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# The degradation and local tissue effects of collagen hydrogel and sponge implants in muscle

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ABSTRACT

Collagen products will degrade and evoke certain local tissue reaction after being implanted. ISO 10993.6 is the international standard to evaluate local tissue reaction for biomaterials. However, even in the present version, it does not define the specific view fields for scoring local tissue reaction in the sections, which may influence the fair evaluation for the degradable polymer, such as collagen. In this study, 4 kinds of collagens and 2 kinds of bio-inert products were implanted into the rabbit muscle for 26 weeks, and two different scoring methods (uniform distribution scoring method and area locating scoring method) were applied to evaluate the local tissue reaction for each implant. The results showed that the tissue reaction on the interface was active, but the various scores in the implant and surrounding areas could help us to understand the differences of the degradation process. An area locating scoring method which selected the fields in the implant, interface and surroundings respectively showed a higher total score and offered more detailed information compared to the uniform distribution scoring method. Since the degradation and tissue reaction degree in the different areas are ever changing for the degradable implants, this area locating scoring method is sensitive, effective and applicable for the semi-quantitative evaluating system of ISO 10993.6.



## 1. Introduction

Because of its wide supply, promising plasticity, formability, mechanical properties and biological activity, collagen is widely used for tissue filling, tissue engineering and hernia repair, etc [1–4]. However, although collagen is believed holding low immunogenicity, its degradation products can be recognized by host immune system, and lead to the recruiting of immune cells, and further raise inflammatory reactions [5–7]. After the implantation of the collagens, the degradation begins at the interface and the degradation speed may be related to the properties such as preparation methods and structures. On the other hand, many researches have proved that the cytokines derived from the immune cells, included granulocytes, macrophages, etc, can accelerate the degradation [8,9]. With different interface degradation process, the implanted collagens may lead to different local tissue reactions, and finally different performances in the body [10,11]. Therefore, to investigate the interface tissue effects will be helpful to design better implantable collagens, and be a key biological evaluation index for their safety and effectiveness.

The biological evaluation of the local tissue reactions after the implantation based on ISO 10993.6 international standard is routine before the entrance of the biomaterials to the market [12,13]. The annex E of the present version of ISO 10993.6 offered the semi-quantitative evaluating system on the sections, but did not define how to select the high powered microscope fields for the scoring. Besides, as the guideline for all kinds of the medical devices and biomaterials, ISO 10993.6 does not consider the particularity of the degradable natural polymers, such as collagens especially. Because of the existing of degradation interface between the implanted collagens and the surrounding tissue, the scores getting from the areas at, in or out the interface will be far different [14,15]. The subjective choice of the observer will be greatly impact on the evaluation output and thereafter on the fair assessment of these biomaterials.

In this study, 4 kinds of collagens and 2 kinds of bio-inert products were implanted into the muscles of rabbits to evaluate the local tissue effects. The test animal management, periods,

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surgery and testing conditions were followed ISO 10993.6 standard. The semi-quantitative evaluating system in the annex E was applied, but two different scoring methods were compared. One method scored on selected areas in the implants, the interface and the surrounding tissue respectively. Another method scored on uniformly distributed high powered fields on the sections, which was frequently used for many bio-inert biomaterials. We hope this study can further improve the applicability of the semi-quantitative evaluating system of ISO 10993.6 on the degradable implants, and offer some clues for the better designation, evaluation and application of the degradable implants.

## 2. Material and methods

### 2.1. Preparation of collagen and bio-inert products

2 in the 4 kinds of collagen implants were prepared by the same collagen solution, which were constructed to hydrogel and sponge as present report [16,17]. The brief process showed below.

Type I collagen was extracted from calf skin with pepsin (Sigma-Aldrich, St. Louis, MO, USA) in acetic acid (0.5 mol/L, Kelong chemical industry, Chengdu, China). The solution that contained the extracted collagen was sterilized by filtration through MILLEX®GP Filter Unit (0.22 μm, PES Membrane; Millipore, Harrow, UK). After the sodium chloride fractionation, the collagen fibrils were reconstructed. The collagen hydrogel was prepared by neutralizing the solution by the 1 M sodium hydroxide and 4-(2-hydroxyethyl)-1-piperazineethane-sulfonic acid (HEPES) buffer. 0.5 mL of the neutralized collagen solution was dispensed into 48-well plates (Corning Coster) and incubated at 37 °C for 10 min to form hydrogels.

Collagen sponge was prepared by the following procedures. Neutralized collagen solution (7 mg/mL) was transferred into Petri dishes (Corning Coster, Cambridge, MA, USA), frozen at −80 °C for 24 h, and freeze-dried at −44 °C under 10–30 Pa in a sterile condition for 12 h.

The other 2 collagens were COOK medical Biodesign Surgisis 4-layer tissue graft SIS and Duramax Artificial Duramater (Beijing TianXinFu Medical Appliance Co.,Ltd). The 2 bio-inert products were GORE MYCROMESH Biomaterial (e-PTFE, produced by W. L. Gore & Associates, Inc) and DynaMesh-SIS direct soft (PVDF). In this study, all of the implants were numbered: Sample 1, prepared hydrogel; Sample 2, prepared sponge; Sample 3, Duramax Artificial Duramater; Sample 4, COOK Biodesign Surgisis; Sample 5, GORE MYCROMESH Biomaterial; Sample 6, DynaMesh-SIS.

### 2.2. Muscle implantation experiments

Before the implantation, 6 kinds of the samples were prepared into 10 mm × 4 mm × 2 mm shape approximately. 72 Japanese white rabbits were divided randomly into 6 groups for 4 implant periods (2, 4, 12 and 26 weeks, 3 animals for each group in each period). Each animal (offer 4 implant sites in paraspinal muscle) were implanted by 1 kinds of sample. At each termination of the period, the rabbits were sacrificed by pentobarbital sodium overdose.

The implants and the tissue around the implants were retrieved and fixed by 10% formalin. After gradient ethanol dehydrated and embedded by paraffin, they were sectioned to 5 μm and stained by hematoxylin-eosin (H.E).

The use of all the animals in this study was followed the guidelines for the care and use of laboratory animals of Sichuan University and the standard of ISO 10993-2.

### 2.3. Selection of scoring fields for semi-quantitative evaluation

The semi-quantitative evaluation system of ISO 10993-6 annex E was used to evaluate and compare the local tissue reaction. 2 methods for the selections of scoring fields were used in the study. In method 1 area locating scoring method, the high powered( × 400) fields were located in implant (2 fields, including only the implant area in the field as possible), interface (4 fields, including the implants area and the surrounding areas as possible equally) and surrounding areas (4 fields, including the surrounding tissue areas only, but it should near the interface as possible) in a lower powered ( × 50) field of the section. In method 2 uniform distribution scoring method, the high powered( × 400) fields were uniformly distributed on the lower powered ( × 50) field. The average scores in different scoring fields selecting methods were used for the evaluation of the changes of the local tissue effects with the implanted periods and compared the different fields selecting methods.

### 2.4. Statistical analyses

The score data derived from the experiments were presented as the mean ± SD, and were analyzed with SPSS 11.0 (SPSS, Chicago, IL, USA). Differences between groups were analyzed by ANOVA. A *P*-value of less than 0.05 was considered significant.

## 3. Results

### 3.1. The gross and lower powered fields observation of the implants

The obtained hydrogel blocks appeared white and smooth. At the edge of the blocks, it showed certain translucent (Fig. 1a). The collagen sponge was obtained by freezing the neutral collagen solution and subsequent lyophilizing. The sponges appeared loose and porous and easy to be sheared to desired shapes (Fig. 1b).

The post-operative observation showed all the animals recovered well. No any local and systemic abnormalities were found. The macroscopic observation of retrieved articles showed all implants were still in site (data not show).

For each sample, at least 10 sections contained the implantation sites were examined. The lower powered field observation of the H.E stained sections gave gross information for the change of the implant and surrounding tissue (Fig. 2). The implants, interface and surrounding tissue areas could clearly be distinguished on the lower powered fields ( × 50). For sample 1, a typical degradation and inflammatory process of implanted natural polymer in muscle was observed. With the trauma of the operation, some inflammatory cells appeared around the implants. The implants gradually

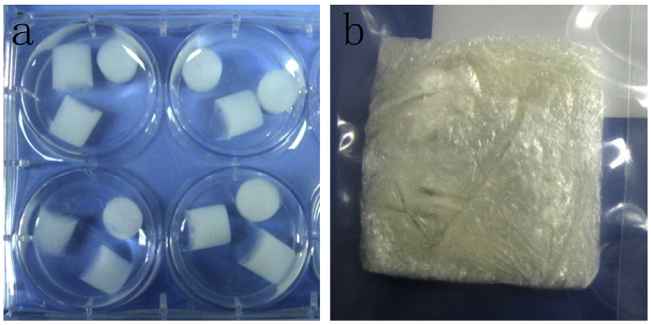


**Fig. 1. The constructed collagen hydrogel and sponge from a collagen solution.** (a) The hydrogel blocks appeared white and smooth. (b) The sponges appeared loose and porous and easy to be sheared to desired shapes.