.2 ± 1.2
marrow	1.7 ± 0.5	16.2 ± 1.4	14.9 ± 1.7	12.3 ± 2.4
p	0.47	0.11	0.15	0.07
Soft tissue				
cort. bone	48.8 ± 1.0	38.2 ± 2.0	38.9 ± 3.0	25.8 ± 4.0
marrow	46.2 ± 0.8	36.7 ± 1.0	47.5 ± 5.2	51.9 ± 1.9
p	0.28	1.0	0.28	0.07
Osteoid + OB				
cort. bone	0.8 ± 0.4	5.3 ± 0.5	5.0 ± 1.1	5.9 ± 1.4
marrow	2.1 ± 0.6	2.1 ± 0.2	2.9 ± 0.8	1.7 ± 0.6
p	0.15	0.11	0.15	0.07

bone, which is measured by osteoid covered by osteoblasts, is higher in the cortex than in the marrow ($p = 0.07$). This tendency is also supported by more soft tissue in the marrow than in the cortex at 28 days ($p = 0.07$).

4. Discussion

The data from this study indicate the importance of the biomechanical situation in the implantation site in bone. After drilling a hole in the diaphysis of the rat femur, the distribution of the normal strain is disturbed. Mechanisms of repair and regeneration lead to bone development with direct apposition on the natural coral as early as seven days after implantation to restore normal strain history. Similar results were recently shown [7] using coral hydroxyapatite in cortical defects of the canine radius. Bone ingrowth increased from 52% after 16 weeks to 74% after 1 year, and in biomechanical tests bending strength and stiffness increased simultaneously.

On the other hand, a minor increase of bone developed on natural coral from week 1 to week 3 and a slight decrease was observed at week 4 in the bone marrow site. This may be the result of the non-weight-bearing situation in the bone marrow cavity, where only the osteoconductive influence of the natural coral governs the reaction. The study [7] showed maximum bone ingrowth of 38% after 4 weeks, falling monotonically to 17% after 1 year in non-weight-bearing cancellous bone.

Furthermore, the data support the idea that the implant material is preferentially absorbed where it is not needed for substitution of load-bearing structures, i.e. in the bone marrow cavity. In this region, the mechanisms for coral absorption either by cells or extracellular processes are enhanced in order to replace the coral by bone marrow. Whether the absorption is mainly by dissolution or by cellular activity, i.e. by osteoclasts, cannot be clearly stated, insofar as the aims of the study were not met.

Natural coral is a very suitable material for cell attachment, e.g. of human fibroblasts [8] which produce a matrix with fibrils. In this study, osteoblasts and osteoclasts were observed very closely on natural coral surfaces with evidence of bone forming by deposition of a mineralizing matrix and resorption of the natural coral leaving resorption lacunae. This fact indicates a very close and precise regulation and cooperation of these cells. The given dynamic underlines the existence of processes which govern the two basic processes, namely bone deposition via osteoblast recruitment and activation and at the same time, but at another site, the recruitment and activation of absorbing cells, osteoclasts or other cells. The cytokines which are mediators of these processes have to be identified for further understanding of the described processes.

5. Conclusions

Bone deposition at natural coral surfaces and absorption of coral and bone are influenced by biomechanics at cortical and bone marrow sites.

Acknowledgement

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