Introduction:
Several growth factors (GFs) participate in the regulation of cell proliferation, migration, differentiation and apoptosis. GFs such as VEGF-A and FGF-b are essential to trigger the angiogenic cascade that is crucial for the subsequent steps of new tissue formation. Human Platelet’s lysate (hPL) has been used as an autologous source of GFs. Herein, we investigated whether marine-origin polysaccharides are able to attract and stabilize pro-angiogenic GFs from PL and activate endothelial cells by providing pro-angiogenic cues.

Materials and methods:
κ-κ, λ-κ-carrageenan (Car), alginate, chitosan and heparin were purchased from Sigma-Aldrich. Human PL was obtained as described elsewhere. The interaction of the mentioned PE with diluted PL (1, 3 and 6 bilayers), in the absence of ECGS and with 10% serum. The effect of the VEGF-A and FGF-b binding quantified by ELISA (Peportech). Human umbilical vein endothelial cells (HUVECs) were cultured in M199 culture media supplemented with 20%FBS and ECGS. Cells were seeded on 48-well plates previously modified with the nanocoatings prepared by Layer-by-Layer assembling, i.e. by the alternating deposition of each of the mentioned PE with diluted PL (1, 3 and 6 bilayers), in the absence of ECGS and with 10% serum. The effect of the VEGF-A and bFGF on HUVECs was assessed in cultures established with 150 or 200 nM of FGF/VEGF Receptor Tyrosine Kinase Inhibitor (Santa Cruz Biotechnology).

Results:
The thickness of the nanocoatings was measured by ellipsometry and VEGF-A and FGF-b binding quantified by ELISA (Peportech). Human umbilical vein endothelial cells (HUVECs) were cultured in M199 culture media supplemented with 20%FBS and ECGS. Cells were seeded on 48-well plates previously modified with the nanocoatings prepared by Layer-by-Layer assembling, i.e. by the alternating deposition of each of the mentioned PE with diluted PL (1, 3 and 6 bilayers), in the absence of ECGS and with 10% serum. The effect of the VEGF-A and bFGF on HUVECs was assessed in cultures established with 150 or 200 nM of FGF/VEGF Receptor Tyrosine Kinase Inhibitor (Santa Cruz Biotechnology).

Discussion and conclusions:
Nanocoatings composed by sulfated marine-origin polysaccharides and hPL bio-active endothelial cells inducing the formation of tube-like structures. The formation of the tube-like structures, which depended on the PE and number of bilayers, was achieved after 20 hours of incubation and was mediated by the VEGF/FGF. The combination of hPL with these PEs may be an efficient and simple method to introduce pro-angiogenic cues in any 2D/3D cell-material interface and improve tissue regeneration.

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References: