

Assessment of survival and microscopic changes in porcine skin flaps undergoing immediate intraoperative tissue expansion

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The technique of rapid intraoperative tissue expansion has been used with increasing frequency in the clinical setting over the last several years. This technique takes advantage of the skin's ability to immediately stretch and increase in surface area when expanded under a constant load. Sixteen random-pattern, rapidly expanded skin flaps on 10 domestic male pigs were studied to assess the predictive value of the fluorescein test for flap viability after rapid intraoperative tissue expansion. Partial fluorescence was found to be a more accurate predictor of flap survival in the experimental rapidly expanded flaps when compared to full fluorescence. Partial fluorescence was found to under-predict flap survival by 0.3 to 0.5 cm, whereas full fluorescence was found to under-predict flap survival by 2.5 cm. Additionally, histologic and ultrastructural changes were examined in rapidly expanded skin from the hip region in three pigs. The only microscopic change noted between control and experimental flaps was dilated capillaries in the dermis of expanded skin, which was noted by electron microscopy. Collagen and elastic tissue changes were not demonstrated in rapidly expanded pig skin by electron microscopy, direct immunofluorescence, collagen, and elastic tissue stains. (OTOLARYNGOL HEAD NECK SURG 1993;109: 926-32.)

The concept of tissue expansion was first reported by Neumann¹ in 1957 when he used an inflated rubber balloon for skin expansion over a 2-month period to reconstruct an avulsed auricle. Approximately 20 years later, Radovan^{2,3} refined skin expansion techniques for breast reconstruction and correction of adjacent defects, again using controlled or long-term expansion. Subsequently, it has been shown by Cherry et al.⁴ that controlled expansion for 5 weeks before creation of a random cutaneous flap results in increased survival length, similar to that with a bipedicle delay flap. Sasaki and Pang⁵ revealed an

increase in capillary blood flow in skin gradually expanded over 4 to 5 weeks, as well as in delayed flaps when compared to nondelayed control flaps. The most significant changes in soft tissue as a result of controlled expansion occur in the dermis and adipose layer, where a decrease in overall thickness occurs.^{6,8} There is considerable angiogenesis and neovascularization, leading to increased flap survival.

However, inherent disadvantages to conventional controlled tissue expansion exist. There is a need for two operations—one for insertion of the expander and another to remove it and complete the reconstruction. Controlled expansion can lead to several months of cosmetic and functional deformities associated with the buried expanders and their valves, as well as possible implant exposure and infection. Also, multiple office visits are required to infuse the saline solution into the expander, often on a weekly or bi-weekly basis over a several-week period. In 1987, Sasaki⁹ reported 120 cases in which he used rapid intraoperative tissue expansion, or what he termed intraoperative sustained limited expansion, 50 cases of which were in the head and neck. Several reports using this technique have subsequently been published.¹⁰⁻¹⁴ This technique takes advantage of the skin's ability to immediately stretch and increase in surface area, allowing for recruitment of adjacent tissue to accomplish closure of soft tissue defects. The indication for rapid intraoperative tissue expansion lies in those cases in which a small amount of tissue gain allows the recon-

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structive surgeon to perform a simpler closure, or a closure with less tension that may not have been achieved without a small amount of extra tissue. This study was undertaken to: (1) assess the predictive value of flap survival with the fluorescein test in rapidly expanded, random pattern porcine skin flaps, and assess tissue gain compared to control nonexpanded flaps and (2) to study the microscopic and ultrastructural effects after rapid intraoperative tissue expansion.

METHODS AND MATERIAL

Ten domestic young male pigs, whose individual weights ranged from 16 to 24 kg, were studied. Two flaps (one expanded, one control) were harvested in four pigs. Six pigs were large enough to harvest four flaps (two expanded, two control). In total, thirty two dorsally based, random-pattern flaps measuring 4 x 15 cm were divided into two groups. Group 1 consisted of flaps undergoing rapid cyclic intraoperative expansion and Group 2 contained control flaps to compare tissue gain and flap viability. All flaps were designed to necrose distally on the basis of their excessive length-to-width ratio. Care was taken to always place the control flap directly opposite the experimental flap, to ensure symmetry of flap design. The flaps were elevated in the subcutaneous plane, superficial to the panniculus carnosus. All surgery was done using halothane anesthesia, with no injection of vasoconstrictors. The protocol was approved by the Animal Care Department at the Oregon Health Sciences University and all procedures were performed in the Animal Care Department.

Part I

Once flaps were accurately marked with ink, a 5 cm incision was made along the dorsal aspect of the vertical limb of the experimental flap and a 250cc tissue expander was inserted into a pocket between the subcutaneous tissue and the deep fascia. The flap underwent three cycles of expansion, each consisting of 10 minutes of inflation with 5 minutes of deflation (Fig. 1). The volumes injected into the expanders increased with each cycle (100cc, 300cc, and 420cc). After the third expansion cycle, the flaps were elevated before the balloon was deflated. The experimental flaps were elevated and trimmed appropriately to fit into the recipient bed with no tension and sewn in place with a simple, running 4-0 nylon suture. The control flap was elevated simultaneously and sutured in place in an identical fashion. The length of tissue gain for the expanded flap vs. control flap was measured. Five cc of 10% fluorescein was then injected intravenously. Twenty minutes later the length of full and partial fluorescence in expanded flaps was assessed under ultraviolet A (UVA) light and both points were tattooed with indelible ink. Full fluorescence (FF) was defined as the longest flap length in which the entire flap width fluoresced brightly.

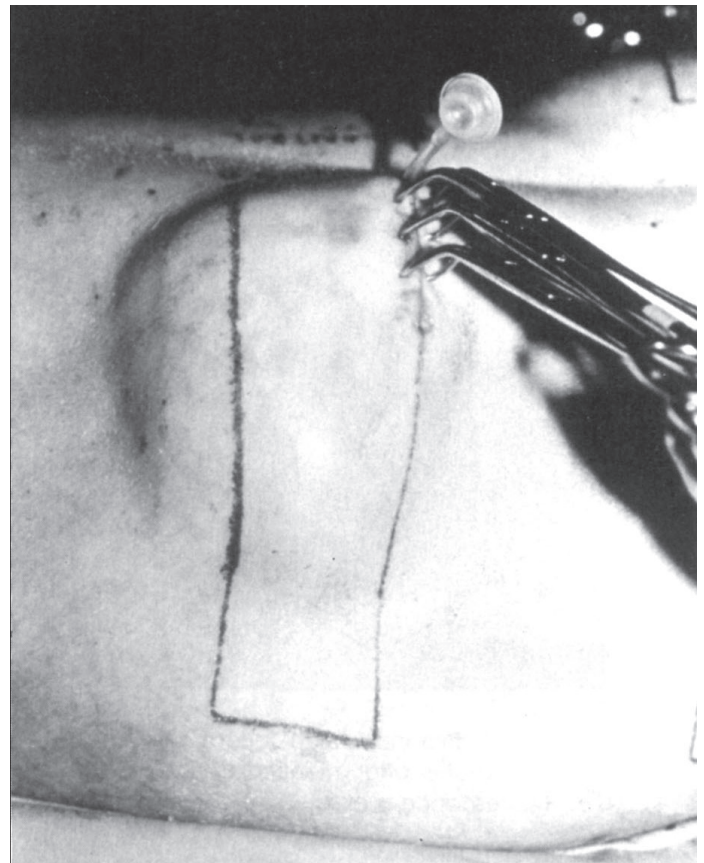


Fig. 1. Expanded flap undergoing cyclic expansion with 250 cc tissue expander.

Partial fluorescence (PF) was the longest flap length showing any fluorescence at all (spotty fluorescence) (Fig. 2). Seven days later the viability of the expanded flaps was assessed and compared to the level predicted by FF and PF.

The strength of the relation between the length of full and partial fluorescence and the length of expanded flap surviving at 7 days was assessed with the Pearson product-moment correlation coefficient. The determination of the best predictor and the corresponding equation was performed with a least squares multiple linear regression analysis using a forward stepwise variable selection procedure. All p values are two-sided.

Part II

Three pigs from part I were used to study the histologic and ultrastructural changes in rapidly expanded pig skin. Three 4 mm punch biopsies were obtained from nonexpanded hip skin for controls. One biopsy was processed for histopathology, one for direct immunofluorescence, and one for electron microscopy. An incision was then made through the biopsy sites and subcutaneous pocket was developed. Cyclic rapid intraoperative expansion was performed using a 75cc Foley catheter balloon with successive inflation volumes of 60cc, 120cc, and 180cc, respectively, for each progressive expansion

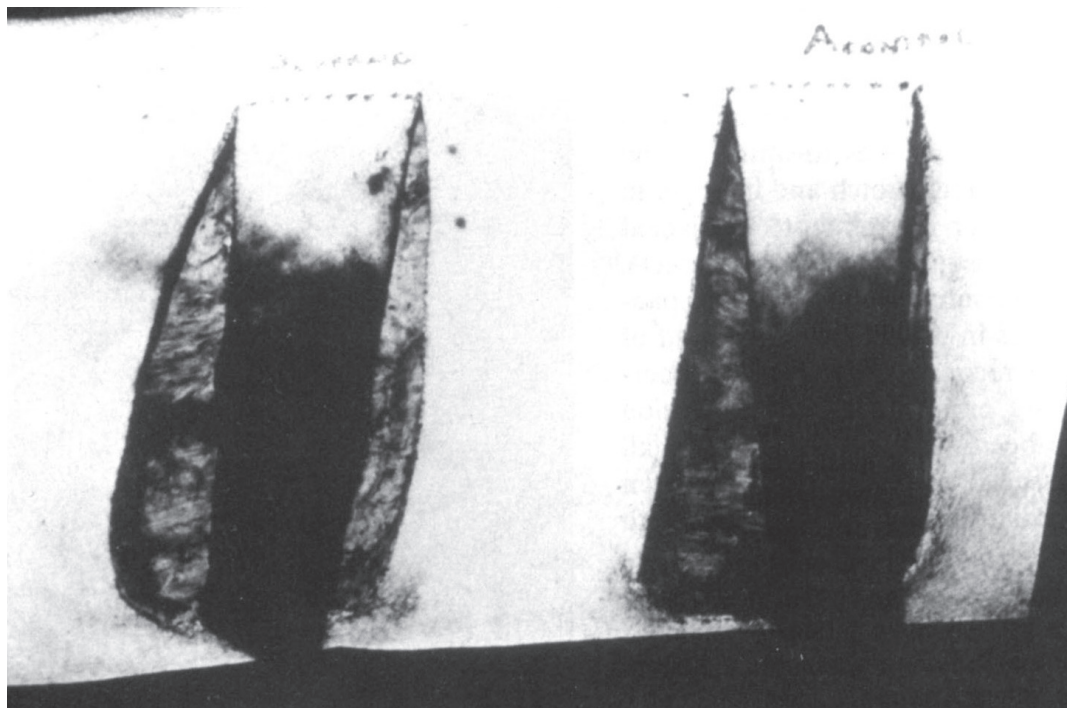


Fig. 2. Expanded flap (after cyclic expansion) and control flap photographed under UVA light 20 minutes after injection of 5 cc of 10% fluorescein. Full fluorescein is proximal (white), no fluorescence is distal (black), and partial fluorescence is in between.

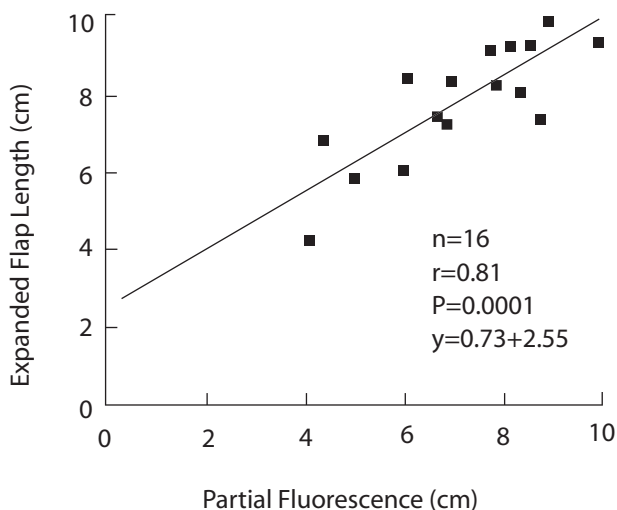


Fig. 3. Scattergram representation of partial fluorescence in expanded flaps.

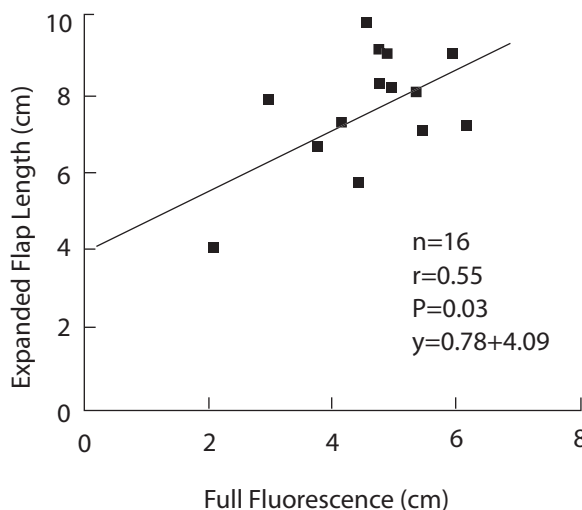


Fig. 4. Scattergram representation of full fluorescence in expanded flaps. Regression analysis revealed that both partial and full fluorescence significantly correlated with flap survival, but that partial fluorescence was the better predictor of the two variables.

cycle, using the inflation/deflation time sequence described previously. After the final deflation process, three 4 mm punch biopsies were immediately obtained over the expanded skin, adjacent to the control biopsies. The three sets of biopsies were used to compare rapidly expanded pig skin to nonexpanded control pig skin. One set of biopsy specimens was processed for routine hematoxylin-eosin stain and special stains for collagen (Masson Trichrome) and elastic tissue (Verhoff Van Gieson).¹⁵ The second set of specimens was processed for di-

rect immunofluorescence studies for collagen and elastic tissue. Four immunofluorescent antibodies were identified with known positive staining qualities in pig skin. Elastin and fibrillin antibodies were used to study elastic tissue while type VI and type VII collagen antibodies were used to study collagen.¹⁶ The third set of specimens was processed and stained with a 1% solution of osmium tetroxide for ultrastructural studies by electron microscopy.¹⁷

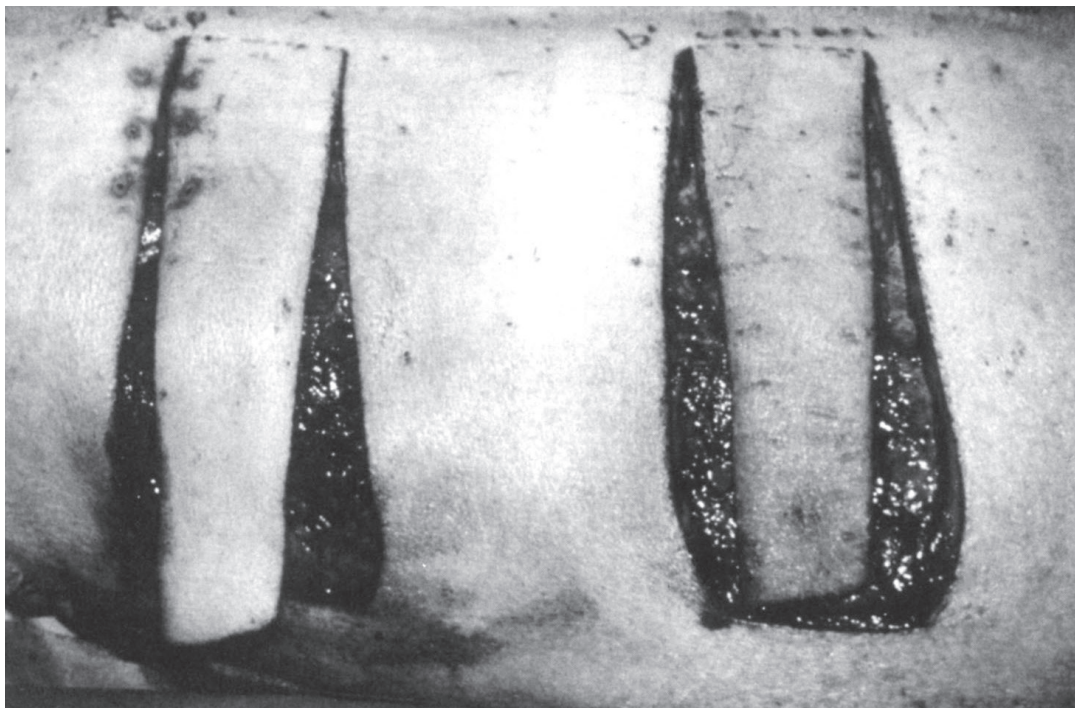


Fig. 5. Expanded flaps (left) resulted in a 5.3% tissue gain of original flap length after cyclic expansion, compared to control flaps (right).

RESULTS

Part I

Regression analysis revealed that partial and full fluorescence were both significantly correlated with surviving length of the expanded flap but that partial fluorescence was the better predictor of the two variables ($r = 0.81$; $p = 0.0001$ vs. $r = 0.55$; $p = 0.03$, respectively) (Figs. 3 and 4). Further, it was found that the inclusion of full fluorescence into the equation already containing partial fluorescence did not add significantly to the predictive ability. In addition, FF consistently underpredicted flap survival when compared to PF by approximately 2.5 cm. In general, PF underpredicted flap survival by 0.3 to 0.5 cm.

After intraoperative expansion, an average of 0.8 cm (5.3% of the original flap length) of the expanded flap required excision to fit appropriately into its recipient site without tension (Fig. 5). However, at 7 days postoperatively there was no significant difference in flap viability when comparison was made of control vs. rapidly expanded flaps (group 1 vs. group 2).

Part II

Biopsy set 1. There was no significant difference in collagen, elastic tissue, or skin thickness noted between group 1 (expanded flaps) and group 2 (control flaps) when evaluated by routine hematoxylin-eosin and special collagen and elastic tissue stains.

Biopsy set 2. A more detailed comparison of the control specimen, relative to the expanded specimens using immunofluorescent antibody staining of elastin, fibrillin, and collagen,

again revealed no significant collagen, elastic tissue, or skin thickness changes. There was no reorientation of the collagen fibers and no microfragmentation of the elastic tissue in the expanded tissue.

Biopsy set 3. The only ultrastructural change noted when expanded skin was compared to nonexpanded skin was dilated capillaries in the dermis of the expanded skin (Figs. 6 and 7). Again, no significant collagen, elastic tissue, or skin thickness differences were noted. No changes were noted at the basement membrane zone.

DISCUSSION

Controlled tissue expansion over a 5-week period results in an increase in capillary blood flow and in surface area similar to that seen in bipediced delay flaps.⁴ Numerous changes occur in the skin and soft tissues during controlled tissue expansion. The changes usually slowly return to their pre-expansion state after discontinuation of the expansion process. Tissue expansion over a 4- to 5-day period demonstrates an initial increase in blood flow, only to return to near-normal pre-expansion levels within 24 hours after deflation.¹⁸ Sasaki⁹ showed an initial decrease in blood flow during expansion with recovery to normal levels within a minute after deflation after rapid intraoperative tissue expansion in human beings. In Sasaki's 120 cases⁹ undergoing intraoperative expansion, 50 were in the head and neck, with a 2% incidence of marginal flap necrosis.



Fig. 6. Electron micrograph of control nonexpanded skin. Normal dermal capillary is shown (C) in the papillary dermis.



Fig. 7. Electron micrograph of micrograph of rapidly expanded skin. Papillary dermal capillaries (C) are greatly dilated.

Marginal flap necrosis can be minimized by a procedure that could accurately predict flap survival intraoperatively. The fluorescein test was used to evaluate viability because of its simplicity, accuracy, and low toxicity. Partial fluorescence in the expanded flaps was the most accurate predictor of viability. PF, however, generally underpredicted flap survival by 0.3 to 0.5 cm. FF underpredicted flap survival, on the average, by as much as 2.5 cm. This finding is similar to other studies demonstrating a 0.3 to 0.5 cm difference between surviving flap lengths and the prediction based on partial fluorescence.¹⁹

Several authors have noted that the most effective way to recruit extra tissue is by cyclic stretching of the skin.^{9,10,12,20-22} This forms the basis for multiple expansion cycles used during rapid intraoperative tissue expansion. Hoffman and Baker¹² have observed a 15% to 20% increase in forehead flap width in human skin undergoing immediate intraoperative expansion. Machida et al.¹¹ showed an average gain in length of approximately 14%. Sasaki⁹ has noted 0.5 cm to 2.5 cm tissue gain after intraoperative expansion in human skin, depending on the site of expansion. In this study with pigs, an average tissue gain of 0.8 cm (5.3% of the original flap length) was noted. The de-

crease in the amount of tissue gain in this study may be partially explained by the use of pig skin vs. human skin. Pig skin is the animal model that most closely resembles human skin in terms of elastic tissue and other qualities. Still, the elastic tissue content in pig skin is only about 10% of that found in human skin.

The amount of tissue gained by conventional controlled tissue expansion is certainly much greater than by rapid expansion. During conventional expansion numerous biologic events result in genesis of new tissue. These events, collectively known as biologic tissue creep, account for the majority of tissue gained during conventional expansion. In contrast to conventional expansion, the skin's ability to immediately stretch and increase in surface area during rapid intraoperative expansion is partly explained by a phenomenon termed mechanical tissue creep.^{20,21} Mechanical tissue creep is described as the lengthening of skin that occurs when it is stretched under a constant load. It is thought to be secondary to: (1) a relative dehydration of tissue due to displacement of fluid and ground substance, (2) a realignment of randomly positioned collagen fibers into a paralleled fashion, (3) elastic fiber microfragmen-

tation, and (4) migration of adjacent tissue into the field as a result a stretching force.

The only significant difference noted between the control and rapidly expanded pig skin was the marked dilated capillaries in the expanded skin noted by ultrastructural studies. This corresponds to the commonly seen vascular congestion (blue color) noted immediately postoperatively in an intraoperative expanded flap, and is probably a temporary phenomenon. There was no reorientation of the collagen fibers or microfragmentation of the elastic tissue. These findings support the hypothesis that the tissue gained from rapid expansion in pig skin is primarily the result of relative tissue dehydration resulting from displacement of ground substance and fluid and recruitment and migration of adjacent skin as a result of a stretching force. These findings are similar to those found by Sasaki⁹ in which he also found no significant routine histologic changes in any level of human skin or subcutaneous tissue after rapid cyclic expansion.

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