

EVALUATION OF KERATINISED GINGIVAL FIBROBLAST PROLIFERATION AFTER PROTEFIX® GEL APPLICATION, IN THE ASPECT OF WOUND HEALING IN ELDERLY PATIENTS

Barbara Sterczala¹, Julita Kulbacka², Jolanta Saczko², Marzena Dominiak¹

¹Department of Dental Surgery, Wrocław Medical University, Poland

²Department of Molecular and Cell Biology, Wrocław Medical University, Poland

ABSTRACT

INTRODUCTION: Atrophic mucous membranes in elderly patients become more susceptible to all kinds of injuries: chemical, biological, and mechanical, including iatrogenic. Thus, the healing processes are difficult and challenging. Therefore, it is important to implement preparations to support tissue regeneration in postoperative recommendations. Acceleration of the recovery process would significantly improve the quality of life of elderly patients.

AIM: The aim of the pilot study was the evaluation of the keratinised gingival fibroblast proliferation process after the use of Protefix® gel *in vitro*.

MATERIAL AND METHODS: An *in vitro* pilot study of human fibroblasts obtained from healthy donors and isolated from keratinised gingival tissues. The primary cell line of human fibroblasts was stimulated by 5%, 50%, and 100% Protefix® gel for 20 min and 24 h.

RESULTS: In this *in vitro* pilot study, it was shown that Protefix® gel significantly stimulated the proliferation of human fibroblasts from primary culture.

CONCLUSIONS: Summarising, the conducted pilot study showed significant stimulation of proliferation of human fibroblasts after exposure to Protefix® gel at a concentration of 5%. The obtained results indicate that the tested product may promote the healing of surgical wounds, which is especially important in elderly patients in whom the healing process is more complicated.

KEY WORDS: gerodontology, cell culture, Protefix®.

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INTRODUCTION

The quality of life and the activity of geriatric patients depends to a large extent on the condition of the oral cavity. Xerostomia, burning mouth syndrome (BMS), oral candidiasis, prosthetic stomatitis, and mucous injury due to: poor denture adhesion, mucosal part rough-

ness, and thermal or allergic reaction (caused by the release of free monomer) are some of the more common reasons for elderly patients to report to the dental office [1, 11, 18]. As well as the pain, discomfort of elderly patients is caused by the lack of teeth; therefore, because of rehabilitation of the stomatognathic system, they are often referred to the dental surgeon. Pre-prosthetic pro-

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ADDRESS FOR CORRESPONDENCE: Barbara Sterczala, Department of Dental Surgery, Wrocław Medical University, 26 Krakowska St., 50-425 Wrocław, Poland, e-mail: Barbara.sterczala@umed.wroc.pl

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cedures preparing for treatment include procedures in the field of muco-gingival surgery, including: fraenoplasty of lip or cheek, and vestibuloplasty [4, 6]. Patients also report for the removal of papillary hypertrophy of the palatal mucosa and fibromuscular hypertrophy of the mucous membrane, and gingiva in the form of fissile granulomas, which are the result of unfavourably distributed chewing forces of the lower teeth directed to the upper total denture causing so-called combination syndrome [7, 10].

Geriatric patients are a group requiring a special dental approach, considering the anatomical and physiological changes resulting from the aging process, accompanying psychosomatic diseases, and taken medications. This is often interdisciplinary care. Diabetes, hypertension, hormonal changes in the menopausal period, and deficient diseases such as anaemia or Sjögren syndrome are the cause of symptoms such as dry mouth and burning mucous. These symptoms are persistent in everyday life. In addition, renewal processes are slower. Therefore, it is important to implement in the post-treatment recommendations preparations that will support tissue regeneration.

Currently, there are numerous products available that locally relieve the pain caused by damage to the mucous and have a protective effect against the action of mechanical, biological, or chemical stimuli. Depending on the application and the time of their use, we can distinguish different forms of preparations, e.g.:

- pastes administered immediately after the treatment, having a fibrous, self-hardening, or elastic consistency: Peripac[®], Coe Paki[®] and Coe Pak[®], Septo-pack[®]; they are usually administered for 8-10 days provided that the patient respects the oral hygiene regimen;
- rinses containing chlorhexidine, e.g. Eludril[®], Corso-dyl[®], Curasept[®], KIN[®];
- gels: Elugel[®], Perio-KIN[®], to provide proper oral hygiene.

We can also distinguish preparations according their origin:

- animal like: Solkoseryl Dental Adhesive Paste[®] containing a composition of protein-free dialysate calf blood or Collgel[®] derived from the skin of silver carp;
- plant like: Baikadent[®], Protefix[®].

Protefix[®] is an easy-to-apply preparation due to its gel form. It contains in its composition clove oil, which has an antiseptic effect, which is of colossal importance in elderly people susceptibility to bacterial, fungal, or viral infections; it acts also as an analgesic. Due to its glyceroloxidriester content, the preparation has an extended period of penetration of active substances into the damaged tissue [12, 14].

The aim of our pilot study was to evaluate the keratinised gingival fibroblast proliferation after the use of Protefix[®] gel *in vitro*.

MATERIAL AND METHODS

CELL CULTURE

In this *in vitro* pilot study, the human fibroblasts obtained from healthy donors and isolated from keratinised gingival tissues were used according to the procedures described in Patent No. P 3812045 (Saczko, 2008) [16]. The cells were grown in Dulbecco Modified Medium (DMEM, Sigma-Aldrich) containing 10% foetal bovine serum (FBS) and enriched in a 5% antibiotic/antifungal solution (Sigma). Serum is crucial for cell cultivation because it contains valuable growth factors, mineral salts, lipids, hormones, and adhesion factors. It stimulates cell growth, has a positive effect on the buffering properties of the medium, and protects the cells against mechanical damage. The culture medium was changed twice a week. Cell culture and all procedures were performed under sterile conditions. The cells were maintained in a humidified atmosphere at 37°C and 5% CO₂. For further experiments, fibroblast cells were subjected to trypsinisation (0.25% trypsin-EDTA, Sigma-Aldrich) and seeded into 96-well plates for further testing.

MITOCHONDRIAL ACTIVITY OF FIBROBLASTS IN THE MTT TEST

The primary cell line of human fibroblasts was re-suspended into transparent 96-well plates (Nunc). After 24 hours, Protefix[®] gel was added at the following dilutions: 5%, 50%, and 100%. Then the cells were incubated for 20 minutes or 24 hours. After incubation, an MTT assay (Sigma-Aldrich) was performed to evaluate the cell viability. The method is based on the detection of mitochondrial enzyme activity - succinate dehydrogenase. It converts the orange water-soluble tetrazolium salt (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) to formazan. This product has the form of dark purple, insoluble crystals. Due to their dissolution with isopropanol (they are not soluble in water), a coloured solution is formed. The intensity of the coloured reaction is verified spectrophotometrically at 570 nm. The amount of the resulting coloured product indicates the oxidative activity of the mitochondria in the cells, and thus the number of viable cells in the entire population (the more reduced the MTT, the greater the number of metabolically active cells.) The measurements were performed on a Multiskan[™] FC microplate photometer (Thermo Scientific).

RESULTS

In this *in vitro* pilot study it was shown that Protefix[®] gel significantly stimulated the proliferation of human fibroblasts derived from primary culture. After exposi-

tion to the Protefix® gel at a concentration of 5%, fibroblasts revealed a significant increase in the colour reaction of formazan crystals, in particularly after 24 hours of incubation. The cell viability increased by as much as 50% in comparison to untreated control cells. With the increasing concentration of the preparation, i.e.: 50% and 100%, a proportional decrease in the proliferation of the examined cells was observed. The results of cell viability are shown in Figure 1.

DISCUSSION

The aging process, systemic diseases, or medicines that are taken cause the mucous membrane of elderly patients to become dry and smooth. Cytologically, an increased number of eosinophils is observed. The processes of apoptosis dominate. In the connective tissue the number of cells decreases, including fibroblasts. This leads to destruction of the capillaries. Vascular walls are damaged, which causes the weakening of ion transport, and this determines the reduction of alkaline and acid phosphatase [5, 8]. Atrophic mucous in elderly patients becomes more susceptible to all kinds of injuries: chemical, biological, and mechanical, including iatrogenic.

Mediators of inflammatory and immunological reactions are responsible for the wound healing process, including regulating molecules, i.e. cytokines, which are produced by cells of the immune system but also by fibroblasts. Fibroblasts release growth factors that affect various biochemical processes such as mitogenesis, chemotaxis, angiogenesis, and morphogenesis, which are involved in the tissue regeneration process. At the wound site, fibroblasts that are unable to perform their functions are also damaged. In the healthy and efficient body, fibroblasts migrate from neighbouring areas. However, systemic diseases, bacterial, viral, or fungal infections limit cell migration. Another factor necessary for proper wound healing is angiogenesis from the vascular bed, allowing nutrition and constant delivery of systemic growth and cellular factors [3, 9, 13].

It is therefore important to implement a post-treatment preparation, especially for geriatric patients, which will: be easy to use, be biocompatible, show adhesion to tissues, and be anallergic. In addition to the soothing effect, it will also help to regenerate damaged tissue, thus minimising the risk of parathypical processes. Protefix® from the preparations available on the market, thanks to the glyceroloxdytriesters in the composition, creates a lipid film on the gingivae, without causing excessive pressure of the mucosa, thus protecting the affected oral mucosa against abrasion. At the same time, it does not cause the anaemisation of the damaged site and creates space for cell proliferation. In addition, the included clove oil has antifungal and antiviral activity. After application, it soothes irritations resulting from the use

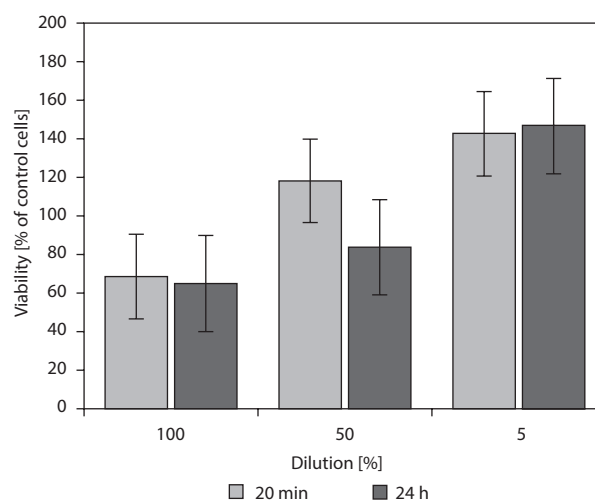


FIGURE 1. The effect of 5%, 50%, and 100% Protefix gel on human gingival fibroblasts after 20 minutes and 24 hours of the exposure. The data represent the mean values of the minimum repetition from three independent repetitions

of newly made prosthetic restorations and relieves pain [12, 14]. It shows antibacterial activity, especially for *Escherichia coli*, *Mycobacterium phlei*, *Bacillus subtilis*, *Streptococcus aureus*, which certainly prevents infection of the wound, especially in people with dry oral mucosa, thus supporting the migration of cells from areas adjacent to the wound [15, 17]. Clove oil also has a local effect to improve blood circulation through antioxidant activity and improves cell nutrition.

Our pilot study confirms the stimulation of fibroblast proliferation by Protefix® gel, and therefore the appropriateness of using the product immediately after damage to the mucosa. Proliferation of fibroblasts stimulated with the preparation supports the release of fibroblast growth factor (FGF), which induces collagen synthesis. This connection was confirmed in the histological studies by Žurek *et al.* [20]. Also, the correlation between the amount of FGF and the proliferation of periodontal regeneration cells was confirmed in the research by Yu *et al.* [19]. The extracellular matrix (EMC) modulated in this way may affect periodontal regeneration. However, Dominiak *et al.* showed that the numerous growth factors that last up to four days affect the reduction of pain and swelling after surgical procedures [4].

CONCLUSIONS

Summarising, the conducted pilot study showed a significant stimulating effect on human fibroblast proliferation after exposure to Protefix® gel at a concentration of 5%. The obtained results indicate that the tested product may considerably enhance the healing of surgical wounds, which is especially important in elderly patients in whom the healing process is more problematic.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interests with respect to the authorship and/or publication of this article.

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