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Control of Simultaneous Osteogenic and Adipogenic Differentiation of Mesenchymal Stem Cells

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Abstract

Mesenchymal stem cells (MSCs) are pluripotent cells which can differentiate into several distinct lineages such as osteoblasts and adipocytes. Osteogenesis and adipogenesis of MSCs induced with specific induction factors have been well exploited. However, it is not clear how simultaneous osteogenesis and adipogenesis of MSCs is balanced by the composition of induction medium. In this study, MSCs were cultured in the mixture medium at different ratio of osteogenic medium (OM) and adipogenic medium (AM) to investigate the simultaneous osteogenesis and adipogenesis of MSCs. Differentiation was investigated by histological staining and real-time PCR analysis. The results showed that adipogenesis was dosage dependent on the concentration of adipogenic induction factors, while osteogenesis was not. The balance of osteogenesis and adipogenesis could be control by different ratio of osteogenic and adipogenic media. The results will be useful for controlling stem cell differentiation.

Keywords: Osteogenesis; Adipogenesis; Differentiation balance; Simultaneous differentiation; Mesenchymal stem cells

Introduction

Mesenchymal stem cells (MSCs) are pluripotent with the ability to differentiate into multiple cell lineages such as osteoblasts, adipocytes and chondrocytes [1]. MSCs have been used for tissue engineering and regenerative medicine. *In vitro* expansion and directed differentiation of MSCs are important for fundamental and clinical applications. The culture condition of isolation and *in vitro* expansion of MSCs have been extensively investigated [2-4]. It has been revealed that the composition of expansion medium, oxygen pressure, and culture substrate properties have significant effects on the biological properties of MSCs, such as proliferation, surface marker expression, and differentiation potential [5-7]. A combination of growth factors, extracellular matrix, physical stimuli, and culture conditions has been frequently used to investigate the differentiation of MSCs [8,9]. The soluble factors for MSCs differentiation are well established [1].

Adipogenic differentiation of MSCs occurs in medium containing dexamethasone (Dex), 3-isobutyl-1-methylxanthine (IBMX), insulin and indomethacin (Indo). To promote osteogenesis, Dex and β-glycerophosphate (β-GP) are usually supplemented in induction medium [9]. Although various factors have been used to investigate their effects on differentiation of MSCs, most of the studies focus on the MSCs differentiation into an individual lineage.

Osteogenesis and adipogenesis have been reported to have an inverse correlation [10]. Up-regulation of osteogenic differentiation is associated with down-regulation of adipognic differentiation and *vice versa*. Disruption of the balance between ostogenesis and adipogenesis may lead to some diseases, such as osteoporosis and diabetic bone loss [11-13]. Investigation of simultaneous osteogenesis and adipogenesis will provide some useful information for understanding the development process of the diseases related with osteogenesis and adipogenesis. It is not clear whether osteogenesis and adipogenesis of MSCs can be induced simultaneously and how the medium composition balances simultaneous osteogenesis and adipogenesis in the same medium. Therefore, in this study, MSCs were cultured in a mixture medium of osteogenic and adipogenic induction medium at various ratios and the balance of osteogenesis and adipogenesis of MSCs in this mixture medium was investigated.

Materials and Methods

Preparation of mixture medium

The osteogenic differentiation medium (OM) was prepared by adding 10 nM dexamethasone (Dex, Sigma, St. Louis, MO) and 10 mM β-glycerophosphate (β-GP, Sigma, St. Louis, MO) in Dulbecco's modified Eagle's medium (DMEM, Sigma, St. Louis, MO) with low glucose (1000 mg/L), containing 10% fetal bovine serum (FBS, Equitech-Bio, Kerrville, TX), glutamine (584 mg/L), penicillin (100 units/mL), streptomycin (100 mg/L), nonessential amino acids (0.1 mM), sodium pryuvate (1 mM), proline (0.4 mM) and ascorbic acid (50 mg/L). The adipogenic differentiation medium (AM) was prepared by DMEM with high glucose (4500 mg/L) supplied with Dex (1 μM), 3-isobutyl-1-methylxanthine (0.5 mM, IBMX), insulin (10 μg/mL) and indomethacin (100 mM, Indo). The mixture medium of osteogenic and adipogenic medium was prepared by combining OM and AM at different ratios (OM/AM (v/v)) of 100/0, 95/5, 90/10, 85/15, 80/20, 75/25, 70/30, 65/35, 60/40, 55/45, 50/50, 45/55, 40/60, 35/65, 30/70, 25/75, 20/80, 15/85, 10/90, 5/95 and 0/100. The concentration of induction factors of Dex, β-GP, IBMX, insulin and Indo was calculated from the mixture ratio (Figure 1).

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Simultaneous osteogenic and adipogenic differentiation of MSCs

Human bone marrow-derived mesenchymal stem cells (MSCs, passage 2) were obtained from Lonza (Walkersville, MD) and subcultured twice in growth medium (Lonza, MD). The subcultured MSCs (at passage 4) were seeded in tissue culture plates (TCPS) at a density of 5,000 cells/cm^2 under an atmosphere of 5% CO_2 at 37°C. The mixture medium was used and refreshed every 3 days during culture. The cells were cultured in mixture medium for 3, 7, 14, and 21 days.

Alkaline phosphatase (ALP) staining

After incubation in mixture medium for 1, 2 and 3 weeks, the cells were washed with phosphate-buffered saline (PBS) and fixed with 4% paraformaldehyde in PBS for 10 min. And then the cells were incubated with 0.1% naphthol AS-MX phosphate (Sigma, St. Louis, MO) and 0.1% fast blue RR salt (Sigma, St. Louis, MO) in 56 mM 2-amino-2-methyl-1,3-propanediol (pH 9.9, Sigma, St. Louis, MO) working solution at room temperature for 10 min, washed with PBS twice and then observed under an optical microscope. More than ten bright field images of ALP staining were taken from each sample and at least 3 parallel samples were stained. As reported by Alm et al. [14], the level of ALP activity was quantified by using an ImageJ program analysis. Briefly, a constant threshold value of the reflection intensity was defined from background and the percentage of ALP positively stained area to total area of the cells was calculated.

Alizarin red S staining

To evaluate the deposition level of calcium phosphate, the cultured MSCs were washed with PBS twice, followed by fixation with 4% paraformaldehyde for 10 min at room temperature. The fixed cells were incubated with 0.5% Alizarin red S (Sigma, St. Louis, MO) solution at room temperature for 10 min and extensively washed twice with PBS. The stained cells were observed under an optical microscope. Similarly, Alizarin red S positive areas were quantified by determining the percentage of stained area to total area of the cells using ImageJ.

Oil red O staining

To evaluate the adipogenic differentiation, Oil red O (Sigma, St. Louis, MO) staining of the cytoplasmic droplets of neutral lipids in the differentiated cells was performed according to the previous research [15,16]. The cultured cells were rinsed with PBS and fixed with 4% paraformaldehyde for 10 min. After treatment with 60% 2-propanol solution for 5 min, the cells were treated with Oil red O working solution for 5 min at room temperature. The Oil red O working solution was prepared by mixing three parts of Oil red O stock solution (0.3 g/mL Oil red O powder in 2-propanol) with two parts of MilliQ water. After that, the stained cells were washed with 60% 2-propanol solution once and PBS three times to help remove the background. The photomicrographs of the stained cells were captured by optical microscopy. The percentage of cells positively stained by Oil red O was calculated from the stained area and total area of cells as mentioned above.

Real-time PCR Analysis

Total RNA was extracted from the cells cultured at different conditions for pre-designated periods by using Sepasol solution

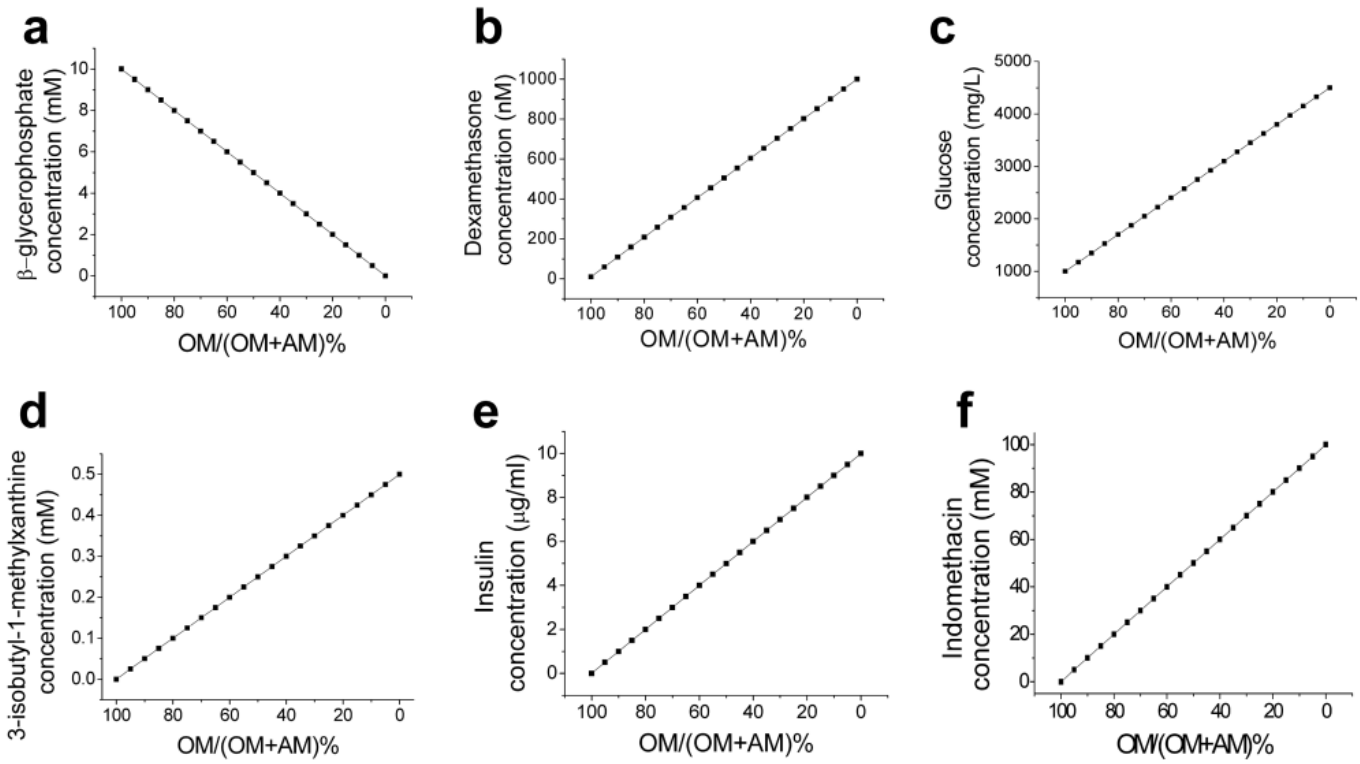

Figure 1: The concentration of osteogenic and adipogenic induction factors. Osteogenic differentiation cocktails contain (a) β-glycerophosphate (β-GP) and (b) dexamethasone (Dex). The concentration of (c) glucose was also changed according to different mixture medium. Adipogenic differentiation cocktails share the same inducing factor, dexamethasone. Besides that, adipognic induction factors used in this study contain (d) 3-isobutyl-1-methylxanthine (IBMX), (e) insulin, and (f) indomethacin (Indo). Among these soluble factors, only the concentration of β-GP decreased with the increasing percentage of adipogenic medium.

according to the manufacturer's instruction (Nacalai Tesque, Kyoto, Japan). Total RNA (1.0 μg) was used as a first strand reaction that included random hexamer primers and murine leukemia virus reverse transcriptase (Applied Biosystems, CA). Real-time PCR was used to quantify 18S rRNA, glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), Alkaline phosphatase (*ALP*), secreted phosphoprotein 1 (*SPP1*, osteopontin), bone sialoprotein 2 (*IBSP*), SP7 transcription factor (*SP7*; osterix), peroxisome proliferator-activated receptor gamma (*PPARG*), lipoprotein lipase (*LPL*), fatty acid binding protein 4 (*FABP4*) and CCAAT/enhancer binding protein (*CEBPA*), and fatty acid synthase (*FASN*). The reaction was performed with 10 ng of cDNA, 90 nM PCR primers, 25 nM PCR probe and FastStart TaqMan Probe Master (Roche Diagnostics Japan, Tokyo, Japan). The expression level of 18S rRNA was used as an endogenous control and gene expression level relative to *GAPDH* was calculated using the comparative Ct method. The sequences of primers and probes are based on our previous studies as listed in Table 1 [15,17]. The primers and probes were obtained from Applied Biosystems and Hokkaido System Science (Sapporo, Japan).

Statistical analysis

All data are presented as mean ± standard deviation (SD), with 3 biological replicates. The statistically significant effects were detected by one-way analysis of variance (ANOVA) with Tukey's post hoc test. All the results were performed in at least three independent experiments. A significant difference is marked as *(p<0.05), **(p<0.01), and ***(p<0.001).

Results

Osteogenic differentiation of MSCs in mixed osteogenic/adipogenic medium

Specific induction factors are necessary to commit MSCs toward osteogenic and adipogenic differentiation. To investigate how the osteogenic induction medium (OM) and adipogenic induction medium (AM) in the mixture medium affect the osteogenic differentiation of MSCs, cells were cultured in mixture medium at various ratios for 3, 7, 14, and 21 days. ALP was stained acting as the early stage maker of osteogenesis [18-21]. The ALP staining was negative when MSCs were cultured in growth medium after 3, 7, 14, and 21 days (Figure 1a), while the cells cultured in mixture medium were positively stained. For the first 3 days of culture, the cells were stained weakly. ALP staining became gradually stronger when the culture time increased. After 21 days, cells cultured in medium at all the mixture ratios were positively stained.

mRNA		Oligonucleotide
18 S rRNA		Hs99999901_s1
GAPDH		Hs99999905_m1
ALP		5'-GACCCTTGACCCCCACAAT-3'
		5'-GCTCGTACTGCATGTCCCCT-3'
		5'-TGGACTACCTATTGGGTCTCTTCGAGCCA-3'
IBSP	Forward	5'-TGCCTTGAGCCTGCTTCC-3'
	Reverse	5'-GCAAAATTAAAGCAGTCTTCATTTTG-3'
	Probe	5'-CTCCAGGACTGCCAGAGGAAGCAATCA-3'
SPP1	Forward	5'-CTCAGGCCAGTTGCAGCC-3'
	Reverse	5'-CAAAAGCAAATCACTGCAATTCTC -3'
	Probe	5'-AAACGCCGACCAAGGAAAACTCACTACC-3'
SP7	Forward	Hs00541729_m1
PPARG	Reverse	Hs01115510_m1
LPL	Probe	Hs00173425_m1
FABP4		Hs00609791_m1
FASN		Hs00188012_m1
CEBPA		Hs00269972_s1

Table 1: Primers and probes for real-time PCR analysis.

The intensity of ALP staining was also dependent on the ratio of OM and AM. The cells cultured in mixture medium with an OM/AM ratio in the range of 90/10~60/40 were more positively stained than the cells cultured in other mixture medium. The ALP staining was further quantified by image analysis of the percentage of positively stained areas (Figure 1b) [14]. The intensity of ALP staining was almost zero after 3 days of culture and increased with culture time. After being cultured in the mixture medium for 14 and 21 days, the ALP intensity was very high, and the dependence of ALP staining on the composition of mixture medium became remarkably obvious after 14 and 21 days culture. The degree of ALP staining increased when the OM/AM ratio was changed from 100/0 to 90/10, remained maximum at the same level from OM/AM ratio of 90/10 to 60/40, and further decreased below a ratio of 55/45. At mixture ratios of 5/95 and 0/100, the cells were only slightly stained by ALP, even after being cultured for 21 days.

Calcium deposition was examined by Alizarin red S staining, as calcium deposition is a late stage indicator of osteogenic differentiation [20]. MSCs cultured in growth medium showed no calcium deposition. MSCs cultured in the OM/AM mixture medium showed no calcium deposition until the cells were cultured for 21 days (Figure 2a). At day 21, the Alizarin red S staining was strong for the cells cultured in mixture medium with OM/AM ratio of 100/0, 95/5, 90/10, 85/15, 80/20, 75/25, 70/30, moderate for cells culture at OM/AM ratio of 65/35, 60/40, 55/45, 25/75, 20/80, and 15/85, and low for the cells cultured at OM/AM ratio of 50/50, 55/45, 60/40, 65/35, 30/70, 90/10, 95/5, and 0/100. The Alizarin red S staining intensity was quantified by image analysis of the percentage of Alizarin red S stained areas (Figure 2b). The Alizarin red S staining was at the same high level when MSCs were cultured in mixture medium with an OM/AM ratio of 100/0 to 70/30. It showed a decrease from an OM/AM ratio of 65/35 to 30/70 and was much lower when the OM/AM ratio was 55/45, 40/60, 35/65, and 30/70. There was a small peak of Alizarin red S intensity when OM/AM ratio was in the range of 25/75~15/85. The intensity of Alizarin red S staining showed a quite low level again when the OM/AM ratio was 10/90, 5/95 and 0/100.

Based on the ALP and Alizarin red S staining results, a few mixture ratios (100/0, 95/5, 85/15, 70/30, 50/50, 20/80, and 0/100) where MSCs showed distinctly high or low intensity of staining were selected for gene expression experiments. Expression of osteogenic genes *ALP, SPP1, SP7,* and *IBSP* were measured after MSCs were cultured for 21 days (Figure 3). Compared with that of cells cultured in growth medium, expression level of these osteogenic genes increased significantly when MSCs were cultured in mixture medium. Expression of the osteogenic genes was shown to be dependent on the composition of the mixture medium. Expression level of *ALP* by MSCs cultured at OM/AM mixture ratios of 100/0, 95/5, 85/15, and 70/30 were higher than that of cells cultured at OM/AM mixture ratios of 50/50, 20/80, and 0/100. Expression level of *SPP1*, *SP7*, and *IBSP* was high in cells cultured in mixture medium with OM/AM mixture ratio of 100/0, 95/5, 85/15, and 70/30. The expression levels were moderate for cells cultured at OM/AM mixture ratio of 20/80, and low for cells cultured at OM/AM mixture ratio of 50/50 and 0/100. *ALP* and *SPP1* are the early stage genes of osteogenesis, while *SP7* and *IBSP* are the late stage genes. High expression of late stage marker genes was in good agreement with the calcium deposition results. Together with the ALP and Alizarin red S staining, gene expression results indicated that MSCs cultured in mixture medium with OM percentage greater than 70% showed strong osteogenic differentiation, and moderate osteogenic differentiation when the OM percentage was in a range of 70%~55%. The OM percentage lower than 50% was not good for osteogenic differentiation,

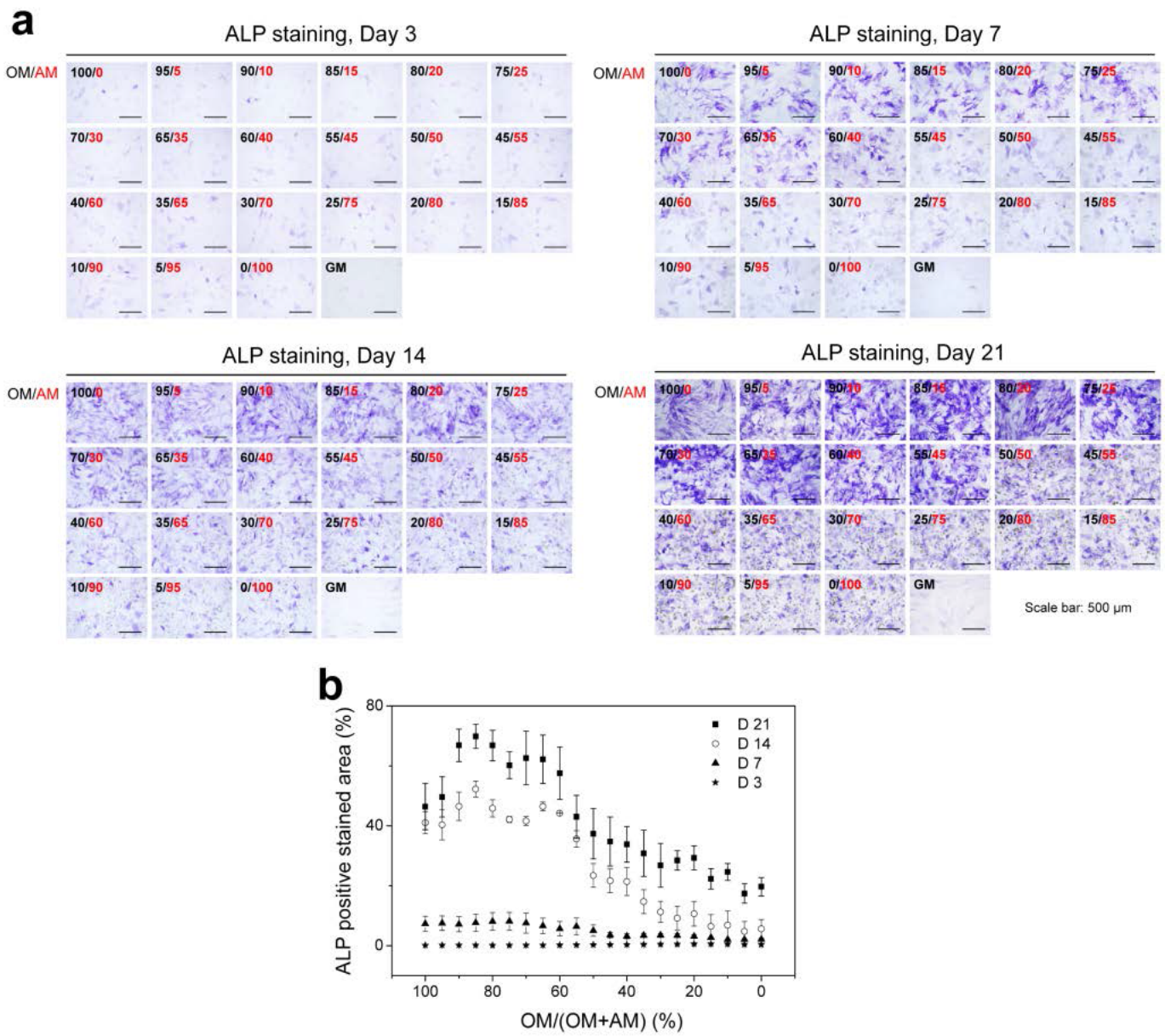

Figure 2: (a) Photomicrographs of ALP staining of MSCs after culture in different ratio of osteogenic medium (OM) and adipogenic medium (AM) for 3, 7, 14, and 21 days. 100/0 indicates the medium containing 100% OM and 0% AM, and so forth. GM indicates the growth medium. Scale bar=500 μm. (b) Percentage of cells that were positively stained with ALP after culture in different ratio of OM and AM for 21 days. Data represent mean ± SD (n ≥ 30, n represents the total number of images taken from 3 triplicate wells with more than 10 images from each well).

except the range between 25% and 15%. During the range between 25% and 15%, MSCs also showed moderate osteogenic differentiation.

Adipogenic differentiation of MSC in mixed osteogenic/adipogenic medium

Adipogenic differentiation of MSCs in the OM/AM mixture medium was investigated by Oil red O staining and real-time PCR. Oil red O staining of cells was negative for 3 days culture and slightly positive for 7 days culture, and became strongly positive after 14 and 21 days culture (Figure 4a). Few cells were positively stained in mixture medium with an OM/AM ratio of 100/0, 95/5, and 90/10. The number of stained cells increased with an increase in AM percentage, and a considerable amount of cells were stained when AM percentage was higher than 50%. MSCs cultured in growth medium were not positively stained.

Quantification of the positive stained area showed the staining intensity of the cells cultured in mixture medium (Figure 4b). The positive stained area was almost zero when MSCs were cultured in the mixture medium for 3 days and very low after 7 days of culture. The positive stained area showed a significant increase after 14 and 21 days of culture, respectively. The positive stained area was dependent on the composition of the mixture medium. It increased with increasing AM percentage and became plateau when the AM percentage was higher than 75%.

Expression of adipogenesis related genes *LPL*, *FABP4*, *PPARG*,

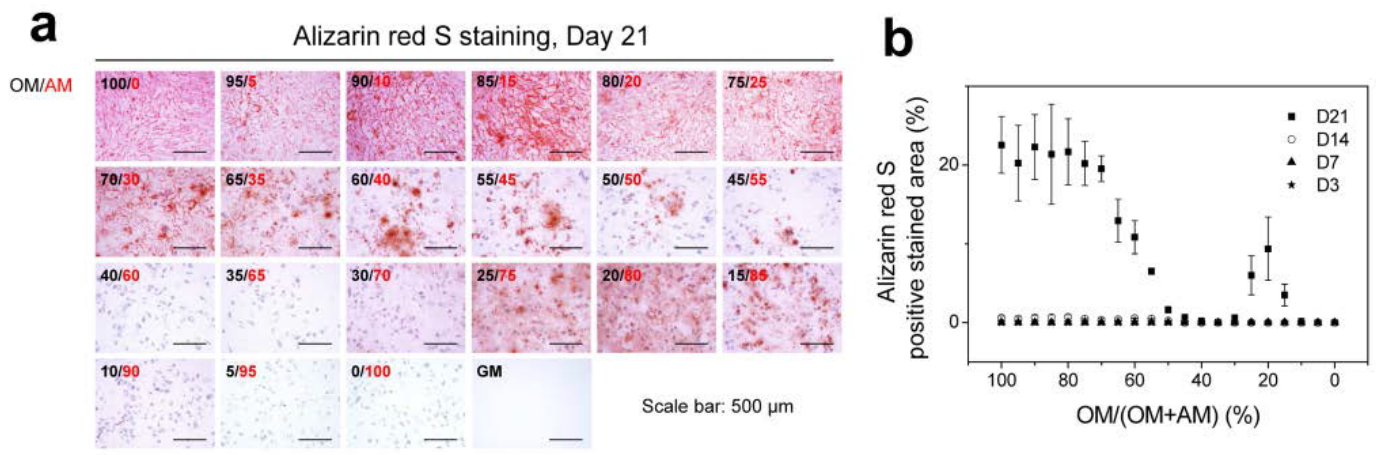

Figure 3: (a) Photomicrographs of Alizarin red S staining of MSCs after culture in different ratio of OM and AM for 3, 7, 14, and 21 days. 100/0 indicates the medium containing 100% OM and 0% AM, and so forth. GM indicates the growth medium. Scale bar=500 μm. (b) Percentage of cells that were positively stained with Alizarin red S after culture in different ratio of OM and AM for 21 days. Data represent mean ± SD (n ≥ 30, n represents the total number of images taken from 3 duplicate wells with more than 10 images from each well).

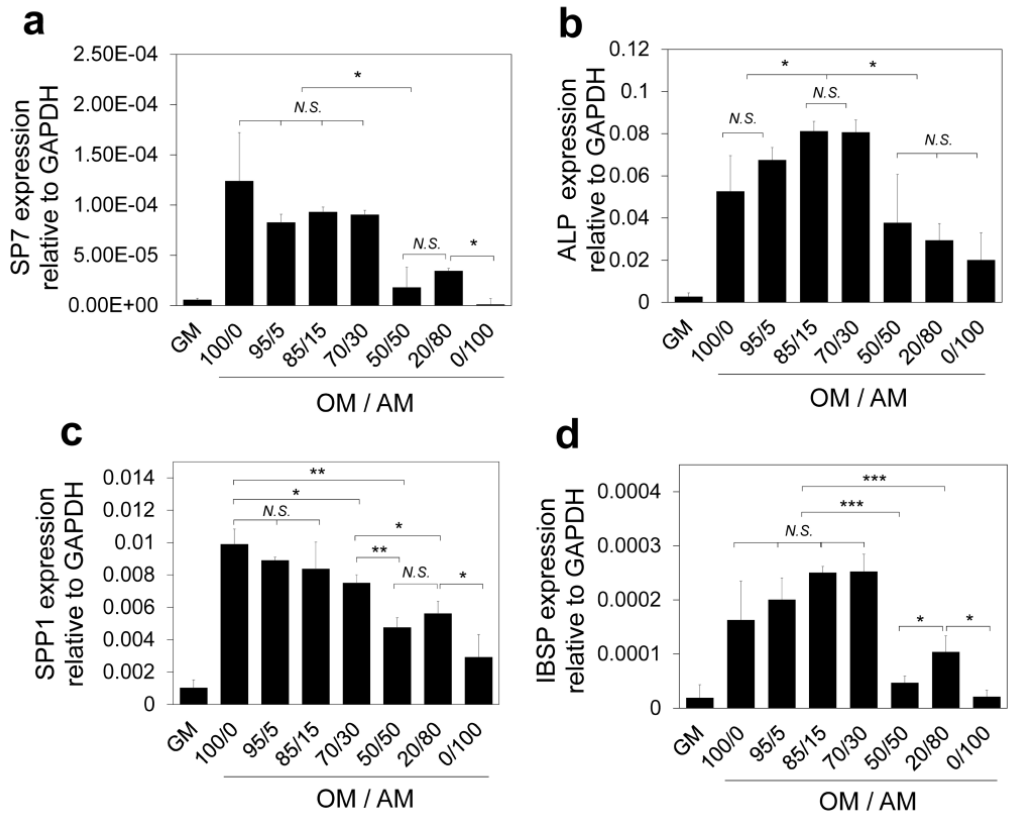

Figure 4: Expression of (a) *SP7*, (b) *ALP*, (c) *IBSP*, and (d) *SPP1* genes after MSCs were cultured in GM and mixture medium of OM and AM at a ratios of 100/0, 95/5, 85/15, 70/30, 20/80, and 0/100 for 21 days. The gene expression was investigated by real-time PCR analysis. Data represent mean ± SD (n=3, n represents the number of duplicate samples of cultured cells at the same condition). *, $P<0.05$, **, $P<0.01$, ***, $P<0.001$.

CEPBA, and *FASN*, was quantified by real-time PCR (Figure 5). Expression of the genes was very low when MSCs were cultured in growth medium. All the genes showed low expression in mixture medium containing less AM, and higher expression in mixture medium containing more AM. The expression of the genes increased with the AM percentage. For *PPARG* and *LPL*, expression levels were high when AM percentage was greater than 70%. For *CEBPA*, *FABP4*, and *FASN*, their expression reached the highest level when AM percentage was greater than 80%.

Discussion

Osteogenic and adipogenic differentiation are the most important

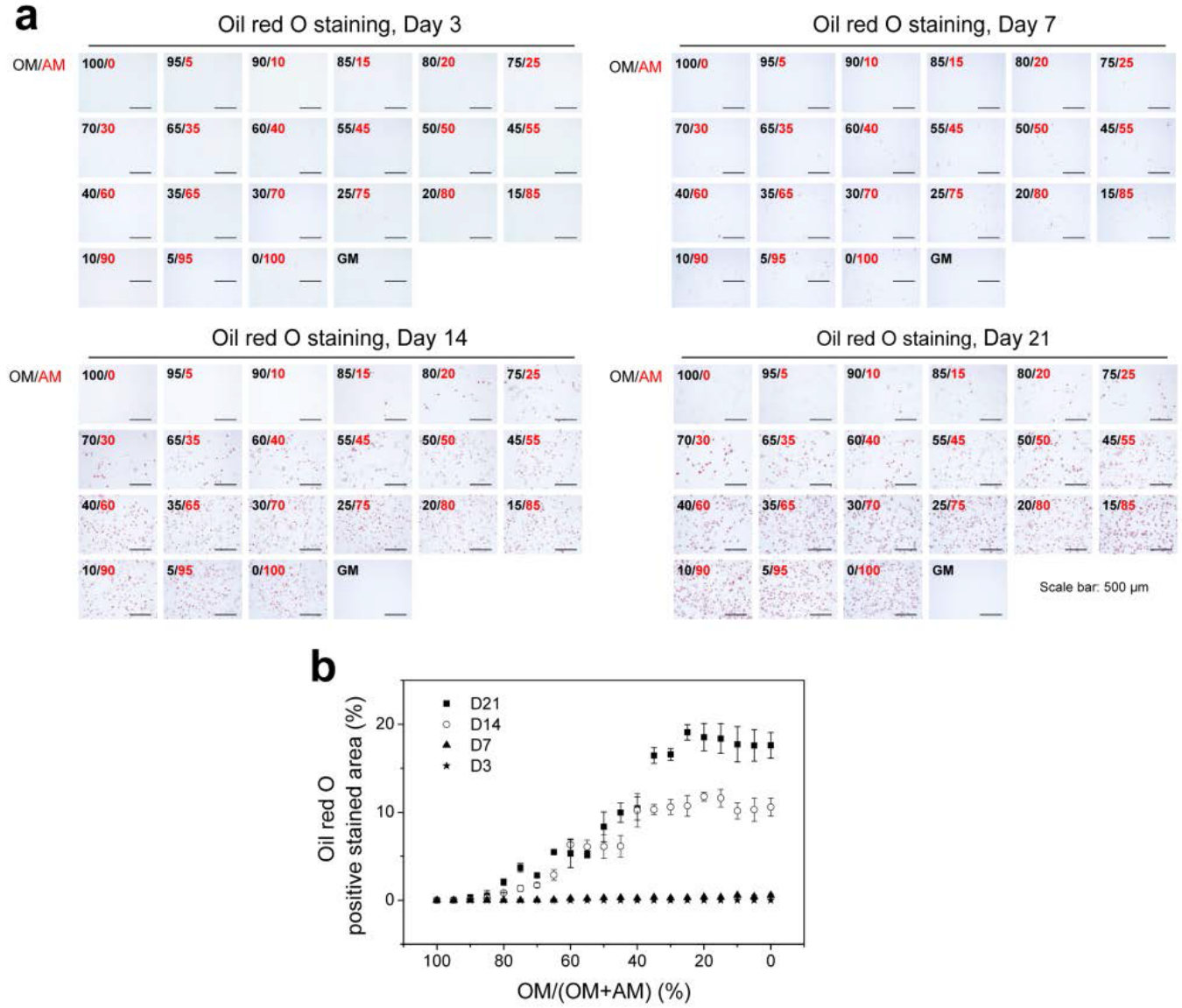

Figure 5: (a) Photomicrographs of Oil red O staining of MSCs after culture in different ratio of OM and AM for 3, 7, 14, and 21 days. 100/0 indicates the medium containing 100% OM and 0% AM, and so forth. GM indicates the growth medium. Scale bar=500 μm. (b) Percentage of cells that were positively stained with Oil red O after culture in different ratio of OM and AM for 21 days. Data represent mean ± SD (n ≥ 30, n represents the total number of images taken from 3 duplicate wells with more than 10 images from each well).

characteristics of MSCs. Control of their osteogenesis and adipogenesis is very important for tissue engineering and regenerative medicine [12,22,23]. Usually, differentiation of MSCs is studied by culturing them in a specific differentiation medium. The classical cocktail of induction factors for osteogenic and adipogenic differentiation of MSCs *in vitro* has been well established. The composition of medium has significant effect on the differentiation of MSCs. The osteogenic induction medium and adipogenic induction medium have some similar components.

However, it is unclear whether MSCs can simultaneously differentiate to osteogenic and adipogenic lineages and whether the medium components can balance the osteogenesis and adipogenesis of MSCs. In this study, the effect of these two types of induction media in mixture on balance of the osteogenic and adipogenic differentiation of MSCs was investigated. Simultaneous osteogenic and adipogenic differentiation of MSCs was induced by culturing MSCs in the mixture medium with different ratio of OM and AM. After they were cultured in the mixture medium for 21 days, MSCs showed strong osteogenic differentiation in the mixture medium containing an OM/AM ratio from 100/0 ~ 70/30. Moderate osteogenic differentiation was observed at OM/AM ratio of 70/30~50/50 and 25/75~15/85. OM/AM ratios of 50/50~25/75 and 15/85~0/100 showed low levels of osteogenic differentiation. Adipogenic differentiation of MSCs in the mixture medium showed a simple dependence on the AM percentage. High ratio of AM resulted in strong adipogenic differentiation, while at low ratio adipogenic differentiation was weak.

Therefore, the mixture medium can be divided into 5 ranges to balance the osteogenic and adipogenic differentiation of MSCs. They are OM/AM ratios of 100/0~70/30 for strong osteogenic differentiation and weak adipogenic differentiation, 70/30~50/50 for moderate

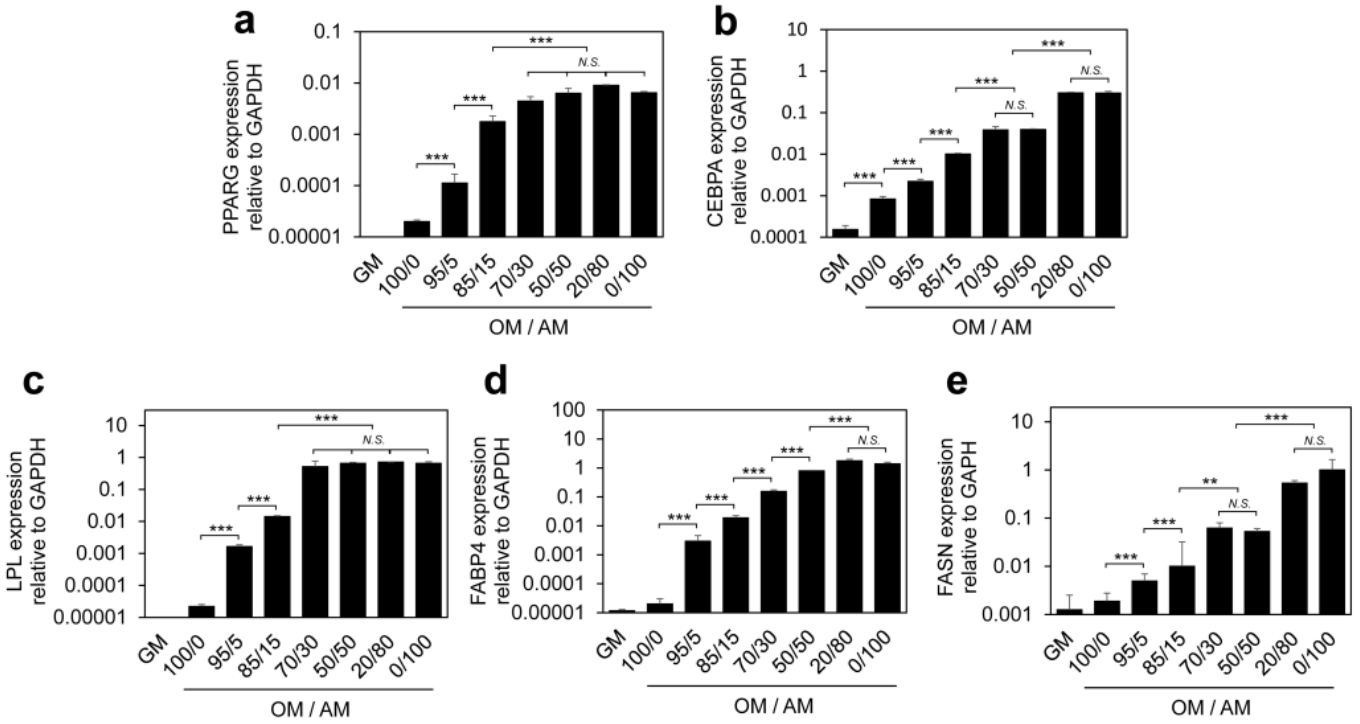

Figure 6: Expression of (a) *PPARG*, (b) *CEBPA*, (c) *LPL*, (d) *FABP4* and (e) *FASN* genes after MSCs were cultured in GM and mixture medium of OM and AM at a ratios of 100/0, 95/5, 85/15, 70/30, 20/80, and 0/100 for 21 days. The gene expression was investigated by real-time PCR analysis. Data represent mean ± SD (n=3, n represents the number of duplicate samples of cultured cells at the same condition). *, $P<0.05$, **, $P<0.01$, ***, $P<0.001$.

osteogenic differentiation and moderate adipogenic differentiation, 50/50~25/75 for weak osteogenic differentiation and moderate adipogenic differentiation, 25/75~15/85 for moderate osteogenic differentiation and strong adipogenic differentiation, 15/85~0/100 for weak osteogenic differentiation and strong adipogenic differentiation.

A large number of evidence suggests that an inverse correlation exists between osteogenesis and adipogenesis [11,24,25], mainly based on different culture supplements. The up-regulation of osteogenic differentiation is associated with down-regulation of adipogenic differentiation, and *vice versa* [10]. The general trend of osteogenic and adipogenic differentiation in the mixture medium was consistent with the previously reported results. However, the results in this study showed more detailed information on the balance of simultaneous osteogenic and adipogenic differentiation by the mixture medium.

Typically, the culture supplements regulate key transcription factors that control the balance of osteogenesis and adipogenesis. Dex and β-GP are the two inductors for osteogenic differentiation of MSCs [26]. Dex, IBMX, insulin, and Indo are the main inductors for adipogenic differentiation. The concentration of these inductors in the OM/AM mixture medium is shown in Figure 6. The favorable osteogenic differentiation at high percentages of osteogenic induction medium and favorable adipogenic differentiation at high percentages of adipogenic induction medium should be due to the appropriate concentration range of their respective induction factors.

Dex, a synthetic glucocorticoid, has been reported to be an essential soluble factor for osteogenesis of MSCs, but it has also been recognized as a adipogenesis promoter at a higher concentration [9]. Many reports found that Dex modulates osteogenesis and adipogenesis in MSCs by regulating *SP7* expression [25], *Wnt* signaling [27], *TAZ* [28], and *SPP1* [29] expression. The Dex concentration in the mixture medium with an OM/AM ratio of 100/0~70/30 was 10~300 nM. Alm et al. [14] reported that treatment with 10 nM Dex was not as effective as with 100 nM Dex.

For mineralization, the late stage of osteogenesis, the presence of both calcium and phosphate ions is necessary. β-GP serves as a crucial source of calcium phosphate. Furthermore, the calcium phosphate deposition is highly dose dependent on the concentration of β-GP. The β-GP concentration decreased with the change of OM/AM ratios from 100/0 to 0/100. The increase of Dex concentration and decrease of β-GP concentration in the mixture medium explains the best osteogenesis-stimulation effect of mixture medium with an OM/AM ratio of 100~70/30. The reason for the stimulation effect at an OM/AM ratio of 25/75~15/85 is not clear. It probably is due to the synergistic effects from all the combined components in the mixture medium.

During the adipogenesis of MSCs, high concentration of Dex usually induces expression of *CEBPA* and *PPARG*, which act as two key transcription factors of adipogenic differentiation [30]. The positive effect of Dex on adipogenic differentiation of MSCs can be amplified by cAMP-elevating agents such as IBMX [31]. On other hand, IBMX can up-regulate the expression of *PPARG* and *LPL* and also down-regulate osteogenic gene makers such as *RUNX2* and *SPP1* by activation of protein kinase A. Therefore, Dex and IBMX play important roles in modulating the balance between osteogenesis and adipogenesis. Beside these two adipogenic factors, insulin and indomethacin can also promote adipogenic differentiation by stimulating *PPARG* or binding with *PPARG* [32].

We also analyzed the main transcription factor for osteogenic differentiation of MSCs, *SP7*. *SP7* can induce preosteoblasts to differentiate into mature osteoblasts that mineralize and express high level of osteogenic-specific genes, such as bone sialoprotein (*IBSP*).

Frequently, *LPL* expression and observation of oil droplets are used as the early and late markers of adipogenesis, respectively. During the terminal differentiation of adipogenesis, adipocytes markedly increase the expression of proteins related to lipid metabolism, such as *FABP4*, an adipocyte-specific fatty acid protein, also identified as *aP2*. All the adipogenesis related genes were up-regulated with an increase in the AM percentage. This should be attributed to the increase of the adipogenesis induction factors.

These results established an important relationship between osteogenesis and adipogenesis. In bone marrow, osteoblasts and adipocytes are derived from the mesenchymal stem cells, transcription factors and genes involved in these two lineages could be manipulated by induction factors to favor osteogenic or adipogenic differentiation of MSCs. It has been reported that *PPARG* is proposed as a major factor in lineage skewing, promoting adipogenesis at the expense of osteogenesis [13]. *PPARG* activating led to ROS accumulation cause apoptosis of osteoblasts [12], and *PPARG* silencing stimulated a higher level of matrix mineralization [24]. Our results indicate that when the normal osteogenesis of MSCs is disrupted under interventions, adipogenesis dominates the differentiation process, and the reverse is also true. Shifting in balance between these two processes is regulated by involved transcription factors, which can be activated or silenced by soluble factors.

Conclusion

In conclusion, the simultaneous osteogenic and adipogenic differentiation of MSCs was studied by culturing MSCs in mixture medium. The composition of the mixture medium showed a significant effect on the osteogenesis and adipogenesis of MSCs. Adipogenic differentiation became stronger with the increase of adipogenic differentiation medium percentage and decrease of osteogenic differentiation medium percentage in the mixture medium. Osteogenic differentiation happened at two different ranges of compositions. It was very strong when the percentage of osteogenic differentiation medium was high and decreased to very weak levels when the percentage decreased to 50%. However, osteogenic differentiation became strong again when the percentage of osteogenic differentiation medium was in the range of 25% to 15%. Balancing of simultaneous osteogenic and adipogenic differentiation of MSCs by the mixture medium should provide useful information for stem cell studies and tissue regeneration.

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