proliferation of endothelial cells when exposed to the IDPs of the bioactive glass-ceramic scaffolds 45S5.5Li (M199 + 45S5.5Li) and it would be expected to be due to the presence of Li^+ in the culture medium.

On the other hand, after stimulation with the IDPs of the bioactive glass-ceramic scaffolds 45S5.5Li (M199 + 45S5.5Li), the migratory response of HUVECs showed a statistically significant increase, managing to close the gap in approximately 40% of the total area at 8 h poststimulation, this response being equivalent to using M199 medium supplemented with VEGF (Figs. 7.3 and 7.4).

In addition, at 6 h poststimulation using M199 + 45S5.5Li, the capacity of HUVECs to form endothelial tubules in Matrigel also showed a statistically significant increase, with a similar response to that achieved with culture medium supplemented with VEGF (Figs. 7.5 and 7.6).

At 24 h poststimulation, the expression of β-catenin in HUVECs treated with M199 + 45S5.5Li showed a statistically significant increase in comparison to the control, with an equivalent response to that obtained with the use of M199 + 0.60 mmol LiCl and M199 medium supplemented with VEGF (Fig. 7.7A and B). In line with these results, Zeilbeck et al. (2014) showed that the incubation of human dermal microvascular endothelial cells with LiCl caused an increase in the levels of β-catenin and their translocation to nuclei, indicating an activation of the Wnt/β-catenin signaling pathway.

7.2.2 In Vivo Evidence

Little has been done to investigate the in vivo angiogenic effects of the Li-containing BGs. The proangiogenic potential was studied on chorioallantoic membranes (CAMs) of quail (*Coturnix Coturnix japonica*) embryos following the methodology of Parsons-Wingerter et al. (1998). Fertilized eggs, which were incubated in ovo at 38°C for 2 days and 60% relative humidity, were used. Their content was poured carefully in six-well culture plates. At 7 days of incubation, 0.5 mL of embryonic medium (EM) enriched with IDPs (EM + 45S5; EM + 45S5.5Li) was applied to the CAMs (Fig. 7.8). EM with 10 μg mL^{-1} bFGF (EM + bFGF) was used as a positive control and EM without IDPs or bFGF was used as a negative control. The effect of EM enriched with 0.20 mmol LiCl or NaCl was also evaluated, considering unstimulated CAMs as the absolute control (AC).

Integrin αvβ3 is expressed at low levels on quiescent endothelial cells, but is significantly upregulated on proliferating endothelial cells during angiogenesis (Haro Durand et al., 2015). The ELISA assay showed a statistically significant increase (29%) in the levels of expression of the β3 subunit of integrin αvβ3 in CAMs 2 days after treatment with the IDPs of 45S5.5Li (EM + 45S5.5Li) in comparison to the AC, negative control (EM), and CAMs treated with EM enriched with IDPs of the 45S5 glass (EM + 45S5) (Fig. 7.9). However, the greatest expression (76%) of the β3 subunit was observed with the use of