Chapter 3

Results

1 Cell viability

Patterns of cell growth are shown in Figure 8. At 24 hours after cell seeding, numbers of cells in all groups were similar. Two phases of cell growth in log and plateau phases were demonstrated. A sharply increase of cell growth in log phase could be observed in the first 12 culture-days. Growth of cells entered the plateau phase on days 15 – 27. Comparing among the groups of study, cell proliferation on chitosan sponge (group A) on days 12 and 15 was significantly higher than the other groups. (Day12: Scheffe; group B ($p=0.01$), group C ($p=0.005$), and group D ($p=0.008$); Day 15: Dunnette T3; group B ($p=0.000$), group C ($p=0.001$) and group D ($p=0.007$)). On culture-day 18, the growth of cells on chitosan sponges was significantly higher than the growth of cells on 1:1 and 1:2 chitosan-collagen sponges (Scheffe: group C ($p=0.046$) and group D ($p=0.003$)). On culture-days 21, 24 and 27 the growth of cells on all sponges was not significantly different ($p>0.05$). On culture-day 27, the growth of cells on 1:2 chitosan-collagen sponges (group D) was in the highest level compared to the other groups, but it was not significantly different ($p>0.05$).

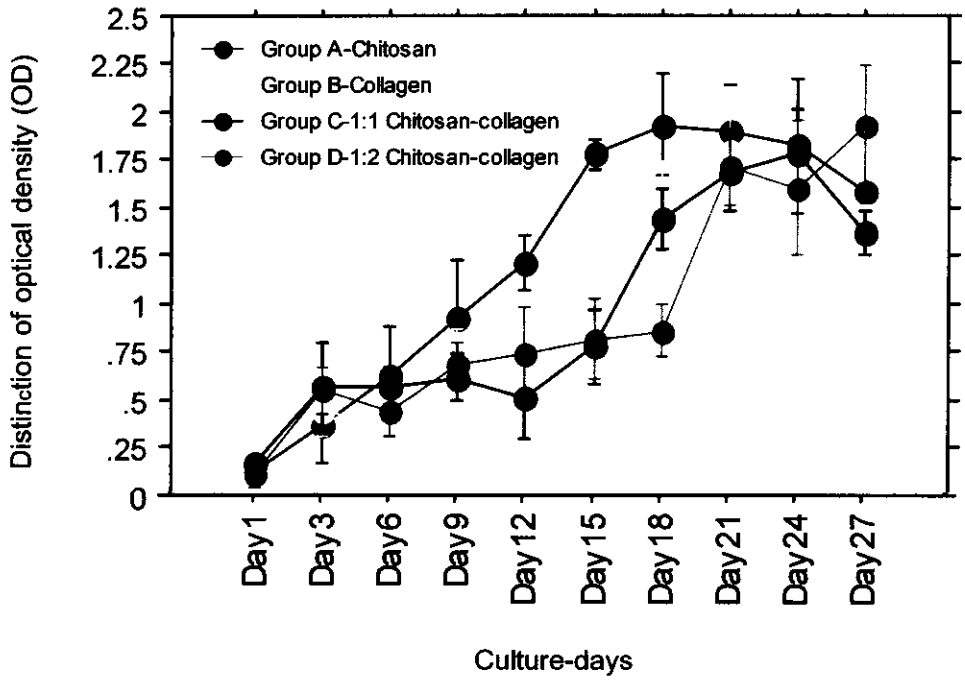

Figure 8 Growth of cells (MTT assay) on three-dimensional sponges (Mean ± SD)

2 Alkaline phosphatase activity (ALP)

Levels of ALP activity of MC3T3-E1 on three-dimensional sponges in groups A-D are demonstrated in Figure 9. Levels of ALP activity of cells on collagen sponges (group B) from days 15–27 were not significantly higher than the activity of cells on chitosan-collagen sponges (groups C and D) ($p>0.05$). Throughout cell culture period levels of ALP of cells on 1:1 and 1:2 chitosan-collagen sponges were in similar levels ($p>0.05$) and were in the middle levels between the activity of cells on collagen sponges (group B) and chitosan sponges (group A).

On culture-days 9–15 and 21–27 levels of ALP activity of cells on chitosan sponges (group A) were in the lowest levels compared to other groups ($p<0.05$). On culture-days 21, 24, and 27, levels of ALP activity of cells on chitosan sponges (group A) were significantly lower than the activity of cells on collagen sponges (group B) (Scheffe: $p=0.048$, 0.039, and 0.015, respectively) but they were not significant difference from group C and D. On day 24 the ALP activity of cell on chitosan (group A) was significantly lower than those of cells on collagen (group B) and 1:2 chitosan-collagen sponges (group D) only (Scheffe: $p=0.039$ and 0.020, respectively).

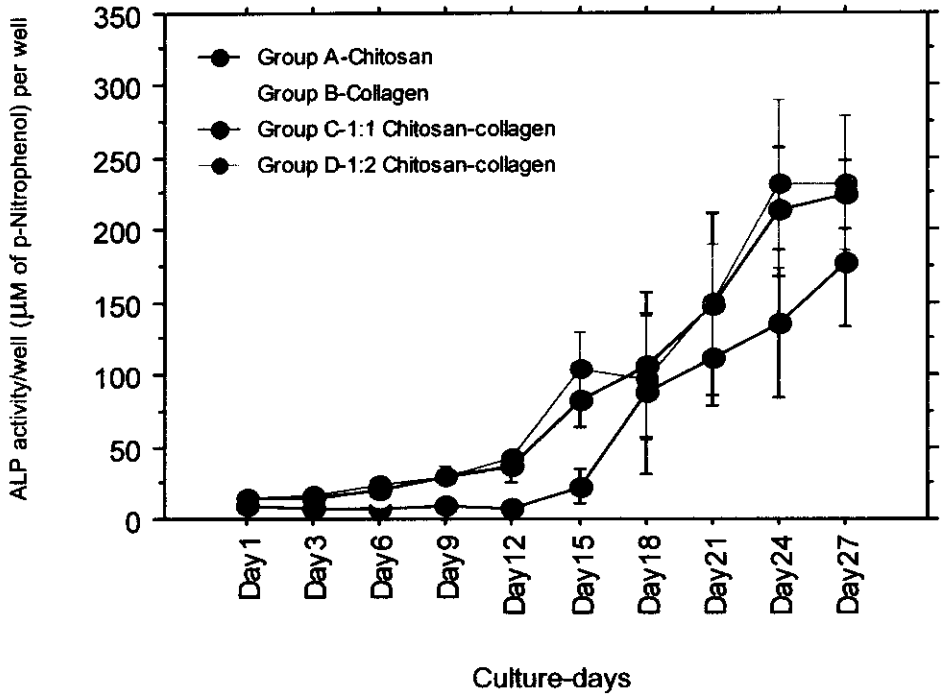

Figure 9 Alkaline phosphatase activity of cells on three-dimensional sponges (Mean ± SD)

3 Calcium content

Levels of calcium content are demonstrated in Figures 10 and 11. On culture-days 15-27 the highest levels of calcium content was consistently found in a group of cells on collagen sponges (group B) which was markedly higher than the other groups (Scheffe: $p=0.000$). The concentration of calcium content in group B was in a range of 200–550 mg/L (Figure 10). Levels of calcium content among groups A, C and D were not significantly different ($p>0.05$) and were relatively stable in a low level of less than 15 mg/L (Figure 11).

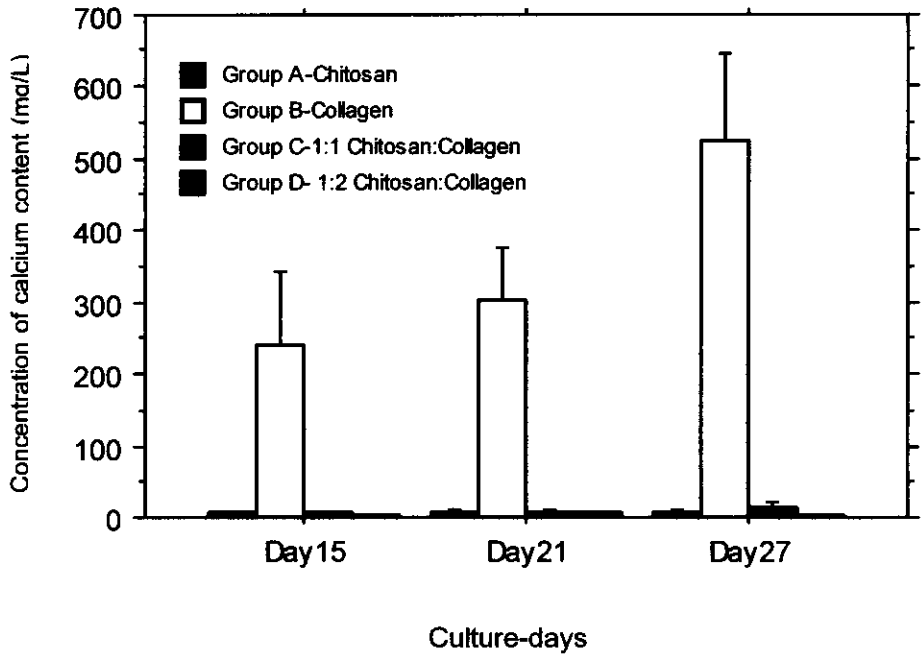

Figure 10 Levels of calcium content in extracellular matrices of MC3T3-E1 on three-dimensional sponges, groups A–D (Mean ± SD)

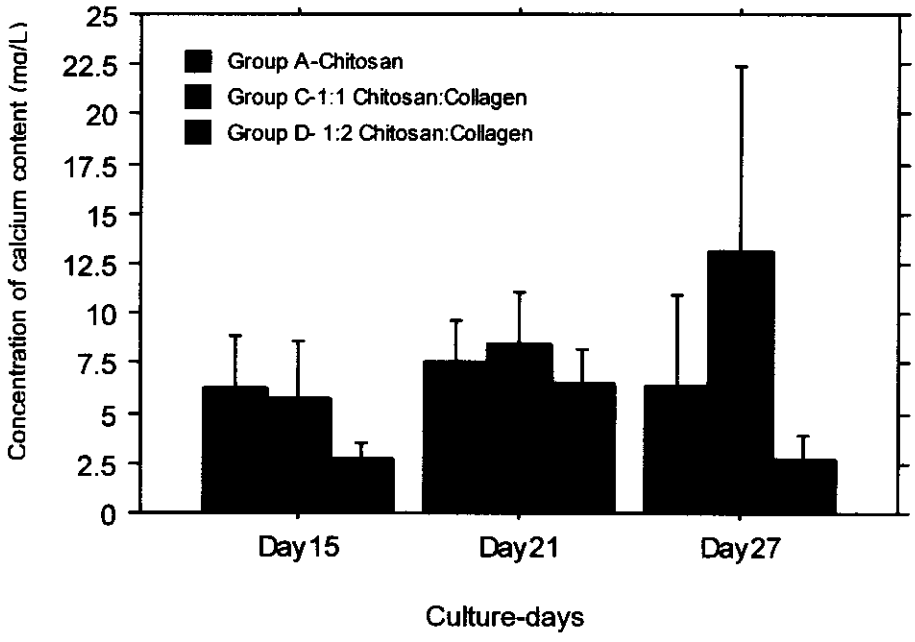

Figure 11 Levels of calcium content in extracellular matrices of MC3T3-E1 on three-dimensional sponges of groups A, C and D (excluding group B) (Mean ± SD)

4 Scanning electron microscopic observation

SEM demonstrated microstructure of sponges before cell seeding and morphology and growth of cells on sponges (Figures 12-16). Collagen and 1:1 and 1:2 chitosan-collagen sponges (groups B – D) showed internal porous microstructure with pore sizes ranging from 107.11-198.84, 111.34–262.29, and 132.61-193.63 μm in diameter respectively (Table 3). Chitosan sponges demonstrated collapse internal structure (Figure 12).

Table 3 Pore size (μm) of collagen, 1:1 and 1:2 chitosan-collagen sponges

Pore sizes / Area	Collagen	1:1 chitosan-collagen	1:2 Chitosan-collagen
1	162.15	177.63	144.73
2	128.81	113.67	149.03
3	117.29	198.93	193.63
4	160.58	111.34	133.36
5	175.48	175.87	132.61
6	107.11	175.42	142.53
7	113.80	170.74	190.32
8	121.00	200.16	156.54
9	198.84	262.29	180.52
10	188.03	138.60	145.37
Mean $\pm$ SD	147.31 $\pm$ 33.63	172.47 $\pm$ 44.63	156.86 $\pm$ 22.90

On culture-day 9 the attachment of cells and spreading of cytoplasmic process of cells on the surface of sponges could be seen in all groups (groups A-D) and migration of cells into the internal porous structures of sponges was found in groups B-D. Spreading of the cytoplasmic process established intercellular contact and formed three-dimensional growth on three-dimensional structures of sponges (Figure 13).

On culture-day 15 increasing in the size of the cells on the surface of the sponges could be observed. Cell growth reached initial confluence establishing a network of intercellular contact and multi-layers of cell growth (Figure 14).

On culture-days 21 and 27 it was clearly demonstrated that the cells were larger in size and elongated. Increasing in the density and layers of cells on the surface of sponges were clearly demonstrated. Cells grew in multi-layers and fully covered external surfaces of sponges (Figures 15 and 16). On culture-day 27 rod-shape crystallites could be seen depositing on surface of cell layers in all groups (Figure 16). Differences in morphology, growth and differentiation of cells on each type of sponges could not be differentiated from SEM images (Figures 13-16).

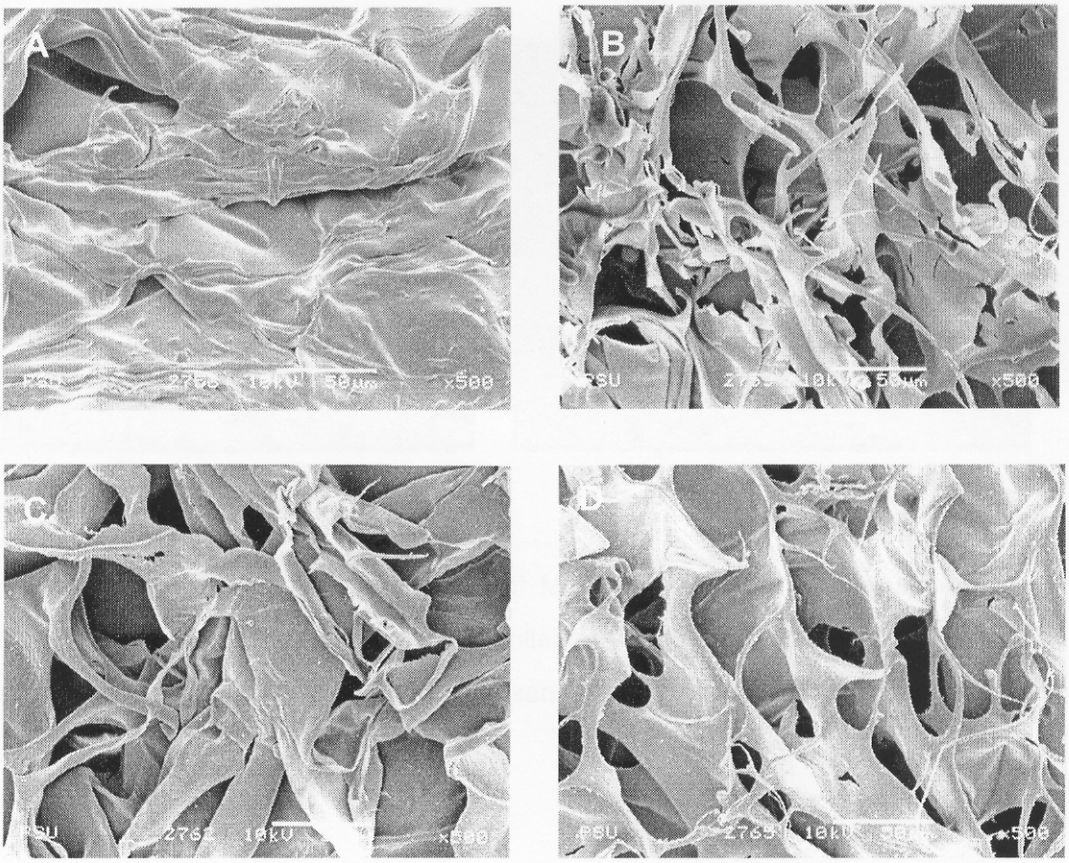

Figure 12 Scanning electron micrographs of sponges before cell seeding; Group A: chitosan sponge (A), Group B: collagen sponge (B), Group C: 1:1 chitosan-collagen sponge (C) and Group D: 1:2 chitosan-collagen sponge before seeding cells. (Original magnification x500)

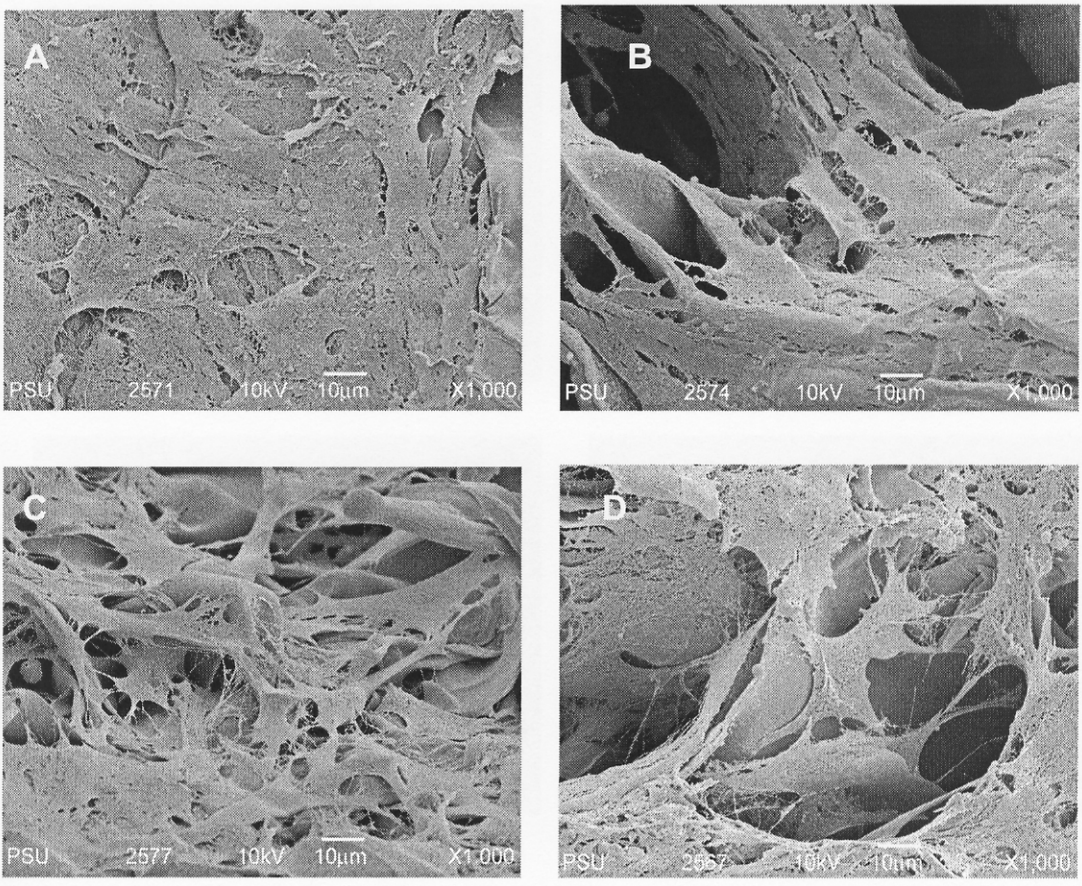

Figure 13 Scanning electron micrographs of MC3T3-E1 on sponges on culture-day 9; Group A: chitosan sponge (A), Group B: collagen sponge (B), Group C: 1:1 chitosan-collagen sponge (C) and Group D: 1:2 chitosan-collagen sponge. (Original magnification x1000)

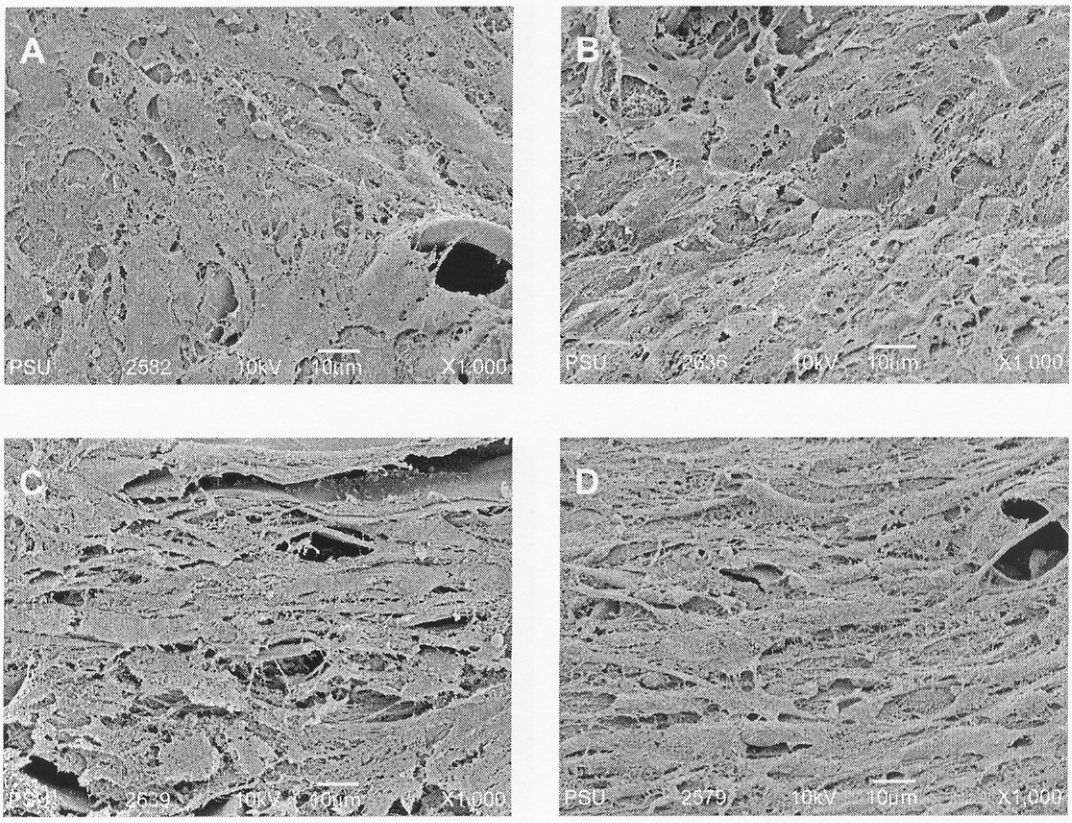

Figure 14 Scanning electron micrographs of MC3T3-E1 on sponges on culture-day 15; Group A: chitosan sponge (A), Group B: collagen sponge (B), Group C: 1:1 chitosan-collagen sponge (C) and Group D: 1:2 chitosan-collagen sponge. (Original magnification x1000)

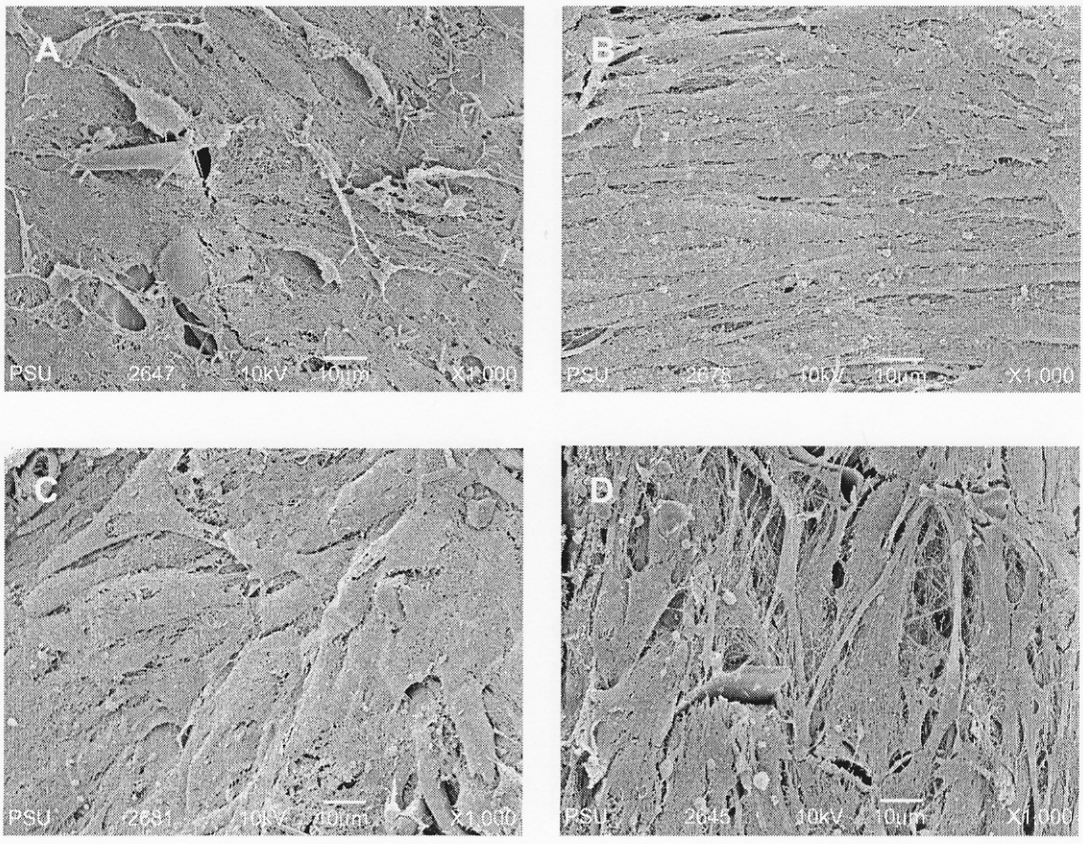

Figure 15 Scanning electron micrographs of MC3T3-E1 on sponges on culture-day 21; Group A: chitosan sponge (A), Group B: collagen sponge (B), Group C: 1:1 chitosan-collagen sponge (C) and Group D: 1:2 chitosan-collagen sponge. (Original magnification x1000)

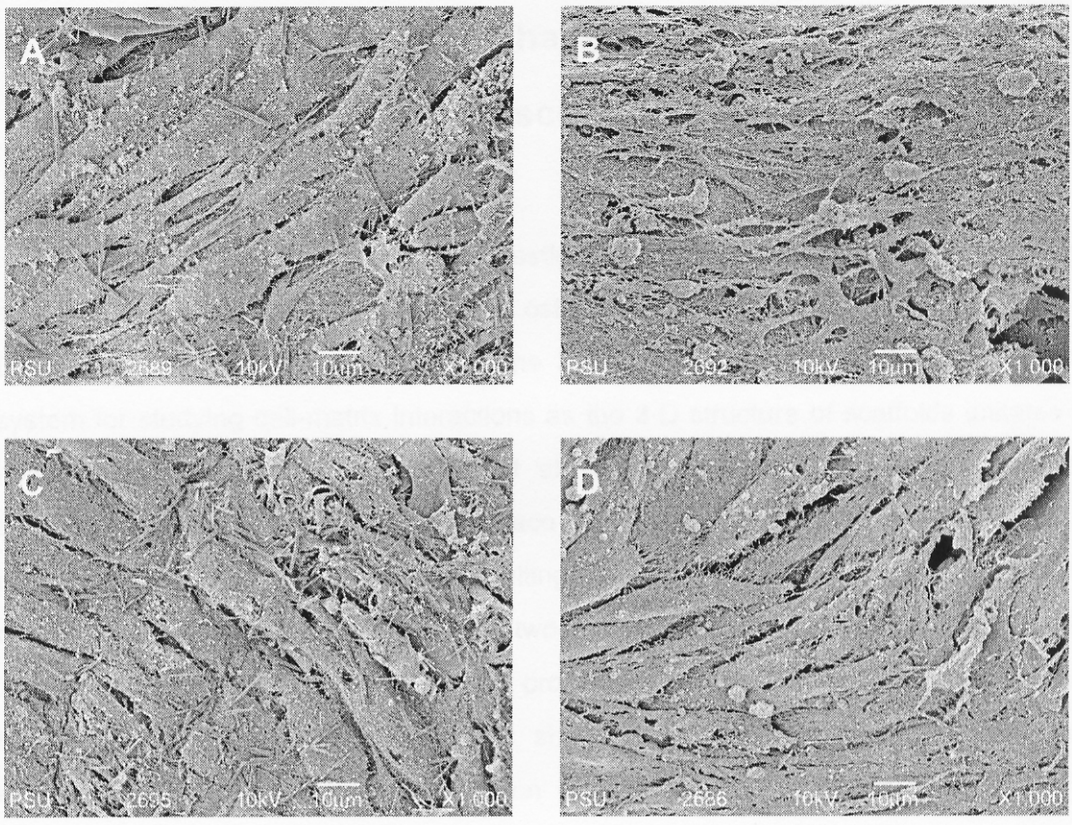

Figure 16 Scanning electron micrographs of MC3T3-E1 on sponges on culture-day 27; Group A: chitosan sponge (A), Group B: collagen sponge (B), Group C: 1:1 chitosan-collagen sponge (C) and Group D: 1:2 chitosan-collagen sponge. (Original magnification x1000)