



Osmotically induced tissue expansion with hydrogels: a new dimension in tissue expansion? A preliminary report

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SUMMARY. The possibility of using an osmotically-driven system for effective tissue expansion has been verified. As a biocompatible and efficient system, a copolymer of methylmethacrylate and vinylpyrrolidone is used in animal research (rats). This hydrogel was able to generate, in vitro, a physical swelling pressure of approximately 235 mm Hg. The range of possible volume expansion in solutions of differing concentrations was 3 to 30 times its water-free gel form. In animal experiments a high biocompatibility and efficiency in tissue expansion can be observed.

INTRODUCTION

Skin expansion using subcutaneous silicone balloons is a well established method used in plastic surgery. However, there are still some shortcomings to this conventional method. Usually, the skin overlying the expander is repeatedly stretched to the extent that it is blanched (Sasaki and Pang, 1984). Due to intraluminal pressure spikes, decreased blood perfusion and hypoxia of the skin were found (Pietilä, 1990) that may result in necrosis and perforation of the skin and mucous membrane when expanded. Furthermore, the permeability of the silicone membrane led to perimplant seroma formation which, in connection with the percutaneous inflation technique, may increase the risk of infection.

Therefore, the idea of a pressure sensitive, self-inflating device using osmosis as the driving force for expansion seems to be obvious.

A self-inflating expander was first described by Austad and Rose (1982) using a permeable silicone balloon filled with a saturated NaCl-solution. Unfortunately this device had two major disadvantages: firstly, there were relatively long inflation periods of 8 to 14 weeks and, secondly, rupture of the balloon in the early stages of expansion resulted in necrosis of the overlying tissue caused by the hypertonic solution. To eliminate the disadvantages, it was necessary to develop a new device which is completely independent of the use of hypertonic solution. Such a system does not necessarily need to be a balloon-like device; it can also be a solid body, a so-called hydrogel.

But the first and essential question to answer is, does such a system generate enough physical pressure to expand tissues such as skin sufficiently and does it work when implanted in living tissue? In this investigation the efficacy of a hydrogel as a tissue expander and its biocompatibility was proved.

THEORY

Collagen and gelatin are natural hydrogels, synthetic ones are polymethacrylates, polyacrylamids and polyvinylpyrrolidone (Ratner and Hoffman, 1976). Hydrogels (HG) consists of 2 components: the polymer network, which is constant in quantity, and the aqueous component, which is variable (Refojo, 1976). The dry, water-free gel (exogel) absorbs water from the tissue extracellular space. The water uptake causes swelling of the gel. The amount of swelling can be expressed by the swelling coefficient (V_s/V_0) i.e. the volume of the swollen hydrogel (V_s) divided by the volume of the water-free exogel (V_0). The swelling pressure transferred to the surrounding tissue expands the tissue. The osmotic pressure (π) attributed to the polymer network is the driving force of the swelling. The swelling process distends the network and is opposed by the elastic contractility (p) of the stretched polymer chains (Refojo, 1976). The swelling pressure (P) of a non-ionic HG can be defined as:

$$P = \pi - p \quad (1)$$

At the swelling equilibrium:

$$\pi = p \quad (2)$$

and the swelling pressure (P) is zero.

Swelling pressure can be described as the pressure exerted by a gel when swelling is constrained but swelling solvent is available (Refojo, 1976). An empirical relationship for the swelling pressure

$$P = k c^n \quad (3)$$

was introduced by Posnjak (1912), where k and n are constants whose values are between 2 and 3, and c is the concentration of the polymer network. This formula demonstrates the rapid increase in the physical pressure (P) by higher concentrations of the

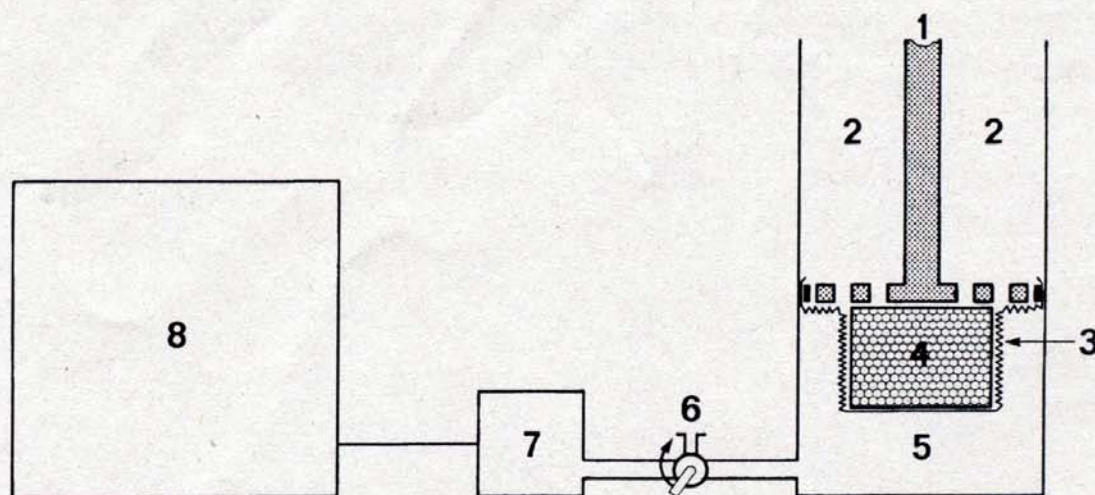


Fig. 1 – Schematic drawing of the pressure registration unit: (1) perforated piston with peripheral rubber sealing and lockable piston rod; (2) chamber with 0.9% NaCl-solution; (3) folded, waterproof membrane; (4) gel cylinder in expansion chamber; (5) measuring chamber; (6) triple way stopcock to evacuate the measuring chamber and approximate the membrane to the gel cylinder; (7) pressure transducer and (8) monitoring and recording system.

polymer network. P can also be increased by adding ionic components such as carboxyl groups to cross-linkers of the network, because the dissociation of the entrapped ions raises the osmotic pressure. HGs can be regarded as an osmotic system, where the permeable membrane and the solute are identical. Calculating of the osmotic pressure of such a gel is a complicated procedure, because concentration measurement of the solute is not as simple as in normal solutions. The hydration of a hydrogel cannot be completely compared with the characteristics of a sponge. Indeed, a fully hydrated gel releases water under mechanical pressure like a sponge does, but also a gel shrinks if the osmotic pressure (solute concentration) of the surrounding fluid increases and imbibes water if the fluid osmotic pressure decreases. This explains why the swelling coefficient of HGs is usually higher in distilled water than in a solution.

MATERIAL AND METHOD

A copolymer of methylmethacrylates (MMA) and N-vinyl-2-pyrrolidone (PV) (Geaflex 70, Wöhlk Corp., Kiel, Germany) was used as the basic material. In the first step of the experiment, which includes recording the *in vitro* swelling pressure and testing the biocompatibility of the expander material, a gel has been used with a linear expansion factor of 3.61 in 0.9% sodium chloride solution and 4.1 in Aqua dest. In a second step the, time-dependent expander volume and the gain of the expanded skin was investigated using a gel with an expansion coefficient of 20 to 30 (to be reported in a further paper) (Fig. 2).

In vitro swelling pressure

To obtain the *in vitro* swelling pressure generated by the gel, the exogel was equilibrated in isotonic sodium chloride solution. The *in vitro* swelling pressure of 10 gel cylinders was measured in a special chamber in

which 2 compartments were separated from each other by a folded, nonelastic and waterproof membrane which allows the gel to swell without absorption of pressure. The expansion chamber has on its opposite side a perforated rigid wall that allows the gel to absorb water from an additional space above but not to expand in this direction. The measuring chamber registered the differences in pressure and volume due to gel swelling via membrane elongation. A pressure transducer (Gould, Statham PD 23 ID, Oxnard, USA) converted the pressure into an electric signal which was monitored and recorded (Sirecust 610, Siemens Corp., Erlangen, Germany) (Fig. 1). After evacuation of the measuring chamber the system was zeroed prior to adding 0.9% NaCl-solution to the expansion chamber. From the median values, the time-dependent relationship of the pressure curve was calculated by a computer-assisted curve fitting program (SigmaPlot, Jandel Scientific, Corte Madera, USA).

Biocompatibility and verification of swelling

Biocompatibility and verification of the *in vivo* swelling was tested in rats. The research was registered and licensed by the state government of Lower Saxony, No. 504.42502/01-39.91.

Water-free gel cylinders weighing 1.5 g and with a diameter of 1.4 mm and a height of 0.9 mm (volume = 1.4 cm³) were implanted on the dorsal surface of 20 adult Wistar rats (average weight = 200 g). Prior to the insertion of the expanders all animals were anaesthetised by a combination of Rampun (6 mg/kg) and Ketanest (75 mg/kg). After a 2.5 cm incision on the middle of the back, subcutaneous tunnelling in a caudal and cranial direction was performed and 2 cylinders were placed between the dorsal fascia and panniculus muscle, one in the cranial third and one in the caudal third of the back. The wounds were closed with 4-0 polyamid sutures. The skin area overlying the expanders was tattooed with India ink.

The 5 animals, in a single step investigation, were sacrificed after 84 days. Parts of normal, unexpanded and expanded skin and the underlying fascia and muscle of the 10 expanders were taken for histological evaluation. All biopsies were fixed in buffered formalin solution (4% formaldehyde) for 24 h and dehydrated in a graded ethanol series. Following dehydration, the tissue was embedded in paraffin. From these paraffin blocks, sections of 3 to 5 μm in thickness were made and stained with haematoxylin/eosin, toluidine blue, cresyl, elastica and Goldner methods.

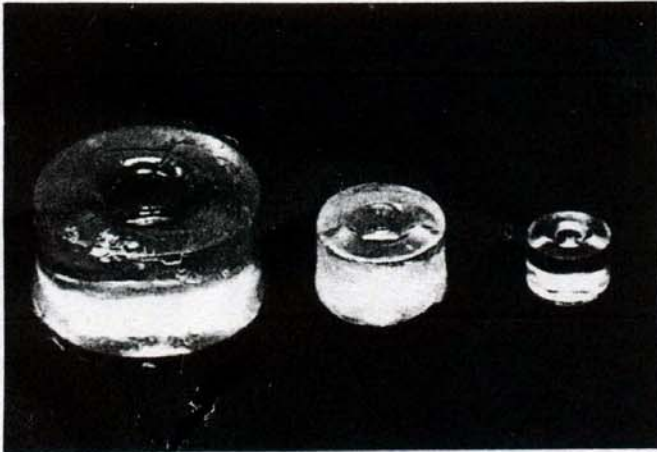


Fig. 2 - Gels with a water content of 96%, 70% and the water-free exogel equivalent of a swelling ratio of about 22, 4 and 0.

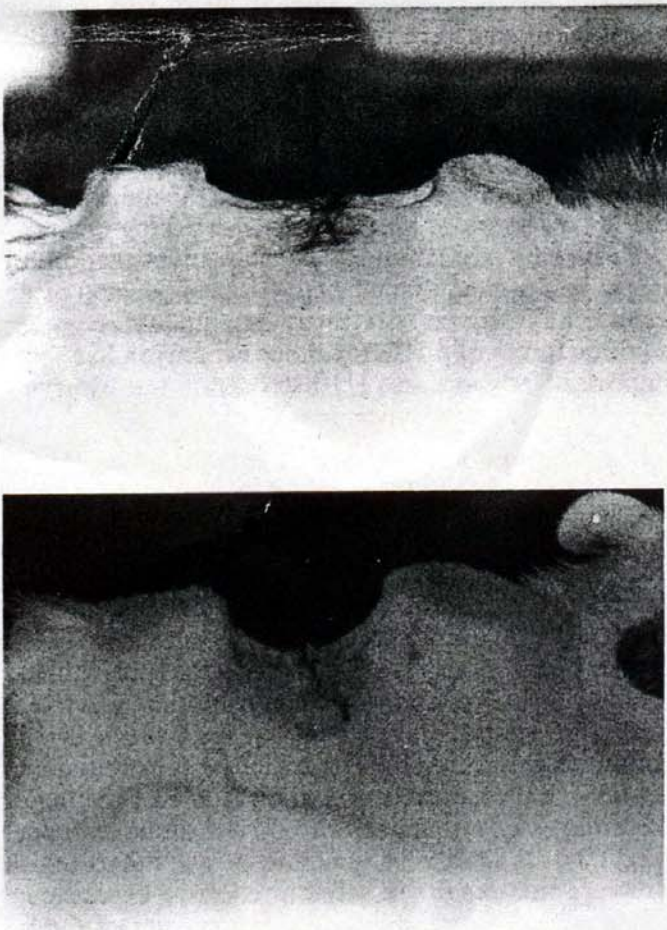


Fig. 3 - Exogel (top) after implantation and the swelling hydrogel 3 days postoperatively (bottom) in situ.

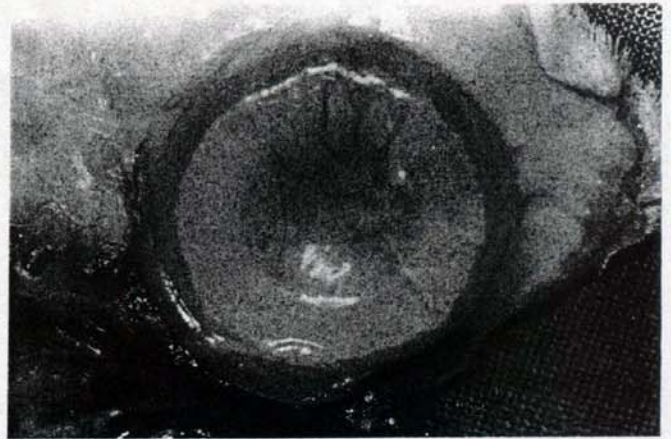


Fig. 4 - Specimen with skin and fibrous capsule containing an expander with a swelling coefficient of 5.5, 11 days postoperatively. The skin was dissected from the capsule to show the effect of suction.

RESULTS

The in vitro swelling pressure

After 72 h the hydrogel generated a maximal pressure of 235 mm Hg. Further equilibration time did not result in a change of the pressure values. From the median values the following relationship was calculated by a computer assisted curve fitting program.

$$P = 221.7 (1 - e^{-0.16t}) + 1.2 \quad (4)$$

This exponential function characterizes the time-dependent development of the swelling pressure and is derived from its basic function

$$P = P_{\max}(1 - e^{-bt}) + P_0 \quad (5)$$

where the swelling pressure (P) is a function of the time (t) and P_{\max} is the maximal generated swelling pressure and P_0 the pressure at the time $t = 0$ (Fig. 2).

Verification of swelling and biocompatibility

Clinical trial

In all 5 cases, no clinical signs of inflammation, perforation or loss of the expander were observed. After 2 days postoperatively the skin was relaxed but closely attached to the gel cylinders during the whole period of the investigation. The volume of the expanders increases between 250 and 300% (Figs 4 and 5).

Histology

Macroscopically, there was no remarkable difference in the texture of expanded and normal skin. The expanded skin was tightly related to the gel cylinder, which could be easily removed after incision and was not fixed to the tissue.

Compared with the rats normal skin there was only an insubstantial change in the thickness of the epidermis, dermis and subcutaneous layer in all slides taken 84 days postoperatively. The gel cylinder was surrounded by a small dense fibrous capsule in which multinucleated giant cells and other inflammatory

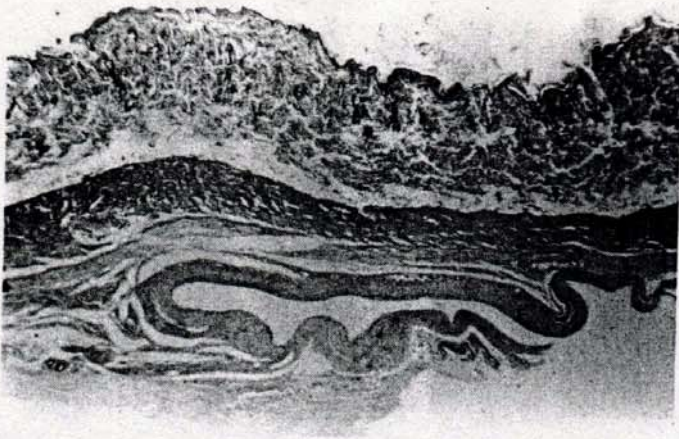


Fig. 5 – Cross section of skin with the panniculus muscle and the fibrous capsule (middle of the picture) 84 days postoperatively; HE staining. Note: the normal muscle thickness and stretching of the muscle at the boundary of the expanded tissue area (middle, right).

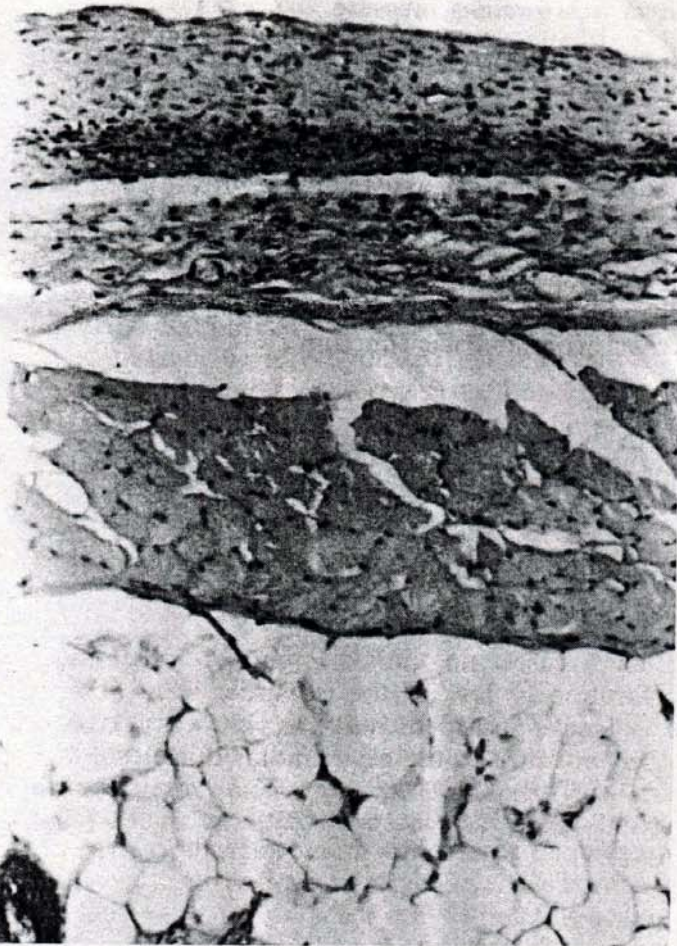


Fig. 6 – Fibrous tissue capsule, panniculus carnosus muscle and subcutaneous fat of the expanded skin 84 days postoperatively; HE staining.

muscle and consists of dense connective tissue, collagen fibres and a higher amount of blood vessels than the inner layer (Fig. 6).

DISCUSSION

HGs may have a great potential as osmotically driven self-inflatable devices in tissue expansion because they have some substantial advantages in comparison with conventional balloon-like devices. As observed by the *in vitro* measuring of the swelling pressure, the HG system is able to generate the necessary physical force for efficient tissue expansion.

In discussing the biocompatibility of polymers a number of problems are encountered in properly defining the terminology used to describe the response of a living system to an implanted foreign material (Ratner and Hoffman, 1976). The term 'tissue compatible', for the purpose of an expanding device, will be used in the sense of Ratner and Hoffman (1976) to describe a material, that shows a normal acute inflammatory reaction and then rapidly 'heals in' to a passive state wherein the implant is surrounded by a thin, uniform fibrous capsule in which multinucleated giant cells and other inflammatory cells are generally absent. Concerning this definition, the copolymers of methacrylate derivatives and vinylpyrrolidone, as HGs, have been testified highly biocompatible by several authors (Höh, 1985; Nagaoka et al., 1990) reporting on contact lens material consisting of the same basic material. In accordance with our findings, the same histological structure of the fibrous capsule was also described by Pasyk et al. (1982) and Mustoe et al. (1989) for silicone balloon expanders. Lew and Fuseler (1991) have proved that the thickness of the capsule is related to the expander pressure. So, the thin capsule observed in our investigation may be the indication of the low pressure of an osmotically-working device.

Regarding the findings of this investigation, it might be assumed that efficient tissue expansion by an osmotically driven device is possible. Using the osmotic forces of a HG for tissue expansion has some important advantages: (1) necrosis of the overlying tissue caused by a hypertonic solution as mentioned by Austad and Rose can be avoided, because a HG is completely independent of a hypertonic solution; (2) accelerated expansion to shorten expansion time may be possible by infiltration of isotonic NaCl-solution adjacent to the device to increase the fluid available for expansion; (3) the implanted exogel is a solid body that can be manufactured in every shape and size. It is also much more resistant to physical forces than a balloon-like expander; (4) when osmotically active it sucks the tissue against itself so that the skin will follow its contour. This fact might be substantial and useful in ear reconstruction and (5) one of the major advantages of an osmotically driven tissue expander might be its ability to generate a low but constant pressure that results in safe but effective expansion.

The *in vitro* measured swelling pressure of the gel represents the initial pressure when the exogel starts to imbibe water. An equilibrium pressure of more than

cells were absent and it can be divided into an inner and outer layer. The inner layer adjacent to the expander was thicker than the outer one and consists of fibrocytes and collagen fibres and a few blood vessels. The outer capsule was attached to the hyperdermal connective tissue under the panniculus

200 mm Hg cannot be achieved in living tissue. The development of pressure in tissue is related to the ultrafiltration rate of extracellular fluid from the capillary blood vessels. If expander swelling pressure reaches the capillary blood pressure value (20–35 mm Hg for capillaries of a diameter of 5 to 12 μm (Lipowsky, 1987) ultrafiltration stops and the HG is not able to imbibe fluid from the extracellular space. References to pressure data for 'classical tissue expansion' range between 29 mm Hg for initial and 100 mm Hg for post inflation values (Maxwell and Falcone, 1992; Schmidt et al., 1991) depending on the inflation procedure and measurement.

The discontinuity of pressure and volume expansion of a self-inflating device in infinitesimal small steps allows the tissue to recover from the stress and normalize its function. The biomechanical equivalent of this fact is the skins relaxation, which, according to Mustoe et al. (1989), minimizes, in conventional tissue expansion, any tissue ischaemia and allows further inflation of the balloon and subsequent 'creep' of tissue and skin expansion.

Concerning these facts, a negative feedback mechanism of tissue reaction and swelling pressure of the device may be postulated, avoiding complications due to extensive tissue stretching and high expander pressure. This assumption correlates with clinical trials in the animal experiments. Unfortunately, we were not able to register the *in vivo* swelling pressure to verify the assumption of the feedback mechanism, so further research will be necessary.

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