# The Effect of Transforming Growth Factor Beta (TGF-β3), HCl and BSA/HCl on Trypsinisation of Bone Cells Monolayer

F. Sefat, M. Youseffi, R.F. Berends, S.A. Khaghani, and M.C.T Denyer

***Abstract*- In order to investigate wound healing in bone it is essential to know the process of bone cell attachment and detachment to the surface. Cells are not found in isolation and they usually adhere to other cells or surrounding extracellular (ECM) environment in vivo and substrate or a surface in vitro. Trypsinisation was carried out in order to investigate its effect on cell detachment, in the presence of TGF-β3, HCl or BSA/HCl solutions. Trypsin was therefore added to four groups of bone cells with addition of TGF-β3, HCl, HCl/BSA solutions and additional flask as control. These results further confirmed that application of TGF-β3, HCl and HCl/BSA at 50ng/ml decreased the degree of cell attachment on surface of culture flasks.**

**Cell detachment in control is about 43% after 6 minutes, which is slow. Bone cells in presence of BSA/HCl have start detaching from the surface faster than control (about 4-5 minutes after applying trypsin). Cell detachment is about 63% after 6 minutes which is faster as compared to the control. Bone cells in presence of HCl alone have start detaching from the surface faster than control and BSA/HCl (about 2 minutes after applying trypsin). Cell detachment is about 69% after 6 minutes which is faster compared to the BSA/HCl and control.**

**Trypsinisation experiments for bone cells cultured with TGF-β3 shows that cells started to detach from the surface about 1 minute after application of trypsin and were completely detached by the third minute. Cell detachment is about 85% after 4 minutes, which is faster as compared to the control, HCl and BSA/HCl. Trypsinisation results indicated that application of TGF-β3 at 50ng/ml decreased the degree of cell attachment.**

***Index Terms*: Bone cells monolayer, BSA/HCl, HCl, TFG-β3, Trypsinisation**

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## I. Introduction

Cells are not found in isolation and they usually adhere to other cells or surrounding extracellular matrix (ECM) environment in vivo and substrate or a surface in vitro [1,2].

The effect of TGF-β3 is mediated by a range of signalling pathways. The interaction of bone cells with their surrounding ECM environment influence some physiological function and pathological processes [3]. These interactions are mediated by integrins. Integrins are capable of transducing the signals from ECM to the cells in which results in migration, differentiation and specific protein synthesis. To determine which integrins are involved flow cytometric analysis and immunoprecipitation need to be carried out.

## II. Aims and objectives

The aim of this study was to investigate the effect of TGF-β3, HCl and BSA/HCL on bone cell detachment via Trypsinization process.

## III. Materials and Methods

Trypsinisation was carried out to investigate the effect of TGF-β3 on cell detachment. To establish the appropriate dilution at which to plate cells, a 1 in 3, 1 in 6 and 1 in 12 dilution was plated into the three rows of a 12 well plate. For TGF-β3 to have sufficient time to influence cells in culture, cells were grown for at least 2 days prior to the attachment assay. The 1 in 3 dilution was confluent by day 3 and was therefore chosen for this assay.

In order to reconstitute the vile containing TGFβ3, a solution of HCl (4mM), BSA (1mg/ml) and Distilled water was prepared.

Trypsin was added to four groups of cultured bone cells with four different solutions including TGF-β3, HCl, HCl/BSA solution and bone cell only as control to study the effect of these solutions on cell detachment. HCl and HCL/BSA solutions were used, as they are carrier for TGF-β3.

Bone cells were cultured in a 12 well petridishes and left for 3 days to become confluent with three different cell dilution. Three wells were labeled as A1-A3 on the left of culture dish was seeded with 1:3 ratio of cell to DMEM

known as control. Three wells were labeled as B1-B3 seeded with 1:3 ratio cell and DMEM with addition of 50ng/ml BSA/HCl.

Another three wells were labeled as C1-C3 seeded with 1:3 ratio cell and DMEM with addition of 50ng/ml HCl. The other wells were labeled as D1-D3 on the right of culture dish which was seeded with 1:3 ratio cell and DMEM with addition of 50ng/ml TGF-β3. Cells were checked after 3 days to observe their confluency. Cells were imaged every 20 second for duration of 15 minutes (45 frames in total). The 12 well cultured dishes were placed under microscope.

Old media aspirated and cells were washed with Hank's balanced salt solution (HBSS) and microscope was focused. Trypsin (0.5ml) was added and recording was carried out for 15 minute for duration of 20 second each.

This method was repeated for groups A, B, C and D. The speed of cells detaching from surface is important and it is possible to find out which group (A, B, C or D) detach faster. Figure 1 shows the schematic drawing of the 12 well.

Trypsin was added to three groups of cultured bone cells with four different solutions including TGF-β3, HCl, HCl/BSA solution and bone cell only as control to study the effect of these solutions on cell detachment. HCl and HCl/BSA solutions were used, as they are carrier for TGF-β3.

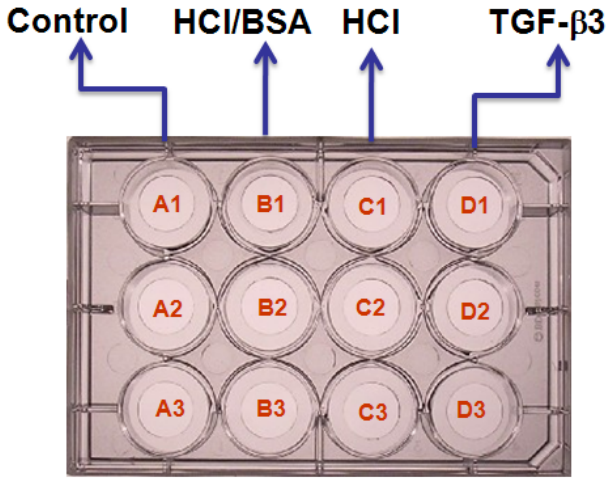


**Figure 1. 12well culture dish for trypsinisation with four different cell dilutions**

## IV. Results and Discussion

Figure 2a is the control trypsinisation process for the period of 14 minutes, showing that cells started detaching from the surface about 6 minutes after applying trypsin. Cell detachment about 43% was after 6 minutes, which is slow. It became clear that cells detached very slowly for the control.

Figure 2b shows the trypsinisation process of bone cell line in presence of BSA/HCl. Cells started detaching from the surface faster than control about 4-5 minutes after applying trypsin. Cell detachment was about 63% after 6 minutes which was faster as control.

Figure 2c shows the trypsinisation experiments for bone cells cultured with HCl alone which shows that cells started to detach from the surface about 2 minutes after application of trypsin. Cell detachment is about 69% after 6 minutes which was faster as compared to the BSA/HCl and control. Cell detachment was about 85% after 4 minutes, which is faster as compared to the control and BSA/HCl.

Figure 2d shows the trypsinisation experiments for bone cells cultured with TGF-β3 which shows that cells started to detach from the surface about 1 minute after application of trypsin and were completely detached by the third minute.

For comparison, as shown in Figures 3a and 3b, a completely different response was recorded with the bone cells plated without TGF-β3, and that the rate of detachment was much slower in control even after 6-8 minutes. These results further confirmed that application of TGF-β3 at 50ng/ml decreased the degree of cell attachment on surface.

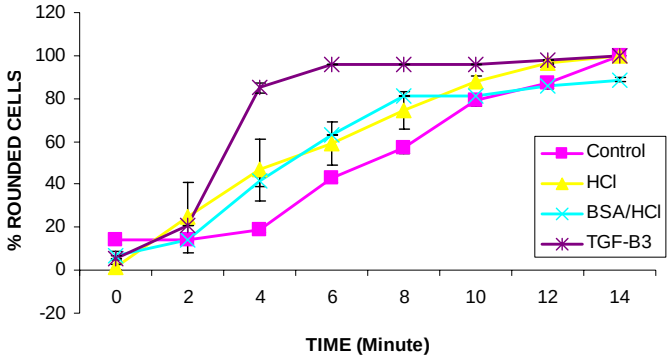


**Figure 3a. Comparison between percentages rounded cells during trypsinisation process for control, HCl, BSA/HCl and TGF-β3 additions.**

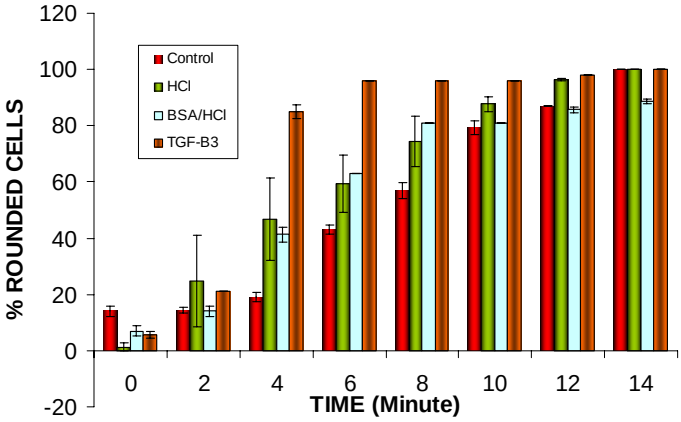


**Figure 3b. Comparison between percentages rounded cells during trypsinisation process for control, HCl, BSA/HCl and TGF-β3 additions.**

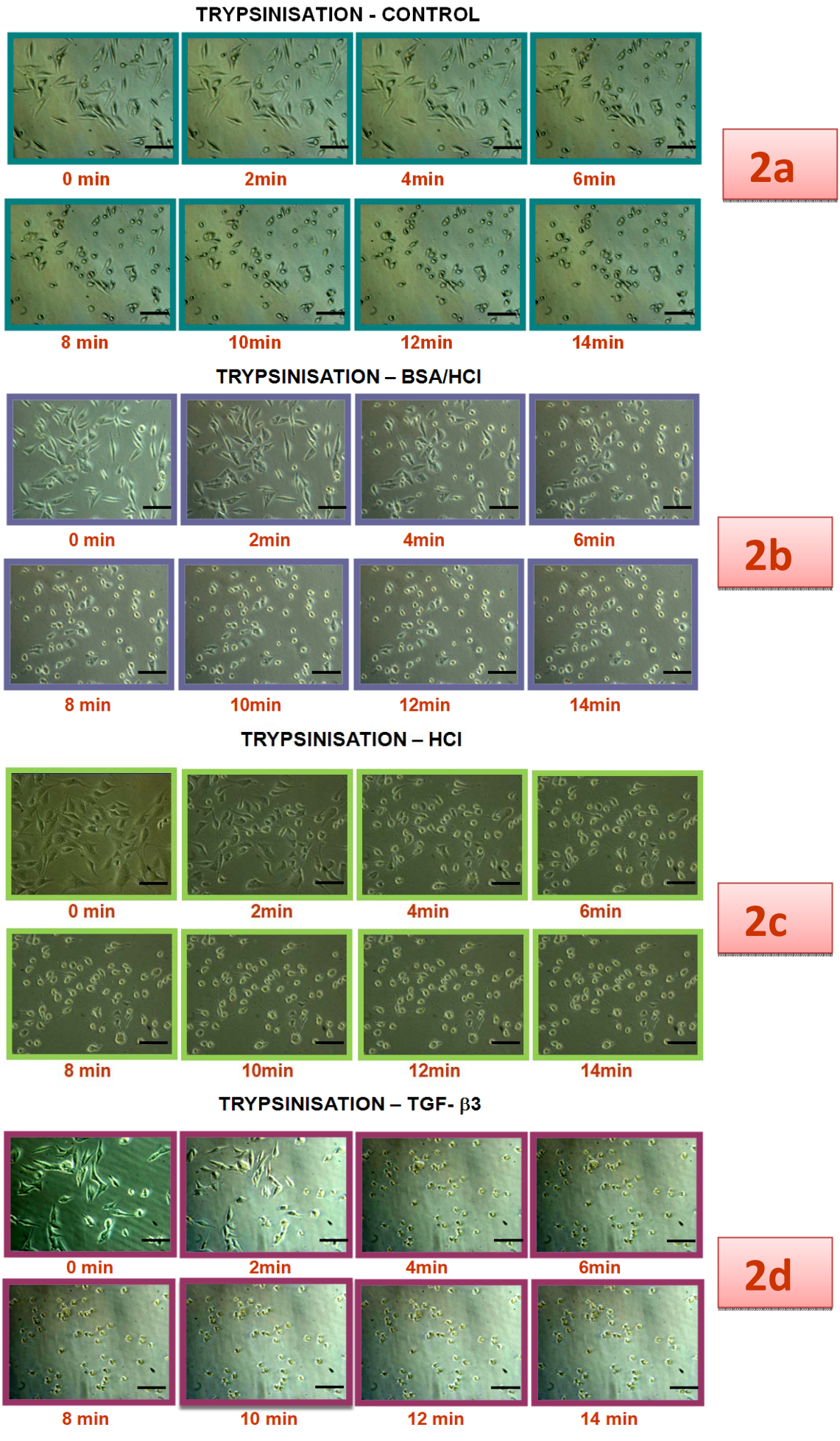


**Figure 2. Trypsinisation process; (a) Control, (b) BSA/HCl, (c) HCl and (d) TGF-β3; (Scale bar=100 μm).**

## V. STATISTICAL ANALYSIS

HCl and HCl/BSA showed similar results with significant differences ($P < 0.05$) compared to the control. TGF-β3 showed significant difference ($P < 0.05$) compared to the other treatments. Multiple comparisons with respect to different treatments and at each time point were performed.

Comparative results were given as means ±SE. The significant values of the difference were tested by one-way ANOVA followed by the Bonferroni adjustment.

TGF-β3 appeared to detach faster with more stimulatory action than HCl and HCl/BSA. TGF-β3, HCl and HCl/BSA are significantly different ($P < 0.05$) compared to the control. Error bars = 95% CI.

## VI. CONCLUSIONS

Trypsinisation was carried out in order to investigate its effect on cell detachment, in the presence of TGF-β3, HCl, BSA/HCl and control solutions. Trypsin was therefore added to four groups of bone cells with addition of TGF-β3, HCl, HCl/BSA solutions and also control. These results further confirmed that application of TGF-β3 at 50ng/ml decreased the degree of cell attachment on surface of culture flasks.

In conclusion, TGF-β3, HCl and HCl/BSA enhanced the rate of cell detachment in relation to the negative controls, indicating perhaps that TGF-β3 does not act alone in the trypsinisation, but instead functions synergistically with signalling pathways that are dependent on the availability of hydrogen ions. Such a mechanism would depend on signalling molecules undergoing a conformational change on binding hydrogen ions, which is not a new concept.

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