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Original Articles

BMP and FGF enhance alkaline phosphatase activity for rat mesenchymal stem cell

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The goal of periodontal treatment may be recognized regeneration of periodontal tissue. The regeneration is composed cells, growth factors and scaffolds. This study was to evaluate the alkaline phosphatase (ALP) activity of mesenchymal stem cell(MSC) cultured with bone morphgenic protein (BMP) or fibroblast growth factor (FGF). The MSC was cultured with BMP or FGF for up to three weeks. The cultured glass slides were stained for ALP. The area of ALP positive on the glasses was measured. The total area of ALP positive was the highest in BMP group among the groups. In FGF group, the positive area was lower than BMP group, but significantly higher than that in the control group. BMP and FGF can enhance the osteogenic activity for MSC. FGF can be a perdicatble material for periodontal regeneration.

Key words: mesenchymal stem cell, fibroblast growth factor, bone morphogenic protein, alkaline phosphate activity

Introduction

Mesenchymal stem cells (MSC) have been considered as a potential therapy for the treatment of periodontal defects[1-3]. Recently, bone marrow derived MSC have emerged as a promising new therapeutic option for the regeneration of periodontal tissues[4].

Polypeptide growth factors such as epidermal growth factor, fibroblast growth factor(FGF), plateletderived growth factor and bone morphogenetic proteins(BMP) have been used to facilitate periodontal regeneration[5].

The combination of MSC and growth factors may be important materials for the periodontal regeneration[6].

This study was to evaluate the alkaline phosphatase (ALP) activity of MSC cultured with BMP and FGF.

Materials and methods

Cell culture

Rat MSC (KE-400, DS Pharma biochemical, Suita, Japan) were cultured in conditioned medium, 10% FCS (USA), 50μg/ml ascorbic acid, 10mM/ml β-glycerophosphoric acid and dexamethazon containing D-MEM (USA). ϕ12mm cover glass (Matsunami glass, Kishiwada, Japan) were setlled on the bottom of the well in 12 wells culture plate (Becton Dickinson Franklin Lakes, NJ, USA). 1x10^4 cell/ well were seeded on the cover glass. 10ng of rhBMP (BMP-2, Wako, Osaka, Japan) or rhFGF (FGF-2, Cosmobio, Tokyo, Japan) were added in the medium. The control were cltured without growth factors.

The culture were incuvated up to 3 weeks in the 5% CO_2 at 37C°. The culture medium was chaged every 3 days. The cultures were stopped at 1 week, 2 weeks and 3 weeks.

After culture, the glass slides were fixed with 10% formaline PBS.

Staining for ALP

The galsses were stained with ALP-staining kit (Takarabio, Kyoto, Japan). The area of ALP positive reaction were measured with Adobe® Photoshop®6.0 (San Jose,CA,USA) under the microscope.

The statistical analyse was used ANOVA and significanse defferent were analysed with Turky-Kramer.

Results

Culture for 1 week

The number of ALP positive cells in BMP group and in FGF group were increased compare to the control group (Fig.1)

Culture for 2 weeks

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ALP positive activity was higher than culture for 1 week in all group.

The number of ALP positive cells in BMP group and in FGF group were increased compare to the control group (Fig.2)

Culture for 3weeks

ALP positive activity was more stronger than culture for 1 week and 2 weeks in all group.

The number of ALP positive cells in BMP group and in FGF group were increased compare to the control group (Fig.3)

The area of ALP positive reaction.(Fig.4)

The BMP group showed the most highest ALP positive area during the the culture period. FGF group showed higher reaction than control group.

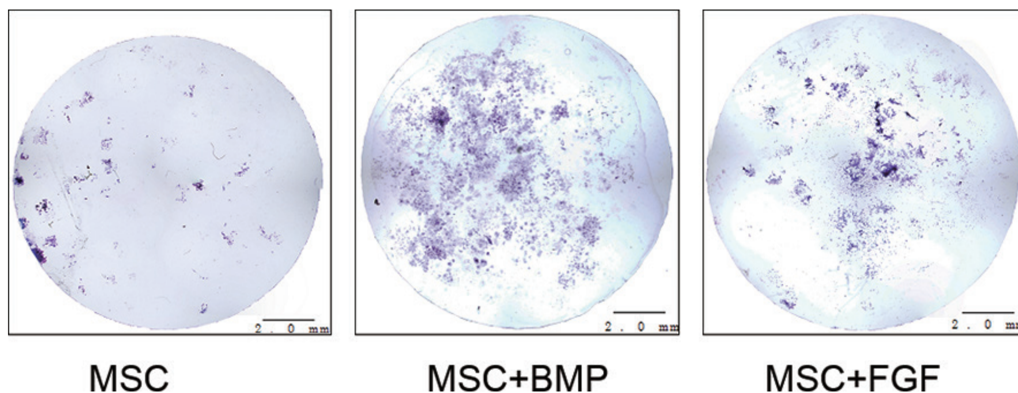
DISCCUSION

Stem cells are the foundation cells for every organ and tissue in the body, including the periodontium[7]. The most common source of stem cell is the bone marrow (hematopoietic stem cells) or bone marrow stromal cells (mesenchmal stromal stem cells). These last can be potential candidates for periodontal regeneration[8]. They can differentiate into endothelial, perivascular, neural, bone or muscle cells[9].

BMPs are glycoproteins accounting for the recruitment of osteoprogenitor cells to sites of bone formation[10]. BMPs are proteins found in high amounts in bone tissues and are considered as responsible for inductive and regenerative abilities of demineralized bone grafts used in periodontal therapy[11].

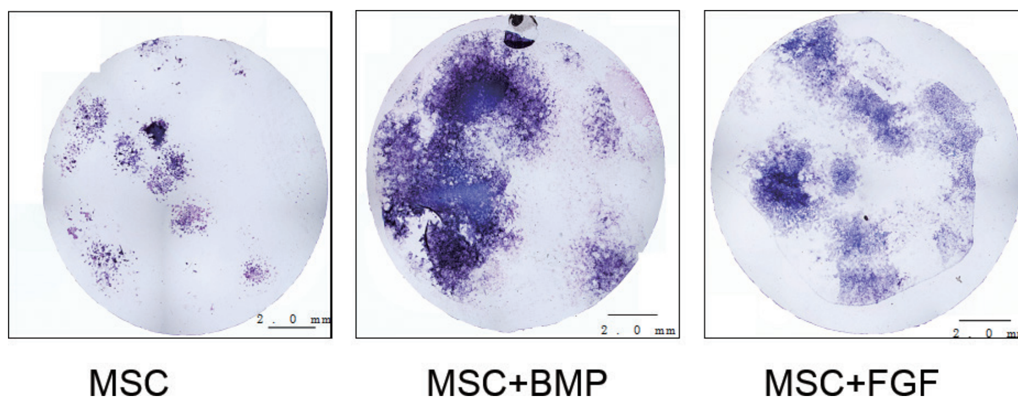
Hughes et al.[12] demonstrated through in vitro tests with rats osteoprogenitor cells that the BMP-6, BMP-4 and BMP-2 can stimulate osteoblasts differentiation. They suggested that an early osteoprogenitor cell is an important cell for BMPs action during bone induction.

FGF is a representative growth factor which has shown the potential effects on the repair and



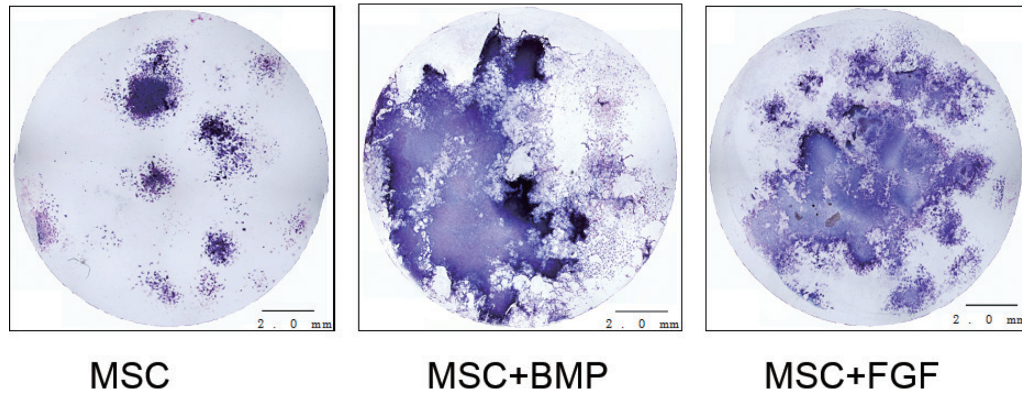
(Fig.1) Culture for 1 week

The number of ALP positive cells in BMP group and in FGF group were increased compare to the control group.

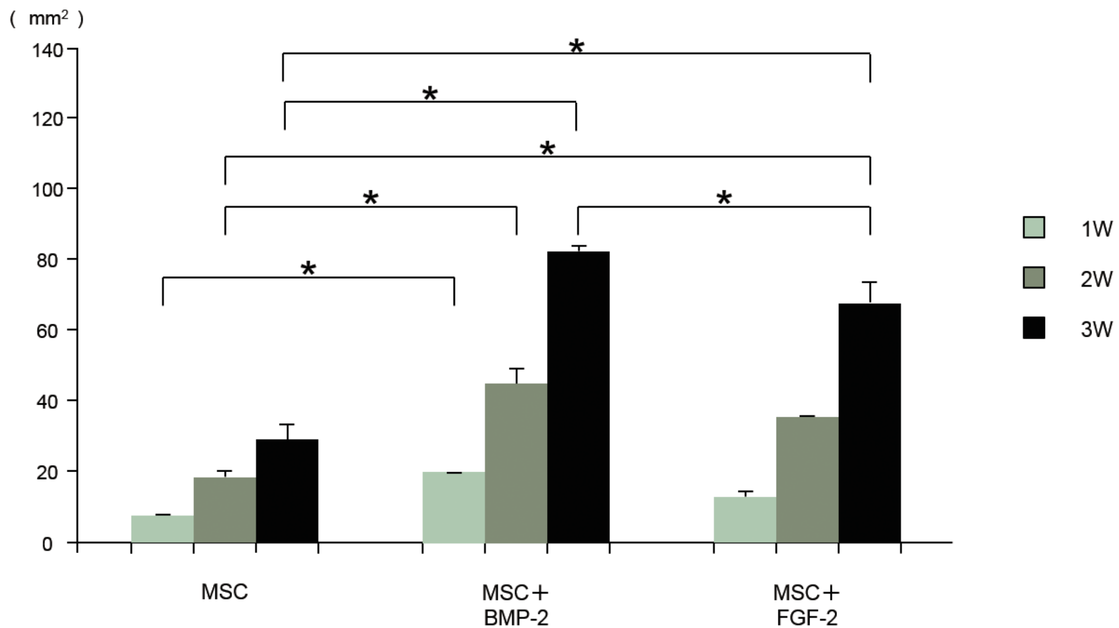


(Fig.2) Culture for 2 weeks

ALP positive activity was higher than culture for 1 week in all group. The number of ALP positive cells in BMP group and in FGF group were increased compare to the control group.



(Fig.3) Culture for 3weeks
ALP positive activity was more stronger than culture for 1 week and 2 weeks in all group.
The number of ALP positive cells in BMP group and in FGF group were increased compare to the control group.



(Fig.4) The area of ALP positive reaction.
The BMP group showed the most highest ALP positive area during the the culture period. FGF group showed higher reaction than control group.
Average +/- SD, * $p<0.05$

regeneration of tissues[13,14]. FGFs have been utilized for the regeneration of damaged tissues, including skin, blood vessel, muscle, adipose, tendon/ligament, cartilage, bone, tooth, and nerve[14,15]. Cell proliferation by FGFs has been reported in many cell types, including endothelial cells, stem cells, and epithelial cells[16].

Biomaterial-based systems, including delivery carriers of FGFs and scaffolds of stem cells regulated by the FGFs functions, have recently been potentially developed and shown to have many good results *in vivo*[14]. Future clinical applications of FGFs in the regeneration of periodontal tissues will be realized when their biological functions are maximized by the appropriate use of biomaterials and stem cells[4]. Morerecently, the clinical studies of rhFGF-2 using for periodontal regeneration material have been reprted[4].

Osteoblast differentiation in vitro and in vivo can be characterized in three stages, cell proliferation, that secrete and mineralize the bone matrix maturation, and matrix mineralization[17]. In vitro, matrix maturation and mineralization are usually enhanced by growing the cells to complete confluency and by adding specific osteogenic factors[18]. During proliferation, several extracellular matrix proteins can be detected. The matrix maturation phase is characterized by maximal expression of ALP. Finally, at the beginning of matrix mineralization genes for proteins such as OC, BSP, and OPN are expressed and once mineralization is completed, calcium deposition can be visualized using adequate staining methods. Analysis of bone cell-specific markers like AP, OC, and collagen type I or detection of fuctional mineralization is frequently used to characterize osteoblasts in vitro[18].

Proliferating Osteoblasts show alkaline phosphatase (AP) activity, which is greatly enhanced during in vitro bone formation[17]. AP activity is therefore a feasible marker for osteoblast. AP can easily be detected using BCIP/NBT as a substrate, which stains cells. blue-violet when AP is present[17].

This study has demonstrated that the BMP and FGF can diffenrnciated the MSC to osteogenic cells. This study was supported by pervious studies that BMP can enhance the MSC osteogenic activity[4]. In addition, in FGF group, the ALP activity was lower than BMP group, but significantly higher than that in the control group. FGF can enhanse the MSC osteogenic activity in this study. Human MSC may also have potential to differenciate to osteobast. The BMP and FGF can be a candidate for regeneration of periodontal tissue.

The complex of MSC and growth factors will be necessary the carrier or scafforld to deliver the specific tissue. Further studies will be necessary for complete regeneration material.

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BMPとFGFによる培養間葉系幹細胞のアルカリフォスファターゼ活性への影響

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間葉系幹細胞（MSC）は骨髄中に存在する体性幹細胞で，造血システムを維持するうえで重要な役割を果たしている．MSCは多彩な分化能を有し，生体内投与を行うと，組織傷害部位に集積する性質があることから再生医療への応用が期待されている．

本研究は培養MSCに成長因子のBMPやFGFを添加することによってMSCのALP活性におよぼす影響を検討した．BMPまたはFGFを添加した培養液でガラススライド上に播種したMCSを培養した．培養終了後にALP染色を行いALP陽性面積を測定した．BMPはMSCの多くの報告と同様にALP活性を顕著に促進した．またFGFにおいてもBMPより低いものの，ALP活性を有意に増加させた．これらの結果はBMPと同様にFGFもMSCのALP活性を向上させる能力があり，歯周組織再生療法への応用が期待されると考えられる．

キーワード：間葉系幹細胞，線維芽細胞成長因子，骨造成因子，アルカリフォスファターゼ活性

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