Modulation of Tissue Ingrowth Into Porous High-Density Polyethylene Implants With Basic Fibroblast Growth Factor and Autologous Blood Clot

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**Objective:** To investigate the effect of direct application of biologic materials normally present in wounds (basic fibroblast growth factor [bFGF] and autologous blood clot [ABC]) to accelerate the bony and soft tissue ingrowth into porous high-density polyethylene implants.

**Methods:** We conducted a prospective, blinded animal histological study. Disks made of porous high-density polyethylene impregnated with bFGF or ABC were implanted into adult Sprague-Dawley rats in both subcutaneous and subperiosteal locations. Animals were killed and implants were harvested at 2, 4, and 10 weeks postimplantation and examined histologically for fibroblast invasion, collagen deposition, and inflammatory reaction. The results were compared with control (untreated) implants.

**Results:** As a group, the histological results showed significantly more fibroblasts within the ABC-treated implants than control implants or bFGF-treated implants. This difference in the number of fibroblasts between ABC-treated implants and bFGF-treated and control implants was also statistically significant 2 weeks after implantation.

**Conclusions:** At the concentration of bFGF of 1 µg/10 µL, no acceleration of tissue ingrowth into porous high-density polyethylene implants was noted. However, when porous high-density polyethylene implants were treated with ABC, the implants were invaded to a greater degree by soft tissue, particularly in the early postoperative period (first 2 weeks). Bioactive substances associated with the coagulation and platelet cascades present in the ABC may be responsible for this accelerated incorporation of the porous implant and may have clinical implications.
MATERIALS AND METHODS

Pathogen-free Sprague-Dawley rats weighing between 300 and 350 g (Charles River Labs, Wilmington, Mass) were chosen for this experiment based on their large size, which allowed several PHDPE disks (model 6330 [1.5-mm-thick] and model 7210 [0.85-mm-thick] Medpor sheets) to be surgically implanted in each animal. This study consisted of 21 animals divided into 3 groups based on the time from implantation to histological analysis: group 1 had 9 animals, group 2 had 7 animals, and group 3 had 5 animals.

SUBCUTANEOUS IMPLANT PLACEMENT

Each rat was anesthetized with sodium pentobarbital (50 mg/kg) intraperitoneally to allow a total anesthesia time of approximately 45 minutes. The backs of all animals were shaved, prepared with povidone-iodine (Betadine; Purdue Frederick, Norwalk, Conn), and draped. Three paramedian incisions were made on the back of each animal: 2 incisions were made on the left side and 1 incision on the right side. One PHDPE disk (1.5 mm thick and 10 mm in diameter) was inserted subcutaneously below the panniculus carnosus at each incision site, with each animal receiving 3 disks in the soft tissues of the back in distinct pockets separated by at least 2 cm (Figure 1).

Bleeding was not encountered in the dissection in the plane beneath the panniculus carnosus. One disk served as a control (untreated), a second disk was manually impregnated with ABC, and the third disk was soaked in bFGF (recombinant human bFGF; Intergen Co, Purchase, NY). The wounds were closed with surgical staples, which were removed on postoperative day 7.

SUBPERIOSTEAL IMPLANT PLACEMENT

In 15 animals (5 from each group), the posterior occiput was shaved and prepared with povidone-iodine. A transverse incision was made at the vertex of the cranium to allow access to the pericranium. The pericranium was incised and reflected. One PHDPE disk (0.85 mm thick and 0.3 mm in diameter) was inserted beneath the pericranium, and the wound was closed in one layer using absorbable sutures. In each group, 2 animals received bFGF-treated disks, 2 animals received ABC-treated disks, and 1 animal received a control disk. Subperiosteal disks were prepared in the same fashion as the subcutaneous disks. All animals were tagged, placed in habitats, and given standard rat feed and water ad libitum.

PREPARATION OF bFGF

Lyophilized bFGF was reconstituted in 10 mmol/L Tris (pH 7.6) to a concentration of 1 µg/µL. The solution was further diluted to a concentration of 1 µg/10 µL using Tris buffer. The bFGF disks were prepared by placing a 10-µL aliquot of bFGF solution (1 µg/10 µL) on the implant. If the bFGF solution seeped over the edge of the implant, it was aspired with a micropipette and placed on the disk after implantation and prior to closure.

PREPARATION OF ABC

The hair on the ventral surface of the neck was shaved over the area of the left internal jugular vein. Percutaneous access to the vein was attempted with a 22-gauge needle. If this was unsuccessful, the vein was accessed by cutting directly down into the area. A total of 0.3 mL of the animal’s blood was withdrawn, mixed with 0.5 mL of topical thrombin, and placed in a sterile nonporous container. Once congealed, the clot was manually impregnated into the implant pores under sterile conditions. Excess clot at the implant surface was removed.

HISTOLOGICAL ANALYSIS

The animals were examined daily for signs of infection, exposure, or rejection of the implants. Group 1 animals were killed at 2 weeks, group 2 at 4 weeks, and group 3 at 10 weeks postimplantation, using a lethal dose of sodium pentobarbital. The PHDPE disks were removed along with the surrounding soft tissue or skeletal tissue.

The specimens were preserved in formalin, stained with hematoxylin-eosin, and sectioned for histological analysis. A section of each slide containing the implant material and tissue was viewed at low power (×10) and high power (×40). Each histological slide of an implant was examined at 3 separate areas. During each viewing, the total and mean number of fibroblasts, blood vessels, and multinucleated giant cells per high-power field and extent of collagen deposition were recorded. Collagen deposition was estimated as a percentage of the viewing field. Each specimen was reviewed by 2 examiners (P. S. and R. C.) on 2 separate occasions and the average was used; examiners were blinded to the treatment given to each specimen.

This study was approved by the Animal Care Committee of New York Medical College, Valhalla.

RESULTS

SUBCUTANEOUS IMPLANTS

Implant extrusion or infection was not noted in any of the animals. At the time the implants were harvested, all the disks were anchored within the surrounding soft tis-
Subcutaneous and subperiosteal implant location in the rat. Implants were disks of porous high-density polyethylene (PHDPE) treated with either autologous blood clot (ABC) or basic fibroblast growth factor (bFGF) or untreated (control). Five rats received one of the implant types above in the pericranial region.}

Of the 63 disks implanted in the subcutaneous tissues, 60 were available for histological examination. Three disks (2 in group 1 and 1 in group 3) were damaged during laboratory processing and were unreadable. The findings of the histological analysis revealed fibrous connective tissue coursing throughout the implant pores. Blood vessels were seen within the implant pores, and no acute inflammatory changes were noted.

Comparing the histological results of all the disks with one another, ABC-treated disks had an increased number of fibroblasts compared with bFGF-treated disks and control disks (analysis of variance, \( P < .05 \)). Likewise, the histological results showed an increase in collagen deposition in ABC-treated disks compared with the deposition found in the control disks and bFGF-treated disks (analysis of variance, \( P < .05 \)). Significant differences in number of blood vessels and multinucleated giant cells were not noted among any of the disks (Figure 2 and Figure 3).

Implants from each harvest time were also analyzed separately (Figures 4, 5, and 6). The disks examined 2 weeks after implantation (group 1) had a mean of 49.3 fibroblasts per high-power field for the ABC-treated disks, 33.5 for the control disks, and 32.2 for the bFGF-treated disks. The differences in the mean number of fibroblasts per high-power field between ABC-treated and control disks and between ABC-treated and bFGF-treated disks were statistically significant (Tukey HSD test, \( P < .05 \)). The collagen deposition was estimated to be a mean of 65.5% in the group 1 ABC-treated disks, whereas it was 48.1% and 47.5% for the control and bFGF-treated dishes, respectively. The differences between ABC-treated and control disks and between ABC-treated and bFGF-treated disks were statistically significant (Tukey HSD test, \( P < .05 \)). The collagen deposition was estimated to be a mean of 65.5% in the group 1 ABC-treated disks, whereas it was 48.1% and 47.5% for the control and bFGF-treated disks, respectively. The differences between ABC-treated and control disks and between ABC-treated and bFGF-treated disks were statistically significant (Tukey HSD test, \( P < .05 \)).

For the remaining 2 harvest times (groups 2 and 3), histological results of the ABC-treated disks showed an increase in fibroblast ingrowth and collagen deposition compared with the control and bFGF-treated disks; however, these differences were not statistically significant (\( P > .05 \); Figures 2 and 3).

**SUBPERIOSTEAL IMPLANTS**

Implant extrusion or infection did not occur in any of the animals. Each of the 15 disks inserted into subperiosteal pockets was available for histological examination. In 7 animals, the disks were loose in the soft tissue of the scalp. Soft tissue ingrowth occurred; however, the disks were not adjacent to the cranium. The remaining 8 disks stayed in the subperiosteal envelope, adjacent to the cranium.

Of the 5 disks harvested 2 weeks after surgery (group 1), 4 remained adjacent to the cranium. The control disk and one of the ABC-treated disks demonstrated abundant bony ingrowth. One of the bFGF-treated disks and one of the ABC-treated disks demonstrated minimal bony ingrowth.

Of the 5 disks harvested 4 weeks after surgery (group 2), 3 were in the subperiosteal pocket. The control disk and 1 bFGF-treated disk demonstrated bony ingrowth, and 1 ABC-treated disk revealed minimal ingrowth.

Of the 5 disks harvested 10 weeks after surgery (group 3), 1 ABC-treated disk remained in the subperiosteal pocket and demonstrated bony ingrowth. (Figure 7).
The search for an ideal implant is motivated by a desire to reconstruct the facial framework in a safe and efficient manner. Autologous tissue is perfectly suited to reconstruction; however, it often requires a second operative site. Harvesting this tissue often entails a risk, which may be unacceptable to both the patient and the surgeon. Alloplastics offer the surgeon a readily available material but are notorious for extruding months or years after implantation. Degradation of the implant has also been a problem with some materials.\textsuperscript{10,11} However, not all alloplasts are alike and, in many cases, an acceptable compromise can be found between risks and benefits.

Porous high-density polyethylene has been used in facial reconstruction for more than 50 years, and it has been shown to be well tolerated.\textsuperscript{12} It is a porous (mean pore, 150 µm) alloplastic material that is not degraded in vivo and allows for fibrous ingrowth of soft tissue. Also, bony ingrowth has been well documented in animal models.\textsuperscript{13,14} Once ingrowth occurs, the implant is stabilized and implant infection and extrusion is less likely.\textsuperscript{15} Sclafani et al\textsuperscript{2} demonstrated the ability of exposed PHDPE implants to heal by second intention and to tolerate skin grafting once fibrovascular ingrowth had occurred. In the same animal model, they found a relative resistance to infection among implants with fibrovascular ingrowth. In a review of the use of PHDPE in 187 patients undergoing nasal reconstruction, Romo et al\textsuperscript{5} found a complication rate of less than 2.4%. In 5 of the patients, implants were removed secondary to infection or extrusion and a paucity of fibrovascular ingrowth was noted. Clearly, PHDPE is capable of supporting soft tissue.

**Figure 4.** Representative histological sections of soft tissue implants harvested 2 weeks after implantation. A, Disk treated with basic fibroblast growth factor. B, Disk treated with autologous blood clot (ABC). C, Untreated (control) disk. Note the increased number of fibroblasts and the denser deposition of collagen in the ABC-treated disk (hematoxylin-eosin, original magnification ×40).

**Figure 5.** Representative histological sections of soft tissue implants harvested 4 weeks after implantation. A, Disk treated with basic fibroblast growth factor. B, Disk treated with autologous blood clot. C, Untreated (control) disk (hematoxylin-eosin, original magnification ×40).
tissue and/or bony ingrowth. However, in the instances when ingrowth does not occur, the implant is vulnerable to infection and extrusion.

Manipulation of the healing wound has been a practice of surgeons throughout history. Ambrose Pare noted improved healing and survival in soldiers whose vascular injuries were ligated compared with soldiers who underwent cauterization with hot oil. Furthermore, Joseph Lister, MD, advanced the practice of medicine with his emphasis on antiseptic treatment of wounds. With increasingly effective antibiotics and strict practice of sterile techniques, wound healing was allowed to progress uninhibited. Researchers began to focus their efforts on understanding and controlling the molecular environment of the wound with the goal of accelerating healing and increasing the overall strength of the wound. Growth factors emerged as the clinical tools for this job. Despite the identification and replication of many of these peptides, a precise understanding of their behavior eludes us. Nevertheless, a somewhat chaotic method of adding a growth factor to a wound and observing the result has begun.

Unfortunately, dosages and long-term mutagenic potential of growth factors have not been thoroughly elucidated in humans.

Basic fibroblast growth factor is a peptide, 154 amino acids in length, that acts as a potent mitogen toward a wide range of cell types, including fibroblasts, osteoblasts, and endothelial cells. It is one of several growth factors present in human tissue after a wound occurs. Vogt et al documented an increase in levels of bFGF in split-thickness skin graft donor sites vs serum levels in 16 patients. Nevertheless, the precise concentration of bFGF in the healing wound of a human is not known. It follows that if we do not know the concentration of bFGF in a normal wound we cannot be certain of either the dose needed to augment healing or whether an overdose is detrimental. Limited data from human studies suggest that metaplasia does not occur after the use of a growth factor in a healing wound. For instance, in a study by Brown et al, biopsy specimens of wounds 1 year after treatment with epidermal growth factor showed no evidence of metaplasia.

Animal studies have revealed a broad range of doses and effectiveness of bFGF. A review of 7 studies using bFGF in Sprague-Dawley rats revealed an improvement in bFGF-treated animals at 7 different doses. The methods of delivery also varied from topical application to subcutaneous injection to time-released distribution from a hyaluronate gel carrier system. In our study, bFGF-treated disks did not demonstrate a statistically significant increase in several parameters of wound healing: fibroblast invasion, angiogenesis, and collagen deposition. Several factors may explain this observation. First, we administered bFGF at the time of wounding and did not apply it subsequently. The half-life of most angiogenic growth factors is several hours; therefore, continuous infiltration may be necessary to induce measurable changes. In a single-infusion model, Hayward et al failed to observe angiogenesis after injection of bFGF into the panniculus carnosus of Sprague-Dawley rats, but they did observe marked proliferation of fibroblasts in the connective tissue stroma of the bFGF-treated animals vs control animals. Unfortunately, they did not quantify the fibroblast response,
so it is unclear if this was statistically significant. Phillips et al. used a single-infusion model in rats with diabetes mellitus and reported statistically significant increases in wound strength in bFGF-treated animals.

Second, we used a dose of bFGF of 1 µg/10 µL in all animals. This concentration may have been either more or less than an effective dose compared with other reports. Wang and Aspensberg, using a bone chamber model in rat tibias, reported an increase in bony ingrowth in bFGF-treated animals vs control animals after application of a bFGF dose of 0.04 µg; however, no difference between control and treated animals was noted at a dose of 1.0 µg. Although we used a dose that others have reported effective, the use of a variety of doses in our experiment might have clarified this issue of an effective dose.

Third, neovascularization is histologically detectable on the third day postinjury under normal conditions. In a single-infusion model, the bFGF might function more effectively if it is injected at this time. But bFGF is not simply an angiogenic growth factor, it also recruits fibroblasts to the wound. Fibroblasts appear on the third day of healing, so presumably the chemotactic stimuli are present before the cells appear. Therefore, application of an appropriate dose of a known chemotactic agent, such as bFGF, should theoretically augment this process.

Finally, we did not assess the biological activity of the bFGF used in this experiment. It is conceivable that the lack of effectiveness of bFGF was related to steps in its reconstitution or in the lyophilized bFGF used in this study.

Although there have been reports of abnormal healing after application of growth factors, untoward effects were not observed in this model. The bFGF did not promote an inflammatory reaction nor did it enhance the multinucleated giant cell response to PHDPE.

In addition to the use of bFGF, we enlisted ABC, a naturally occurring compound, in its naturally occurring dose. The formation of a blood clot is critical not only for hemostasis but also for the initiation of healing. Polymerized fibrin forms the matrix over which wound healing takes place. However, other molecules present during clot formation, such as thrombin, transforming growth factor-β, and platelet-derived growth factor, appear to function as chemotactic stimuli. The precise mechanism has not been clarified, but several authors reported improved healing in both animal models and surgically treated patients in whom an ABC was applied to the wound. While a large blood clot disrupts clinical healing because of the pressure generated within the closed space and the resulting ischemia, with the direct application of a blood clot, normal healing can be augmented beyond the healing that inevitably occurs within a “dry” postoperative wound. In fact, the presence of some degree of clot seems to be necessary for proper healing. In an animal model, Okamoto et al. demonstrated how removal of the blood clot in a tooth socket immediately after suturing the wound resulted in a pronounced delay in healing. In our model, we noted a statistically significant increase in the presence of fibroblasts and collagen in the wounds of animals treated with an ABC. We introduced topical thrombin into the model because it helped to create a clinically more adherent clot.

Ingrowth of bone was not observed consistently in our model. This inconsistency may be related to the lack of effectiveness of our dose of bFGF or it may be a result of lack of fixation of the implant to the cranium. The subperiosteal pocket was not large, but at harvesting it appeared that several of the disks did not remain within the pockets. The migration of implants may be explained by the skulls of the Sprague-Dawley rats, which lacked large flat surfaces and had an uneven contour, preventing direct apposition of bone to implant necessary for tissue ingrowth.

In the presence of an ABC, there was an increase in fibroblast ingrowth and collagen deposition into a PHDPE disk compared with control disks and bFGF-treated disks. This improved fibroblast invasion and collagen deposition occurs early, 2 weeks after implantation, and the difference appears to decline with increasing time after wounding. Whether this creates a more secure and infection-free environment for the implant could not be determined. However, this early time is when a porous implant is most vulnerable to extrusion and infection. Accelerating ingrowth into these implants by treating them with an ABC may diminish the complication rates associated with the PHDPE material.

Our current understanding of growth factors is not yet sophisticated enough to reliably use them with facial implants. While they are promising clinical tools, growth factors require further study with respect to mechanism, dosage, and timing of administration before they can be used to manipulate healing wounds.

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