RESTORATION OF MEIBOMIAN GLAND FUNCTIONALITY WITH NOVEL MESENCHYMAL STEM CELL-DERIVED PRODUCT "DERIVED-MULTIPLE ALLOGENEIC PROTEINS PARACRINE SIGNALING (D-MAPPS)": A CASE REPORT

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ABSTRACT

Meibomian gland dysfunction (MGD) results in the increased tear film osmolarity and leads to the development of dry eye disease. Results obtained in several experimental and clinical studies suggested that mesenchymal stem cells (MSCs) could promote repair and regeneration of injured meibomian glands. We recently developed a new biological product "derived-Multiple Allogeneic Proteins Paracrine Signaling (d-MAPPS)" which activity was based on the effects of immunosuppressive and trophic factors secreted by MSCs. Herewith, we report a case of MGD treated by d-MAPPS containing eye drops, demonstrating therapeutic potential of d-MAPPS in regeneration of injured meibomian glands and in the attenuation of MGD. D-MAPPS containing eye drops significantly attenuated MGD-related symptoms (foreign body sensation, burning, pain in the eye and eye fatigue) and remarkably improved quality of life. The analysis of meibomian glands demonstrated restoration of meibomian gland morphology, structure and function, after the 3-weeks of d-MAPPS based therapy. MGD patient did not report any adverse effects related to the d-MAPPS administration, indicating that d-MAPPS containing eye drops were safe for intraocular application.

Keywords: *Meibomian gland dysfunction; therapy; mesenchymal stem cells; d-MAPPS*

INTRODUCTION

Meibum is an oily substance which, due to the high content of phospholipids, cholesterol and wax esters, protects the ocular surface against microbial pathogens and environmental hazards (1). It is produced by meibomian glands, holocrine sebaceous glands located in the upper and lower eyelids (2). Through the production of lipid-rich meibum that reduce aqueous tear evaporation, meibomian glands provides tear film stability and protects the ocular surface against desiccation (3). Accordingly, both congenital and acquired meibomian gland dysfunction (MGD) results in the increased tear film osmolarity and leads to the development of evaporative dry eye disease (DED) (4).

Meibomian gland dropout and altered meibum secretion were usually seen in the patients suffering from MGD (5). Hyperkeratinization of the ductal epithelium and obstruction of the meibomian gland orifice, accompanied by the stasis of the gland, cystic dilatation and atrophy of the excretory acini, have been considered as the most important pathogenic mechanisms responsible for the development of MGD (6). Additionally, as suggested by the several recently published studies, dysfunction of PPARγ gene and the depletion of meibomian gland stem cells might be also involved in the pathogenesis of MGD (5). PPARγ is highly expressed in meibomian gland acinar cells where regulates lipogenesis and meibum composition (7). Therefore, PPARγ dysfunction results in altered lipid synthesis, higher protein/lipid ratio, increase in meibum viscosity and tear film instability (5, 7). Meibomian gland stem cells are small population of self-renewable cells located at the interface between the ductal and acinar basal epithelium (8). Throughout life, ocular surface and meibomian glands undergo many periods of stress and injury that provoke meibomian stem cells to proliferate and differentiate into meibocytes in order to re-establish their number and function (5). Accordingly, aging and stress-driven exhaustion of meibomian stem cells leads to the meibomian gland dropout, resulting in the development of MGD (5).

Dryness, grittiness, scratchiness, soreness, irritation, and burning are the most usually symptoms reported by the patients suffering from MGD (9). Currently, there is no cure for MGD and the treatments are directed towards improving the symptoms rather than towards eliminating the cause of the disease (10). Therefore, new therapeutic approaches should be focused in the modulation of the main pathological mechanisms which are responsible for an altered function of meibomian glands.

Results obtained in several experimental and clinical studies suggested that mesenchymal stem cells (MSCs), self-renewable stem cells that reside in almost all postnatal tissues, including the corneal stroma, trabecular meshwork and periorbital fat of the eye, could be considered as new therapeutic agents in the treatment of MGD (11). MSC-based therapy significantly improved tear volume and tear film stability which resulted in the attenuation of MGD and DED (11). Beneficial effects of MSCs were attributed to the immunomodulatory and regenerative properties of their secretome (12). MSCs produce large amount of immunosuppressive factors that may suppress inflammation-driven injury of meibomian glands and, at the same time, MSCs secrete trophic factors which support proliferation of meibocytes and promote regeneration of injured epithelial cells (11).

In line with these findings, we recently developed a new biological product “derived-Multiple Allogeneic Proteins Paracrine Signaling (d-MAPPS)” which activity was based on the effects of immunosuppressive and trophic factors secreted by MSCs (13). D-MAPPS efficiently suppressed generation of inflammatory phenotype in activated peripheral blood mononuclear cells and promoted regeneration of injured corneal epithelial cells (14). Continuous administration of d-MAPPS containing eye drops significantly alleviated ocular discomfort and pain in 131 DED patients during the 12-month follow-up (15). Herewith, we report a case of MGD treated by d-MAPPS containing eye drops, demonstrating therapeutic potential of d-MAPPS in regeneration of injured meibomian glands and in the attenuation of MGD.

CASE REPORT

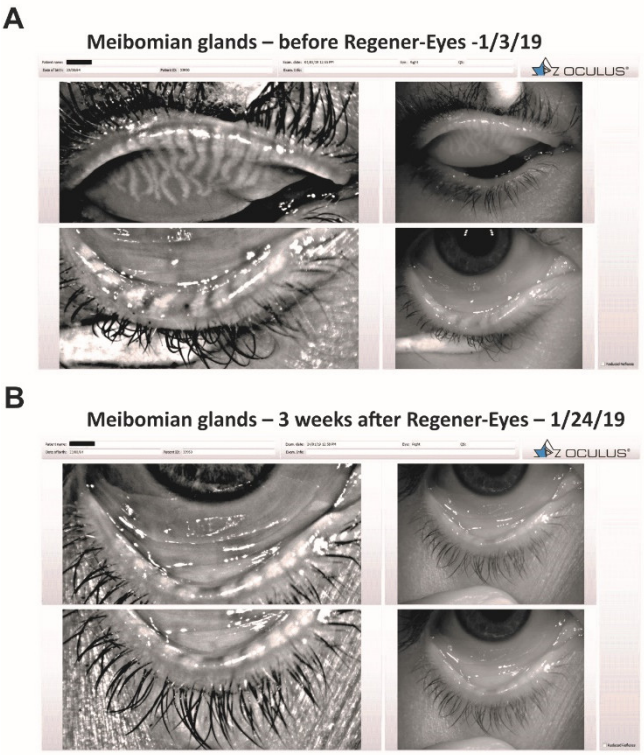
A 55-year-old woman reported symptoms characteristic of MGD: dryness, scratchiness, tearing and burning. She noted that her symptoms first occurred several years prior to her visit and were increasing in severity during the last few months. Although during the past few years she used different types of artificial tears, ointments and intense light pulse therapy, the symptoms become worsen and affected her daily activities. She reported no history of autoimmune and chronic inflammatory diseases and denied use of medications.

At the time of visit, a MGD patient reported foreign body sensation and the pain in the eyes, which were accompanied with grittiness, soreness, irritation, burning and eye fatigue. The slit lamp examination revealed inflamed and rough eyelids with mild telangiectasia. Lid margin debris, which was easily wiped away with a cotton-tipped applicator, was also observed, while pouting material from the meibomian gland orifices were not seen in MGD patient.

D-MAPPS containing eye drops significantly attenuated MGD-related symptoms and remarkably improved quality of life, during the 3-weeks of follow up. The analysis of meibomian glands demonstrated restoration of meibomian gland morphology and structure, after the 3-weeks of d-MAPPS based therapy. As it is shown in Figure 1A, before application of d-MAPPS containing eye drops, meibomian ducts of MGD patient were dilated while meibomian glands were enlarged and tortuous with abnormal structure. Morphology of meibomian glands was significantly improved after 3 weeks of d-MAPPS based therapy (Figure 1B). Meibomian glands were observed as hypoilluminescent grape-like clusters showing normal morphology and structure. Similarly,

hyperilluminescent ducts and underlying tarsus indicated beneficial effects of d-MAPPS containing eye drops (Figure 1B).

Figure 1. Restoration of meibomian gland morphology and structure after the 3-weeks of d-MAPPS-based therapy

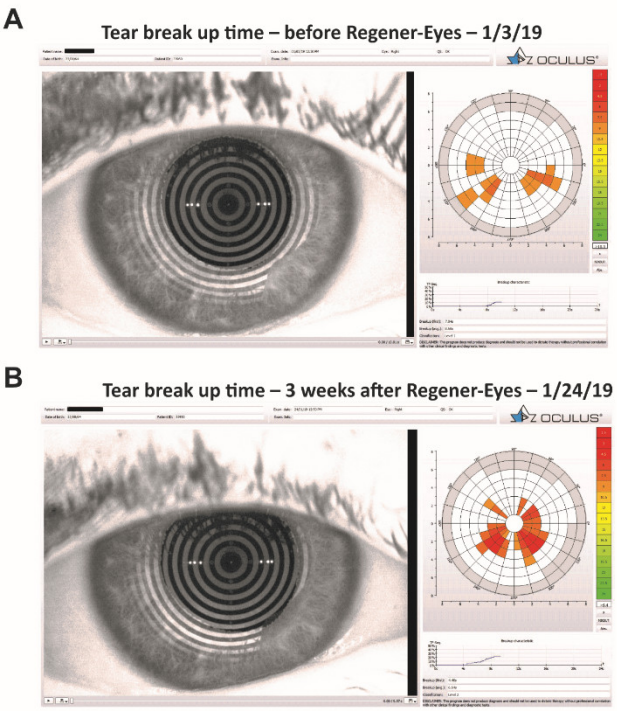


Meibomian ducts of MGD patient were dilated and meibomian glands were enlarged and tortuous with abnormal structure before d-MAPPS-based therapy (A). Meibomian glands had normal morphology with and structure 3-weeks of d-MAPPS-based therapy. (hypoilluminescent grape-like clusters of glands with hyperilluminescent ducts and underlying tarsus) (B).

The tear film breakup time (TBUT) was measured in order to confirm the efficacy of d-MAPPS based therapy. Due to the altered contents of lipids, tear film in patient suffering from MGD was unstable and rapidly evaporated (4). TBUT measured tear film stability and was used to indirectly assess the function of meibomian glands (16). TBUT determines the time elapsed from the last complete eyelid blink until appearance of the first dry spot on the cornea (16). As it is shown in Figure 2B, significantly improved TBUT was noticed 3 weeks after d-MAPPS based therapy, confirming d-MAPPS induced restoration of meibomian gland function.

Complications such as ocular pain, persistent bleeding, and infections were not observed during or after the administration of d-MAPPS containing eye drops. Additionally, MGD patient did not report any adverse effects related to the d-MAPPS administration, indicating that d-MAPPS containing eye drops were safe for intraocular application.

Figure 2. Improved function of meibomian glands after the 3-weeks of d-MAPPS-based therapy



Tear film breakup time (TBUT) measured in a patient suffering from MGD before d-MAPPS-based therapy (A). Significantly improved TBUT noticed 3 weeks after d-MAPPS-based therapy, suggesting d-MAPPS-induced restoration of meibomian gland function (B).

DISCUSSION

MSCs are, due to their potential to provide trophic support to the injured meibomian glands and due to their ability to suppress detrimental T cell-driven immune response in the eye, considered as potentially novel agents in cell-based therapy of MGD (11). As demonstrated by Beyazyıldız and colleagues (17), administration of MSC containing eye drops significantly alleviated DED symptoms in experimental rats. MSCs express various number of chemokine receptors which enable them to migrate to the site of injury and inflammation both after local and systemic application (18). Accordingly, topical administration of MSCs resulted in their higher presence both in injured meibomian glands and in inflamed conjunctival epithelium (17). MSC based therapy significantly increased presence of secretory granules and promoted regeneration and expansion of goblet cells, which resulted in improved tear volume and tear film stability in MSC treated DED rats (17). Additionally, MSCs were capable to increase tear production, resulting in alleviation of eye inflammation and DED-related symptoms in a mouse model of Sjögren's syndrome (19).

However, results obtained in several animal models suggested that transplanted MSCs, in response to the growth factors produced in the local microenvironment, may differentiate into undesired tissues, mainly bone and cartilage (20, 21).

Although MSCs have low expression of human leucocyte antigens (HLA), several studies reported allogeneic immune responses in HLA mismatched recipients of allogeneic MSCs (22-25). Therefore, safeness of MSCs in cell-based therapy of autoimmune and degenerative diseases is still a matter of debate (26).

Majority of MSC-mediated beneficial effects in alleviation of inflammatory eye diseases relied on immunosuppressive and regenerative capacity of MSC-derived factors (15). Accordingly, with aim to avoid safety concerns related to unwanted differentiation of transplanted MSCs (26), we designed d-MAPPS, an ophthalmic MSCs-derived soluble product, which contained MSC-derived immunomodulatory factors that are capable of suppressing detrimental immune response in the eye, and to alleviated MGD (sTNFRI, sTNFRII, IL-1Ra) (13).

TNF-α and IL-1β play important pathogenic role in the development and progression of intraocular inflammation, including DED and MGD (27-29). An elevated concentration of these inflammatory cytokines in serum samples and tears of patients suffering from MGD and DED correlated with the severity of symptoms and clinical parameters (30). Soluble TNF receptors (sTNFRI and sTNFRII) suppress TNF-α-driven inflammation in the eye (31). Accordingly, an up-regulation of sTNFR1 in inflamed ocular surfaces provide protection to the corneal epithelium and ocular surface (31). In similar manner, MSC-derived IL-1Ra, a naturally occurring cytokine, acts as a competitive inhibitor of IL-1β and attenuates IL-1βdriven inflammation in the eye by preventing accumulation of circulating leucocytes in injured corneal epithelium (32). IL-1Ra binds to IL-1 receptor on endothelial cells and prevents pro-inflammatory events initiated by IL-1β:IL-1R interaction, including enhanced influx of neutrophils, macrophages, and lymphocytes in inflamed eyes (33). Furthermore, MSCs, in IL-1Ra dependent manner inhibited activation of inflammasome and reduced consequent production of IL-1β in macrophages, leading to the attenuation of corneal injury, acute and chronic inflammatory diseases in the eye, including DED and MGD (32). In line with these findings, we assume that high concentration of sTNFRI, sTNFRII and IL-1Ra in d-MAPPS containing eye drops and consequent inhibition of TNF-α and IL-1βdriven inflammation may be crucially responsible for enhanced regeneration of meibomian glands and alleviation of MGD in d-MAPPS treated patients.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was conducted in accordance with the ethicalstandards of the committee responsible for human experimentation (institutional and national) and the Helsinki Declaration of 1975, as revised in 2013. Voluntary writtenand informed consent was obtained from the patient prior to enrollment in the study.

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