4 RESULTS

4.1 Dynamic cell culture conditioning

The bioreactor could be easily assembled and placed in the humidified incubator. During the dynamic conditioning period, the whole system was compact without any sign of leakage and functioned stably. HE stain showed that there was no microbiological contamination in any experiments.

4.2 Morphology

Macroscopically, all cell-seeded patches were covered by tissue (Figure 13). Gross appearance showed that all TE constructs were intact without any rupture of structure, and both sides of patches exhibited a confluent and smooth surface.

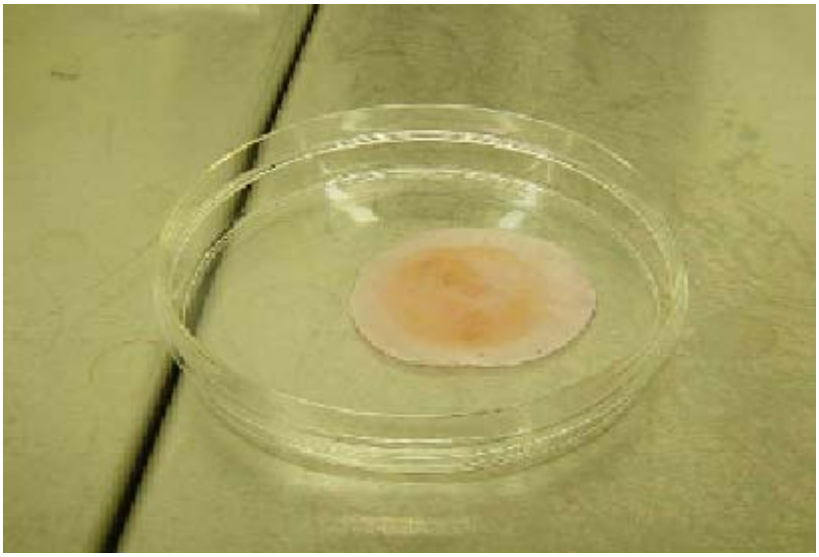

Figure 13 MSC based tissue engineered patch after 7 days in the pulse duplicator *in vitro* system (bioreactor). The central area of scaffold construct was covered by yellow tissue; the circumference of the patch (the fixation area) lacked tissue. The TE construct was intact and pliable.

4.2.1 Histology

Microscopically, all tissue engineered patch constructs were covered by tissue. HE staining of bioreactor conditioned TE patch sections demonstrated cellular tissue organized in a layered fashion with a dense outer layer and lesser cellularity in the deeper portions (Figure 14). The histologic examination showed mutiple, confluent cell layers on both sides of the P4HB patch scaffold. Additionally, after conditioning in the pulsatile flow bioreactor, the cells were mostly viable and grew into the pores and formed tissue inside the scaffold. Static controls showed a loose, less organized tissue formation with irregular cellular ingrowth (Figure 15).

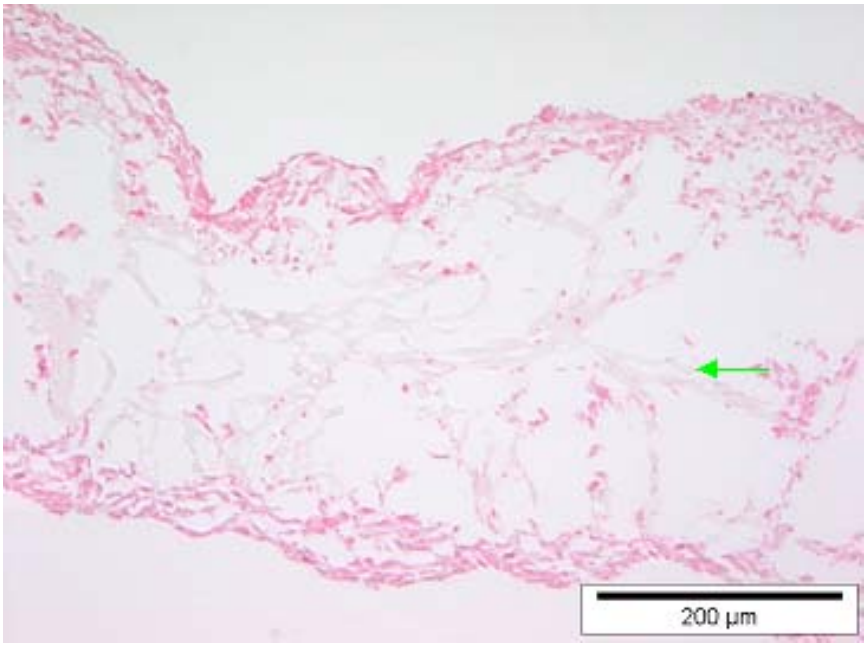

Figure 14 Hematoxylin and eosin staining of bioreactor conditioned TE patch showed that both sides of the P4HB patch scaffold were covered by cellular tissue in a mutiply and confluently layered fashion. Cells attached well to the polymer and had grown into the deeper sections of patch. At 4 weeks each polymeric scaffold was partially absorbed and replaced with cells and extracellular matrix. Degradation of scaffold was demonstrated by multiple breakages and fragmentation of polymer fibers (green arrow).

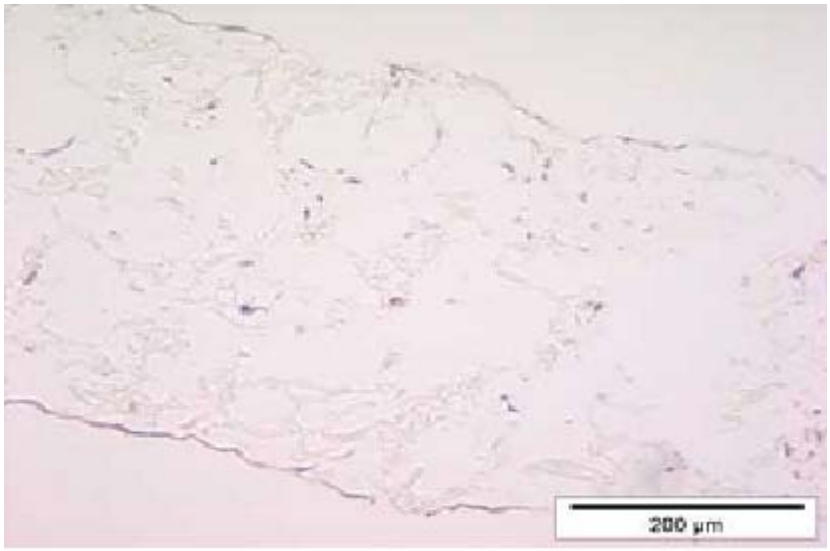

Figure 15 Hematoxylin and eosin staining of static control demonstrated a loose, less organized cellular tissue formation on both sides of the scaffold construct.

4.2.2 Immunohistochemistry

Immunohistochemistry showed positive staining for collagen types I (Figure 16), a-SMA (Figure 17) and fibronectin (Figure 18) throughout the TE constructs, whereas staining for CD31 and CD34 was negative.

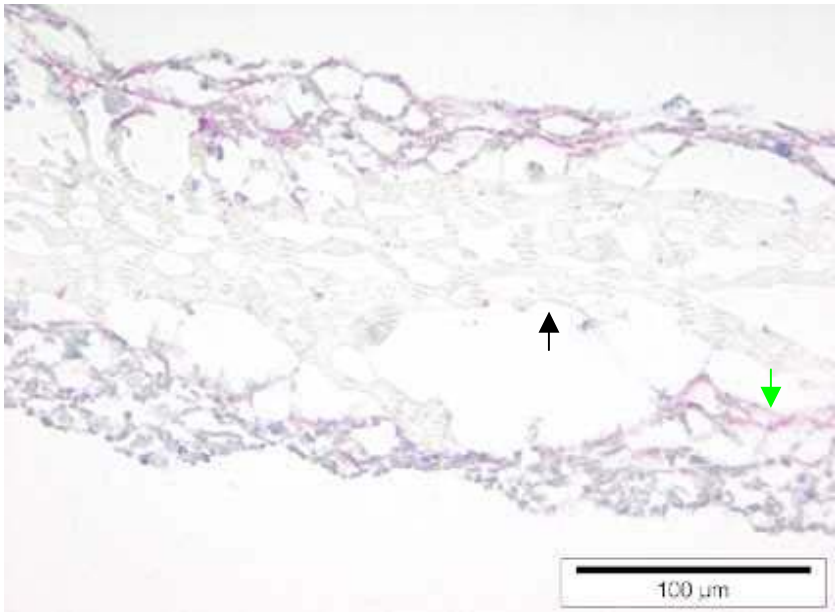

Figure 16 Positive immunohistochemical staining for collagen I (pink in color, green arrow) of human SMC-seeded tissure engineered P4HB patch. Polymeric scaffold was partially absorbed (gray in color, black arrow)

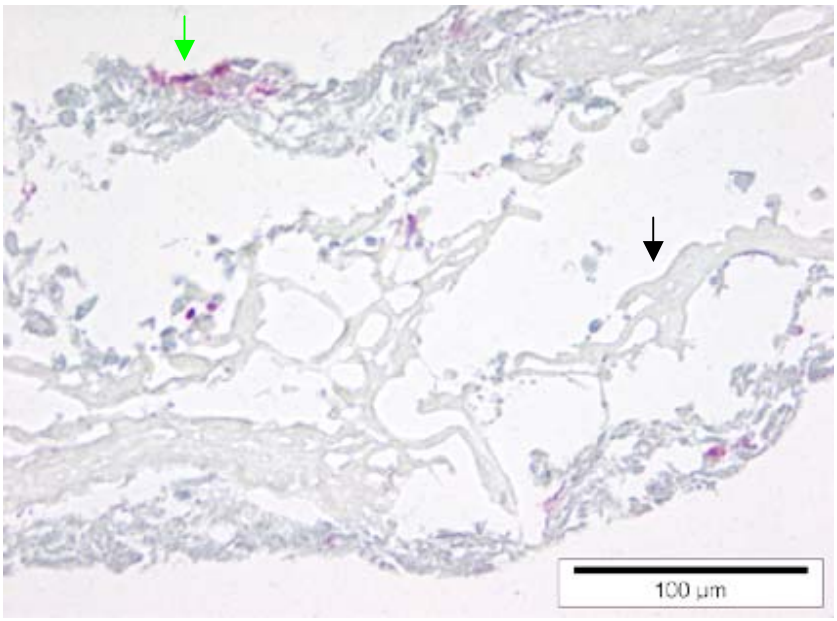

Figure 17 Immunohistochemical staining for a-SMA of TE patch was positive (pink in color, green arrow). Polymeric scaffold was not stained (gray in color, black arrow).

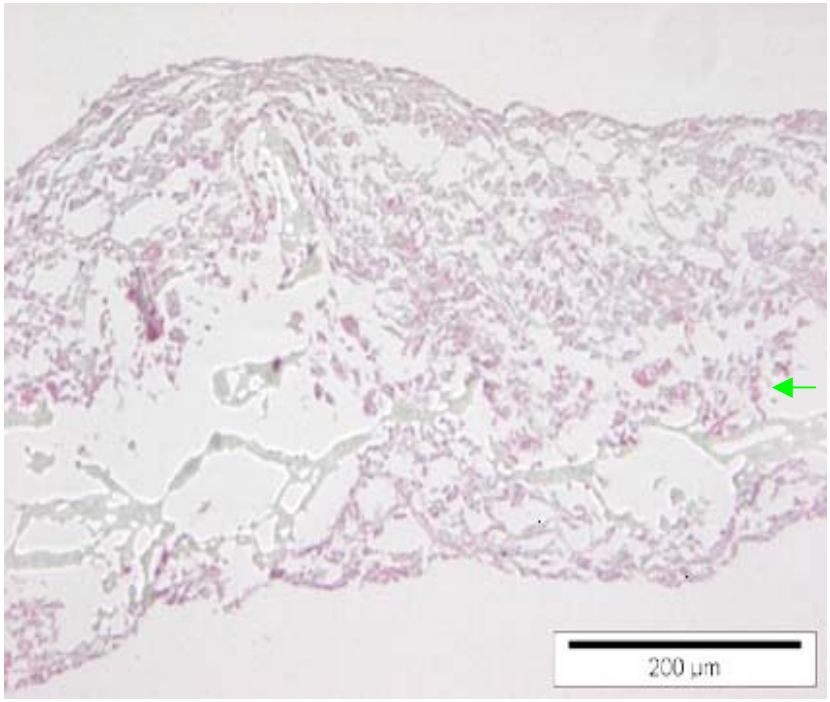

Figure 18 Staining for fibronectin of TE patch was positive (pink in color, green arrow).

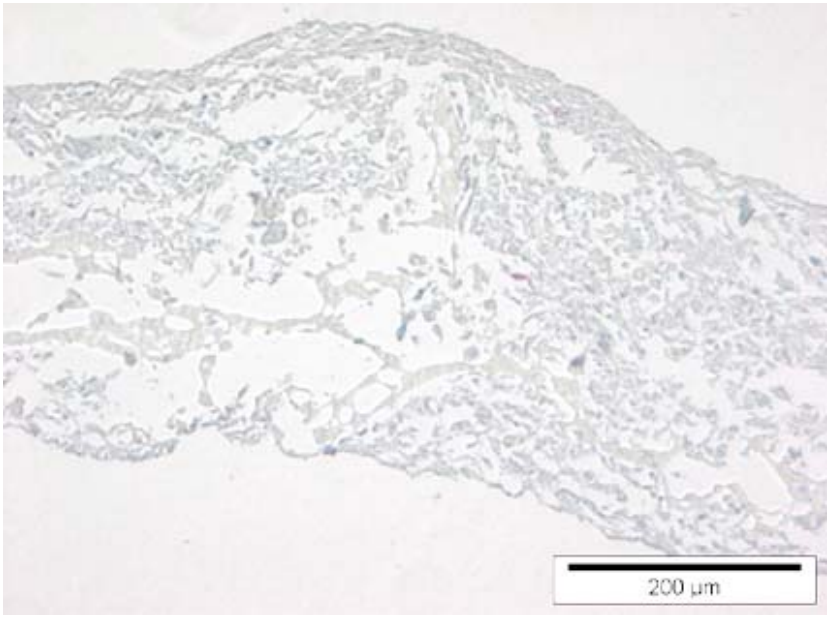

Figure 19 Staining for CD31 of TE patch was negative.

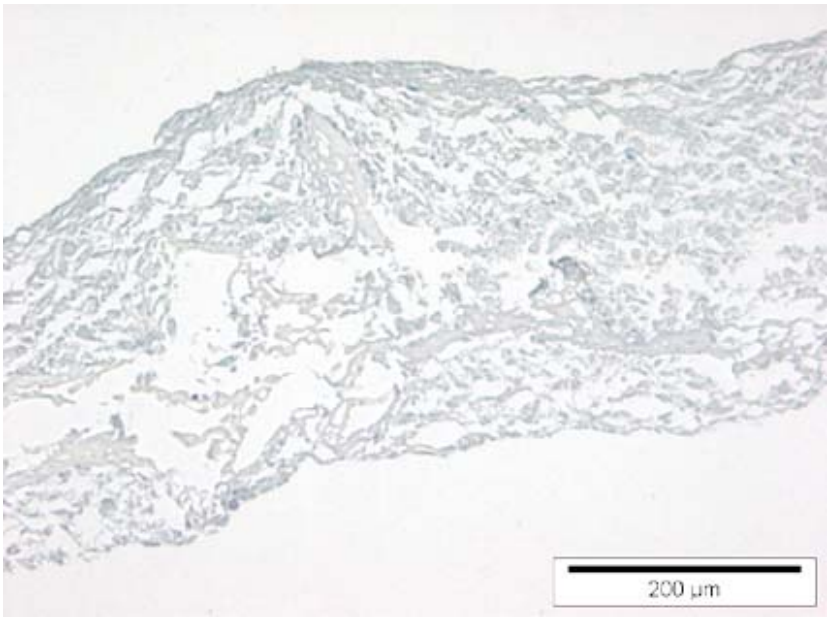

Figure 20 Immunohistochemical staining for CD34 was negative.

4.2.3 Scanning electron microscopy

The scanning eletron microscopical examination showed that cells attached well to the surface of the P4HB scaffold and formed a homogenous confluent smooth surface after conditioning in the bioreactor system (Figure 21), whereas the static controls showed a less confluent, inhomogenous surface (Figure 22).

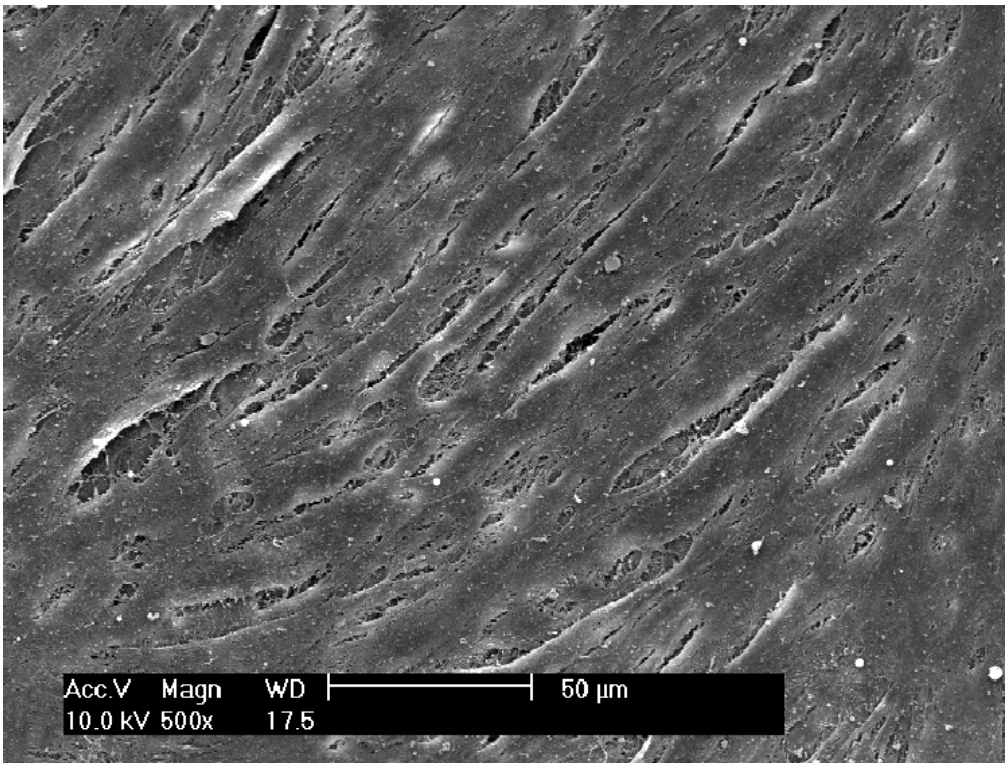

Figure 21 ESEM of the TE patch demonstrated homogenous tissue and confluent smooth surfaces with cell orientation in the direction of flow exposition.

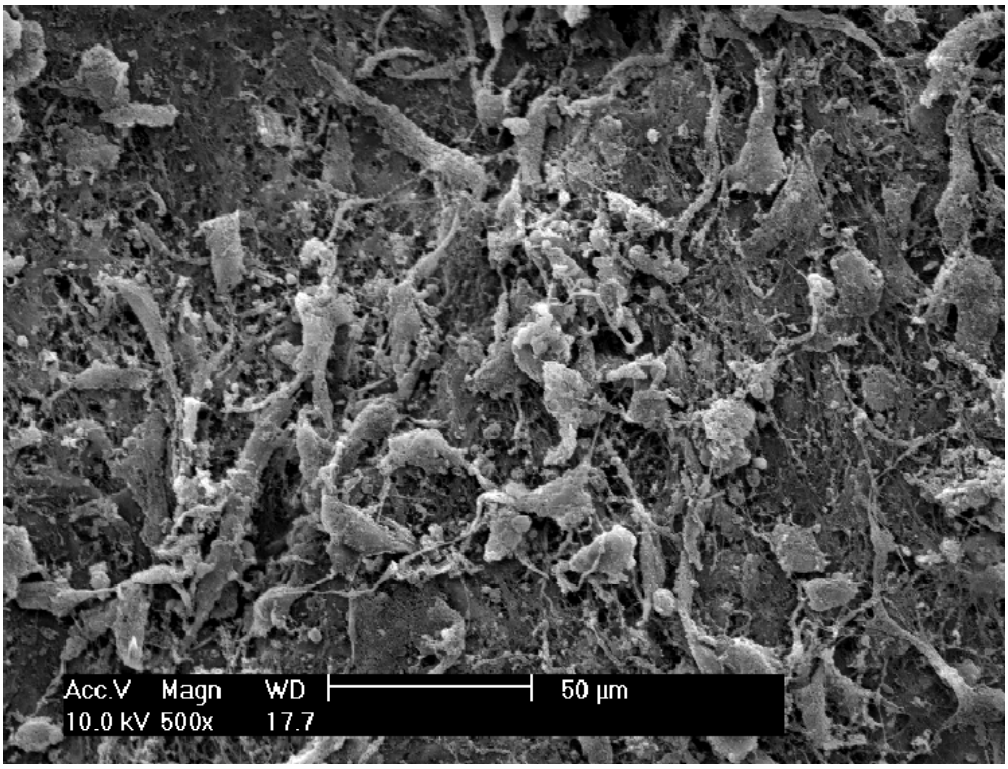

Figure 22 The static controls showed a less confluent, inhomogenous surface without cellular orientation.

4.3 Extracellular matrix formation

Additionally, we demonstrated the capacity to generate collagen and elastin under *in vitro* pulsatile flow conditions in our biochemical examination.

Collagen and elastin formation occurred under both static and flow conditions. The collagen of the conditioned TE patch showed significantly higher values compared with the static TE patch ($p < 0.05$) (Figure 23). The levels of elastin were found to be more increased in conditioned TE patches compared with the static controls, but the difference was not significant ($p > 0.05$) (Figure 24).

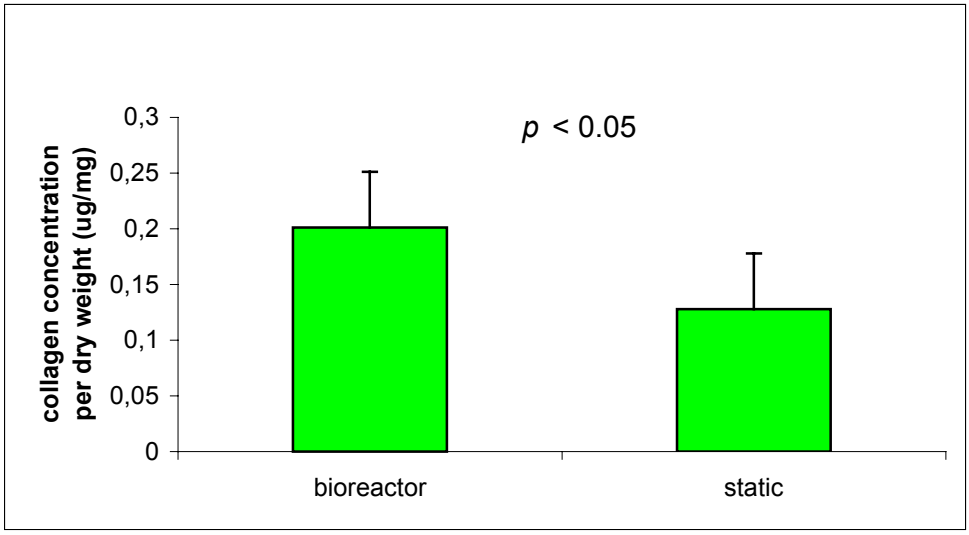

Figure 23 Collagen concentration of the dynamic vs. the static TE patch. Quantitative biochemical assays of collagen of the conditioned TE patch showed higher values compared with the static controls ($p < 0.05$).

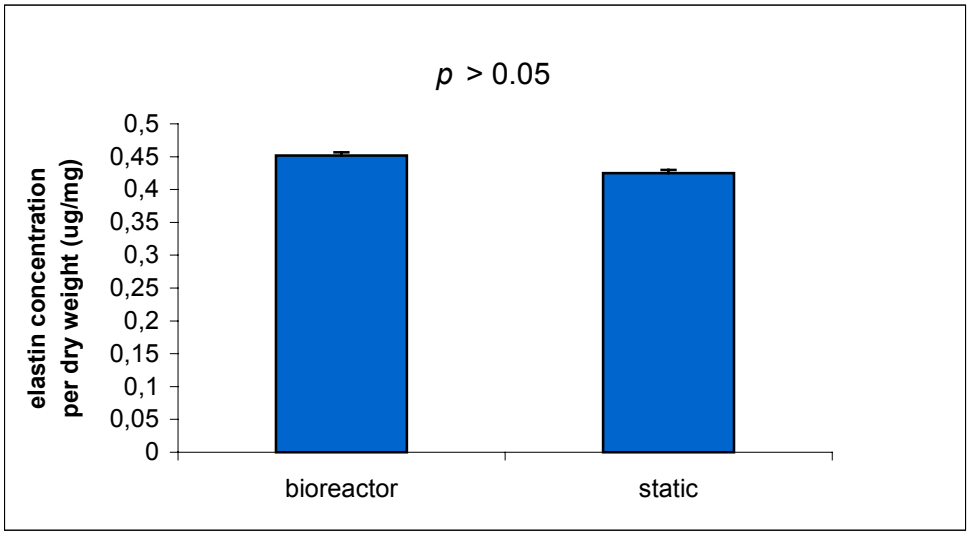

Figure 24 Quantitative biochemical assays of elastin of the conditioned TE patch showed higher values compared with static control, but the difference was not significant ($p > 0.05$).