Histologic changes in capsule formation around silicone implants after a single dose injection of amniotic fluid

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Summary. Excisional fetal wounds exposed to amniotic fluid do not contract as opposed to those not exposed. In adult rabbits, the effect of amniotic fluid on capsule formation around silicone rubber cubes was investigated. The results showed that a single dose injection of amniotic fluid can modify the fibrous capsule formation histologically in the adult animal.

Key words: Capsule formation – Silicone implants – Amniotic fluid – Fetal wound healing

Fetal response to tissue injury, including rapid healing without inflammation, fibrosis or scar formation, has been an attractive focus of recent wound healing research. Some reports have addressed the role of the fetal environment; namely, the amniotic fluid [6, 7, 9, 11]. It contains high levels of hyaluronic acid (HA) which may have, among others, a significant effect in healing, with less scarring [8, 10]. The efficacy of amniotic fluid, if any, when placed in an adult wound environments is not yet known.

This study is aimed to investigate the effect of amniotic fluid on the formation of capsules around silicone implants in an adult rabbit model.

Materials and Methods

Obtaining the amniotic fluid

At 14–24 days gestation, New Zealand female rabbits were anesthetized by intramuscular injection of a cocktail consisting of atropine sulfate 0.1% (1mg/kg), xylazine 2% (Rompun, 4 mg/kg), and ketamine hydrochloride 50 mg/kg; and this was followed by a laparotomy.

After hysterotomy was performed, 1–3 cc’s of amniotic fluid was aspirated from each animal using a syringe. The laparotomy incision was then closed, and those rabbits were kept apart from this study.

Placement of silicone cubes

In ten male New Zealand rabbits weighing 3–3.5 kg, anesthesia was obtained as described above.

Male rabbits possess two ventral rows of breasts (left and right) consisting of 4–5 nipples each, and extending from the axillary region above to the inguinal area below.

In each animal, two abdominal nipples, one on the left and the other one on the right, were prepared. Through a small incision, 1 cm medial to the selected nipple, a pocket was created below the breast and a silicone cube was placed in the pocket. The silicone cubes, 1 x 1 x 1 cm in size were cut and fashioned during surgery. Following closure of the incision, 1 cc of amniotic fluid was injected into the right-side pocket and 1 cc saline into the left-side pocket in each animal.

The experimental group consisted of the right-side silicone cubes which were injected with amniotic fluid, and the control group consisted of the left-side silicone cubes which were injected with saline.

Silicone cubes, including their capsules, were harvested at 7, 10 and 21 days postoperatively. The specimens were fixed in 10% formalin, embedded in paraffin, stained with H&E and also with trichrome, and examined with light-microscopy.

Results

One animal was excluded from the study because of wound infection. Nine animals were evaluated. Upon exploration at either 7, 10 or 21 days, a thin, semitransparent, membrane-like capsule was observed around all silicone cubes in both groups.

Histologically, fibroblasts, macrophages, polymorphonuclear leucocytes, lymphocytes and plasma cells were found in all capsules. The number of fibroblasts were less in the capsule specimens of the experimental group on days 7, 10 and 21, when compared to those of the control, and the difference was statistically significant (p < 0.001) (Table 1). The number of macrophages were less in the capsules of the experimental group on
Table 1. Comparison of Fibroblast numbers between two groups

<table>
<thead>
<tr>
<th>Day</th>
<th>Number of fibroblasts (mean)</th>
<th>Significance**</th>
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<tbody>
<tr>
<td></td>
<td>Experimental</td>
<td>Control</td>
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<tr>
<td>7</td>
<td>4</td>
<td>12</td>
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<td>10</td>
<td>5</td>
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<td>21</td>
<td>7</td>
<td>16</td>
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* Counted under X1000 magnification. Means were calculated at least from the areas examined
** Significant if *p* < 0.05

Table 2. Comparison of macrophage numbers between two groups

<table>
<thead>
<tr>
<th>Day</th>
<th>Number of macrophages (mean)</th>
<th>Significance**</th>
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<td>21</td>
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* Counted under X1000 magnification. Means were calculated at least from ten areas examined
** Significant if *p* < 0.05

days 7, 10 and 21, when compared to those of the control; however, the difference between the two groups was statistically significant at 21 days postoperatively (*p* < 0.001), as seen in Table 2.

There was no significant difference between the two groups when the numbers of other inflammatory cells, such as PMN leucocytes, lymphocytes and plasma cells were compared. On day 7 postoperatively, the stroma of capsules in both groups consisted of inflammatory cells within a loose edematous matrix without identifiable collagen bundles. On day 10, however, in the control group, thin collagen bundles infiltrated the stroma of the capsules and numerous fibroblasts were found to be arranged in groups, while those of the experimental group showed neither collagen bundles nor fibroblast groups. On day 21 in the control group, dense collagen bundles were found to occupy the capsular stroma in the control group, while those in the experimental group showed a less amount of collagen bundles, thus, representing a loose stroma.

Discussion

The specific effects of amniotic fluid on granulation tissue is not yet known. Enthusiasm in understanding events of fetal wound healing lead to the consideration of the possible role of amniotic fluid in scarless healing. Amniotic fluid is known to be rich in hyaluronic acid and in growth and trophic factors [5, 8, 9]. In the rabbit fetus, excisional wounds contract and reepithelialize when excluded from amniotic fluid, but expand when in contact with amniotic fluid [4, 6].

Longaker, et al further showed that, besides the direct effect of the high level exogenous HA within the amniotic fluid on the fetal skin wound, there is also a factor which stimulates the wound to increase the production of its endogenous HA [7]. Chiu, et al showed that HA is undetectable by day 7 in the adult wound, but levels of HA in fetal wound remain elevated for three weeks [1]. A prolonged presence of HA in the fetal wound may provide the mesenchymal signal for healing by regeneration, rather than scarring and fibrosis. It was also shown that the lack of fetal wound contraction is not due to the inability of fetal fibroblasts to cause contraction, but is related to exposure of the cells to amniotic fluid [6]. Early studies in experimental animals showed that intraperitoneal administration of amniotic fluid lessened post-taparotomy abdominal adhesions [6]. This finding suggests that amniotic fluid would also be efficacious in the adult. An implant capsule model in the rabbit was used in this study, and histological alterations in forming capsules around implants following a single injection of amniotic fluid showed that amniotic fluid could act as a potent agent to influence granulation tissue and fibroblasts.

The histological features of the fibrous capsule which forms around silicone implants have been reported in detail [2]. Ginsbach, et al identified by light-microscopy two distinct histological layers in human: 1) a superficial layer (situated next to the prosthesis) contained several different cellular elements, which in some areas formed an epithelium-like layer, and 2) a fibrous layer which consisted of longitudinally oriented, thick bundles of collagen fibers [3]. The latter determines the thickness of the capsule. Experimental evidence, however, showed that there is no relationship between capsule thickness and capsule contraction [2].

Gayou emphasized that the contracted capsule in the human was considerably more cellular than the noncontracted one, i.e. histologically, the number of fibroblasts, macrophages and also plasma cells were higher in the contracted capsule [2]. Interestingly, we found less cellularity in the capsules around the silicone cubes, which were injected with amniotic fluid. Our results showed that amniotic fluid clearly inhibited the increase in number of fibroblasts and macrophages, but not that of other inflammatory cells. Amniotic fluid also caused thin fibrous capsule wall formation with less cellularity in this study.

Despite its effect in decreasing the cellularity of the capsule, this study cannot provide an answer as to how effective amniotic fluid is in preventing a capsular contracture, nor the specific action of amniotic fluid on specific cells, their activities and also functions. But, this study clearly demonstrates that amniotic fluid has the potential to modify granulation tissue in the adult. Direct and/or indirect effects of different substances within the amnion on specific cells of the granulation tissue will provide more insight in modifying adult wound healing.