Pathological Analysis on Tissue Reactions to Absorbable Monofilament Suture -Using GFP Bone Marrow Transplanted Rat Model

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Abstract

In the examination, using the experimental rats of GFP (Green florescence Protein) bone marrow transplanted model, we embedded an absorbable monofilament suture Monocryl®, into the subcutaneous tissue and examined the related forming foreign body granuloma tissues. It was also examined the main cellular components, namely as macrophages. Histopathologically, the embedded the Monocryl suture thread was seen as a circular in void surrounded by the proliferation of macrophages. However, there were almost no multinucleated foreign body giant cells. Fibrous connective tissue was interposed among large masses. Even after 6 months, some clusters considered to be residues of macrophages that grew on the embedded suture thread, were still remained. Immunohistochemical experiment of GFP indicated that almost all proliferating macrophages were GFP-positive. However, the relatively thin fibrous tissues formed on the outermost layer of the granulation tissue growth were mostly GFP-negative. The results suggest that numerous macrophages were derived from the mesenchymal tissue of transplanted bone marrow tissues.

Keywords: Absorbable monofilament suture, Monocryl®, Foreign body granuloma, Macrophage, Bone marrow derived cells, GFP bone marrow transplanted rat model

Introduction

There are many examination results clinically used absorbable suture threads, such as poly glycolic acid/lactic acid polyester (polyglactin): Vicryl® and Vicryl rapide®, and so on [1-3]. These absorbable suture threads are commonly used in various parts of inside the body in the medical and dental fields. Regarding the oral regions in dentistry, these absorbable suture threads are clinically often used. However, there are physico-chemical examinations data are numerous published, there are some in histopathological fate of the absorbable suture threads and the elicited tissue reaction data are almost no data published. Foreign body reaction is expected to naturally occur during the dissolution process due to the breakdown and absorption of the suture. Therefore, we examined the histopathological response of rat subcutaneous tissue after Vicryl® absorbable suture was implanted until 6 months and reported the results [4-6]. Furthermore, using GFP rat bone marrow transplanted model, we examined and the results showed that the foreign body granuloma including some giant cells are supplied from then bone marrow derived cells [7,8].

Monocryl® is one of the famous absorbable suture threads and was also clinically used in the medical and dental areas. The related examination data was published [8]. However, there were few pathological analytic data in the literature. In this examination, we examined the absorbable suture thread Monocryl®, which is also used in the dorsal subcutaneous tissues using the same surgical procedures [4-7].

Materials and Methods

GFP bone marrow transplanted rats were used as experimental animals. The procedures are nearly the same as our former examination [4-7]. That is as followings: five-week old female SD rats (Charles River Laboratories Japan, Inc., Yokohama, Japan) and 6-week old female GFP transgenic rat SD-Tg (CAG-EGFP) (Shimizu Laboratories Supplies Co., Kyoto, Japan) were used to prepare the GFP bone marrow transplanted rats. Bone marrow cells from GFP transgenic rats were collected, and washed with RPMI 1640 medium containing antibiotics and then replaced with HBBS. Five-week old female rats syngeneic to GFP rats were irradiated with 10 Gray of X-rays and transplanted with 1×10⁷ bone marrow cells or more from the tail vein. Grafted rats

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with transplanted bone marrow at 4 weeks after bone marrow transplantation were used in this examination.

Using these rats, an ultra-pliable absorbable monofilament suture called Monocryl® (#4-0; Johnson and Johnson Japan Co., Tokyo, Japan) was bundled and implanted in the dorsal subcutaneous tissue. That is, under general anesthesia using pentobarbital injection, the back of the rats was shaved, incised and the suture Monocryl® was implanted in the subcutaneous tissue. After 2 weeks, 1 month, 3 months and 6 months, the embedding site-tissues were removed as one block, and 10% neutral buffered formalin fixed paraffin-embedded sections were made and examined histopathologically.

For immunohistochemistry, the specimens were pretreated using citrate buffer (citrate buffer pH 6.0, LSI Medience Co., Tokyo, Japan) in an autoclave at 121°C for 10 min. This was followed by blocking with Protein Block Serum-free (Dako Japan Co., Tokyo, Japan) for 30 min at room temperature. Anti-GFP antibody (ab290: Abcam, Cambridge, UK; 1/2000) was used as the primary antibody and the reaction was carried out at 4°C overnight. After secondary reaction with anti-rabbit immunoglobulin antibody for 30 min, the slides were washed with PBS and then developed with DAB. Hematoxylin was used for nuclear staining.

This present examination was planned according to the Matsumoto Dental University Animal Experiment Committee and Okayama University Animal Experiment Guidelines and was conducted under the review and approval of both Animal Experimental Committees.

Results

Histopathological Examination

Two-week-specimen showed the suture Monocryl® was observed as elongated, circular and/or oval shape vacuoles, according to its direction. A lot of granulation tissue cells were proliferated around the vacuoles showing Monocryl® (Figure 1a). In these granulation tissues, macrophages (Mφ) were evident. However, very few multinucleated giant cells were observed (Figure 1b). One-month-specimen revealed that the histopathological features were also the same of two-week-specimens. The thin fibrous tissues were formed on the outermost layer of the granulation tissue. Fibrous connective tissue including fibroblasts was interposed around the spaces showing Monocryl®. One-month-specimen showed the circular/oval vacuoles left by the suture decreased in size (Figure 1c). Mφ filled the space for the most of parts. That is, Mφ almost slightly disappeared forming clusters of residues. However, they were fairly reduced number of nuclei decreased compared to those seen at one to tree month. Six-month-specimens showed the numerous clusters of residual Mφ were still observed (Figure 1d). That is observed as formy cells.

Immunohistochemical Examination

Two-week-specimens showed that many macrophages (Mφ) were positively reacted by GFP (Figure 2a). Furthermore, the Mφ and related proliferating cells were almost all GFP-positive (Figure 2a). In one-month-specimens, the voids became smaller. The proliferative tissue was filled with Mφ which was GFP-positive (Figure 2b,c). The stain of the cell membrane and cytoplasm in Mφ was more intense compared to two weeks. However, the intracellular substances resulting from phagocytosis lightly reacted to GFP compared to two weeks (Figure 2c). Three-month-specimens showed the residual Mφ that formed clusters were still observed. The cell membrane was densely positive to GFP and the cytoplasm was lightly stained with hematoxylin. Some of the fibroblasts in the fibrous tissues surrounding the clusters of residual Mφ and the around tissues were sometimes positive to GFP. However there are very small amount of Mφ. Six-month-specimens showed the clusters of residual very small amount of
Mφ that proliferate around the Monocryl was GFP-positive. The cell membrane was stained with GFP and the cytoplasm was stained with slightly hematoxylin in the periphery of the cytoplasm (Figure 2d).

**Discussion**

Variety types of absorbable suture thread are widely utilized in the medical field, and also used in the dental fields during surgical procedures [7]. Furthermore, there are a number of literatures regarding the bioabsorbable medical materials used inside the body; such as PLLA, PGA, PLA and PLGA, and so on [9-12]. In the literatures there were much results of physiological and chemical examinations were mentioned and discussed. However, there were very little descriptions about the tissue reactions in the literatures [9]. Regarding description of the tissue reaction to the absorbable materials Ji W, et al. [12] reported the histopathological features and immunohistochemical result of CD68 and histochemical results of TRAP. According to the manuscript, the almost proliferating cells were determined as macrophages with small amount of giant cells meaning foreign body giant cells, and TRAP positive cells were present near the scaffolds. However, the all photographs were very small for determination of the cell morphology.

Although it can be assumed that a foreign body reaction occurs during the process of disintegration or absorption of the suture, details on the mechanism have not been cleared. Thus, we conducted an experiment on the histopathological examination of polyglycolic acid/lactic acid polyester (polyglactin) absorbable suture threads Vicryl® and have published the histopathological features (HE) with some histochemical (Azan staining) and immunohistochemical results of CD68 [4].

Our research group has some examination results the cytological movement from the bone marrow derived cells using GFP bone marrow transplanted model animals, especially using mouse [13-19].

Regarding the published data, the Monocryl was monofilament sutures based on segmented block copolymers of ε-caprolactone and glycolide. Monocryl® provide an in vivo breaking strength retention of about 20-30% after 2 weeks. Absorption is complete between 3 to 4 months after inside the body; such as PLLA, PGA, PLA and PLGA, and so on [9-12]. Thus, we plan to examine the Monocryl® using GFP transplanted rat model. In this time, regarding the present examination, we have focused the suture thread Monocryl®. Histopathologically, Monocryl was observed as a void surrounded by Mφ and very few multinucleated giant cells. Peripheral fibrous tissues were observed interspersed among the granulation tissues. Six months after implantation, some clusters of residual Mφ that showing mixomatous structure could be observed. Mononuclear cells were also reported to be positive to CD68 in immunohistochemical study [4]. Similar histopathological findings were obtained in this study meaning the proliferating cells are Mφ.

GFP bone marrow transplantation rat model was used due to our assumption that the cells that proliferated around the embedded suture Monocryl® were supplied by the bone marrow derived cells, as same as Vicryl® [6]. As mentioned our literatures [6,13-19], in GFP transplanted rat model, all the cells migrated from bone marrow derived ones constituting the tissue expressed the GFP protein and even if the transplanted bone marrow-derived cells differentiate into other cell types, tracking of the differentiated cells was possible because of the GFP protein they carry. So that, our research group examined the pluriptency of bone marrow-derived cells by using this technique and learned that the cells migrated and differentiated into constituent cells of the tooth and the periodontal tissues [14]. Our research group also examined using these model animals [14-19].

We also examined using cholesterine as one of experimentally induced foreign body granuloma, and histological responses, as well as some immunohistochemical analysis [13]. The present results observed on the granulation tissue were roughly similar to the findings using cholesterin crystals implanted in the subcutaneous tissues in GFP-transplanted model mice [13].

Based on the present results of the immunohistochemical examination, nearly all cells (Mφ) of the granulation tissue which proliferated at the site where the suture Monocryl® implanted were GFP-positive. Furthermore, within the granulation tissue, small amounts of fibroblasts and collagen fibers were interspersed and they were almost GFP-negative. The staining feature of the Mφ cytoplasm was similar to the peripheral stain of round structures in the center of the cells. Some of them stained pale violet and some were almost devoid of stain. These structural features suggesting Mφ phagocytosis, meaning the change in stain was considered to be due to the degree of hydrolysis of the absorbable suture Monocryl®.

The present examination results determined the completely absorbable period of time in side body. However, our present examination became the nearly the same examination images of other literature of chemical and physiological characteristics [9-12].

In conclusion, our present examination showed that Monocryl® was almost disappeared in the dorsal embedded site after 3 months; some are still remained in the 6-month specimens as formy cells. Next, both Mφ and small amount of FBGC were both positive to GFP while the fibrous tissues including the fibroblasts interspersed between Mφ and FBGC were negative to GFP. The results suggest that the Mφ and FBGC were derived from the transplanted bone marrow mesenchymal cells as same as the Vicryl® case [6].

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**Conflict of Interest**

The authors have declared that no conflict of interest exists.

**References**


