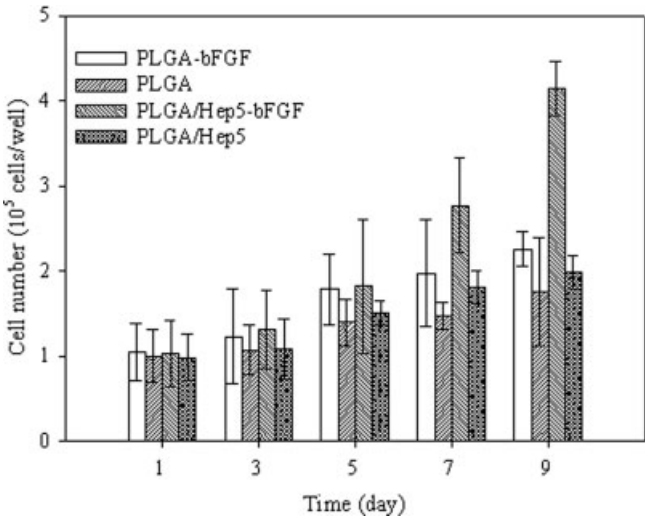

Figure 3. Proliferation of HUVECs cultured with different PLGA scaffolds having different amounts of immobilized heparin.

of bFGF from the specifically interacted bFGF fraction. The dissociation rate of free bFGF from bFGF-heparin affinity complexes was primarily governed by the concentration difference between free bFGF species in the release medium and bFGF species bound to heparin-immobilized on the surface.[29] Because specific bFGF binding to heparin is dictated by a thermodynamic equilibrium with a binding constant of 0.47 μM, decreasing the concentration of free bFGF in the release medium would shift the equilibrium toward the dissociation of bFGF from the bFGF-heparin complexes. Hence, the sustained release pattern observed after the burst release was most likely due to the gradual dissociation of free bFGF from the bFGF-heparin complexes with decreasing concentration of free bFGF in the medium. Since the release medium was replenished with fresh buffer solution at predetermined time intervals, the concentration of free bFGF in the medium could be maintained low enough throughout the release experiment. This led to continuous dissociation of bFGF from the complexes, ultimately resulting in the sustained bFGF release pattern. From this point of view, the initial burst release could also be caused in part from the subtle bFGF dissociation from the complexes upon incubation of bFGF-loaded heparin-immobilized scaffolds in fresh buffer medium, besides the nonspecific binding effect as mentioned above. Because most bFGF species was specifically bound on the surface of porous PLGA scaffolds, the observed controlled release was not likely to occur by a passive diffusion mechanism or a polymer erosion enhanced diffusion mechanism. Similar release patterns were observed for various heparin conjugated hydrogel systems such as alginate and hyaluronic acid.[9,25] Hence, the immobilization of heparin onto the inner surface of porous PLGA scaffolds was a more effective way in controlling bFGF release rate over an extended period, compared to other methods that directly incorporated bFGF into the inner pore space of the scaffold or entrapped PLGA microspheres loaded with bFGF.[23,24]

For bioactivity assay of the released bFGF fractions, HUVECs were cultured with the four different PLGA scaffolds. As shown in Figure 3, the proliferation of HUVECs cultured with bFGF-loaded heparin-immobilized PLGA scaffolds (PLGA/Hep5-bFGF) was significantly higher when compared with other PLGA scaffolds. During the culture period, the cell number of HUVECs cultured with PLGA/Hep5-bFGF increased to fourfold of initially seeded cell number, while bFGF-adsorbed PLGA scaffolds (PLGA-bFGF) exhibited modest increase in cell number. This result verified that bFGF fractions released from heparin-immobilized PLGA scaffolds preserved their bioactivities to a greater extent. The above results additionally suggest that sustained release of bFGF from the scaffolds was very important to stimulate cell proliferation.[21,22] Considering the fact that the half-life of bFGF in serum conditions is very short, it is conceivable that bFGF slowly released from the scaffolds could maintain its threshold concentration in the cell culture medium required for cell proliferation. The proliferation profiles of HUVECs with different bFGF loading amounts are shown in Figure 4. It can be seen that the proliferation of HUVECs was accelerated by increasing the

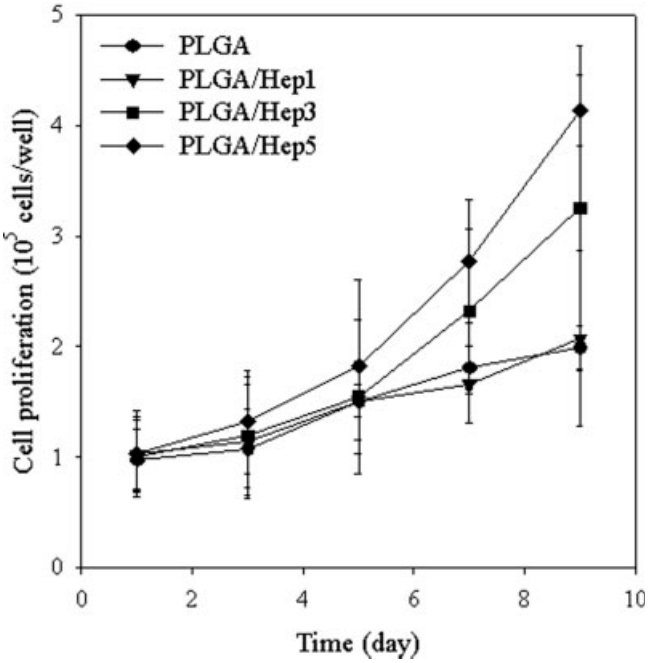

Figure 4. Proliferation of HUVECs cultured with heparin-immobilized scaffolds having different bFGF loading amounts.