Supporting Information

to

A Novel Plasma-based Bioink Stimulates Cell Proliferation and Differentiation in Bioprinted, Mineralized Constructs

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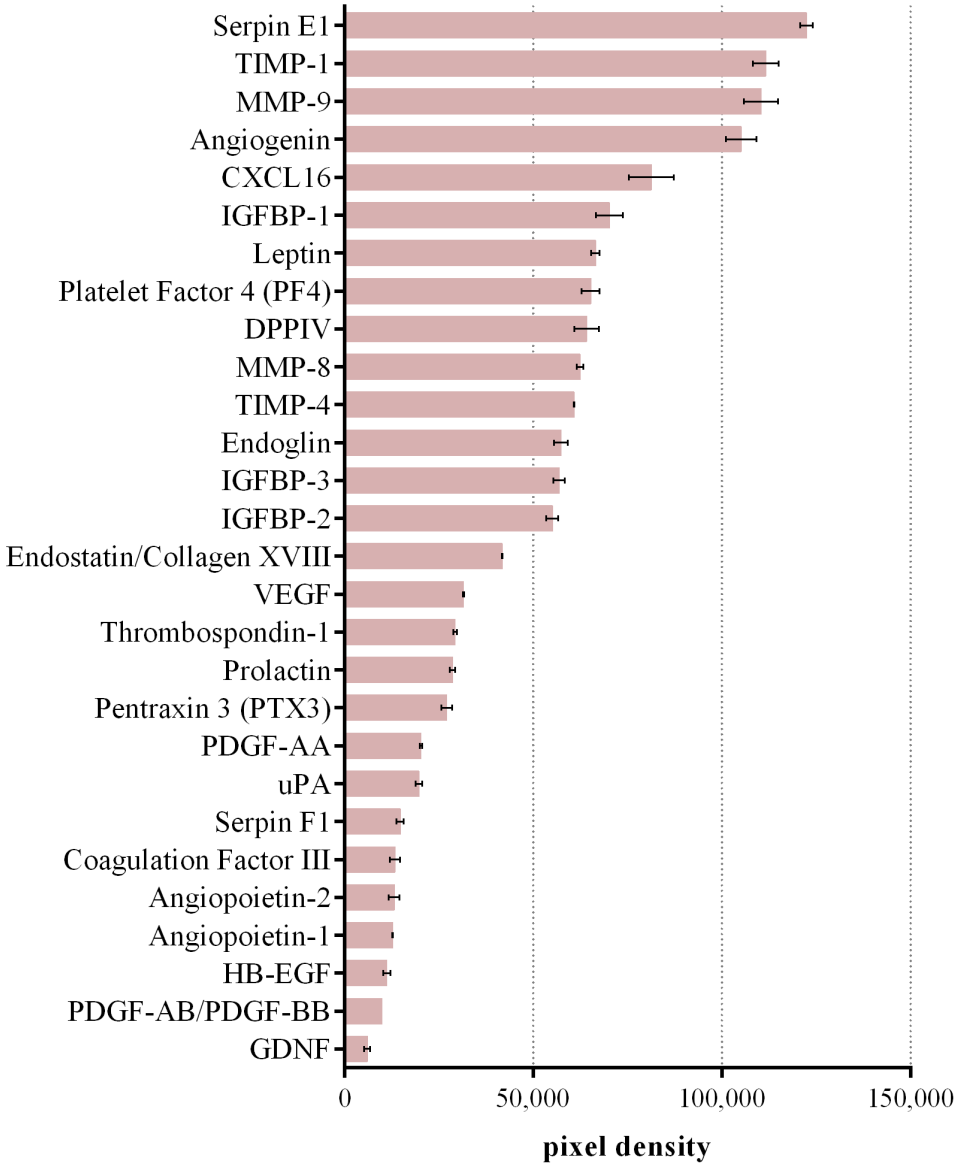

Figure S1. Full angiogenic protein array of human blood plasma (in total 50 tested proteins). The array revealed 28 angiogenic proteins.

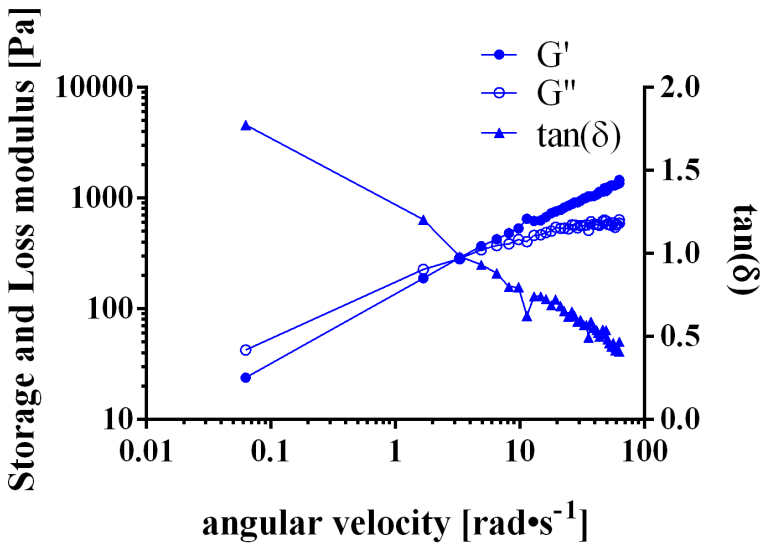

Figure S2. Frequency sweep analysis of the plasma-alg-mc ink. The test revealed that G'' was higher than G' at low angular velocities indicating that it is not crosslinked in zero-shear state.

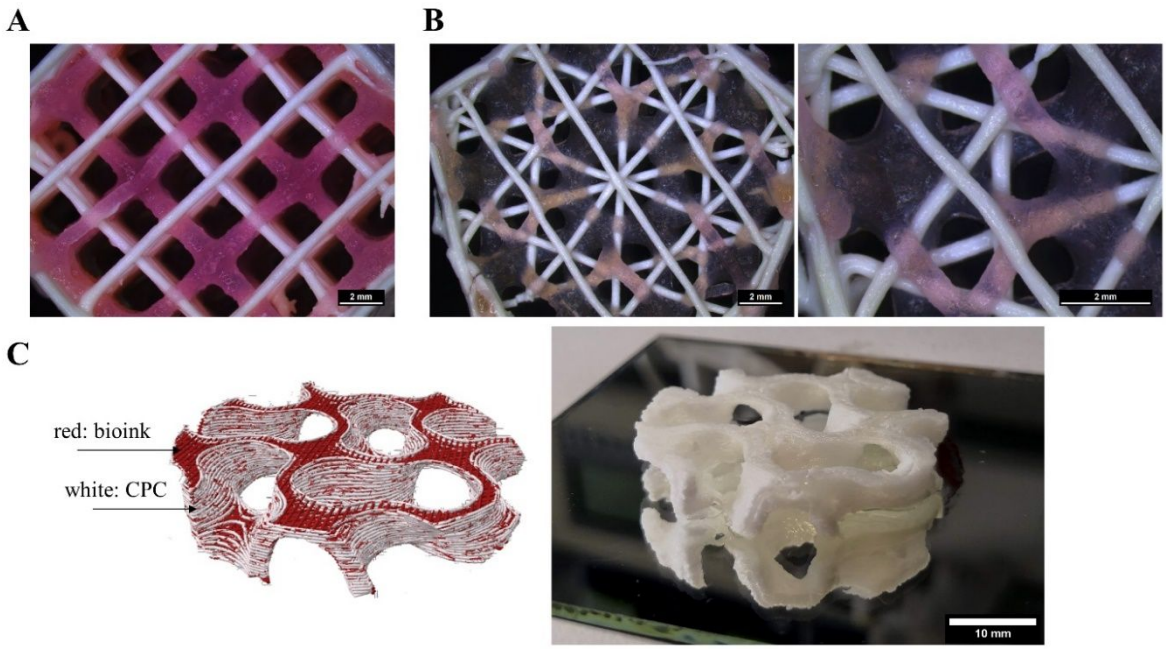

Figure S3. (A) Microscopic picture of a biphasic scaffold consisting of CPC/plasma-alg-mc, plotted in a 0°/90°. (B) A more complex layer orientation of 72° changed the pore morphology of the biphasic constructs. (C) Biphasic gyroid structure of CPC and plasma-alg-mc, which could be fabricated with high shape fidelity.

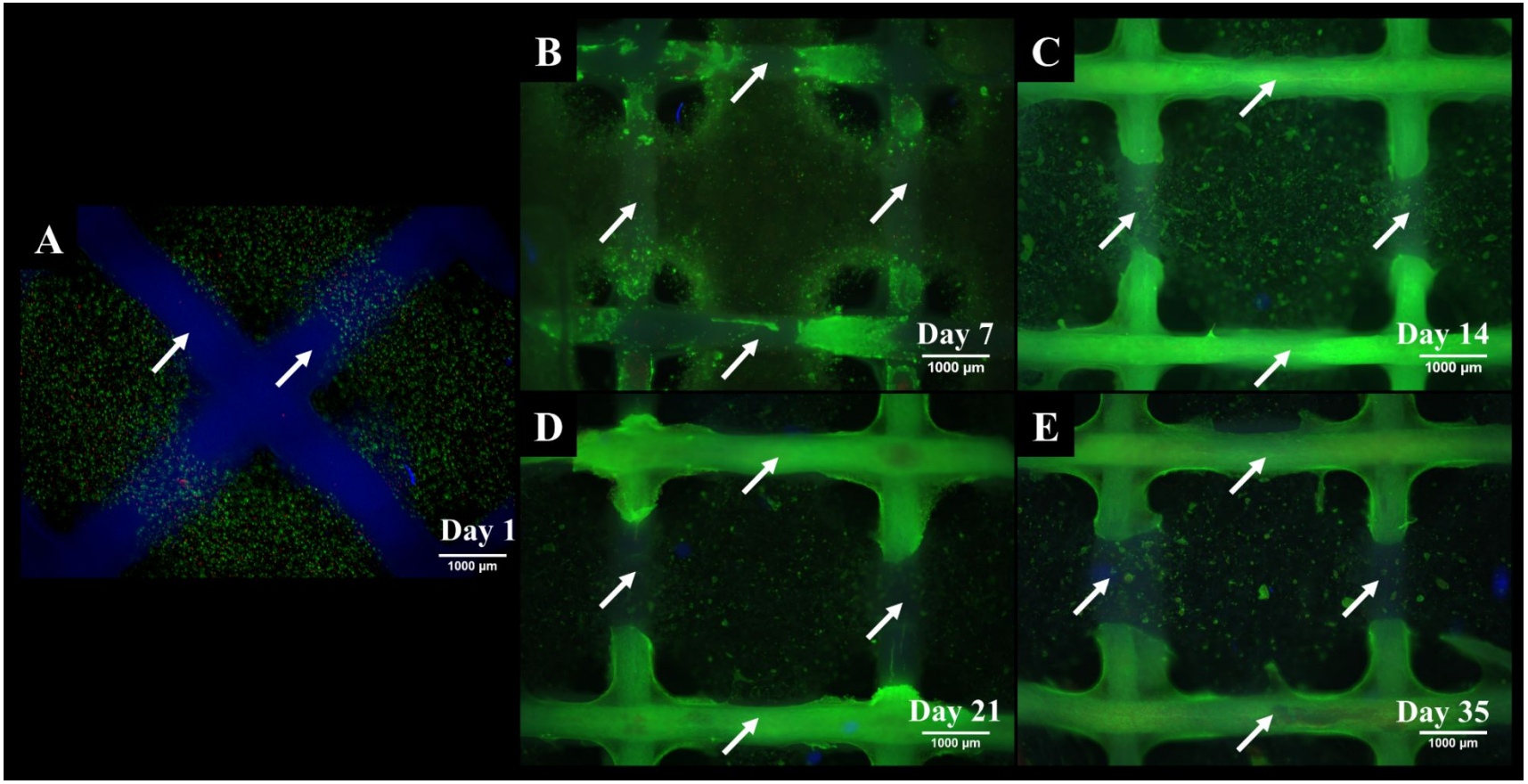

Figure S4. Live/Dead images of bioplotted MSC over 35 days (green – metabolically active cells, red – dead cells, blue - autofluorescent CPC, denoted by the white arrows). MSC migrated onto CPC strands in the first week of culture (A,B). Afterwards, the proliferated strongly, covering the CPC strands by a very strong cell layer (C). Then, the MSC started to bridge the pores (day 21, D). The cell layer did not detach, but stayed in shape and cells started growing back inside the plasma-alg-mc bioink (E).

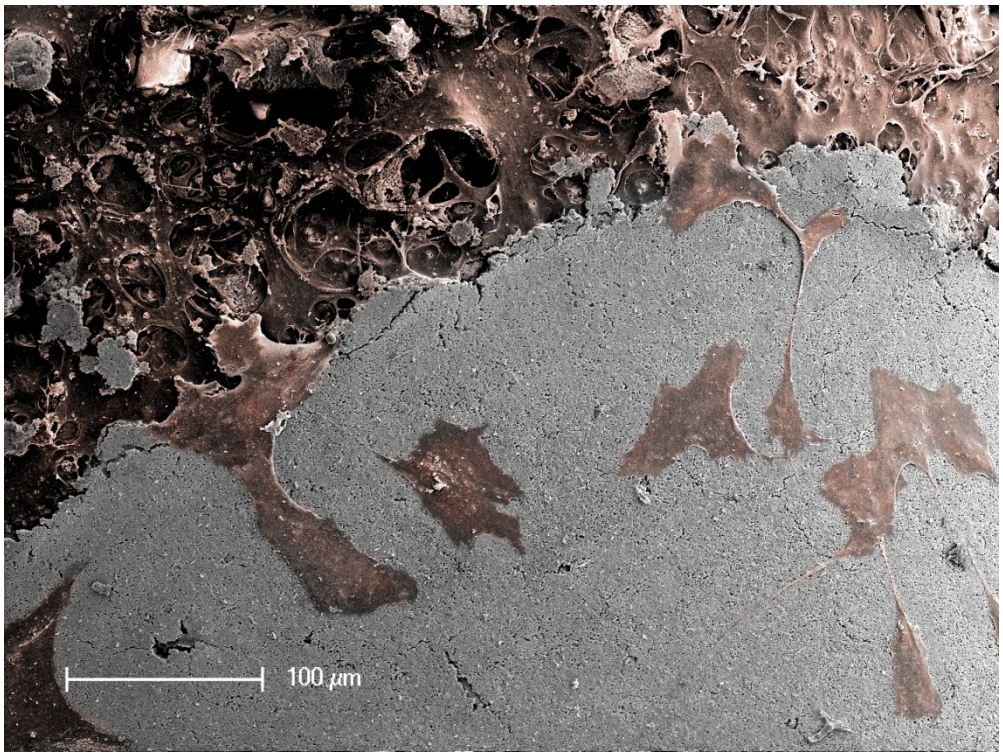

Figure S5. Colored SEM image of MSC migrating from the bioink onto the smooth CPC surface at day 7.

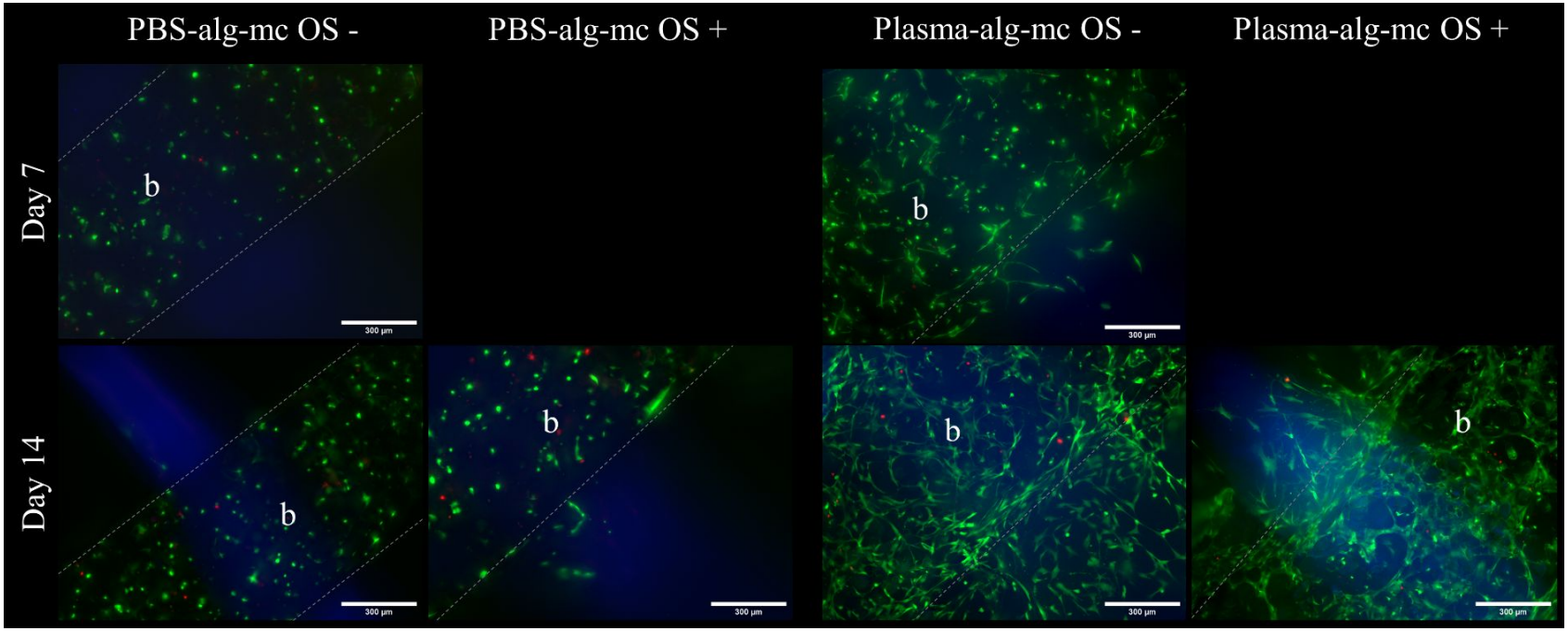

Figure S6. Bioplotting of hOB preosteoblasts in combination with CPC. All images demonstrate live/dead stainings at the CPC/bioink interface with either PBS-alg-mc or plasma-alg-mc as bioink (green - metabolically active hOB, red – dead hOB, blue – autofluorescent CPC; the bioink is marked by the letter b). In the biphasic CPC/PBS-alg-mc structures, hOB demonstrated a roundish shape. In biphasic plasma-alg-mc constructs, hOB were able to spread within the bioink and migrated on top of the CPC strands after 7 d. There, they strongly proliferated covering the entire CPC surface. Scale bars represent 300 µm.

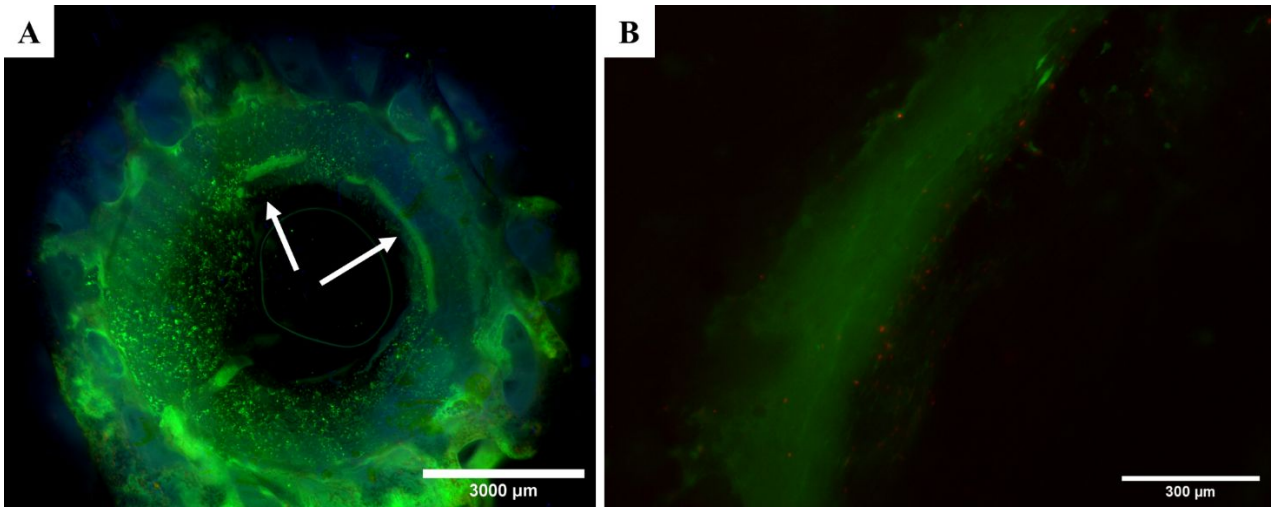

Figure S7. Bioplotted model structure of a HUVEC/MSC co-culture after 35 days, shown is a fluorescence microscopic image of a representative live/dead stainings (green cells are alive, red cells are dead, CPC has a blue autofluorescence). (A) The plotted plasma-alg-mc channel did not close over the culture period of 35 days, furthermore cells started to cover the outer surface of the channel (white arrows). (B) Higher magnification image of the cells covering the surface of the plotted channel-like structure.

Video S1. 3D plotting of a scaphoid bone. The CAD file was reconstructed from CT data. Afterwards a three-channel bioplotter was equipped with methylcellulose (transparent) as support material, plasma-alg-mc (yellowish) and CPC (white). The video demonstrates the layerwise plotting process.