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Original Article

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In vitro effects of dynamic strain on the proliferative and metabolic activity of human osteoblasts

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Abstract

Aim of the study : It has been well shown by human and animal studies that mechanical load is an important regulator of skeletal mass and architecture. However, cellular reactions which adapt bone tissue to the mechanical environment are not definitively determined. For this purpose we studied the cell activity of human bone derived cell cultures after mechanical stimulation by cyclic, uniaxial strain at a magnitude occurring in normal loaded bone tissue. *Materials and Methods :* Human osteoblasts were isolated from cancellous bone biopsies of 5 different donors. Cell seeding was made in DMEM in a density of 10.000 cells/cm^2 on deformable culture dishes for three days prior to initiating cell stretching at 1000 μstrain, 1Hz for 1800 cycles for two subsequent days with an especially developed cell stretching device. 48h after the second stimulation cells were harvested and cell number was determined with a Coulter Counter. Cell bound alkaline phosphatase activity was analyzed in cell lysates by a colorimetric assay, osteocalcin and CICP (procollagen I propeptide) production were analyzed in cell supernatants with ELISAs. Three parallel cultures were tested. Statistics: Wilcoxon. *Results:* In all experiments mechanical stimulation resulted in a significant increase in cell number (10-48%) and CICP release (7-49%). Simultaneously a significant decrease in alkaline phosphatase activity (9-25%) and osteocalcin release (5-32%) could be observed. *Conclusions :* The results demonstrate that cyclic strain at physiologic magnitude leads to an increase of early osteoblast activities related to matrix production while those activities which are characteristic for the differentiated osteoblast and relevant for matrix mineralization are decreased. These new findings confirm *in vivo* observations about the importance of dynamic strain for bone formation during fracture healing and bone remodeling and could contribute to the optimization of fracture healing.

Keywords: Osteoblast, Dynamic Strain, Proliferation, Differentiation

Introduction

The ability of bone tissue to functionally adapt to the mechanical environment depends on the property of bone cells to model and remodel bone architecture in response to mechanical strain. The responsiveness of bone derived cells to mechanical strain has been confirmed by several in vitro investigations[1-8]. In summary the results indicated that mechanical stimuli elicit the proliferation of bone derived cells and it is assumed that under suitable mechanical conditions matrix production by increased number of bone cells and/or matrix synthesis leads to an increased bone mass in response to mechanical loading.

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Structural adaptations provided by modeling and remodeling activities seem to depend on threshold ranges of mechanical usage or corresponding strains or stresses. Frost (1992) has described a window of mechanical usage which is defined by an upper boundary (1500 μstrain), called the minimum effective strain above which bone will undergo modeling and change its structure to reduce the local strains and a lower threshold (50 μstrain) below which bone tissue will be resorbed until the local strains are increased[9]. These specifications are based on strain data of Lanyon et al. (1975) who recorded strains of 400 μstrain during normal walking by fixing rosette strain-gauges to human tibial shafts[10]. During strenuous activities strains may be of the order of more than 3000 μstrain[11].

Previous cell culture experiments clearly demonstrated that dynamic cell stretching at 10.000 μstrain stimulates osteoblast proliferation[7]. In the same study, strain magnitudes between 10,000 and 88,000 μstrain either did not affect or decreased the proliferation of human osteoblasts.

We therefore expect the optimal strain magnitude for cell growth stimulation at even lower levels.

In the present study we investigated whether bone formation by human osteoblasts in vitro is stimulated at low strain levels. For this purpose we measured the effect on cell proliferation by cell counting and on matrix formation by the assessment of collagen type I carboxyterminal propeptide. Matrix maturation was determined by the measurement of alkaline phosphatase activity and mineralisation was assessed by the measurement of osteocalcin release.

Methods

The cell substrate consisted of rectangular, optically clear, elastic culture dishes[7]. For the application of strains at physiological levels monolayer cell cultures on silicone dishes were subjected to cyclic, homogenous stretching by 4-point bending driven in a sinusoidal strain cycle pattern by computer controlled linear actuators[12].

Osteoblast cultures derived from bone samples were taken from the tibia or femur of healthy patients between 28 and 81 years of age undergoing surgery for fracture repair. Bone cell cultures were established as described by Neidlinger-Wilke et al[7].

Cells were seeded at a density of 10,000 cells/cm^2 in 5 ml DMEM supplemented with 10% FCS, penicillin (100 U/ml) and 1% l-glutamine (all compounds from Biochrom, Berlin, Germany) and maintained for 3 days until subconfluent cell density (65-75% confluence) was reached. Then FCS concentration was reduced to 2%. 24 h (= t_0) and 48 h after serum reduction cyclic strain was applied. Unstimulated cultures were used as controls. 48 h after the last stimulation cycle the assessment of biochemical marker was performed. We used respectively 6 culture wells (3 stimulated and 3 controls) for the analysis of the various cell activities.

Proliferation was determined with a Coulter Counter 48 h after the last stimulation cycle. Cell bound alkaline phosphatase (AP) activity was analyzed by a colorimetric assay in cell lysates prepared 48 h after the last cell stimulation cycle. Osteocalcin (OC) concentration was determined in the cell culture supernatants with an ELISA. Collagen type I propeptide (CICP) concentration was determined in cell culture supernatants with an ELISA. The measured AP activity, OC and CICP concentrations were normalized to the cell number of the same culture wells.

Results

The cellular responses were altered in five different cell populations after the application of low level dynamic strain at a frequency of 1 Hz (fig. 1). A significant increase (10-48%) of cell proliferation was found in all cell populations isolated from cancellous bone after strain application at 1000 μstrain compared to unstimulated control cultures (One way-ANOVA, $p < 0.0001$). The same mechanical conditions led to a significant increase of CICP concentration in cell culture supernatants of stretched cell cultures (7-49%) (ANOVA, $p < 0.0001$). In contrast, AP activity (9-25%) and osteocalcin concentration (5-32%) were both significantly reduced (ANOVA: OC: $p < 0.017$; AP: $p < 0.0001$).

Discussion

In our study, cyclic strain at a physiologic magnitude of 1000 μstrain led to an increase of proliferation and early osteoblast activities related to matrix production (CICP). In contrast, activities that are characteristic for the differentiated osteoblast (AP activity) and relevant for matrix mineralization (OC release) were decreased. This phenomenon has also been observed for cell growth, OC release and AP activity by Stanford et al[13]. in the osteoblast-like cell line MC3T3 after stimulation with cyclic stretching[13]. Concurrent with the increased proliferation the release of CICP was stimulated. An increase of collagen type I has also been observed by Jones et al[5]. after stretching bovine osteoblasts with low strains.

Osteoblasts are located on the surface of cancellous and cortical bone on the yet unmineralized cell matrix. Therefore, the deformation of the cell substrate to which these cells adhere would be a proper signal for the stimulation of these cells. This situation was simulated in our experiment by culturing osteoblasts as a monolayer on flexible cell substrate surfaces. The 4-point bending device

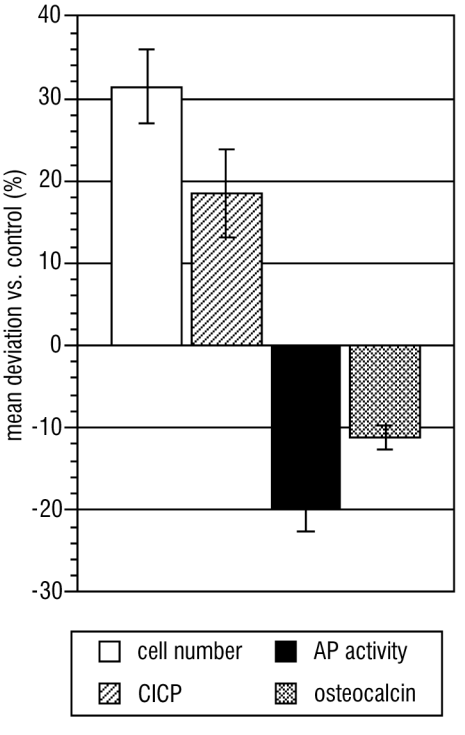

Figure 1. Effects of mechanical strain on cell proliferation, CICP release, alkaline phosphatase activity and osteocalcin synthesis of five different osteoblast populations. Bars represent the mean percentage of change of all five osteoblast populations with respect to the control (=0). The error bars represent the standard error of the mean (SEM) of five osteoblast populations.

produced homogenous, predominantly uniaxial strains of the cell culture substrate such that every cell was subjected to the same deformation. However, we cannot exclude that shear stresses were produced by hydrostatic pressure from the weight of the medium and the inherent forces required to move the medium up and down during mechanical cycling. We cannot exclude the contribution of fluid flow in our system. However, other investigators found effects of mechanical strain on osteoblasts in systems where there is no participation of fluid flow[6,14]. Further it could be shown that shear stresses that occur at 0.25 Hz and 1.2 % axial strain have been shown to be less than 0.2×10^{-6} N/cm^2[2,15]. Effects of fluid flow on cells have only been observed at higher shear stresses[16,17].

A large variability even in control cultures was observed with regard to absolute values as well as differences between stimulated and control cultures. This phenomenon has already been observed previously and may be associated with individual differences between donor patients[7,18]. Even sequential outgrowths from the same explants of the same patients were shown to react with distinct responsiveness to mechanical strain[14]. It is therefore striking in our experiments that the same trends of increasing early osteoblast activities (proliferation and CICP) and decreasing late developmental functions (AP and OC) were observed for all osteoblast populations.

Proliferation, AP, OC and CICP were determined because these markers represent the different stages of osteoblast development. Osteoblasts show a strongly regulated, sequential gene expression pattern which consists of a reciprocal and functionally coupled relationship between proliferation and differentiation[19]. At the transition between proliferation and differentiation, the down regulation of genes for cell proliferation and collagen synthesis coincides with the onset of gene expression for matrix maturation which gives rise to mineralization. Based on the findings of the current study it can be assumed that mechanical strain intensifies the proliferation phase by a prolongation of cell growth stage or by an inhibition of cell differentiation. Similar effects have been shown for TGF-ß that was added to osteoblast cultures during the proliferation stage[20]. TGF-ß has also been shown to be induced in osteoblasts by mechanical signals[21,22] and is therefore suggested to be a regulator of mechanically induced cell reactions. These findings fit well into the concept of bone modeling and remodeling. The reinforcement of the proliferation phase would lead to an increased matrix production that is necessary to functionally adapt bone tissue to mechanical stress. Matrix mineralization, on the other hand, is reduced by mechanical loading and does not recommence until the local strain magnitude is decreased.

Our experiments showed a consistent effect of physiologic mechanical strain on the cell activity of five different human osteoblast populations. This suggests that we have a reliable system to investigate strain influences on bone cell activity. The data presented in this paper show slight differences between osteoblast activities of cells stimulated by cyclic mechanical strain and unstimulated cells. Although modest, these in vitro effects confirm in vivo observations about mechanically influenced bone tissue adaptation.

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