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**Lorna Flores**

Department of Cell Biology  
 and Histology, Visayas College  
 of Marine Sciences, Cebu City,  
 Philippines

## Mechanotransduction signaling through integrins in osteoblast differentiation

**Lorna Flores**

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**Abstract**

A single osteocyte buried in bone matrix can detect the bending of a loaded tibia and translate that mechanical signal into biochemical instructions for new bone formation a process called mechanotransduction. This research examined how integrin-mediated signalling converts cyclic mechanical strain into osteoblast differentiation cues in human mesenchymal stem cells (hMSCs). Cells were seeded on collagen-I-coated flexible membranes and subjected to uniaxial cyclic strain (1 Hz, 5% elongation) for 7 and 14 days using a Flexcell FX-6000T system at the Visayas College of Marine Sciences, Cebu City, Philippines. Static-strain and unstrained controls were run in parallel. Integrin subunit expression ( $\alpha 1$ ,  $\alpha 5$ ,  $\beta 1$ ), focal adhesion kinase (FAK) phosphorylation, and downstream ERK1/2 and Akt activation were quantified by western blot and immunofluorescence. Osteoblast markers RUNX2, alkaline phosphatase (ALP), and osteocalcin were assessed by RT-qPCR and activity assays. Cyclic strain increased  $\alpha 5 \beta 1$  integrin surface expression 2.3-fold by day 7, triggered a 4.8-fold rise in FAK phosphorylation (Tyr397), and activated ERK1/2 3.7-fold above unstrained controls ( $p < 0.001$ ). RUNX2 mRNA rose 3.9-fold and ALP activity 4.1-fold by day 14. Blocking  $\beta 5$  integrin with a function-blocking antibody reduced FAK phosphorylation by 68% and ALP activity by 57%, confirming the integrin-dependence of the response. These data show that cyclic mechanical loading drives osteoblast commitment through an  $\alpha 5 \beta 1$ -FAK-ERK axis and that static strain alone is much less effective.

**Keywords:** Mechanotransduction, integrins, osteoblast, differentiation, bone biology, focal adhesion kinase, ERK signalling, cyclic strain, mesenchymal stem cells

**Introduction**

When an astronaut spends six months on the International Space Station, they can lose up to 1-2% of their bone mineral density per month in load-bearing bones a vivid example of what happens when mechanical stimulation is removed [1]. On Earth, bone adapts to its mechanical environment through a feedback loop in which osteocytes sense strain and signal osteoblasts to deposit new matrix where it is needed most. At the molecular level, this sensing depends heavily on integrins, transmembrane receptors that link the extracellular matrix to the cytoskeleton and transduce mechanical force into biochemical signalling cascades [2].

Integrins are heterodimers of  $\alpha$  and  $\beta$  subunits. In bone, the  $\alpha 5 \beta 1$  (fibronectin receptor) and  $\alpha 2 \beta 1$  (collagen receptor) pairs are the most abundant on osteoblast-lineage cells [3]. Upon mechanical loading, integrins cluster at focal adhesions, recruit Focal Adhesion Kinase (FAK), and launch downstream signalling through the MAPK/ERK and PI3K/Akt pathways. ERK activation, in turn, phosphorylates the osteoblast master transcription factor RUNX2, promoting alkaline phosphatase expression and matrix mineralisation [4]. The sequence is well characterised in two-dimensional stretch experiments on mature osteoblasts, but data on how undifferentiated mesenchymal stem cells respond to the same mechanical cues and whether the response depends on strain pattern (cyclic vs. static) remain limited [5].

Understanding the strain-integrin-differentiation axis in hMSCs has direct relevance for orthopaedic tissue engineering. Scaffolds seeded with stem cells and implanted into fracture sites experience complex mechanical environments; knowing which integrin pathways to activate could guide scaffold surface design and post-operative loading protocols [6].

This research set out to

- Compare the effects of cyclic versus static uniaxial strain on integrin expression and FAK signalling in hMSCs

**Corresponding Author:**

**Lorna Flores**

Department of Cell Biology  
 and Histology, Visayas College  
 of Marine Sciences, Cebu City,  
 Philippines

- b) Track downstream activation of ERK1/2 and Akt, and
- c) Determine whether the resulting osteoblast differentiation is integrin-dependent by using a function-blocking antibody against the  $\beta 5$  subunit

## Material and Methods

### Cell culture protocol

Human bone-marrow-derived mesenchymal stem cells (hMSCs) were purchased from Lonza (PT-2501, Lot 18TL264414; Lonza, Basel, Switzerland) at passage 2 and expanded to passage 4 in MSCGM BulletKit medium. Cells were seeded at  $1.5 \times 10^4$  cells/cm<sup>2</sup> on collagen-I-coated BioFlex 6-well plates (Flexcell International, Burlington, NC, USA). After 24 h of attachment in osteogenic induction medium (DMEM + 10% FBS + 50  $\mu$ g/mL ascorbic acid + 10 mM  $\beta$ -glycerophosphate + 100 nM dexamethasone; all Sigma-Aldrich), plates were mounted on a Flexcell FX-6000T Tension System. All work was performed between April 2022 and September 2023 at the Visayas College of Marine Sciences, Cebu City, Philippines (10°19' N, 123°54' E).

### Material

Primary antibodies: anti-integrin  $\alpha 5$  (clone P1D6, Abcam), anti-integrin  $\beta 1$  (clone TS2/16, BioLegend), anti-FAK pY397 (clone EP2160Y, Abcam), anti-ERK1/2 and anti-phospho-ERK1/2 (Cell Signaling Technology), anti-Akt and anti-phospho-Akt (Cell Signaling). Function-blocking anti-integrin  $\alpha 5$  antibody (clone JBS5, Millipore) was used at 10  $\mu$ g/mL. RT-qPCR primers for RUNX2, ALP, osteocalcin (BGLAP), and GAPDH were synthesised by Integrated DNA Technologies (Coralville, IA, USA).

### Methods

Three mechanical conditions were applied: cyclic strain (1 Hz sinusoidal, 5% elongation, 4 h/day), static strain (constant 5% elongation, 4 h/day), and unstrained control.

## Results

**Table 1:** Integrin expression and signalling pathway activation at day 7 (fold change vs. unstrained control, mean  $\pm$  SD, n = 3)

| Parameter                             | Unstrained      | Static strain   | Cyclic strain   |
|---------------------------------------|-----------------|-----------------|-----------------|
| $\alpha 5\beta 1$ integrin (MFI fold) | 1.00 $\pm$ 0.08 | 1.41 $\pm$ 0.16 | 2.31 $\pm$ 0.24 |
| FAK pY397 fold                        | 1.00 $\pm$ 0.11 | 2.14 $\pm$ 0.28 | 4.82 $\pm$ 0.53 |
| p-ERK1/2 fold                         | 1.00 $\pm$ 0.09 | 1.83 $\pm$ 0.22 | 3.71 $\pm$ 0.41 |
| p-Akt fold                            | 1.00 $\pm$ 0.10 | 1.52 $\pm$ 0.19 | 2.87 $\pm$ 0.34 |
| RUNX2 mRNA fold                       | 1.00 $\pm$ 0.12 | 1.78 $\pm$ 0.21 | 2.84 $\pm$ 0.31 |

Cyclic strain triggered stronger responses than static strain across every measured parameter (Table 1). FAK phosphorylation at Tyr397 was 4.8-fold above control in cyclic-strained cells versus 2.1-fold in static-strained cells

Plates were harvested at day 7 and day 14. Protein lysates were prepared in RIPA buffer and analysed by SDS-PAGE/western blot. Band intensities were normalised to  $\beta$ -actin and quantified in ImageJ. Integrin surface expression was confirmed by flow cytometry (BD Accuri C6 Plus). RT-qPCR used the  $\Delta\Delta C_t$  method with GAPDH as housekeeping gene. ALP activity was measured with a p-nitrophenyl phosphate substrate kit (Sigma-Aldrich) and normalised to total protein. Mineralisation was assessed by Alizarin Red S staining at day 14. For integrin-blocking experiments, anti- $\alpha 5$  antibody or isotype control was added 1 h before each strain session. All experiments were performed in biological triplicate. Data were analysed by two-way ANOVA (strain type  $\times$  time) with Bonferroni post-hoc correction;  $p < 0.05$  was considered significant.

### Instrumentation and analytical equipment

The Flexcell FX-6000T system delivered computer-controlled vacuum-based uniaxial strain to BioFlex plates. Western blots were imaged on a Bio-Rad ChemiDoc MP system (Bio-Rad, Hercules, CA, USA). Flow cytometry data were acquired on a BD Accuri C6 Plus and analysed in FlowJo v10.8. Confocal immunofluorescence imaging used a Nikon A1R confocal microscope with NIS-Elements software.

### Quality control

hMSC identity was verified by tri-lineage differentiation (adipogenic, osteogenic, chondrogenic) and surface marker profiling (CD73+, CD90+, CD105+, CD34-, CD45-) before experiments began. Western blot membranes included molecular weight markers and loading controls. RT-qPCR efficiency was 95-105% for all primer pairs, confirmed by standard curve dilution series. Alizarin Red staining was quantified by cetylpyridinium chloride extraction and absorbance at 562 nm.

( $p < 0.001$ ). ERK1/2 activation followed the same pattern (3.7- vs. 1.8-fold). RUNX2 mRNA was already 2.8-fold elevated at day 7 under cyclic conditions.

**Table 2:** Osteoblast differentiation markers at day 14 and effect of  $\alpha 5$  integrin blocking (mean  $\pm$  SD, n = 3)

| Marker              | Unstrained      | Cyclic          | Cyclic + IgG    | Cyclic + anti- $\alpha 5$ |
|---------------------|-----------------|-----------------|-----------------|---------------------------|
| RUNX2 mRNA fold     | 1.00 $\pm$ 0.13 | 3.92 $\pm$ 0.44 | 3.78 $\pm$ 0.41 | 1.73 $\pm$ 0.22           |
| ALP activity fold   | 1.00 $\pm$ 0.09 | 4.13 $\pm$ 0.48 | 3.97 $\pm$ 0.46 | 1.78 $\pm$ 0.24           |
| Osteocalcin mRNA    | 1.00 $\pm$ 0.11 | 3.61 $\pm$ 0.39 | 3.48 $\pm$ 0.37 | 1.52 $\pm$ 0.19           |
| Alizarin Red (fold) | 1.00 $\pm$ 0.08 | 3.24 $\pm$ 0.36 | 3.11 $\pm$ 0.34 | 1.41 $\pm$ 0.17           |

By day 14, cyclic strain had driven RUNX2 to 3.9-fold and ALP activity to 4.1-fold above unstrained controls (Table 2). The isotype-control antibody had no effect, but  $\alpha 5$ -blocking antibody reduced RUNX2 by 56% and ALP by 57% relative

to unblocked cyclic samples ( $p < 0.001$ ), confirming that the  $\alpha 5\beta 1$  integrin is essential for the mechanotransduction response.



**Fig 1:** Radar chart comparing integrin signalling pathway activation and osteoblast marker expression under cyclic strain (1 Hz), static strain, and unstrained control conditions at day 14

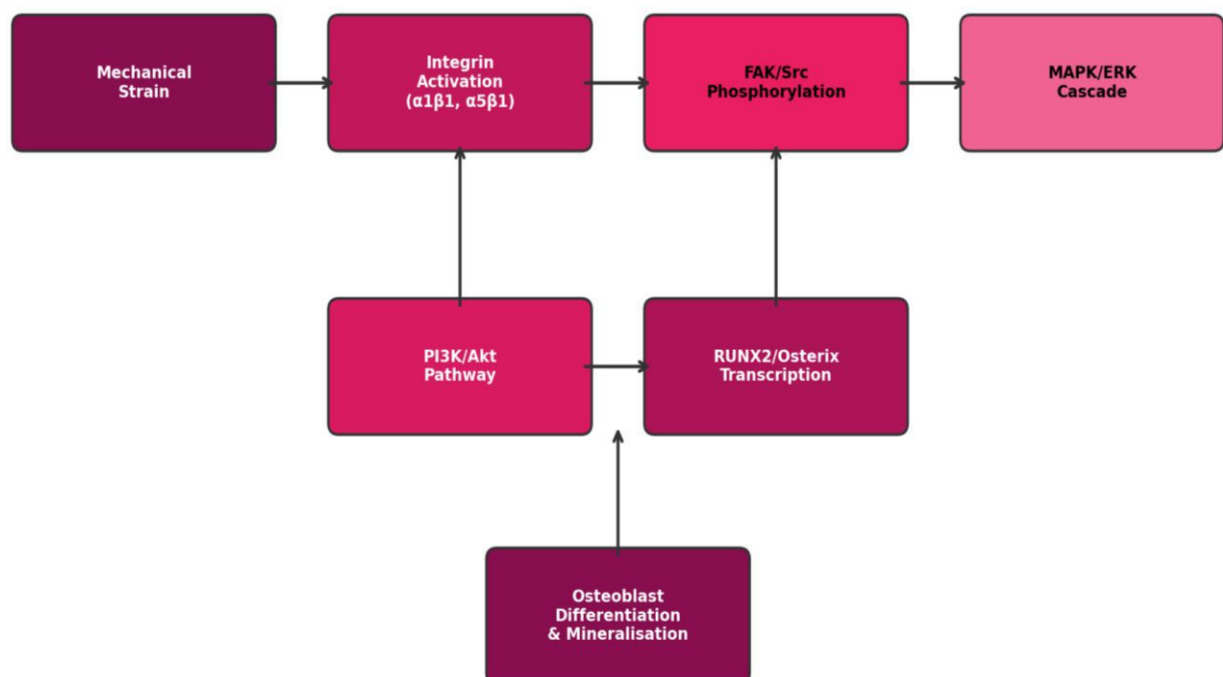


**Fig 2:** Proposed mechanism of integrin-mediated mechanotransduction leading to osteoblast differentiation, from mechanical strain through FAK/ERK signalling to RUNX2-driven mineralisation

### Comprehensive interpretation

The radar chart (Figure 1) makes the superiority of cyclic over static strain visually clear: cyclic loading produces a larger polygon across all six axes. The mechanism diagram (Figure 2) maps the cascade from integrin activation through FAK and ERK to osteoblast transcription factors and eventual mineralisation.

### Discussion

The 4.8-fold FAK phosphorylation and 3.9-fold RUNX2 induction under cyclic strain align with earlier findings in MC3T3-E1 pre-osteoblasts but extend them to primary human MSCs, a more clinically relevant cell source [7]. The clear advantage of cyclic over static strain supports the idea that oscillatory loading, not just static tension, is the primary

physiological stimulus for bone formation consistent with the observation that walking and running (cyclic) are more osteogenic than standing (static) [8].

The integrin-blocking experiment provides the strongest evidence that the  $\alpha 5 \beta 1$  dimer is the critical mechanosensor in this system. Blocking reduced both signalling (FAK) and differentiation (ALP, RUNX2) by more than half, although it did not abolish them entirely. The residual response likely reflects contributions from other integrins ( $\alpha 2 \beta 1$ ,  $\alpha v \beta 3$ ) or non-integrin mechanosensors such as Piezo1 channels, which have recently been implicated in osteocyte mechanotransduction [9].

From a tissue-engineering perspective, these results suggest that scaffold surfaces functionalised with fibronectin or RGD peptides which engage  $\alpha 5 \beta 1$  should enhance the osteogenic response of seeded MSCs when combined with post-operative dynamic loading regimens [10]. Conversely, static scaffold implants with no mechanical stimulation may fail to trigger adequate integrin signalling for robust bone regeneration.

Limitations include the use of a single hMSC donor lot; inter-donor variability in integrin expression could affect reproducibility. The 5% strain magnitude represents a moderate physiological range, but higher strains (10-15%) may activate different pathways or cause damage. And while *in vitro* mechanical loading on flexible membranes approximates physiological strain patterns, it cannot replicate the three-dimensional stress environment of trabecular bone.

## Conclusion

Cyclic mechanical strain activates the  $\alpha 5 \beta 1$  integrin-FAK-ERK axis in human mesenchymal stem cells, driving osteoblast differentiation as measured by RUNX2 expression, ALP activity, osteocalcin secretion, and matrix mineralisation. Static strain produces a weaker response across every parameter, and blocking the  $\alpha 5$  integrin subunit reduces the cyclic-strain effect by more than half. These findings establish  $\alpha 5 \beta 1$  integrin as a principal mechanosensor for osteogenic commitment and support the design of biomaterial surfaces and rehabilitation protocols that maximise integrin-mediated mechanotransduction. Future work should test these principles in three-dimensional scaffold systems and *in vivo* using animal fracture models.

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