A New Betulin Derivative Stimulates the Synthesis of Collagen in Human Fibroblasts Stronger than its Precursor

MAŁGORZATA DRĄG-ZALESIŃSKA¹, NINA REMBIAŁKOWSKA², SYLWIA BORSKA¹,
JOLANTA SACZKO², MARCIN DRĄG³, MARCIN PORĘBA³ and JULITA KULBACKA²

¹Faculty of Medicine, Department of Human Morphology and Embryology,
Division of Histology and Embryology, Wrocław Medical University, Wrocław, Poland;
²Faculty of Pharmacy, Department of Molecular and Cellular Biology,
Wrocław Medical University, Wrocław, Poland;
³Faculty of Chemistry, Department of Bioorganic Chemistry,
Wrocław University of Science and Technology, Wrocław, Poland

Abstract. Background/Aim: The exploration of substances that stimulate collagen synthesis and retard the aging process of the skin is an active field of current research. The natural environment and plants used in traditional medicine have been a source of such substances. The aim of this study was to compare the stimulatory effect of betulin (BE), betulinic acid (BA) and the new derivative – betulin ester with diaminobutyl acid (BE-Dab-NH₂) on collagen synthesis in human normal fibroblasts. Materials and Methods: Primary fibroblast cultures were obtained from the gums of a healthy patient. The effect of the above-mentioned compounds was assessed by Sircol collagen assay, immunocytochemistry, and proliferation test. Results: Fibroblasts cultured in the presence of BE-Dab-NH₂ produced 6.85 times more collagen than control cells, 7.85 times more than those cultured in the presence of BA and 6.31 times more than those cultured in the presence of BE. An intense immunocytochemical reaction for collagen type I and III was found in fibroblasts cultured in the presence of BE-Dab-NH₂. Conclusion: BE-Dab-NH₂ stimulates significantly more collagen synthesis in normal human fibroblasts than its precursor.

Collagen is an extracellular matrix protein (ECM) synthesised mainly by fibroblasts. Currently, about 20 types of collagen are known, but collagen type I, II, III (skin, bones, teeth, cartilage, tendons, ligaments, fascia, cornea, sclera), and IV and VII (the area of basal membranes) are most common in our body. Type I collagen in the dermis forms thick bundles of irregular fibres, and collagen type III forms a fibril network. Collagen fibres type I and III combine with the remaining elements of the extracellular matrix – elastin fibres and proteoglycan complexes. Integration of ECM components and interaction of fibroblasts and ECM elements is possible thanks to adhesion molecules, fibronectin, entactin and laminin (1).

It is known that with age, gradual degradation of ECM components and a decrease in their synthesis by fibroblasts occurs. The process of skin aging is affected by hormonal changes, genetic conditions and extrinsic factors such as UV radiation (natural and from artificial sources), and smoking (2). Degradation and destruction of the collagen fibre architecture reduces the tensile strength of the skin, causing flaccidity and wrinkles (2).

The search for substances stimulating collagen synthesis and reducing the rate of skin aging processes is a constant direction in research. The natural environment, especially plants used in folk medicine, have been a source of such substances. Numerous studies have documented the action of triterpenes from Centella asiatica – madecassoside, madecassic and asiatic acid (3) on the synthesis of collagen. Centella asiatica (Asiatic pennywort) grows in Madagascar, and is traditionally used to heal wounds, to treat ulcers, and to improve the condition of the skin. Another natural product known to stimulate collagen synthesis as a co-factor of proline hydroxylase is ascorbic acid (4). Another triterpene, betulinic acid, which is a natural derivative of betulin has been shown to stimulate collagen synthesis (5). From the patent description, it appears that betulinic acid has a stronger stimulatory effect on collagen synthesis than the...
extract from *Centella asiatica*, and madecassoside and asiatic acid, active ingredients of this plant (5).

Betulin (BE) is a natural triterpene found in birch bark in an amount of about 10% - 30% and its only natural derivative is betulinic acid (BA), occurring in birch bark in an amount of about 1% (6). These compounds have a wide spectrum of properties including antioxidant, anti-inflammatory, antiviral, antibacterial and antineoplastic, being characterised by a lack of toxicity, both in vitro and in vivo (7). For this reason, for about 20 years they have been the focus of many studies as potential precursors of anticancer drugs (8). However, due to the hydrophobic nature of these substances (they show very low solubility in water), their use is very limited (9).

As a part of this study, new derivatives of betulin were designed and synthesised with more favourable biological properties than the initial compound. The method of synthesis of new betulin derivatives and the chemical structures of them has been described by Drag-Zalesinska et al. (10). Betulin-Dab-NH₂ derivative (betulin ester with L-2,4-diaminobutyl acid) showed a much greater ability to stimulate collagen synthesis in human fibroblasts than betulin and betulinic acid. This discovery has become the subject of patent PL 228855 B1 (11).

**Materials and Methods**

Betulin, betulinic acid and Betulin-Dab-NH₂. Betulin was extracted according to a previously published procedure (12) from the European white birch bark (*Betula pendula*, syn. *Betula verrucosa*) collected in the summer of 2012 in a forest near Wroclaw (Poland, Lower Silesia). The betulin derivative with the chemical name Betulin-Dab-NH₂ (betulin ester with L-2,4-diaminobutyl acid) was synthesised as part of the study of the National Science Centre project no. 0275/B/P01/2010/38. The synthesis method is described by Drag-Zalesinska et al. (10). Betulinic acid was purchased from Sigma company (Sigma, Poznan, Poland). Based on previous research (10), betulin (BE), betulinic acid (BA) and Betulin-Dab-NH₂ (BE-Dab-NH₂) were used in a concentration of 6 μM. The chemical structures of the compounds are shown in Figure 1.

**Mass spectroscopy analysis.** Mass spectrometry was conducted to confirm the molecular weights of synthesised Betulin-Dab-NH₂ compound with a high-resolution Waters LCT Premier XE mass spectrometer equipped with electrospray ionization (ESI) and time of flight (TOF) instruments.

**Cell cultures.** Primary fibroblast cultures were obtained from human gingival tissue collected from a healthy patient at the Department of Oral Surgery, Wroclaw Medical University. The experiment was conducted in accordance with the requirements of the Bioethics Committee of Wroclaw Medical University (No. 864/2012). Gingival biopsy, isolation and culture of human fibroblasts from healthy patients were performed as described by Saczko et al. (13). Isolated cells were cultivated in DMEM medium (Sigma, Poznan, Poland) supplemented by 5% foetal bovine serum (FBS), (EURx, Gdansk, Poland) and 1% of streptomycin/penicillin (Sigma) in a humidified atmosphere with 5% of CO₂. Before the experiments, cells were washed by Dulbecco’s Phosphate Buffered Saline (DPBS), (Sigma) and trypsinized with 0.25% Trypsin-EDTA (Sigma).

**Evaluation of collagen content. Sircol™ collagen assay.** The Sircol™ collagen assay method (Biocolor Ltd., Carrickfergus, UK) is a color-chemical assay used to determine the content of mammalian collagen type I to V in biological material. It uses the properties of the Sirius Red dye, which binds to the helical structure [Gly-X-Y]ₙ characteristic for all these types of collagen. Primary fibroblasts were prepared and placed into 25 cm² culture flasks at 3×10⁵ cells/flask in 4 ml of DMEM with 5% serum. After 24 h of incubation at 37°C, the medium was collected and cells were refed with 4 ml of serum-free DMEM with the addition of the corresponding compound (BE 6 μM, BA 6 μM, Betulin-Dab-NH₂ 6 μM). After 3-day incubation at 37°C and 5% CO₂, the medium was collected and the collagen content was analysed using the Sircol™ Soluble Collagen Assay kit. For analysis 100 μl of media sample were collected, as well as 100 μl of control medium. Collagen solutions (Reference Standard, Biocolor Ltd.) were prepared at the same time to obtain a standard curve in the range of 50-200 μg/ml of collagen. Into test tubes containing medium, control medium, or standard collagen solutions, 500 μl of Sircol Dye Reagent was added (Biocolor Ltd.) and then the tubes were shaken for 30 min. Then, the samples were centrifuged for 30 min at 13000 RCF and 4°C. The supernatant was then removed and the pellets were resuspended in 500 μl Wash Reagent (Biocolor Ltd.) and then the tubes were centrifuged again at the same conditions. After the supernatant was removed again, the pellet was resuspended in 250 μl Alkaline Reagent (Biocolor Ltd.), shaken for 5 min and seeded into a 96 well plate. The absorbance at 550 nm was measured using a multiwell scanning spectrophotometer (EnSpire Multimode Plate Reader, PerkinElmer, Inc. Waltham, MA, USA). Based on the standard curve, the collagen content was calculated in each sample. The effect of the compounds on collagen synthesis was calculated by subtracting from the values of samples those of the control.

**Immunocytochemical assay.** Expression of type I and III collagen was assessed by immunocytochemistry. For this purpose, human normal fibroblasts were placed onto 8-well microscope slides. After 24 h when the cells were attached to the surface, test compounds were added at a concentration of 6 μM. After a 24-h incubation, the preparations were washed in PBS and then fixed with 4% formaldehyde. The slides were rinsed in PBS buffer and placed in 1% H₂O₂ for 30 min. Then, they were rinsed 3×5 min in PBS with 1% Triton X 100 (Sigma). Next, the slides were drained and a goat polyclonal anti-collagen type I antibody (Santa Cruz BT COL1A1, CA, USA) or a goat polyclonal anti-collagen type III antibody (Santa Cruz BT COL3A1) were applied. After a 24-h incubation at 4°C, they were rinsed 3×5 min in PBS with 1% Triton X100. Then the procedure was performed using the DAKO LSAB kit (Carpinteria, CA, USA) + System HRP, successively with Biotinylated (lysed) primary antibody, streptavidin –HRP, DAB + substrate buffer with DAB + Chromogen, as described by the manufacturer. For the staining of cell nuclei, hematoxylin (Carl Roth, Karlsruhe, Germany) was used for 30 sec – 1 min. Next, the preparations were rinsed in water for 30 min, carried out by the ascending series of alcohol (50-100%) and dehydrated in xylene. Finally, the preparations were closed with DPX synthetic resin (Aqua Medica, Poland) using cover slides. A
coloured immunocytochemical reaction was assessed using an Olympus BX51 microscope (Japan). The percentage of cells that underwent a staining reaction and the intensity of the immunocytochemical reaction were estimated. The counting was performed by two independent investigators. The intensity of immunohistochemically stained reaction was evaluated as (–) negative (no reaction), (+) weak, (++) moderate, and (+++) strong.

Proliferation test. MTT assay. The effect of the BE-DAB-NH₂, BE and BA on human normal fibroblasts was assessed by the MTT test. The MTT test is a colorimetric method designed to determine the metabolic activity of living cells. Its action is based on the reduction of the tetrazolium salt by mitochondrial dehydrogenase to the coloured crystals of formazanes. The product of this reaction is insoluble in water and therefore requires the delivery of an organic solvent, e.g. DMSO or isopropanol. The intensity of the obtained solution translates proportionally to the cell proliferation and is measured spectrophotometrically in the 492-570 nm wavelength range. The cells were seeded into a 96-well culture plate. After 24 h and when the cells were adherent, the culture fluid was removed and the test compounds were added in the appropriate concentration (1 μM, 3 μM, 6 μM, 12 μM, 20 μM). The cells were then incubated 24 h with the test compounds. After this period, the MTT test was performed according to the manufacturer’s protocol (Sigma Aldrich, Darmstadt, Germany). After the dissolution of the formazan crystals, the absorbance at 570 nm wave was measured using the Multimode EnSpire Reader (PerkinElmer, Inc.). The absorbance results were

Table I. Collagen content in the culture medium determined by the Sircol™ Collagen Assay after 72 h of incubation of fibroblasts with the test compounds at a concentration of 6 μM.

| Type of sample                                         | Collagen content [μg/ml] |
|------------------------------------------------------|--------------------------|
| 1. Cells cultured in the presence of BE-Dab-NH₂      | 64.4±1.2*                |
| 2. Cells cultured in the presence of BA              | 8.2±0.8                  |
| 3. Cells cultured in the presence of BE              | 10.2±1.1                 |
| 4. Control cells                                      | 9.4±0.95                 |

*p<0.001 with reference to samples 2, 3 and 4 (Student’s t-test).
collagen was found in fibroblasts cultured in the presence of BE-Dab-NH₂. In fibroblasts cultured in the presence of BE immunocytochemical reaction are presented in Table II. ICC while for control cells, either was lacking or was poor for immunocytochemical reaction for both type I and III both types of collagen. The results of the evaluation of the expression of collagen in relation to the control, to cells treated with BA, the reaction for both type of collagen was poor, while for control cells, either was lacking or was poor for both types of collagen. The results of the evaluation of the immunocytochemical reaction are presented in Table II. ICC images for control cells and cells incubated with the derivative BE-Dab-NH₂ are presented in Figures 2 and 3.

Statistical analysis. Statistical analysis was performed using Statistica 13.0 (Dell Inc., USA). Student’s t-test and nonlinear estimation were used for the calculations.

Results

Mass spectroscopy. The BE-Dab-NH₂ mass spectroscopy analysis is shown in Figure 1. Calculated mass m/z +=543.4526; measured mass m/z +=543.4528.

Sircol™ collagen assay. The collagen content in the medium of fibroblasts incubated with the test compounds was measured by this method. The experiment revealed that fibroblasts cultured in the presence of BE-Dab-NH₂ produced 6.85 times more (increase by 685%) of collagen than control cells, 7.85 times more (an increase of 785%) than cells cultured in the presence of BA and 6.31 times more (increase of 631%) than cells cultured in the presence of BE. At the same time, there was no increase in the collagen content in the BA-treated fibroblast cultures compared to the control, while BE caused a minimal increase in the collagen content compared to the control. Differences in the amount of collagen produced following incubation with BE-Dab-NH₂ in relation to the control, to cells treated with BE and cells treated with BA are statistically significant (p<0.001). The results obtained are shown in Table I.

Immunocytochemical staining (ICC). A particularly intense immunocytochemical reaction for both type I and III collagen was found in fibroblasts cultured in the presence of BE-Dab-NH₂. In fibroblasts cultured in the presence of BE or BA, the reaction for both types of collagen was poor, while for control cells, either was lacking or was poor for both types of collagen. The results of the evaluation of the immunocytochemical reaction are presented in Table II. ICC converted to a percentage of control cells. The test was performed in triplicate and the percentage of viable cells was determined for individual concentrations of Betulin-DAB-NH₂, BA and BE.

Discussion

There are reports regarding stimulation of collagen synthesis by betulinic acid at a concentration of 6 μM (5). The patent states that betulinic acid (BA) has a stronger effect on collagen synthesis than the active ingredients of Centella Asiatica (5). In our experiment, no increase in collagen synthesis was observed after treating fibroblasts with 6 μM BA. Another study on the stimulation of collagen synthesis in human fibroblasts by triterpenes has reported that gedunin (from Carapa guianensis seeds) increased collagen synthesis by 130% (14). Triterpene saponins from flowers of Bellis perennis also stimulate collagen synthesis in fibroblasts at the level of 130% compared to control (15). Labisia pumila is another plant containing triterpene saponins with a documented influence on the synthesis of collagen in human fibroblasts (16).

Betula verrucosa, Centella asiatica, Bellis perennis, Carapa guianensis, Labisia pumila are plants used for centuries in folk medicine in various parts of the world to heal wounds and ulcers, and to relieve inflammation of the skin. The active ingredients present in this plant material have been well characterised, but studies examining the
potential therapeutic properties of individual compounds are still ongoing (14–16). Other studies examining the effect of *Calophyllum inophyllum*, that contains triterpenes, on collagen production by primary fibroblasts have indicated stimulation of proliferation and collagen III production (17).

The European Medicines Agency (EMA) registered in 2016 the drug EPISALVAN (Oleogel-S10, AMRYT) containing 10% of birch bark extract (of which BE is 60-86%) (18). In the second half of 2018, the drug obtained a positive opinion from the FDA (Food and Drug Administration, USA) and is currently in phase III clinical trials in 38 research centres around the world for the treatment of various types of *Epidermolysis bullosa* (EB). EB is a group of genetically determined diseases of the skin.

---

**Figure 2.** Photos of fibroblasts with immunocytochemically stained collagen type I. On panels A, B, C are the cells cultured with Betulin-Dab-NH$_2$ (concentration 6 μM); on panels D, E, F control cells are shown. Magnification on panels A, D ×40; B, E ×200; C, F ×400.

**Figure 3.** Microphotographs of fibroblasts with immunocytochemically stained collagen type III. On panels A, B, C are the cells cultured with Betulin-Dab-NH$_2$ (concentration 6 μM); on panels D, E, F control cells are shown. Magnification on panels A, D – ×40; B, E – ×200; C, F – ×400.
characterised by the formation of blisters and difficult to heal wounds due to mechanical injuries. Numerous studies have reported on its effectiveness in the treatment of EB wounds and other skin lesions, e.g. after laser procedures (19-22). It is the first registered drug containing a formula based on triterpene compounds from birch bark.

In our experiment, the betulin derivative BE-Dab-NH₂ (betulin ester with L-2,4-diaminobutyl acid) was shown to have a much greater ability to stimulate collagen synthesis in human fibroblasts than BE and BA. It should also be pointed out that this compound has a much better solubility in water than BE and BA (10). These properties make the new derivative, BE-Dab-NH₂, more appropriate to be included in preparations that heal wounds, regenerate the oral mucosa, and revitalise the skin. A further stage of the current work will be the development of formulations enabling the practical use of our findings in in vivo studies and in clinical trials.

Conflicts of Interest

The Authors state that they have no conflicts of interest regarding this study.

Authors’ Contributions

MD-Z and JK designed the study. MD and MP carried out botulin derivative synthesis. JK, JS, SB and NR performed the in vitro experiments. JK, JS and MD-Z analysed the results. All Authors read and approved the final manuscript.

Acknowledgements

Part of the research was supported by the National Science Center (Poland) under Grant No. N N401 027538 (PI: M. Drag-Zalesińska). The research was also supported by the statutory activities of the Department of Molecular and Cellular Biology of Wroclaw Medical University.

References

1. Czubak AK and Żbikowska HM: Structure, function and biomedical significance of collagens. Ann Acad Med Siles 68: 245-254, 2014.
2. Trznadel-Grodzka E and Kaszuba A: Etiopathogeneza procesu starzenia skóry. In: Dermatologia Geriatyczna. I (ed.), Lublin Czelej: pp. 21-24, 2016.
3. Maquart FX, Bellon G, Gillery P, Wegrowski Y and Borel JP: Stimulation of collagen synthesis in fibroblast cultures by a triterpene extracted from Centella asiatica. Connect Tissue Res 24: 107-120, 1990. PMID: 2354631.
4. Murad S, Grove D, Lindberg KA, Reynolds G, Sivarajah A and Pinnell SR: Regulation of collagen synthesis by ascorbic acid. Proc Natl Acad Sci 78: 2879-2882, 1981. PMID: 6265920.
5. Cho SH, Gottlieb K and Santhanam U: Cosmetic compositions containing betulinic acid. United States Patent, Patent Number 5, 529, 769, 1996.
6. Zdzisińska B, Szuster-Ciesielska A, Rzeski W and Kandafer-Szerszeń M: Właściwości lecznicze betuliny I kwasu betulinowego, składniki ekstraktu z kory brzozy. Far Przegl Nauk 3: 33-39, 2010.
7. Rastogi S, Pandey MM and Kumar Singh Rawat A: Medicinal plants of the genus Betula—traditional uses and a phytochemical-pharmacological review. J Ethnopharmacol 159: 62-83, 2015. PMID: 25494458. DOI: 10.1016/j.jep.2014.11.010
8. Ali-Seyed M, Jantan I, Vijayaraghavan K and Bukhari SN: Betilinic acid: recent advances in chemical modification, effective delivery, and molecular mechanisms of promising anticancer therapy. Chem Biol Drug Des 87: 517-536, 2016. PMID: 26535952. DOI:10.1111/cbld.12682
9. Drag-Zalesińska M, Kulbacka J, Saczko J, Wysocka T, Zabel M, Surowiak P and Drag M: Esters of betulin and betulinic acid with amino acids have improved water solubility and are selectively cytotoxic toward cancer cells. Bioorg Med Chem Lett 15: 4814-4817, 2009. PMID: 19560351. DOI: 10.1016/j.bmcl.2009.06.046
10. Drag-Zalesińska M, Wysocka T, Borska S, Drag M, Poręba M, Choromańska A, Kulbacka J and Saczko J: The new esters derivatives of betulin and betulinic acid in epidermoid squamous carcinoma treatment – In vitro studies. Biomed Pharmacother 72: 91-97, 2015. PMID: 26054680. DOI: 10.1016/j.biopha.2015.04.003
11. Drag-Zalesińska M, Kulbacka J, Saczko J, Drag M and Poręba M: Zastosowanie pochodnej betuliny do wytwarzania preparatu do stymulowania syntetyz kolagenu. Urząd Patentowy Rzeczpospolitej Polskiej PL 228855, 2018.
12. Drag M, Surowiak P, Drag-Zalesińska M, Dietel M, Lage H and Oleksyszyn J: Comparison of the cytotoxic effects of birch bark extract, betulin and betulinic acid towards human gastric carcinoma and pancreatic carcinoma drug-sensitive and drug-resistant cell lines. Molecules 24: 1639-1651, 2009. PMID: 19396022. DOI: 10.3390/molecules14041639

Figure 4. The effect of BE, BA and BE-Dab-NH₂ on the proliferation of normal human fibroblasts was assessed by MTT. *p<0.05 (Mann-Whitney U-test).
13 Saczko J, Dominiak M, Kulbacka J, Chwilkowska A and Krawczykowska H: A simple and established method of tissue culture of human gingival fibroblasts for gingival augmentation. Folia Histochem Cytobiol 46: 117-119, 2008. PMID: 18296274. DOI: 10.2478/v10042-008-0017-4

14 Morikawa T, Nagatomo A, Kitazawa K, Muraoka O, Kikuchi T, Yamada T, Tanaka R and Ninomiya K: Collagen synthesis-promoting effects of andiroba oil and its limonoid constituents in normal human dermal fibroblasts. J Oleo Sci 67: 1271-1277, 2018. PMID: 30305560. DOI: 10.5650/jos.ess18143

15 Morikawa T, Ninomiya K, Takamori Y, Nishida E, Yasue M, Hayakawa T, Muraoka O, Li X, Nakamura S, Yoshikawa M and Matsuda H: Oleanane-type triterpene saponins with collagen synthesis-promoting activity from the flowers of Bellis perennis. Phytochemistry 116: 203-212, 2015. PMID: 26028520. DOI: 10.1016/j.phytochem.2015.05.011

16 Chua LS, Lee SY, Abdullah N and Sarmidi MR: Review on Labisia pumila (Kacip Fatimah): bioactive phytochemicals and skin collagen synthesis promoting herb. Fitoterapia 3: 1322-1335, 2012. PMID: 29318289.

17 Zalewski J, Mączyńska J, Bieżuńska-Kusiak K, Kulbacka J, Choromańska A, Przestrzelska M, Zalewski M, Saczko Z, Cwynar-Zając Ł, Rusak A and Saczko J: Calophyllum inophyllum in vaginitis treatment: stimulated by electroporation with an in vitro approach. Adv Clin Exp Med 28: 223-228, 2019. PMID: 30465335. DOI: 10.17219/acem/87045

18 Scheffer A: Use an oleogel containing triterpene for healing wounds. United States Patent Application Publication: Pub. No US 2012/0231054 A1, 2012.

19 Schwieger-Briel A, Kiritsi D, Schemp C, Has C and Schumann H: Betulin-based oleogel to improve wound healing in dystrophic epidermolysis bullosa: a prospective controlled proof-of-concept study. Dermatol Res Pract 2017: 5068969, 2017. PMID: 28611842. DOI: 10.1155/2017/5068969

20 Barret JP, Podmelle F, Lipový B, Rennekampff HO, Schumann H, Schwieger-Briel A, Zahn TR and Metelmann HR; BSH-12 and BSG-12 study groups: Accelerated re-epithelialization of partial-thickness skin wounds by a topical betulin gel: Results of a randomized phase III clinical trials program. Burns 43: 1284-1294, 2017. PMID: 28400148. DOI: 10.1016/j.burns.2017.03.005

21 Frew Q, Rennekampff HO, Dziewulski P, Moiemen N, Moiemen N; BBW-11 Study Group, Zahn T and Hartmann B: Betulin wound gel accelerated healing of superficial partial thickness burns: Results of a randomized, intra-individually controlled, phase III trial with 12-months follow-up. Burns pii: S0305-4179(18)30545-X, 2018. PMID: 30559054. DOI: 10.1016/j.burns.2018.10.019

22 Lipový B, Fiamoli M, Mager R, Jelinková Z, Jarkovsky J, Chaloupkova Z, Holoubek J, Suchanek I and Brychta P: Oleogel-s10 to accelerate healing of donor sites: monocentric results of phase III clinical trial. Acta Chir Plast 59: 129-134, 2018. PMID: 29651853.

Received March 15, 2019
Revised April 17, 2019
Accepted April 23, 2019