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Experimental study of new bionic bone in repairing goat skull defects

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ABSTRACT: Objective In order to verify the biological safety and the effect of inducing osteogenesis of two different proportions of collagen based biomimetic bone materials, the model of goat skull defect was designed. **Methods** First, we used trephine to make circular defect in goat skull, then the bionic bones with different proportions of collagen were implanted into the defect areas. Finally, the inflammatory response and osteogenesis in the bone defect areas were observed after implantation. **Results** Most of the A group materials with high collagen content were mostly degraded and partly induced into bone, while the B group with low collagen content was not degraded and had no effect on bone formation. After the two groups of materials were implanted into the goats, there were no inflammatory reactions, which meant that the collagen based biomimetic bone material had good biocompatibility. **Conclusions** The collagen based biomimetic bone material has good biocompatibility, good biodegradability, and a certain effect on bone formation.

KEYWORDS: Skull defect; Artificial bone; Induced osteogenesis; Degradation; Biocompatibility

0 Introduction

In neurosurgical patients, many types of cranial defects requiring bone graft repair occur, such as defects in various parts of the cranial cap and combined cranio-ocular defects[1–3]. Cranial defects are mostly secondary to lesions due to open cranial injury, surgical intracranial decompression and tumor resection, congenital malformations and progressive skeletal diseases[4–6]. Such diseases are increasing year by year, for example, the number of open craniocerebral injuries due to car accidents, natural or man-made disasters, etc. is about 40,000 per year, the number of deaths from various malignant tumors of the brain is up to 30,000 per year, and the number of patients with cerebral hemorrhage and cerebral infarction is increasing year by year[7–9]. Therefore, it is urgent to find a new material that can effectively solve the aftercare of patients with such diseases.

Although a variety of materials are clinically used for cranial repair, so far there is no ideal technique or material that can make the cranial defect morphologically, structurally and functionally complete[10]. At present, in cranial bone repair, the main clinical applications are metallic materials, polymer materials and artificial bone materials, but all these materials have certain problems in the clinical application of cranial bone repair. metallic materials[11] cannot be insulated after their implantation due to their good thermal and electrical conductivity, resulting in poor postoperative body sensation in patients However, because of their physiological inertness, they will exist in the patient's body as a long-term foreign body after implantation, and their composition is different

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from that of human tissue, so there may be risks of rejection and tissue encapsulation; Most of the artificial bone materials now used in neurosurgery clinics are inorganic salts with high mechanical strength, but degradation is too fast to achieve long-term shaping[13]; while the mechanical strength of artificial bone materials synthesized from organic and inorganic components cannot meet the demand of cranial bone repair.

With the in-depth study of bone tissue development and metabolism, the function and role of collagen in bone growth and development have gradually become clear. Collagen is the most important structural protein in spinal animals and accounts for about 1/3 of the total protein in human body, mainly existing within the intercellular space, with both common and different points in structure. Its main role in the human body is to act as a tissue support, giving tissue tension. At the same time, the molecules and fibers of collagen play an important role in the growth and development of the organism, as well as in the differentiation, adhesion and movement of cells[14]. Collagen has good biocompatibility and low immunogenicity and is completely degradable in living organisms; therefore, in recent years, collagen is being used as a novel biomaterial in various clinical applications such as soft tissue repair, trauma hemostasis, cell growth support, nerve regeneration support, wound repair, corneal scaffold, etc.[15]. As early as the 1960s, collagen was used as a bone repair material, but the mechanical strength required for bone repair could not be achieved after simple collagen implantation, and a single collagen would degrade rapidly after implantation, which could not achieve the expected bone repair effect. In contrast, a large number of basic and clinical studies have confirmed that HA does not resorb after implantation into the human body, has good biocompatibility and bone guidance, and can form an ideal osseous bond with the host bone, and clinical applications have achieved excellent therapeutic results. However, the lack of adhesion between hydroxyapatite particles, which can easily disperse and move in vivo, and the inability to penetrate the spatial structure, also have major limitations.

In the study of the biomineralization process of bone scabs and embryonic bone, it was found that the basic components of natural bone are hydroxyapatite and type I collagen fibers, and the content of collagen fibers is about 35%. It was also found that the secretion of self-assembled inorganic substances by bone cells can induce the deposition of inorganic substances in a certain direction to form a certain shape, size and structure, which means that type I collagen fibers can induce the orderly assembly of hydroxyapatite crystals in a certain environment to form a bionic bone material with a certain structure. The internal structure of HA-collagen self-assembled material was observed by X-ray and scanning electron microscope, and it was found that the self-assembled material could reach the strength of human cortical bone; and a large number of animal experiments showed that HA-collagen artificial bone material has good bio-destructive properties, and can induce osteoblasts to adhere to the material and gradually fuse with the autologous bone. The HA-collagen bionic bone material used in this study was self-assembled by dissolving type I collagen fiber powder extracted from bovine Achilles tendon, giving certain reaction conditions to simulate the biological fluid environment, and adding nano-scale hydroxyapatite crystals. In addition to having most of the characteristics of HA-collagen artificial bone material, it can be processed into any shape by mold freeze-drying process, as well as obtaining artificial bone material with different strengths by adjusting the ratio of inorganic and organic materials. The material used in this study is self-developed collagen-based bionic bone material, which uses macromolecular collagen to construct the spatial structure and uses self-assembled mineralization technology to form hydroxyapatite crystals on the collagen surface, and the ratio of collagen and hydroxyapatite can be adjusted to obtain different mechanical strength and biological properties according to clinical needs, and at the same time, it can be synthesized according to the CT image data of the skull defect area design, with high potential for clinical application. How-

ever, its biological safety and osteogenic ability need further experimental validation.

The most direct way to verify the effect of induced osteogenesis and the biocompatibility of artificial bone materials is to conduct experimental animal studies by establishing an animal experimental model and then simulating the manner and means of clinical use. The practical application of artificial bone materials can be effectively determined by various immunological, imaging, and histological tests.

To this end, in this study, in order to verify the properties related to two independently developed collagen-based bionic bone materials with different ratios, we used the material to repair the defect area by establishing a goat skull defect model, and used gross observation, histological observation, and imaging observation to investigate the induced osteogenic effect, the The material was used for the repair of the defect area. In order to determine whether the material has certain clinical value and significance, and which ratio of the material is more suitable for skull repair.

1 Materials and methods

1.1 Materials and experimental animals

The artificial bone material was derived from the pre-produced bionic artificial bone repair material, and the main components were hydroxyapatite and collagen. Considering the collagen ratio of normal bone[16–17], the collagen content of group A was selected to be 45%; in order to study the osteoinduction effect of different collagen contents, the collagen content of group B was selected to be about 25%[18–19], and the collagen content of group A was greater than that of group B. Titanium was ordered from conventional manufacturers.

Six adult healthy goats, 1 year old, about 15 kg, were selected from the Yantai Animal Experiment Center. three in group A and three in group B. A 10 mm diameter defect area was created in the goat skull using a ring drill, and the artificial bone material was embedded in the skull defect area, as shown in **Figure 1**.

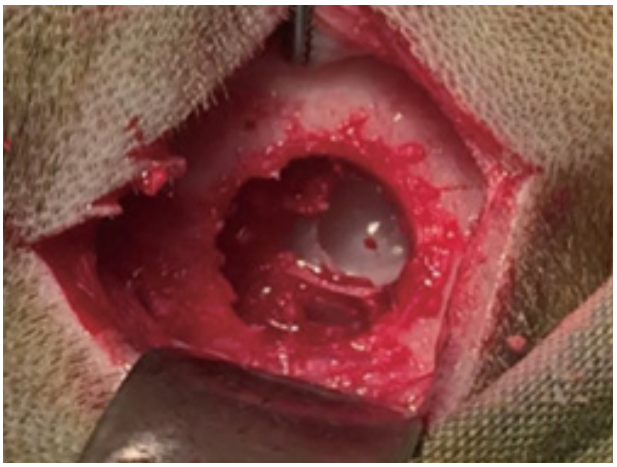


Figure 1 Prepared skull defect model

1.2 Methods

1.2.1 Preparation of skull defect model

The experimental goats were fasted for 12 h. Penicillin 800,000 units was given 30 min before surgery to prevent infection. Preoperatively, anesthesia was induced by intramuscular injection of 0.1 mL/kg of Sulforaphane II, and cardiac monitoring and tracheal intubation were placed. Intraoperatively, the depth of anesthesia was maintained with 10% chloral hydrate intravenously according to the response of the goats.

A longitudinal incision of 3 cm in length was made on the top of both sides of the goat, and the scalp and periosteum were incised to reveal the parietal bone. A circular bone defect of 10 mm in diameter was drilled with a 10 mm diameter ring drill, the outer package of the artificial bone material was opened, the inner sterile package was removed, the inner package was opened, and the artificial bone material was removed with sterile forceps, and the artificial bone material with high collagen content was implanted into the skull defect area of group A goats, and the artificial bone material with low collagen content was implanted into the skull defect area of group B goats. The dura mater was protected during osteotomy, and care was taken not to damage the superior sagittal sinus.

After the operation, the goats were given intramuscular injection of Sulforaphane 0.2 mL/kg, and the tracheal tube was removed after the

goats woke up; they were given intramuscular injection of penicillin 800,000 units per day for 5 d. The goats were routinely kept in captivity, and the males and females were separated into pens and fed with concentrate.

1.2.2 Observation indexes and methods

(1) General condition:Postoperative observation of goat mental status, feeding, activity and incision healing, etc. The postoperative mobility and behavior of the experimental animals can visually reflect the physiological state of the experimental animals, and the impact of surgery and implant materials on the experimental animals can be well judged by real-time feedback from experienced animal keepers on the behaviors of the experimental animals such as eating; in addition, the impact of surgery and implant materials on the experimental animals can also be judged by observing whether there is redness and swelling of the surgical wound, pus oozing, etc. The impact of surgery and implant materials on animals can also be judged.

(2) Imaging observation (CT examination): Computed tomography (CT) can accurately detect small differences in density between various different tissues in a cross-sectional anatomical plane, which is a more ideal way to observe bone tissue as well as soft tissue lesions. In clinical practice, it is often used in the diagnosis of arthritis and soft tissues. Due to its high resolution, various soft tissues, bone and bone joints can be clearly visualized. And CT processing can do axial imaging in addition to higher density resolution than conventional X-ray examination, which greatly facilitates the observation of the cranial region of goats. In this study, we used CT to carry out imaging observation, and in addition to effectively avoiding the obscuration of the defect area by the goat horn and skull, we could also observe the changes of the defect area more accurately. Since the occlusion of the skull and goat horns interfered with the X-ray observation, only CT scans were given for postoperative observation.

At 4, 12 and 24 weeks postoperatively, respectively, cranial CT was performed under anesthesia of 0.1 mL/kg of intramuscularly injected tamsulosin II, with uniform conditions of 120 kV, 150 mA of current, layer thickness of 0.5 mm, and preservation in DICOM format. The cranial defect repair and the absorption and degradation of the implanted materials were observed.

(3) Gross specimen observation: At 12 weeks after surgery, one goat was executed and the material was taken from each of groups A and B. The surgical site was observed for material discharge, local infection and healing of the cranial defect, and the combination of the material with the host bone interface.

(4) Histological observation:At 24 weeks postoperatively, 2 goats in each of groups A and B were executed and sampled, and the cranial specimens and the surrounding 5 mm of normal cranial bone were removed. The specimens were fixed with 10 times the volume of 10% neutral formalin for 1 week. The specimens were sectioned in hard tissue and stained with conventional HE, Masson stain and toluidine blue (TB). The bone graft area was observed under light microscopy for inflammatory cell infiltration, osteogenesis, and material degradation to evaluate its local osteogenic activity and ability to repair bone defects.

2 Results

2.1 General Information

The goats were awakened within 60 min after surgery, and they could stand and move freely on the day after surgery, and they ate basically normally. The incision was free of infection, healed well, and no material came out, and the incision sutures fell off by themselves. There was no death of the goats.

2.2 Imaging observation

Group A: At 4 weeks after surgery, the edges of the defect were clear, the implant material was completely developed, and no new bone was formed around it, as shown in **Figure 3** (a). At 12 weeks after surgery, the edges of the defect were gradually blurred, most of the implant material was developed, and a small amount of new bone was formed around the defect area on the medial

side of the skull, as shown in **Figure 3** (b).

At 24 weeks after surgery, the edges of the defect were blurred, the material covering the defect area was significantly reduced, and there was degradation and absorption of the material, but the general morphology was still recognizable, as shown in **Figure 3** (c).

Group B: At 4 weeks postoperatively, the edges of the defect were clear, the implant material was completely developed, and no new bone was formed in the periphery, as shown in **Figure 4** (a). At 12 weeks after surgery, the edges of the defect were clear, the implant material was completely developed, and there was also a small amount of

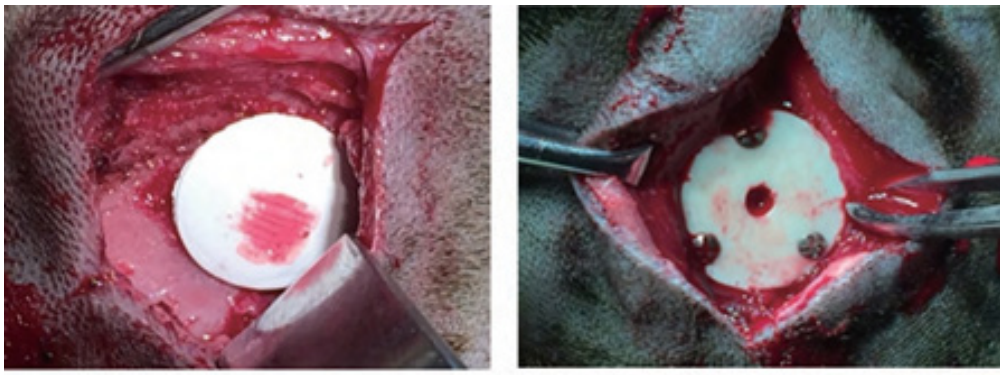


Figure 2 Implantation with artificial bones

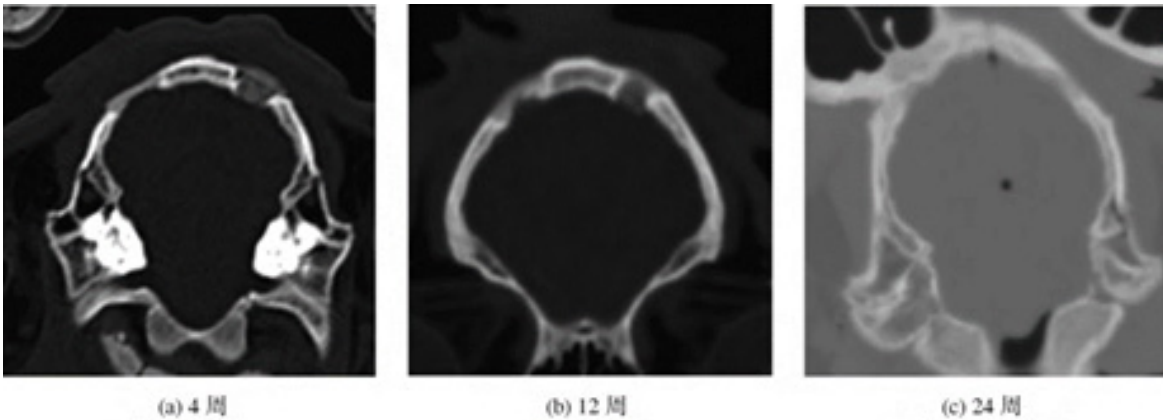


Figure 3 CT results of A group

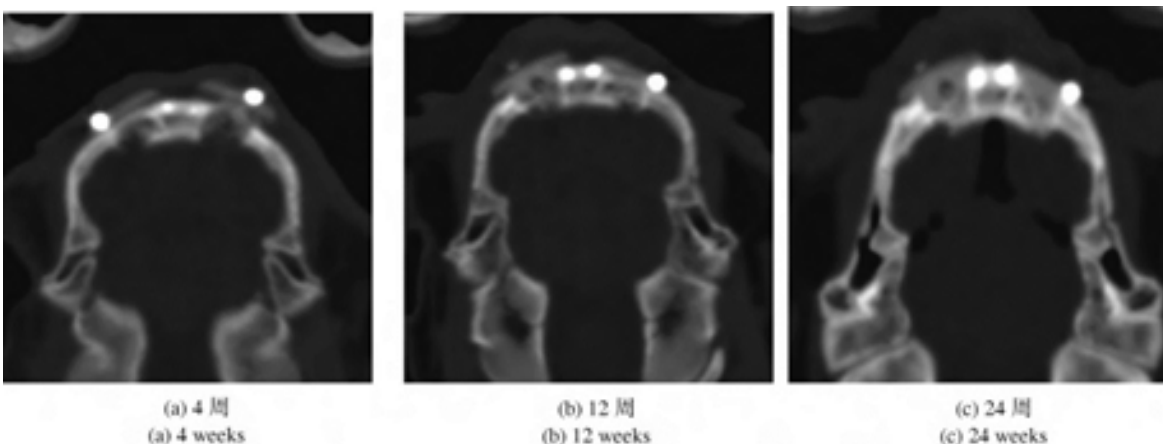


Figure 4 CT results of B group

new bone formation on the inner side of the skull and the edges of the defect area, but the amount of bone formation was significantly lower than that in group A, as shown in **Figure 4** (b). At 24 weeks postoperatively, there was no significant change in the material from before, as shown in **Figure 4**(c).

As shown by the CT results, the biodegradation rate of group A with high collagen content was higher than that of group B with high hydroxyapatite content, and the effect of induced osteogenesis was higher than that of group B.

2.3 Gross specimen observation

In the 12-week cut specimen, the scalp soft tissue of each group of goats showed no abnormality after incision; in group A, there was mild resorption on the surface and periphery of the material, and the general shape of the material was clearly discernible; the material on the inner side of the skull had fused with the surrounding bone tissue and could not be removed completely; there was new bone formation on the inner side, and the bone defect area had not been completely repaired, as shown in **Figure 5**; in group B, there was no resorption on the front side of the material, and the shape of the material was clear after removal of the titanium nail. The material can be removed completely after removing the titanium nail, and the interface between the material and the host bone is clear; on the reverse side, there is no new bone formation on the medial side, and the bone defect area has not yet been repaired, as shown in **Figure 6**.

In 24 weeks, the original surgical incision was found to leave only a linear scar, and there was no abnormality in the soft tissue of the scalp of each group of goats after incision. In the frontal view, there was no resorption of the material, the morphology was the same as before surgery, the material could be removed completely after removing the titanium nail, and the interface be-

Figure 5 Skull defects of A group(12 weeks)

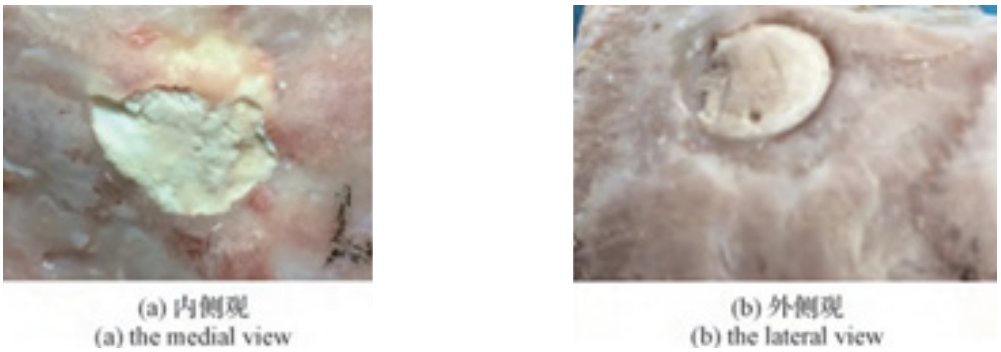


Figure 6 Skull defects of B group(12 weeks)

(a) 内侧观
(a) the medial view

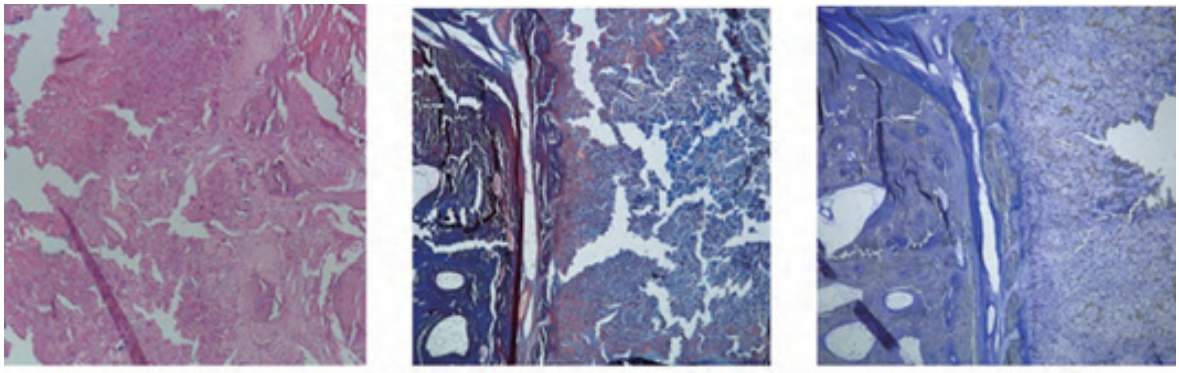
(b) 外侧观
(b) the lateral view

Figure 7 Skull defects of A group(24 weeks)

(a) 内侧观
(a) the medial view

(b) 外侧观
(b) the lateral view

Figure 8 Skull defects of B group(24 weeks)



(a) HE 染色
(a) HE staining

(b) Masson 染色
(b) Masson staining

(c) TB 染色
(c) TB staining

Figure 9 Staining results of A group

tween the material and the host bone was clear; in the reverse view, there was new bone formation on the medial side, and the bone defect area was not yet repaired, as shown in **Figure 8**.

The gross observations were basically consistent with the CT observations, but the gross observations also revealed that the induced osteogenesis in group A was better than that in group B, which might be related to the ratio of collagen and hydroxyapatite.

2.4 Histological observation

In order to better reflect the histological changes in the defect area, three staining methods frequently used in current bone tissue studies, namely HE staining, Masson staining and TB staining, were chosen for histological observation in this paper.

The material was taken at 24 weeks postoperatively, and the specimens were stained with hard tissue sections, HE staining, Masson staining and TB staining, and observed under light microscopy, and no inflammatory reaction of the surrounding soft tissues was found in all specimens, as shown in **Figure 9** and **Figure 10**. From the staining results, it can be seen that most of the material was degraded and absorbed, and there was some new bone formation in the skull defect area.

3 Discussion

3.1 General information and general observation of specimens

After surgery, the goat did not die, did not show any disease, all behavioral abilities were normal, and the wound did not show any redness, pus, or tissue fluid exudation, which can initially indicate good surgical results, good aseptic treatment of the implant material, and good biocompatibility of the implant material. The animal specimen, as the most intuitive way of display, is more important in such defective area repair experiments. After removing the specimen, the difference between the defective area and the surrounding tissues can be observed, and the state of the defective area can be seen very clearly. After execution of the goat, the skull containing the defective area was removed, and the changes in the defective area could be observed very clearly and the differences between the groups could be visualized. Based on the general observation of the specimens, it can be seen that group A with high collagen content induces osteogenesis better than group B with low collagen content, and the degree of degradation is higher in group A than in group B. This is similar to the findings of Katthagen et al[20], who conducted a quantitative study on the osteogenic effect of HA-collagen after implantation in animals, and experimentally demonstrated that after implantation of the material, osteogenesis was fast, and also found that after implantation of HA-collagen, new bone was formed mainly around hydroxyapatite, thus concluding that HA-collagen hydroxyapatite particles can act as a scaffold during the osteogenesis process, and collagen fibers can promote the growth of granulation tissue and induce the growth of osteoblasts into it. Therefore, the combination of collagen and hydroxyapatite must be in a reasonable ratio to achieve the best osteogenic effect. Too much collagen may cause the material to degrade too quickly in vivo to match the rate of osteogenesis, while too much hydroxyapatite may lead to poor osteogenic effect and slow degradation in vivo. However, the specific ratios have to be adjusted according to the different clinical needs.

3.2 Imaging observation

The trends of changes in the cranial defect area at 4, 12 and 24 weeks were effectively demonstrated by CT observation. The gradual replacement by new bone tissue after implantation by group A and HA-collagen artificial bone materials initially proved that group A materials had good osteoinductive effect, while no significant changes were observed in the defect area of group B, which also reflected that the osteoinductive effect of

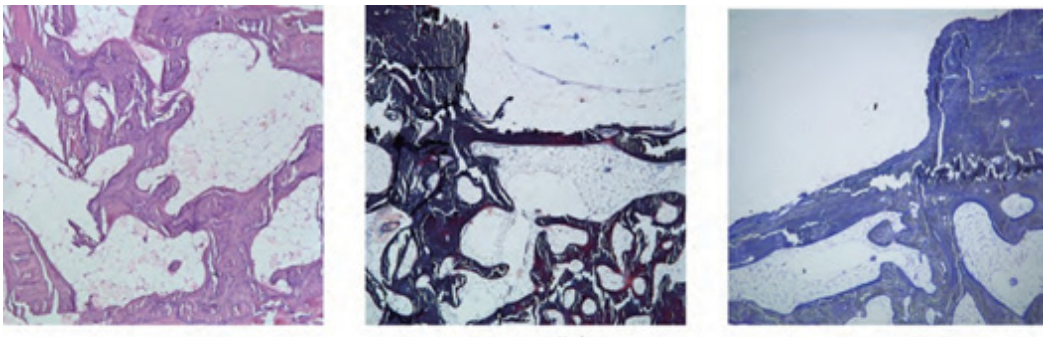


Figure 10 Staining results of B group

group B materials was average or no osteoinductive effect. From this, it can be tentatively judged that the biodegradation rate of artificial bone materials with high collagen content is higher than that of artificial bone materials with low collagen content, while the high content of hydroxyapatite may make the material less osteoinductive and less biodegradable. These are similar to the results of previous studies, Shen et al[21] found in animal experiments that fibroblasts and macrophages, and occasionally multinucleated giant cells, were visible in the defect area after HA and collagen composite implantation, and a small amount of collagen was visible in the implantation area at 4 weeks, and collagen was completely resorbed at 8 ~ 16 weeks. in a study by Bell et al[22], collagen alone was partially resorbed at 4 weeks after implantation and was completely replaced by In a study by Bell et al[22], collagen alone was partially absorbed at 4 weeks and completely replaced by fibrous connective tissue at 12 weeks.

The preliminary judgment can be made through imaging observation that the self-assembled artificial bone materials of hydroxyapatite and type I collagen studied in the preliminary stage can repair cranial defects to a certain extent, and different ratios of raw materials have different osteogenic effects of induction, and the appropriate ratios can be adjusted later according to clinical needs.

3.3 Histological observation

In this study, through the combination of 3 staining methods, each taking the advantages of the other, the advantages complement each other. It can be seen by HE staining and toluidine blue staining that in all the grouped tissues, there was no inflammatory cell infiltration at the edges of the defect area, the defect area, the new bone tissue, and the interior of the material, indicating that the material This indicates that the material has good biocompatibility and did not cause immune rejection in goats; and by observing the growth of osteoblasts and calcium salt deposition inside the tissues of group A and group B, it indicates that these two groups of materials have the function of partially inducing the growth of osteoblasts, while no growth of osteoblasts and other osteogenic features were found around the material and inside the material of group C, which indicates that the material of this group is weaker than the other two groups in inducing osteogenesis. The results of Masson staining showed that the collagen in groups A and B showed different degrees of degradation and was gradually replaced by autologous bone tissue, indicating that these two groups of materials have good biodegradability and will be partially degraded within a certain period of time until complete degradation after implantation, while group C has a low collagen content and the hydroxyl It can be speculated that the materials of this ratio may remain in the body for a long time after implantation and cannot be degraded or require a long degradation process.

A large number of clinical studies have shown that the traditional bone tissue repair materials currently used, after implantation into the human body, are not well matched with the speed of new bone formation due to the large differences in composition, structure and composition of the bone tissue, and cannot achieve "biological fusion "This will inevitably lead to loosening, detachment, aging and corrosion of the implant material in the host body, which will affect the effect of bone repair. In this study, we found that the HA-collagen artificial bone material gradually degraded, and the area was gradually replaced by new bone tissue while the material degraded, which indicates that the degradation rate of the material has a certain match with the rate of cranial bone regeneration, and if a suitable ratio of inorganic and organic materials can be found, through a reasonable experimental design, it should be possible to formulate an artificial bone with a degradation rate matching the rate of cranial bone regeneration. If we can find a suitable ratio of inorganic and organic materials, we should be able to formulate an artificial bone material that matches the rate of degradation and the rate of cranial regeneration through a reasonable experimental design.

4 Conclusion

In this paper, we found that two different ratios of collagen-based bionic bone materials were used to repair cranial defects in goats, and both ratios of collagen-based bionic bone materials had good biocompatibility. The biodegradability of the artificial bone material with high collagen content is also higher than that of the artificial bone material with low collagen content. In view of the special characteristics of poor cranial bone regeneration ability, it will be of great clinical and social significance if this study is coupled with cell engineering and the introduction of stem cell technology to design new artificial bone materials more suitable for the regeneration of cranial bone defects.

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