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Shape-dependent regulation of differentiation lineages of bone marrow-derived cells under cyclic stretch

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Abstract

Multipotent stem cells are considered as a key material in regenerative medicine, and the understanding of the heterogeneity in the differentiation potentials of bone marrow-derived cells is important in the successful regenerative tissue repair. Therefore, the present study has been performed to investigate how the differentiation of post-harvest, native bone marrow-derived cells is regulated by cyclic stretch *in vitro*. Bone marrow-derived cells were obtained from mouse femur of both hind limbs and categorized into the following five categories: amebocytes, round cells, spindle cells, stellate cells and others. The cells were seeded on a silicone-made stretch chamber, and subjected to cyclic stretch with an amplitude of 10% at a frequency of 1 Hz for 7 days for cell shape analysis and for 3 days for the analysis of the expression of marker proteins of osteogenic (osteocalcin), vascular smooth muscle (α-smooth muscle actin and smooth muscle myosin heavy chain) and neurogenic (neurofilament) differentiation. When disregarding the differences in the cell shapes, there was an overall trend that the application of 10% cyclic stretch inhibited osteogenic and neurogenic differentiation, but enhanced smooth muscle differentiation. Close examinations revealed that round cells were influenced the most by cyclic stretch (significant up- or down-regulation in all the four marker protein expressions) while amebocytes and spindle cells were only influenced by cyclic stretch

for vascular smooth muscle and/or neurogenic differentiation. As far as the authors know, this is the first study reporting the shape-related differences in the fate decision criteria for mechanical strain in bone marrow-derived cells.

Introduction

Stem cells have a multipotent differentiation capability and have been considered as a key material in the success of regenerative medicine. Bone marrow is a primary source of the stem cells among other tissues such as blood and adipose tissue. Bone marrow-derived stromal cells, also named as bone marrow mesenchymal stem cells (bMSCs), have been studied extensively for their multipotent differentiation capability. Moreover, an application of mechanical loading has been demonstrated to be effective in directing differentiation towards cells in load-bearing tissues such as blood vessel (vascular smooth muscle cells), bone (osteoblasts) and articular cartilage (articular chondrocytes) (Steward and Kelly, 2015).

Among a variety of mechanical loading regimens, cyclic stretch has been adopted in a number of studies in an attempt to lead bMSCs into specific lineages. It has been demonstrated that the application of cyclic stretch to bMSCs *in vitro* resulted in changes in the expression of markers for osteogenic, chondrogenic, smooth muscle and tenogenic differentiations. The direction of the change (i.e. upregulation or downregulation) depends on the amplitude of cyclic stretch. In general, cyclic stretch with a low amplitude up to 5% induces an upregulation of osteogenic differentiation (Byrne et al., 2008; Haudenschild et al., 2009;

Kearney et al., 2010; Koike et al., 2005; Qi et al., 2008; Rui et al., 2011; Ward Jr. et al., 2007), whereas that with an amplitude over 10% resulted in the enhancement of the differentiation towards smooth muscle cells (Ghazanfari et al., 2009; Jang et al., 2011). Tenogenic differentiation is inducible by cyclic stretch with an amplitude ranging from 1% to 10% (Kuo and Tuan, 2008; Morita et al., 2019, 2013).

It is known that bone marrow contains cells with a variety of shapes. Two types of cell shape were reported first (Mets and Verdonk, 1981): fibroblast-like cells and large, epithelial-like cells. This was followed by a report showing the presence of round-shape cells (Colter et al., 2001; Kobayashi et al., 2004; Vogel et al., 2003). These cell shape seemed to represent different differentiation capabilities; fibroblast-like spindle cells can be differentiated into smooth muscle cells under fluid flow stimulation, while round-shape cells and large cells can be differentiated into adipocytes and osteoblast, respectively (Kobayashi et al., 2004). Chondrogenic and neurogenic differentiation capabilities have also been confirmed (Freeman et al., 2015; Ward Jr. et al., 2007). Despite the presence of such heterogeneity in the population of bone marrow-derived cells, in the most of past studies, cells were isolated from the marrow of long bones, expanded and cultured in tissue culture flasks before the use in subsequent culture experiments for cell differentiation as stem cells (e.g. Both et al., 2007; Farrell et al.,

2006). During these procedures, non-adherent cells, which could include hematopoietic cells, were excluded so that the remaining, cells attaching to a plastic substrate were selectively cultured to confluency, at least once, to make them a homogeneous population of stem cells. Although this could potentially give researchers stable and reproducible materials, such stem cells still demonstrate widely varying differentiation potentials between individual cells (Freeman et al., 2015). In addition, the use of stem cell lines comes with a drawback that the differentiation capability of the original, native cells in bone marrow cannot be analyzed. The heterogeneity of bone marrow-derived stem cells is thought to lead to an unsatisfactory outcome of tissue repair by the administration of these cell types in clinical settings (Huang et al., 2010; Wang et al., 2015). For a better outcome in the therapeutic use of stem cells, understanding of how the native bone marrow-derived cells can be differentiated to various specific lineages would be useful, particularly in the presence of extrinsic signals such as mechanical and/or chemical stimuli. Therefore, the present study has been performed to investigate how the differentiation of post-harvest, native bone marrow-derived cells is regulated by cyclic stretch *in vitro*.

Materials and methods

Bone marrow-derived cell isolation

All animal experiments were approved by the institutional review board for animal care at Nagoya Institute of Technology (Approval No. 17003) and were performed following the *Guide for Animal Experimentation, Nagoya Institute of Technology*. Bone marrow-derived cells were obtained from 6-week old Std:ddY mouse femur of both hind limbs. The femur was harvested aseptically in a clean bench, and cut at both ends. Culture medium, consisting of DMEM (Sigma-Aldrich, Japan) supplemented with 10% fetal bovine serum and penicillin-streptomycin at 1unit/ml (both from GIBCO, USA), was pre-warmed at 37°C and injected into the medullary cavity. Bone marrow containing bone marrow-derived cells was extracted with the medium into a centrifuge tube. In one experiment, a total of eight femurs from four mice was collected and pooled, and centrifuged at 1000 rpm for 5 min. This was followed by the removal of the supernatant, and the remaining cells were seeded on a silicone-made stretch chamber (STB-CH-04, Strex, Japan) (Supplementary Figure 1). The elastic membrane at the bottom of the chamber was pretreated with 47% sulfuric acid (Wako, Japan) for 1 h for

hydrophilization, followed by incubation with 0.1% type I collagen solution for 24 h. The cells in the chamber were incubated another 48 h in an incubator (37°C/5% CO_2), with medium exchange at 24 and 48 h to remove non-adherent cells.

Mechanical stimulation

A mechanical loading system (NS-300, Strex) was used to apply cyclic stretch to the cells in the stretch chamber with an amplitude of 10% at a frequency of 1Hz. For the analysis of the shape, density and alignment of cells, cyclic stretch was provided for 7 days. In each day during the 7-day period, cyclic stretch was applied for 22 h, followed by a 2-h resting period. The system was kept in 37°C and 5% CO_2 in the incubator throughout the 7-day experiment, except for the medium change and/or the imaging of the cells during the resting period. Each experiment was performed with the cells cultured in the chambers in the absence of cyclic stretch, which served as static controls. For cell differentiation analysis, cyclic stretch was provided for 3 days without resting periods. The system was kept in 37°C and 5% CO_2 in the incubator throughout the 3-day experiment. Non-stretched, static controls were included in each cyclic stretch experiment.

Analysis of cell shape, density and alignment

During the resting period of the cyclic stretching experiment, the images of cells were captured from randomly selected 10 locations within the chamber on an inverted microscope (IX71, Olympus, Japan). From these images, the shape and the density of the cells were evaluated. Because the shape of native bone marrow-derived cells was varied, the shape was classified manually into five categories: amebocytes, round cells, spindle cells, stellate cells and others. A representative image of these cells is shown in Figure 1. Amebocyte was defined as cells spreading widely with a large area. Round cell attached to the elastic membrane, but kept the round (spherical) shape. Spindle cell exhibited well-known spindle shape of cells such as fibroblasts. Stellate cells are those attaching to the substrate and spreading with thin and long cell processes. Cells not fallen into these four categories were categorized as others. To determine the density, the number of each of these cell types were manually counted in all the 10 photomicrographs obtained in each day, and the cell density (the number of cells per mm^2) was calculated. The alignment of the cells and actin filaments was examined with images of the cells labeled with rhodamine-phalloidine (ThermoFisher Scientific, USA). Detailed procedures are provided in Supplementary material 1.

Immunofluorescence staining for differentiation markers, fluorescence microscopy and image analysis

At the end of the stretching culture experiment, the cells were analyzed for the differentiation towards following specific lineages: osteogenic, smooth muscle and neurogenic differentiation. Details of the procedures of fluorescence staining, fluorescence microscopy and image analysis are provided in Supplementary material 2.

Statistical analysis

Please see Supplementary material 3.

Results

Cell density

In static culture, the cell density increased with the culture period, reaching to more than double on day 7 compared to day 0 (Fig. 2(a)). The density of the amebocytes showed no marked changes during the 7-day period, while the density of the round cells greatly increased. The

spindle cells increased the density on day 4, but decreased on day 7. In the case of stretch culture, an overall density was at a level of day 0 during the 7-day period (Fig. 2(b)). The density of the amebocytes decreased to half of that of day 0 on day 4, while the density of the round cells showed a small increase. The spindle and stellate cells were not observed on day 0, but there was a small population of the cells observed on days 4 and 7.

The alignment of cells and actin filaments

In the cells examined, only amebocytes with the shape index (defined as $4\pi \times$*cell area* divided by the square of *cell perimeter*) lower than 0.5 were analyzed for the alignment of cell body as well as actin filaments (Figure 3(a)). On day 0, the amebocytes exhibited a wide range of alignment angles, with a mean angle of 51° (Fig. 3(b)). This was shifted to the direction of stretch by the application of cyclic stretch, but only with a small amount; there were no significant differences in the cellular alignment angles among day 0, day 4 and day 7. Actin filaments also exhibited a wide range of the fiber alignment angle, with the mean angle of 47° (Fig. 3(c)). This was slightly but significantly increased to 49° on day 3, and significantly decreased to 25° on day 7.

Osteogenic differentiation

In osteocalcin expression, cyclic stretch exhibited an inhibitory effect on the osteogenic differentiation of bone marrow-derived cells, and the extent of the inhibition was dependent on the cell type (Figs. 4 and 5). The expression in the amebocytes and the spindle cells was not significantly affected by the application of cyclic stretch. On the other hand, the expression in the round cells was significantly inhibited by cyclic stretch. No expression was observed in the static condition in the stellate cells; however, the expression was observed in the stretch condition. Comparison between all stretched and all unstretched cells also resulted in a statistically significant reduction in the stretch group compared to day 0 and the static group. When comparing among cell types, the expression in the amebocytes was significantly lower than the round cells on day 0. In the static condition on day 3, the expression in the amebocytes was also significantly lower than the round and the spindle cells. In the stretched condition, the expression in the spindle cells was the highest among the four cell types. For the frequency of the cell types demonstrating osteocalcin expression, the round cell was the most abundant cell type among the five cell types including “others” in all the three conditions.

Smooth muscle differentiation

Cyclic stretch demonstrated a significant enhancing effect on the expression of smooth muscle cell markers, αSMA and SMMHC (Figs 4 and 6). In the amebocytes, round cells and spindle cells, the expression of both αSMA and SMMHC in the stretch group was significantly higher than that in the static group (except for αSMA in the spindle cells). The expression in the stellate cells was only observed on day 3. A comparison between all strained and all unstrained cells also confirmed the significant enhancing effect. The expression of both markers in the round cells were significantly higher than those in the amebocytes in all three conditions. The expressions in the spindle cells were also significantly higher than those in the amebocytes in the static and stretched groups. In addition, the round cells exhibited significantly higher expression than the spindle cells in αSMA in the static and stretched conditions and SMMHC in day 0 and the stretch conditions. Regarding the frequency of the cell types, the round cells were the most abundant cell type expressing αSMA and SMMHC.

Neurogenic differentiation

Neurogenic differentiation was inhibited by the application of cyclic stretch (Figs. 4 and 7). In the round and spindle cells, the application of cyclic stretch resulted in significantly lower expression of neurofilaments when compared to the level in the static control cells. The

inhibitory effect was also observed in the stellate cells, although the number of cells was very low. No such downregulation was observed in the amebocytes. There were also no statistically significant differences when comparing unstretched and stretched cells as a whole. Round cells exhibited the highest expression in all three conditions. The expression in the round and spindle cells was significantly higher than that in the amebocytes on day 0. The round cells also expressed significantly higher amount of neurofilaments than the amebocytes and the spindle cells in the static and the stretch conditions. Among the five cell types including others, the round cells and the amebocytes were the most of those expressing neurofilaments in day 0, while the round cells were the most abundant type in day 3 static and stretch conditions.

Discussion

The present study investigated mechanical regulation of differentiation capability of native bone marrow-derived cells including large amoebalike cells, round-shape cells, spindle-shape cells. When disregarding the differences in the cell shapes, there was an overall trend that the application of 10% cyclic stretch inhibited osteogenic and neurogenic differentiation, but enhanced smooth muscle differentiation. More interestingly, close examinations revealed that

the responsiveness to the mechanical stimulation depends on the type of cells (amebocytes, round cells, spindle cells or stellate cells). As far as the authors know, this is the first study reporting the shape-related differences in the fate decision criteria for mechanical strain in bone marrow-derived cells.

According to the past studies, there is an appropriate strain range for each specific stem cell differentiation lineage. In osteogenic differentiation, a small amplitude of cyclic strain, up to 5%, was favored, while a high amplitude strain, 10% or more, exhibited an inhibitory effect (Koike et al., 2005). By contrast, both low and high amplitudes were favored in the differentiation into smooth muscle cells (Ghazanfari et al., 2009; Jang et al., 2011; Ward Jr. et al., 2007). Mechanical loading may not be favored by neurogenic differentiation (Ward Jr. et al., 2007).

These differences may represent the mechanical environment of resident cells in tissue which stem cells are differentiated into. For example, stretch generated in human long bones during physiological movements is less than 1000 μstrain (0.1% strain) (Burr et al., 1996). In addition, according to "mechanostat" theory by Frost (Duncan and Turner, 1995; Frost, 2003; Rosa et al., 2015), the range of strain inducing anabolic and adaptive bone modeling is between 1500 and 3000 μstrain (0.15 and 0.3% strain). Therefore, the application

of high amplitude cyclic stretch to bone marrow-derived cells may not be appropriate for osteogenic differentiation.

Deformation (circumferential strain) of arterial walls has been observed as approximately 6% in ascending aorta and 11% in main pulmonary artery in human (Patel et al., 1964), although the deformation varies depending on the anatomical locations and species. This, in turn, deforms smooth muscle cells within the wall. Thus, as smooth muscle cells experience stretching at a relatively high level in vivo, it is reasonable that bone marrow-derived cells exhibit the upregulation of smooth muscle markers when subjected to 10% cyclic stretch.

In contrast, it has been exhibited that the cerebral white matter is under residual tension, while the cerebral gray matter is under residual compression (Xu et al., 2009), indicating that neurons are under mechanical loading statically, but not dynamically. In addition, the growth of neurons prefers on soft substrates (Georges et al., 2006) and axon tracts can grow under a quasi-static tensile loading (Pfister et al., 2004). Therefore, neurogenic differentiation favors static mechanical stimulation, and thus was inhibited by cyclic stretch in the present study.

It has been suggested that mesenchymal stem cells in an unstimulated condition

express genes for at least five fates: osteogenic, chondrogenic, adipogenic, myogenic and neurogenic (Ward Jr. et al., 2007). In the present study, we used bone marrow-derived cells without expansion and passaging. It has been demonstrated in the present study that round-shape cells exhibited the highest fluorescence signals for marker proteins of osteogenic, smooth muscle and neurogenic differentiation on day 0 (before the application of cyclic stretch). This may indicate that differentiation potential is heterogeneous in bone marrow-derived cells. Indeed, in our additional qualitative analysis, the synthesis of stem cell markers, Sox-2 and Oct-4, was examined (Fig. 8). The synthesis of Sox-2 was high in round cells and relatively high in spindle cells and stellate cells, whereas that was markedly low in amebocytes. Oct-4 was also synthesized in those expressing Sox-2, and the difference in the synthesis level between round cells and stellate and spindle cells were more evident. Oct-4 synthesis was also very low in amebocytes. These differences could be relevant to such heterogeneity in the differentiation potentials bone marrow-derived cells.

In recent years, the heterogeneity of stem cells has gained much attention. To maintain the homeostasis of adult tissues, stem cell proliferation and differentiation must be balanced; when one stem cell divides, one daughter cell stays in the stem cell compartment, while the other is committed to differentiation towards specific lineage, so that an overall stem

cell population size remains constant (Krieger and Simons, 2015), suggesting that all undifferentiated cells in bone marrow are not equally committed to differentiation towards specific lineages. In addition, while the expression of genes associated with stem cell multipotency is high in bone marrow-derived stem cells, the expression levels of genes involved in osteogenic, chondrogenic adipogenic, neurogenic and vascular smooth muscle differentiation are varied between individual cells (Freeman et al., 2015). Such differences could be reflected in cell morphology (Post et al., 2008). These findings are relevant to our observation that cells with different morphologies exhibited different levels of initial expression of differentiation markers as well as different responses in the determination of differentiation lineages by cyclic stretch. Namely, round cells are influenced the most by cyclic stretch (significant up- or down-regulation in all the four marker protein expressions) while amebocytes and spindle cells were only influenced by cyclic stretch for αSMA (amebocytes), SMMHC (amebocytes and spindle cells) and neurofilaments (spindle cells). This heterogeneity in mechano-response is attributed to the varied quality of the repair of load-bearing tissues when bMSCs are used for therapeutic applications in clinical settings (Huang et al., 2010; Wang et al., 2015). It also highlights that round cells which seem to have a small and weak adhesion to the substrate are more prone to respond to mechanical stretch than amebocytes

with a large and strong adhesion to substrate. This contradictory finding guarantees future investigation.

Interestingly, although cells with large, flatten shape or spindle shape have been considered as the main population of cells derived from bone marrow, the presence of small, round-shape cells in bone marrow-derived cells was first reported by Colter et al (Colter et al., 2001). They have found the round cells in cell colonies raised from single cells isolated as adherent cells from bone marrow extracts and shown that round cells exhibited a superior proliferation and multi-directional differentiation potential. The presence of such round cells has also been reported in other past studies (Kobayashi et al., 2004; Vogel et al., 2003). In the present study, it was demonstrated that round cells exhibited the highest expression of differentiation markers (osteocalcin in day 3 static, smooth muscle markers in day 3 stretch and neurofilament in day 0). In addition, the proliferation of round cells was faster than other types of cells in static condition (Fig. 2(a)). This well coincides with these previous findings.

However, the mechanisms of how the cells with different shapes exhibited different mechanoresponse are still unclear. It has been suggested that mechanosensitive ion channels are involved in the mechanically-stimulated differentiation of stem cell species (He et al., 2019). Transient receptor potential (TRP) channels are responsible for the compression and fluid shear

stress-induced osteogenic differentiation (Liu et al., 2015; Xiao et al., 2015). Piezo1 channel is reported to be involved in the cell differentiation through mechanical interaction between cell and extracellular matrix (Pathak et al., 2014). In the present study, it is likely that cells with different shapes possess different levels of tension in the plasma membrane, reflecting actin filament organization, as well as different type/distribution of the mechanosensitive ion channels. Therefore, such differences would be a regulatory factor in the mechanoresponse of bone marrow-derived cells in different shape. In addition to cytoskeleton-mediated signal transduction, mechanically-induced molecular mechanotransduction pathways (Song et al., 2018; Zhang et al., 2019) as well as direct deformation of cell nucleus by cyclic stress (Rahimpour et al., 2019) should play significant roles in the mechanoresponse.

In the present study, three differentiation lineages were selected for the examination. However, it does not indicate that these differentiation lineages were only upregulated by 10% cyclic stretch. Other differentiation lineages, such as differentiation to tendon ligament cells, could also be upregulated by the same mechanical stimulation. Thus, it is necessary to investigate other possible effects of cyclic stretch on bone marrow-derived cells in future work.

In conclusion, we have examined mechanical regulation of differentiation lineages of bone marrow-derived cells and have shown that the application of 10% cyclic uniaxial strain

significantly enhanced smooth muscle differentiation, significantly inhibited osteogenic differentiation and possessed an only small influence on neurogenic differentiation. In addition, among the five cell types we classified, round cells exhibited a superior proliferation and responsiveness to mechanical stimulation. The findings obtained will be useful in the preparation of bone marrow-derived stem cells for an effective tissue repair.

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Conflict of interest statement

The authors have neither financial nor personal relationships with other people or organizations that could inappropriately influence the present work.

References

Both, S.K., Muijsenberg, A.J.C. van der, Blitterswijk, C.A. van, Boer, J. de, Bruijn, J.D. de,

2007. A Rapid and Efficient Method for Expansion of Human Mesenchymal Stem Cells. Tissue Eng. 13, 3–9. doi:10.1089/ten.2005.0513

Burr, D.B., Milgrom, C., Fyhrie, D., Forwood, M., Nyska, M., Finestone, A., Hoshaw, S., Saiag, E., Simkin, A., 1996. In vivo measurement of human tibial strains during vigorous activity. Bone 18, 405–410. doi:10.1016/8756-3282(96)00028-2

Byrne, E.M., Farrell, E., McMahon, L.A., Haugh, M.G., O'Brien, F.J., Campbell, V.A., Prendergast, P.J., O'Connell, B.C., 2008. Gene expression by marrow stromal cells in a porous collagen-glycosaminoglycan scaffold is affected by pore size and mechanical stimulation. J. Mater. Sci. Mater. Med. 19, 3455–63. doi:10.1007/s10856-008-3506-2

Colter, D.C., Sekiya, I., Prockop, D.J., 2001. Identification of a subpopulation of rapidly self-renewing and multipotential adult stem cells in colonies of human marrow stromal cells. Proc. Natl. Acad. Sci. 98, 7841–7845. doi:10.1073/pnas.141221698

Duncan, R.L., Turner, C.H., 1995. Mechanotransduction and the functional response of bone to mechanical strain. Calcif. Tissue Int. 57, 344–358.

Farrell, E., O'Brien, F.J., Doyle, P., Fischer, J., Yannas, I., Harley, B.A., O'Connell, B., Prendergast, P.J., Campbell, V.A., 2006. A Collagen-glycosaminoglycan Scaffold Supports Adult Rat Mesenchymal Stem Cell Differentiation Along Osteogenic and

Chondrogenic Routes. Tissue Eng. 12, 459–468. doi:10.1089/ten.2006.12.459

Freeman, B.T., Jung, J.P., Ogle, B.M., 2015. Single-cell RNA-Seq of bone marrow-derived mesenchymal stem cells reveals unique profiles of lineage priming. PLoS One 10. doi:10.1371/journal.pone.0136199

Frost, H.M., 2003. Bone's mechanostat: A 2003 update. Anat. Rec. Part a 275A, 1081–1101. doi:10.1002/ar.a.10119

Georges, P.C., Miller, W.J., Meaney, D.F., Sawyer, E.S., Janmey, P.A., 2006. Matrices with compliance comparable to that of brain tissue select neuronal over glial growth in mixed cortical cultures. Biophys. J. 90, 3012–3018. doi:10.1529/biophysj.105.073114

Ghazanfari, S., Tafazzoli-Shadpour, M., Shokrgozar, M.A., 2009. Effects of cyclic stretch on proliferation of mesenchymal stem cells and their differentiation to smooth muscle cells. Biochem. Biophys. Res. Commun. 388, 601–605. doi:10.1016/j.bbrc.2009.08.072

Haudenschild, A.K., Hsieh, A.H., Kapila, S., Lotz, J.C., 2009. Pressure and distortion regulate human mesenchymal stem cell gene expression. Ann. Biomed. Eng. 37, 492–502. doi:10.1007/s10439-008-9629-2

He, L., Ahmad, M., Perrimon, N., 2019. Mechanosensitive channels and their functions in stem cell differentiation. Exp. Cell Res. 374, 259–265. doi:10.1016/j.yexcr.2018.11.016

Huang, A.H., Farrell, M.J., Mauck, R.L., 2010. Mechanics and mechanobiology of mesenchymal stem cell-based engineered cartilage. J. Biomech. 43, 128–136. doi:10.1016/j.jbiomech.2009.09.018

Jang, J.-Y., Lee, S.W., Park, S.H., Shin, J.W., Mun, C., Kim, S.-H., Kim, D.H., Shin, J.-W., 2011. Combined Effects of Surface Morphology and Mechanical Straining Magnitudes on the Differentiation of Mesenchymal Stem Cells without Using Biochemical Reagents. J. Biomed. Biotechnol. 2011, 1–9. doi:10.1155/2011/860652

Kearney, E.M., Farrell, E., Prendergast, P.J., Campbell, V.A., 2010. Tensile strain as a regulator of mesenchymal stem cell osteogenesis. Ann. Biomed. Eng. 38, 1767–1779. doi:10.1007/s10439-010-9979-4

Kobayashi, N., Yasu, T., Ueba, H., Sata, M., Hashimoto, S., Kuroki, M., Saito, M., Kawakami, M., 2004. Mechanical stress promotes the expression of smooth muscle-like properties in marrow stromal cells. Exp. Hematol. 32, 1238–1245. doi:10.1016/j.exphem.2004.08.011

Koike, M., Shimokawa, H., Kanno, Z., Ohya, K., Soma, K., 2005. Effects of mechanical strain on proliferation and differentiation of bone marrow stromal cell line ST2. J. Bone Miner. Metab. 23, 219–225. doi:10.1007/s00774-004-0587-y

Krieger, T., Simons, B.D., 2015. Dynamic stem cell heterogeneity. Development 142, 1396–1406. doi:10.1242/dev.101063

Kuo, C.K., Tuan, R.S., 2008. Mechanoactive Tenogenic Differentiation of Human Mesenchymal Stem Cells. Tissue Eng. Part A 14, 1615–1627. doi:10.1089/ten.tea.2006.0415

Liu, Y.S., Liu, Y.A., Huang, C.J., Yen, M.H., Tseng, C.T., Chien, S., Lee, O.K., 2015. Mechanosensitive TRPM7 mediates shear stress and modulates osteogenic differentiation of mesenchymal stromal cells through Osterix pathway. Sci. Rep. 5, 1–13. doi:10.1038/srep16522

Mets, T., Verdonk, G., 1981. In vitro aging of human bone marrow derived stromal cells. Mech. Ageing Dev. 16, 81–89. doi:10.1016/0047-6374(81)90035-X

Morita, Y., Sato, T., Higashiura, K., Hirano, Y., Matsubara, F., Oshima, K., Niwa, K., Toku, Y., Song, G., Luo, Q., Ju, Y., 2019. The optimal mechanical condition in stem cell-to-tenocyte differentiation determined with the homogeneous strain distributions and the cellular orientation control. Biol. Open 8, bio039164. doi:10.1242/bio.039164

Morita, Y., Watanabe, S., Ju, Y., Xu, B., 2013. Determination of optimal cyclic Uniaxial stretches for stem cell-to-tenocyte differentiation under a wide range of mechanical

stretch conditions by evaluating gene expression and protein synthesis levels. Acta Bioeng. Biomech. 15, 71–79. doi:10.5277/abb130309

Patel, D.J., Austen, W.G., Greenfield, J.C., Tindall, G.T., 1964. Impedance of certain large blood vessels in man. Ann. N. Y. Acad. Sci. 115, 1129–1139. doi:10.1111/j.1749-6632.1964.tb50685.x

Pathak, M.M., Nourse, J.L., Tran, T., Hwe, J., Arulmoli, J., Le, D.T.T., Bernardis, E., Flanagan, L.A., Tombola, F., 2014. Stretch-activated ion channel Piezo1 directs lineage choice in human neural stem cells. Proc. Natl. Acad. Sci. 111, 16148–16153. doi:10.1073/pnas.1409802111

Pfister, B.J., Iwata, A., Meaney, D.F., Smith, D.H., 2004. Extreme Stretch Growth of Integrated Axons. J. Neurosci. 24, 7978–7983. doi:10.1523/JNEUROSCI.1974-04.2004

Post, S., Abdallah, B.M., Bentzon, J.F., Kassem, M., 2008. Demonstration of the presence of independent pre-osteoblastic and pre-adipocytic cell populations in bone marrow-derived mesenchymal stem cells. Bone 43, 32–39. doi:10.1016/j.bone.2008.03.011

Qi, M.C., Hu, J., Zou, S.J., Chen, H.Q., Zhou, H.X., Han, L.C., 2008. Mechanical strain induces osteogenic differentiation: Cbfa1 and Ets-1 expression in stretched rat mesenchymal stem cells. Int. J. Oral Maxillofac. Surg. 37, 453–458.

doi:10.1016/j.ijom.2007.12.008

Rahimpour, E., Vahidi, B., Mollahoseini, Z., 2019. A computational simulation of cyclic stretch of an individual stem cell using a nonlinear model. J. Tissue Eng. Regen. Med. 13, 274–282. doi:10.1002/term.2790

Rosa, N., Simoes, R., Magalhães, F.D., Marques, A.T., 2015. From mechanical stimulus to bone formation: A review. Med. Eng. Phys. 37, 719–728. doi:10.1016/j.medengphy.2015.05.015

Rui, Y.F., Lui, P.P.Y., Ni, M., Chan, L.S., Lee, Y.W., Chan, K.M., 2011. Mechanical loading increased BMP-2 expression which promoted osteogenic differentiation of tendon-derived stem cells. J. Orthop. Res. 29, 390–396. doi:10.1002/jor.21218

Song, Y., Tang, Y., Song, J., Lei, M., Liang, P., Fu, T., Su, X., Zhou, P., Yang, L., Huang, E., 2018. Cyclic mechanical stretch enhances BMP9-induced osteogenic differentiation of mesenchymal stem cells. Int. Orthop. 42, 947–955. doi:10.1007/s00264-018-3796-z

Steward, A.J., Kelly, D.J., 2015. Mechanical regulation of mesenchymal stem cell differentiation. J. Anat. 227, 717–731. doi:10.1111/joa.12243

Vogel, W., Grünebach, F., Messam, C.A., Kanz, L., Brugger, W., Bühring, H.-J., 2003. Heterogeneity among human bone marrow-derived mesenchymal stem cells and neural

progenitor cells. Haematologica 88, 126–33.

Wang, Y., Yuan, M., Guo, Q.-Y., Lu, S.-B., Peng, J., 2015. Mesenchymal Stem Cells for Treating Articular Cartilage Defects and Osteoarthritis. Cell Transplant. 24, 1661–1678. doi:10.3727/096368914X683485

Ward Jr., D.F., Salasznyk, R.M., Klees, R.F., Backiel, J., Agius, P., Bennett, K., Boskey, A., Plopper, G.E., 2007. Mechanical Strain Enhances Extracellular Matrix-Induced Gene Focusing and Promotes Osteogenic Differentiation of Human Mesenchymal Stem Cells Through an Extracellular-Related Kinase-Dependent Pathway. Stem Cells Dev. 16, 467–480. doi:10.1089/scd.2007.0034

Xiao, E., Yang, H.Q., Gan, Y.-H., Duan, D.-H., He, L.-H., Guo, Y., Wang, S.Q., Zhang, Y., 2015. Brief Reports: TRPM7 Senses Mechanical Stimulation Inducing Osteogenesis in Human Bone Marrow Mesenchymal Stem Cells. Stem Cells 33, 615–621. doi:10.1002/stem.1858

Xu, G., Bayly, P. V., Taber, L.A., 2009. Residual stress in the adult mouse brain. Biomech. Model. Mechanobiol. 8, 253–262. doi:10.1007/s10237-008-0131-4

Zhang, L., Wang, Y., Zhou, N., Feng, Y., Yang, X., 2019. Cyclic tensile stress promotes osteogenic differentiation of adipose stem cells via ERK and p38 pathways. Stem Cell

Res. 37, 101433. doi:10.1016/j.scr.2019.101433

Figure legends

Figure 1

Bright-field image showing representative shapes of five types of cells isolated from bone marrow: (a) amebocytes, (b) round cells, (c) spindle cells, (d) stellate cells and (e) others.

Figure 2

Temporal changes in cell density of the cells isolated from bone marrow in the static culture condition (a) and in the stretch culture condition (b). The number of experiments was 1 and 2 in the static and the stretch condition, respectively. The data in the stretch condition present mean value.

Figure 3

The alignment of cells and actin filaments in response to cyclic stretch. (a) Representative images of amebocytes on day 4. (b) The angle of the long axis of cells to the direction of stretch. (c) The angle of actin filaments to the direction of stretch. The number of cells analyzed was 17, 21 and 7 on day 0, day 4 and day 7, respectively. In both angles, 0° corresponds to the direction of stretch.

Figure 4

Representative photomicrographs of the expression of differentiation markers identified by immunofluorescence staining. To highlight the difference between static and stretch conditions, the brightness of the fluorescence images of the two conditions was elevated to the same degree in each marker proteins. Fluorescence images with the original brightness are provided in Supplementary material 4.

Figure 5

The expression of osteogenic marker osteocalcin from bone marrow-derived cells in four cell types. (a) Average intensities of fluorescence signals obtained from immunofluorescence staining, corresponding to the expression levels. 'Total' presents the data pooled from the four cell types for each of day 0, day 3 static and day 3 stretch group. (b) The frequency of the cell types in all cells expressing osteocalcin in each condition. The number of experiments was 4, 2 and 4 for day 0, day 3 static and day 3 stretch group, respectively.

Figure 6

The expression of smooth muscle markers α-smooth muscle actin (αSMA) and smooth muscle myosin heavy chain (SMMHC) from bone marrow-derived cells in four cell types. (a, b) Average intensities of fluorescence signals for αSMA (a) and SMMHC (b) obtained from

immunofluorescence staining, corresponding to the expression levels. ‘Total’ presents the data pooled from the four cell types for each of day 0, day 3 static and day 3 stretch group. (c) The frequency of the cell types in all cells expressing both markers in each condition. The number of experiments was 3, 3 and 5 for day 0, day 3 static and day 3 stretch group, respectively.

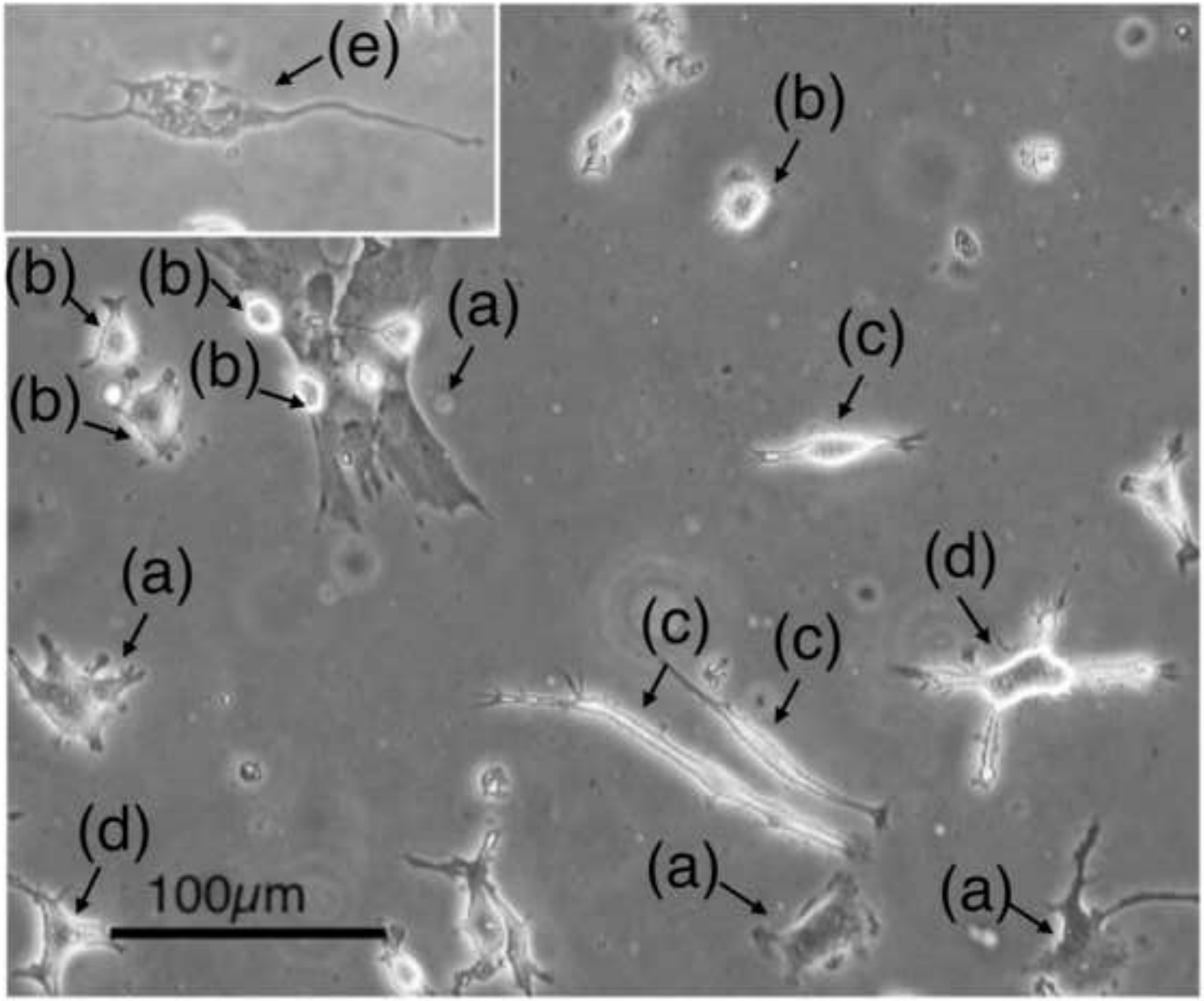
Figure 7

The expression of neurogenic marker neurofilament from bone marrow-derived cells in four cell types. (a) Average intensities of fluorescence signals obtained from immunofluorescence staining, corresponding to the expression levels. ‘Total’ presents the data pooled from the four cell types for each of day 0, day 3 static and day 3 stretch group. (b) The frequency of the cell types in all cells expressing neurofilament in each condition. Number of experiments was 4, 2 and 4 for day 0, day 3 static and day 3 stretch group, respectively.

Figure 8

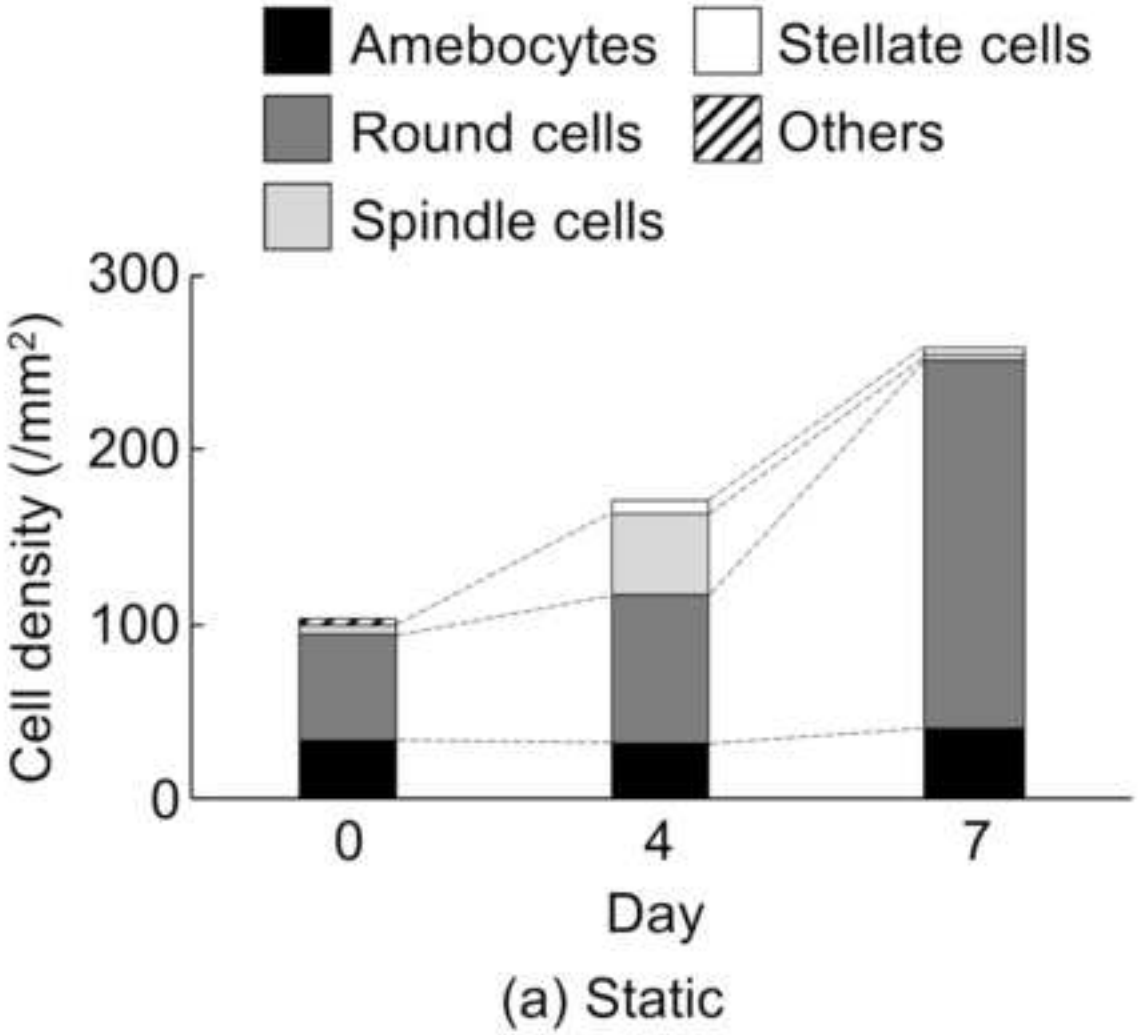
Immunofluorescence images of bone marrow-derived cells labeled for stem cell markers, Sox-2 (green) and Oct-4 (red), along with cell nucleus (blue). White arrowheads in the Sox-2 image for round cells indicate representative round cells in the image. The letters “st”, “sp”, “r” and “am” indicate stellate cell, spindle cell, round cell and amebocyte in the Sox-2 images in the top row. Please refer to Supplementary material 5 for the experimental procedures.

Figure 1

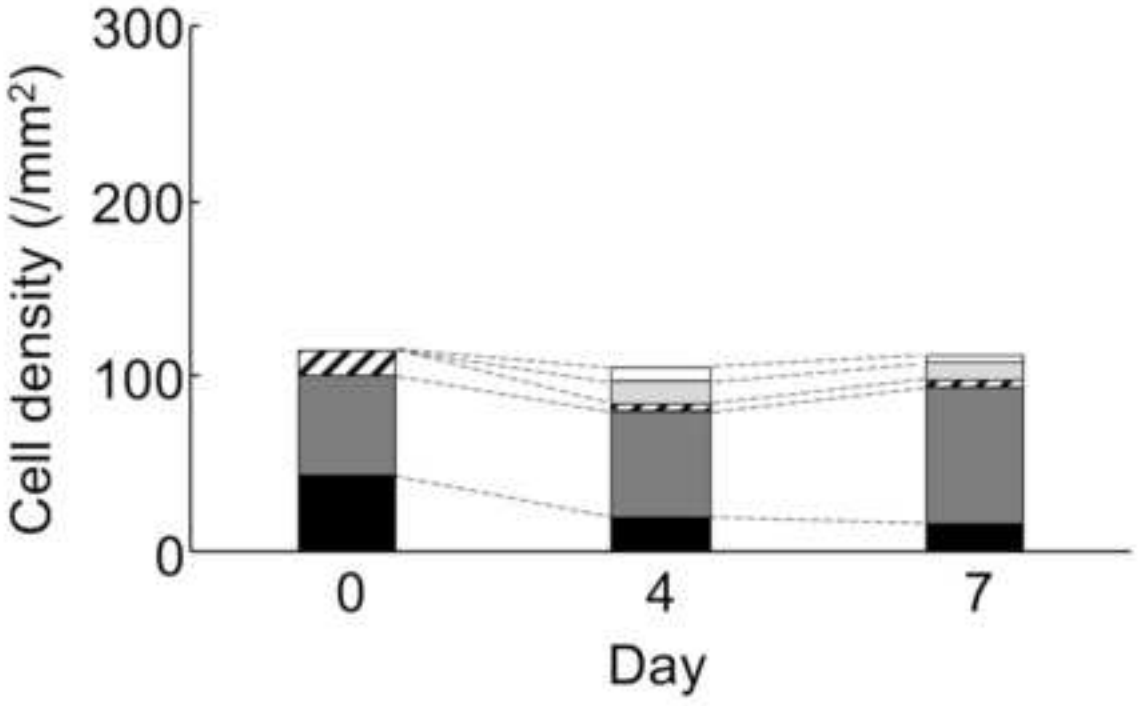


(a) Amebocytes, (b) Round cells, (c) Spindle cells,
(d) Stellate cells, (e) Others

Figure 2

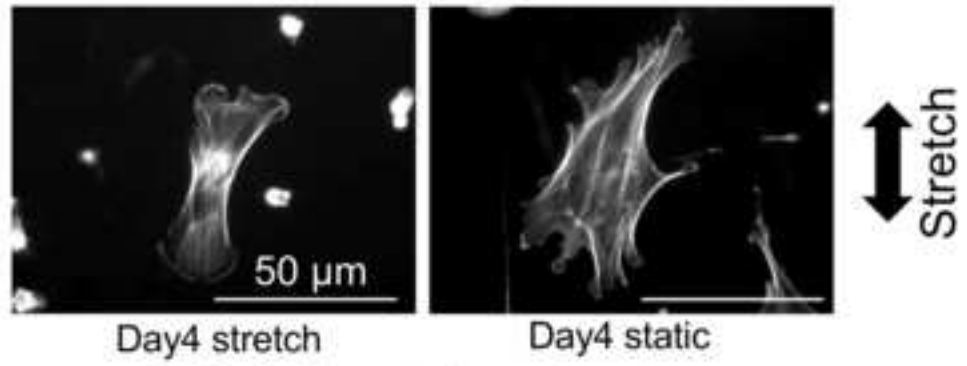


(a) Static

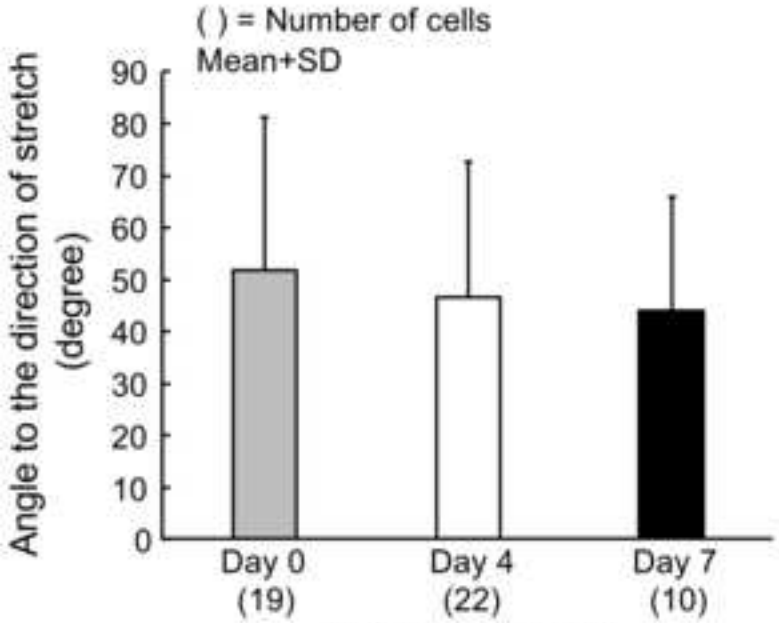


(b) Stretch

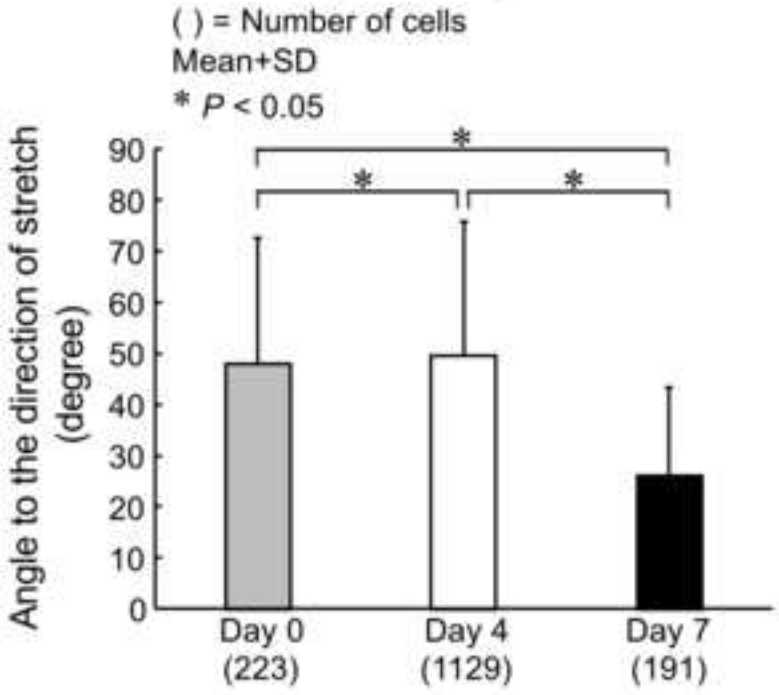
Figure 3



(a) Actin filaments in amebocytes

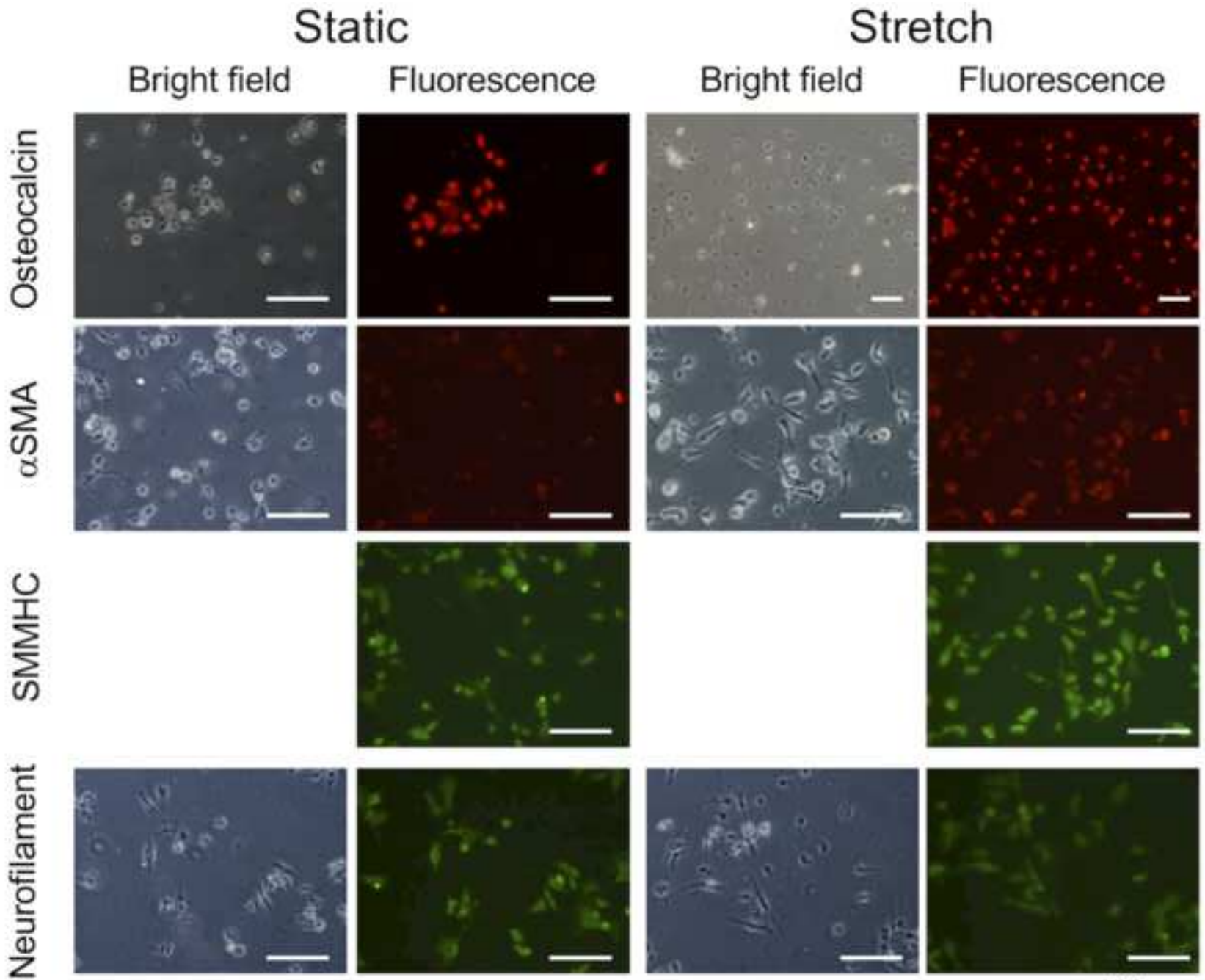


(b) Cell alignment



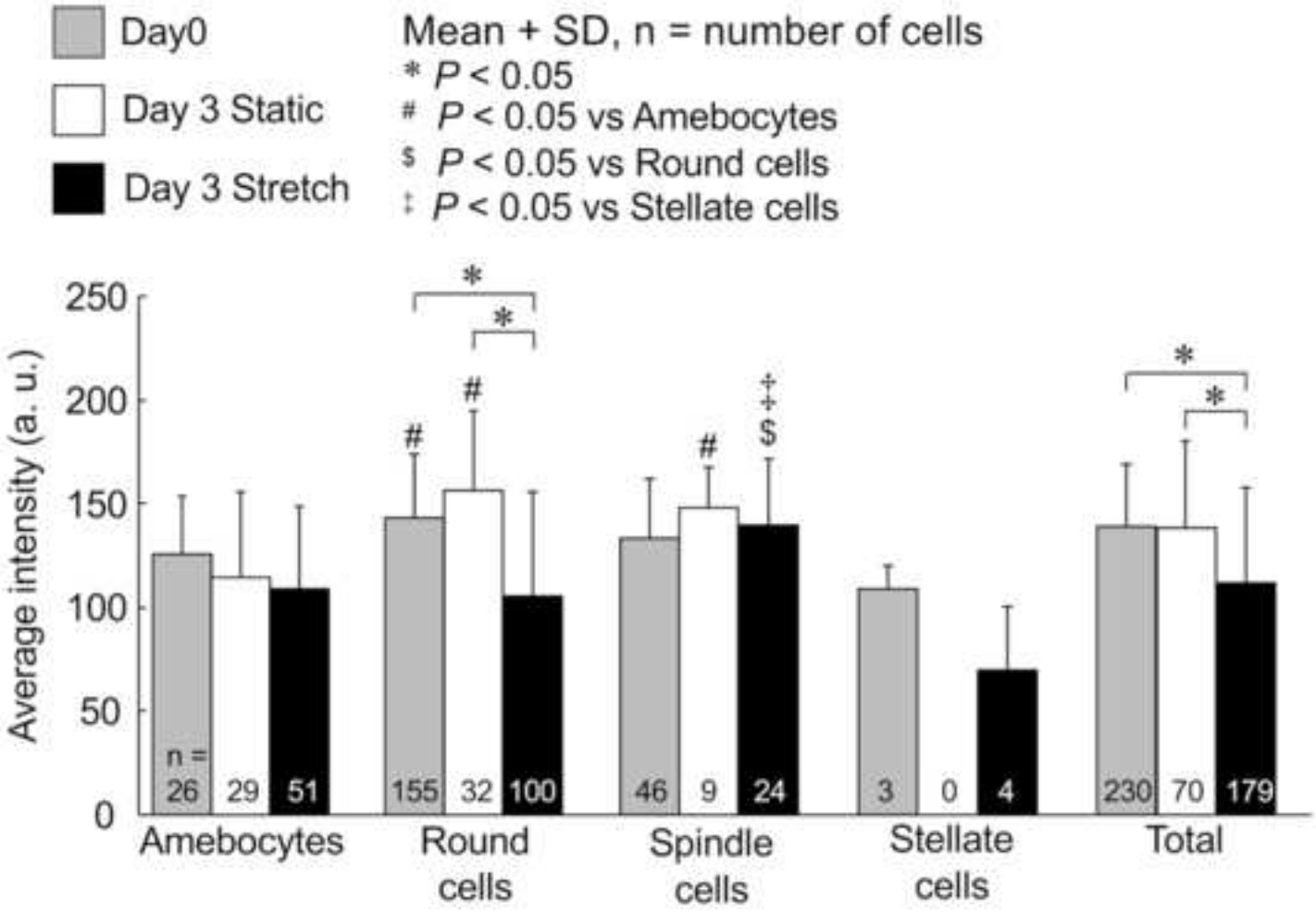
(c) Actin filament alignment

Figure 4

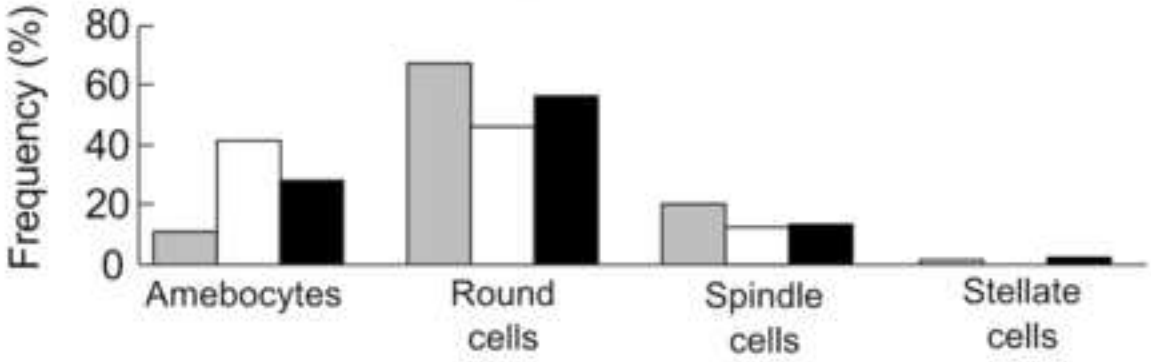


αSMA, a-smooth muscle actin; SMMHC, smooth muscle myosin heavy chain

Figure 5



(a) Osteocalcin



(b) Frequency of type of cells

Figure 6

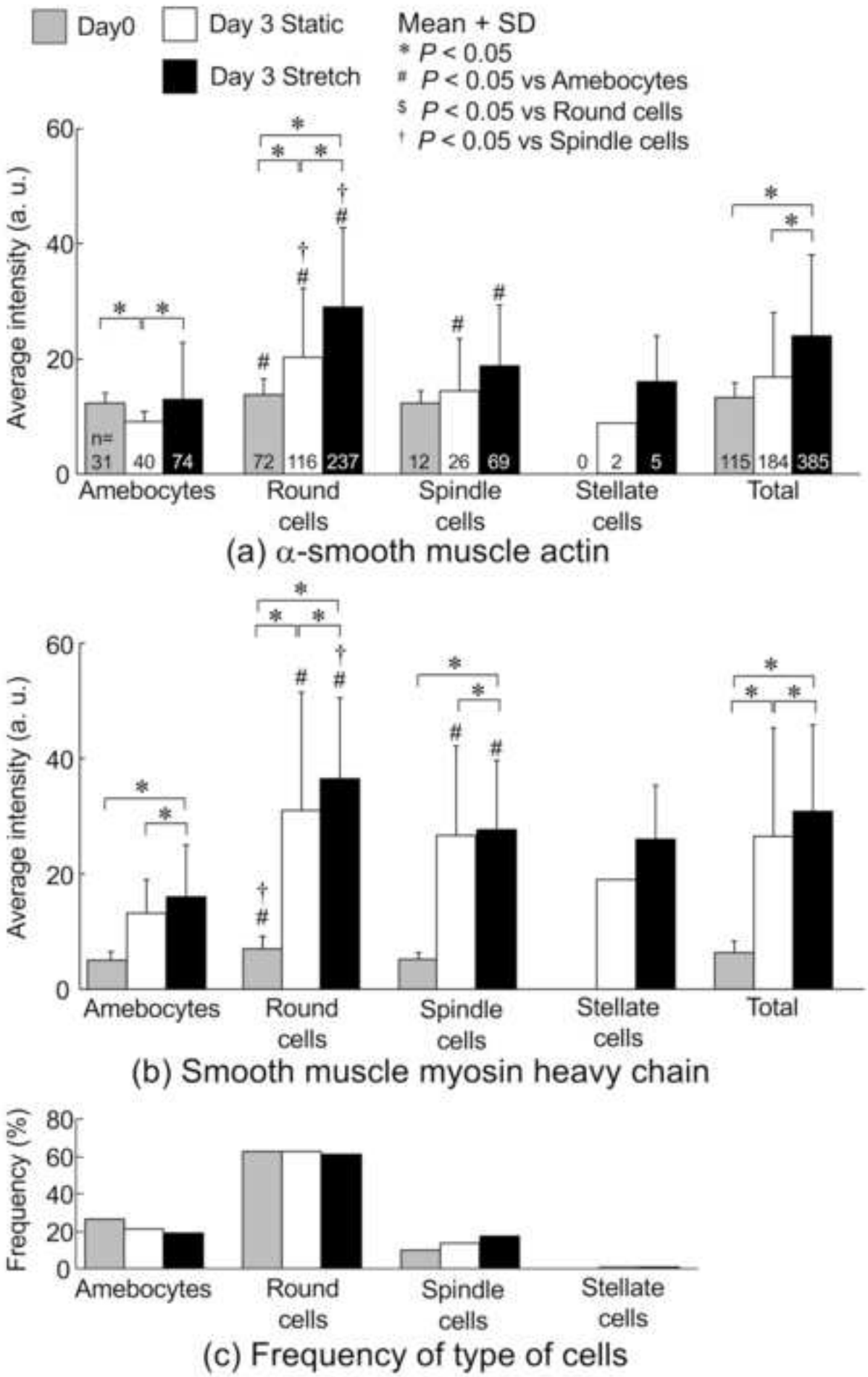


Figure 7

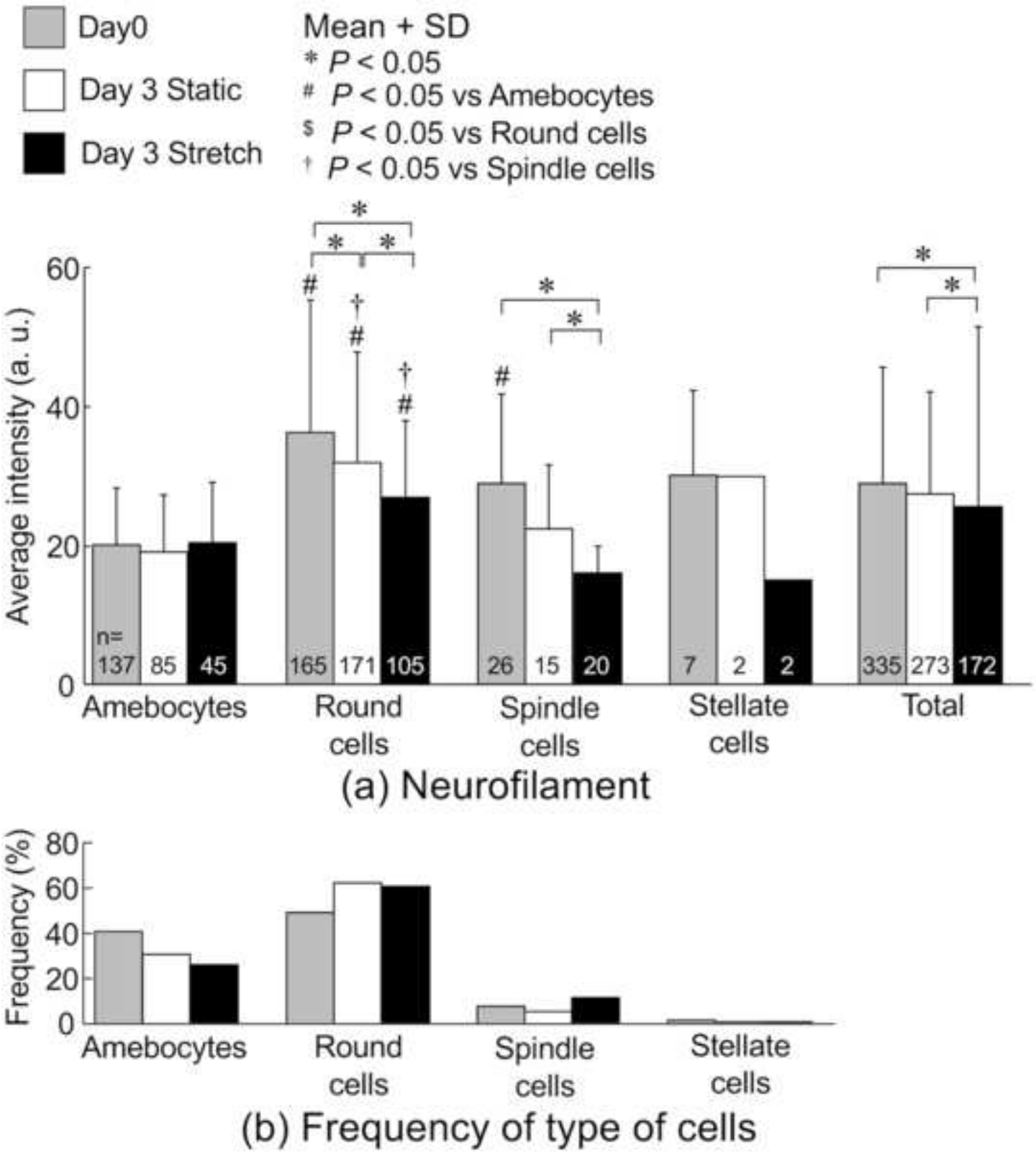
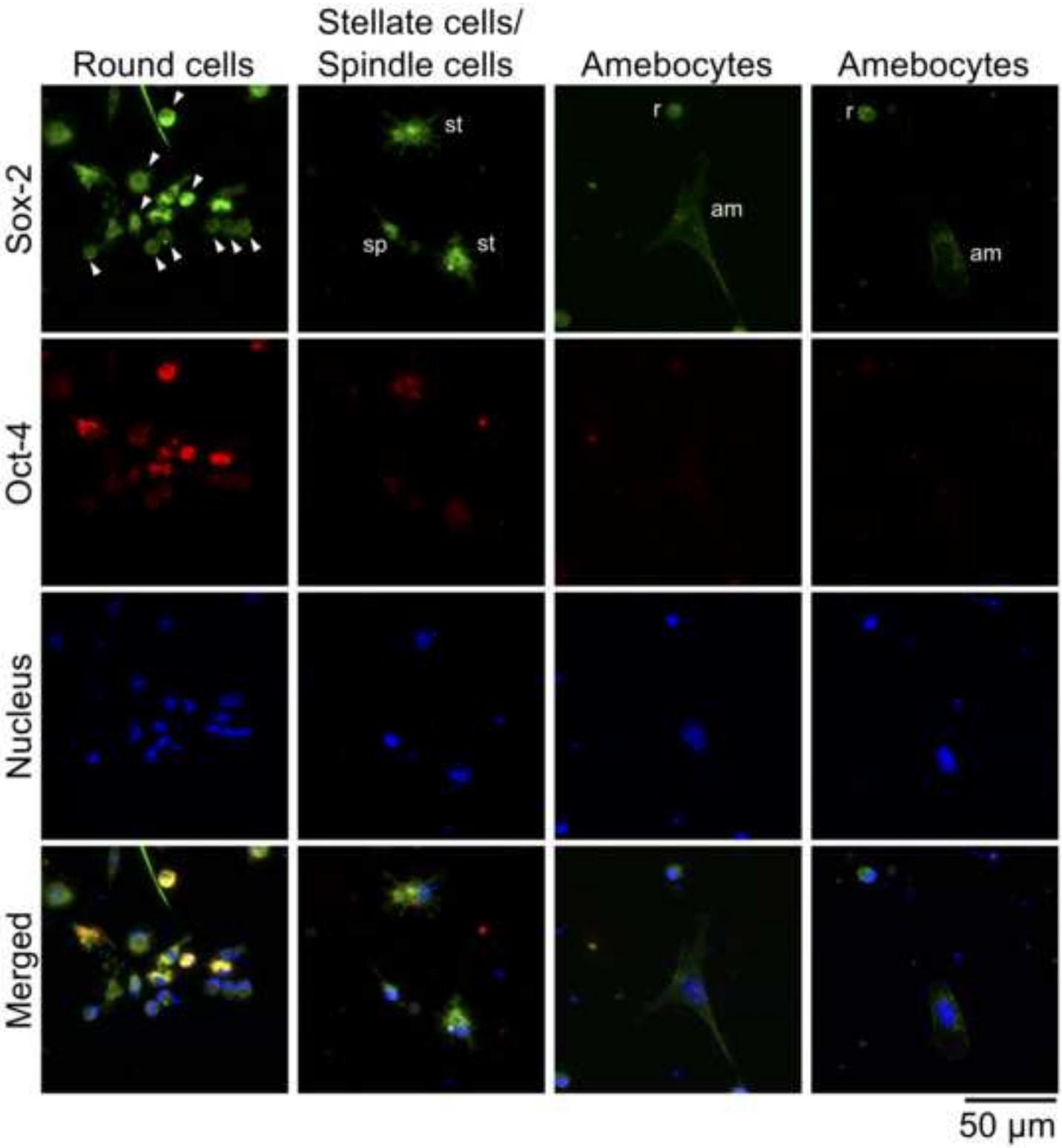


Figure 8



Supplementary Figure 1

CONFLICT OF INTEREST STATEMENT

The authors have neither financial nor personal relationships with other people or organizations that could inappropriately influence the present work.