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The effect on osteogenesis of type I collagen applied to experimental bone defects

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Abstract – The purpose of the present investigation was to evaluate the effects of type I collagen sponge on the healing of bone defects. In this study, six adult male rabbits were used. After the induction of general anesthesia with intraperitoneal kethamine, the anterior surfaces of tibias of the rabbits were surgically exposed, and two holes with 4 mm in diameter were prepared on each tibia for the investigation. Only one hole in each tibia was filled with type I collagen, the other unfilled hole was used as control. During the study, radiopacity changes in the radiographs of the tibias of the rabbits were evaluated. The animals were killed on the 28th day, and histologic sections of the tibias were prepared. On the 28th day, it was histopathologically observed that collagen cavities were filled with new bone. In addition, it was determined that there was an increase in radiopacity of the defect areas from 14 to 28 days in both groups, and there were statistically a significant difference between control and collagen groups ($P = 0.0001$). In this study, consequently, it was determined that type I collagen sponge in the experimental cavities provides a more rapid regeneration of bone defects compared with non-filled cavities.

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Key words: osseous defect; bone regeneration; type I collagen

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Collagen is the main structural protein component of connective tissue, and makes up the majority of the body proteins (1). In the previous studies; collagens had been shown to be effective stimulators of osteogenesis (2, 3). For that reason, various studies have been carried out to evaluate the effects of collagens on bone defects healing (4–14). However, it is not easy to determine, in terms of controlled clinical studies, the real effectiveness of a biomaterial, even if there are potentially several parameters of clinical evaluation. Thus, to determine whether these materials are effective in tissue regeneration (especially in bone wound healing), additional studies are always needed. The purpose of this investigation was to evaluate the effects of type I collagen on the healing of bone defects.

Material and method

In this study, six adult male New Zealand rabbits were used, each weighing from 1.5 to 2 kg. After the induction of general anesthesia with intraperitoneal kethamine HCL (20 mg kg^{-1}) (Pfizer, Istanbul, Turkey), the anterior surfaces of tibias of the rabbits were surgically exposed, and two holes with 4 mm in diameter were prepared on each tibia for the investigation. Only one hole in each tibia was filled with Conderss (Euroresearch, Milano, Italy), the other unfilled hole was used as control, and the incision region was sutured.

For radiographic investigation, the radiographs of the tibias of the rabbits were exposed together with a pure (99.90%) aluminum step-wedge with six 0.5 mm incremental steps from 0.5 to 3.0 mm.

soon after surgery (day 1) and on the 14th, 21st and 28th postoperative days. Nanodor 2 tip dental X-ray unit set (Siemens, Pittsburgh, PA, USA) was used for all exposures with a target-to-film distance of 82 cm and exposure time of 0.10 s at 50 kVp and 1 mA. The optical density of osseous defects was measured, using a digital densitometer (Densoquick 2 Model 1696 Digital Densitometer; Pehamed, Sulzbach, Germany). The densitometer was calibrated and zeroed according to the manufacturer's recommendation before each reading. Calibration was rechecked before the density of each new radiograph was measured. All readings were blindly conducted by the same investigator and were repeated three times in each measuring site and the average results were recorded. The optical density of the step-wedge in each radiograph was measured, and a graph representing density vs. millimeters of aluminum was plotted. The density of osseous defect was expressed in equivalent millimeters of aluminum. Student's *t*-test was used to analyze the data.

For the histopatologic investigation, the animals were killed on the 28th day. Immediately after death, their tibias were removed. The tibias of rabbits were placed in 10% formalin. After decalcification in 5% formic acid, all specimens were serially sectioned longitudinally at 5-μ intervals and stained with Masson's trichrome.

Results

Histopathologic results

On 28th day, in the defects of the control group, mononuclear cell infiltration, fibroblastic activity, heavy collagen development and mild osteoblastic activity and calcification were observed, whereas the defects filled with collagen was filled with new bone (Figs 1 and 2).

Radio-densitometric results

On the 14th day, it was observed that cavity borders almost disappeared, radiopacity increased and bone forming started in the collagen cavities, and on the 28th day, it was observed that the collagen cavities were filled with new bone which was in the same density as normal bone and which showed radiopacity while periosteal bone formation continuing. The density changes in control and collagen cavities are shown in Table 1. It was determined that there was an increase in radiopacity of the defect areas from 14 to 28 days in both groups, and there were statistically a significant difference between control and collagen groups ($P = 0.0001$) (Figs 3 and 4).

Fig. 1. At the 28th day, it is shown that control site has mononuclear cell infiltration, fibroblastic activity, heavy collagen development and mild osteoblastic activity and calcification (Massons' Trichrome, original magnification ×100).

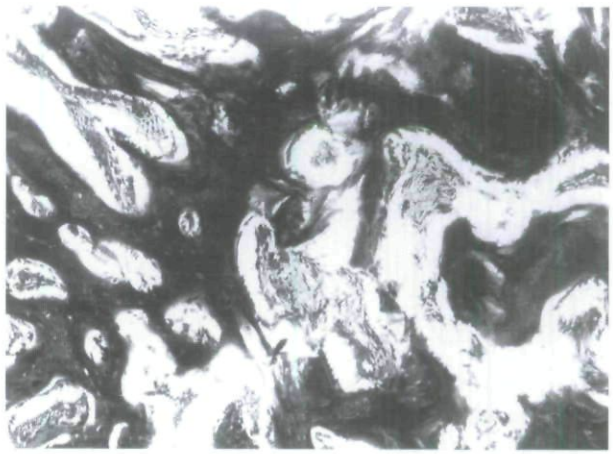

Fig. 2. At the 28th day, it is shown that collagen site is filled with new bone (Massons' Trichrome, original magnification ×100).

Table 1. The density changes in between control and collagen groups

Day	Collagen group (mean ± SD)	Collagen group (mean ± SD)	*t*-value	*P*
1	1.160 ± 0.10	1.303 ± 0.14	2.01	>0.05
14	0.836 ± 0.77	1.269 ± 0.54	11.29	0.0001
21	0.797 ± 0.63	1.038 ± 0.52	6.60	0.0001
28	0.774 ± 0.39	1.000 ± 0.71	6.71	0.0001

Discussion

In previous studies, it has been confirmed that collagen is a nontoxic and biocompatible material and is well tolerated by the body (3–7). In addition, it has been know that collagen applied to bone defect quickened the healing process and caused the

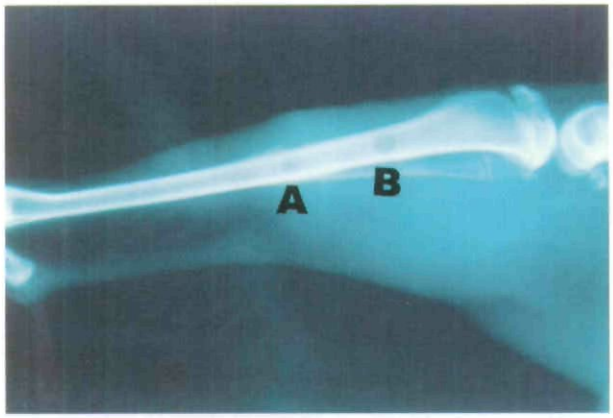

Fig. 3. At the 14th day, it is seen that cavity borders almost disappeared, radiopacity increased in collagen cavity (A: collagen; B: control).

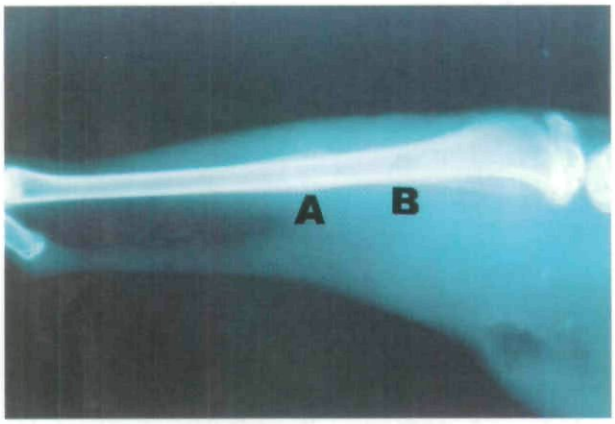

Fig. 4. At the 28th day, it is seen that the experimental cavities were filled with new bone that was in the same density as normal bone and which showed radiopacity while periosteal bone formation was continuing (A: collagen; B: control).

bone defects to be filled new bone tissue in a shorter time (3–5, 7–14). Therefore, collagens have been used in the treatment of various pathologic conditions, such as the closure of oral antral communications, alveolar augmentation, alveolitis, guided-tissue regeneration, maxillary cyst and tumors, apicoectomies, and healing of extraction wounds (4, 5, 8, 9).

However, bone formation is an important clinical consideration in oral and maxillofacial surgery. The early phase of bone defect healing as during which organization of the clot is completed can be followed clinically, whereas bone formation and remodeling can only be determined histologically, biochemically or radiologically (3, 9–14). Thus, radiography is the major non-surgical method for detecting bone formation in a healing osseous wound. Bone healing is radiologically expressed as an increase in radiopacity, resulting in a higher optical density of the bone image. To determine the process of a healing bone wound, the radiopacity changes between radiographs should be compared. In the present study, on the 14th day, it was established that cavity borders almost disappeared, radiopacity increased and bone forming started in the collagen cavities, and on the 28th day, it was determined that the collagen cavities were filled with new bone which was in the same density as normal bone and which showed radiopacity while periosteal bone formation continuing. In addition, there was statistically a significant difference from 14 to 28 days between control and collagen groups, and it was observed that experimental bone cavities were histopathologically filled with new bone in collagen group on the 28th day. As corresponding with these findings, Gungormus and Kaya (9), evaluating the effect of type I collagen on the healing of bone defects in an experimental animal model and in humans, shown that that heterologous type I collagen provides a more rapid regeneration of bone defects. DeVore et al. (12), in an experimental study in rabbits, demonstrated that collagen initiated or aided in the total replacement of a surgically created mandibular bone defect at the inferior border. In an animal study, Cobb et al. (13) and Mannai et al. (14) noted that collagen implants accelerated the osseous repair of the extraction socket.

In this study, consequently, it was determined that type I collagen sponge in the experimental cavities provides a more rapid regeneration of bone defects compared with non-filled cavities.

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