Supporting Information

Tuning cell behavior on 3D scaffolds fabricated by atmospheric plasma assisted additive manufacturing

Maria Cámara-Torres[1], Ravi Sinha[1], Paolo Scopece[2], Thomas Neubert[3], Kristina Lachmann[3], Alessandro Patelli[4], Carlos Mota[1], Lorenzo Moroni[1*]

[1] Complex Tissue Regeneration Department, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Universiteitssingel 40, 6229 ER Maastricht, The Netherlands.

[2] Nadir S.r.l., Via Torino, 155/b, 30172 Venice, Italy.

[3] Fraunhofer Institute for Surface Engineering and Thin Films IST, Bienroder Weg 54E, 38108 Braunschweig, Germany.

[4] Department of Physics and Astronomy, Padova University, Via Marzolo, 8, 35131 Padova, Italy.

* Corresponding author at: Maastricht University, MERLN Institute for Technology-Inspired Regenerative Medicine, Complex Tissue regeneration Department, Universiteitssingel 40, 6229 ER Maastricht, The Netherlands. Email address: l.moroni@maastrichtuniversity.nl

Table S1. Primer sequences used for qRT-PCR.[a]

gene	forward primer	reverse primer
B2M	ACAAAGTCACATGGTTCACA	GACTTGTCTTTCAGCAAGGA
ALP	ACAAGCACTCCCACTTCATC	TTCAGCTCGTACTGCATGTC
RUNX2	TCAACGATCTGAGATTTGTGGG	GGGGAGGATTTGTGAAGACGG
OCN	TGAGAGCCCTCACACTCCTC	CGCCTGGGTCTCTTCACTAC
SP7	CCTCTGCGGGACTCAACAAC	AGCCCATTAGTGCTTGTAAAGG
BSP	CCCCACCTTTTGGGAAAACCA	TCCCCGTTCTCACTTTCATAGAT
BMP2	ACTACCAGAAACGAGTGGGAA	GCATCTGTTCTCGGAAAACCT

[a] B2M: β-2-microglobulin, ALP: alkaline phosphatase, RUNX2: Runt-related transcription factor 2, OCN: osteocalcin, SP7: osterix, BS: bone sialoprotein, and BMP2: bone morphogenetic protein-2

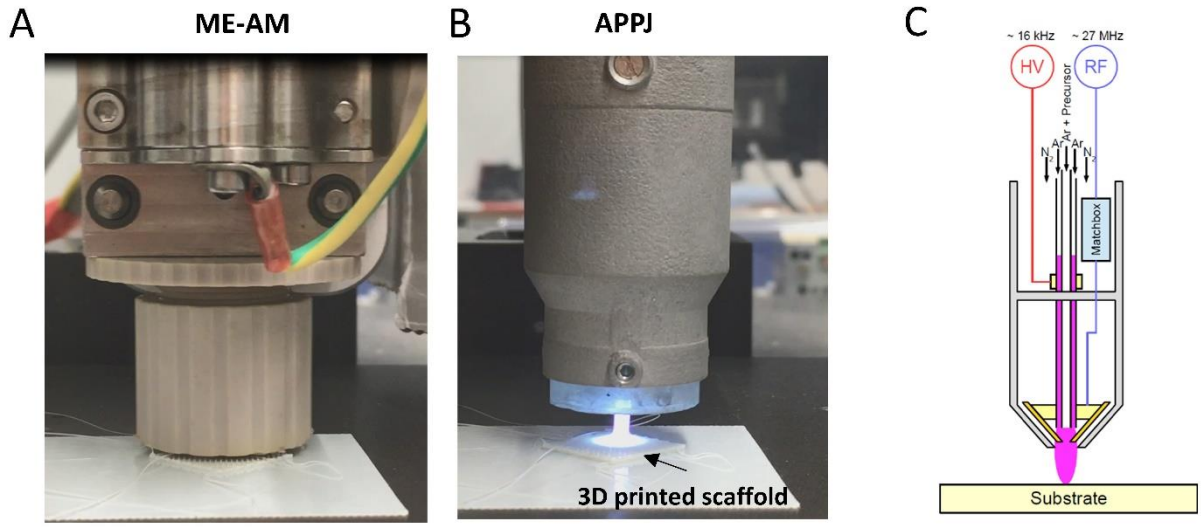

Figure S1. Hybrid additive manufacturing platform consisting of (A) a melt extrusion printhead and (B) an atmospheric pressure plasma jet (APPJ), mounted on a 3D axis stage. [1-2] (C) Schematics of the APPJ, which is based on a dielectric barrier discharge scheme simultaneously powered by a high-voltage (HV) generator and a radio-frequency (RF) generator. Electrodes are positioned externally to an alumina duct where argon is fluxed and plasma is ignited. Previously mixed precursors in vapor phase are carried by argon and mixed with the ignited plasma right before the RF generator through an inner coaxial tube, which is connected to external bubblers with precursors in liquid (APTMS, VTMOS) or solid (MA) phase. Nitrogen is carried through an outer coaxial duct for shielding and cooling at the exit of the torch.

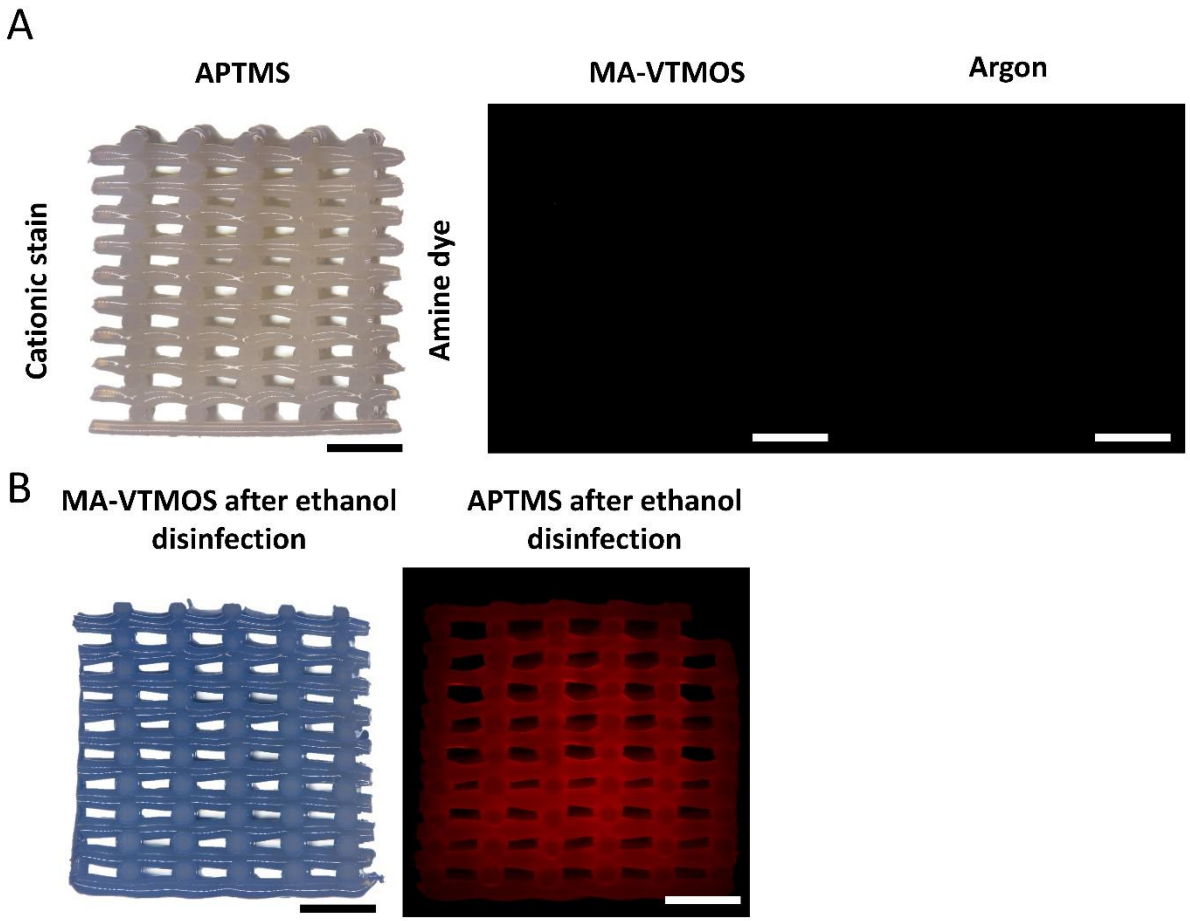

Figure S2. (A) Stereomicroscopy image of APTMS scaffold stained with the cationic dye methylene blue, and fluorescent microscopy images of MA-VTMOS and Argon scaffolds stained with amine specific dyes, to prove the specificity of the staining. (B) Stereomicroscopy image of MA-VTMOS scaffold and fluorescent microscopy image of APTMS scaffold stained with coating specific dyes after ethanol disinfection to verify the maintenance of the treatment before cell culture experiments. Scale bars 1mm.

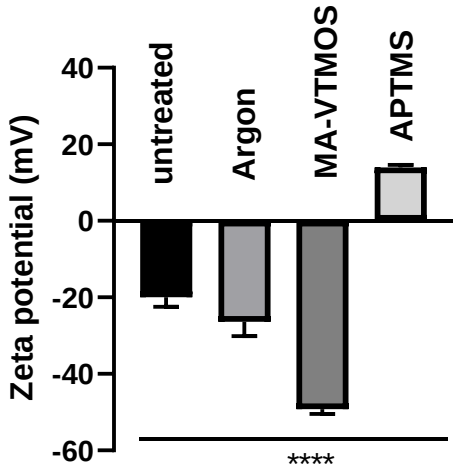

Figure S3. Zeta potential measurements of untreated, and Argon, MA-VTMOS and APTMS treated 2D substrates (melt-pressed films). Data presented as average ± s.d. and statistical significance performed using one-way ANOVA with Tukey's multiple comparison test (****. $p<0.0001$).

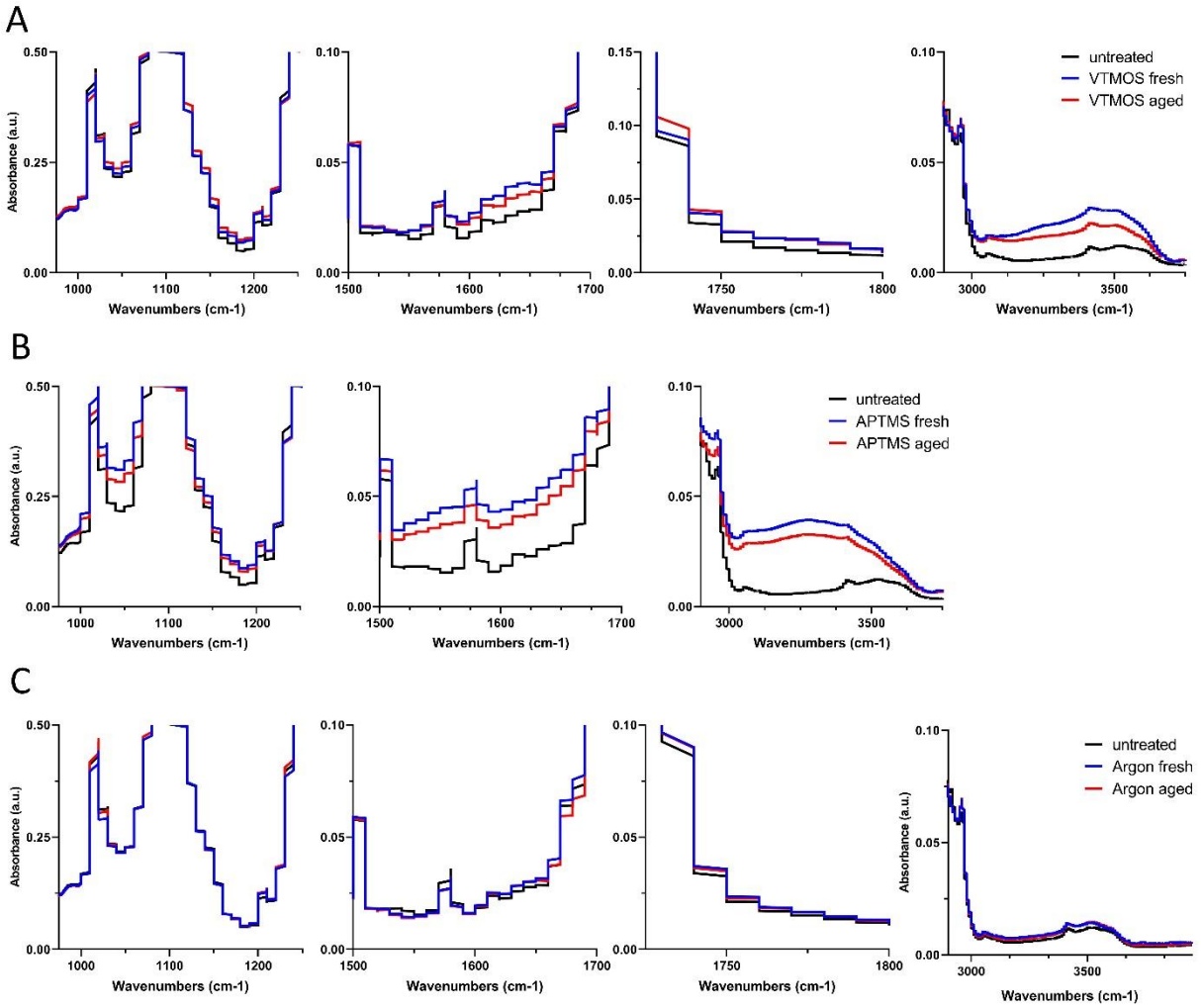

Figure S4. Regions of interest of the FTIR spectra of (A) MA-VTMOS, (B) APTMS and (C) Argon plasma treated 2D substrates (melt-pressed PEOT/PBT films).

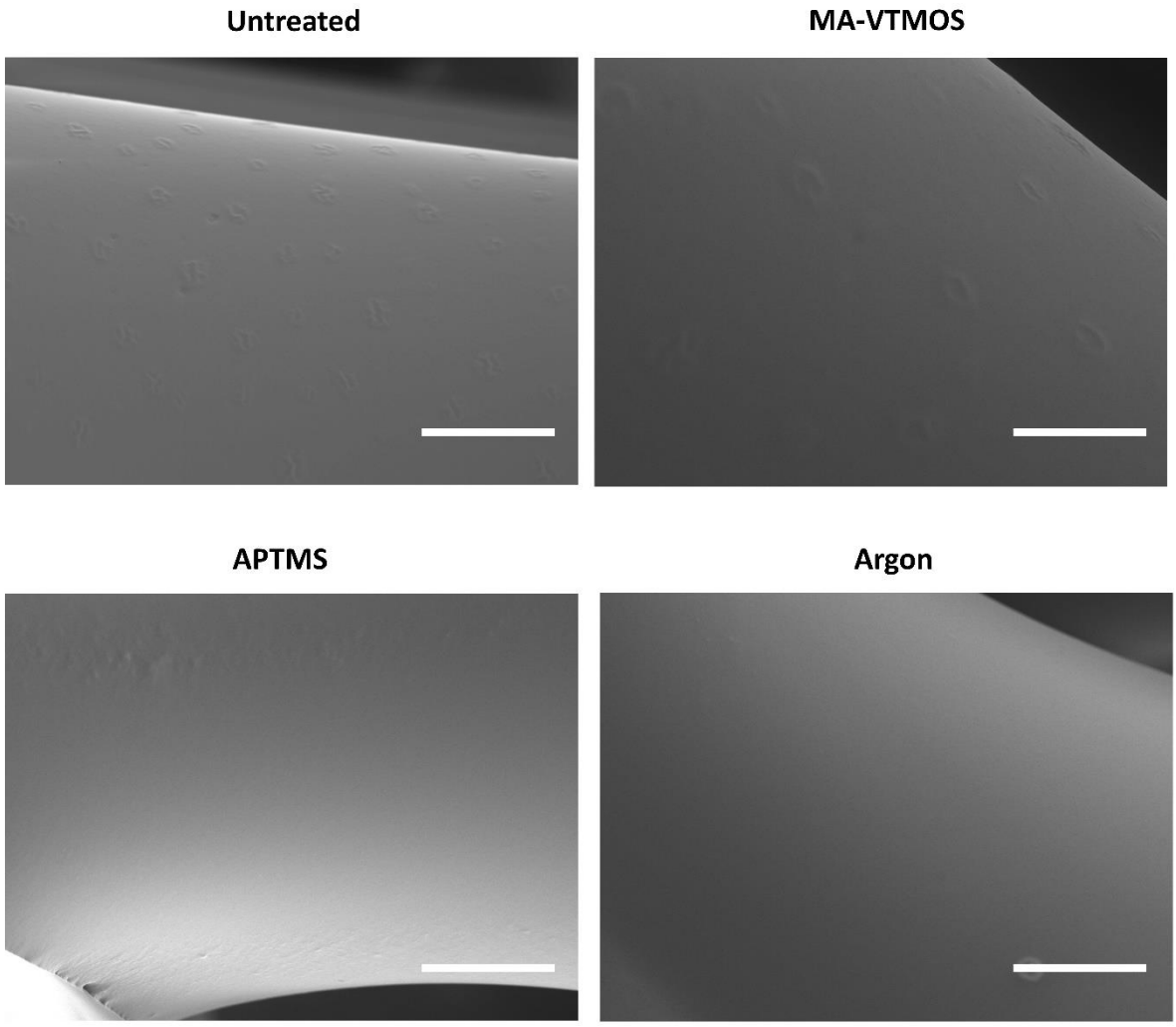

Figure S5. SEM micrographs the surface of untreated and MA_VTMOS, APTMS and Argon plasma treated scaffolds. Scale bars 50 µm.

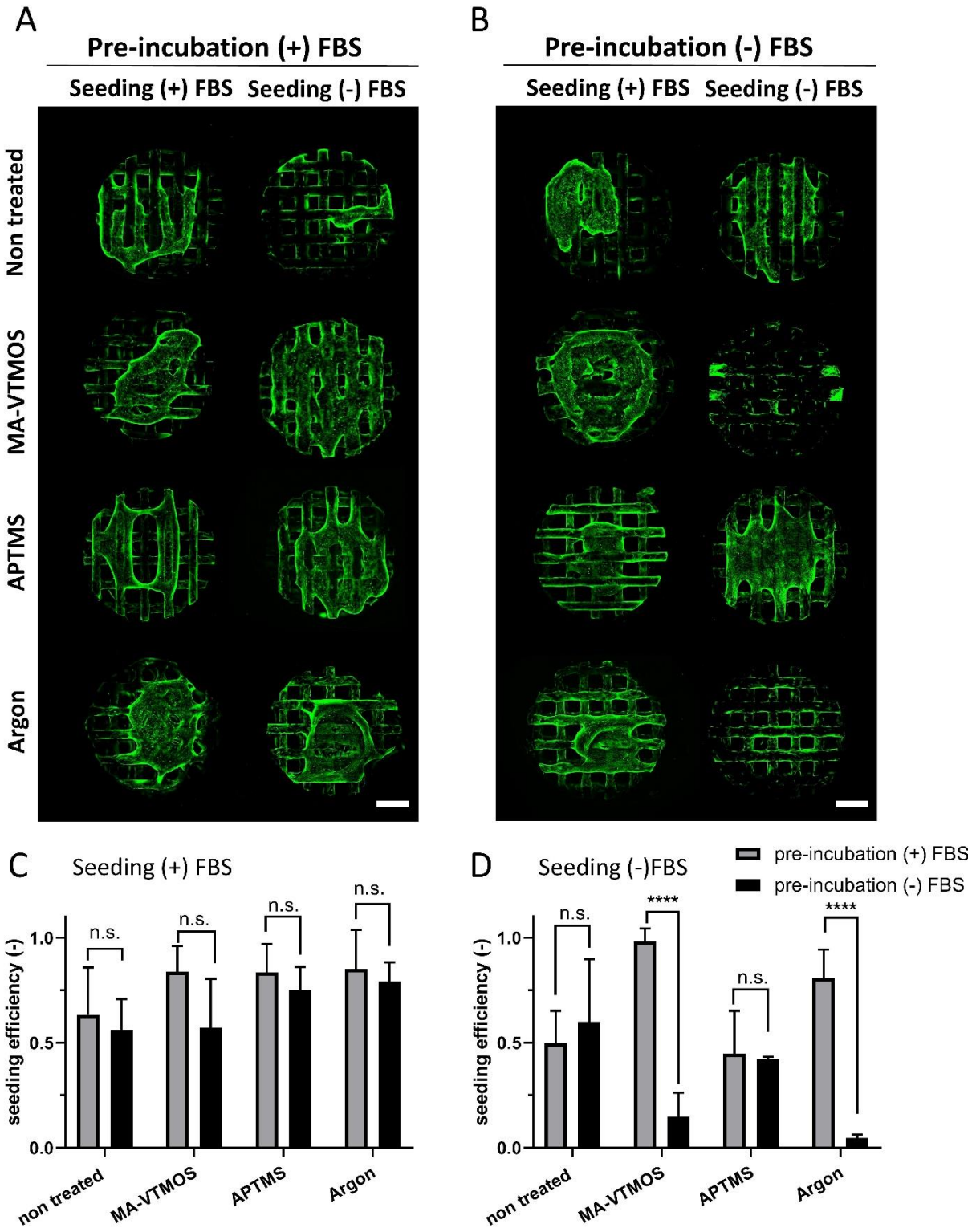

Figure S6. Fluorescence microscopy images (F-actin, green) of hMSCs in the bottom lids of scaffolds after 24h of culture and pre-incubated with (A) (+)FBS or (B) (-) FBS and seeded with (+)FBS or (-)FBS. Quantification of cell seeding efficiency on scaffolds (C) seeded with (+)FBS and (D) seeded (-)FBS. Data presented as average ± s.d. and statistical significance performed using two-way ANOVA with Tukey's multiple comparison test (n.s. $p > 0.05$; **** $p < 0.0001$).

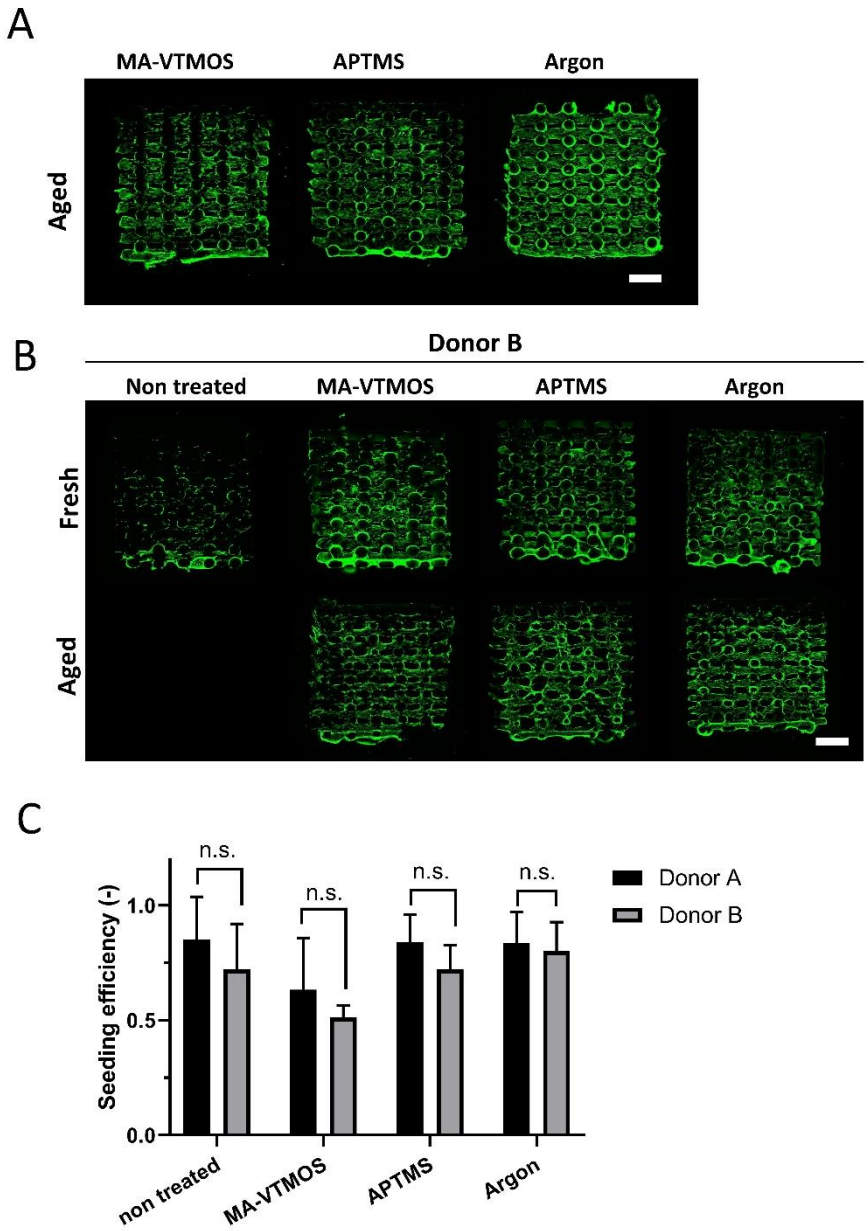

Figure S7. (A) Fluorescence microscopy images (F-actin, green) of hMSCs on the cross section of aged plasma treated scaffolds after 24h of culture, and pre-incubated and seeded with (+)FBS. Scale bars 1mm. (B) Fluorescence microscopy images (F-actin, green) of hMSCs from a different donor (donor B) on the cross section of fresh and aged plasma treated scaffolds. Scaffolds were pre-incubated and seeded with (+)FBS. Scale bars 1 mm. (C) Seeding efficiency on plasma treated scaffolds comparing hMSCs from 2 different donors. Data presented as average ± s.d. and statistical significance performed using two-way ANOVA with Tukey's multiple comparison test (n.s. $p > 0.05$).

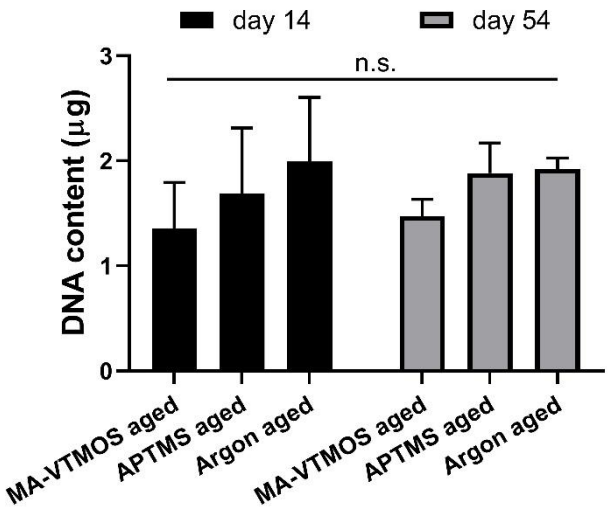

Figure S8. DNA content on aged plasma treated scaffolds after 14 or 54 days of culture (7 days or 47 days in MM, respectively). Data presented as average ± s.d. and statistical significance performed using two-way ANOVA with Tukey's multiple comparison test (n.s. $p > 0.05$).

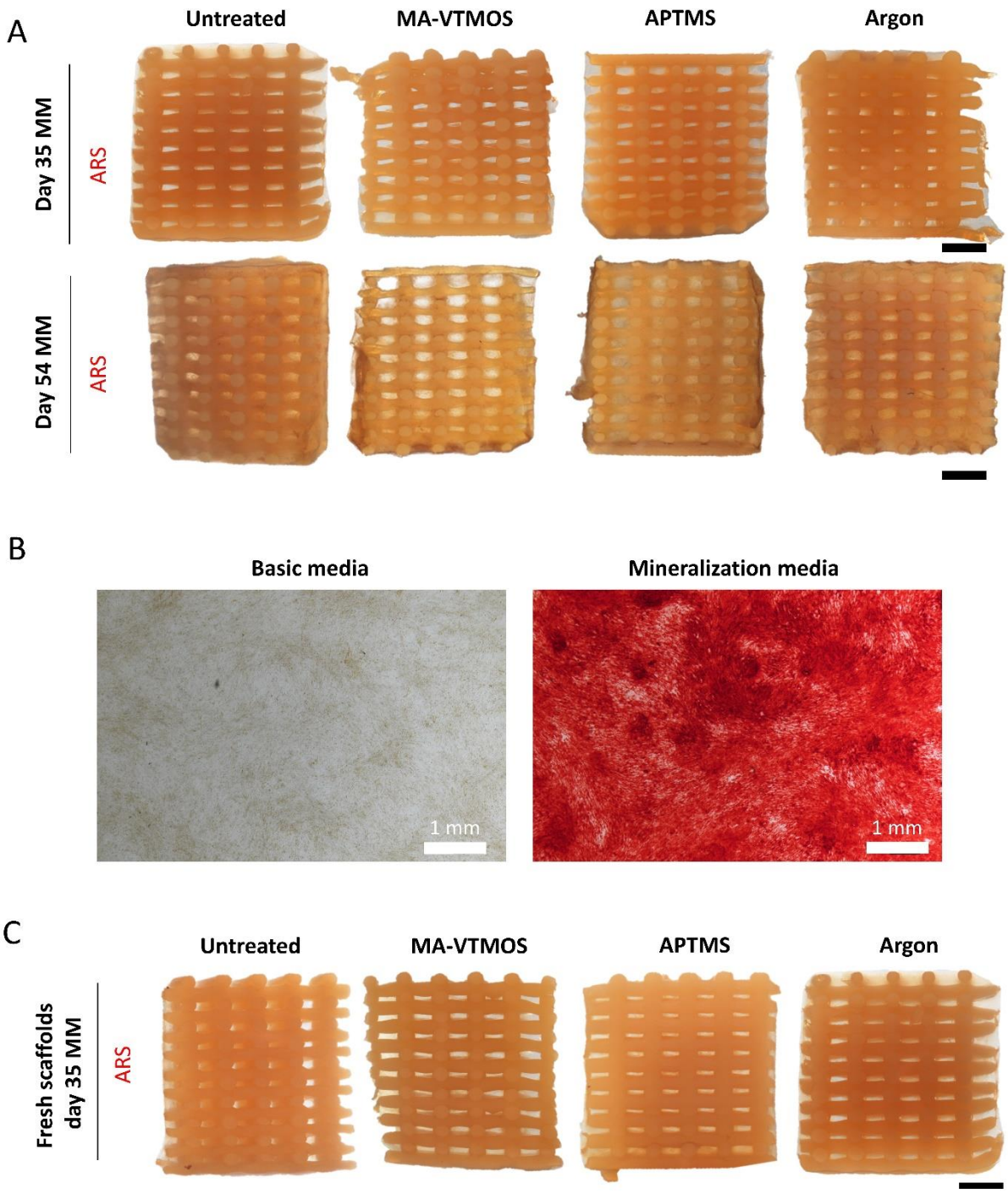

Figure S9. (A) Stereomicroscopy images of aged (plasma treatment) scaffold cross sections stained with alizarin red S after 35 and 54 days of culture (28 and 47 days in MM, respectively) with hMSCs. (B) Stereomicroscopy images of hMSCs monolayers (osteogenic differentiation in 2D) stained with alizarin red S after 35 days of culture (28 days in MM). (C) Stereomicroscopy images of fresh (plasma treatment) scaffold cross sections stained with alizarin red S after 35 days of culture (28 days in MM) with hMSCs. Scale bars 1 mm.

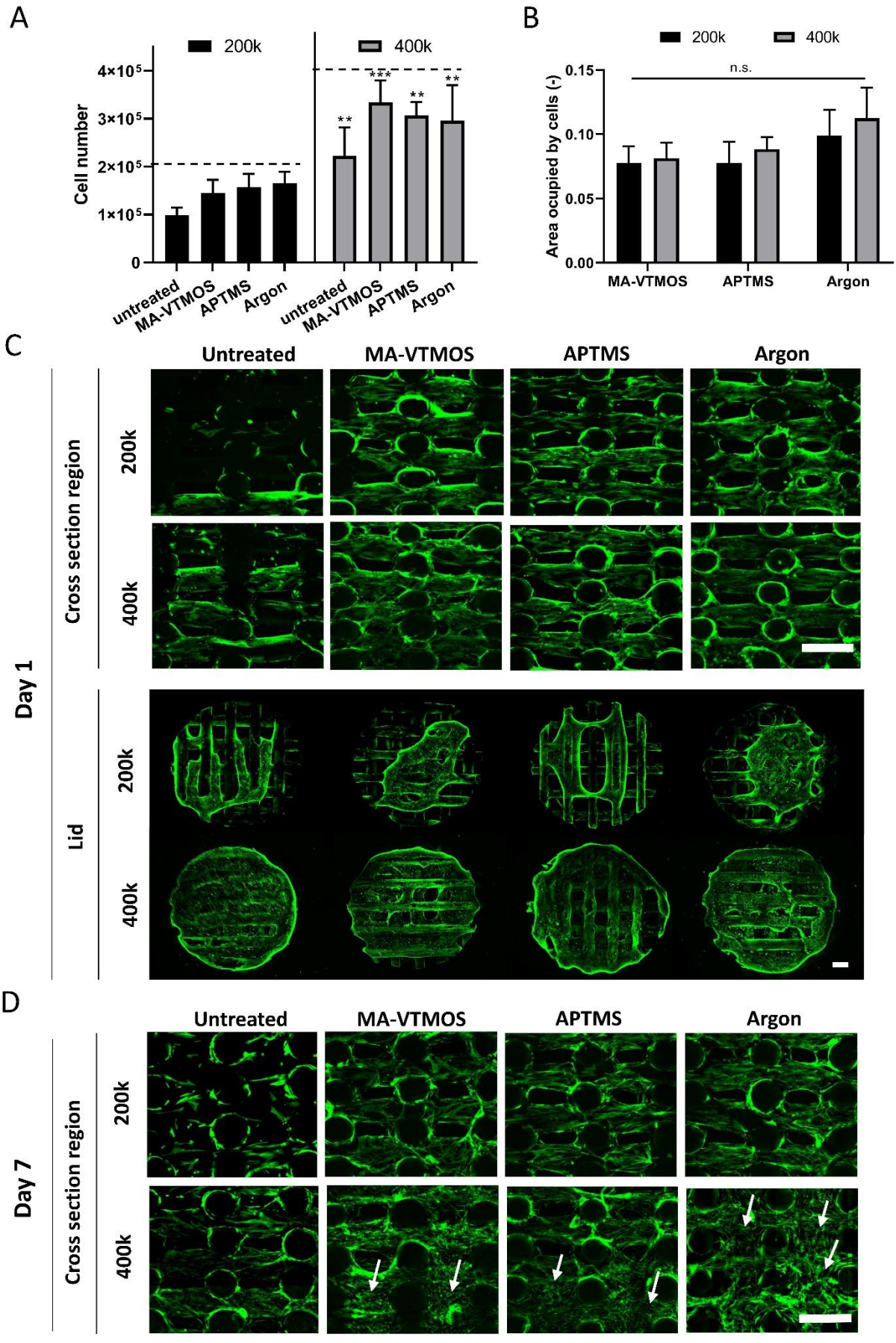
A
200k
400k
Cell number
4×10⁵
3×10⁵
2×10⁵
1×10⁵
0
**
***
**
**
untreated
MA-VTMOS
APTMS
Argon
untreated
MA-VTMOS
APTMS
Argon
B
200k
400k
n.s.
Area ocupied by cells (-)
0.15
0.10
0.05
0.00
MA-VTMOS
APTMS
Argon
C
Untreated
MA-VTMOS
APTMS
Argon
Day 1
Cross section region
200k
400k
Lid
200k
400k
D
Untreated
MA-VTMOS
APTMS
Argon
Day 7
Cross section region
200k
400k

Figure S10. (A) DNA content on plasma treated scaffolds after seeding with two different cell densities: 200,000 cells (200k) or 400,000 cells (400k) per scaffold. Data presented as average ± s.d. and statistical significance performed using two-way ANOVA with Tukey's multiple comparison test (** $p < 0.01$; *** $p<0.001$, for comparisons between 200k and 400k for each plasma treatment). (B) Quantification of cell coverage on plasma treated scaffolds seeded with 200k or 400k cells after 7 days of culture in BM. Data presented as average ± s.d. and statistical significance performed using two-way ANOVA with Tukey's multiple comparison test (n.s. $p> 0.05$). (C) Comparison of cell coverage among plasma treated scaffolds seeded with 200k or 400k cells per scaffold: (C) Fluorescent microscopy images of hMSCs in a representative cross sectional area and in the bottom lids of scaffolds after 1 day of culture. (D) Fluorescent microscopy images of hMSCs in a representative cross sectional area of scaffolds after 7 day of culture in BM. White arrows signing scaffolds' pores coverage by cells Scale bars 500µm.

References

1. Sinha, R.; Cámara-Torres, M.; Scopece, P.; Falzacappa, E. V.; Patelli, A.; Moroni, L.; Mota, C., A Hybrid Additive Manufacturing Platform to Create Bulk and Surface Composition Gradients on Scaffolds for Tissue Regeneration. *bioRxiv* **2020**, 2020.06.23.165605.
2. Patelli, A.; FALZACAPPA, E. V.; Scopece, P.; Pierobon, R.; Vezzu, S., Method for Generating an Atmospheric Plasma Jet and Atmospheric Plasma Minitorch Device. Google Patents: 2017.