ABSTRACT
Relatively few attempts have been made in the past to isolate and expand lymphatic endothelial cells (LECs). Recently this task has become feasible thanks to the identification of new lymphatic markers such as Podoplanin, Lyve-1, Prox-1 and D2-40. Using a two-step purification method based on the sorting of endothelial cells with Ulex Europaeus Agglutinin 1-coated beads followed by purification with monoclonal antibody D2-40, we were able to purify and in vitro expand human derived LECs from tissues such as lymph-node, spleen, thymus, palatine tonsil and iliac lymphatic vessels. The isolated LECs were expanded on collagen type 1 and fibronectin coated flasks for up to 8-10 passages and then analyzed for phenotypic and functional properties. LECs were able to form a capillary like network, when seeded on Cultrex BME, indicating their capability to form lymphatic vessels in vitro. Comparative studies were performed, and we found that specific lymphatic and vascular markers were differentially expressed by LECs prepared from different sources, clearly demonstrating the phenotypic heterogeneity of LECs from different organs and different segments of the lymphatic vasculature. We here propose a new technique to make available ready sources of abundant well-characterized human LECs to examine normal profiles and behavior to compare with abnormal conditions.