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Assessment of the subcutaneous degradation process of insoluble hyaluronic acid in rats

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ABSTRACT

Insoluble hyaluronic acid (IHA) may prevent adhesions by forming a physical barrier during the period when postoperative adhesions form. This study was performed to verify the changes that a solid IHA membrane undergoes as it is degraded *in vivo*, and to ascertain the swelling rate of IHA required for it to function as a physical barrier during the postoperative adhesion formation period. Nine female WI rats weighing 300–400 g were used. Discs 8 mm in diameter were cut out of dry IHA membranes made of IHA with a swelling rate (wet weight/dry weight) of either 2.47 (high-swelling IHA) or 1.94 (low-swelling IHA). They were placed in saline to swell and then washed with saline before subcutaneous implantation in four pockets in each rat. The high-swelling IHA started to degrade more rapidly than the low-swelling IHA. There was no evidence of degradation of the low-swelling IHA until day 7, but once it had started, the speed of degradation tended to be similar to that of the high-swelling IHA. The present results showed that, when IHA is implanted subcutaneously in rats, it is degraded over time in a phased process. The swelling rate required for the use of IHA as a postoperative adhesion barrier was also suggested.

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1. Introduction

Adhesions are extremely common after thoracotomy, and they increase the operative risk if thoracotomy is performed again [1] [2] [3]. Although materials to help prevent post-thoracotomy adhesions have been under development for many years, none has yet reached the market, and they remain in the developmental stage [4] [5]. We have previously reported that the use of an insoluble hyaluronic acid (IHA) membrane is extremely effective in preventing post-thoracotomy adhesions in dogs [6] [7]. Adhesions are believed to form during the 7 days following surgery [8] [9]. IHA may prevent adhesions by forming a physical barrier during the period in which postoperative adhesions form.

However, it is known that hyaluronic acid is rapidly metabolized *in vivo* [10]. The form taken by IHA while it is being metabolized *in vivo* during the postoperative adhesion formation period and whether or not it undergoes a process of degradation remain unclear. Hyaluronic acid undergoes both nonenzymatic and enzymatic degradation *in vivo*. One factor that has been shown to affect the speed of this degradation is the swelling rate of hyaluronic acid [11]. The effects of differences in the swelling rate on changes in its form as a physical barrier *in vivo* have yet to be elucidated.

Although the distribution of hyaluronic acid *in vivo* [12] and its degradation process are known [13], these provide little information about the degradation of extracorporeally introduced IHA, particularly concerning changes in its form during the initial stage of degradation. The metabolism of subcutaneously injected gel filler in mice has been described, but only over the short observation period of 3 days [11]. Previous studies of hyaluronic acid degradation provide insufficient knowledge of the degradation

Abbreviations: IHA, insoluble hyaluronic acid.

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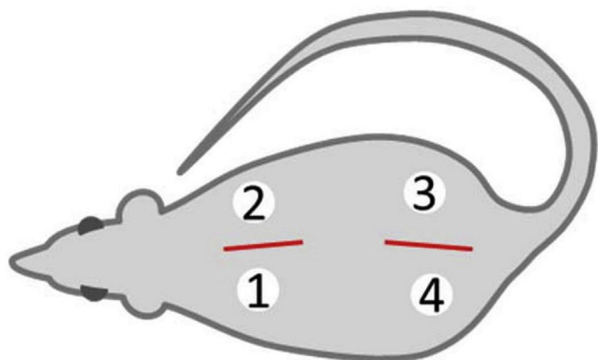

Fig. 1. The implantation sites of the rats. The implantation sites were subcutaneous pockets created on the left and right sides of the backs of the rats. Four pockets were created in each rat (a craniad and a caudad pocket on each of the left and right sides).

Table 1
Observation of test membrane degradation when placed subcutaneously in rats over time.

Criteria for Scoring	
Score	Criteria
7	Remain unchanged
6	Bubble diameter less than 1 mm
5	Bubble diameter 1–2 mm
4	Bubble diameter 2–3 mm
3	Bubble diameter over 3 mm
2	Partly tubular or collapse
1	Completely tubular or collapse (come apart but visible fragment)
0	Non solid

process undergone by membranous IHA inside the body. Seprafilm®, an anti-adhesive barrier material widely used to prevent abdominal adhesions, does not consist of hyaluronic acid alone, but is a composite formulation that also contains carboxymethyl cellulose [14]. Its metabolism *in vivo* may therefore differ from that of IHA membranes, which consist of hyaluronic acid alone. In previous studies, we have reported that IHA membranes exhibit a variety of forms on autopsy. In some dogs 2 weeks post-thoracotomy, there was no sign at all of the IHA membrane within the chest cavity, whereas in other animals, a liquefied substance was evident [6] [7]. A study of repeated thoracotomy 10 weeks after initial surgery in dogs found no sign of the IHA membrane in any animal [15]. These studies investigated the state of the IHA membrane after the postoperative adhesion formation period had passed, and they provide no information on the form of this membrane during that period.

The objective of this study was twofold: to verify the changes that the solid IHA membrane undergoes as it loses its form and is degraded *in vivo*, and to ascertain the swelling rate of IHA required for the membrane to function as a physical barrier during the postoperative adhesion formation period.

2. Materials and methods

2.1. Animals and experimental materials

Nine female WI rats weighing 300–400 g were used. Discs 8 mm in diameter were cut out of dry IHA membranes made of IHA with a swelling rate (wet weight/dry weight) of either 2.47 (high-swelling IHA) or 1.94 (low-swelling IHA). They were placed in saline to swell and then washed with saline before implantation.

2.2. Experimental membrane implantation and removal

Anesthesia was induced by placing the rats in a sealed vessel containing isoflurane (Isoflu; DS Pharma Animal Health Co., Ltd., Osaka, Japan). The gas velocity for inhalation anesthesia was 1.25 L/min. The isoflurane concentration was started at 2.0% in room air and maintained at 1.5% intraoperatively.

The implantation sites were subcutaneous pockets created on the left and right sides of the backs of the rats. Four pockets were created in each rat (a craniad and a caudad pocket on each of the left and right sides) (Fig. 1). The anesthetized animals were immobilized in the prone position on the operating table, and their backs were shaved at the locations where incisions were to be made. These locations were then disinfected with 70% alcohol solution and 7.5% povidone iodine (Neojodin Scrub 7.5%, Iwaki Seiyaku Co., Ltd.). An incision was made in the dorsal midline, and the pockets were created by blunt dissection beneath the skin on both sides. Forceps were used to place the high-swelling or low-swelling IHA in the bottom of the pockets created, after which the incisions were sutured by the usual method.

The number of post-implantation days was counted from the date of implantation as day 0. Rats were euthanized by isoflurane anesthesia overdose on post-implantation days 3, 5, 7, 9, 11, 14, and 26. Incisions were then made in the sutured areas, the skin was dissected, and the conditions of the inserted experimental materials and the implantation sites were observed. When feasible, the IHA membrane was removed, washed in purified water, stained with methylene blue solution, and photographed.

2.3. Assessment and evaluation

The state of degradation of the IHA membrane was scored on an 8-point scale ranging from no change (7 points) to no visible solid (0 points) (Table 1). Because IHA membrane easily ruptures and splits when damaged, if the membrane had broken up into fragments, they were reassembled, and the material was scored as a single piece.

3. Results

For the high-swelling IHA, on day 3, the degradation score was 6 in one animal and 2 in three (median score 2). On day 5, the score was 1 in three animals (median score 1).

For the low-swelling IHA, on day 3, the degradation score was 7 in four animals (median score 7). On day 5, it was 7 in three animals (median score 7), and on day 7, it was 7 in three animals (median score 7). On day 9, it was 7 in one animal, 4 in one, and 2 in two (median score 3). On day 11, it was 7 in two animals and 2 in two (median score 4), and on day 14, it was 1 in four animals (median score 1) (Figs. 2 and 3). On day 14, in all four animals, the experimental material consisted of encapsulated bubbles of liquid beneath the skin. On day 26, no solid experimental material was evident in any of the four animals, and the score was 0 in all cases. Vesicles were present at the implantation sites in two of the four animals. At one of these sites, tiny vesicles containing approximately 20 μL of diluted blood were present, and there was no solid IHA within the vesicles (Fig. 4A). At the other site, the vesicles contained approximately 100 μL of colorless, clear liquid with almost no viscosity (Fig. 4B). There were no vesicles at the remaining two sites, and no vestiges of the implanted experimental material remained (Fig. 4C and D).

Both the high-swelling and low-swelling IHA were degraded over time when implanted subcutaneously into rats. The high-swelling IHA started to degrade more rapidly than the low-swelling IHA. There was no visible evidence of degradation of the

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