Use of Autologous Bone Marrow Mesenchymal Stem Cells for Healing of Free Full-Thickness Skin Graft in a Zone with Pronounced Hypoperfusion of Soft Tissues Caused by Arteriovenous Shunting

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A combined graft consisting of a free full-thickness skin flap and cultured autologous fibroblast-like bone marrow mesenchymal stem cells was effectively implanted and healed on the facial soft tissue defect after removal of a pathological vascular conglomeration in a female patient with congenital arteriovenous macrofistulous dysplasia. In order to reduce bloodflow intensity and arteriovenous shunting, repeated endovascular occlusion and transcutaneous ligature of regional vessels from the carotid artery basin feeding the pathological zone was carried out followed by resection of this tumor-like vascular formation.

Key Words: *arteriovenous anastomoses; free dermatoplasty; NO therapy; bone marrow; mesenchymal stem cells*

According to published data, arteriovenous macrofistulous angiodysplasia of soft tissues and skin (congenital arteriovenous shunts, anastomoses or fistulas, Parkes—Weber—Rubashov syndrome) are a congenital defect of vascular development, manifesting by multiple arteriovenous shunts of different diameters to a level of arterioles and venules [1,2, 5,6,8,11,14]. Dumping of the arterial blood into the venous system through these anastomoses without passing the capillary network appreciably depletes tissue perfusion causing the so-called "stealing" syndrome. The clinical picture is characterized by enlargement of soft tissues, alteration of the color, temperature, and texture of the skin above the formation because of hypoperfusion, and pathological pulsation of vascular tumor-like formation. The disease progresses as a result of formation of new shunts and expansion of the zone with pathological bloodflow, which can involve vital organs into the process and lead to overexercise of the right heart with subsequent development of organ and cardiac insufficiency.

This means that the treatment of macrofistulous angiodysplasia, carried out by one of previously developed methods [1,2,12-14] should be combined and include a complex of X-ray endovascular and surgical methods [1,7,9,15]. The aim of X-ray endovascular interventions (embolization and occlusion) is to reduce or stop blood flow in the shunting zone. The main task of surgical methods is maximally possible separation of arteriovenous anastomoses (skeletization of vessels) or removal of the entire angiomatous conglomeration with suturing of the vessels feeding it. Recently, a combination of X-ray endovascular and surgical

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methods is used more and more often, which leads to better results, reduces the risk of profuse intraoperative bleeding, and allows the maximum resection of the pathological tissues.

Removal of vast tumor-like vascular formations leads to the appearance of large skin defects. The underlying tissues are also exposed to long-term hypoperfusion and partially contain arteriovenous shunts. Adequate blood supply to the recipient zone and accelerated cell regeneratory processes (stimulation of neoangiogenesis, improvement of microcirculation, and formation of connective tissue matrix) in these patients are an obligatory condition for healing of free full-thickness skin flap.

We present a complex approach to the treatment of patients with arteriovenous macrofistulous vascular malformations of intricate location. The method consists in step-by-step invasive interventions aimed at reduction of the arteriovenous shunting and arterial blood supple via endovascular embolization and occlusion and transcutaneous suturing of afferent vessels and small branches, at maximally possible resection of the pathological ischemic vascular formation, closure of the defect by combined transplantation of autologous cultured bone marrow fibroblast-like mesenchymal stem cells (FMSC) and autologous free full-thickness skin graft (FFSG). The use of FMSC for repair of soft tissue defect results in rapid uncomplicated healing of the skin autograft due to the formation of new vessels, improvement of microcirculation [3,4], and production of collagen matrix (collagen I and III) [10] forming the substrate for FFSG and preventing the growth of arteriovenous anastomoses from the recipient pathological zone into the transplant.

Patient K. (female), 15 years, was hospitalized in Pediatric Clinical Hospital on January 27, 2003 (case history No. 1110-s/2003). The patient had a tumor-like formation of soft consistency with bright pink-colored skin of the nose spreading to the forehead. She complained headache, swelling and pulsation sensations in the bridge of the nose and orbits. The patient suffered from the disease since birth. Photodestruction of the vascular formation on the face was carried out not once at local clinic; the treatment was ineffective, because it promoted the formation of superficial cicatricial tissue, but did not reduce the pathological conglomeration.

Ultrasonic examination with duplex scanning of facial vessels was carried out on January 29, 2003. It showed that soft tissues in the projection of the nose and forehead were thickened (10-12 mm) and actively vascularized. Bloodflow in the vascular formation was mixed, predominantly from the basin of both temporal and orbital arteries. X-ray examination (12.02.03) of the skull bones in two projections showed moderately pronounced hypertensive hydrocephalic syndrome.

On February 7, 2003, carotid angiography in the lateral projection (left and right) was carried out. Based on its results, extensive arteriovenous shunting (2 sec) in the orbital, nose ridge, and frontal areas was carried out during examination of the carotid artery basins. It was found that soft tissues are primarily supplied from hyperplastic orbital and facial arteries.

Repeated ultrasonic examination with duplex scanning of the facial vessels (May 12, 2003) showed that the arteriovenous dumping occurred mainly in the projection of the bridge of the nose, with the bloodflow shunting from two basins: via the branches of the external carotid (temporal and maxillary) arteries and internal carotid (orbital) arteries.

Based on the results of examination, arteriovenous macrofistulous dysplasia of facial soft tissues was diagnosed (Fig. 1). A complex of X-ray endovascular and surgical interventions was planned, consisting of three successive stages: endovascular occlusion of vascular branches feeding the formation, transcutaneous suturing of the residual afferent branches, macro- and microsurgical ligature and resection of the remaining vascular branches involved in bypass bloodflow, and maximally possible removal of the pathological angiomatous conglomeration with changed skin covering it, followed by repair of the tissue defect by combined transplantation of FMSC and FFSG. According to the protocol developed and used in the hospital, transcutaneous suturing of the vessels was planned for days 3-7 after occlusion (maximum ischemization of the angiomatous formation allowing surgical intervention is attained by this period). Hypoperfusion of the future recipient zone of the defect after removal of vascular conglomeration prompted us to carry out combined cell and tissue transplantation. Skin flap in our case closed the defect, while FMSC served as the factor stimulating neoangiogenesis, improving microcirculation, and forming the connective tissue matrix. With this aim in view, 30 days before transplantation, red bone marrow (RBM) was to be collected for *in vitro* culturing of FMSC during this period until the production of the needed cell volume.

In accordance with the plan of treatment, on May 20, 2003 endovascular occlusion of maxillary artery branches on the right and left was carried out. Catheters were intravascularly introduced through the right femoral artery into the maxillary artery on the left and maxillary artery on the right. Occlusion of smaller vessels was carried out with hydrogel

cylinders (0.5 mm in diameter), after which Gianturko spirals were inserted into large branches on both sides. Simultaneous control angiography showed an essential decrease in vascularization of the formation. No postoperative complications were recorded.

Because of impossibility of embolization of the orbital arteries (from the system of internal carotid arteries), on May 26, 2003 additional transcutaneous suturing of the angular arteries of the eye, maxillary arteries at the site of their release from the skull, and of temporal artery branches was carried out through the thickness of the formation from the wings of the nostrils to the bridge of the nose with subsequent fixation of sutures on rolls. Decreased tension, lesser volume and pulsation of the angiomatous formation were noted during the postoperative period.

Ultrasonic examination with duplex scanning of facial vessels (04.08.03 and 08.10.03) showed hypoechogenic Doppler-negative incorporations: dilated (to 2-3 mm) soft tissue vessels, solitary skin vessels of up to 1.0-1.3 mm in diameter, signs of arteriovenous dumping in the bridge of the nose area, and collateral bloodflow against the background of soft tissues thickening to 8-10 mm. These data indicated hemodynamic signs of positive effect of embolization of large vessels, presenting as the development of collateral bloodflow and decreased volume of the arteriovenous dumping. However, arteriovenous anastomoses retained in the bridge of the nose region necessitated repeated endovascular occlusion before radical removal of the formation.

Puncture of the upper wing of the ileac bone at the site of the anterior upper spine on the left was carried out on November 5, 2003. A total of 100 ml of RBM cell suspension was evacuated by puncturing; these cells were subjected to purification and culturing under laboratory conditions.

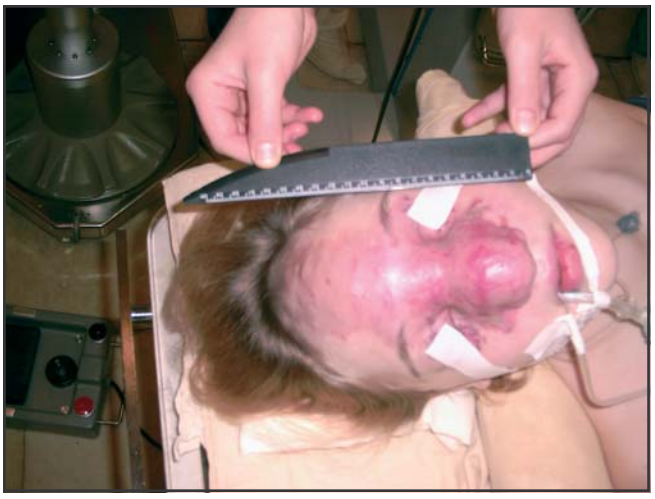

Fig. 1. Patient K. with arteriovenous macrofistulous dysplasia of facial soft tissues before surgery.

Fibroblast-like mesenchymal (stromal) progenitor cells were derived from the suspension of mesenchymal (stromal) cells. After isolation and purification of RBM from hemopoietic fraction cells the mesenchymal cells were seeded in a concentration of 1.5×10^6 cell/ml into Petri dishes in IMDM (Iskov modified Dulbecco medium) with supplements and cultured at 37°C, 5% CO_2, and 95% humidity (Fig. 2, *a*, *b*). The medium was replaced every third day. After 4 weeks the cell material was immunohistochemically tested for mesenchymal cell structures: vimentin (100% staining) and CD133 (20% staining), and was considered ready for transplantation (Fig. 2, *c*, *d*).

After culturing RBM stromal progenitor cells were washed twice from growth medium in Mg^{2+}, Ca^{2+}-free Hanks' solution and treated with Trypsin. Cell suspension was centrifuged at 800 rpm for 5 min and resuspended in Hanks' solution. The resultant cells were counted in a Goryaev chamber. Isolated cells were suspended in saline to a concentration of 10^7 cell/ml and stored in ice for 4 h until application to the wound surface. Cell viability before transplantation was 94±2% (trypan blue staining).

On December 4, 2003 the orbital, frontal, and maxillary arterial branches were ligated on both sides, arteriovenous shunts and pathologically changed skin were resected, and combined transplantation of FMSC and FFSG was carried out. In order to reduce the risk of intraoperative bleeding, preventive vasoconstriction was created by injecting adrenaline hydrochloride solution (1:1000) into soft nasal tissues. An incision of about 2 cm was made in the corners of the left and right orbits in the projection of the left and right orbital arteries, the proximal parts of the arteries were mobilized on both sides, and the arteries were ligated. Comitant veins were also mobilized (the bulk of the aneurysm 1×1 cm), sutured, ligated at some distance, and crossed. Maxillary arteries with concomitant veins on the left and right were similarly mobilized. Visually the veins were significantly dilated and had arteriovenous anastomoses of up to 1.0-1.5 cm in size. Anastomoses in the proximal parts of the vessels were separated, the distal parts of vessels were ligated and crossed. Frontal arteries were mobilized through additional incisions, ligated and crossed. The entire conglomeration with pathologically changed skin was mobilized and resected at the interface of the malformation, after which a skin defect

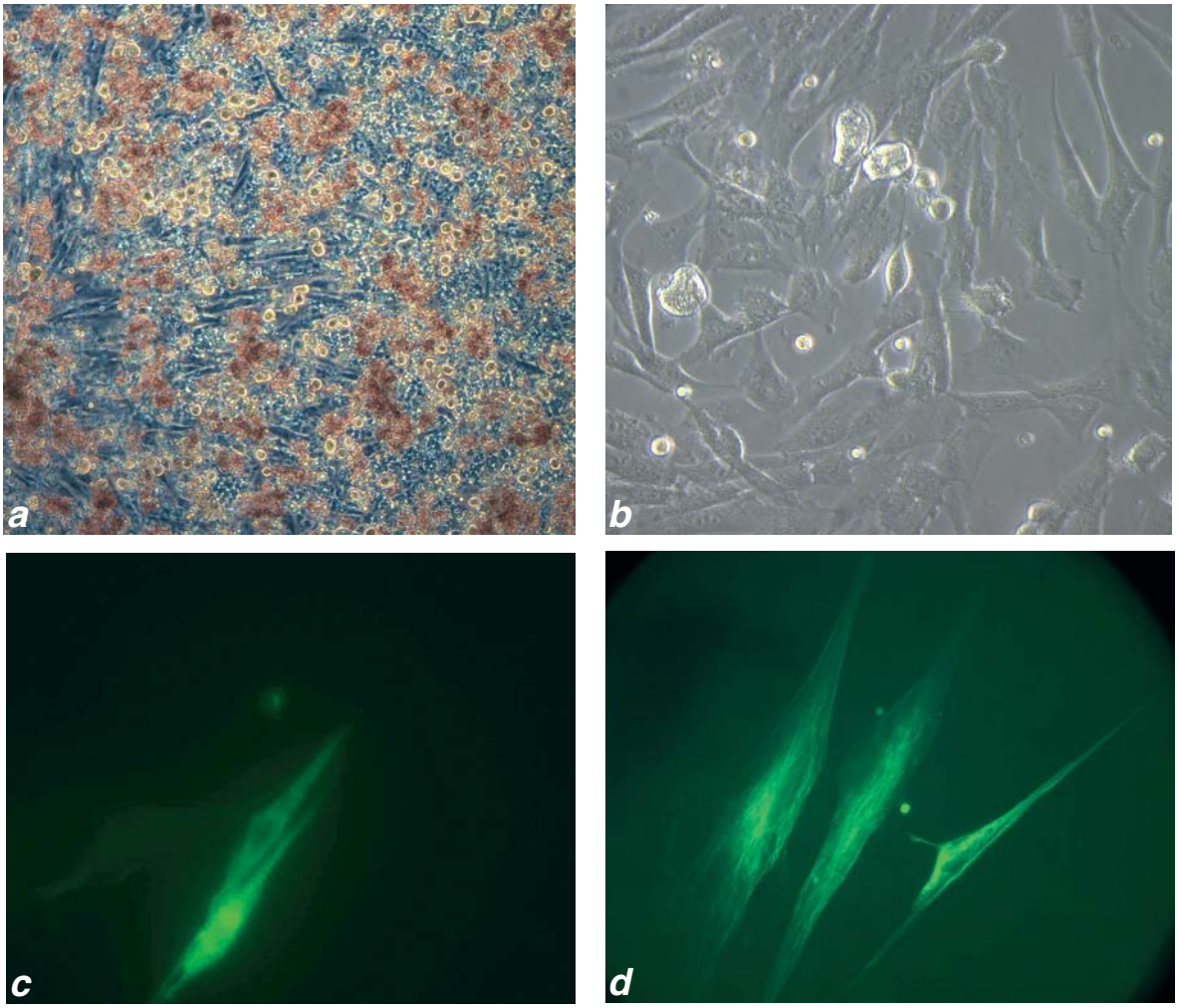

Fig. 2. Bone marrow FMSC culture from patient K. *a*) day 6 of culturing, phase contrast, ×100; *b*) day 28 of culturing, phase contrast, ×400; *c*) day 28, immunofluorescent staining for CD133, ×1000; *d*) day 28, immunofluorescent staining for vimentin, ×1000.

of irregular shape up to 15 cm long and about 7-8 cm wide formed. Hemostasis with an electrocoagulator and cleansing of the wound were carried out. Autologous FMSC (20×10^3 cell/cm^2; a total of 2.25×10^6 cells) were applied onto the wound with a pipette. A free full-thickness skin transplant was then resected from the anterior abdominal wall, treated after Krasovitov, transplanted to the site of defect, and fixed by nodular sutures (Fig. 3, *a*).

On day 11 after surgery there was no discharge from under the skin transplant, but the graft was hyperemic at some sites (Fig. 3, *b*). Visually on day 18 partially detached nonviable epidermal layer on the forehead (2×2 cm) formed; it was removed. Aluminum hydroxyapatite gel was applied onto this site of the full-thickness skin transplant, in order to reduce the risk of cicatrices and hyperpigmentation of the skin. Slight hyperemia was observed on the rest surface of the transplant. On day 25 after surgery the autotransplant site on the forehead was covered with a thin dry crust, no inflammatory phenomena were seen. The transplanted flap was considered to be viable.

In addition to infusion, antibacterial therapy, and wound dressing, the patient received a course of NO therapy consisting in local exposure of the postoperative sutures and the transplant to a hot air-plasmic flow containing NO. The aim of local NO therapy was to stimulate the regeneratory processes at the site of intervention by additional stimulation of neoangiogenesis, restoration of the connective tissue and tissue immunity. NO therapy was carried out with a Plason device operated in A mode. Ten 5-min sessions were carried out every 2-3 days on days 13-32. The sutures were removed on days 25 and 32; no suture incompetence was observed. No side effects of NO therapy were recorded.

The autotransplant had sufficient blood supply during the entire period of observation, due to which there were no cicatricial deformations even

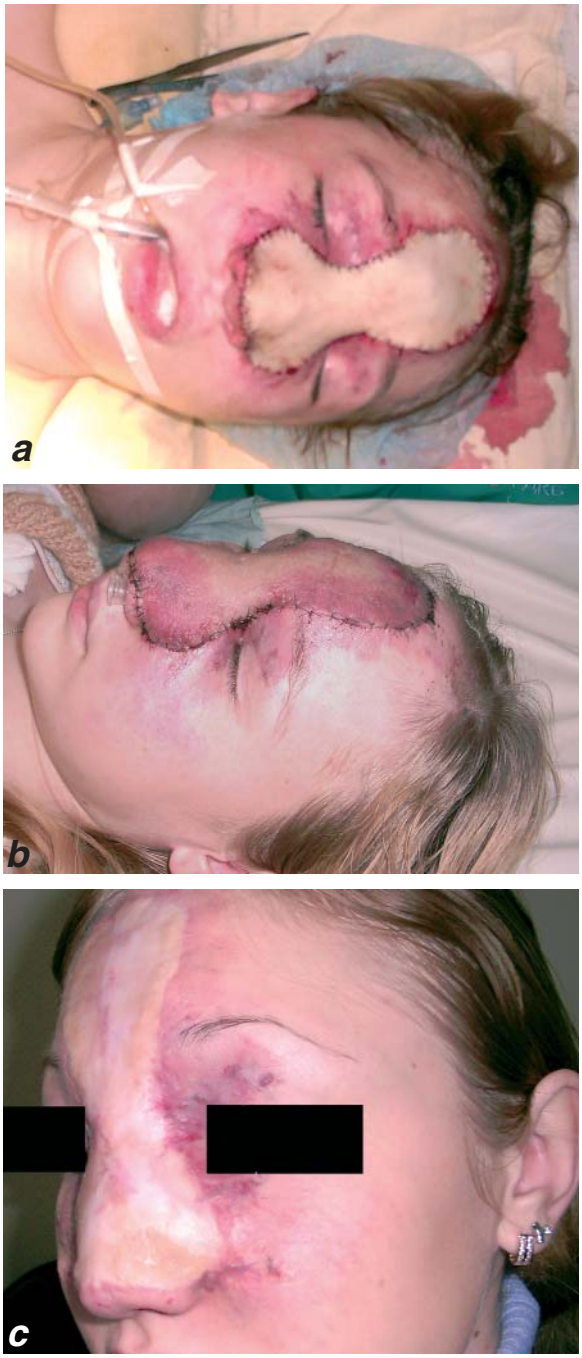

Fig. 3. Patient K. after operation. *a*) after transplantation of FFSG from the anterior abdominal wall; *b*) on day 11 after transplantation: edema and hyperemia of some sites of tissue; *c*) skin transplant 5 months after operation.

at the site of nonviable epidermal layer detachment; the color of the transplant approached that of the adjacent tissues.

However, later a trend to the formation of arteriovenous shunts in the underlying tissues under the transplant developed, mainly at the periphery because of arteriovenous anastomoses in the deeper layers of tissues; these anastomoses were particularly well discernible outside the autodermotransplant (Fig. 3, *c*).

Ultrasonic examination with duplex scanning of facial vessels 5 months after the operation (8.04.04) showed thickened soft tissues on the forehead and slight hypervascularization. A solitary arterial stem of 1.5-2.0 mm in diameter was detected in the projection of the bridge of the nose at a depth of 3 mm from the skin surface, with bloodflow of low peripheral resistance (arteriovenous dumping). Signs of arteriovenous dumping to the left of the wing of the nostril were detected (one of the facial artery branches was dilated). Maxillary arteries demonstrated no signs of shunting on both sides. Hence, hemodynamic signs of arteriovenous anastomoses were detected at the transplant periphery (in the bridge of the nose and to the left of the wing of the nostril), but not in the thickness of the transplant and tissues under it, where no signs of arteriovenous dumping were detected.

The results of combined transplantation of FMSC and FFSG onto a large skin defect under conditions of adjacent tissue hypoperfusion indicate that FFSG can completely take in even under conditions of hypoperfusion and pathological trophics of the recipient tissues of the graft bed. Good healing of FFSG can be provided by autologous predifferentiated FMSC preimmobilized on the wound surface. FMSC create adequate conditions for healing of a skin transplant, as they promote the development of granulation tissue at the expense of neoangiogenesis, production of collagen matrix (types I and III collagen), growth factors, and other factors of stromal structure regeneration. After combined transplantation of FMSC and FFSG the autologous predifferentiated stromal cells not only create conditions for the healing of a skin flap, but can form stromal structures later providing adequate feeding of FFSG and preventing the growth of arteriovenous anastomoses from the pathological tissues of recipient zone into the transplant.

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