Introduction: Mesenchymal stem cells (MSCs) in bone marrow are heterogeneous and exist at low-end ratio (1 in 1X105 bone marrow mononuclear cells (BM MNCs)), which further decreases along with the increase in the age of donors. MSCs have a self-renewal capacity so that they can be easily expended in vitro cultivation, but it also decreases over repeated passages[1]. For these reasons, it is highly limited to obtain large number of pure MSCs for clinical application. Many researches have been performed to obtain as many MSCs as possible by controlling the experimental microenvironment such as addition of specific growth factors (e.g. basic-fibroblast growth factor) in basal media, or coating extra cellular matrix (ECM) molecules (e.g. fibronectin, collagen) on culture plate[2, 3]. In addition, many studies have reported on the modulation of MSCs function by applying mechanical stimuli (e.g. stretch, shear stress and ultrasound)[4]. Ultrasound at low intensities (LIUS), in particular, is a special type of sound that can generate mechanical stimuli and has been reported to regulate proliferation and differentiation of cells via cellular signaling pathways. LIUS is known to enhance DNA synthesis, and expression of integrins and cytoskeletal proteins[4, 5]. Recently, many studies have investigated molecular mechanism of the LIUS effect on cells but it is still largely unclear. In the present study, we examined the effect of LIUS on adhesion and proliferation of BM MSCs during the early cultivation period via the colony forming unit-fibroblasts (CFU-Fs) assay. The cellular and molecular changes in cells by LIUS was also investigated.

Materials and Methods: BM MNCs were collected from the aspirates of the femurs of 9-week-old Sprague-Dawley rats (about 250~300g) by flushing several times with 6ml phosphate-buffered saline (PBS). MNCs were suspended in MSC medium consisting of α-MEM supplemented with 10% FBS, and plated with density of 8X104 cells/cm2 in 60 nm culture dish. After 6 days, non-adherent cells were discarded and the adherent cells were replenished with fresh medium after washing. Cells were then culture for about 12 days with the culture medium replaced every 3 days. For LIUS stimulation, cells were seeded at a density of 8X104 cells/cm2 in 60 mm culture dish stimulated with LIUS for 10 min per day over 6 days. LIUS stimulation was performed using Nobelife (Duplogen) at 1 MHz and an intensity of 1, 100 and 200 mW/cm2. Control plates were also treated same way only without applying the LIUS. After LIUS stimulation, cells were cultured for another 6 days, and stained with 5% crystal violet solution in 100% methanol for 10 min for CFU-F assay. Colonies greater than 2 mm in diameter were counted. Western blotting and immunostaining analyses were also performed for FAK, pFAK, actin and paxillin at 12 days.

Results: When first examined at 100 and 200 mW/cm2, LIUS showed positive effect on CFU-F at 100 mW/cm2 but not at 200 mW/cm2, control(Fig.1.A). In an in depth study with more samples, LIUS at 100 mW/cm2 increased both the number and size of colonies by about 1.5 folds, when compared with those of untreated control(Fig.1.B). Immunostaining showed that LIUS at 100 mW/cm2 induced expression of F-actin and the number of focal adhesions by expression level and localization of paxillin. Western blot analysis also showed that LIUS at 100 mW/cm2 clearly induced phosphorylation of FAK and expression of paxillin(Fig.2). In the following studies, LIUS stimulation showed no effect on the surface marker profile and multiple differentiation potential of MSCs such as to osteogenic, adipogenic and chondrogenic lineages (data not shown).

Discussion: This study demonstrated that LIUS stimulation promoted adhesion and colony forming capacity of MSCs during the early stage of primary culture, without affecting their stemness and differentiation capacity. These LIUS activity was probably mediated via its regulation of molecules and signal pathways involved in the cell adhesion and cytoskeletal remodeling such as F-actin, FAK and paxillin. These results suggest that LIUS could be a useful tool to increase the recovery and yield of MSCs in the mixed population of BM MNCs, particularly for clinical application[4, 5].

References:
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