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The Effect of Human Recombinant Erythropoietin (rHuEPO) and Tacrolimus (FK506) in Autologous and Homologous Full Thickness Skin Graft (FTSG) Take and Viability in a Rat Model

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1. Introduction

The use of autologous skin grafts comprises a well established method of reconstruction in large areas involving partial thickness defects. Additionally, when skin availability is limited due to associated trauma or injury (e.g. burns) the use of homologous skin grafts from a compatible donor appears to be a valid alternative. The effort to maximize effectiveness of skin grafting in terms of increasing graft take, protecting viability, reducing full healing time and achieving improved postoperative outcomes without impairing function has also been constant especially in cases of impaired general condition.

Human recombinant erythropoietin (rHuEPO) is a glycoprotein with primarily a hemopoietic role due to the inhibition of precursor erythrocytes and the promotion of their proliferation. Its genetic expression is being regulated by hypoxia. It is primarily synthesized by the fetal kidneys and liver and by the adult kidneys (Paschos et al. 2008).

The biological activity of rHuEPO is promoted by its interaction with the cell surface erythropoietin receptor (EPOR) (Brines and Ceramic 2008) which is a type I cytokine expressed by precursor erythrocytes as well as in other non hemopoietic systems such as the central and the peripheral nervous system in response to trauma (Galeano et al. 2004), in cardiac muscle cells, vascular endothelial smooth muscle cells and in subcutaneous mast cells with potent anti-ischaemic action (Brines and Ceramic, 2008; Galeano et al. , 2004).

Current research has focused on its non-hemopoietic role. Many studies report the local production of erythropoietin (EPO) in response to trauma prior to the local concentration of pre-inflammatory cytokines and EPO acting antagonistically to them by reducing edema, inhibiting cellular apoptosis, promoting neovascularization by enhancing endothelial cell mitosis and promoting wound healing (Paschos et al. 2008).

Since endogenous local EPO production is inadequate, it has been shown that the exogenous administration of rHuEPO enhanced wound healing and revascularization in experimental

studies on healthy rats, rats with genetically induced diabetes mellitus and rats with burn injuries (Galeano 2006). In clinical studies including human subjects, the wound healing promoting properties of rHuEPO have been reported following γ -knife for brain surgery and recently in patients who underwent destruction osteogenesis (Mihmanli et al. 2009)

Vascular endothelial growth factor (VEGF) comprises a heparin-binding, dimeric glycoprotein which initiates the proliferation and migration of endothelial cells to participate in the development of new vascular lumens, and increases the penetration and extravasation of plasma macromolecules (Hom et al., 2005; Paschos et al., 2008). VEGF receptors are found in endothelial cells and are expressed under conditions of hypoxia and following endothelial damage. Two high-affinity endothelial cell receptors, KDR/Flk-1 and flt-1 mediate VEGF effects on tissue physiology (Lantieri et al. 1998)

VEGF has been shown to participate in wound healing enhancement, embryo development, growth of certain solid tumors, and ascites formation (Isogai et al., 2006; Schultze-Mosgau et al., 2003). In this study, the immunohistochemical detection of VEGF expression is used as a method of skin graft take evaluation.

Tacrolimus (FK-506), has recently been established as a valid immunosuppressant and comprises a well-known calcineurin (a serine/threonine phosphatase) inhibitor. It has been shown that FK-506 inhibits TGF- β induced VEGF production by antagonizing calcineurin which leads to the attenuation of the activation and translocation of the nuclear factor that activates T-cells (nft) (Mori et al 1997). FK-506 also inhibits: a) interleukin-2 induced interleukin-5 production by CD4+ T cells and b) T-cells proliferation stimulated by interleukin-2 and interleukin-7 (Mori et al 1997). Confirmation was provided by similar studies which showed that rapamycin, another immunosuppressant which does not inhibit calcineurin, did not have any effect on TGF- β induced VEGF production while cyclosporine A, which is also a calcineurin inhibitor, resulted in reduction of VEGF production (Marumo et al. 1995). The action of FK-506 is mediated by immunophilins, a class of proteins which bind to immunosuppressive drugs, giving to FK-506 the ability to interact with calcineurin and to interfere with its access and dephosphorylation of various substrates. The primary immunophilin was shown to be FKBP 13 which is localized to the endoplasmic reticulum lumen, where processing and presentation of antigen in the immune system are thought to take place (Nigam et al. 1993). The liver and the intestinal mucosa are the main sites of FK-506 metabolism by the cytochrome P4503A4 enzyme. Subsequently, FK-506 is eliminated through biliary excretion. (Lampen et al., 1995; Nakazawa et al., 1998; Plosker & Foster, 2000). FK-506 has been widely used systemically, maintaining a profile of safety during the long-term experience with the drug. Most side effects appear to be related to whole-blood concentrations and refer to nephrotoxicity, cardiovascular toxicity, metabolic and neurotoxicity. However, almost all of the reports of FK-506 toxicity are derived from its use in solid-organ transplantation, in which much higher doses are used compared to those for skin transplantation (Mayer et al., 1997; Pirsch et al. 1997). The aim of this animal study is to investigate the effect of human recombinant erythropoietin (rHuEPO) and tacrolimus (FK-506) on autologous and homologous full thickness skin grafts in an experimental research design including Wistar rats.

2. Methods and material

Thirty adult inbred female Wistar rats (n=30), with weights ranging from 220 to 300 gr, were selected for this study. Two control and 3 experimental groups were formed, each consisting

of 6 subjects. This experimental study followed the National Research Council's Guide for the Care and Use of Laboratory Animals and was approved by the National Bioethics Committee. This study used the minimum number of animals needed in order to establish a valid conclusion and statement regarding the specific subject. All necessary refinements and design methods have been performed and planned according to accredited established protocols. All surgical procedures were performed under general anesthesia with the use of ketamine hydrochloride (80 mg/kg intramuscularly) and xylazine (2 mg/kg intramuscularly).

After induction of general anesthesia the surgical site was shaved and prepped with povidone iodine and chlorhexidine solution. The animals were covered with sterile surgical drapes with only the operative site exposed.



Fig. 1. Preoperative design of the FTSG on the back of the subject



Fig. 2. Dissection and harvesting of the FTSG and then rotation of the FTSG on the wound bed by 180° so as the previously caudal edge becomes rostral and the donor site can be used as the recipient site



Fig. 3. Cleaned wound area and dressing application on the FTSG

A full thickness skin graft was harvested from the area between the two scapula bones on the dorsum of each rat. The skin graft was elliptical in shape, with a long diameter of 2 cm and a small diameter of 1 cm (Figure 1). During harvesting, the panniculus layer was carefully removed from the bed of the skin graft and recipient site. The skin graft was kept in phosphate-buffered saline before being transferred to the recipient site. In this experimental model, each skin graft was repositioned in the same area from which it was harvested after being rotated by 180 degrees in the vertical axis, so as the severed vascular axons in the skin graft remain misaligned to the vascular axons of the recipient bed. In this way each donor site also serves as a recipient site since revascularization and new vascular connections between skin graft and recipient bed are still required due to the preserved vascular misalignment in the new graft position. When a homologous FTSG was required, animals underwent surgery in pairs and a FTSG was harvested in the aforementioned way from each animal and it was then transplanted into the donor site of the other after being similarly rotated by 180 degrees (see subject groups below).

The location of the recipient bed is conveniently situated between the scapular bones, unreachable by the rats and well fixated on a minimally mobile area causing negligible irritation to the rats. No animal involved in this project was subjected to discomfort, pain, or

distress. Any such discomfort, pain, or distress was alleviated with the appropriate approved medication (Figure 1-3). Nylon 5-0 non-absorbable sutures were invariably used. The animals were placed in a warm environment with the aid of heating lamps and were continuously monitored. Pain management was carried out with the use of Buprenorphine at 0.05-0.10 mg/Kg, SC, and then twice daily. The animals were observed for their pattern of breathing, alertness, ability for food and drink uptake. After surgery rats were carefully observed for dehydration and diarrhea. Rats were kept separately in their housing cages which were standard filter top secured cages.

The animals were randomized into the following groups according to the treatment that was used:

Group A (First control group, n=6): An autologous FTSG was harvested and repositioned. Local infiltration with water for injection was also performed as a control.

Group B (Second control group, n=6): Animals underwent surgery in pairs. Consequently there were 3 pairs in this group. A homologous FTSG was harvested from each animal of each pair and transplanted into the donor site of the other. FK506 (tacrolimus) was administered systematically to prevent rejection. Local infiltration with water for injection was also performed as a control.

Group a (experimental, n=6): An autologous FTSG was harvested and repositioned. Local infiltration with rHuEPO was performed.

Group b (experimental, n=6): Animals underwent surgery in pairs as in group B, forming a total of 3 pairs. A homologous FTSG was harvested from each animal and it was transplanted into the donor site of the other while FK506 (tacrolimus) was administered systematically (local infiltration) to prevent immunologic response. Local infiltration with rHuEPO was performed.

Group c (experimental, n=6): An autologous FTSG was harvested and repositioned and local infiltration with FK506 was performed. Table 1 summarizes the details regarding each animal group.

Group	Type	Number of subjects	FTSG	FK506	rHuEPO	wfi
A	control	6	autologous	no	no	yes
B	control	6	homologous	for immunosuppression	no	yes
a	experimental	6	autologous	no	factor	no
b	experimental	6	homologous	for immunosuppression	factor	no
c	experimental	6	autologous	factor	no	no

Table 1. The animal groups in the study. FTSG: full thickness skin graft, for immunosuppression: FK506 is used in order to prevent rejection, not as tested factor, factor: FK506 or rHuEPO are used as local infusion around the recipient bed and their effect on graft viability and healing is tested, wfi: water for injection used for local infiltration as a placebo, when one of the previously mentioned factors were not used

FK-506 (Fujisawa USA, Inc.) was dissolved in 80% ethanol and 20% cremaphor to create a stock solution with a concentration of 10 mg/ml. This stock was then diluted with 75%

propylene glycol and 25% water to a final concentration of 0.5 mg/ml. The diluted solution was replaced every 3 days.

In groups B and b, FK-506 was subcutaneously administered daily at a dose of 2mg/Kg. The use of FK-506 either as a systematic immunosuppressant (groups B and b) or as a local agent (group c) always involved local, subcutaneous infiltration. In groups a and b, 400UI/kg in a 100µL solution of rHuEPO were subcutaneously administered, daily. Local infiltration of agents was invariably performed around and under the recipient bed.

The effect of FK-506 and rHuEPO administration in the aforementioned experimental groups was investigated. The investigation consisted of the clinical, histological and immunohistochemical evaluation of the grafts and their viability-take on the 10th post grafting day. All subjects were euthanized on the 10th post grafting day by prolonged inhalation in a closed diethyl-ether inhalation chamber.

Skin graft take area and viability were assessed using digital photographs and manual measurements. The photographs were processed by digital image surface area analysis software (Pixcavator Image Analysis Software 2.3). Specimens of autologous and homologous skin grafts were then harvested together with a marginal recipient bed skin, along the perimeter of the graft for histological examination and immunohistochemistry. The formalin fixed, paraffin embedded tissue sections were treated with hematoxylin-eosin stain as well as by two antibodies. Standard procedures as recommended by the manufacturer were performed.

The antibodies used in our study were:

Monoclonal Mouse Anti-Human Vascular Endothelial Growth Factor, Clone VG1, isotype: IgG1, kappa antibody was used to label the VEGF-121, VEGF-165, and VEGF-189 isoforms of vascular endothelial growth factor (VEGF) Clone VG1.

Monoclonal Mouse Anti-Human CD31, Endothelial Cell, Clone JC70A, isotype: IgG1, kappa, (Dako A/S, Glostrup

Denmark Autostainer/Autostainer Plus) antibody was used to label endothelial cells of newly formed vessels to determine angiogenesis.

Histology findings were evaluated for vascular presence and endothelial condition, lymphocytic infiltration, dermal/epidermal interphase reaction (spongiosis, incomplete, complete epidermal separation) and necrosis. More specifically, due to the relatively variable macroscopic presentation of homologous graft rejection, histology findings and criteria were solely used to describe and categorize potential rejection according to the established method of Tatsuya et al (1997) which includes the following three grades:

Grade 1: intraepidermal blister formation,

Grade 2: incomplete epidermal separation from the dermis;

Grade 3: complete epidermal separation from the dermis.

Immunohistochemistry analysis aimed at demonstrating the degree of angiogenesis in the skin grafts as a direct sign of vascular network rearrangement and development, viability and effective graft take.

Manual measurement and clinical assessment of graft take was performed using light microscopy and corresponded adequately with the digital image analysis.

Spleens were harvested and subjected to the mixed lymphocyte reaction, to assess the degree of immunosuppression. Liver and kidneys were also harvested and examined for signs of potential hepato- and nephrotoxicity.

3. Statistical analysis

Areas of graft take comprise numeric data which are presented with approximation of two decimal digits. Means are presented \pm SD where applicable, and the *t* test for independent samples has been used for statistical analysis of the results. SPSS 17.0 (IBM Corporation Somers, NY 10589) was used. Statistical significance was considered for $p<0.05$.

4. Results

Clinical results were classified according to the mean area of inadequate graft take - graft necrosis (N). In control group A $N=40.98\text{ mm}^2$, in control group B $N=71.2 \pm 4.67\text{mm}^2$, in experimental group a $N= 15.22 \pm 4.76\text{ mm}^2$ in experimental group b $N= 29.2\pm 4.70\text{ mm}^2$, in experimental group c $N=46.52 \pm 4.60\text{ mm}^2$. Manual measurements corresponded well with the aforementioned digital image analysis measurements. The corresponding percentages (over the total area of each graft which was $20 \times 10\text{ mm}^2= 200\text{ mm}^2$) of mean graft necrotic areas were: group A: 20.49%, group B: 35.6%, group a: 7.6%, group b: 14.6%, group c: 23.26% ($p<0.001$ in all group comparisons: A-a, B-b and A-c) (Figures 4-9).

Histological findings using hematoxylin-eosin stain showed the differences in graft revascularization and take among groups (10th post-grafting day) (Figures 10-19)

Immunohistochemistry resulted in significant observations regarding graft revascularization in the each group (Figures 20-27).

Clinical, histological and immunohistochemical findings consistently show the resulting quality of graft take and degree of revascularization among the various groups: graft take in group a>group b> group A> group c> group B.

No signs of hepato- and nephro-toxicity were found.

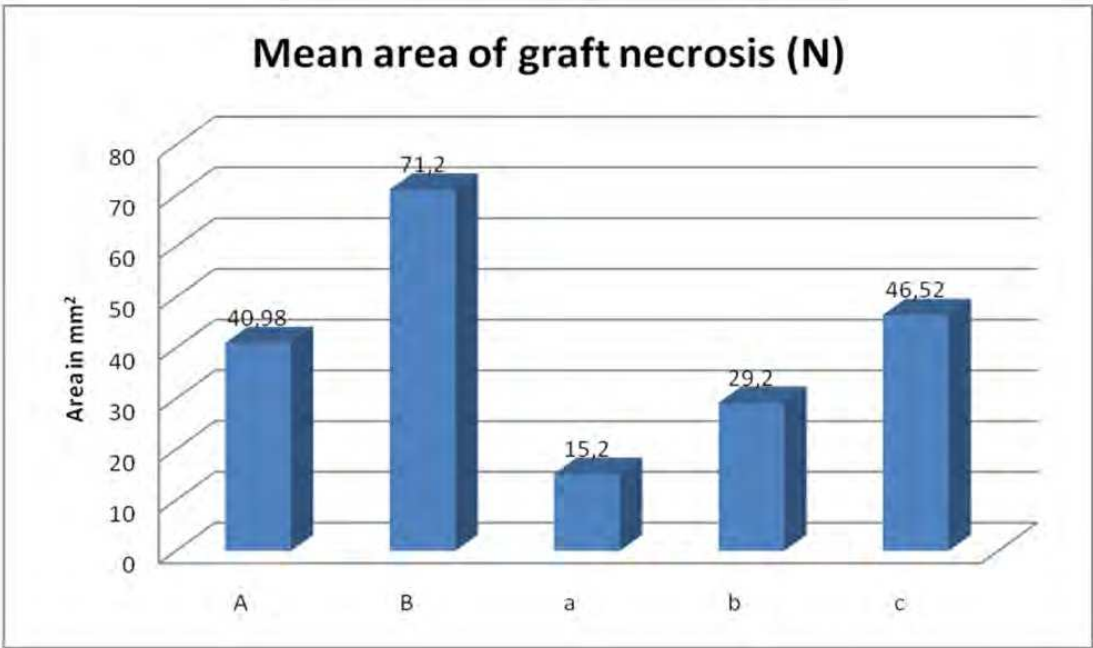


Fig. 4. Mean inadequate graft take-necrosis percentage per group. A, B, a, b and c correspond to animal groups



Fig. 5. Group A macroscopic presentation. Mild peripheral necrosis, good overall take



Fig. 6. Group a macroscopic presentation. Full graft take, no signs of necrosis, excellent graft viability



Fig. 7. Group B macroscopic presentation. Considerable peripheral graft rejection. Central graft take and viability



Fig. 8. Group b macroscopic presentation. Minimal peripheral rejection and nearly complete graft take.



Fig. 9. Group c macroscopic presentation. Partial graft necrosis, not uniform graft take compared to control group A.

Groups A-a

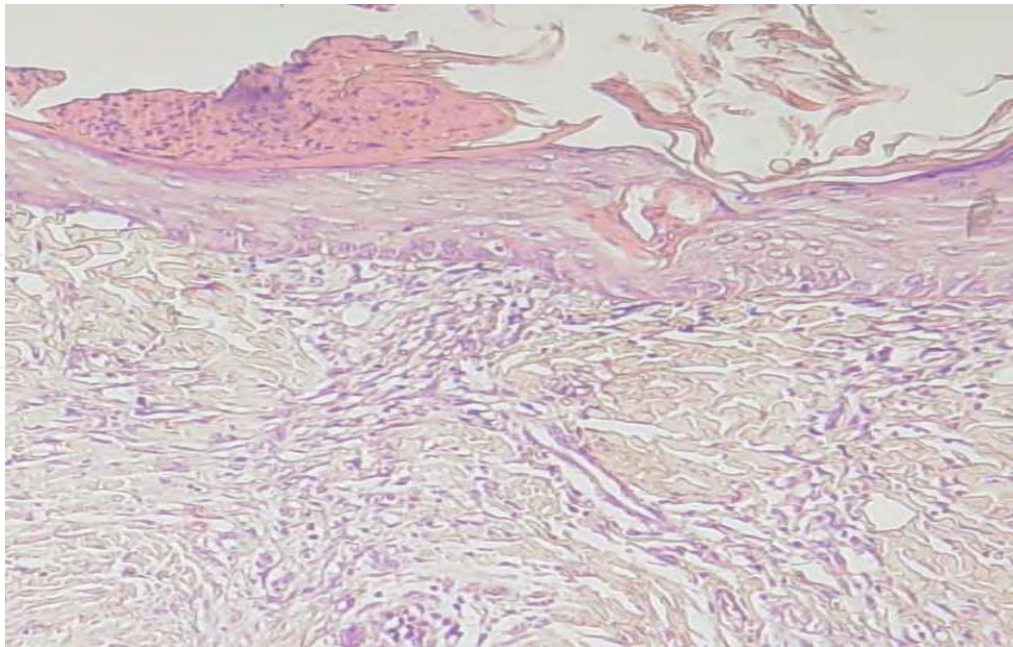


Fig. 10. Formalin fixed, paraffin embedded FTSG x50 in group A. Normal graft take with intermediate inflammatory inflammation demonstrating FTSG take without the use of exogenous active factors.

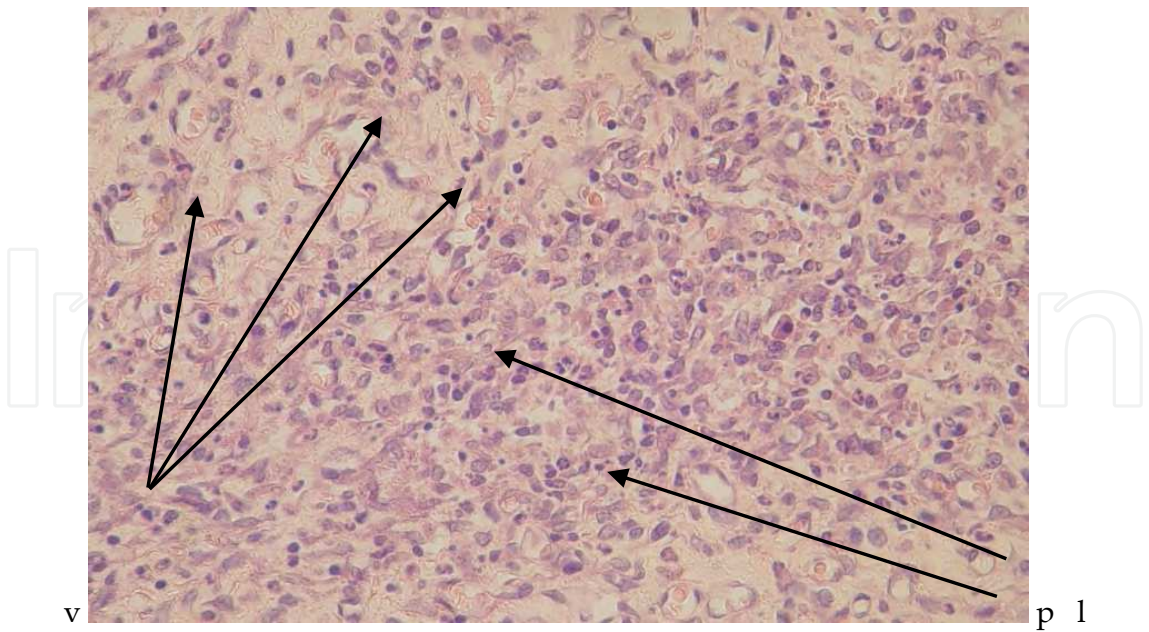


Fig. 11. Formalin fixed, paraffin embedded autologous FTSG x200 in group A. Vascular lumens with erythrocytes (v), polymorphonuclear cells(p), lymphocytes (l) and other inflammatory cell intermediate concentrations are also shown. The number of vessels is indicative of the quality of graft take and viability

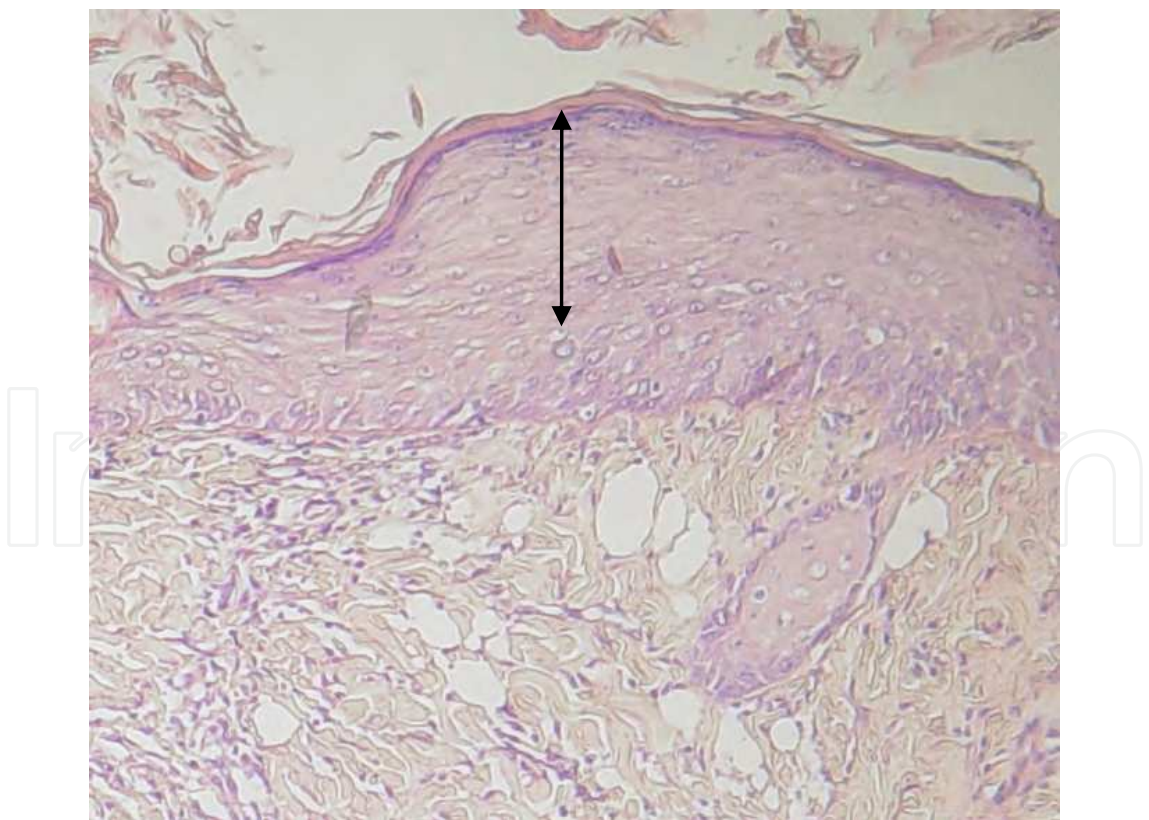


Fig. 12. Formalin fixed paraffin embedded autologous FTSG x50 in group a. Higher vessel concentration, more uniform and higher epidermal layer (double arrow line), less inflammatory infiltration than in control group A

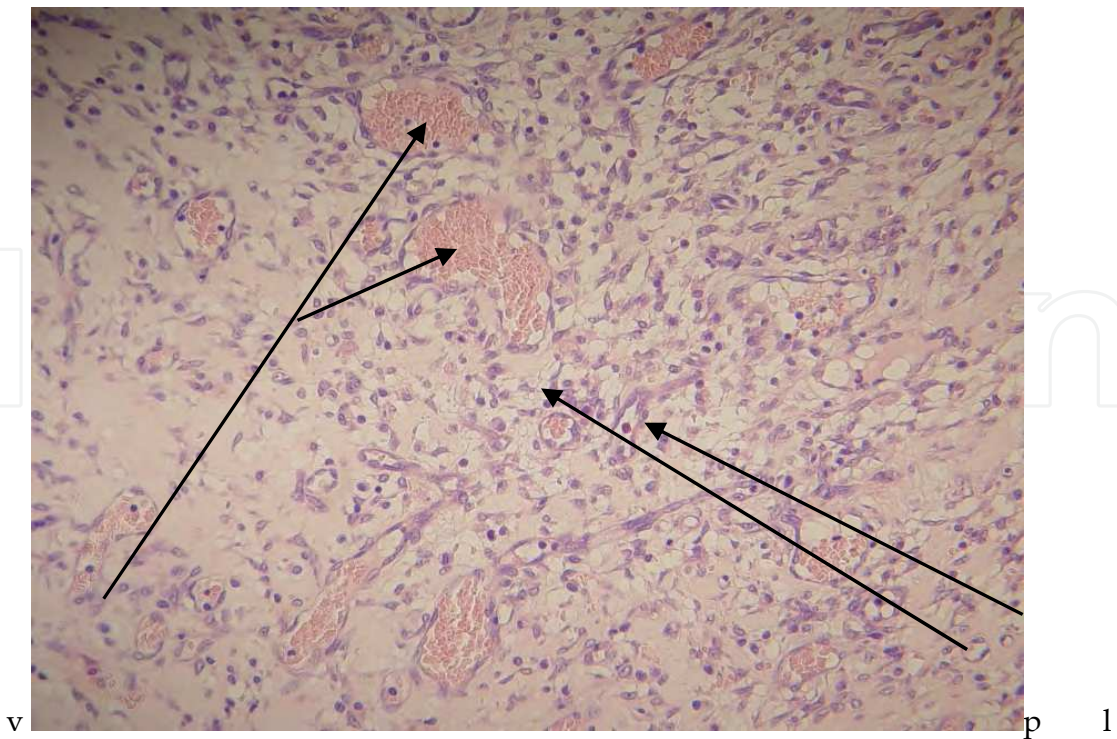


Fig. 13. Formalin fixed, paraffin embedded autologous FTSG x200 in group a. Vascular lumens with erythrocytes (v), polymorphonuclear cells(p), lymphocytes (l) and other inflammatory cell concentrations are also shown. Note the increased number of vessels and the milder inflammatory response which are indicative of better quality of graft take and viability than in the control group

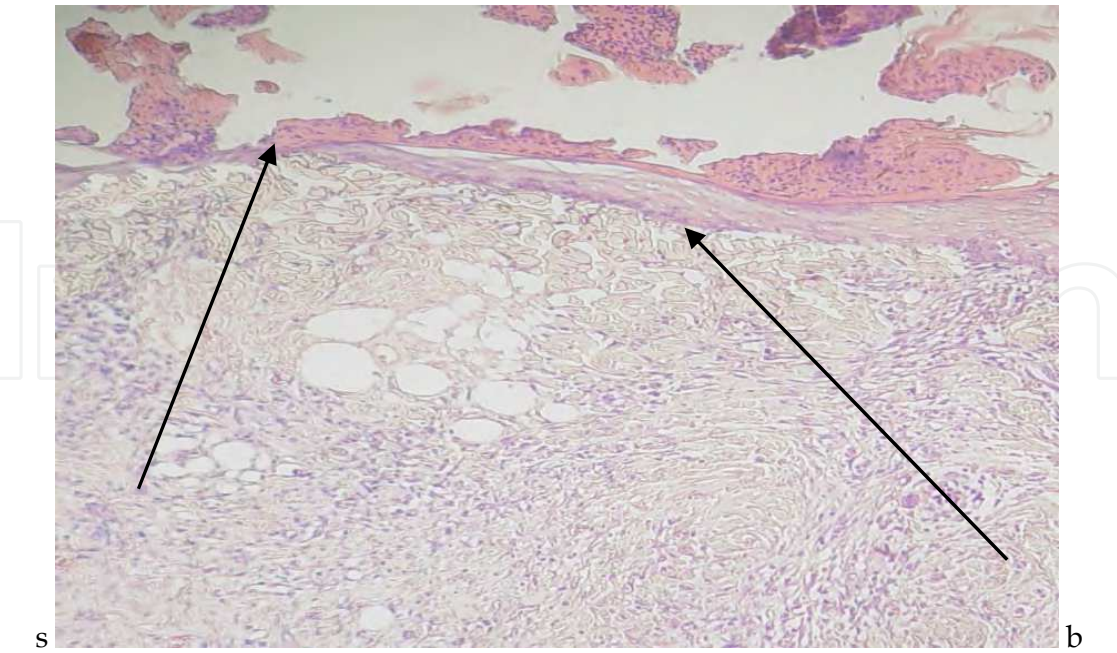


Fig. 14. Homologous FTSG x50 in group B. Increased intraepidermal blisters (b), dermoepidermal lack of cohesion (s) and increased inflammatory infiltration compatible with grade 2-3 of rejection according to Tatsuya et al (1997).

Groups B-b

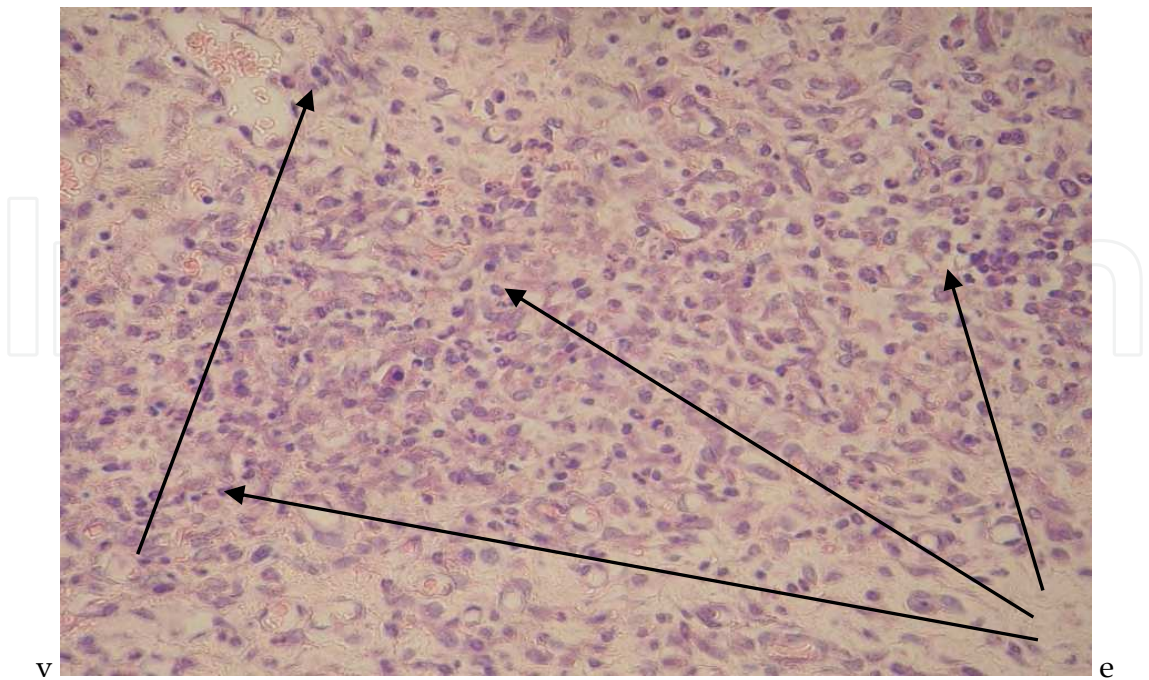


Fig. 15. Formalin fixed, paraffin embedded homologous FTSG x200 in group B. Inflammatory response and mild revascularization compatible with the initial stages of rejection, impairing the ongoing course of graft revascularization (v) and take. Inflammatory cells include large eosinophils concentrations (e) characteristic of the aforementioned response type.

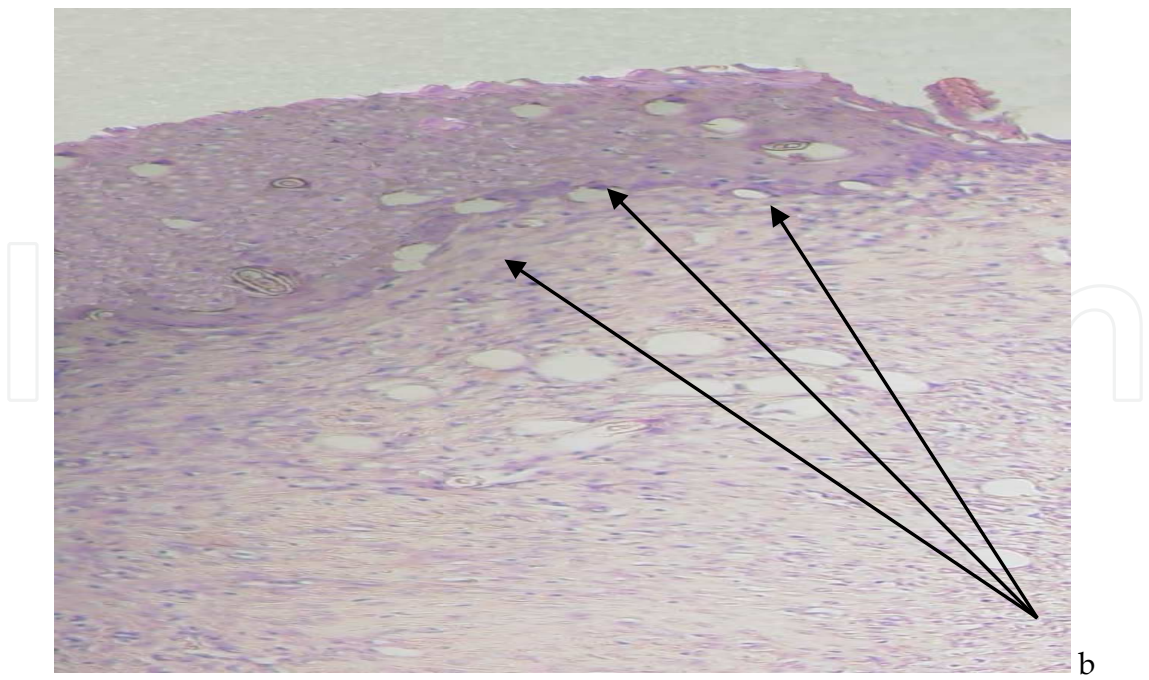


Fig. 16. Formalin fixed, paraffin embedded homologous FTSG x50 in group b. Intraepidermal blisters (b) but lack of partial dermoepidermal separation. Better quality of graft take and minimal rejection response than in control group B.

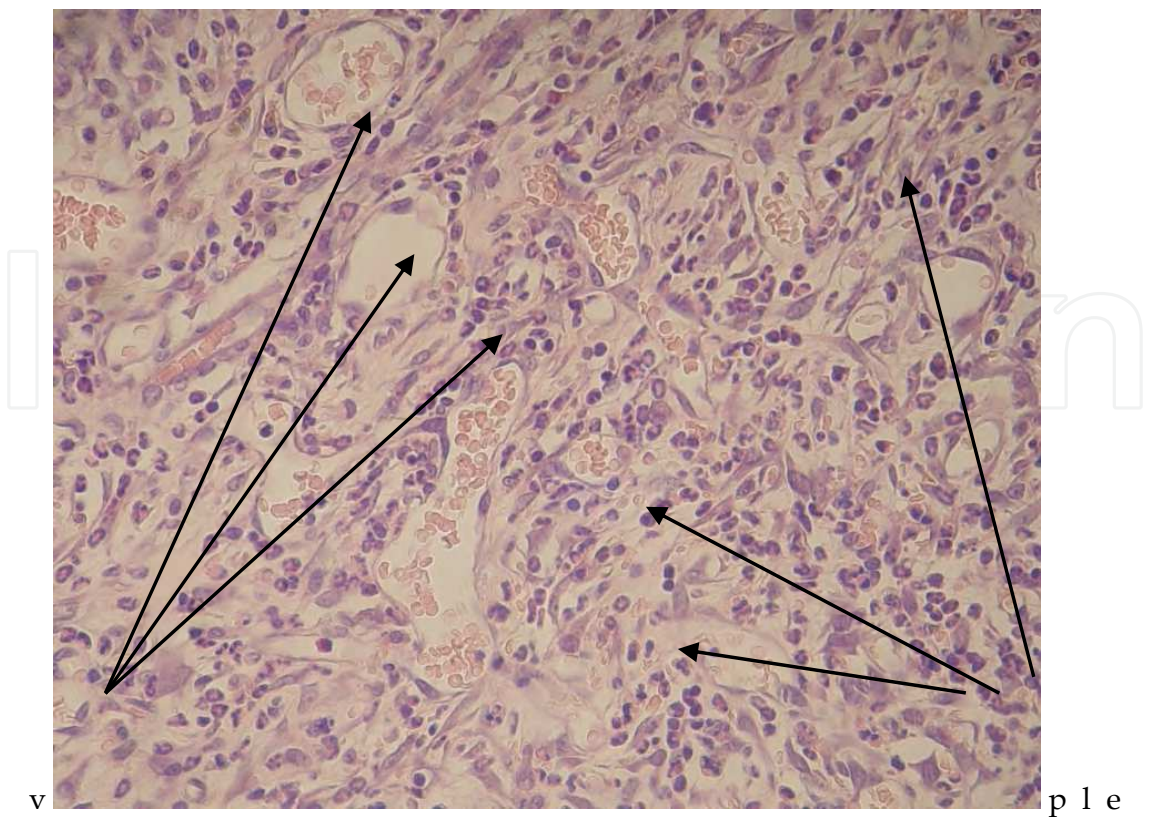


Fig. 17. Formalin fixed, paraffin embedded homologous FTSG x200 in group b. Inflammatory response with concentrations of eosinophils (e), lymphocytes (l) and polymorphonuclear cells (p) but also nearly unimpaired revascularization (v) demonstrating a considerably better quality of graft take and milder rejection response than in control group B.

Group c

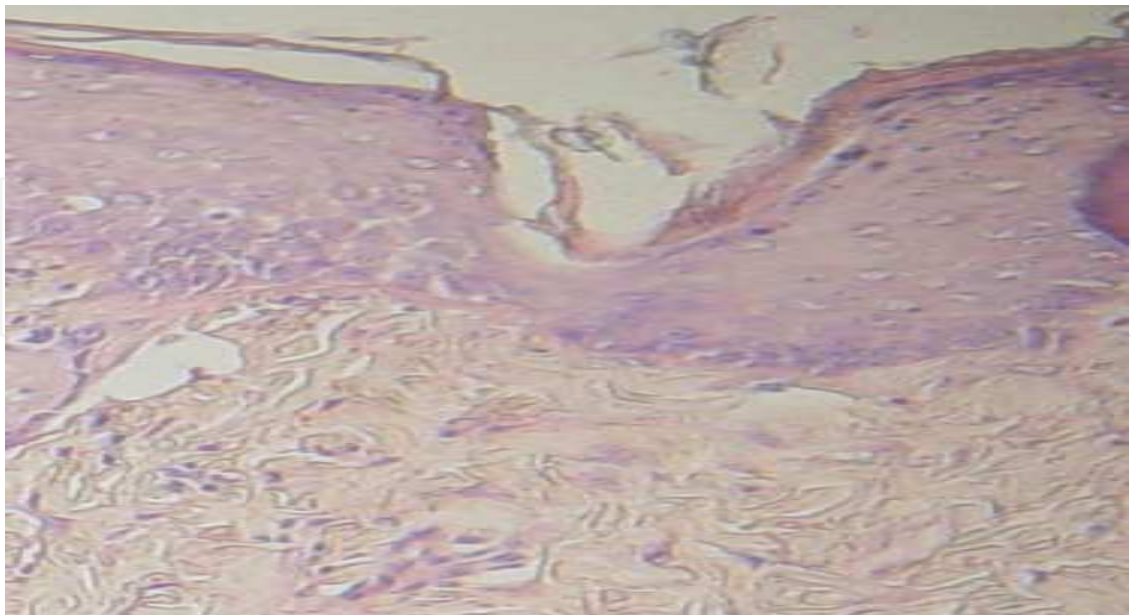


Fig. 18. Formalin fixed, paraffin embedded autologous FTSG x50 in group c. Nearly normal graft take with minimal inflammatory response, firm dermoepidermal cohesion but marginally decreased new vessels development than in control group A.

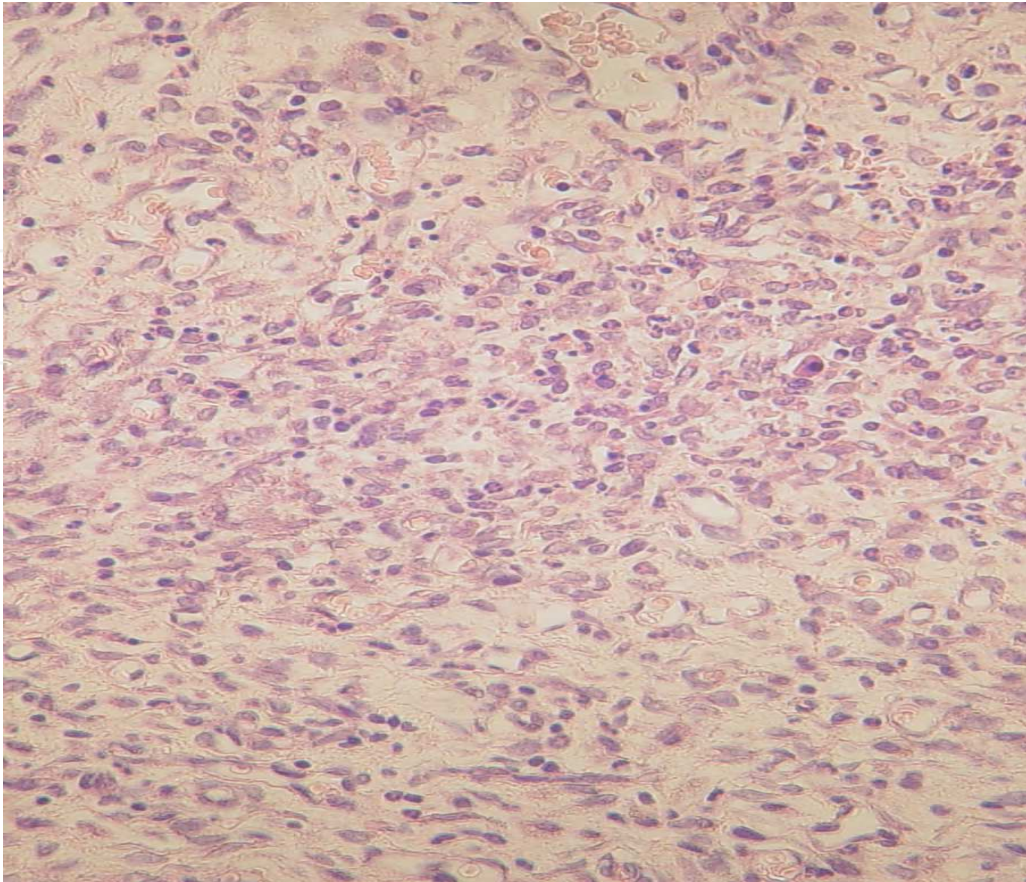


Fig. 19. Formalin fixed, paraffin embedded autologous FTSG x200 in group c. Nearly normal graft take with minimal inflammatory response and decreased revascularization compared to group A.

Groups A-a

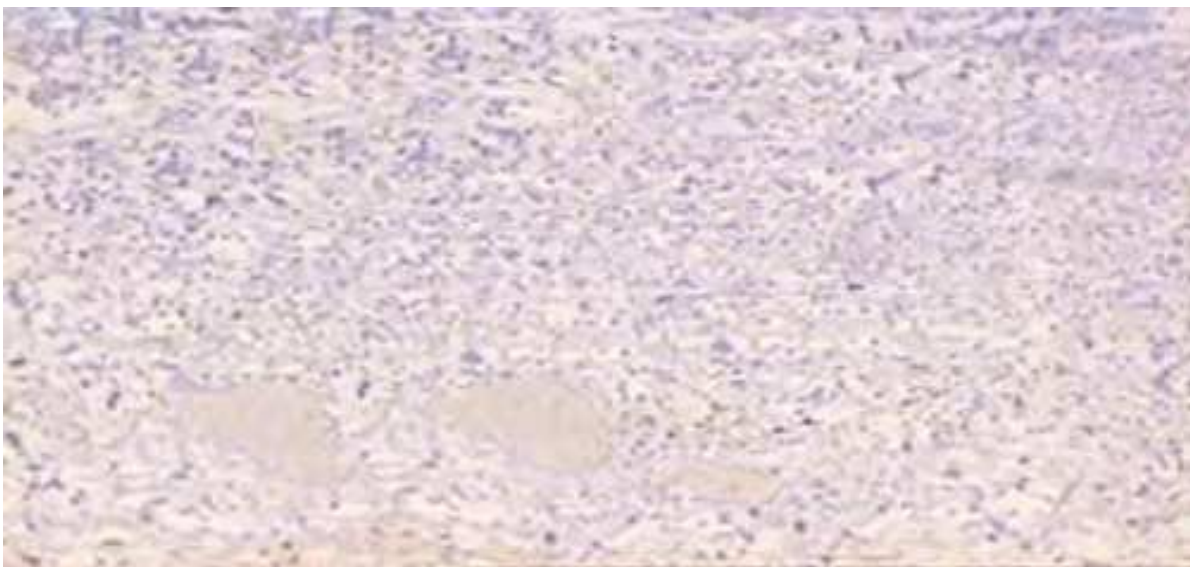


Fig. 20. VEGF expression in group A, as detected by treating tissues with human VEGF monoclonal antibody. Low or absent stain in the normal autologous grafting group.

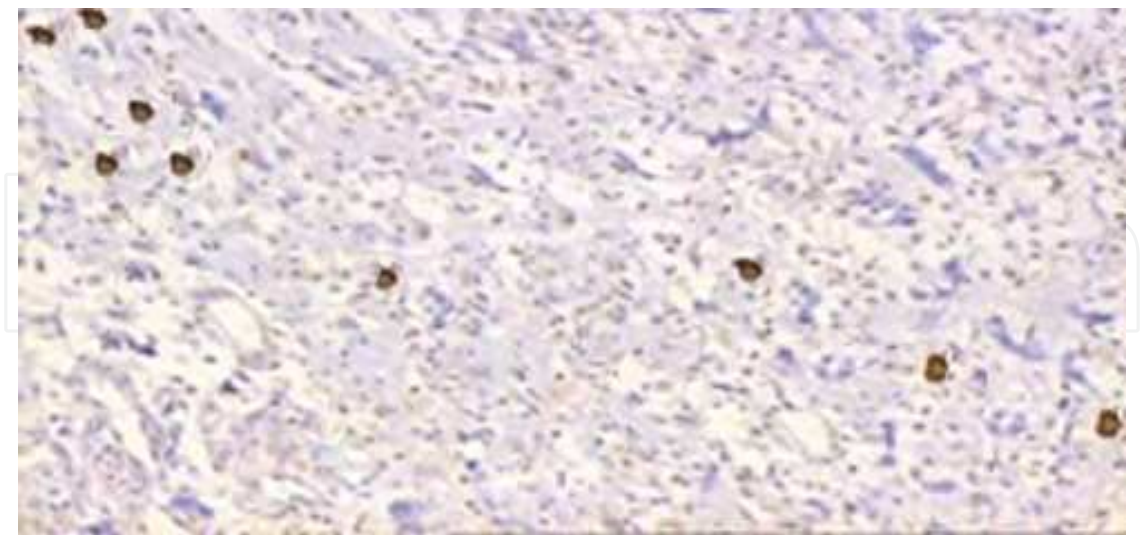


Fig. 21. VEGF expression in group a, as detected by treating tissues with human VEGF monoclonal antibody. Stained-activated macrophages (brown) are noted indicating an increase in revascularization process compared to the control group A

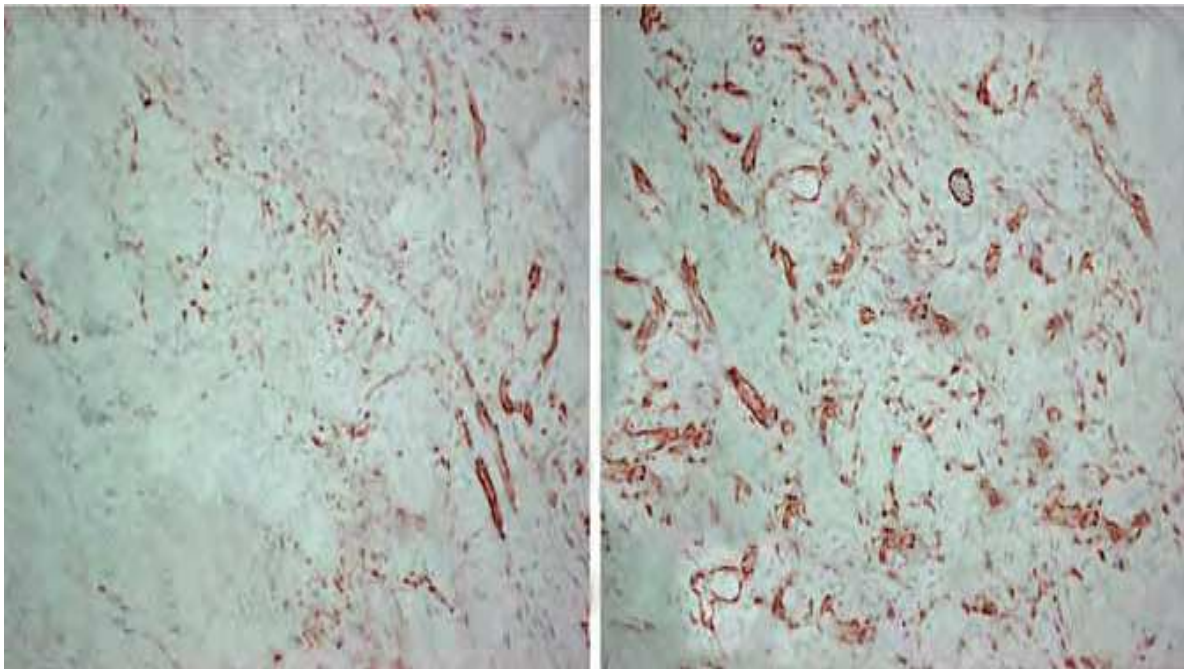


Fig. 22. CD31 stained endothelial cells of newly formed vessels (copper color) in control group A (left) and group a (right). Notably increased angiogenesis is shown in group a

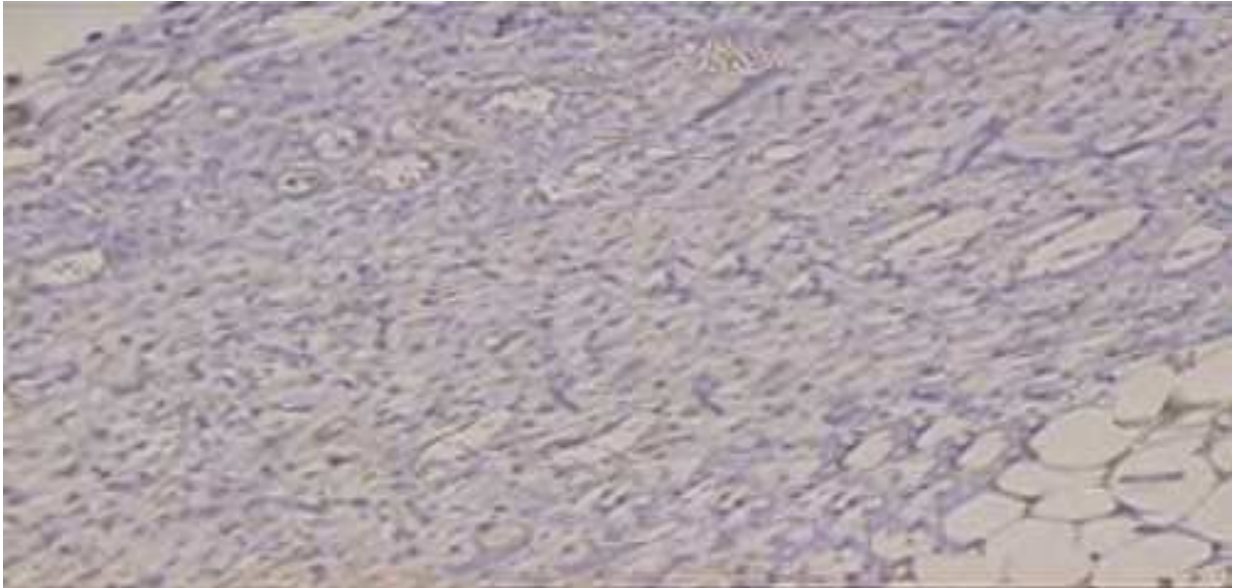
Groups B-b

Fig. 23. VEGF expression in group B, following tissue treatment with human VEGF monoclonal antibody. Low or absent stain in the homologous grafting group. Blisters are also noted, indicative of early rejection response signs.

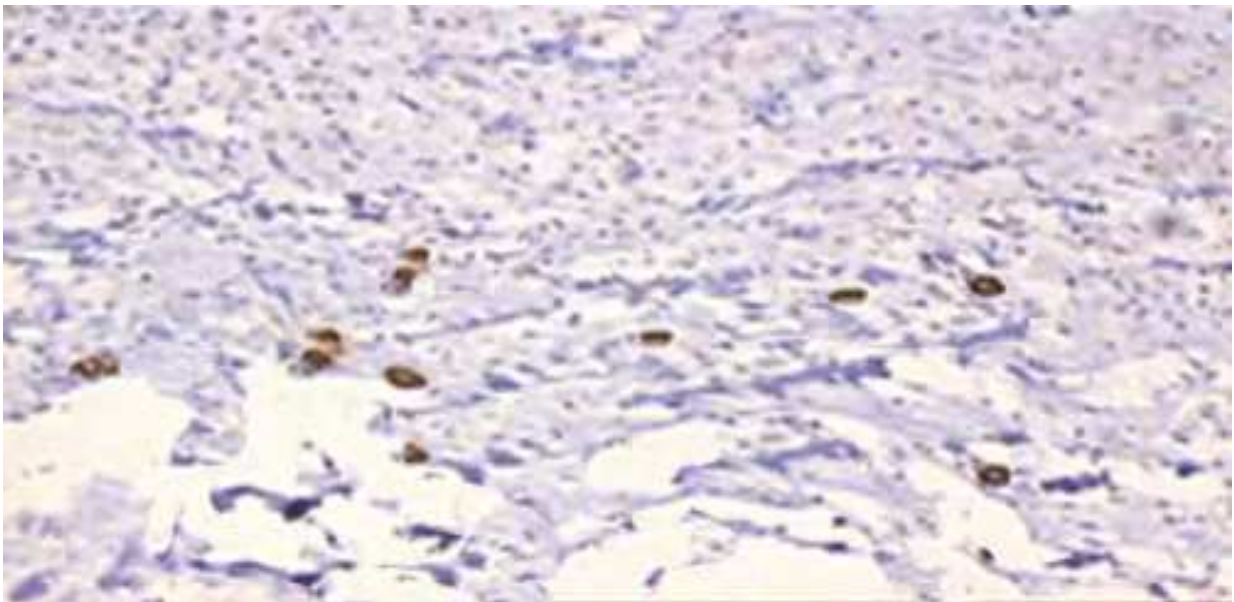


Fig. 24. VEGF expression in group b after the use of VEGF monoclonal antibody. Stained-activated macrophages (brown) are noted compared to their absence in group B tissues, indicative of increased angiogenesis.

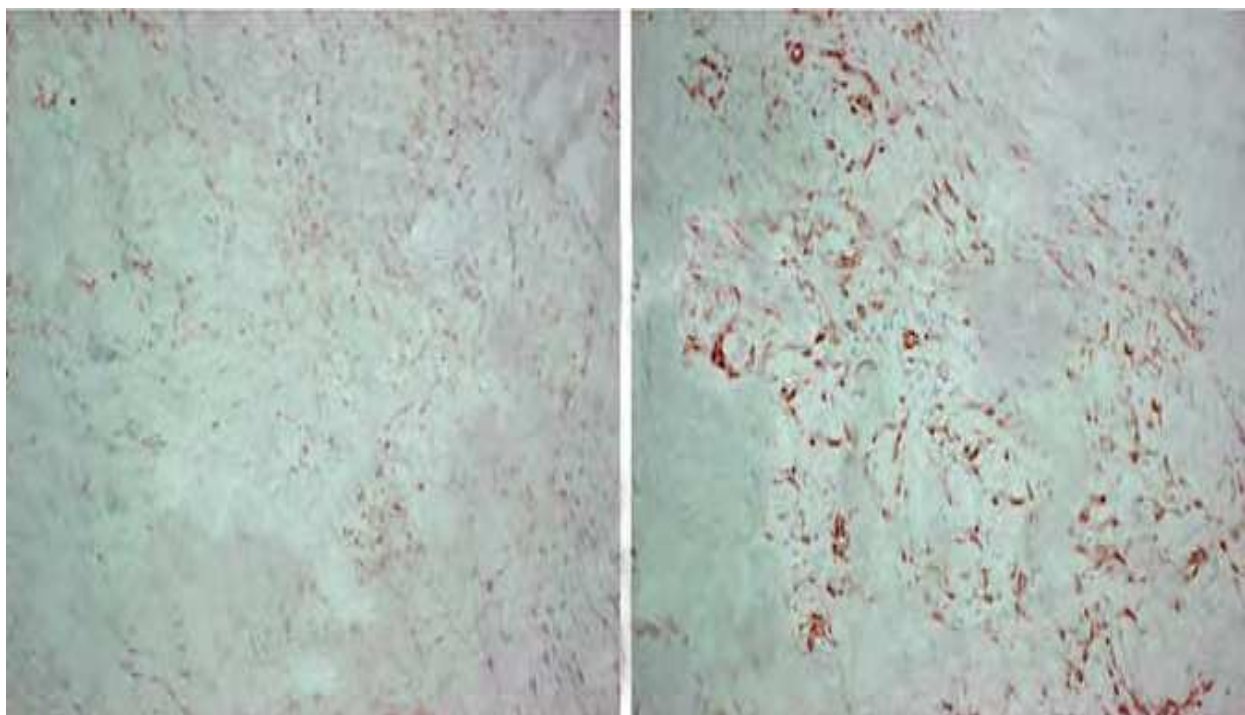


Fig. 25. CD31 stained endothelial cells of newly formed vessels (copper color) in control group B (left) and group b (right). Note the absence of endothelial concentrations in group B and the increased angiogenesis in group b

Groups c

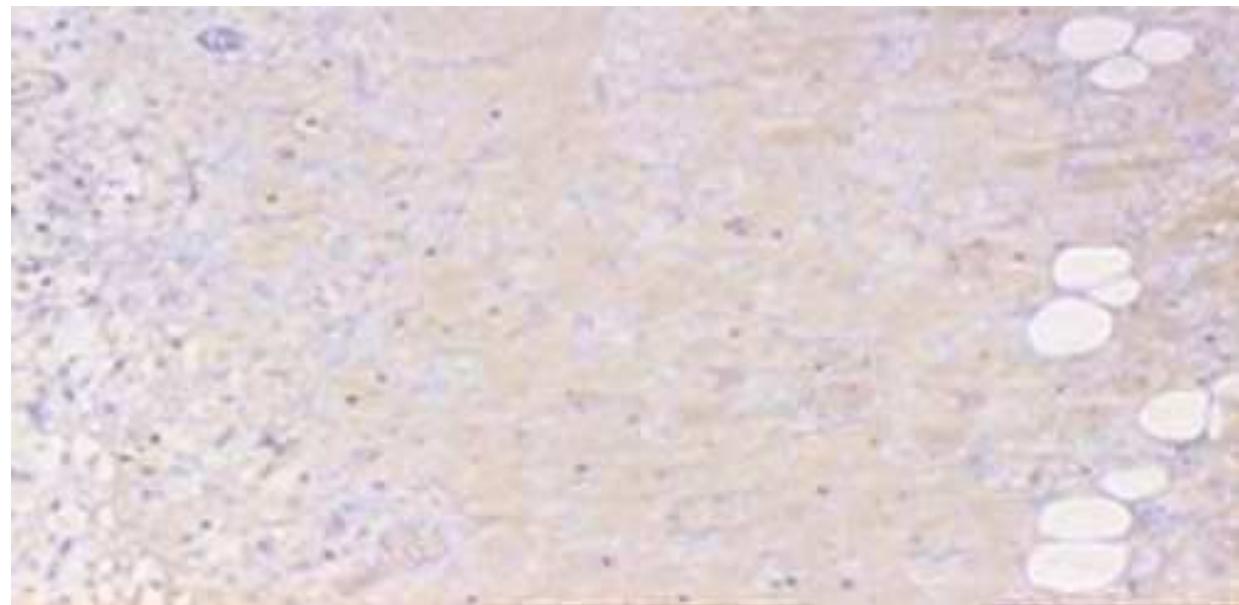


Fig. 26. VEGF expression in group c, after treating tissues with human VEGF monoclonal antibody. Minimal stain in the autologous grafts included in group c.

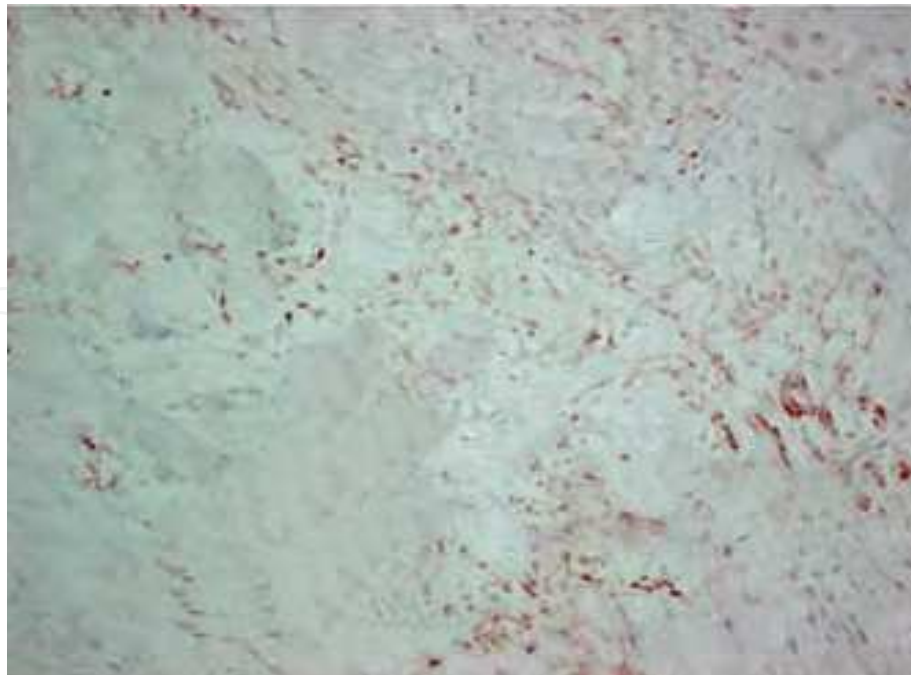


Fig. 27. CD31 stained endothelial cells of newly formed vessels in group c. Considerable decrease in angiogenesis compared to control group A

5. Discussion

Between days 3 and 8 after grafting, the transient formation of spherical protrusions in the graft capillaries resembling angiogenic buds is taking place in what appears to be an angiogenic response of the autochthonous graft capillaries (Lindenblatt et al 2010). Proangiogenic molecules in the recipient bed and the graft tissue were revealed by immunohistochemical analysis which showed that the wall of the aforementioned buds expressed CD31 and desmin, indicating the presence of both endothelial cells and pericytes (Gerhardt & Betsholtz 2003). A docking of sprouting vascular formation takes place from the recipient bed which connects to the aforementioned spherical protrusions of the pre-existing graft vessels unlike the previously suggested neovascularization theory which requires weeks to complete. Additionally, there is rather no vessel inosculation from the recipient bed to the graft as it was previously accepted but there is what appears to be a gradual angiogenic response leading to newly formed vascular buds (Goretsky et al., 1995; Okada, 1986; Young et al., 1996).

The traditional vessel inosculation theory implies vessel re-formation and lumen restoration of already existing vessels via direct approximation of vessels of the recipient bed and the ones in the graft. Since it is nearly impossible to assume that simple graft positioning leads to an effective number of successful graft-bed vessel couplings, the aforementioned angiogenetic process has been recently described. Conversely, it has also been suggested that pre-existing channels in the graft allow vessels from the recipient bed to invade the graft from the periphery replacing a considerable number of graft vessels (Capla et al. 2006). In order to provide the physiologic mechanism of graft take process it could be suggested that during the initial stages (hours) following transplantation, blood, nutrients and angiogenic factors cover the graft area and bed. Hypoxia along with the substrate of available factors creates the signal for the angiogenic response which commences at 2-3 postgrafting days. According to our findings it was noted that the center of the graft

contains a higher density of vascular buds and vessels suggesting that angiogenesis begins there and continuous outwardly towards the periphery of the graft. It is controversial whether angiogenesis takes place before the 2-3rd postgrafting day since there are authors who claim that the graft on its own has the physiologic potential to perform angiogenesis much earlier (Laschke et al., 2008; Shepherd et al., 2004). However, it is generally accepted that revascularization is a process that involves the presence of both the recipient bed and the graft itself since it is their interaction which leads to effective angiogenesis. Additionally, after the initial angiogenic response, vessels from the recipient bed gradually take over and invade-merge with the existing vascular infrastructure of the graft and with the newly formed vascular buds. Following that, endothelial cells initially migrate into the perivascular space creating gradually enlarging vascular lumens which in turn are covered by a migrating pericytes from the inner side of the vascular wall. Pericytes control and further organize the formation of effective vascular structure by producing VEGF and by signalling the end of aberrant angiogenesis where it takes place.

Wang et al (1996) in their study including 22 Sprague-Dawley rats, used human platelet derived wound healing factor (HPDWF) from burn patients and porcine pituitary extract on the recipient bed of 6 pieces of FTSG placed 1 cm apart on the back of each rat and investigated their effect on wound healing. The recipient site was actually the bed of a previously raised dermocutaneous local flap which was subsequently re-positioned on top of the grafts after interposing a completely occlusive sheet between the flap and the grafts. They found approximately 14.4% for the HPDWF and 13.16% for the PPE group improvement in terms of the time needed to bridge the gaps between the FTSGs. However, limitations in their study are the fact that there was no account of the role of the overlying flap in graft wound healing despite its suggested occlusive nature by adding for example a control group without the flap covering technique as well as the lack of evidence regarding the histochemical compatibility of the used factors with the subjects and the potential adverse effects of their use. Additionally the study refers to autologous skin grafts only.

Tatsuya et al (1997) found that the topical application of FK-506 on rat skin allografts may prolong their viability and prevent the rejection cascade phenomena. Nevertheless there was no attempt to further improve graft take results like in our study where the additional use of rHuEPO provided additional graft healing improvement in terms of lowering the percentage of post-grafting necrosis by 21% compared to the control group where only FK-506 was used (35.6% vs 14.6). In our study, rHuEPO had a considerably favorable effect on graft take and viability by achieving 12.89% less graft necrotic area (20.49%-7.6%). According to Kaemmer et al. (2010) the formation of granulation tissue in soft tissue wounds is promoted by rHuEPO primarily under tissue hypoxia. More specifically, rHuEPO promotes cell migration, proliferation, myo-fibroblasts and VEGF production, solely under hypoxia but not under normoxia. Consequently, hypoxia in the graft tissue comprises the signal for the initiation of rHuEPO promoting effect on the graft healing process.

In group b, the additional administration of rHuEPO achieved 21% smaller area of graft necrosis. The favorable effect of rHuEPO was shown in homologous skin grafting. New drugs have been developed in order to achieve prevention of the rejection process without causing toxicity is a goal in transplantation with calcineurin inhibitors (CNIs) comprising one of them. Rejection was prevented by the use of FK-506, which is a CNI, while rHuEPO promoted gradual healing of the graft area due to its effect on cell migration and new vessel formation. It could be suggested that preventing excessive inflammatory response and tissue rejection 'bought' the necessary time for rHuEPO to have its favorable effect in graft and wound healing. Additionally, it has been demonstrated that rHuEPO inhibits the production of pro-inflammatory cytokines by inflammatory cells, antagonizing the

activation of local mechanisms which trigger potential innate injury response (Strunk et al. 2008). Thus it could be suggested that the tissue protective action of rHuEPO complemented indirectly the rejection preventing role of FK-506.

Graft take and revascularization were lower in group c where FK-506 was administered in subjects with autologous skin grafts than in the control group A. According to Mori et al (1997) the explanation could be suggested to be the multiple inhibitory action of FK-506 to many wound healing mediator molecules, primarily to TGF- β induced VEGF, leading to angiogenesis impairment and consequently to graft take interference. However, it was shown that graft take obstruction by FK-506 is neither irreversible nor detrimental since the process of graft take appears to progress achieving optimum or nearly optimum levels. It could therefore be suggested that FK-506 creates a new equilibrium, unfavorable to graft revascularization, prolonging the required time for complete graft take and increasing the risk of impaired viability for a part of the graft tissue. Nevertheless, more studies are required to clarify the exact FK-506 mechanism of action in graft take and healing.

The potential clinical investigations that the finding of this study could include are the use of rHuEPO in immunologically challenged patients (such as patients with extensive trauma, burn injuries, transplanted organs, cancer, immunocompromising infectious disease, special medication treatments, radioactivity injuries, hematological diseases etc) in order to promote or/and sustain good quality skin graft take during the course of their hospitalization or even permanently, as a definitive treatment. In our knowledge this is the first time rHuEPO has been used in conjunction with FK-506 in homologous skin grafting with favorable results.

When it comes to the potential use of skin graft promoting factors to patients, considerations regarding adverse effects also concern the use of rHuEPO as its systemic administration has been associated with strong pro-coagulant and hemodynamic effects which are likely to lead to thrombotic complications especially in patients with cancer, infection, or trauma. Exacerbating the abnormal retinal angiogenesis in patients presenting with retinopathy and the tumor growth supporting effect of rHuEPO have also been reported (Bennett et al., 2008; Corwin et al., 2007). Nevertheless, there is not enough evidence to support any of the aforementioned adverse effects. Cautious use in patients with history of diabetes, heart disease, cancer and extensive trauma is always required.

6. Conclusion

Human recombinant erythropoietin promotes autologous FTSG take and viability in rats. It also appeared to have a favorable effect on homologous FTSG in rats, with the use of FK-506 as an immunosuppressant. When used alone in rats with autologous skin grafts, FK-506 was shown to have a moderately unfavorable effect on graft take. Overall, rHuEPO was shown to promote graft take and viability in rats. Clinical considerations regarding both the favorable and the adverse effects of special factors when used in patients, require future studies which could provide additional insight and feedback.

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The procedure of skin grafting has been performed since 3000BC and with the aid of modern technology has evolved through the years. While the development of new techniques and devices has significantly improved the functional as well as the aesthetic results from skin grafting, the fundamentals of skin grafting have remained the same, a healthy vascular granulating wound bed free of infection. Adherence to the recipient bed is the most important factor in skin graft survival and research continues introducing new techniques that promote this process. Biological and synthetic skin substitutes have also provided better treatment options as well as HLA tissue typing and the use of growth factors. Even today, skin grafts remain the most common and least invasive procedure for the closure of soft tissue defects but the quest for perfection continues.

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