PROMOTED BONE HEALING WITH THE BONE GRAFTING MATERIALS BASED ON COLLAGEN-HYDROXYAPATITE/TRICALCIUM PHOSPHATE MICROSPHERES CONTAINING RECOMBINANT HUMAN TRANSFORMING GROWTH FACTOR- BETA 1

*INTRODUCTION* Congenital or acquired bone defect is a major problem in the orthopedic surgery. The use of autogenous and banked allogeneic bone grafts to induce the formation of bone in bone defects has become common (Urist et al., 1994). Autografts are preferably used because of their superior efficacy and capability of avoiding transmission of infection. However, it does have drawbacks including donor site morbidity and limited availability, particularly in children. The effectiveness of allogeneic bone graft is limited by the problems of high cost of bone banking, potential of graft-related disease transfer, the high rates of nonunion and infection, and allograft fractures. Despite the efficacy of these conventional methods, problems associated with their use have led to a search for alternatives.

The growth factors in the TGF beta superfamily are critical regulators of many cellular processes (Roissier et al. 1998). Produced by all skeletal tissues, TGF betas potently regulate cell proliferation, differentiation, and matrix synthesis. Using different models, several investigators have analyzed the stimulation of bone healing by TGF beta. Studies of animals have validated the efficacy and the safety of TGFs for bone repair. However, a satisfactory delivery system for rhTGF must be developed before it can be used in humans. In this study, we used the strategy of collagen-containing microspheres as a delivery system for rhTGF, which did not induce immune response of the host (Hsu et al. 1999). The purpose of this study was to examine the effect of collagen-hydroxyapatite/tricalcium phosphate microspheres (Col-HA/TCP) containing rhTGF-beta 1 to repair a critical-sized defect in the rabbit femur. A model for the defect at the distal femur was used. Bone formation and healing at the site of the defect were evaluated with use of the histological techniques.

**MATERIALS AND METHODS** Microspheres (Sized 200 – 300 μm) comprised of biphasic particulate hydroxyapatite/tricalcium phosphate dispersed in fibrous collagen matrices were prepared as described previously (Hsu et al. 1999). Twelve New Zealand white male rabbits were used in this study. The rabbits were anesthetized and then a bone defect was created by a 6 mm drill. These defects were then completely filled with the implant materials. The periosteum, fascia and skin were sutured layer by layer. After 5, 7, 9, 11, 13 and 15 weeks, the animals were killed with an overdose of intravenous pentobarbital. A total of 12 rabbits divided into six groups for the above experimental time periods was used in the study. The defect at both the left and right sides were implanted with Col-HA/TCP microspheres before the closure of the wound, while in the right side 10 μg recombinant human transforming growth factor- beta 1 (rhTGF-beta 1) was injected into the bone defect to mix with Col-HA/TCP microspheres. The hindlimbs were harvested from the treated animals at the mentioned time periods after operation. Implants and surrounding tissues were removed en bloc, fixed, decalcified, and embedded in paraffin. Sections were cut and stained with hematoxylin and eosin. Representative sections were photographed using light microscope.

**RESULTS** The formation of spotty new bone within the metaphyseal defect was observed in the group treated with Col-HA/TCP microspheres. Over the duration of the study, spotty new bone formation was observed with the fibrous tissue filled the most of the defects treated with zero micrograms of rhTGF. The large part at the center of the defect remained free of bone during the entire course of the test up to 15 weeks, and was filled with fibrous tissue and inflammatory cells infiltration. The implanted collagen fibers were still visible at this stage and showed evidence of degeneration. Marked fibrous tissue proliferation between the areas of the Col-HA/TCP microspheres with only scanty bony trabeculae formation were visible at 15 weeks after implantation; the chronic inflammatory cells infiltration was observed in addition to the large multinucleated giant cells proliferation. A dose of ten micrograms of rhTGF-beta1 produced numerous isotropically oriented trabeculae. Active new bone formation was evident in the group treated with Col-HA/TCP microspheres with rhTGF-beta1 addition. After 7 weeks after implantation, when bone regeneration was found in the defect cavity in which active bone marrow formation was observed. During 9 to 11 weeks after implantation, the breakdown and dissolution of Col-HA/TCP microspheres and bone marrow formation were observed in the histological sections. At 13 weeks after implantation, the breakdown and dissolution of the Col-HA/TCP microspheres was quite obvious. At the same time, the regenerated bone increased in size and the maturation of bone marrow was even more pronounced. At 15 weeks after implantation, the implant gradually dissolved and was replaced by the bony structure. The laminar bone appeared, the Col-HA/TCP microspheres continued to be dissolved, digested and replaced by the physiological bony marrow tissue which was filled with active bone marrow cells. It was apparent that bone regeneration was occurring in connection with, and influenced by, the rhTGF-beta1 as evidenced by the incorporation of Col-HA/TCP microspheres into the bone trabeculae.

**DISCUSSION** Several other experimental studies have validated the effectiveness of TGF for the stimulation of bone formation. Locally delivered TGF-beta1 is the most potent stimulator of bone ingrowth tested to date, exceeding the amount of bone formation obtained following autogenous cancellous bone grafting (Kienapfel et al. 1992) and many other tested agents (Kienapfel et al. 1999). The importance of the delivery system was noted in all of these studies.

Despite our efforts to optimize the polymer delivery system, multinucleated giant cells were a component of the wound-healing response. They appeared to be more multinucleated giant cells and lymphocytes in the defects filled with a Col-HA/TCP microspheres implant without rhTGF-beta than in the defects filled with an implant with rhTGF-beta. Despite the inflammatory reaction to Col-HA/TCP microspheres, the results of the present study show that rhTGF-beta1 delivered in Col-HA/TCP elicits bone formation in metaphyseal bone defects. There was clear morphological evidence that rhTGF-beta1 stimulated the formation of new bone. Furthermore, the rhTGF-beta1 and Col-HA/TCP microspheres were clinically convenient to use, biocompatible, and biodegradable, thus increasing the potential therapeutic value of this combination for the stimulation of new-bone formation in bone defects in a clinical setting. We conclude that TGF-beta1-containing collagen/hydroxyapatite/tricalcium phosphate microspheres show promise as an agent to promote bone regeneration of subcritical size bone defects.

**REFERENCES**


**ACKNOWLEDGMENTS**  
The authors sincerely thank the Industrial Technology Research Institute (ROC) for their financial support of this research.  
**[enter an institution], [enter city and state].

48th Annual Meeting of the Orthopaedic Research Society  
**Poster No: 0486**