Transactions of the Materials Research Society of Japan 29[6] 2943-2947 (2004)

Bone Regeneration with Organic/Inorganic Composite Materials

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New bioabsorbable organic/inorganic composite materials were designed for the bone regeneration. The one was β-TCP/PLGC membranes which matrix is copolymer of L-lactic acid, glycol acid and ε-caprolactone, and filler is β-tricalcium phosphate. The other is HAp/Col, which is the composite of hydroxyapatite and type I collagen. They were experimentally applied to the canine bone defects. Although no biological enhancement techniques of tissue engineering such as cell transplantations or cytokine applications were utilized, the bone defects of critical size were successfully treated with the materials alone. These facts might prove that the treatment with biomaterials would be extremely effective and they would be used for bone regeneration, if the materials and the methods of usages were optimized.
Key words: Bone regeneration, Bioabsorbable materials, L-Lactic acid, hydroxyapatite, Collagen

1. INTRODUCTION

Recently, academic and clinical interests on regeneration of living tissues are significantly increasing. Among many fields of regenerations, bone regeneration is one of the most active and advanced fields of application, and we already have many proposed technology of regeneration. We already have a lot of reports how well we could regenerate bone tissues with many kind of bone substitute. We also have more recent tissue engineering technologies such as the application of the growth factors and cell transplantation. Furthermore, we can mention that the most effective bone reconstructive surgery at this present time is based on the distraction osteogenesis. Here we focused on the bone regeneration technology in which we utilize biomaterials alone.

Biomaterials are not drugs or living cells, and have not any magical functions. If they are applied to the bone defects, they are never turned into bone tissues. On the other hand, the living tissues have self-healing functions, and the biomaterials properly utilized may enhance this self-healing ability. The enhancement may be through the provision to the tissues a good milieu for regeneration, which the materials certainly could do. Such a method of usage of biomaterials will be the only one possible way we can make use of biomaterials in the regeneration medicine.

The basic concept above looks reasonable, however, it involves a serious dilemma. If we use materials, the materials occupy some space and in which the regeneration never take place. Hence the regeneration cannot be perfect if we use non-absorbable materials. The applications of bioabsorbable materials had been proposed, and many works on the scaffolds or carriers made from biomaterials had been carried out in relation to tissue engineering. But the difficult situation is not so changed. If materials' absorption is too slow, the presence of materials will be obstacles for regeneration. If materials' absorption is too rapid, it would be the same as the case we do not use materials at all. The process of materials' absorption and that of tissue regeneration should take place in harmony, but the realization of such process is extremely difficult.

We do not employ the strategy of scaffolds or carriers, but reconsider the self-healing process of tissues, and make trials to develop optimum methods and materials for bone regeneration. Here we introduce two new bioabsorbable organic/inorganic composites. The one is β-TCP/PLGC, the composite which matrix is copolymer of L-lactic acid, glycol acid and ε-caprolactone, and filler is β-tricalcium phosphate. And the other is HAp/Col, the composite of hydroxyapatite and type I atelocollagen. We would discuss the strategies for the bone regeneration with the use of these materials and show some promising evidences for the effectiveness of the application of these materials in bone regeneration.

2. BARRIER MEMBRANES

2.1. Strategy

The strategy we adopt first for bone regeneration is based on usages of barrier membranes. The basic principle is shown schematically in Figure 1 for the case of bone defect. If the bone defect is so small, the defect is filled with blood clot. The osteogenic cells from surrounding bone tissue might migrate into the blood clot, then they would proliferate and make bone tissue.

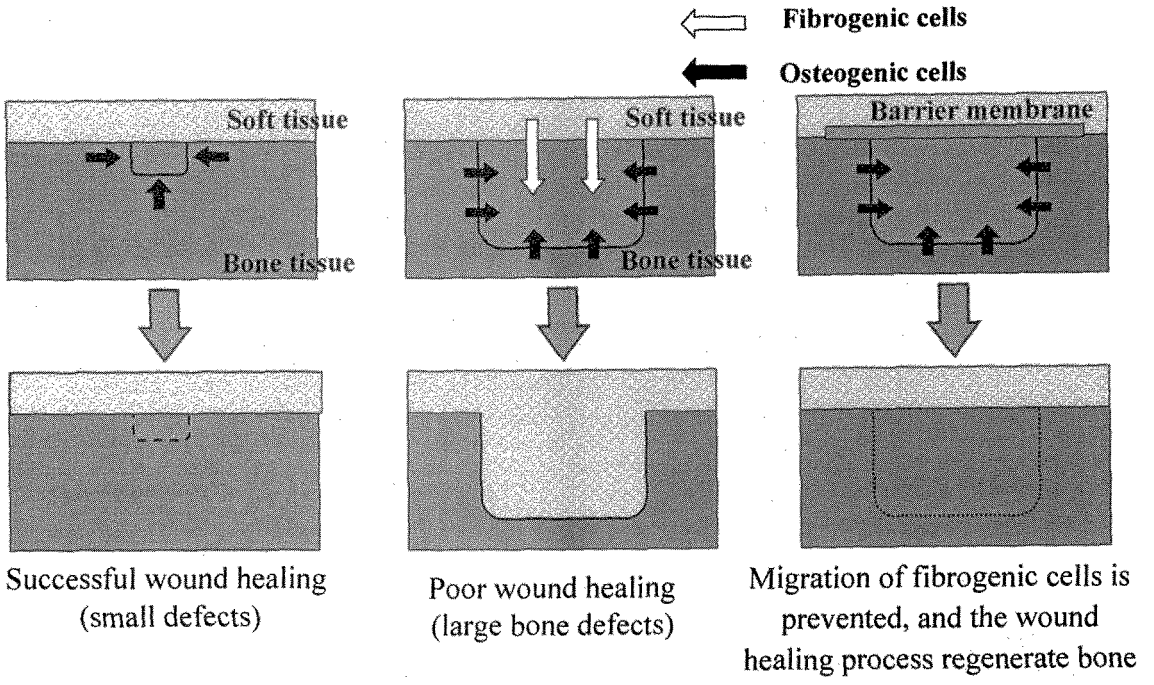

Fig.1 Use of membrane as a barrier

This is the case of successful wound healing process. But if the bone defect were large, fibrogenic cells would come from the covering soft tissues and invade into the blood clot filling the bone defect. If this invasion took place, and it always take place if the defect is large enough, the bone defect will be filled with scar tissue and bone regeneration would never take place.

The biomaterials may be successfully applied to prevent the invasion of fibrogenic cells and make desirable milieu for bone regeneration. If the barrier membrane were placed over the bone defect, the membrane would seal the blood clot filling the defect, and prevent the migration of fibrogenic cells. Then the wound healing function of the bone might regenerate bone tissues. Actually this strategy is clinically proven to be successful and known as the guided bone regeneration. Conventionally inabsorbable materials as PETF membranes are utilized as the barriers, so we are forced to do secondary operations in order to remove the membranes. Furthermore, large defects were not treated by these membranes. Hence we have to make use other materials to improve the technique.

2.2. Materials[1,2]

β-TCP/PLGC was designed to have ideal properties for the barrier membranes. Its matrix is copolymer of L-lactic acid, glycolic acid and ε-caprolactone (PLGC), and its filler is β-tricalcium phosphate (β-TCP). The molecular weight of PLGC is ca. 2.5×10^5 and its *Tg* is about 50℃. β-TCP was synthesized through a wet method and sintered at 800℃ for 3 hours. The β-TCP obtained was palletized to about 125 μm. PLGC was melted in a hot mill at 180℃, then β-TCP particles were added with the weight ratio of 60/40 and mixed for 10 minutes. The composite obtained was formed into membranes of 200 μm thickness under a hot-press at 180℃.

The membranes thus manufactured have desirable thermoplasticity to shape them in a hot bath according to the bone defects, and they become rigid at the body temperature. The membranes also have suitable hardness for manipulation but do not have brittleness. The membranes are bioabsorbable but they keep mechanical strength until 8 weeks after implantation. The speed of degradation was found to be depended on the implantation cites and believed to be resolved within the period not longer than a year. The biocompatibility of the materials was proved through culture tests and implantation tests in vivo.

2.3. Experiments

Animal experiments on bone regeneration in bone defects of canine mandibles were carried out. Experimental animals were five male Beagle dogs of 1 year old and weighting 9 to 12 kg. All premolars were extracted 3 months before the operations. Bone defects were made on both sides of alveolar bone of mandibles of each dog. The defects were of full thickness from the buccal to the lingual surface of the mandibular bone, 10 mm in length in mesial-distal direction and 10 mm in depth. The β-TCP/PLGC membrane was formed to fit the bone in a hot water bath, applied to cover the bone defect of the right side and the wound was closed with gingival suturing. The left defect was simply closed by suturing and used as the control. The bone formations within the bone defects were examined with X-rays in every 2 weeks. The retrieved tissues after sacrifices were examined histologicaly.

2.4. Results

In the cases of membranes were applied to the bone defects in the mandibular bones, significant bone tissues were regenerated in the defects. The bone regeneration took place from the walls of the defects around 4 weeks.

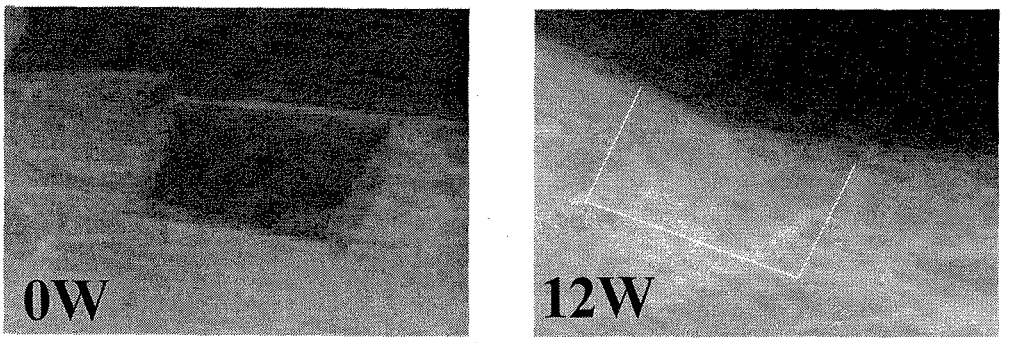

Fig.2 Bone defect in canine mandibular treated with the β-TCP/PLGC membrane

Although the bone seemed immature, the bone growth was so rapid that it reached as high as the neighboring cortical bone till 8 weeks. Then the density of the trabecular bone seemingly was ncreased around 12 weeks as Figure 2 shows. In the control defects where the membrane was not applied, only little bone generation was observed even at 12 weeks late. The surface bone of the defect remodeled to a concaved cortical bone, which suggested that the filling of the defect with bone tissue might require extremely long period if it were possible.

In conclusion, the novel β-TCP/PLGC membranes developed were found to significantly enhance the bone regeneration. They can be used to treat the bone defects as big as 10×10 mm in mandible, of which size of defects conventional membranes cannot be applied.

3. SPACE FILLERS

3.1. Strategy

The bone regeneration strategy making use of the barrier membrane is considerably effective. Nevertheless there are the cases the strategy does not go well. The barrier membranes prevent the invasion of fibrogenic cells and thus make the opportunity for the cells out of the adjacent bone tissues migrate into the defects. It implies that the tissues induced in the defects might be the bone tissues hopefully but there remains the possibility that the tissues differentiate into the cartilage tissues. In reality, we found that the segmental defects of 20mm length in the canine tibia treated with the membranes were turned into the very evidential examples of such phenomena. The calcified tissues spread out from both of the proximal and distal stamps, but the translucent layers were induced in the middle of the defects that were found to be the cartilage tissues. Although we believe the cartilage tissues there would be replaced with the bone tissues after remodeling under functional mechanical stimulations, rapid bridging with bone tissues between the stumps is more desirable in clinical situation. To realize this we have to make use of another strategy.

That is the use of bioabsorbable materials as space fillers. The strategy is illustrated in Figure 3. If the defect is filled with materials, and if the surface layers of the materials gradually degraded spontaneously or were resolved by in vivo surroundings, there would be

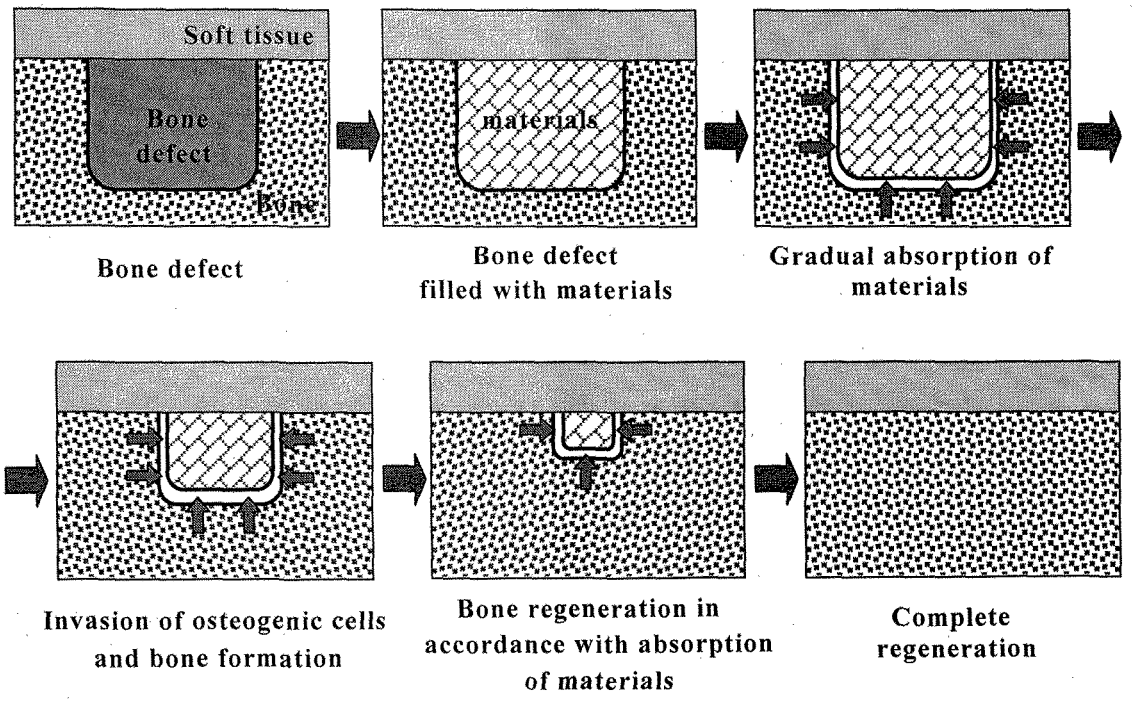

Fig. 3 Use of filling materials

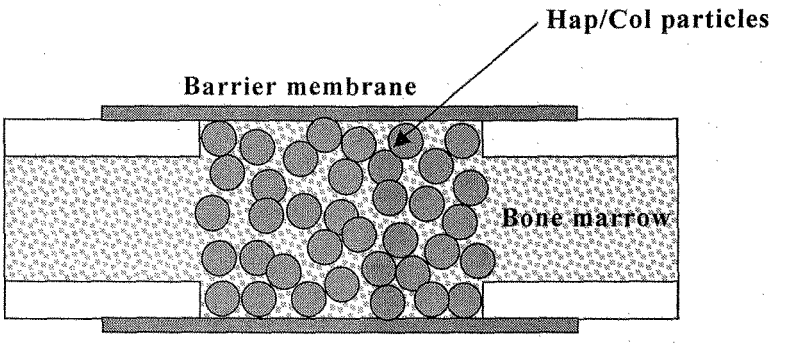

Fig. 4 Combined use of HAp/Col particles and β-TCP/PLGC membranes

generated the small space between the materials and the bone tissues. Since the space generated are small and might be occupied by the bone tissues. Next the surface layers of the materials would be removed again and the process would continue until the original defects were filled with the regenerated bone tissues.

Unfortunately, this strategy could no be realized since the no known materials could be made utilized for this purpose. For example, the bioabsorbable materials such as PLLA do not degrade from the surface layers but from the inner part of the materials. Furthermore, the strategy requires that the materials' degradation or the resorption takes place at the surface facing against the bone tissues but not at that against the fibrous tissues surrounding the bone. The materials for the new strategy should have very peculiar material properties such that the materials are removed only with the resorption by osteoclasts, the cells that bare the task of the bone resorption. We did need new materials but no materials fulfill the required condition, instead we used HAp/Col the new composite of hydroxiapatite and collagen that have virtually optimum properties. Unfortunately they do not have the ability to prevent the invasion of soft tissues, we used the β-TCP/PLGC membranes together. The combined usages of these materials enable us to realize the ideal bone regeneration devices.

3.2. Materials[3,4]

In the makings of HAp/Col, 199.1 mmol/L water dispersion of $Ca(OH)_2$ and 59.7 molar solution of H_3PO_4 containing 5g/L type I atelocollagen from swine skin was mixed in the reaction vessel at 40℃ and pH 9. The precipitate was filtrated and formed into a cylindrical shape with a uniaxial press, and consolidated under extremely high isostatic pressure of 200MPa for 15 hours. The HAp/Col blocks thus obtained were crushed and particles sizes of ca 2 mm were obtained.

HAp/Col is designed to have potent bioactivity as the materials that might be useful in the process of bone regeneration. We found that HAp/Col is osteoconductive and is resorbed by osteoclasts, the ideal manner of material resorption from the surface. We also found the significant enhancement of the bone formation by osteoblasts, through which process the materials are replaced by new bone tissues.

3.3. Experiments

We carried out animal experiments on bone defects in the canine tibia. Experimental animal was a male Beagle dog of 1 year old and weighting 10.5 kg. Right hind limb of the dog was set in Ilizarov external fixture. The segmental bone defects as long as 20 mm in length were made with a bone saw and the β-TCP/PLGC membrane was formed in a hot water bath, applied to the exposed bone and made to cover the bone defect. Then the HAp/Col particles were placed to fill the bone defects. The bone formations were examined with X-rays in every 2 weeks. The retrieved tissues after sacrifices were examined histologicaly.

3.4. Results

At 12 weeks after the operation, the experimental

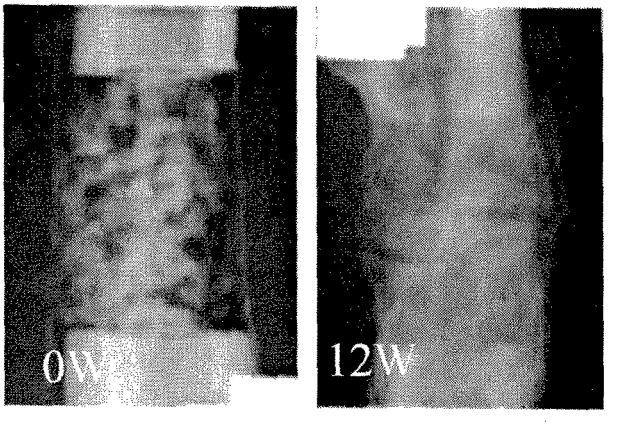

Fig.5 Bone defect in canine tibia treated with HAp/Col particles and β-TCP/PLGC membranes

animal could walk and run freely after removal of the ring external fixture. X-ray observation revealed as Figure 5 shows that bone tissues were regenerated within the 20 mm long bone defect and the calcified bone tissue had bridged between the proximal and the distal stamps of the defect. The histological observation showed that the calcified tissue was the bone tissue and it was in the process of remodeling under the weight bearing condition. On the other hand, the segmental defect which are not treated with the membranes are not bridged with bone or cartilage tissues but the defects were filled with fibrous tissues.

The fact that the animal treated with the biomaterials could walk without the fixation device is the evidence that the regenerated bone tissue had satisfactory strength. In conclusion, the treatment involving the combined application of the newly developed biomaterials β-TCP/CPLA membranes and HAp/Col particles is one of the most effective treatments for the bone defects as large as the segmental defects of 20mm length in canine tibia.

4. CONCLUSION

New bioabsorbable biomaterials were designed for the bone regeneration. They were β-TCP/PLGC membranes and HAp/Col particles, and were applied for the experimental bone defects. Although no biological enhancement techniques of tissue engineering such as cell transplantations or cytokine applications were utilized, the bone defects of critical size were successfully treated with the materials alone. These facts might prove that the treatment with biomaterials would be extremely effective and they would be used for bone regeneration, if the materials and the methods of usages were optimized.

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(Received November 30, 2003; Accepted February 29, 2004)