Chapter 3

Results

1. Study model development

1.1. Viability of cells on titanium disks

The growth curve of MC3T3-E1 cells on titanium disks cultivated in complete culture medium demonstrated three phases of cell growth, *static*, *log* and *plateau* phases. The *static* phase is in between cell seeding and culture-day 3, *log* phase: culture-days 3 – 9 and *plateau* phase: culture-days 9 - 15. Viability of cells on culture-day 9 was significantly higher than on culture-day 3 ($p<0.01$). The pattern of cell growth on a titanium disk was comparable to the pattern of a cell culture plate (Fig.17).

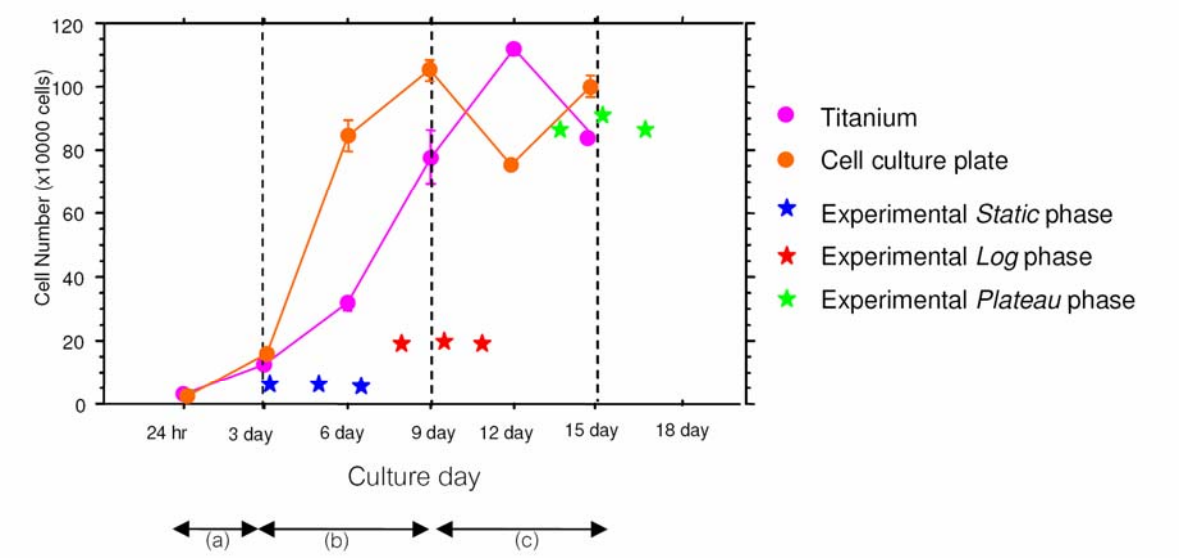

Fig. 17 Growth of MC3T3-E1 on ● titanium disk and ●cell culture plate; growth of cells in (a) *static*, (b) *log* and (c) *plateau* phases; levels of growth in a serum free culture medium without treatment in experimental ★ *static*,★ *log* and ★ *plateau* phases

1.2. Serum free culture medium condition

MC3T3-E1 cells wereable to grow on a titanium surface in a serum free culture medium in any experimental phase for at least 7 days, as it can be seen that growth of cells was stable during 1 – 6 days after cell seeding. On day 15 the growth of cells was at the lowest level and significantly less than growth of cells on day 6 ($p<0.05$) (Fig. 18). Additionally, in all the experimental phases of *static*, *log* and *plateau* phases, the growth of cells in a control group (Group E: serum free culture medium without treated NSAIDs) and growth of cells on supplementation-days 1 and 5, was not significantly different ($P>0.05$) (Fig. 27 – 29).

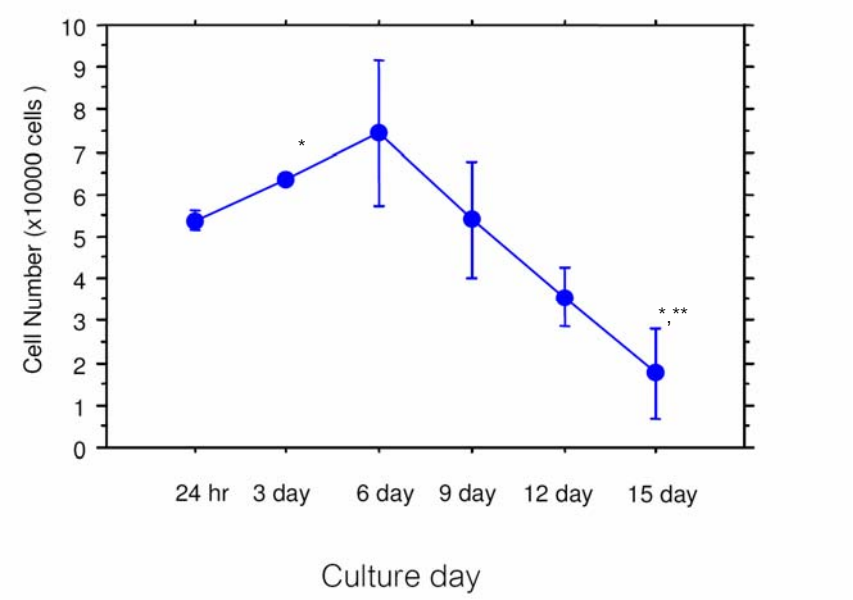

Fig. 18 Growth of MC3T3-E1 on a titanium disk cultivated in serum free culture medium for 15 days; * = $p<0.05$ from 24 hr, ** = $p<0.05$ from 6 day

Growth of cells in serum free culture medium during medication treatment for 1 – 5 days in each experimental phase, *static*, *log* and *plateau* phases, were monitored in comparison to growth of cells cultivated in culture medium supplemented with bovine serum. It was found that growth of cells in serum free culture medium was significantly less than cells cultivated in culture medium with serum ($p<0.05$), exceptionally on treatment day 5 in the *plateau* phase ($p>0.05$). Growth of cells on treatment days 1 and 5 was not significantly different ($p>0.05$) (Fig.19).

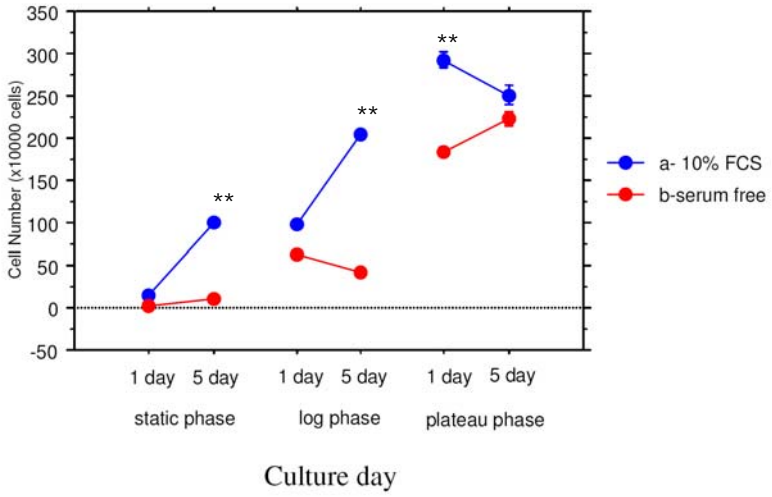

Fig. 19 Comparisons of growth of MC3T3-E1 on a titanium disk cultivated in experimental *static*, *log* and *plateau* phases in ● 10% FBS and ● serum free culture medium; * = $p<0.05$, ** = $p<0.01$

2. Effects of fetal bovine serum in culture medium on attachment and morphology of cells on titanium disks

Cells were able to grow and proliferate on the surface of titanium disks. Osteoblasts were polygonal in shape with round shape nucleus and multiple extending filopodias. Cells sprout their cytoplasmic processes on the titanium surface and secreted extracellular matrix to establish intercellular contact and form cell nodules. Cytoplasmic processes had numeral filopodia extending in multiple directions (Fig.20-25). A deposition of extracellular matrix and growth of cells in multiple cell layers on the titanium surface can be observed in the *log* and *plateau* phases (Fig.22-25).

It was demonstrated that size of cells increased with stages of cell growth or experimental phases, *static*, *log* and *plateau* phases (Fig.20-25). Scanning electron micrographs (SEM) demonstrated superior cell attachment and growth on the titanium surface of cells cultivated in culture medium with serum. Size of cells and spreading of cell cytoplasm and filopodias were markedly greater in culture medium with serum than serum free culture medium. It can be noticed that adverse effects of serum free culture medium on cell growth and attachment were greatest on cells in the *static* phase, followed by cells in *log* and *plateau* phases, repectively. Attachment and morphology of cells on treatment days 1 and 5 were not markedly different (Fig.20-25).

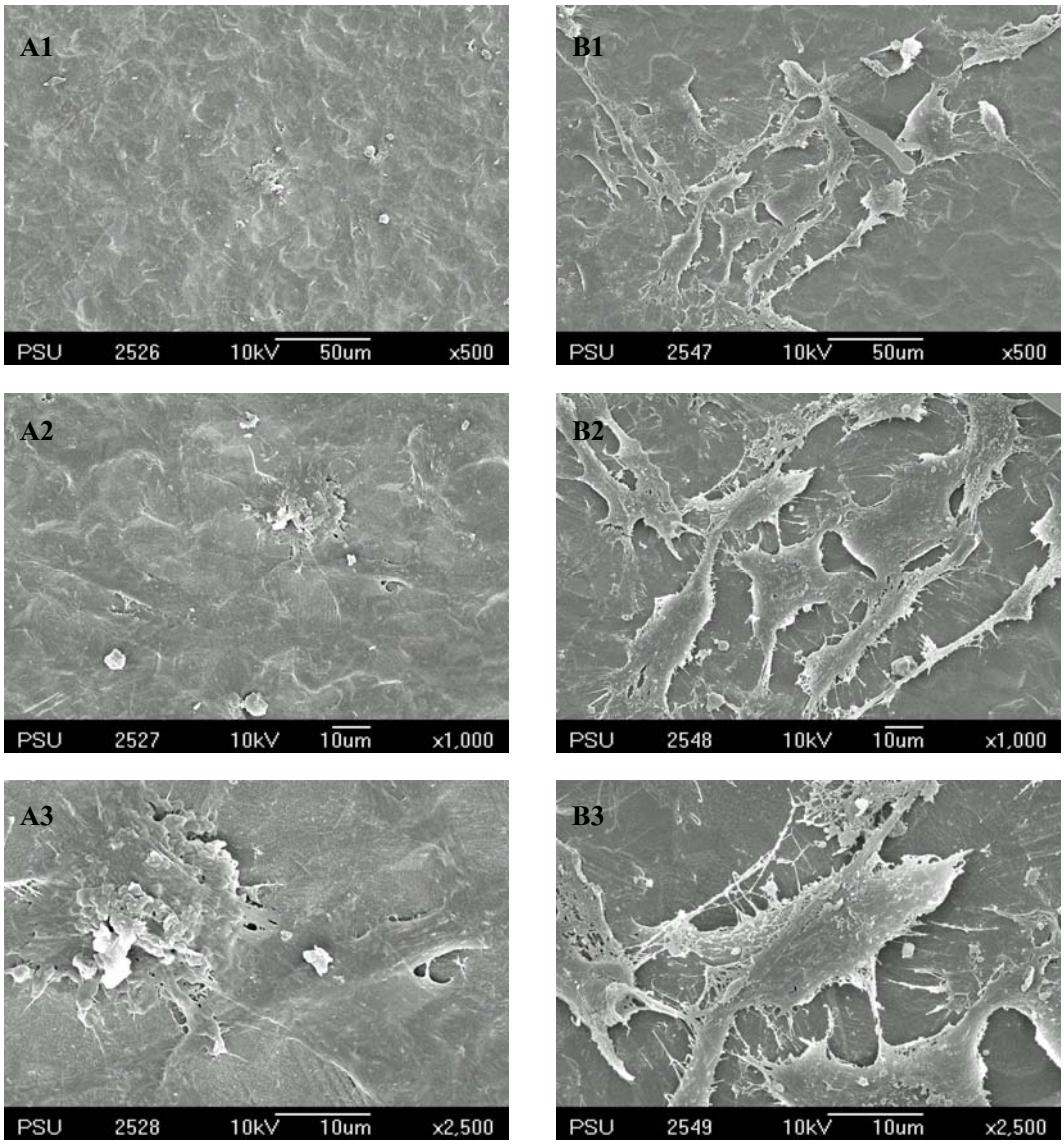

Fig. 20 SEM images of MC3T3-E1 cells on the surface of titanium disks in experimental *static* phase on **treatment day 1** (A) in culture medium with serum and (B) in serum free culture medium. Original magnifications (1) x500, (2) x1000 and (3) x2500

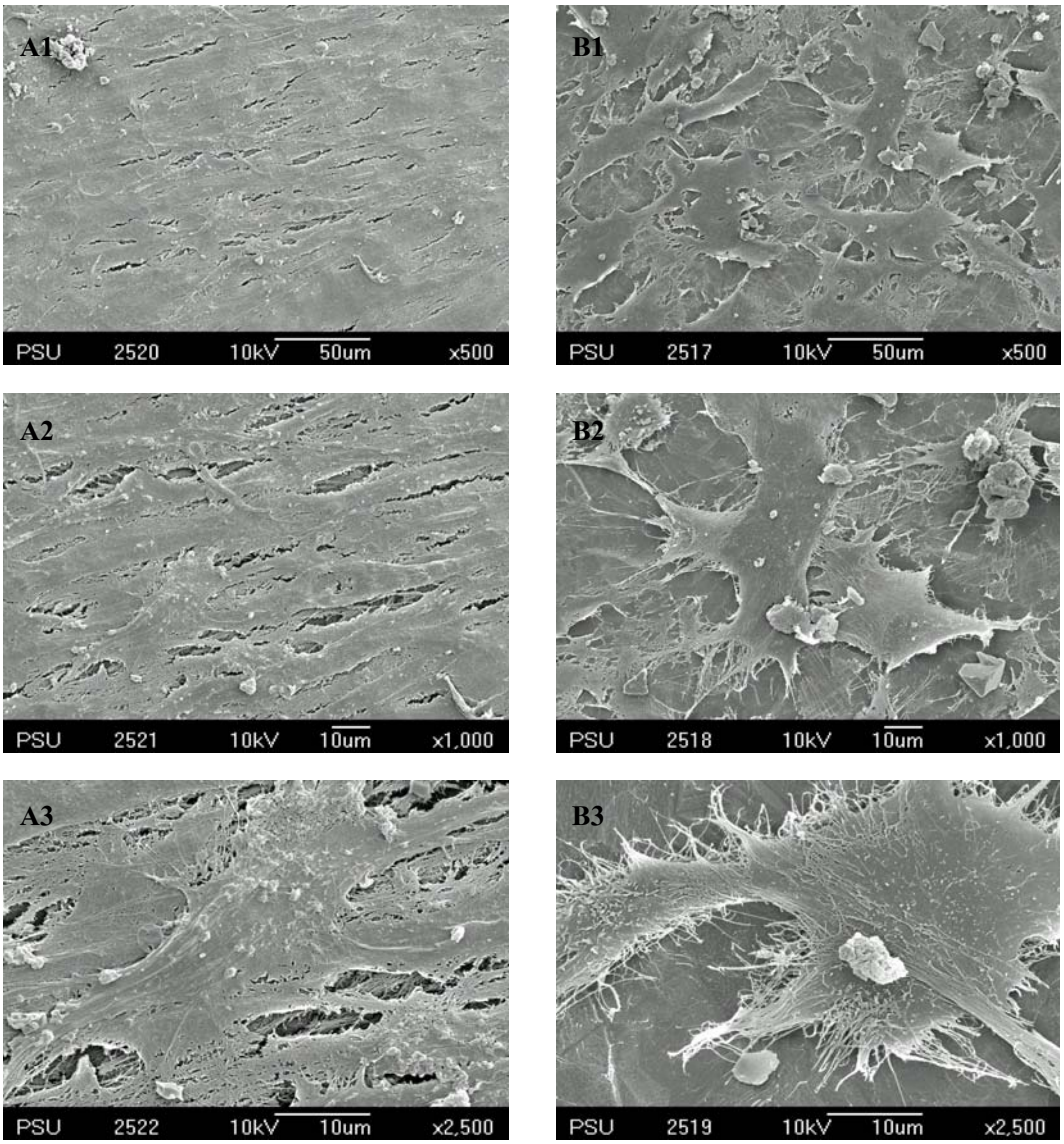

Fig. 21 SEM images of MC3T3-E1 cells on the surface of titanium disks in experimental *static* phase on **treatment day 5** (A) in culture medium with serum and (B) in serum free culture medium. Original magnifications (1) x500, (2) x1000 and (3) x2500

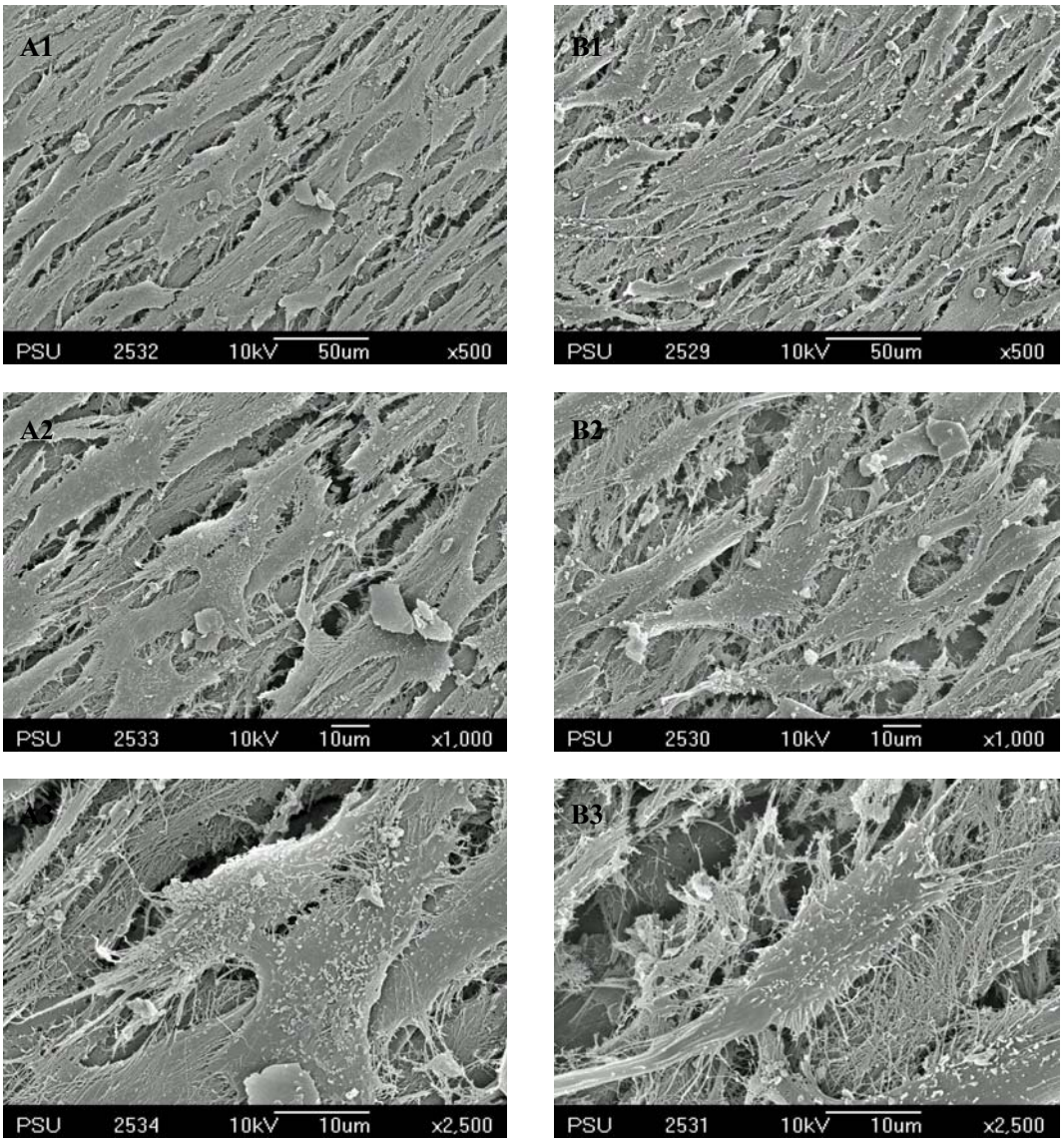

Fig. 22 SEM images of MC3T3-E1 cells on the surface of titanium disks in experimental *log* phase on **treatment day 1** (A) in culture medium with serum and (B) in serum free culture medium. Original magnifications (1) x500, (2) x1000 and (3) x2500

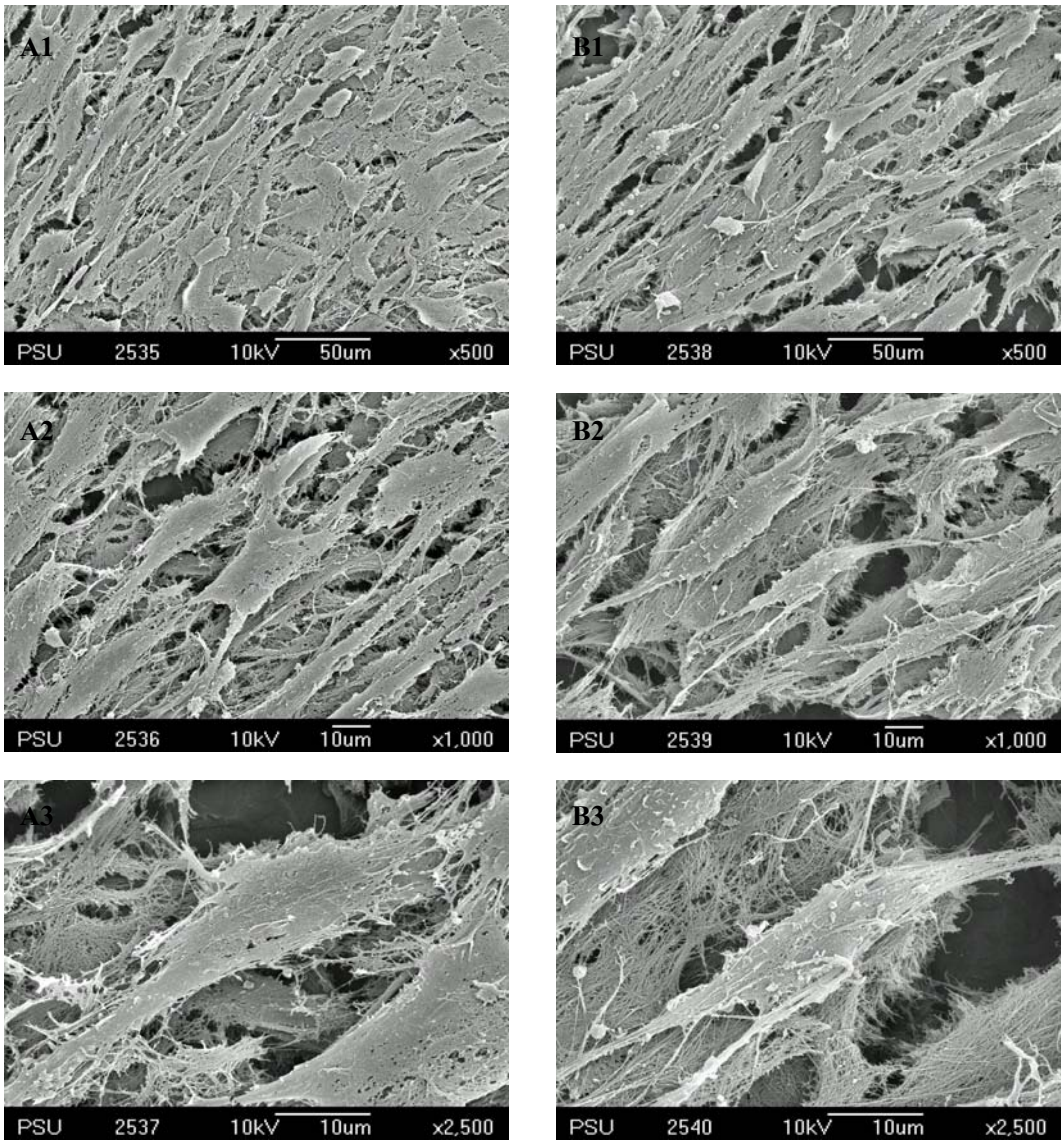

Fig. 23 SEM images of MC3T3-E1 cells on the surface of titanium disks in experimental *log* phase on **treatment day 5** (A) in culture medium with serum and (B) in serum free culture medium. Original magnifications (1) x500, (2) x1000 and (3) x2500

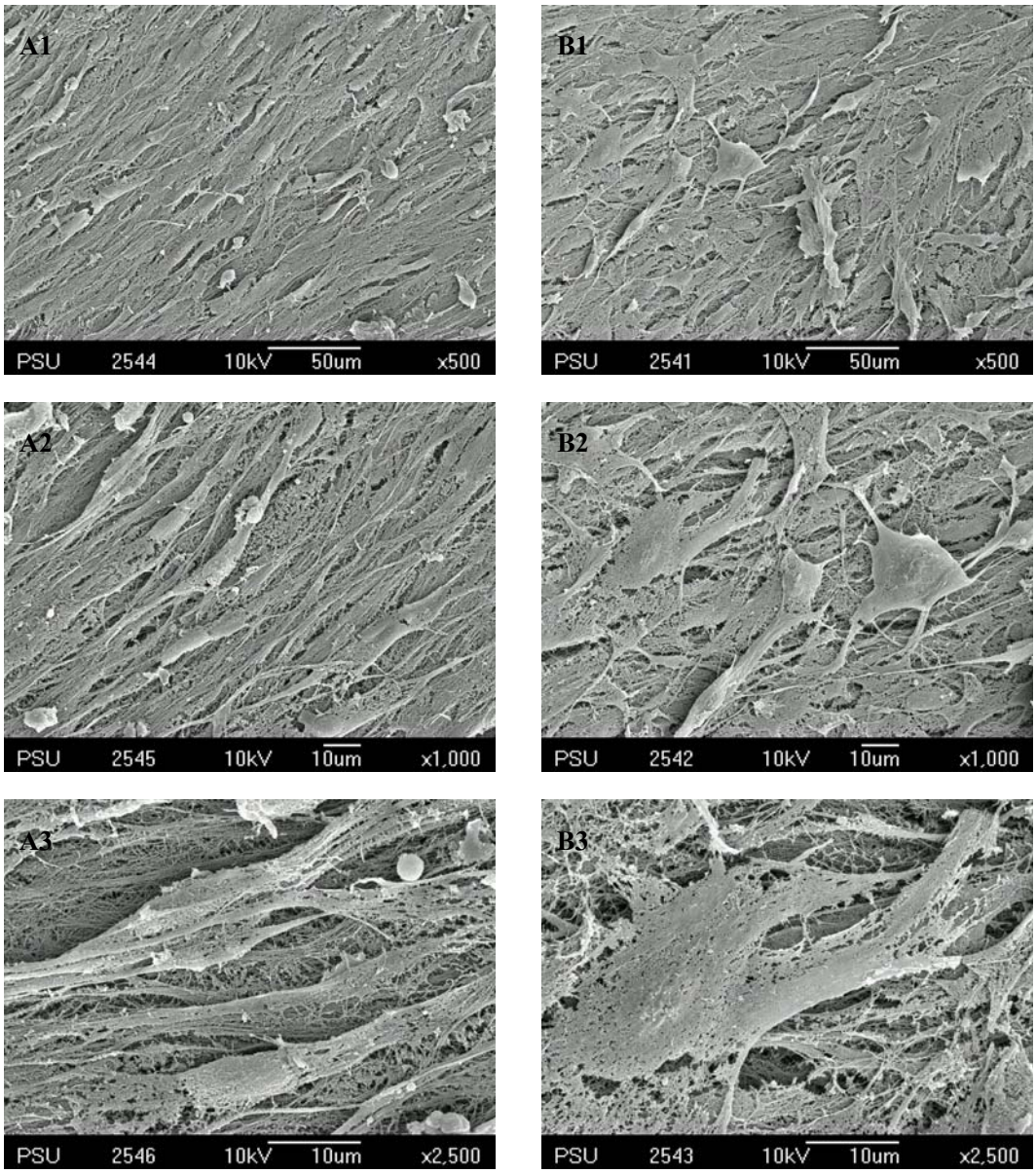

Fig. 24 SEM images of MC3T3-E1 cells on the surface of titanium disks in experimental *plateau* phase on **treatment day 1** (A) in culture medium with serum and (B) in serum free culture medium. Original magnifications (1) x500, (2) x1000 and (3) x2500

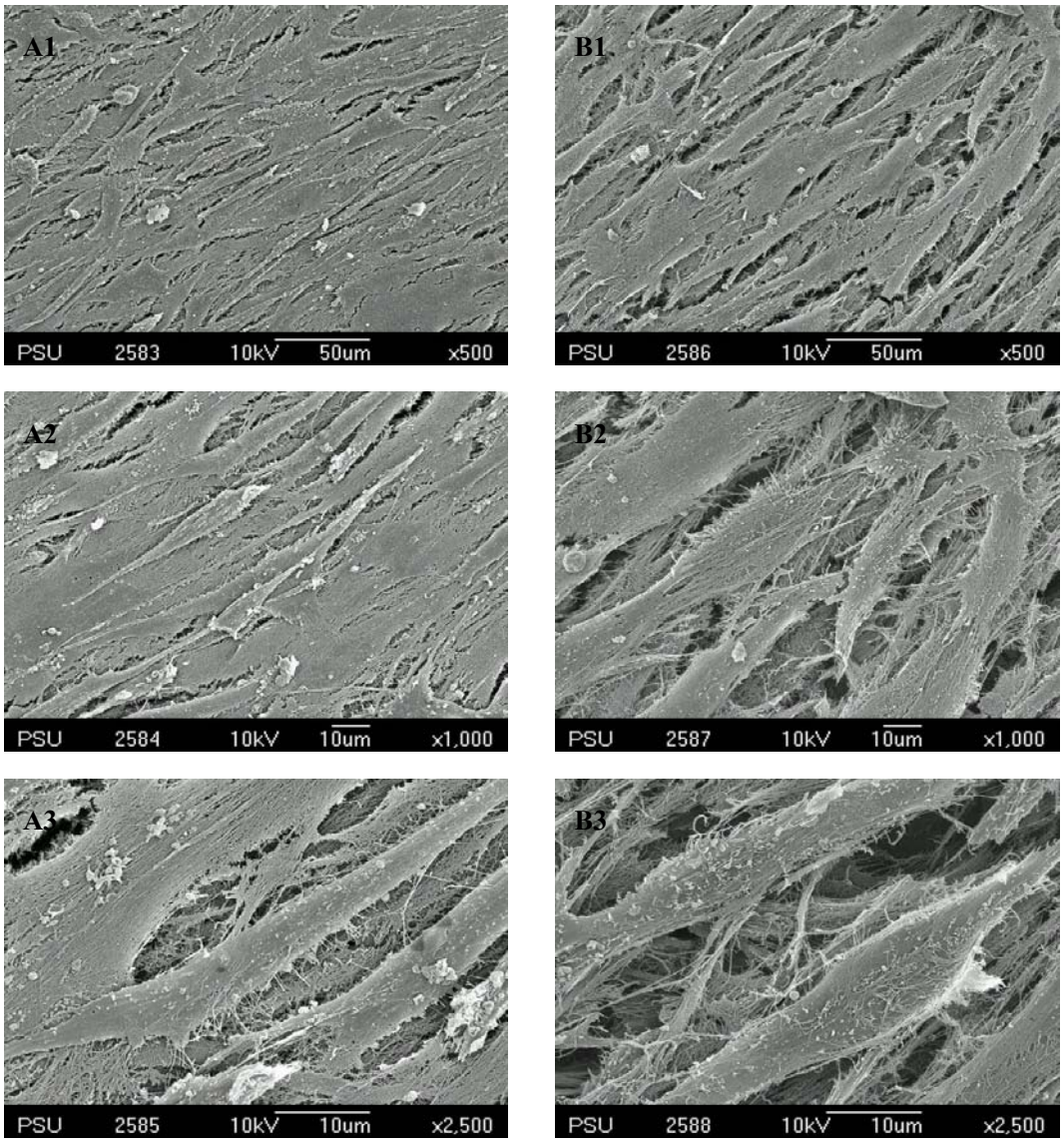

Fig. 25 SEM images of MC3T3-E1 cells on the surface of titanium disks in experimental *plateau* phase on **treatment day 5** (A) in culture medium with serum and (B) in serum free culture medium. Original magnifications (1) x500, (2) x1000 and (3) x2500

3. CLSM images of attachment and distribution of cells on titanium disks

Confocal fluorescence micrographs showed that on the treatment day 5 cells cultivated in serum free culture medium reached initial confluence establishing a network of intercellular contact to cover approximately 80-90% of the surface of titanium disks. These osteoblasts were polygonal in shape. Cells extended their filopodia to create intercellular contact. They attached well and spread their cytoplasmic process on the titanium surface in all groups (Fig.26 A3-C3, A4-C4). A high cell density was found in the central region of the disks (Fig.26 A1-C1, A2-C2). Effects of 0.1 μM indomethacin and 3 μM celecoxib on attachment and morphology of cells can not be distinguished from confocal fluorescence micrographs (Fig.26).

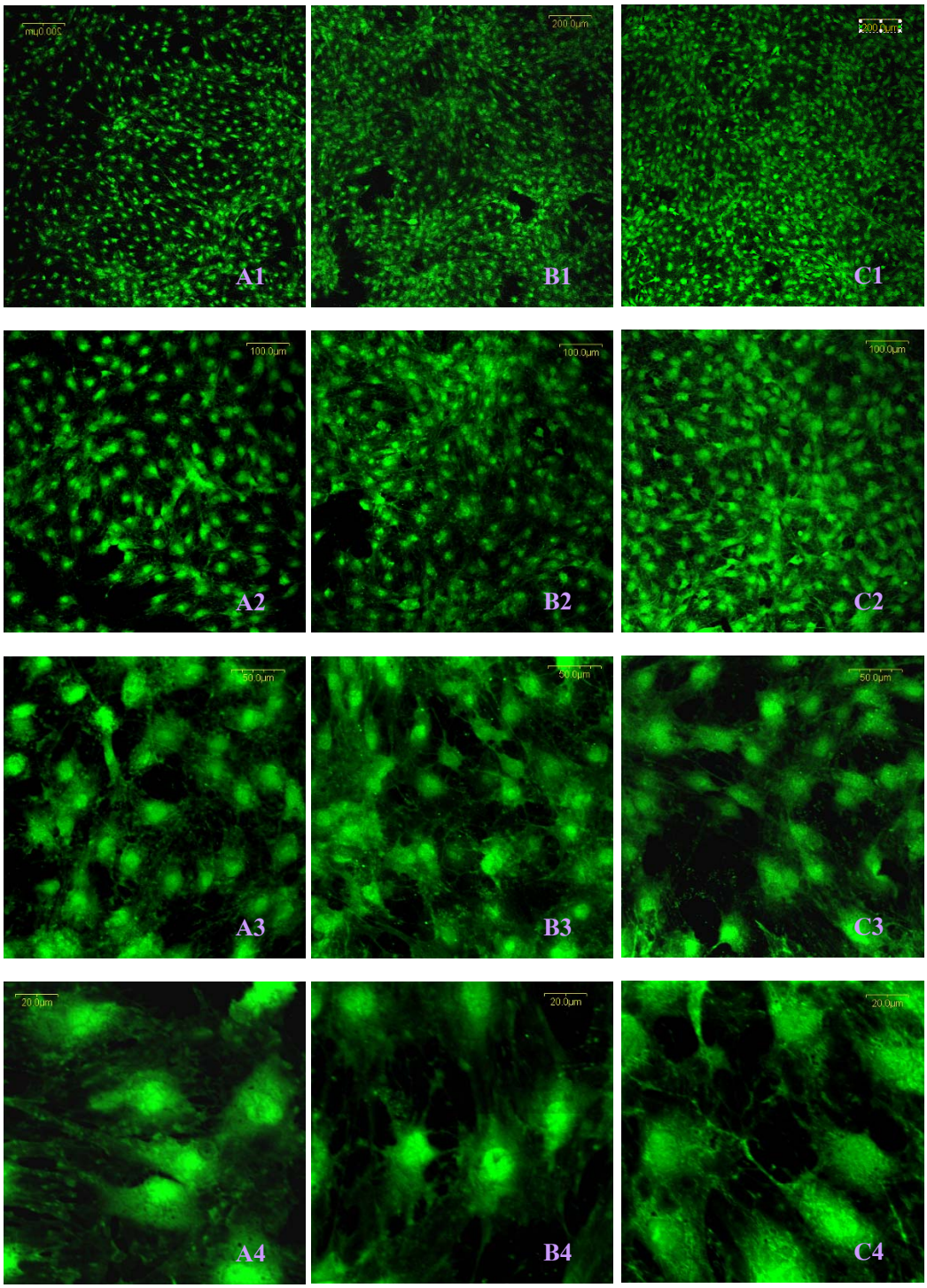

Fig. 26 Fluorescence images of MC3T3-E1 cells stained with FDA in experimental *static* phase on treatment day 5 on the surface of a titanium disk in (A) Groups A, (B) Group C and (C) Group E (original magnifications (1) x100, (2) x200, (3) x500 and (4) x1000)

4. Effects of medication treatments on cell viability

4.1. An overview

The levels of cell growth in each experimental phase, *static*, *log* and *plateau* corresponded with levels of the natural growth curve of cells on titanium disks (Fig.17). Therefore, celecoxib and indomethacin were treated in the three different stages of cell growth, as stated in the study design (Chapter 2, Fig.10).

Effects of therapeutic doses and time of celecoxib (1-9 µM) on growth of cells in *static*, *log* and *plateau* phases were minimal (Fig.27). An inhibitory effect of treated celecoxib was found in a high dose group of 9 µM celecoxib with a long incubation time of 5 days of medication treatment. A significant suppression of cell growth was found in Group D (9 µM celecoxib), on treatment day 5 in the *plateau* phase, when viability of cells in Group D was significantly lower than viability of cells in the control group, Group E ($p<0.05$) (Fig.27).

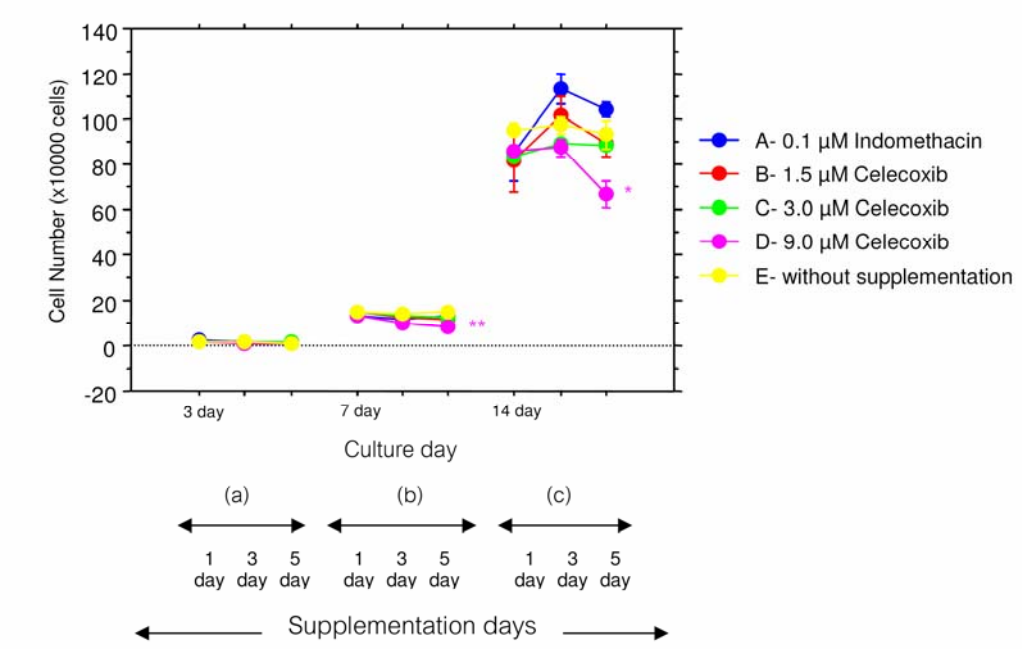

Fig. 27 An overview of growth of cells during medication treatment in experimental (a) *static*, (b) *log*, and (c) *plateau* phases; * = $p<0.05$ from without treatment, ** = $p<0.01$ from without treatment (mean ±SE, n=3)

4.2. Effects of medication treatments on cell viability in a *dose-dependent* manner

In all experimental groups and treatment times, growth of cells in Groups A – C was not significantly different from growth of cells in Group E, a control group of cells on a titanium surface without treatment ($p>0.05$), and there was no difference among these groups of study ($p>0.05$). It should be noticed that a marked decrease of cell growth was found in Group D, a treatment of celecoxib 9 μM, in all experimental phases. There was significant difference in Group D, 9.0μM celecoxib and Group E, control group of cells on a titanium surface without treatment on treatment day 5, in the *log* phase ($p<0.01$) and *plateau* phase ($p<0.05$) (Fig.27, Table 10).

In the experimental *static* phase, the growth of cells in Group D on treatment day 5 was significantly lower than Group A: treatment of 0.1 μM indomethacin ($p<0.001$) and Groups B and C: treatments of low concentrations of celecoxib, 1.5μM and 3.0 μM celecoxib ($p<0.05$) (Fig.28, Table 10).

In the experimental *log* phase, the growth of cells in Group D on treatment day 5 was significantly lower than other group ($p<0.05$) but not Group B, 1.5μM celecoxib (Fig.29, Table 10).

In the experimental *plateau* phase, the growth of cells in Group D on treatment day 5 was significantly lower than other group ($p<0.05$), Group A ($p<0.01$), Group B and Group E ($p<0.05$), but it was not significantly different from viability of cells in Groups C, 3.0 μM celecoxib (Fig.30, Table 10).

4.3. Effects of medication treatments on cell viability in a *time dependent* manner

A time dependent effect was clearly demonstrated in the experimental *static* phase, and growth of cells on treatment day 5 in Group D was significantly lower than growth of cells on treatment day 1 ($p<0.01$) (Fig.27,28, Table 11). In the experimental *log* phase, a significant decrease of cell growth between treatment days 1 – 5 was found in Groups A ($p<0.001$), B and E ($p<0.05$). In the *plateau* phase, the growth of cells in Groups C and D on treatment day 5 was significantly lower than growth of cells on treatment day 1 ($p<0.05$) (Fig.30 and Table 11).

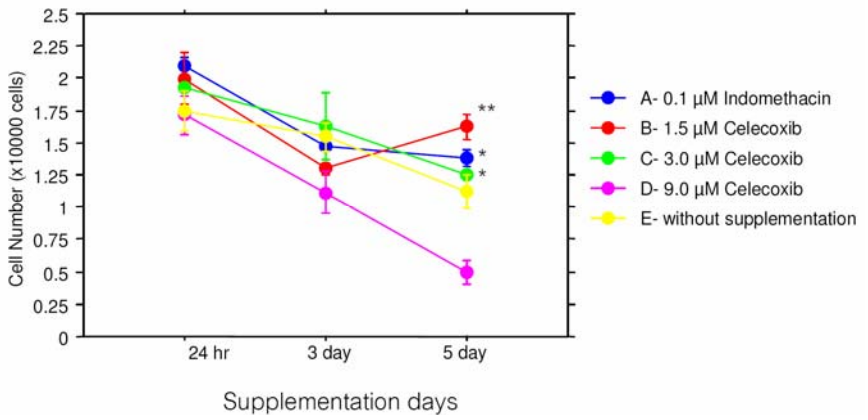

Fig. 28 Growth of cells during medication treatment in the experimental *static* phase; * = p<0.05 from 9.0 µM celecoxib, ** = p<0.01 from 9.0 µM celecoxib (mean ±SE, n=3)

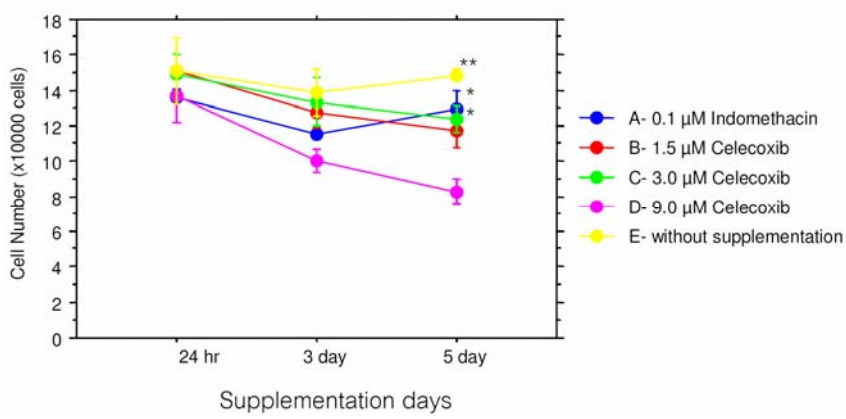

Fig. 29 Growth of cells during medication treatment in the experimental *log* phase; * = p<0.05 from 9.0 µM celecoxib, ** = p<0.01 from 9.0 µM celecoxib (mean ±SE, n=3)

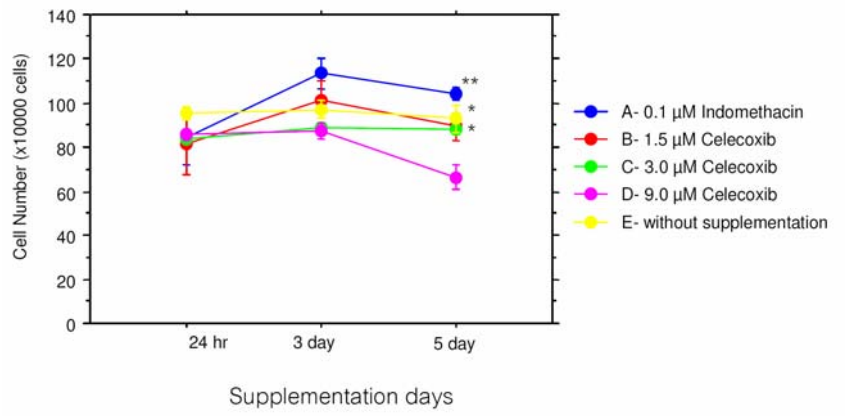

Fig. 30 Growth of cells during medication treatment in the experimental *plateau* phase; * = p<0.05 from 9.0 µM celecoxib, ** = p<0.01 from 9.0 µM celecoxib (mean +SE, n=3)

Table 10 Statistical differences of viability of cells in dose dependent manner

Dose Dependent Effects on Growth of Cells		
On treatment day 5 in experimental	Comparing to	
	Group D: 9 µM Celecoxib	Group E: without treatment
***Static* phase**		
A: 0.1µM Indomethacin	p= 0.014*	p= 0.712
B: 1.5µM Celecoxib	p= 0.008**	p= 0.255
C: 3.0µM Celecoxib	p= 0.047*	p= 0.967
D: 9.0µM Celecoxib	--	p= 0.133
E: without treatment	p= 0.133	--
***Log* phase**		
A: 0.1µM Indomethacin	p= 0.012*	p= 0.532
B: 1.5µM Celecoxib	p= 0.082	p= 0.112
C: 3.0µM Celecoxib	p= 0.028*	p= 0.294
D: 9.0µM Celecoxib	--	p= 0.001**
E: without treatment	p= 0.001**	--
***Plateau* phase**		
A: 0.1µM Indomethacin	p= 0.001**	p= 0.546
B: 1.5µM Celecoxib	p= 0.041*	p= 0.992
C: 3.0µM Celecoxib	p= 0.054	p= 0.974
D: 9.0µM Celecoxib	--	p= 0.016*
E: without treatment	p= 0.016*	--

* = $p<0.05$, ** = $p<0.01$

Table 11 Statistical differences of viability of cells in time dependent manner

Time Dependent Effects on Growth of Cells				
Comparing between treatment times	Groups of Study	Experimental Phases		
		Static (p value)	***Log*** (p value)	***Plateau*** (p value)
Treatment days 1 and 3	A: 0.1µM Indomethacin	p= 0.024*	p= 0.006**	p= 0.133
	B: 1.5µM Celecoxib	p= 0.590	p= 0.127	p= 0.080
	C: 3.0µM Celecoxib	p= 0.386	p= 0.499	p= 0.209
	D: 9.0µM Celecoxib	p= 0.001**	p= 0.087	p= 0.759
	E: without treatment	p= 0.235	p= 0.743	p= 0.613
Treatment days 3 and 5	A: 0.1µM Indomethacin	p= 0.466	p= 0.224	p= 0.929
	B: 1.5µM Celecoxib	p= 0.023*	p= 0.447	p= 0.287
	C: 3.0µM Celecoxib	p= 0.260	p= 0.413	p= 0.829
	D: 9.0µM Celecoxib	p= 0.133	p= 0.213	p= 0.011
	E: without treatment	p= 0.035*	p= 0.496	p= 0.218
Treatment days 1 and 5	A: 0.1µM Indomethacin	p= 0.008**	p= 0.448	p= 0.168
	B: 1.5µM Celecoxib	p=0.112	p= 0.046*	p= 0.647
	C: 3.0µM Celecoxib	p= 0.003**	p= 0.120	p= 0.032*
	D: 9.0µM Celecoxib	p= 0.036*	p= 0.122	p= 0.088
	E: without treatment	p= 0.003**	p= 0.917	p= 0.726

* = $p<0.05$, ** = $p<0.01$

5. Effects of treatments on levels of ALP activity

5.1. An overview

When an overall of ALP activity graph in the three experimental phases was considered, it was found that ALP activity reached the highest level in the *log* phase and decreased in the *plateau* phase. There was no significant difference between Group E, the control group of cells on a titanium surface without treatment and other groups in all experimental phases (Table 12). It should be noticed that levels of ALP activity of Groups B - D (1.5μM celecoxib, 3.0μM celecoxib, 9.0μM celecoxib) were not significantly different from Groups A, 0.1μM indomethacin ($p>0.05$) (Fig.31, Table 12).

5.2. Effects of treatments on levels of ALP activity in dose and time dependent manner

In the *static* phase, a significant increase of ALP activity from treatment days 1 - 3 was found in a control group, Group E ($p<0.001$). At each investigation time on treatment days 1, 3 and 5, levels of the ALP activity of all groups were not significantly different ($p>0.05$) (Fig.32 and Table 13).

In the *log* phase, significant increases of ALP activity from treatment days 1 – 3 was found in Groups A, E and D ($p<0.05$) and Group C ($p<0.01$). ALP activities of Groups C and D from treatment days 3 – 5 tended to increase but they were not significantly different ($p>0.05$). A significant decrease of ALP activity from treatment days 3 – 5 was found in Group C ($p<0.01$). At each investigation time, levels of the activity of each group were stable and not significantly changed ($p>0.05$) (Fig.33 and Table 13).

In the *plateau* phase, levels of ALP activity of groups of low dose celecoxib, Groups B and C tended to increase from treatment days 3 – 5, but they were not significantly different ($p>0.05$). On treatment day 5, levels of ALP activity among groups of study were not significantly different ($p>0.05$). At each investigation time, levels of the activity of all groups of the study were not significantly different ($p>0.05$) (Fig.34 and Table 13).

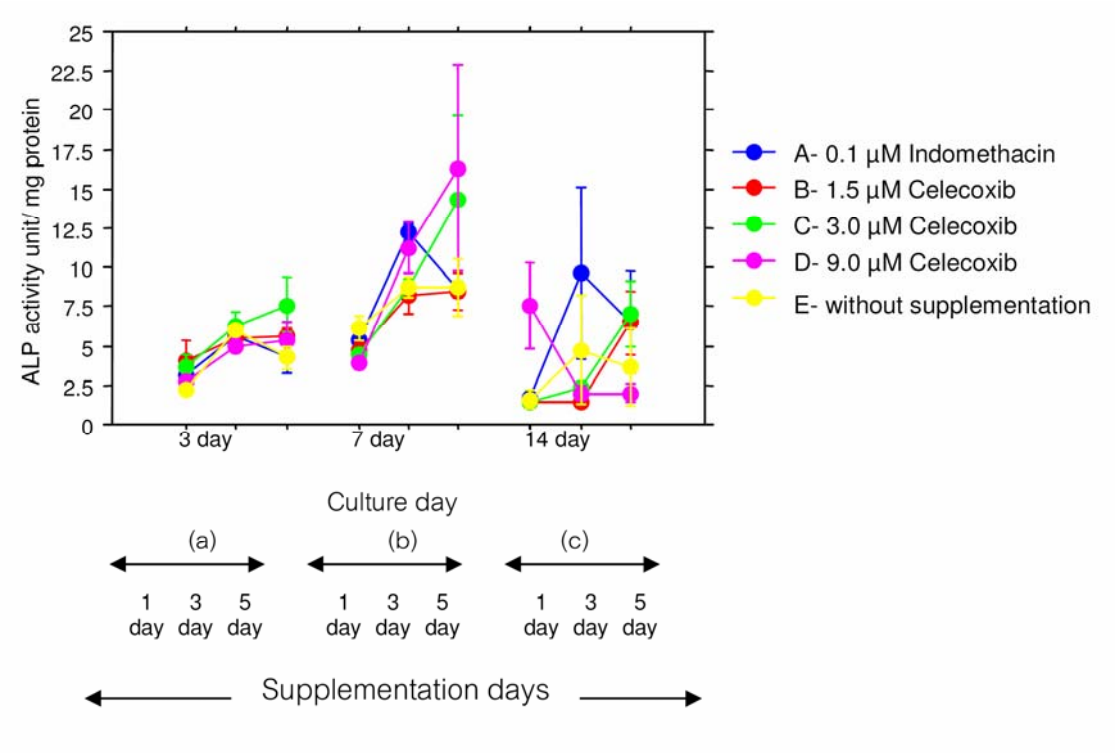

Fig. 31 An overview of ALP activity of cells during medication treatment in experimental (a) *static*, (b) *log* and (c) *plateau* phases (mean ±SE, n=3)

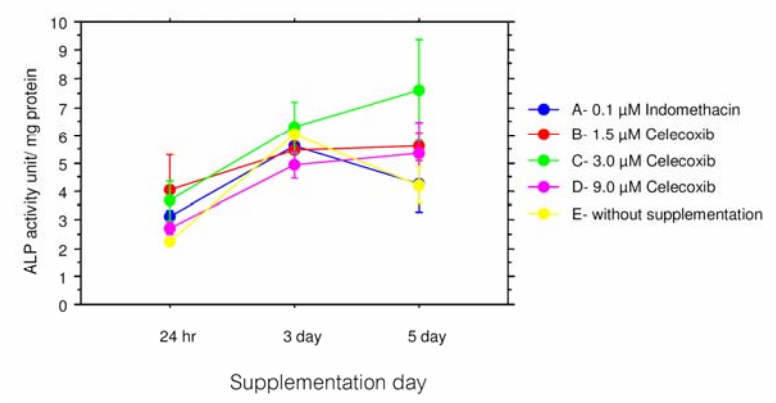

Fig. 32 An ALP activity during medication treatment in the experimental *static* phase (mean ±SE, n=3)

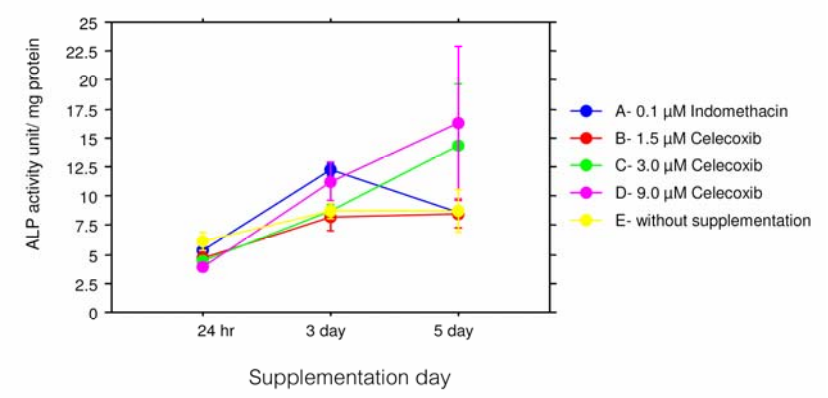

Fig. 33 An ALP activity during medication treatment in the experimental *log* phase (mean ±SE, n=3)

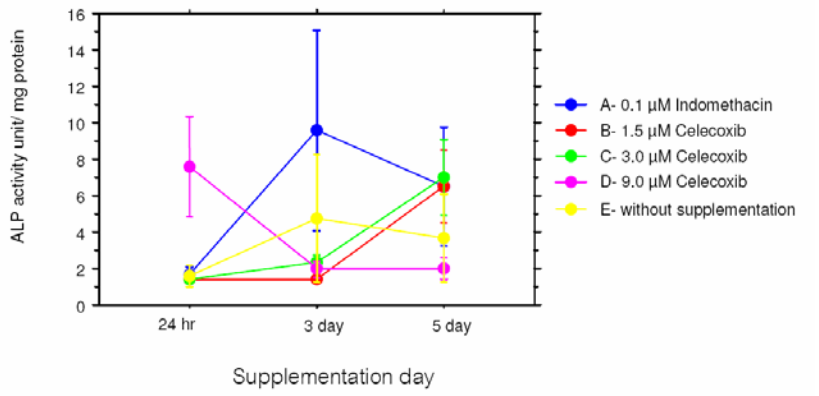

Fig. 34 An ALP activity during medication treatment in the experimental *plateau* phase (mean ±SE, n=3)

Table 12 Statistical differences of ALP activity of cells in dose dependent manner

Dose Dependent Effects on Alkaline Phosphatase Activity		
On treatment day 5 in experimental	Comparing to	
	Group D: 9 μM Celecoxib	Group E: without treatment
***Static* phase**		
A: 0.1μM Indomethacin	p= 0.969	p= 1.000
B: 1.5μM Celecoxib	p= 1.000	p= 0.925
C: 3.0μM Celecoxib	p= 0.629	p= 0.248
D: 9.0μM Celecoxib	--	p= 0.967
E: without treatment	p= 0.967	--
***Log* phase**		
A: 0.1μM Indomethacin	p= 0.916	p= 1.000
B: 1.5μM Celecoxib	p= 0.517	p= 1.000
C: 3.0μM Celecoxib	p= 1.000	p= 0.964
D: 9.0μM Celecoxib	--	p= 0.938
E: without treatment	p= 0.938	--
***Plateau* phase**		
A: 0.1μM Indomethacin	p= 0.851	p= .0998
B: 1.5μM Celecoxib	p= 0.570	p= 0.989
C: 3.0μM Celecoxib	p= 0.510	p= 0.967
D: 9.0μM Celecoxib	--	p= 0.997
E: without treatment	p= 0.997	--

* = $p<0.05$, ** = $p<0.01$

Table 13 Statistical differences of ALP activity of cells in time dependent manner

Time Dependent Effects on Alkaline Phosphatase Activity				
Comparing between treatment times	Groups of study	Experimental Phases		
		Static (p value)	***Log*** (p value)	***Plateau*** (p value)
Treatment days 1 and 3	A: 0.1μM Indomethacin		p= 0.017**	p= 0.298
	B: 1.5μM Celecoxib	p= 0.292	p= 0.081	p= 0.792
	C: 3.0μM Celecoxib	p= 0.153	p= 0.004**	p= 0.173
	D: 9.0μM Celecoxib	p= 0.050	p= 0.047*	p= 0.161
	E: without treatment	p= 0.000**	p= 0.008**	p= 0.490
Treatment days 3 and 5	A: 0.1μM Indomethacin	p= 0.437	p= 0.896	p= 0.688
	B: 1.5μM Celecoxib	p= 0.737	p= 0.434	p= 0.131
	C: 3.0μM Celecoxib	p= 0.609	p= 0.003**	p= 0.101
	D: 9.0μM Celecoxib	p= 0.641	p= 0.587	p= 0.946
	E: without treatment	p= 0.135	p= 0.980	p= 0.853
Treatment days 1 and 5	A: 0.1μM Indomethacin	p= 0.436	p= 0.056	p= 0.308
	B: 1.5μM Celecoxib	p=0.204	p= 0.113	p= 0.130
	C: 3.0μM Celecoxib	p= 0.254	p= 0.219	p= 0.112
	D: 9.0μM Celecoxib	p= 0.136	p= 0.204	p= 0.153
	E: without treatment	p= 0.097	p= 0.263	p= 0.534

* = $p<0.05$, ** = $p<0.01$

6. Effects of medication treatments on levels of osteocalcin in dose and time dependent manner

6.1. Overview

Base line levels of osteocalcin in culture medium of cells on cell culture plate and titanium disks in each experimental phase were demonstrated in Fig.35. Levels of osteocalcin of cells on cell culture plates and titanium disks in all experimental phases were not significantly different ($p>0.05$), and the expression patterns were the same (Fig 36).

On cell culture plates, levels of osteocalcin significantly increased from *static* phase to the *log* phase ($p<0.05$) and significantly decreased from the *log* phase to the *plateau* phase ($p<0.05$), whereas the level of osteocalcin on the titanium disk significantly increased from *static* phase to *log* phase ($p<0.05$), but a decreasing level in the *log* phase to the *plateau* phase was not significant ($p>0.05$). The highest levels of osteocalcin on culture plates and titanium disks were found in the experimental *log* phase. Average concentrations of osteocalcin on titanium disks in experimental *static*, *log* and *plateau* phases were 3.897 $\pm$ 0.5508, 7.275 $\pm$ 1.1747 and 1.151 $\pm$ 0.0431 ng/mg protein, respectively.

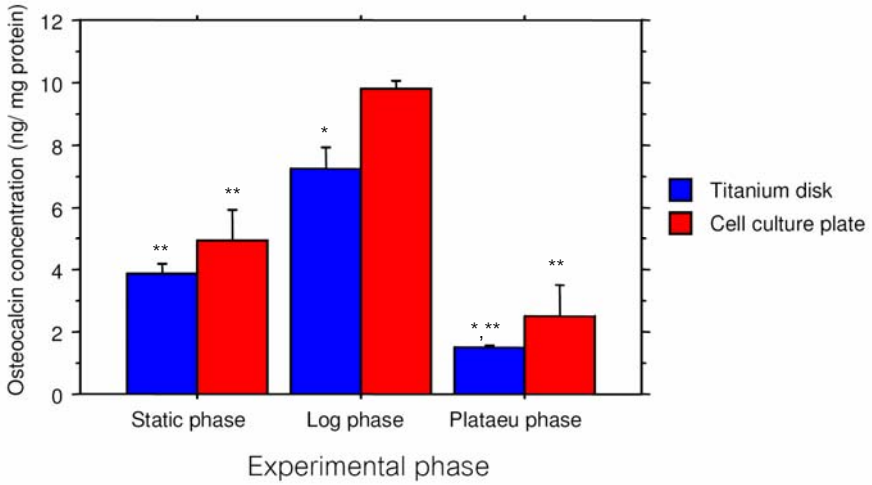

Fig. 35 Levels of osteocalcin in culture medium of cells in control group on treatment day 5 of experimental *static*, *log* and *plateau* phases; * = $p<0.05$ from *static* phase, ** = $p<0.05$ from *log* phase (mean $\pm$SE, n=3)

6.2. Effects of medication treatments on expression of osteocalcin

During medication treatments in each experimental phase, it was found that levels of osteocalcin in all experimental groups were not significantly different from Group E, a control group of cell culture on a titanium disk without treatment ($p>0.05$). Levels of osteocalcin of experimental groups in the *log* phase were higher than the levels in the *static* phase ($p<0.05$), only Group B were not significant different ($p>0.05$). The levels of osteocalcin in the log phase were higher than the experimental *plateau* phase. The levels of osteocalcin of all groups were lowest in the experimental *plateau* phase, and were significantly lower than the levels in the experimental *static* and *log* phases ($p<0.05$).

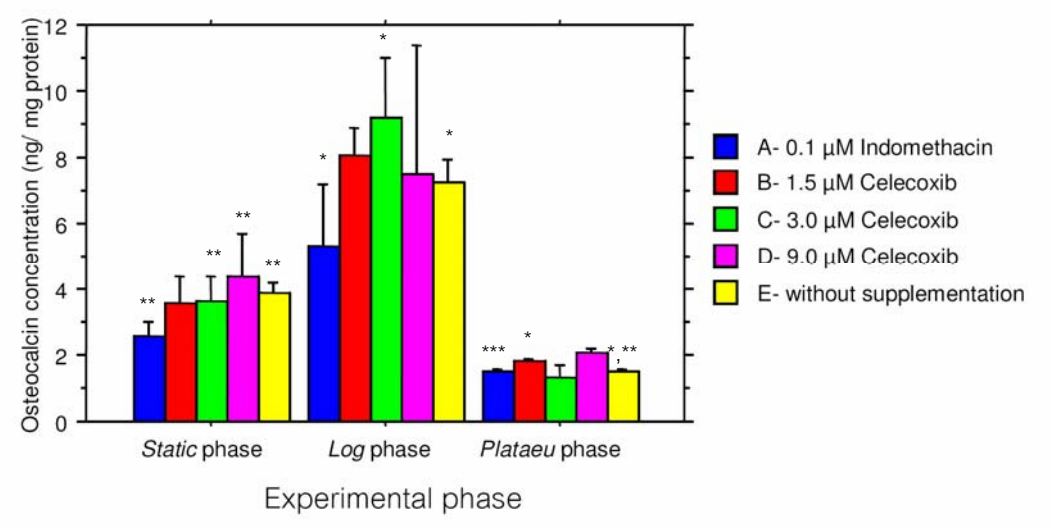

Fig. 36 Levels of osteocalcin in culture medium on treatment day 5 in the experimental *static*, *log* and *plateau* phases; * = $p<0.05$ from *static* phase, ** = $p<0.05$ from *log* phase, *** = $p<0.01$ from *log* phase (mean $\pm$SE, n=3)

Table 14 Statistical differences of osteocalcin concentration of cells in dose dependent manner

Dose Dependent Effects on osteocalcin concentration on each phase		
On treatment day 5 in experimental	Comparing to	
	Group D: 9 μM Celecoxib	Group E: without treatment
***Static* phase**		
A: 0.1μM Indomethacin	p= 0.655	p= 0.860
B: 1.5μM Celecoxib	p= 0.984	p= 1.000
C: 3.0μM Celecoxib	p= 0.984	p= 1.000
D: 9.0μM Celecoxib	--	p= 0.998
E: without treatment	p= 0.998	--
***Log* phase**		
A: 0.1μM Indomethacin	p= 0.805	p= 0.045*
B: 1.5μM Celecoxib	p= 0.988	p= 0.619
C: 3.0μM Celecoxib	p= 1.000	p= 1.000
D: 9.0μM Celecoxib	--	p= 1.000
E: without treatment	p= 0.906	--
***Plateau* phase**		
A: 0.1μM Indomethacin	p= 0.938	p= 1.000
B: 1.5μM Celecoxib	p= 0.998	p= 0.995
C: 3.0μM Celecoxib	p= 0.989	p= 0.999
D: 9.0μM Celecoxib	--	p= 0.931
E: without treatment	p= 0.931	--

* = $p<0.05$, ** = $p<0.01$

7. Effects of medication treatments on levels of PGE_2 in dose and time dependent manner

7.1. Overview

Base line levels of PGE_2 in culture medium of MC3T3-E1 on culture plate and titanium disks cultivated in mineralized culture medium were demonstrated in Fig.37. Levels of PGE_2 in culture medium of cells on disks and plates in *static* and *plateau* phases were not significantly different ($p>0.05$), but in *log* phase levels of PGE_2 of cells on the culture-plate was significantly higher than on titanium disks. Average concentrations of PGE_2 on titanium disks in the experimental *static*, *log* and *plateau* phases were 388 $\pm$ 21.531, 267.883 $\pm$ 291.0347 and 140.669 $\pm$ 20.324 ng/mg protein, respectively.

Titanium disks secretion of PGE_2 gradually decreased as cells were more mature or the growth rate was decreased in the *plateau* pahse. Levels of PGE_2 in the *plateau* phase was significantly lower than in the *static* phase ($p<0.01$). The level in the *log* phase was slightly less than the *static* phase and higher than the *plateau* phase, but the differences were not significant ($p>0.05$).

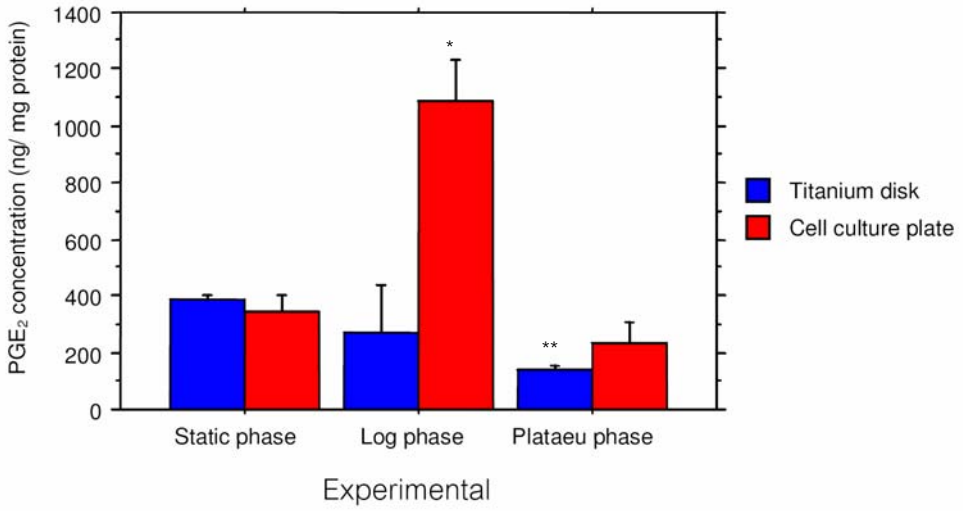

Fig. 37 PGE_2 excretion from cell culture in the control group at treatment day **5** in each phase on ● cell culture plate, and on ● titanium disk; * = $p<0.05$ from *static* phase, ** = $p<0.01$ from *log* phase (mean $\pm$SE, n=3)

7.2. Effects of medication treatments on PGE_2 synthesis

When comparing levels of PGE_2 of cells in experimental groups to the control group, it was found that in the *static* and *log* phases, level of PGE_2 of all experimental groups, Groups A-D, was significantly lower than the control group, Group E ($p<0.05$), but in the *log* phase the differences were not significantly different ($p>0.05$).

When levels of PGE_2 of each group were compared among different experimental phases, the levels of each group in each experimental phase were not significantly different, except Group C and B. It was found that in Group C, levels of PGE_2 significantly increased in the *log* phase ($p<0.05$) and decreased in the *plateau* phase ($p<0.05$). In Group B, a significant difference was found between *static* and *plateau* phases, where the activity significantly decreased ($p<0.05$) (Fig.38).

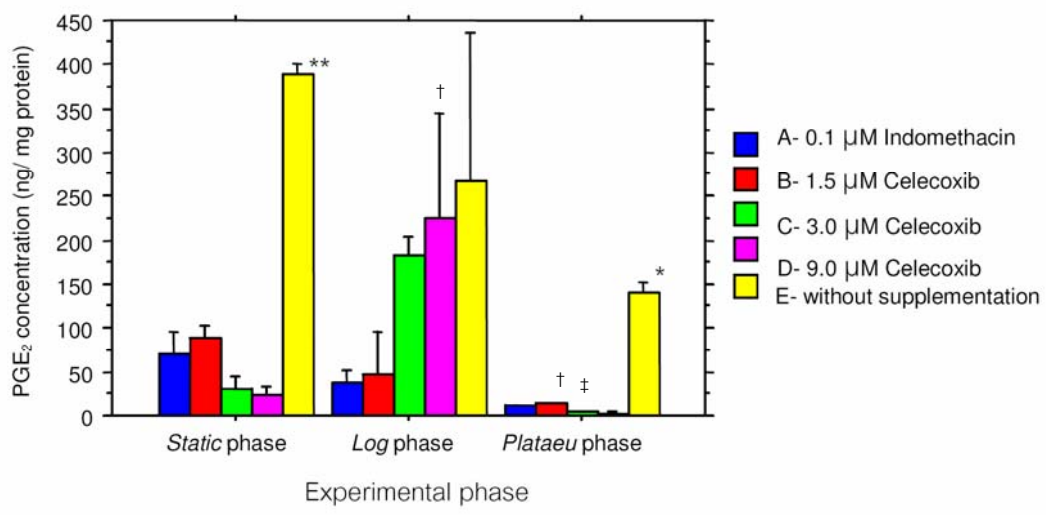

Fig. 38 Levels of PGE_2 in culture medium on treatment day 5 in each experimental phase, *static*, *log* and *plateau*; * = $p<0.05$ from all experimental groups, ** = $p<0.01$ from all experimental groups, † = $p<0.05$ from *static* phase, ‡ = $p<0.05$ from *log* phase (mean $\pm$SE, n=3)

Table 15 Statistical differences levels of PGE_2 of cells in dose dependent manner

Dose Dependent Effects on PGE_2 concentration on each phase		
On treatment day 5 in experimental	Comparing to	
	Group D: 9 μM Celecoxib	Group E: without treatment
***Static* phase**:		
A: 0.1μM Indomethacin	P= 0.844	P= 0.000**
B: 1.5μM Celecoxib	P= 0.617	P= 0.000**
C: 3.0μM Celecoxib	P= 1.000	P= 0.000**
D: 9.0μM Celecoxib	--	P= 0.000**
E: without treatment	p= 0.000**	--
***Log* phase**:		
A: 0.1μM Indomethacin	P= 0.780	P= 0.854
B: 1.5μM Celecoxib	P= 0.857	P= 0.895
C: 3.0μM Celecoxib	P= 1.000	P= 1.000
D: 9.0μM Celecoxib	--	P= 1.000
E: without treatment	p= 1.000	--
***Plateau* phase**:		
A: 0.1μM Indomethacin	P= 0.277	P= 0.034*
B: 1.5μM Celecoxib	P= 0.174	P= 0.034*
C: 3.0μM Celecoxib	P= 1.000	P= 0.031*
D: 9.0μM Celecoxib	--	P= 0.027*
E: without treatment	p= 0.027*	--

* = $p<0.05$, ** = $p<0.01$