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ACTION OF ENAMEL MATRIX PROTEINS ON HUMAN OSTEOGENOUS
PROGENITOR BONE MARROW CELLS, AN EXPERIMENTAL STUDY**

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Summary. Complex treatment of moderate and severe generalized periodontitis includes surgical phase of treatment in aim to provide regeneration and long-term disease remission. The obtained results confirm presence of osteoinductive properties in enamel matrix proteins, this data may be useful during surgical procedures on periodontal tissues.

Key words: generalized periodontitis, regeneration, enamel matrix proteins, Emdogain, osteogenous progenitor human bone marrow cells, osteoinduction.

Резюме. Комплексне лікування генералізованого пародонтиту II, II-III ступенів включає проведення хірургічної фази, яка має на меті регенерацію тканин пародонта з тривалою ремісією захворювання. Отримані дані підтверджують наявність остеоіндуктивних властивостей у емалевих матричних протеїнів, що може бути використано при хірургічних втручаннях на пародонті.

Ключові слова: генералізований пародонтит, регенерація, емалеві матричні протеїни, Emdogain, остеогенні клітини-попередники кісткового мозку людини, остеоіндукція.

Резюме. Комплексное лечение генерализованного пародонтита II, II-III степеней включает проведение хирургической фазы, целью которой является регенерация тканей пародонта с долговременной ремиссией заболевания. Полученные данные подтверждают наличие остеоиндуктивных свойств у эмалевых матричных протеинов, что может быть использовано при хирургических вмешательствах на пародонте.

Ключевые слова: генерализованный пародонтит, регенерация, эмалевые матричные протеины, Emdogain, остеогенные клетки-предшественники костного мозга человека, остеоиндукция.

Introduction. Periodontal diseases (PD) are prevalent diseases among other dental pathologies and are still substantial public burden worldwide [2,3,13,14,36]. Generalized periodontitis (GP) also became to be widely spread disease among other types of periodontal diseases, staying until present times as unsolved clinical problem because of completely untreated [36]. The periodontal tissue complex involves into chronic and progressive destruction mainly the alveolar bone and periodontal ligament (PDL) [36], leading to the tooth loss as frequent result [13,36]. A major discovery was the finding that periodontal tissue regeneration could be achieved by application of enamel matrix proteins (EMPs), and later – by the FDA approved medical device termed Emdogain [9,10]. But the complete periodontal regeneration is a major goal in the treatment and still under plural investigations that needs additional profound investigations to supply understanding of all cell-to-cell regenerative mechanisms [22,24,27,36,38]. It was showed that the bone marrow stromal cells also have the potential to regenerate the periodontium [18]. Current data indicate that amelogenin stimulates a cascade of events enhancing remodelling and regeneration of periodontal tissues [13,14]. Another investigations showed the expression of amelogenin mRNA isoform-M180 in macrophages, suggesting the endogenous expression of amelognin in osteoclasts [3]. Also authors suggested amelogenin might act by increasing bone formation over resorption process [11,12]. Amelogenin expression is highest in bone cells adjacent to PDL and in the periosteal cells compared to bone cells distal to the PDL and the periosteum [11,12]. Amelogenin was highly expressed in endosteal cells, osteoblasts, and cells surrounding blood vessels in the regenerated area and in much less extent in bone cells distal to the regenerated area [12]. It was not clear however, which types of the EMPs induce the regeneration, and what molecular mechanisms are involved [34]. For decades amelogenin was thought to be exclusively an enamel (from epithelial origin) protein. However, in

more recent years, amelogenin has also been detected in dentin matrix [9,10,30], odontoblasts [31], in remnants of Hertwig's root sheath and in PDL cells [36], in long bone cells some of which are multi-potent stem cells [13], osteocytes, osteoblasts, osteoclasts, cartilage chondrocytes, in growth plate cells [14]. *In vitro* and *in vivo* studies showed the chondrogenic and osteogenic activities of recombinant amelogenin polypeptides [39,40]. These findings are in line with those obtained with enamel matrix and amelogenin [37,41]. In contrast, treatment with EMD of human osteoblastic cells (SaM-1) from one patient [28] and of rat femoral bone marrow stroma [20], mouse preosteoblastic cell line [15], significantly stimulated cell proliferation. However, when alveolar bone cells were used, no effect of EMD on cell proliferation was observed, but the bone cells showed the greatest attachment response to EMD [33]. In contrast, it was showed that growth of human mandibular osteoblasts from one patient was significantly increased by EMD [6]. Using an organoid culture system with human primary osteoblasts it was observed a significant increase in cell proliferation [32,38]. It was observed that EMD promotes motility of different osteoblastic cell lines better than the control groups, whereas the proliferation rates depended on the cell type [22,29]. It was showed that EMD stimulated proliferation of human bone marrow stromal cells in a dose-dependent manner [7,16]. Authors suggested that effects of EMD also depend on the local osseous environment [17,19,35]. The increased expression of BMP-2, BMP-7, BSP, cementum attachment protein-23 (CP-23) was noted and two putative cementum markers [21]. EMD exposure to human PDL fibroblasts resulted in significantly enhanced osteocalcin (OC) and osteoprotegerin (OPG) levels [26].

The good clinical results were achieved after treatment with Emdogain [36]. Better tendency for healing in EMD treated part of periodontium was showed, especially in soft tissues [8].

The details of direct influence of Emdogain on osteogenous progenitor cells of human bone marrow are still need to be investigated.

Keywords: generalized periodontitis, regeneration, enamel matrix proteins, Emdogain, osteogenous progenitor human bone marrow cells, osteinduction.

Aim. To investigate the direct action of "Emdogain" (Straumann), "Pref-Gel" (Straumann) on osteogenous progenitor cells - colony-forming fibroblast units (CFFU) of human bone-marrow *ex vivo* and to evaluate its osteoinductive properties.

Materials and methods. Cloning of CFFU of human bone marrow was provided according to methodic of Fridenshtein O.Y. (1973) [5] in modification of Astachova V.S. (1982) [1]. For investigation the cancellous iliac bone was taken from healthy patients out of inflammatory and degenerative-dystrophic lesions during orthopaedic surgical operation.

The cancellous bone for investigation was take in the conditions of operating room, put into container with feeding solution "199". Further processing of the collected material was provided in laboratory box under sterile conditions. The cell cloning procedure was taken under standard conditions during 14 days without changing of feeding solution in Ru-containers (culture containers) under temperature of 37⁰C in gaseous blend containing 5% of CO₂ in atmosphere oxygen with usage of lethally irradiated rabbit bone marrow cells in role of feeder.

For investigation of direct action of "Emdogain" (Straumann), "Pre-Gel" (Straumann) *ex vivo* 4 series experiments of cloning CFFU of human bone marrow were provided. In 1st experimental group into culture container during explantation of bone marrow cells "Pref-Gel" (Straumann) was added. In 2nd experimental group "Pref-Gel" (Straumann) and "Emdogain" (Straumann) were added into culture container during explantation of bone marrow cells. In 3rd experimental group only "Emdogain" (Straumann) was added into culture container. In 4th experimental group, as a control group, the cloning of CFFU was provided without adding of any additional material.

The regeneration potential of bone tissue was assessed according to value of cloning effectiveness of CFFU of human bone marrow among 10⁵ nuclear containing cells.

The cloning effectiveness was assessed according formula:

$$ECFFU = \frac{K}{N} \times 10^5$$

Where K- means amount of colonies that grew up in cultural container $\times 10^5$; N – means amount of cells that were explanted into cultural container.

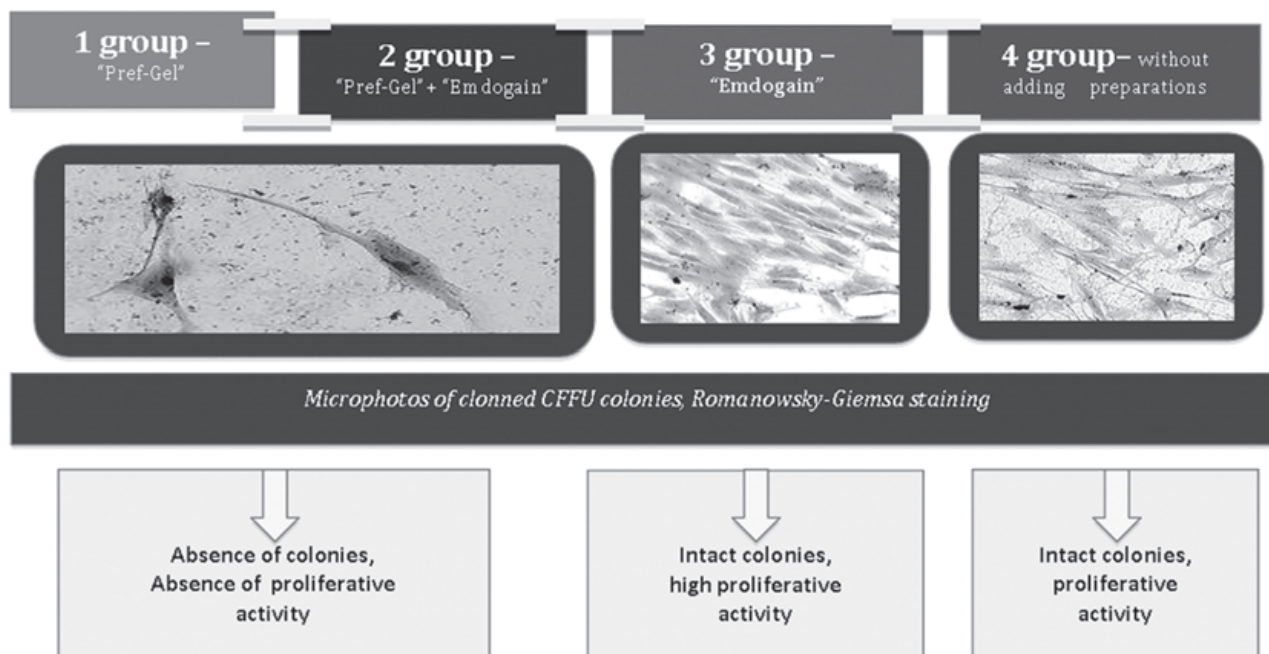
The statistics was provided according computer program analysis “Statistica”. The middle values presented as $M \pm m$, where M - the middle meaning of value, m - standard deviation of middle value meaning.

The 4 experimental series of CFFU cloning of human bone-marrow were provided: 1 group – with adding of etching gel “Pref-Gel”; 2 group – with adding of “Pref-Gel” with “Emdogain”; 3 – with adding of “Emdogain” only and 4 – without adding of any preparations (control). The action was evaluated according to cloning effectiveness of CFFU of human bone marrow among 10^5 nucleus containing cells. 9 experimental and 6 control colonies were cultivated.

Results. The bacterial or fungous overgrowth wasn’t detected in any experimental group. In 6 occasions (66,7%) from 1st and 2nd experimental groups with adding of etching gel “Pref-Gel” and in combination of “Pref-Gel” (Straumann) with “Emdogain” (Straumann), the growth of stromal fibroblasts wasn’t detected. The effectiveness of CFFU cloning=0. In the cultural containers only single stromal fibroblasts were detected, that didn’t formed any colonies. In 3rd experimental group the growth of CFFU was detected in all of cultural containers (33,3%) with adding of “Emdogain” (Straumann) and amount of grew colonies was from 127 till 157, the cloning effectiveness rated from 13,23 till 16,35. In the mean from this group, 145 colonies of CFFU grew up with cloning effectiveness of $15,10 \pm 0,95$ among 10^5 nucleus containing cells. In control group the growth of CFFU colonies was detected in all of cultural containers and the amount was from 69 till 152 colonies in cultural container with cloning effectiveness from 7,19 till 15,83. In the mean amount of CFFU colonies were 120 and the cloning effectiveness of CFFU was $12,48 \pm 1,24$ among 10^5 nucleus containing cells (Table 1). Ad oculus the colonies from control and experimental groups weren’t differ from each other.

Table 1

THE EXPERIMENTAL RESULTS IN MAIN EXPERIMENTAL GROUPS



Conclusions.

In 1st and 2nd experimental groups the growth of stromal fibroblasts wasn’t detected. The effectiveness of CFFU cloning=0. In 3rd group in the mean 145 colonies of CFFU grew up with cloning effectiveness of $15,10 \pm 0,95$ among 10^5 nucleus-containing cells. In control group in the mean 120 colonies of CFFU grew up. The cloning effectiveness was $12,48 \pm 1,24$ among 10^5

nucleus-containing cells. Ad oculus the colonies from control and investigated groups weren't differ from each other. "Emdogain" (Straumann) on 20,8% in comparison with control group, enhances amount of CFFU colonies in bone marrow, increasing specific gravity of multilayer colonies, giving evidence about its osteoinductive properties. The preliminary results of presented investigation showed the direct action of "Emdogain" (Straumann) with additional components on osteogenous progenitor CFFU of human bone marrow taken from iliac bone ex vivo, the certain details of action were determined. The presented results may be useful for practical regenerative periodontology showing the influence of EMD on vital stromal progenitor bone cells.

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