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Platelet lysate and chondroitin sulfate loaded contact lenses to heal corneal lesions

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ABSTRACT

Hemoderivative tear substitutes contain various ephiteliotrophic factors, such as growth factors (GF), involved in ocular surface homeostasis without immunogenic properties.

The aim of the present work was the loading of platelet lysate into contact lenses to improve the precorneal permanence of platelet lysate growth factors on the ocular surface to enhance the treatment of corneal lesions.

To this purpose, chondroitin sulfate, a sulfated glycosaminoglycan, which is normally present in the extracellular matrix, was associated with platelet lysate. In fact, chondroitin sulfate is capable of electrostatic interaction with positively charged growth factors, in particular, with bFGF, IGF, VEGF, PDGF and TGF-β, resulting in their stabilization and reduced degradation in solution. In the present work, various types of commercially available contact lenses have been loaded with chondroitin sulfate or chondroitin sulfate in association with platelet lysate to achieve a release of growth factors directly onto the corneal surface lesions. One type of contact lenses (PureVision®) showed in vitro good proliferation properties towards corneal cells and were able to enhance cut closure in cornea constructs.

1. Introduction

Ocular surface diseases include a lack of mechanical lubrication as well as a reduction in epitheliotropic factors essential for ocular surface health (Nugent and Lee, 2015). Epitheliotropic factors are crucial to maintain the homeostasis of cornea and are normally present in tears. Among them, various growth factors (GF), such as insulin-like GF, nerve GF, epidermal GF, transforming β GF are fundamental to stimulate proliferation and migration of corneal epithelial cells.

When the cornea is damaged, an imperfect wound healing process may cause persistent epithelial defect (PED), leading to a persistent, incomplete or ineffective epithelization of the corneal stroma. PED causes photophobia, foreign body sensation, pain and lowers the patient's quality of life. It can take months to completely heal and has a high recurrence probability. PED is related to various pathologies in particular to dry eye syndrome, neurotrophic keratopathy, post corneal refractive surgery (i.e. LASIK, PRK), chemical burns, graft versus host disease, herpes zoster or herpes simplex infection, dry eye conditions, such as Stevens–Johnson syndrome, ocular mucous membrane pemphigoid and injury from ocular surgery or trauma (Blackmore, 2010). The treatment of ocular surface disorders has a multifactorial approach and a conventional therapy is not often successful.

Hemoderivatives have been used for a wide range of ophthalmic needs, starting from their first use in 1946 (Katzin, 1946). This is supported by the components present in plasma that can also be found in tears. Moreover, hemoderivatives contain immunoglobulins that provide a bactericidal and bacteriostatic effect. There are few reports on the use of serum eyedrops and platelet lysate eyedrops in patients with dry eye syndrome (Nugent and Lee, 2015) and PED (Nugent and Lee, 2015) and in graft versus host disease (Pezzotta et al., 2012). Autologous hemoderivative tear substitutes contain various epitheliotropic factors, such as growth factors, involved in ocular surface integrity without immunogenic

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properties. Among these, the most relevant bioactive molecules are platelet derived growth factors, cell adhesion molecules, and cytokines from alpha granules. In particular, platelet-derived growth factors and cytokines have a pivotal role to maintain corneal integrity, to mediate the renewal of corneal epithelium and the wound healing processes. In particular, epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), transforming growth factor-beta (TGF-ß), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF) and vascular endothelial growth factor (VEGF) are involved in controlling proliferation, differentiation, migration and apoptosis of corneal epithelial cells contributing to epithelial renewal. From a physio-pathological point of view, platelet derived growth factors are the first to appear in the lesions: they increase the number of repaired cells, stimulate angiogenesis, and support the development of new blood vessels and moreover they are able to activate macrophages.

Ideally, the treatment of corneal damage should be local, by means of direct instillation to the precorneal area to reach the corneal epithelium and maintain a prolonged contact. However, ocular drug delivery to the anterior region of the eye is challenging in spite of the easy accessibility (Hsu et al., 2014; Bengani et al., 2013). This is because only a very small fraction of an instilled dose can reach the target tissue: in particular, the drug is diluted upon instillation into the tears and these are rapidly cleared from the precorneal area, also considering that the human tear film contains about 7 μl of fluid.

In recent years, the association between biomaterials and growth factors has become a hot spot in the new era of regenerative medicine and more recently the association of platelet lysate (PL) and mucoadhesive formulations have been developed for treatment of corneal lesions to prolong residence of PL growth factors in precorneal area (Sandri et al., 2012).

A parallel field of PL administration is the cutaneous wound healing. In this perspective, recent results demonstrated PL effectiveness in in vitro, ex vivo and in vivo animal models, both when PL was loaded in solid scaffolds, based on poly(ether) urethane–polydimethylsiloxane material and fibrin with a sustained release of platelet derived growth factors (Losi et al., 2015), and when microencapsulated in an inorganic carrier (porous silicon microparticles) (Fontana et al., 2016).

The use of contact lenses to heal PED is controversial. However the association of soft contact lenses with topical lubrication is a strategy to treat recurrent epithelial erosion. In fact hydrogels and silicone based contact lenses could stabilize the moisture and oxygen supply to the corneal surface even though this could favor corneal dehydration, leading to a competition for hydration between lens and cornea.

Given these premises, the aim of the present work was the PL loading in contact lenses to continuously release platelet growth factors onto the ocular surface and treat corneal lesions. To this purpose, chondroitin sulfate (CS), a sulfated glycosaminoglycan, normally present in the extracellular matrix was associated with PL. In fact, CS is capable of electrostatic interaction with positively charged PDGFs and in particular with bFGF, IGF, VEGF, PDGF and TGF-β, resulting in stabilization and reduced degradation of growth factors in solution. In the present work, contact lenses were loaded with PL/CS mixture or with only CS, as a comparison.

2. Experimental part

2.1. Materials

Chondroitin sulfate sodium salts, bovine, EP 100 (CS) (Bioiberica, Prochifar, Italy).

Platelet Lysate (PL): it was obtained from the Apheresis Service of Immunohaematology and Transfusion Service Center for transplant immunology, by employing a sterile connection technique. Aliquots of hyperconcentrate platelets (high platelet concentration in small plasma volume and minimal leukocyte contamination) were obtained from apheresis, carried out on regular blood donors. The platelet pool was frozen at −80 °C for 5 h and subsequently unfrozen in a sterile water bath at 37 °C. An automated platelet count and tests for aerobic, anaerobic and fungi contamination were performed after saline dilution.

Contact lenses (White, 2010): Focus Dailies Aqua Comfort plus® (Ciba Vision) based on nelfilcon A (non ionic hydrogel): 69% water content; Dk = 26 (group 2, FDA);

Acuvue Oasys® (Johnson & Johnson Vision Care) based on senofilcon A (silicone Hydrogel): 38% water content; Dk = 103 (group 1, FDA);

Optix Night and Day® (Ciba vision) based on lotrafilcon A (silicone hydrogel): 24% water content; Dk = 140 (group 1, FDA);

PureVision® (Baush & Lomb) based on balafilcon A (silicone ionic hydrogel): 36% water content; Dk = 112 (group 3, FDA).

2.2. Methods

2.2.1. In vitro biocompatibility and proliferation properties of platelet lysate associated to chondroitin sulfate

2.2.1.1. Cell cultures. Rabbit corneal epithelial cells (RCEC) Collection (# 95081046, ECACC, Salisbury, Great Britain) were used between the 8th and 12th passage. Normal human dermal fibroblasts (NHDF) from juvenile foreskin (PromoCell GmbH, Germany) and human umbilical vein endothelial cells (HUVEC) (Lonza, Italy) were used between the 2nd and 5th passage in all the experiments (Sandri et al., 2012).

RCEC were grown in standard conditions in Dulbecco's Modified Eagle Medium (DMEM) mixed 1:1 with Ham's nutrient mixture F12, supplemented with *L*-glutamine (1% v/v, 2 mM), a mixture of penicillin (100 IU/ml), streptomycin (0.1 mg/ml) and amphothericin B (0.25 μg/ml), foetal bovine serum (15% v/v), epidermal growth factor (10 ng/ml) and insulin (5 μg/ml) (Sigma) (Sandri et al., 2010).

NHDF were grown in presence of Dulbecco's modified Eagle medium (DMEM, Lonza, I) supplemented with 10% foetal calf serum (FCS, Euroclone, Italy), with 200 IU/ml penicillin, and with 0.2 mg/ml streptomycin (WVR, Italy) (Sandri et al., 2011, 2015).

HUVECs were cultured in endothelial basal medium (EBM2, Lonza, Italy) supplemented with 2% FCS, 0.4% hFGF-B, 0.1% VEGF, 0.1% R3-IGF-1, 0.1% ascorbic acid, 0.1% hEGF, 0.1% heparin and 0.04% hydrocortisone (Lonza, Italy) and with 1% penicillin/streptomycin (Life Technologies).

All the cell cultures were incubated at 37.0 ± 0.5 °C in a humidified atmosphere 95% RH, containing 5% CO_2.

2.2.1.2. Biocompatibility test (Cytotoxicity). RCEC, fibroblasts and HUVEC were seeded in 96-well plates with an area of 0.34 cm^2 at density 35,000 cells/well. 24 h after seeding, 200 μl of each sample, diluted 1:40 with each growth medium, was put into contact with cell substrates for 24 h.

Subsequently MTT assay was performed. Briefly this test is based on the activity of mitochondrial dehydrogenases of vital cells that convert MTT in formazan. 125 μl of MTT solution (Sigma Aldrich, I) at 0.25 μg/ml concentration in HBSS (Hank's Buffered Salt Solution) pH 7.4 was put into contact with each sample for 3 h. MTT solution was then removed from each well and the cell substrates were washed with PBS (150 μl) to remove the samples and the un-reacted MTT solution. After PBS removal, 100 μl of DMSO were put into each well to lysis cell membranes and solubilize formazan crystals. The absorbance of formazan solution was read at 570 nm by means of an ELISA plate reader (Imark