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Research



# Enhancement of Tissue Expansion by Calcium Channel Blocker: A preliminary study

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## Abstract

**Background:** Reconstruction of the defects after surgical resection of tumors is one of the important issues in surgical oncology. It is essential that the defect should be covered with a tissue quite similar to the original one and is best achieved by harvesting tissue from an area adjacent to the defect. Tissue expansion is one of the most frequently used reconstructive techniques. A number of studies evaluated blood circulation, capsule formation, tissue tolerance, histomorphological changes and complications of expander placement. However, only a few attempted to enhance tissue expansion. This study we aimed to evaluate verapamil, a calcium channel blocker, to enhance tissue expansion.

**Material and method:** Twelve New Zealand rabbits weighing between 900 gm and 1200 gm were assigned into study and control groups. High volume expanders (100, 200 or 300 cc) were placed into the subcutaneous tissue. Rabbits in the study group received verapamil. Expanders in the control group were inflated every three days to achieve same pressure as the study group. The size of the flaps was assessed by applying pressure on tip of the flap to demonstrate the contraction. Histopathological examinations were performed.

**Results:** By administering liquid earlier and more quickly less flap retraction was observed in the study group. In the control group expanders were exposed in two rabbits while no complication occurred in the study group. Following extraction of the expanders, the flaps were elevated and less retraction was observed in the study group compared to controls.

**Conclusion:** Verapamil is safe when used topically and provides less retracted flaps. It can be suggested that verapamil acts on the myofibroblasts in the capsule around tissue expanders and thus increases efficiency of the expanders.

## Introduction

Covering of the defect after surgical resection of the primary or metastatic neoplasm is essential and the best tissue for such coverage is the one, which is similar to the original resected tissue. This is best achieved by harvesting a tissue from an area adjacent to the defect. However, harvesting a large local flap from an adjacent tissue will lead to formation of an additional defect at times. In addition, the adjacent area may not provide an appropriate size tissue. The use of tissue expanders may allow an original-like tissue for coverage. Tissue expansion is a mechanical procedure, which expands the surface of an available local area [1]. All living tissues respond to mechanical strength and the mitosis in these tissues increases as the tissues gradually expand. Clinical application of tissue expanders is widespread and the expanders are indispensable for reconstruction of large defects resulting from surgical treatment of tumors, many congenital anomalies and acquired defects. Expanders are most frequently used in reconstruction of breast and scalp defects [2,3]. Schmidt *et al*, summarized the advantages of expanders as: a) best tissue match with defect areas, b) lack of a wound at the donor site, and c) maintenance of vascular supply [4]. It has been demonstrated that a fibrous capsule was formed around tissue expanders and examination of this capsule and the surrounding dermis on electron microscopy has shown an increase in the number of contractile fibroblasts [5]. These fibroblasts contain actin and myosin, which account for not only wound healing but also for formation of a fibrous capsule around the expanders [6]. Verapamil, a safe and widely used calcium chennal blocker, was used in the present study as it has a topical effect on the collagen tissue fibroblasts with an aim to determine whether it can decrease pressure around the expanders and thus increasing efficiency of tissue expanders.

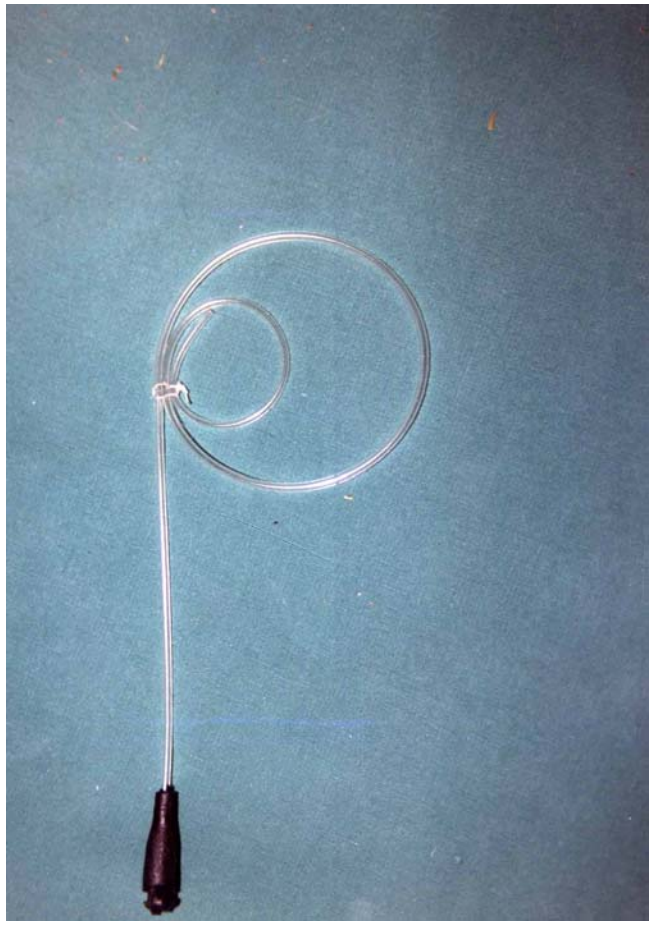

**Figure 1**
Apparatus for administration of verapamil

## Material and method

The study was performed in Experimental Laboratory of Plastic and Reconstructive Surgery Clinic in Izmir Atatürk Teaching Hospital, Turkey, using twelve New Zealand rabbits weighing between 900 gm and 1200 gm. There were six rabbits in verapamil group and six rabbits in control group. Within each group, 100, 200 and 300 cc expanders were used in two animals each. Approval was obtained from the ethical committee of the hospital and the animals were kept in separate cages in temperature, light and airflow-regulated rooms. Experiments were conducted under aseptic conditions. The rabbits were anesthetized with 1 ml ketamine hydrochloride 50 mg/ml and 1 ml of 2% xylazine hydrochloride.

High volume rectangular expanders (McGhan®, Medical Corporation, USA) were used. The rabbits were assigned into a study group to receive verapamil and into a control group and each group was divided into three subgroups based on the volume of the expanders; Group I: 100 cc, Group II: 200 cc and Group III: 300 cc. A reservoir was connected to each expander with a 3 cm silicon tube.

### *Apparatus for administration of verapamil*

Verapamil was administrated using a special cannula: Thirty holes were drilled on an 2 mm diameter 30 cm long intravenous catheter and one end of the catheter was blocked (Figure 1). Thus, the drug was spilled only through the holes and spreads on the base where the expanders were placed. The cannula was twisted to form a spiral and sutured with 5/0 silk. This apparatus was sterilized in a gas autoclave.

The back of the rabbits were shaved and cleaned with povidon iodide and a 3 cm horizontal incision was made 5 cm away from the tail. After dividing the cutaneous and subcutaneous tissue a diverticulum was created on the panniculus carnosus. At this level, a dissection of the same

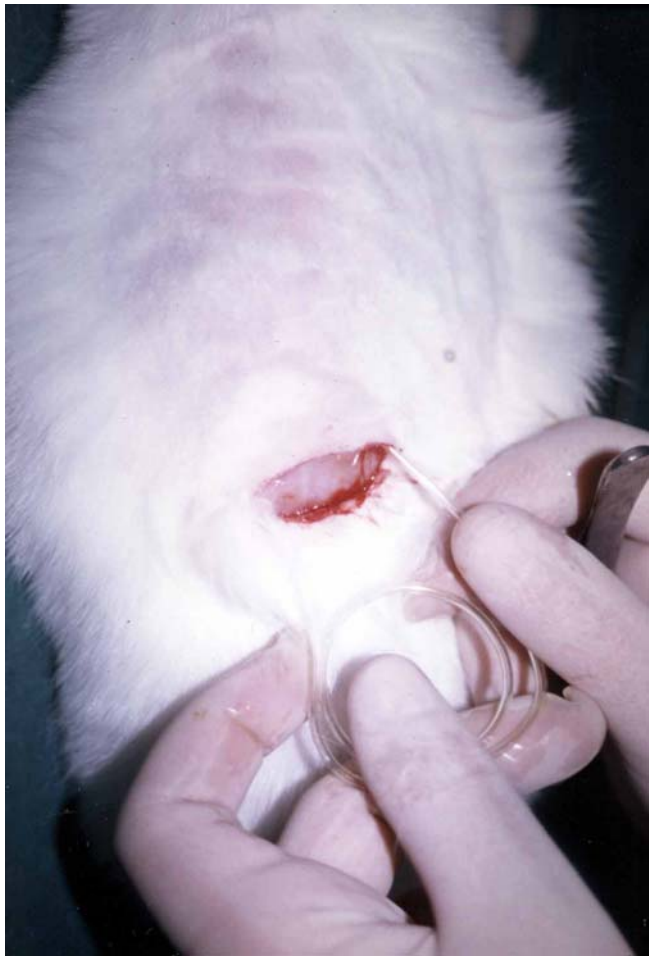

**Figure 2**
Photograph showing application of the apparatus

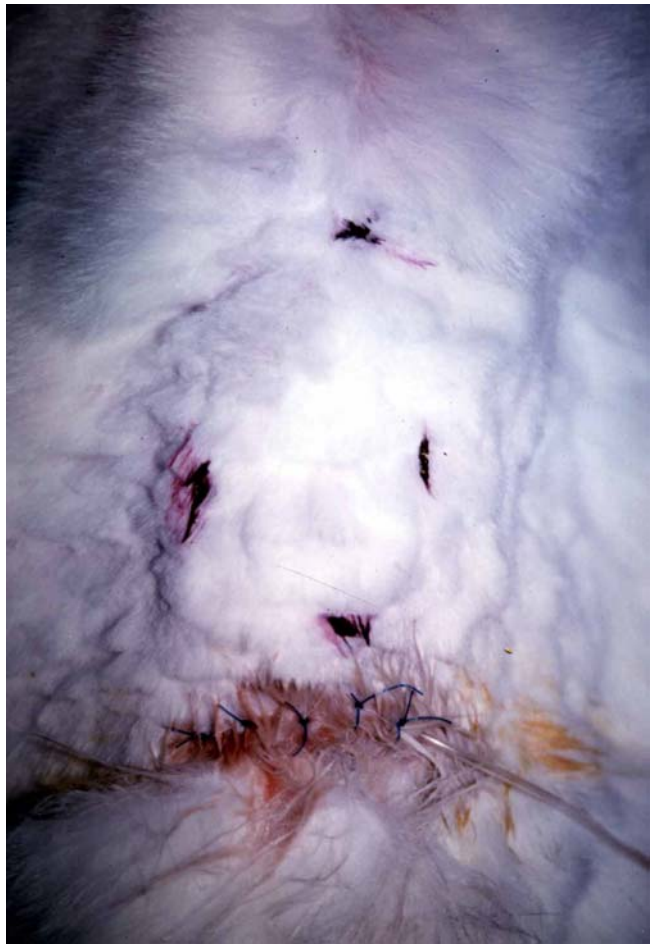

**Figure 3**
Photograph showing AB and XY points of the tissue expansion in rabbit.

size as the base of the expander was made followed by placement of the cannula and the expander into the diverticulum (Figure 2). The reservoir of the expander was placed 2 cm away from the expander in a postero-lateral position. One end of the cannula was left outside the tissue and the skin was sutured with 3/0 prolene. Penicillin was administered once to all animals for prophylaxis.

### *Measurement of expansion*

After the expanders were placed, four spots of crystal violet were created with a caliper on two separate planes on the shaved skin of the rabbits (Figure 3). The length and width of the expanders were labeled as AB and XY respectively. Following each expansion, the distances between the spots was measured.

### *Administration of verapamil*

Immediately after the expanders were placed and every three days thereafter, 2.5 ml verapamil hydrochloride (5 mg/2ml Isoptin®, Knoll Inc.) mixed with 1 cc NaCl was administered in the study group, and only 1 cc NaCl was given in the control group.

### *Expansion of expanders and measurement of intra-expander pressure*

The expanders were not inflated immediately after they were placed, but inflation was started three days later with 15–20% inflation every three days and all expansions took place at the same time postoperatively. At each expansion, first intra-expander pressure was measured in the control group and then the expanders in the verapamil group were inflated with saline equal to this pressure. When the expansions were completed, pressure in each

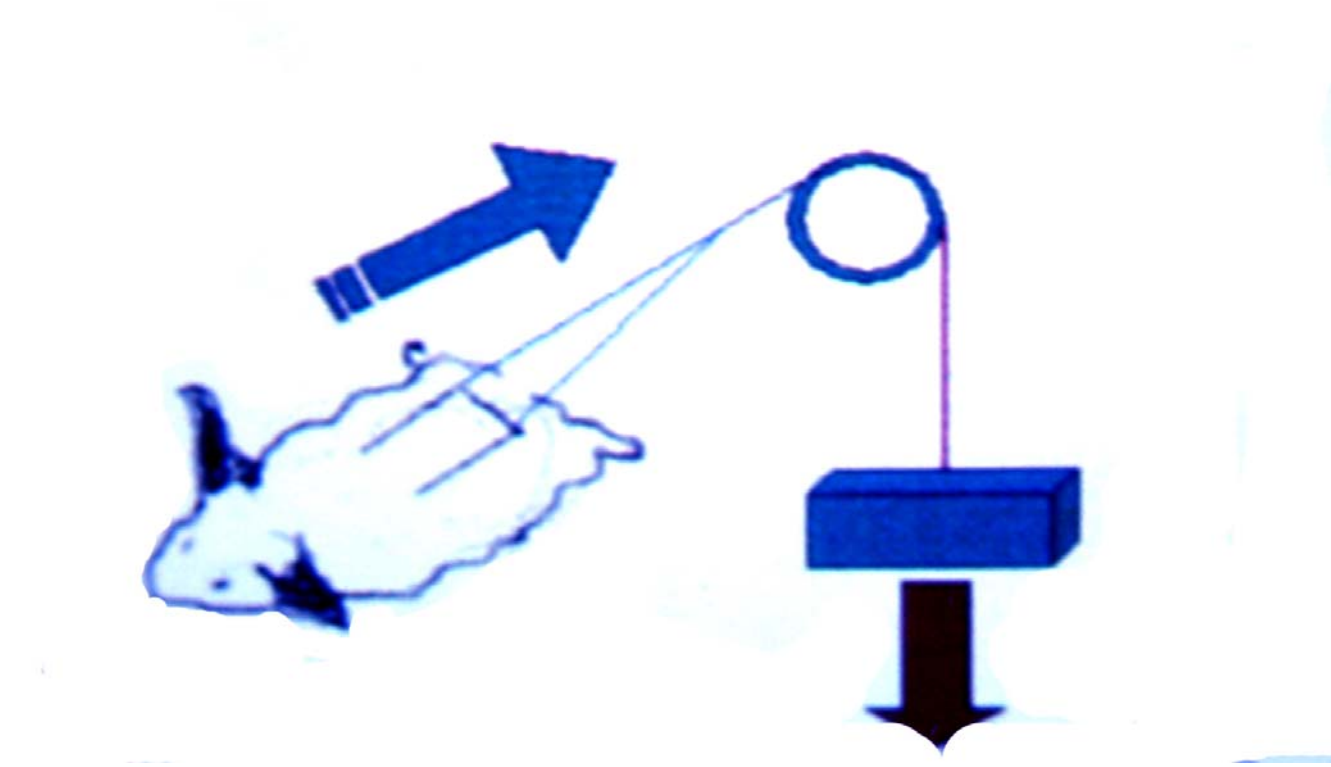

**Figure 4**
Schematic view of the application of 2.46 Newton force to the tip of flaps with pulleys.

expander, amount of expansion and changes in the flaps were recorded.

### *Determination of retraction ratios in flaps*

The length of the flaps was measured twice to determine retraction ratios. The first measurement was made after the procedure of expansion was completed and the second measurement was made after the flaps were elevated. Following the elevation of the flaps, using a system of pulleys, a force of 2.46 Newton was applied on the tips of the flaps and the length of the flaps under the pressure was measured (Figure 4). Thus, the difference between the two values provided retraction ratios.

### *Evaluation*

The differences between AB and XY lengths obtained after the completion of the expansion and those obtained after the elevation of the flaps were determined. In addition, skin specimens were collected from the study and control groups following the completion of the expansions. The specimens were stained with hematoxylin and eosin and were examined. Radiological examinations were performed to determine the apparatus function and its placement (figure 5).

## Results

None of the rabbits died during the experiments. Verapamil injections were well tolerated by the rabbits and it did not cause any systemic changes or systemic or local intoxication in the rabbits. However, in the control group, one rabbit with an expander of 200 cc and one rabbit with an expander of 300 cc experienced exposition of their expanders on day 18 and 15 respectively (figure 6). No infection was detected during the procedures of expansion. However, one rabbit was observed to have immobility in his hindquarters. This may have been due to compression on the nerves.

Details of the expansions are shown in Table 1,2,3. The expansion was completed earlier in all sizes of expanders in the study group than in the control group. The extents (ABs and XYs) in all verapamil groups were longer than those in the control groups. After the expansion was completed, the expanders were removed and superior pedicled expanded flaps 50 × 30 mm in size were harvested. The sizes of the flaps measured after the application of an equal pressure on the tips of the flaps are presented in Table 4. The retraction ratios were lower in the study group than in the control group.

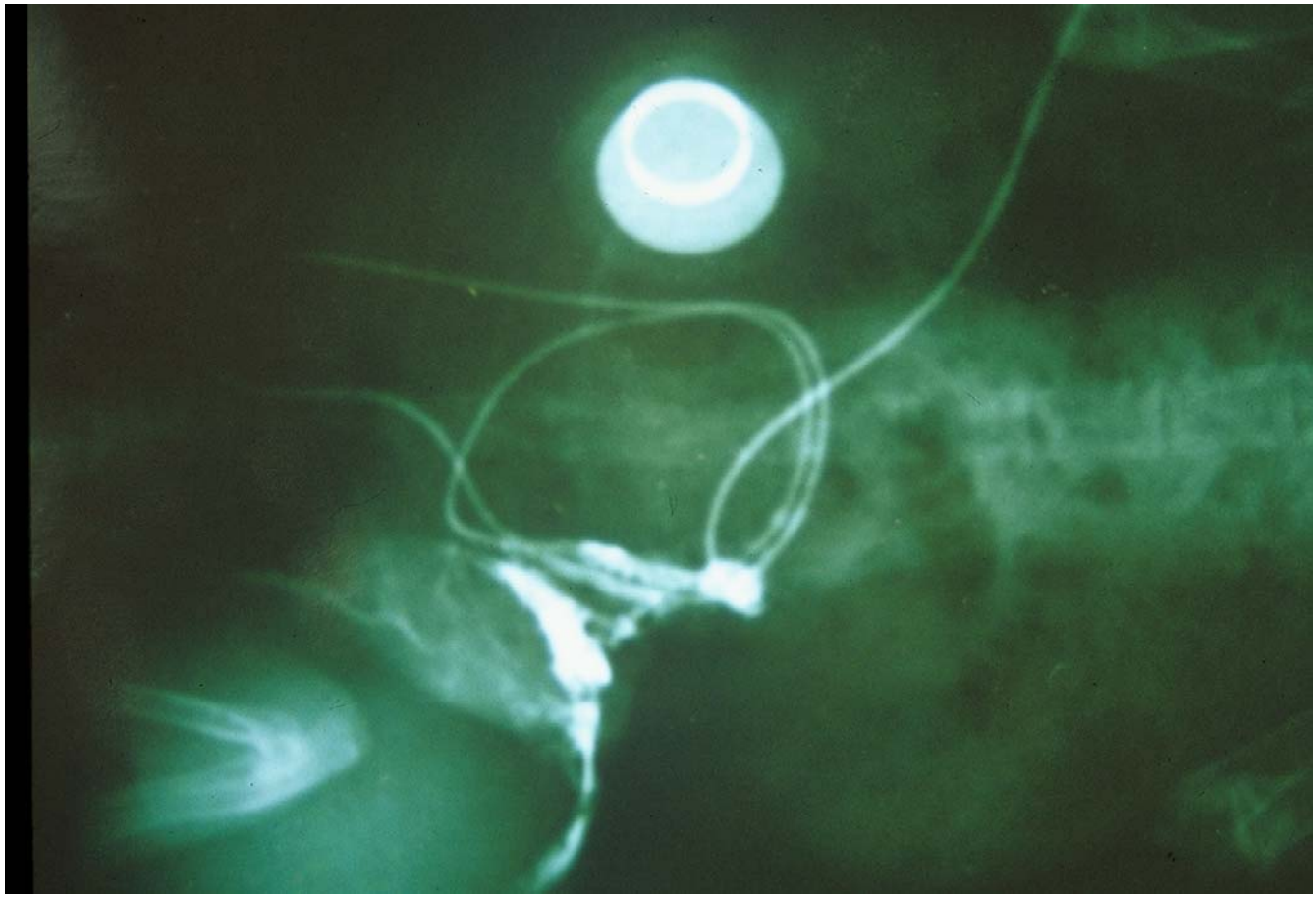

**Figure 5**
X-Ray of the apparatus.

**Table 4: Rate of retractions of the flaps in each group after elevation.**

| Group | Expander volume | Retraction of AB length | Retraction of XY length |
|---|---|---|---|
| Control | 100 cc | 13% | 13.30% |
| Verapamil | 100 cc | 8% | 8.33% |
| Control | 200 cc | 18% | 20% |
| Verapamil | 200 cc | 11% | 15% |
| Control | 300 cc | 22% | 33% |
| Verapamil | 300 cc | 13% | 20% |

There were two rabbits in each group, two rabbits were excluded: one rabbit in control group of the 200 cc expander, one rabbit in control group of 300 cc expander.

The microscopic examination of the specimens taken from each flap revealed that in the study group there was no thickening in the epithelial tissue, the capsule around the expander was thinner, the amount of collagen was less and that cellularity was less and scattered (Figure 7 and 8).

## Discussion

Tissue expansion is a widely used technique in surgical oncology. Many reports have been published in the literature about reconstruction of the breast, head and neck, face, abdominal wall and trunk with tissue expanders after

**Table 1: Details of expansions in subgroup I (100 ml expanders group)**

| Details | group | D0 | D3 | D6 | D9 | D12 | D15 | D18 |
|---|---|---|---|---|---|---|---|---|
| Pressure (mm Hg) | | 0 | 5 | 8 | 10 | 20 | 27 | 30 |
| Inflated volume (percent) | Saline | 0 | 15 | 31 | 45 | 60 | 81 | 100 |
| | Verapamil | 0 | 21 | 47.5 | 74.5 | 103.5 | | |
| AB length (mm) | Saline | 50 | 52 | 55.5 | 58 | 58 | 59.5 | 60.5 |
| | Verapamil | 50 | 53 | 58.5 | 64.5 | 66.5 | | |
| XY length (mm) | Saline | 30 | 32.5 | 34.5 | 37 | 37.5 | 37.5 | 38 |
| | Verapamil | 30 | 35 | 41 | 42.5 | 46 | | |

D-days.

**Table 2: Details of expansions in subgroup II: 200 ml expanders group**

| Details | group | D0 | D3 | D6 | D9 | D12 | D15 | D18 |
|---|---|---|---|---|---|---|---|---|
| Pressure (mm Hg) | | 0 | 3 | 6 | 8 | 30 | 32 | 39 |
| Inflated volume (percent) | Saline | 0 | 15.5 | 31 | 45.5 | 62 | 80 | 99 |
| | Verapamil | 0 | 22 | 42 | 65 | 89 | 106.5 | |
| AB length (mm) | saline | 50 | 52.5 | 55 | 59.5 | 62.5 | 66 | 67 |
| | verapamil | 50 | 54.5 | 61 | 67 | 69 | 75 | |
| XY length (mm) | saline | 40 | 41.5 | 44.5 | 48 | 49.5 | 51 | 53 |
| | verapamil | 40 | 47 | 53 | 57 | 59 | 61.5 | |

There were two rabbits in each group at the beginning of the experiment but one rabbit in saline group was excluded in day 18. D-days

**Table 3: Details of expansions in subgroup III: 300 ml expanders group**

| Details | group | D0 | D3 | D6 | D9 | D12 | D15 | D18 | D0 |
|---|---|---|---|---|---|---|---|---|---|
| Pressure (mm Hg) | | 0 | 8 | 10 | 18 | 22 | 25 | 38 | 45 |
| Inflated volume (percent) | saline | 0 | 15 | 29 | 44 | 60 | 77 | 87 | 100 |
| Inflated volume (percent) | verapamil | 0 | 18 | 38 | 59 | 78 | 95 | 108 | |
| AB length (mm) | saline | 70 | 73.5 | 77 | 80.5 | 84 | 88 | 90 | 94 |
| | verapamil | 70 | 77.5 | 82.5 | 89.5 | 103 | 106 | 108 | |
| XY length (mm) | saline | 50 | 52 | 54 | 56 | 59 | 60 | 62 | 65 |
| | verapamil | 50 | 58.5 | 63.5 | 65 | 70 | 73 | 76 | |

There were two rabbits in each group but one rabbit in saline group was excluded in day 15. D-days

cancer treatment [7–10]. Neumann first reported the concept of tissue expansion in 1957 [11]. He described advantages of this technique as: high quality of tissue match, avoidance of the need for a donor site, and reduction in the number of procedures necessary for reconstruction. In 1976, Radovan used tissue expanders for reconstruction of the breast after mastectomy [12]. Subsequently, the use of tissue expansion has been popularized among plastic surgeons and has become the treatment of choice for many congenital and acquired defects in children and adults [13–16].

Tissue expansion has two disadvantages; one it is time-consuming and second it causes complications. Exposition of the expanders is frequently encountered [17,18]. In practice, the most frequently encountered complication is capsule formation around the expanders. This capsule not only causes difficulties in the procedure of expansion but also decreases the size of flaps obtained

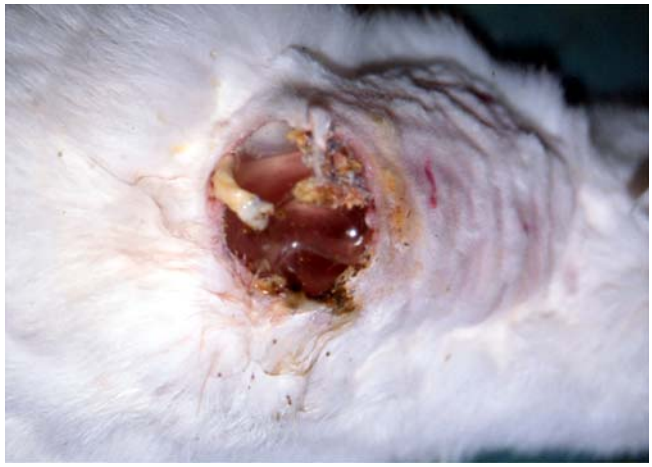

**Figure 6**
Complication in Group III: Exposed tissue expander.

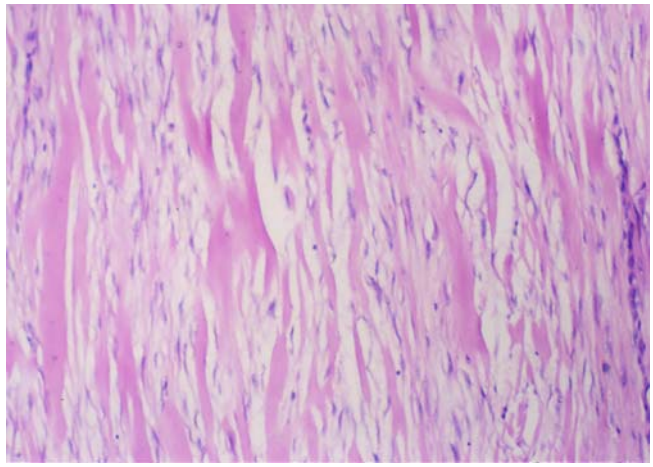

**Figure 7**
Photomicrograph of the subcutaneous tissue from verapamil group showing lesser and scattered cellularity on the subcutaneous tissue. There are no inflammatory cells. (Hematoxylin eosin staining × 100 magnification)

after the expansion. Scar formation around the capsule may result in reduced skin elasticity and impeded flap movement, and expanded skin has a tendency to postoperative retraction [19]. Tension wounds have been shown to demonstrate increased tensile strength as a result of enhanced collagen alignment [20]. The response of the collagen to the expansion has been studied in detail by Timmenga [21]. Accelerated organization of the wound collagen associated with increased tensile strength can be considered as early maturation. The use of drugs affecting collagen synthesis and cross linkage, both in the capsule and in the surrounding dermis, might be of some value in decreasing mechanical resistance of expanded tissues, while administration of topical vasodilator drugs might increase the filling safety margin, thus reducing the risk of ischemia during the expansion [22]. Verapamil is found to be effective on anti-intimal hyperplasia in a number of animal models and decreased the myofibroblast cell proliferation in the intima [23]. Verapamil is widely used topically in the treatment of Peyronie's disease and keloid because of its effects on the collagen metabolism [24–27]. Calcium antagonists increase the extra-cellular matrix collagenase activity as well as decreasing the collagen, and fibronectin synthesis and secretion, altering fibroblastic metabolism. Lee *et al*, speculated that membrane calcium channel blockers could potentially trigger extracellular matrix degradation in dermal scars, resulting in scar volume reduction [28].

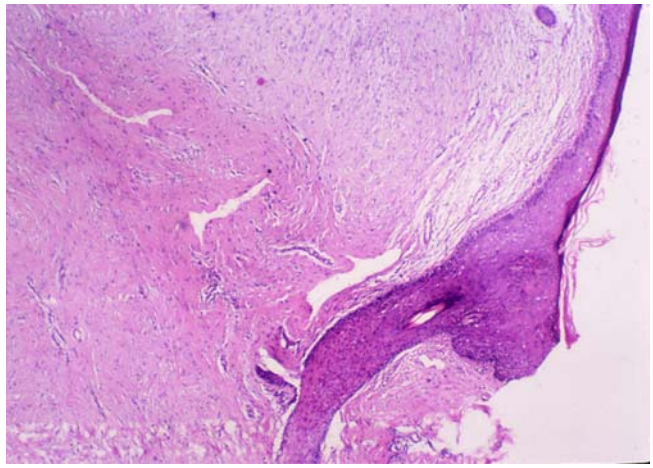

**Figure 8**
Photomicrograph of the subcutaneous tissue from control group showing some inflammatory cells and slightly thickened epidermis with 4 to 10 mitoses. More cellularity was seen than the verapamil group. (Hematoxylin eosin staining × 40 magnification)

Timmenga *et al* concluded in their study that an expander placed between the skin and panniculus carnossus in the rabbit causes considerable skin stretch and affects cutaneous blood supply and therefore that it can be improved for tissue expansion in humans [21]. To date, investigators have used low volume expanders (30–100 cc), while we preferred high volume expanders, which allowed us to evaluate frequently encountered complications in tissue expansion. In the control group in which we used the expanders of 200 cc and 300 cc, two expanders were exposed. We hypothesized that the exposition might be

associated with the pressure to which the skin is exposed and we concluded that verapamil caused relaxation in the tissues around the expander, which provided a safer expansion. Moreover, verapamil allowed the use of high volume expanders in the rabbits. There have been a number of studies on blood circulation to the tissues where expanders are placed [28], formation of the capsule, tolerance of the tissues, histomorphological changes [29] and the complications [18] since 1976. However, there have been only a few studies on the increased efficiency of the expanders. Brobmann and Huber thoroughly investigated the relation between the shape of the expanders and the outcome [30]. Their results showed that less time, pressure, and volume are needed in a larger implant to gain the same surface area as would be attained in several smaller implants. Lee *et al* investigated effects of chemical substances on the efficiency in 1985 [31] using papaverine and cytochalasine D. Other compounds like teophiline [32], dimethyl sulfoxide (DMSO) [33,34] and papaverine too have been used in humans intraoperatively [35]. The agents used to increase the efficiency of the expansion so far, especially cytochalasine D, have been reported to have toxic effects [31]. Lee in his study explained the effects of anti-contractile agents and noted that contractile fibroblasts appeared after application of tension on the dermis for only four days [36]. If the contractile actions of these cells can be diminished, then presumably the dermis will be more easily stretched and epidermal proliferation will maintain an adequate surface layer over the expanding dermis.

We made use of an ordinary catheter and invented an apparatus to administer verapamil. We also demonstrated on radiological examination that the apparatus functioned well. The mean intra-expander pressure was equal in the study and control groups. However, the tissue expansion was greater in the study group. It may be that verapamil, a muscle relaxant, may have prevented the resistance against the expansion.

As mentioned above, we measured the distances between the spots on the skin made with crystal violet, which is a standard method used to evaluate the expansion. However, there is no formula to determine efficiency of expanders precisely. The efficiency depends on the volume and the location of expanders and etiological factors, and it is claimed that an expansion of 30% can be achieved [37]. In this study, we obtained an expansion ranging from 21% to 34% in the control group and an expansion ranging from 33% to 54% in the study group.

Although there has been no study investigating the retraction ratio in the literature, it has been reported that the body responds quickly to the removal of the mechanical effect exerted by the expander. In practice, it is commonly encountered that a little retraction of the flaps is possible after the expander is extracted and until the flaps are transferred into the recipient area. In this study, the retraction ratios determined after the elevation of the flaps were lower in the rabbits administered verapamil.

## Conclusions

The topical usage of verapamil increases the efficiency of expanders, frequently used in reconstructive surgery, and decreases the complications. Thus, it provides less retracted flaps in larger volumes and in a very short time. Verapamil is safer and more potent than the other anti-contractile agents, which increases its use in practice.

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