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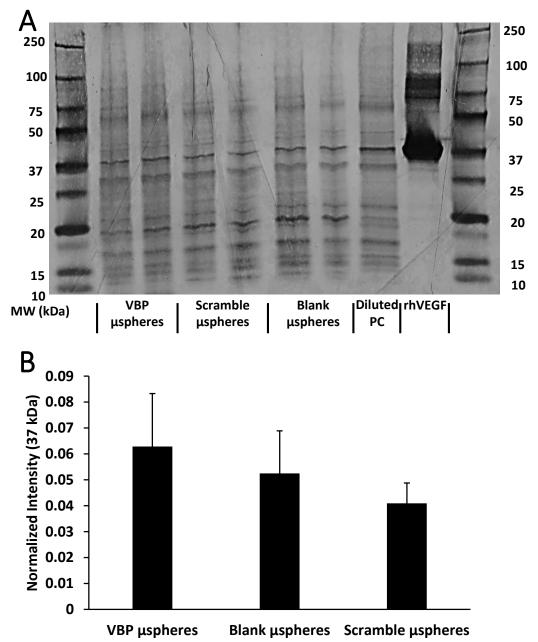


Figure 15. Unique proteins are present in microspheres after incubating in PC. A: SDS-PAGE gel of microspheres (μspheres) after incubation in PC, brief washing, and treatment with Laemli buffer (under non-reducing conditions). After incubation of microspheres (VBP, Scramble, and Blank) with PC as described above, 1 mg microspheres per condition were briefly washed in PBS and suspended in 25 μL PBS and 25 μL Laemli buffer (Biorad) without β-mercaptoethanol (non-reducing conditions) at 55°C for 10 minutes. Control conditions consisted of diluted PC in PBS, recombinant VEGF control (R&D Systems, 100 ng/mL in 0.1 wt.% BSA in PBS), and protein ladder (Biorad) diluted 10-fold in PBS. Subsequently, microsphere suspensions and controls were loaded at 10 μL per well into the wells of a 4-15% gradient Mini Protean TGX precast polyacrylamide gel (Biorad). Gels were then placed in a Biorad electrophoresis chamber which was filled with a running buffer (25 mM Tris-HCl, 192mM Glycine, 0.1% SDS), and gels were run at 110V for 55 min. Subsequently, standard silver stain protocol was performed (GE Healthcare Protein Silver Stain Kit), and the stained gel was imaged using a standard 8 MP camera with backlighting. Subsequent staining with SilverStain demonstrated the presence of several unique proteins that were present in all microsphere types after incubation with PC. B: We performed densitometry analysis in ImageJ of the SDS-PAGE band at 37 kDa, which corresponds to the expected molecular weight of VEGF in non-reducing conditions and to the experimental molecular weight of recombinant VEGF in control (rhVEGF). We calculated the intensity of the 37 kDa band in each lane and normalized the intensity at 37 kDa to the sum of intensity of all peaks (from 10 kDa to 250 kDa). The data are presented as the mean normalized intensity +/- one standard deviation for two lanes per condition. No statistical significance was observed between conditions using one-way ANOVA and Tukey's post-hoc test (p-value > 0.05).