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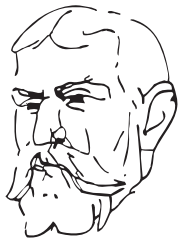

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CONTENTS

ORIGINAL ARTICLES

ORIGINAL ARTICLE

OSTEOPLASTIC PROPERTIES OF MULTIPOTENT MESENCHYMAL STROMAL CELLS OF ADIPOSE TISSUE

DOI: 10.36740/WLek202110103

Andriy Bambuliak, Nataliia Kuzniak, Valentyna Honcharenko, Marianna Ostafiychuk, Alina Palamar
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ABSTRACT

The aim: Determining the ability of samples based on MMSC – AT differentiating in the osteogenic direction.

Materials and methods: The study was conducting at Bukovinian State Medical University, Chernivtsi, Ukraine. Adipose tissue samples were obtaining from the neck of 60 experimental animals (white Wistar rats). Multipotent mesenchymal stromal cells of adipose tissue were obtained by grinding adipose tissue of rats in 0.1% collagenase 1A . Alkaline phosphatase activity was assessing by using the Alkaline Phosphatase Detection Kit (Sigma, USA) according to the manufacturer's protocol. Osteopontin gene expression was determining by immunocytochemical method. To determine the mRNA used the PCR method, which is associated with reverse transcription (RT-PCR) in the area of quantification of gene expression to the marker BGP.

Results: On the 21st day of observations, the expression of mRNA encoding the BGP gene decreased in samples № 1 and № 3 to 35,800 ± 420.0 copies and to 35,000 ± 400.0 copies, $p1<0.01$, $p>0.05$. Also was observing growth of copies of the BGP gene in samples № 2 and № 4 in 2.1, $p<0.01$ and 2.2 times, $p\text{-}p2<0.05$, relative to the data in sample № 1.

Conclusions: Comparative study of osteoplastic properties samples MMSC-AT showed that a larger number of cells differentiate into the osteoblasts in samples containing MMSC-AT + PRP (№ 2) and MMSC-AT + PRP + «Kolapan» (№ 4). This has been proven higher alkaline phosphatase activity, higher levels osteopontin expression, and higher levels BGP gene expression.

KEY WORDS: alkaline phosphatase, osteopontin, multipotent mesenchymal stromal cells of adipose tissue, BGP

Wiad Lek. 2021;74(10 p.I):2374-2378

INTRODUCTION

In medical practice, doctors often face injuries of various aetiologies, which require replacement of bone tissue with biocompatible material [1]. The primary goal of reconstructive surgery is the correct choice of material that has the ability quickly repair damaged tissue in a short period of time [2]. In modern medicine, the use of stem cells in reconstructive surgery and in particular in dental practice is promising. This is possible by phenomenal discoveries in biology and biotechnology, which are basing on the ability of stem cells after their introduction into the recipient's body to get to the site of tissue damage and restore their cellular structure [3, 4].

The optimal source of multipotent stem cells is adipose tissue [5]. This is due to the availability of methods for obtaining material, low invasiveness for the body, the ability to obtain cellular material in sufficient quantities and at the right time [6, 7]. Multipotent mesenchymal stromal cells of adipose tissue are able to differentiate in adipogenic, osteogenic, chondrogenic, endothelial, myogenic, hepatogenic, epithelial and neurogenic directions [8, 9].

The ability to osteogenic differentiation is manifesting by increased levels of markers of bone formation. One of the main markers of bone formation is alkaline phosphatase [10]. It, localized in osteoblasts, plays an important role in the processes of bone mineralization, as it catalyzes the transfer of phosphoric acid ions from the ether to the components of the organic matrix of bone. Increased activity of this enzyme in the blood as a marker of bone formation indicates the activation of bone remodelling [11].

Given the development of modern medicine, in particular dentistry, the search for new materials that would promote osteogenesis and improve bone mineralization processes remains relevant [12]. All of the above prompted us to conduct this study.

THE AIM

Determining the sample based on the MMSC-AT that most differentiates in the osteogenic direction.

MATERIALS AND METHODS

The study was conducting at Bukovinian State Medical University, Chernivtsi, Ukraine. Adipose tissue samples were obtaining from the neck of 60 experimental animals (white Wistar rats) [13]. Multipotent mesenchymal stromal cells of adipose tissue (MMSC-AT) were obtained by grinding adipose tissue of rats in 0.1% collagenase 1A [14]. For the toxicological experiment, which allows to establishing the direct influence of factors in the contact of implantation material at the cellular level, were selected four samples. Sample

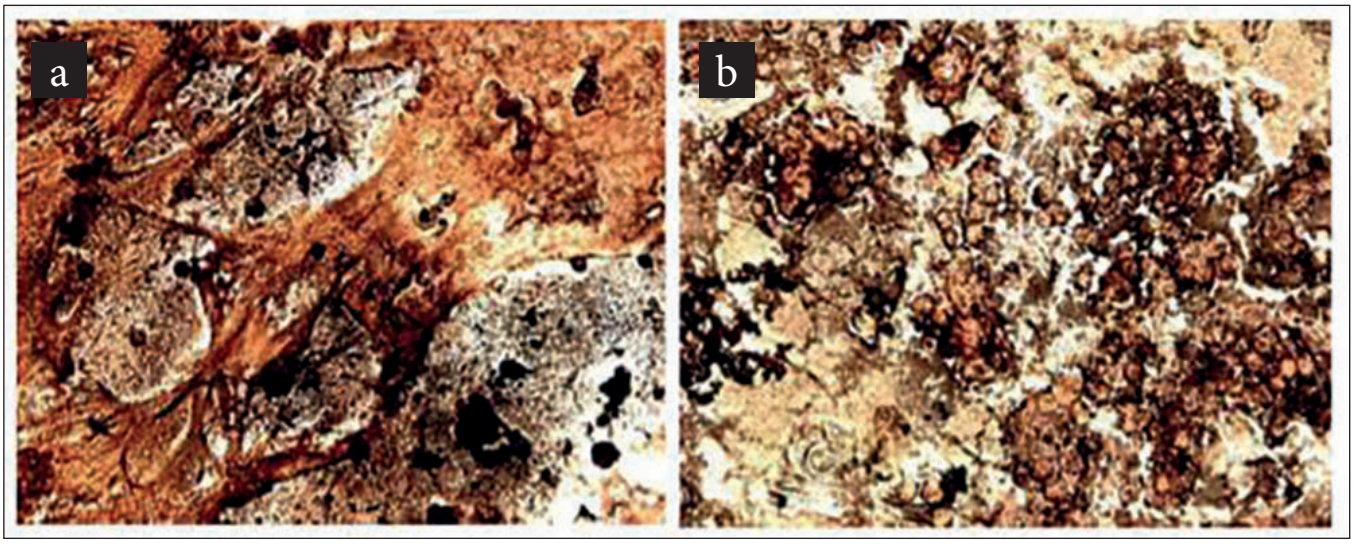

Fig. 1. Osteogenic cell differentiation according to Von Kossa: a – osteoblasts differentiated from MMSC-AT (sample № 1); b – osteoblasts, differentiated from MMSC-AT + PRP + "Kolapan" (sample № 4)

Table I. Alkaline phosphatase activity of osteoblasts in different samples of MMSC-AT

Terms of observation	Sample № 1 (MMSK -AT)	Sample № 2 (MMSK -AT + PRP)	Sample № 3 (MMSK -AT + Kolapan)	Sample № 4 (MMSK -AT + PRP + Kolapan)
7 days	0,25±0,03	0,28±0,04	0,26±0,03	0,27±0,04
14 days	1,38±0,04	1,52±0,05°°	1,37±0,04**	1,54±0,05°,•
21 days	3,56±0,04	3,92±0,09°	3,60±0,08**	4,15±0,08°,•

Notes:
1. °p<0.01; °°p<0,05 – significant difference in values relative to the sample № 1;
2. *p_1<0.01; **p_1<0,05 – significant difference in values relative to the sample № 2;
3. •p_2<0.01; ••p_2<0,05 – significant difference in values relative to the sample № 3.

№ 1 – MMSC-AT, passed osteogenic differentiation; sample № 2 – MMSC-AT+ PRP (platelet-enriched blood plasma); sample № 3 – MMSC – AT + "Kolapan"; sample № 4 – "Kolapan" + MMSC-AT+ PRP. Alkaline phosphatase activity was assessing by using the Alkaline Phosphatase Detection Kit (Sigma, USA) according to the manufacturer's protocol [15]. Osteopontin gene expression was determining by immunocytochemical method [16]. To determine the mRNA used the PCR method, which is associated with reverse transcription (RT-PCR) in the area of quantification of gene expression to the marker BGP [17, 18]. For demonstrating calcium precipitates on the surface of cultured cells was performed Von Kossa analysis. Statistical processing of research results was carrying out using commonly used methods of variation statistics.

RESULTS

One of the biochemical markers of osteoblast differentiation and bone tissue formation is the activity of the alkaline phosphatase (AF) enzyme secreted by osteoblasts and take part in osteoid mineralization. Activity AF in the samples was studying on the seventh, 14th and 21 days of observations (table I).

On the 7th day of the experiment, the activity of AF in the samples studied was the same and ranged from the minimum values of 0.25 ± 0.03 mmol / min • 105 in the sample № 1 to the maximum data of 0.28 ± 0.04 mmol / min • 105 in sample № 2, p – p_2>0,05.

On the 14th day of observations, AF activity increased in all samples, but minimal values was marking in sample № 1 (1.38 ± 0.04 mmol / min • 105) and in sample № 3 (1.37 ± 0.04 mmol / min • 105, p1<0.05). At the same time, in samples № 2, p<0.05 and № 4, p, p2<0.01, p1>0.05, the activity of AF was higher in relation to the data in sample № 1 and № 3, on average by 10.14%.

On the 21st day of the experiment, the highest AF activity was determined in samples № 2 containing a combination of MMSC-AT + PRP – 3.92 ± 0.09 mmol / min • 105, p<0.01, and in sample № 4, which combined MMSK -AT + PRP + "Kolapan" – 4,15 ± 0,08 mmol / min • 105, p, p_2<0,01, p_1>0,05. At the same time, in samples № 1 and № 3 the activity of AF was significantly lower and ranged from 3.56 ± 0.04 mmol / min • 105 to 3.60 ± 0.08 mmol / min • 105, p_1<0, 05, respectively, which indicated on the less metabolic activity of cells in these samples.

To confirm the differentiation of MMSC-AT in the studied samples into osteoblasts, the expression of osteopontin genes was conducting by reverse transcriptase polymerase chain reaction (RT-PCR) in real time. To do this, we used primers for osteopontin – the main protein of bone tissue:

Table II. Expression of osteopontin mRNA in the dynamics of the cultivation of MMSC-AT on the environment with factors of cell differentiation into osteoblasts in different samples

Terms of observation	Sample № 1 (MMSK -AT)	Sample № 2 (MMSK -AT + PRP)	Sample № 3 (MMSK -AT + Kolapan)	Sample № 4 (MMSK -AT + PRP + Kolapan)
7 days	0,87±0,04	1,41±0,06°	0,89±0,04*	1,40±0,06°,•
14 days	1,38±0,04	2,47±0,05°	1,52±0,03°°,*	2,50±0,06°,•
21 days	2,96±0,06	3,45±0,07°	2,73±0,05°°,*	3,49±0,07°,•

Notes:
1.°$p<0,01$; °°$p<0,05$ – significant difference in values relative to the sample № 1.
2.*$p_1<0,01$ – significant difference in values relative to the sample № 2.
3.•$p_2<0,01$ – significant difference in values relative to the sample № 3.

Table III. The number of copies of cDNA encoding BGP bone protein in osteoblasts in different samples

Terms of observation	Sample № 1 (MMSK -AT)	Sample № 2 (MMSK -AT + PRP)	Sample № 3 (MMSK -AT + Kolapan)	Sample № 4 (MMSK -AT + PRP + Kolapan)
7 days	20.000±365,0	24.500±376,0°	22.500±370,0°,*	25.000±385,0°,•
14 days	40.500±560,0	55.000±575,0°	43.000±570,0°,*	60.000±720,0°,*,•
21 days	35.800±420,0	75.000±620,0°	35.000±400,0*	80.500±790,0°,*,•

Notes:
1.°$p<0,01$ – significant difference in values relative to the sample № 1.
2.*$p_1<0,01$ – significant difference in values relative to the sample № 2.
3.•$p_2<0,01$ – significant difference in values relative to the sample № 3.

direct -5'-AAGGCGCATTACAGCAAACACTCA-3' and reverse – 5TCATCGGACTCCTGGCTCTTCAT-3'.

It should be noting that on the 7th day of the experiment (table II) the lowest mRNA level was recorded in sample № 1 – 0.87 ± 0.04 and in sample № 3 – 0.89 ± 0.04, p1<0, 01. At the same time, in sample № 2, which contained a combination of MMSC-AT + PRP, and in sample № 4, which combined MMSC-AT + PRP + "Kolapan", the mRNA level ranged from 1.41 ± 0.06, p<0.01 to 1.40 ± 0.06, p, p2<0.01, p1>0.05, respectively, and was, on average, 62.06% higher than the data in samples № 1 and № 3.

On the 14th day of the study, an increase in the level of osteopontin expression was observing in all experimental samples; however, in the culture of MMSC-AT (sample № 1) the mRNA level was probably lower, with a value of 1.38 ± 0.04 than in the other samples. At the same time, the level of osteopontin expression exceeded the value sample № 1: by 78.98% in sample № 2, p<0.01, by 10.14% in sample № 3, p<0.05 and by 81.16% in samples № 4, p, p2<0.01, p1>0.05.

On the 21st day of observations, a further increase in mRNA levels in the studied cultures was determined. The minimum level of osteopontin expression was investigating in sample № 3, which contained MMSC-AT + "Kolapan" – 2.73 ± 0.05, p<0.05, p1<0.01. The contents of the studied parameter in the sample № 1 – 2.96 ± 0.06 was slightly higher. At the same time, in samples № 2 and № 4 the mRNA level was, on average, 11.22%, p1<0.01 and 27.11% higher, p2<0.01, than in cultures № 1 and № 3, in accordance.

Von Kossa staining (Figure 1) qualitatively confirmed the production salts of calcium and phosphates by osteoblasts, which was differentiationing from MMSC-AT.

It was found that in sample № 1, which contained MMSC-AT with osteogenic differentiation, significantly fewer cells capable of producing calcium precipitates were studied in contrast to sample № 4, which included MMSC-AT + PRP + "Kolapan", where after staining was found massive fragments of mineralized matrix.

Osteogenic differentiation of MMSC-AT into osteoblasts in the studied samples was confirming by quantitative assessment of gene expression to the BGP marker (bone gla-protein). BGP-bone glutamine protein (osteocalcin) is a small protein that is most widely present in the bone matrix, participates in calcification processes and is a marker of osteoblast activity, which is 15% of extracted non-collagenous proteins.

It was found that the expression of mRNA encoding BGP manifests itself as follows (table III): on the 7th day of observations, the minimum number of copies of the BGP gene was examined in sample № 1 – 20,000 ± 365.0 copies and slightly more in sample № 3 – 22,500 ± 370.0 copies, p, p1<0.01. At the same time, the maximum expression of mRNA encoding the BGP gene was investigated in samples № 2 and № 4, which was 1.2, p<0.01 and 1.3 times, p, p2<0.01, p1>0 , 05, higher respectively to the values of the sample № 1.

On the 14th day of research in all studied samples investigated the further increase in the expression of the studied parameter. However, in samples № 2 and № 4, the number of copies of the BGP gene exceeded the data in sample № 1 by 1.4 times, p<0.01 and 1.5 times, p_2 p0.01, respectively.

On the 21st day of observations, the expression of mRNA encoding the BGP gene decreased in samples № 1 and № 3 to 35,800 ± 420.0 copies and to 35,000 ± 400.0 copies,

p1<0.01, p>0.05. Also was observing growth of copies of the BGP gene in samples № 2 and № 4 in 2.1, p<0.01 and 2.2 times, p-p2<0.05, relative to the data in sample № 1.

DISCUSSION

The modern development of biotechnology does not allow the creation of complex organs de novo [19] and is largely limited to the stimulation of the innate abilities of the body's regeneration, which can be supplemented by the replacement of individual tissue sites and the regenerative cascade induction [20]. The existing strategy of tissue engineering usually consists in the in vivo expansion of populations of multipotent cells, such as MMSC, with their subsequent transplantation in the form of a cell suspension or scaffolds inhabited by MSCs into damaged areas [21]. Due to their unique regenerative potential and immunomodulatory properties, MMSC have great prospects in tissue engineering and reconstructive therapy, not only due to their direct participation in tissue regeneration, but also due to a modulating effect on the recipient's immunogenesis in response to the introduction of a foreign body (implant) [22].

Bone is a dynamic tissue characterized by its ability to recover from injury without scarring [23]. The differentiation of MMSC into osteoblasts plays a decisive role in bone regeneration and remodelling. MSCs obtained from bone marrow are considered to be an adequate source for tissue engineering of bones due to their ability for osteogenic differentiation [24]. MMSC can also be isolated from umbilical cord blood, placenta, adipose tissue, etc. The efficiency of osteogenic differentiation of various human MMSC has been demonstrated when populating biocompatible polymer matrices with them. At the same time, it was found that MMSC obtained from bone marrow demonstrated greater efficiency of differentiation into osteoblasts than other types of MMSC [25]. These cells are usually transplanted into three-dimensional porous scaffolds that provide the necessary extracellular environment that contains physical and chemical signals for tissue development and regeneration [26]. Despite the fact that strategies based on various types of biomaterials and stem cells have been developed over the years, modern tissue engineering has not found wide application in clinical settings [27]. Achieving this goal will require a deep understanding of the normal physiological processes of tissue development and the mechanisms underlying the interaction between MMSC and biomaterials during tissue formation, since many important details remain unclear [28]. Biomaterials play a decisive role in the creation of tissue-engineered structures [29]. The material must be able to maintain its structure and integrity for predictable periods of time to allow new tissue formation and maturation even under stress conditions [30, 31].

Thus, MMSC play an important role in bone regeneration, both by regulating the formation of osteoclasts and by negatively affecting the effectors of inflammation and osteclastogenesis [32]. MMSC have the ability to regenerate mesenchymal tissues, regulate bone metabolism, and modulate inflammation, making them attractive candidates for cell technologies in regenerative medicine. Modern strategies for the creation of tissue-engineered constructs are actively using MMSC s to improve the integration of implants and prevent immunological rejection. Preclinical studies have shown that the colonization of biocompatible materials with MMSC significantly increases osteoconductivity and improves implant integration [33, 34]. The first clinical trials of scaffolds inhabited by MMSC confirm their effectiveness [35]. This indicates that the use of tissue-engineered constructs based on biocompatible scaffolds populated with MMSC for bone regeneration is promising.

CONCLUSIONS

So, a comparative study of osteoplastic properties samples MMSC-AT showed that a larger number of cells differentiate into the osteoblasts in samples containing MMSC-AT + PRP (№ 2) and MMSC-AT + PRP + "Kolapan" (№ 4). This has been proven higher alkaline phosphatase activity, higher levels osteopontin expression, and higher levels BGP gene expression. Thus, our study proves the effectiveness of the use of samples №2 and №4 based on MMSC – AT to replace bone defects.

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