



Acceleration of Medpor implant fibrovascularization with local vascular endothelial growth-factor injections: An experimental study

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ABSTRACT

Objective: Medpor is a biocompatible, high-density porous polyethylene implant that is used for multiple indications in plastic surgery. The most frequent complications associated with the Medpor implant are infection and implant exposure. The primary cause of these complications is poor fibrovascularization of the Medpor implant and poor nourishment of the overlying skin. The present experimental study aimed to determine whether vascular endothelial growth factor (VEGF) could accelerate and increase Medpor implant fibrovascularization in vivo, and thereby improve local nourishment and prevent complications.

Materials and Methods: The Medpor implant was inserted under the dorsal skin area in 40 Sprague-Dawley rats. 20 rats receiving local VEGF injections comprised the study group. The control group received saline injections. Fibrovascularization of the Medpor implants was compared.

Results: In the rats injected with VEGF, the Medpor implant fibrovascularized faster, and there were more newly formed blood vessels, as compared with those in the control group.

Conclusion: These findings have led to our use of VEGF-like agents that the accelerate angiogenesis in the Medpor implant as a means to reduce the incidence of such complications as infection and implant exposure.

Key words: Angiogenesis, fibrovascularization, Medpor, vascular endothelial growth factor

Introduction

Medpor (high-density porous polyethylene) is an implant made of alloplastic material with pores 100-250 μm in diameter that has been used safely for many years for multiple indications in plastic surgery [1,2]. It differs from other alloplastic materials due to its larger pores, which facilitate migration of the surrounding soft tissue into the Medpor implant, thus creating a more solid scaffold between itself and the surrounding tissue

[3]. Its non-immunogenic structure allows it to continue functioning for years without causing a foreign body reaction [4]. Yet, even though the Medpor implant has many advantages, it is still associated with such complications as infection and implant exposure [4-7].

Growth factors are pharmacological agents that have been used in a wide range of plastic surgery experimental studies. These include basic fibroblast growth factor, platelet-derived growth factor, endothelial cell

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growth factor, and vascular endothelial growth factor (VEGF). Erdmann et al. reported that VEGF levels increase autogenously in ischemic tissues, and that ischemic tissue produces VEGF as a means to prevent further ischemia and for self-protection [8]. Subsequently, VEGF has been used to increase the viability of various ischemic skin and muscular flaps, and was shown to be effective because it stimulates new vascular formation [9]. Complications associated with the Medpor implant include infection and exposure, which generally occur due to a lack of local nourishment. The present experimental study aimed to determine whether VEGF could accelerate and increase Medpor implant fibrovascularization in vivo, and thereby improve local nourishment and prevent complications.

Materials and Methods

The study included 40 female Sprague-Dawley rats weighing 250-300 g. The study protocol was approved by the Local Ethics Committee. The study was funded in full by the Ankara University Scientific Research Projects Department. The rats were fed standard laboratory feed, and water was supplied ad libitum; a 12-h light-dark cycle was maintained. The rats were obtained from the Gulhane Military Medical Academy Animal Laboratory, and then were kept in cages with their own families.

Anesthesia was achieved using intraperitoneal ketamine (100 mg/kg) and xylazine (20 mg/kg). The rats were divided into two groups (the study group and the control group), each group consisting of 20 rats. Rats that died due to anesthesia or those that were eliminated from the study for various other reasons were immediately replaced.

The middle section of each rat's dorsal area - between the interscapular line and sacral area - was shaved using an electric razor. Then, a 1.5-2 cm horizontal incision penetrating through the skin and subcutaneous tissue was made. A high-density porous polyethylene implant (Medpor® [Howmedica Osteonics Corp., Newnan, GA, USA, catalog number 6330]) measuring 38 mm × 50 mm × 1.5 mm was preoperatively cut into pieces approximately 7 mm × 10 mm × 1.5 mm, each of which was placed inside the pouches constructed superior to the incision using a pair of dissection scissors. To secure the Medpor implant and prevent

it from slipping beneath the incision, the underlying muscle's fascia was also penetrated while suturing the incision with 4-0 prolene.

The rats were isolated until fully awake, and then were returned to their pre-surgery cages. Post surgery, rats in the control group received 1 mL of physiological saline injected subcutaneously in the area overlying the Medpor implant, whereas those in the study group were injected with 1 µg of VEGF in the same area (Sigma-Aldrich, Inc., St. Louis, MO, USA, product number V7259). The product used was stored at -20°C and reconstituted in 10 mL of distilled water at a concentration of 1 µg/mL immediately before use. 1 mL of this solution was injected through the dorsal skin of the rat over the operation pocket area using a 30-gauge needle.

A rat from each group was euthanized on post-implantation days 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, and 34 via a high dose of anesthetics. Biopsy materials were obtained via excision of the Medpor implant and the layer of skin attached to it using 2 cm × 2 cm incisions that penetrated into the muscle tissue. Biopsy materials were stored in 10% formaldehyde solution. To prevent environmental contamination of the portion of the biopsy specimen above the surface of the formol solution, pathology containers were shaken up and down numerous times. Specimens prepared from the biopsy materials at the Ankara University Department of Pathology Laboratory were then sampled from the exact center of each graft following fixation with 10% formaldehyde. The 6 µm sections that were obtained after routine paraffin application were stained with hematoxylin-eosin, and then evaluated under a light microscope to observe tissue changes occurring between the graft's pores. During the evaluation of these materials, the soft tissue components of the Medpor implant's 1.5 mm thickness were taken into consideration. The encounter of the endothelial cells and fibroblasts migrating from both the top and bottom surfaces has been referred to as "bridging," and this has been considered a criterion in this study.

Results

Clinical Observation

The surgical procedure did not cause movement disorder in any of the rats. The rats in both groups were able to eat approximately 4 h post surgery, and weight

Table 1. Microscopic evaluation of the groups.

Post-implantation day of biopsy	Observations at the edge of the Medpor implant	Study group		Control group	
		Bridging within the Medpor implant	Quantity of vascular structures within the bridging (n/ μm^2)	Bridging within the Medpor implant	Quantity of vascular structures within the bridging (n/ μm^2)
Day 3	Edema, vascular proliferation	Significant amount of free erythrocytes	-	-	-
Day 4	Edema, vascular proliferation	Significant amount of free erythrocytes	-	-	-
Day 5	Edema, vascular proliferation	+	3	-	-
Day 6	Edema, vascular proliferation	+	8	-	-
Day 7	Edema, vascular proliferation	+	14	-	-
Day 8	Edema, vascular proliferation	+	24	-	-
Day 9	Edema, vascular proliferation	+	35	-	-
Day 10	Edema, vascular proliferation	+	33	-	-
Day 12	Edema, vascular proliferation	+	29	+	3
Day 14	Edema, vascular proliferation	+	18	+	4
Day 16	Edema, vascular proliferation	+	24	+	8
Day 18	Edema, vascular proliferation	+	26	+	5
Day 20	Edema, vascular proliferation	+	19	+	10
Day 22	Edema, vascular proliferation	+	22	+	14
Day 24	Edema, vascular proliferation	+	29	+	17
Day 26	Edema, vascular proliferation	+	27	+	15
Day 28	Edema, vascular proliferation	+	30	+	19
Day 30	Edema, vascular proliferation	+	31	+	22
Day 32	Edema, vascular proliferation	+	29	+	23
Day 34	Edema, vascular proliferation	+	28	+	24

loss was not observed during the study. During the second post-surgical week, the sutures came undone spontaneously or due to auto-cannibalism, but all the incisions in both groups remained closed. Infection and implant exposure were not observed in either group.

Laboratory Findings

Hair on the biopsy materials obtained from rats in both groups that were euthanized after the second post-surgical week was trimmed using scissors due to difficulty cutting sections because of the re-grown hair. The biopsy sections were stained with hematoxylin-eosin and examined under a light microscope. Preparation of the slides caused Medpor to dissolve, whereas tissues that migrated into Medpor's pores, including fibroblasts, endothelial cells, inflammatory cells, and free erythrocytes, were not affected by the procedure. It was observed that bridging occurred on post-implantation day 5 in the study group (Figure 1); versus day 12 in the control group (Figure 2).

Another parameter considered in this study was the

quantity of new vascular formation per μm^2 (Table 1). Once bridging was observed under low magnification, endothelial-based vascular structures were enumerated, whether or not they contained erythrocytes. Moreover, before bridging was observed, there were more free erythrocytes in the Medpor implant pores (Figure 3a and b), and there was more fibrosis between the subcutaneous tissue and the Medpor implant in the study group than in the control group (Figure 4a and b).

Discussion

VEGF, also known as vasculotropin, is an antigenic, heat- and acid-resistant growth factor. VEGF stimulates endothelial cell growth, angiogenesis, and capillary permeability. It has been shown to increase the growth of endothelial cells isolated from the bovine adrenal cortex, cerebral cortex, and fetal and adult aorta, as well as from the human umbilical vein [10]. Vascular endothelial cells are the only cells specifically targeted by VEGF. It was also reported that VEGF does not have a mitogenic effect on cultured corneal endothelial cells,

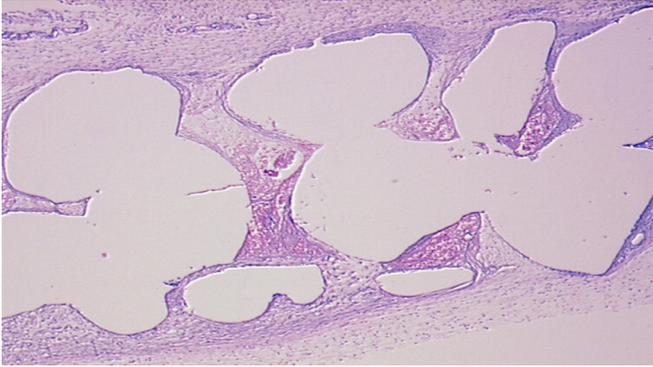


Figure 1. Bridging occurring on post-implantation day 5 in the study group. The physical nature of the implant prevents it from being cut; therefore, the empty spaces seen in the cross-section are the areas in which the implant lies. Fibrin and free blood elements that penetrate through the graft's pores from both surfaces of the implant exhibit bridging (H and E stain, $\times 100$).

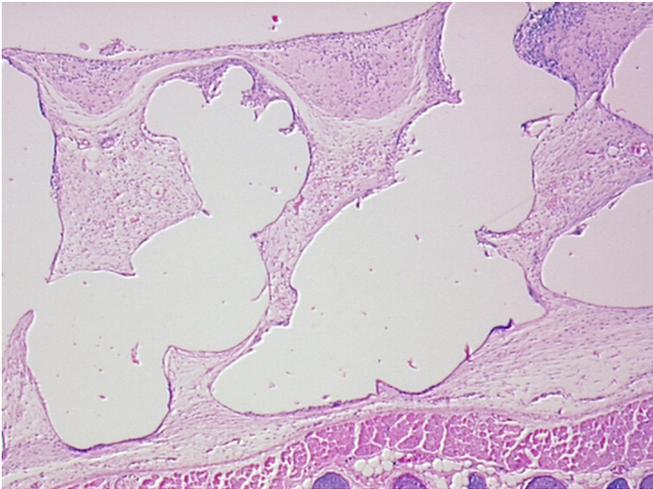


Figure 2. Bridging occurring on post-implantation day 12 in the control group. Elements that create bridging are young, fibrous connective tissue and vascular proliferation (H and E stain, $\times 100$).

vascular smooth muscle cells, keratinocytes, human sarcoma cells, or endothelial cells of the lens [10]. A Northern blot evaluation of 3.7 kb RNA transcripts of human tumor cells, including sarcomas and carcinomas, showed hybridization via the VEGF probe. Mouse sarcoma 180 cells express VEGF mRNA and secrete a VEGF-like mitogen [11]. Human VEGF is a 38.2 kDa protein consisting of two 165 amino acid-containing polypeptide chains.

Numerous studies have shown that the success and stability of reconstruction obtained using Medpor are due its biocompatibility and characteristics. Medpor biocompatibility is the result of its chemical composition, biostability, and surface characteristics. Generally, the least reactive biomaterials are those composed of elements closest to carbon and calcium on the periodic table of elements, as these elements constitute most

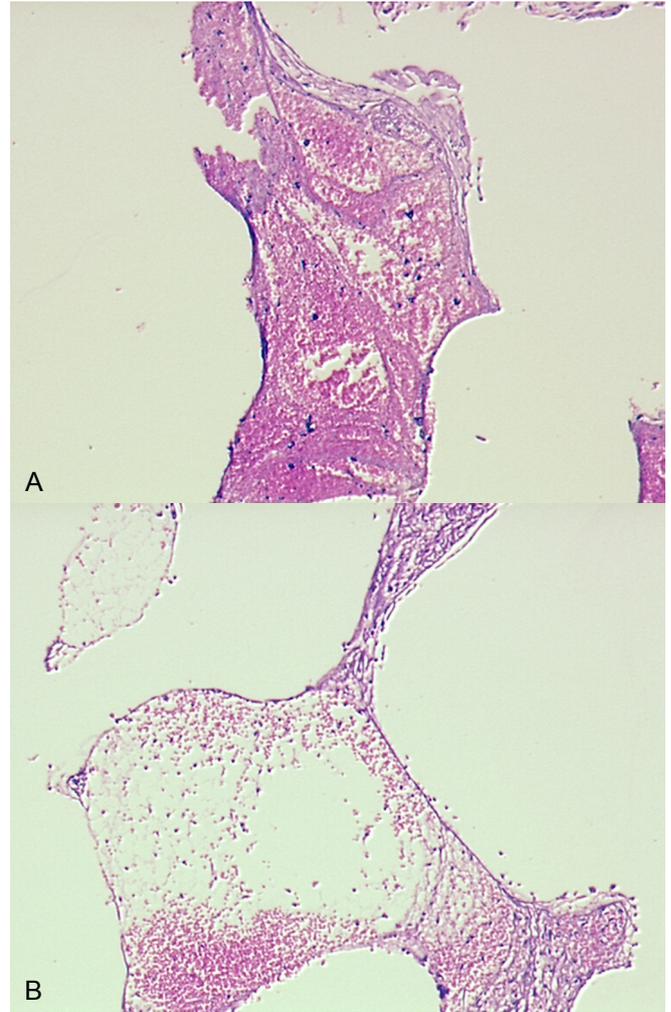


Figure 3. (A) There were significantly more free erythrocytes observed in the study group than in the control before bridging was noted on post-implantation day 4 (H and E stain, $\times 100$) (B) Fewer erythrocytes were noted in the control group than in the study group before bridging occurred on post-implantation day 4 (H and E stain, $\times 100$).

of the human body. Medpor's porous polyethylene structure consists of straight-chained aliphatic hydrocarbons. Because polyethylene is an inert material, it is used as the standard reference material for biocompatibility testing. Foreign body reactions are minimal. The chemical composition and stability of Medpor precludes Type 4 hypersensitivity reactions, and local/systemic malignancies and systemic diseases.

The biocompatibility of Medpor might also be due its porous surface structure. Medpor contains pores that permit the migration of surrounding tissue into the material without the capsule formation that can occur when using implants with a smooth surface. The 100-250 μm wide pores in Medpor create a continuous trabecular system inside the material. The rapid migration of the surrounding host tissue through these channels fills the pores and limits the mobility of the material;

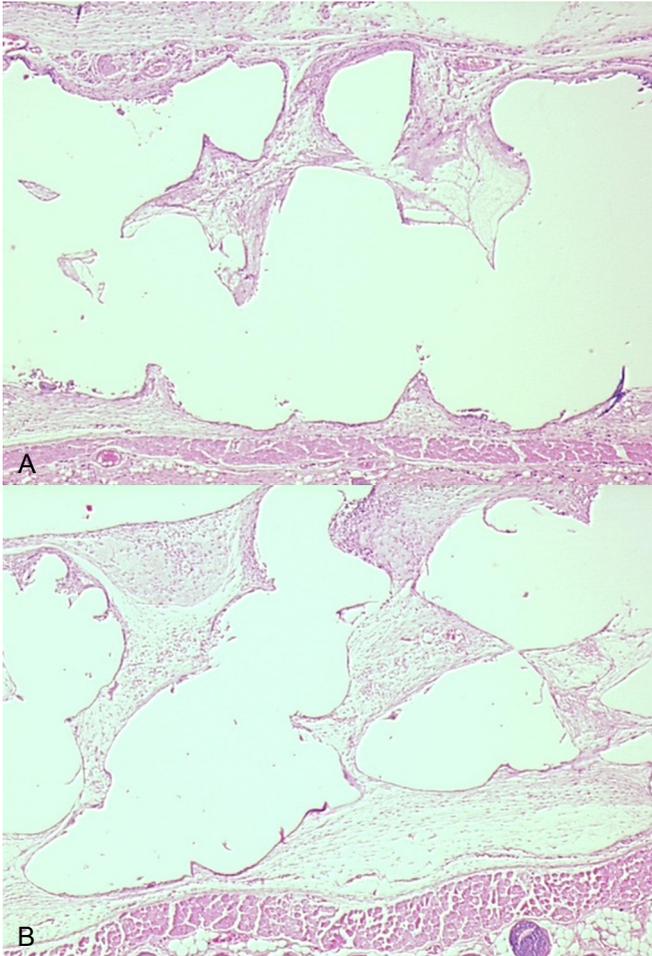


Figure 4. (A) Intense fibrosis and migration of inflammatory cells observed between the Medpor implant and subcutaneous tissue in the study group on post-implantation day 11 (H and E stain, $\times 100$) (B) There was less fibrosis and migration of inflammatory cells observed between the Medpor implant and subcutaneous tissue in the control group than in the study group on post-implantation day 12 (H and E stain, $\times 100$).

therefore, capsulation and continuous mobility of the materials, which are associated with smooth-surfaced materials and result in early and late complications, do not occur with Medpor [12]. Additionally, when Medpor is placed on bone, resorption does not occur in the underlying bone, as bone tissue does not migrate into the material [13].

Complications associated with Medpor are infection and implant exposure, which occur in 3% of cases [12]. The occurrences of these complications are related to the way in which Medpor is placed, poor vascularization of the host tissue, and unstable skin overlying the material. Implant materials without sufficient perfusion will also affect the overlying tissue, decreasing its perfusion and causing the region to break down. Moreover, insufficient perfusion of the implant will limit the ability of immune cells to protect the implant, resulting

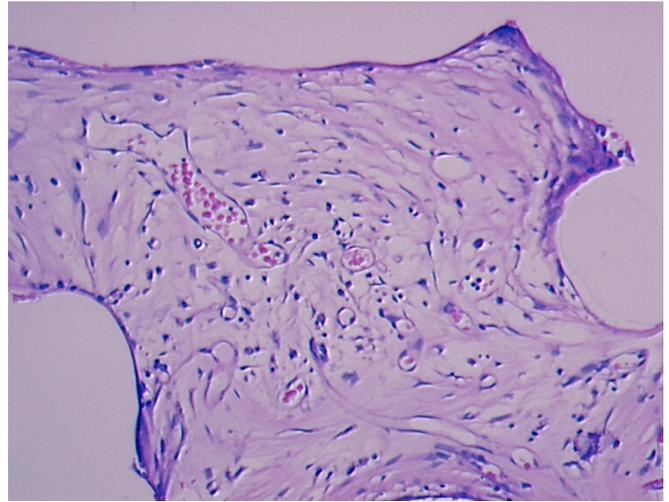


Figure 5. An image of bridging under high magnification on post-implantation day 16 in the study group. Bridging consists of a vast amount of fibroblasts in edematous, loose connective tissue and vascular proliferation. Fibrin and free blood elements are not observed. Collagenization has not fully developed (H and E stain, $\times 400$).

in the spread of infection to the material from an existing local infection. Such cases will usually progress to the exposure of Medpor, creating a vicious cycle in which infection decreases perfusion, resulting in less resistant tissue and increased susceptibility to further infection.

It was reported that Medpor implants were fully fibrovascularized in 2 weeks [14]. We demonstrated that the fibrovascularization started as early as day 3 in both groups. Bridging - the focus the present study - can be defined as an organized vascular structure formed by the migration of endothelial cells, fibroblasts, and inflammatory cells from both surfaces of the 1.5 mm thick Medpor implant (Figure 5).

During the early post-implantation days, there were significantly more free erythrocytes in the Medpor pores in the study group than in the control group, which may have been due to VEGF increasing vascular permeability. In addition, the intense fibrotic tissue formation observed between the Medpor implant and subcutaneous tissue (independent from the operating days) might have been due to VEGF's angiogenic effect and its positive effect on vascular permeability, thereby permitting the migration of various inflammatory cells to the region and inducing fibrotic tissue formation.

The main limitation of this study is the number of rats used. To prevent the sacrifice of many rats, we assumed that each rat reacted to the procedures in a similar way. As it was not possible to take biopsies from the

same rat on 20 different days, we had to take a biopsy from a different rat on each day and compare those.

Conclusion

We think that the difference observed between the study and control groups, in terms of fibrovascularization of Medpor implanted into the dorsal subcutaneous tissue of healthy rats, may be a basis for the future use of VEGF in humans. The present study's findings do not directly indicate that local VEGF administration can reduce the occurrence of infection and implant exposure. Additional research on the rates of implant exposure and infection associated with Medpor implanted in standard, experimentally-induced ischemic tissue in response to local VEGF administration is warranted.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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