Morphologic Changes in the Dermis After the Single Administration of Autologous Fibroblastic Cells: A Preliminary Study

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ABSTRACT

Background. Aging is a multifactorial process defined by an accumulation of damage in all tissues and organs, including the skin, throughout the lifespan of an individual. The reduction of both cellular and extracellular matrix components of the dermis during the aging process is followed by the alteration of the morphology of the skin tissue. This study was conducted to assess skin morphology in men before and 3 months after the intradermal injection of autologous fibroblastic cells.

Methods. Tissue biopsies were surgically obtained before and 3 months after the treatment with autogenously harvested fibroblasts expanded in vitro, as well as after injection of phosphate-buffered saline. The thickness of collagen fiber bundles and number of fibroblasts in the dermis were analyzed in morphometric studies. The morphologic evaluation, using different methods of staining has been performed to analyze of extracellular matrix proteins, including collagen and reticular fibers, fibrillin-1–rich microfibrils, elastic fibers, and hyaluronic acid.

Results. After administration of the cells, we found a noticeable increase in the number of fibroblasts within the dermis, a significant enlargement in diameter of the collagen fiber bundles, and an improvement in the density of reticular fibers, fibrillin-1–rich microfibrils, and elastic fibers compared with the initial, steady-state condition.

Conclusions. The administration of autogenous fibroblasts could be an effective and safe adjunctive therapy to conventional health care treatment to prevent and reduce the age-related accumulation of dermal tissue damage.

AGING is a multifactorial process defined by an accumulation of damage in all tissues and organs, including the skin, throughout the lifespan of an individual [1]. Typical histologic changes observed in chronological skin aging involve gradual atrophy of the epidermis and flattening of the dermal–epidermal junction [2]. However, the most pronounced changes occur in elements of the dermis [2,3], which is divided into the papillary layer and the reticular layer. Both layers are composed of connective tissue and contain the extracellular matrix (ECM), which is a major constituent of the tissue. The ECM consists of a different combination of protein fibers (collagen, reticular, and elastic fibers) and ground substance that fills the majority of the extracellular space within the tissue [4,5]. ECM is produced and secreted by fibroblasts, which are the main cell type in the dermis. Type I and III collagens are by far the most abundant proteins in the dermis [6,7]. These proteins are usually arranged in bundles, often oriented parallel to the epidermis, or are arranged into "basket-weave" bundles [7,8]. The elastic fiber system is an additional group

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of essential skin ECM proteins [9,10]. Adult elastic fibers are composed of an outer mantle of myofibrils (formed mainly by fibrillin-1) and an inner core of cross-linked elastin, both produced by dermal fibroblasts. They form a fine network extending vertically in dermal papillae and surround blood vessels, in the reticular dermis elastic fibers are thicker and run parallel to the skin surface, and surround the larger collagen fibers and skin adnexa [9,10]. Fibrillin-1–rich microfibrils (oxytalan fibers) form a continuous network that extends vertically from the dermal–epidermal junction into the deep dermis [11]. The predominant glycosaminoglycan in the ground substance of the dermis is hyaluronic acid, which constitutes >50% of total body hyaluronan [5].

It is widely accepted that damage to dermal connective tissue caused by the aging process is closely and continuously related to the alteration of its morphology. The histologic features associated with intrinsic (ie, related to non–sun-exposed tissue) and extrinsic (ie, related to sun-exposed tissue) skin aging involves decreased fibroblast number, atrophy of the ECM, reduction and disintegration of collagen and elastic fibers [3], decreased hyaluronan content, and widespread structural modifications [12–14].

Given the important role that skin plays not only as a physiologic barrier for protection against environmental factors but also in interpersonal relations, novel technologies aimed at preventing skin aging in women and, more recently, in men are being extensively investigated in the field of aesthetic dermatology. However, despite the practical evidence, the clinical studies of autologous skin-derived fibroblasts transplantation remain insufficient. To further study the potential therapeutic use of fibroblast transplantation, it is necessary to fully characterize the biologic effects in the human skin setting. Therefore, we designed a pilot study to examine the skin morphology of men and fibrous components production before and 3 months after the intradermal injection of autologous skin-derived fibroblastic cells cultured ex vivo. The studies in vivo were performed with the following particular objectives: (1) to prove the survival and potential proliferation of cultured dermal fibroblasts after autologous transplantation by the cell number analysis of the skin biopsies and (2) to observe in vivo collagen and other fiber secretion activity of the fibroblasts after autologous transplantation by the histologic and immunohistochemical analyses of the skin biopsies.

MATERIALS AND METHODS

Patients

Human skin samples were obtained from 3 volunteers male donors (47, 51, and 52 years of age) in accordance with the Declaration of Helsinki and with the approval of Local Ethics Committee of Pomeranian Medical University. Skin biopsies (0.8 × 0.6 cm) were taken from the postauricular area, which is one of the lower exposed to ultraviolet radiation areas of the head, both before (for fibroblastic cell harvest and for histologic examination) and 3 months after a single injection of in vitro-cultured autologous fibroblasts or phosphate-buffered saline (PBS) (for histologic examination). The cell transplantation and second biopsy were performed near to the initial biopsy site. There were no differences in terms of regional skin specificity between the initial and second biopsy site, owing to the presence of the hair-bearing skin in both biopsy sites. Prior to obtaining the skin fragments, the voluntary patients were locally anesthetized via subcutaneous injection with 1 mL of lignocainum hydrochloricum 1% (Polfa Warszawa, Warsaw, Poland).

Cell Culture

The skin specimens were transported to the laboratory in ice-cold Ca^{2+}/Mg^{2+}-free PBS containing 1:100 penicillin/streptomycin solution (Invitrogen; Life Technologies Warsaw, Poland) and 1 μg/mL Fungizone (both from Gibco, Life Technologies) and then processed immediately. The tissue samples were washed twice with cold Ca^{2+}/Mg^{2+}-free PBS, cut into smaller pieces and incubated in 0.6 U/mL Dispase II (Gibco, Life Technologies) for 1 to 2 hours at 37°C. The epidermis was manually removed from each tissue sample, and the dermis was cut into 1-mm^3 pieces after enzymatic disaggregation with 0.62 Wünsch U/mL Liberase DH (Roche Applied Science, Penzberg, Germany) for 30 to 40 minutes at 37°C. Subsequently, tissue pieces were dissociated by vortexing and then passed through a 70-μm cell strainer (Becton Dickinson, Franklin Lakes, NJ). The dissociated cells were centrifuged at 1500 rpm for 5 minutes. The supernatant was discarded and the pellet was suspended in Medium 199 (Life Technologies) containing 10% human serum isolated from the patients themselves and 0.5% penicillin and streptomycin (Invitrogen). To obtain human autologous serum, 10 mL of whole blood from each patient was collected into plastic tubes containing a serum separator gel with clot activator (Becton Dickinson). Serum separation was completed after centrifugation at 2,000 rpm for 10 minutes. The cells were cultured in a T25 tissue culture flask (Falcon, Becton Dickinson) at 37°C in 5% CO_2 in a humidified atmosphere. The medium was changed 48 hours after plating and every 3 to 4 days thereafter. When the cultures reached 70% confluency, the cells were detached with Accutase (PAA Laboratories, Linz, Austria), washed with PBS and replated in complete medium at 4000 cells/cm^2. The cell cultures were maintained until the fourth passage.

Cells Injection

Cells expanded in vitro were detached with Accutase, washed with PBS, and passed through a 70-μm cell strainer to generate a single-cell suspension. The fibroblasts were diluted in 0.2 mL of PBS and intradermally injected into the right postauricular region of each patient. The series of injections was performed with a 1-mL syringe and a 30-G, 0.5-inch needle into a 1-cm^2 area of superficial dermis using the puncture technique (1 puncture contained 0.05 mL of cell suspension per 0.25 cm^2 of skin area tested). A total of 1.5×10^6 cells were transplanted. An analogous series of injections with PBS only was performed in left postauricular region as an additional control.

Histology

Tissue biopsies were surgically obtained before and 3 months after treatment with autogenously harvested fibroblasts expanded in vitro, as well as after PBS injection. The samples were fixed in freshly prepared 4% paraformaldehyde and embedded in paraffin. For the morphologic analysis, serial slices (3–5 μm in thickness) of skin were mounted onto glass slides and stained with hematoxylin and eosin (H-E). To visualize collagen bundles, the slides were