Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplementary Information

Sustained delivery of growth factors with a high loading efficiency in a layer by layer assembly

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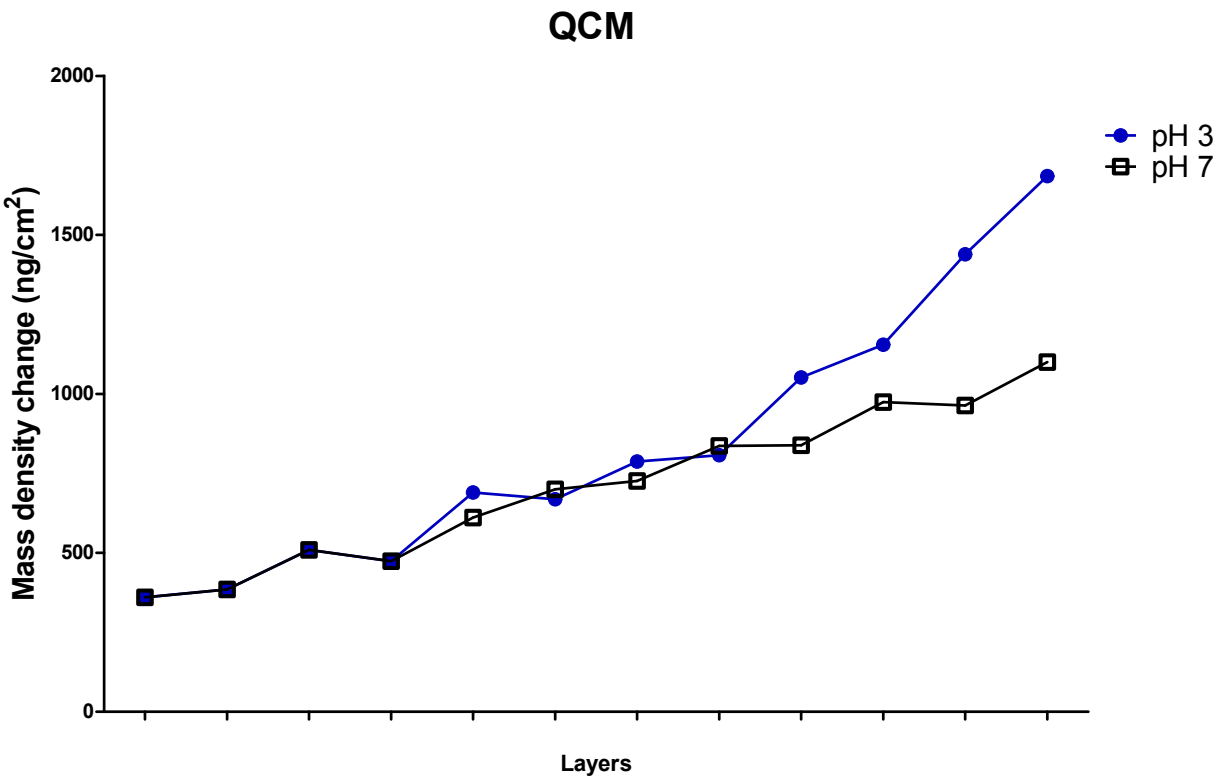
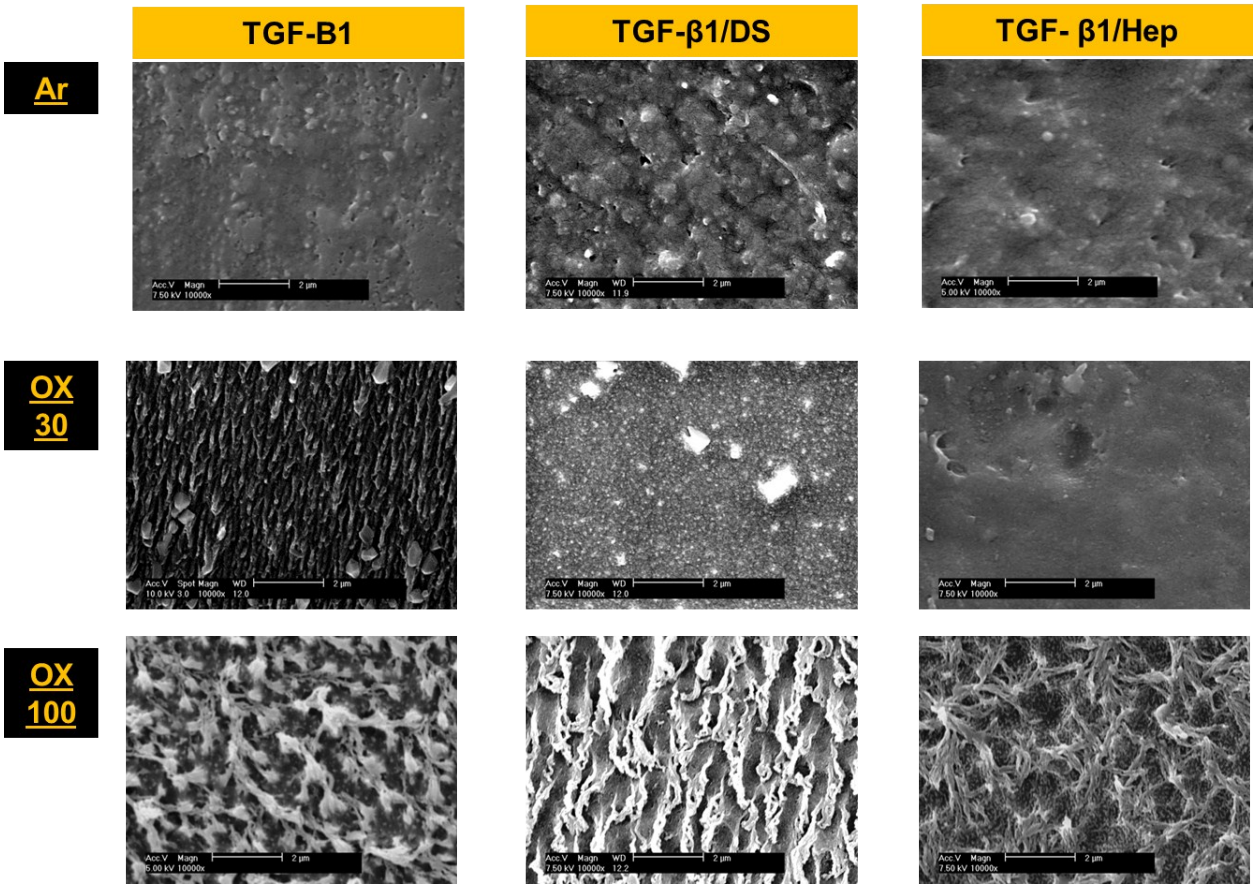
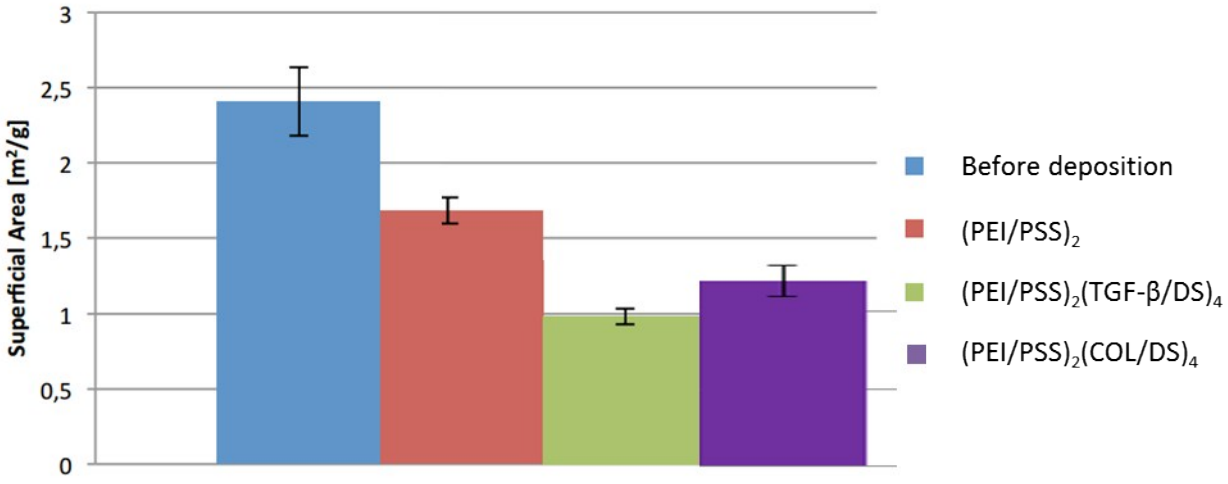


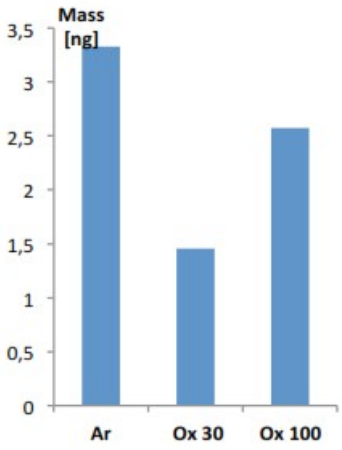
Supplementary Figure 1. Areal mass density of the deposited multilayer $(PEI/PSS)_2(COL/DS)_4$ deposited onto the QCM-D sensor. Increased COL absorption was seen at pH 3 (blue circle) compared to pH 7 (black square).



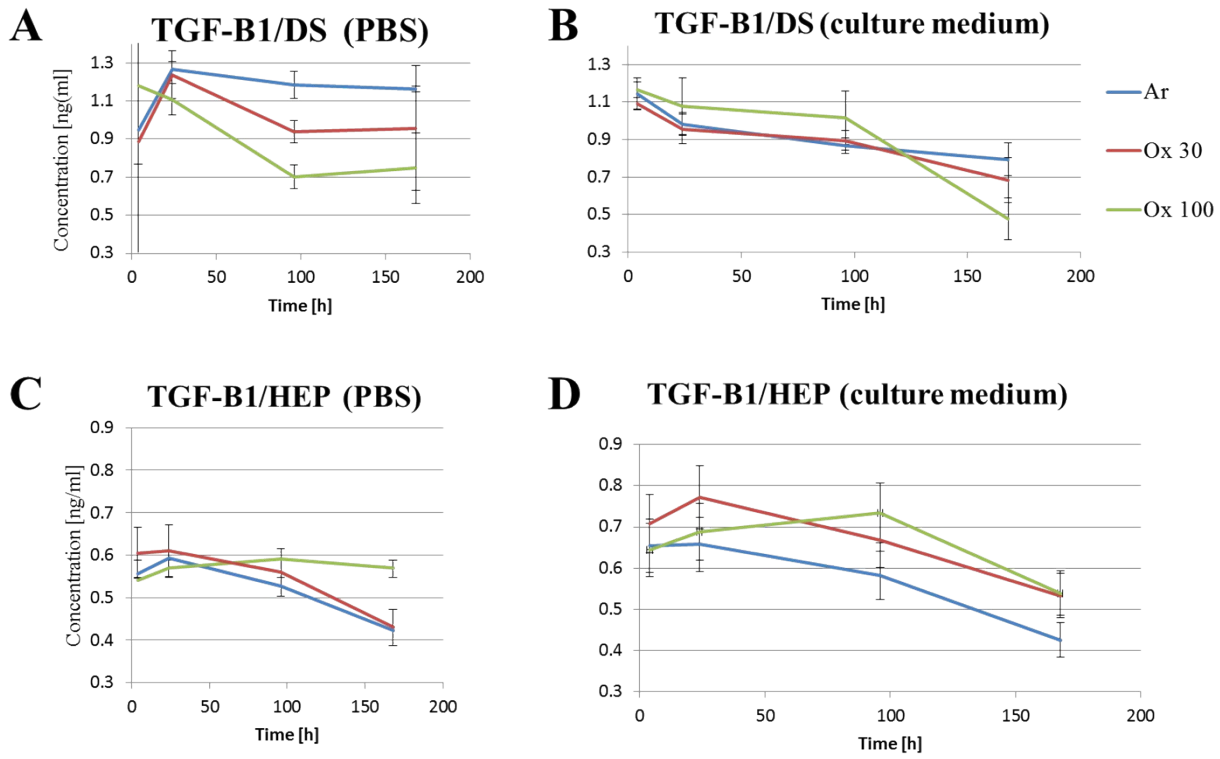
Supplementary Figure 2. SEM analysis of TGF-β1, TGF-β1/DS and TGF-β1/HEP with different surface activations shows different surface topographies. Scale bar: 2 µm.



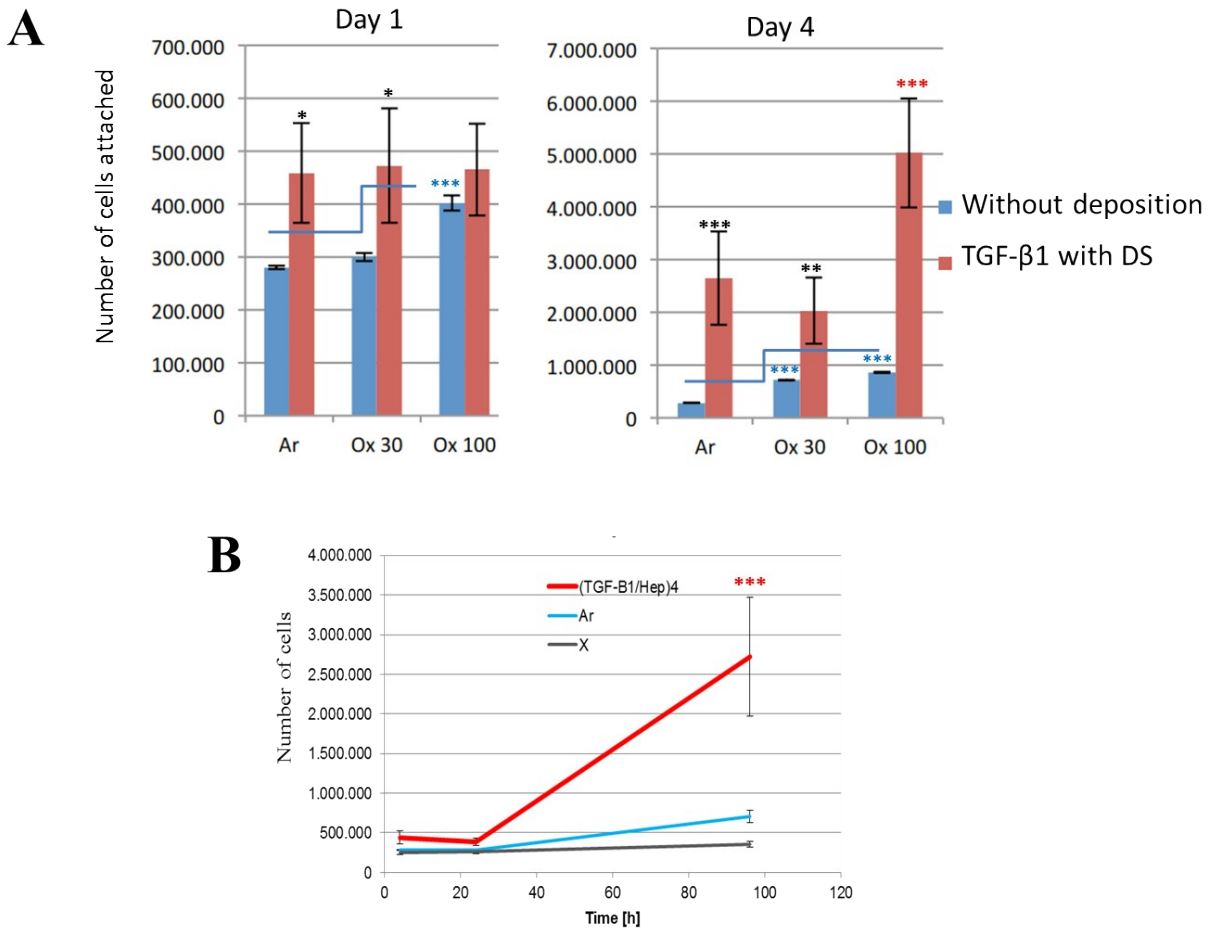
Supplementary Figure 3. BET analysis of surface area before and after $(PEI/PSS)_2$ alone, with $(TGF\text{-}β1/DS)_4$ and $(COL/DS)_4$ deposition. The surface area decreases upon layer build-up.



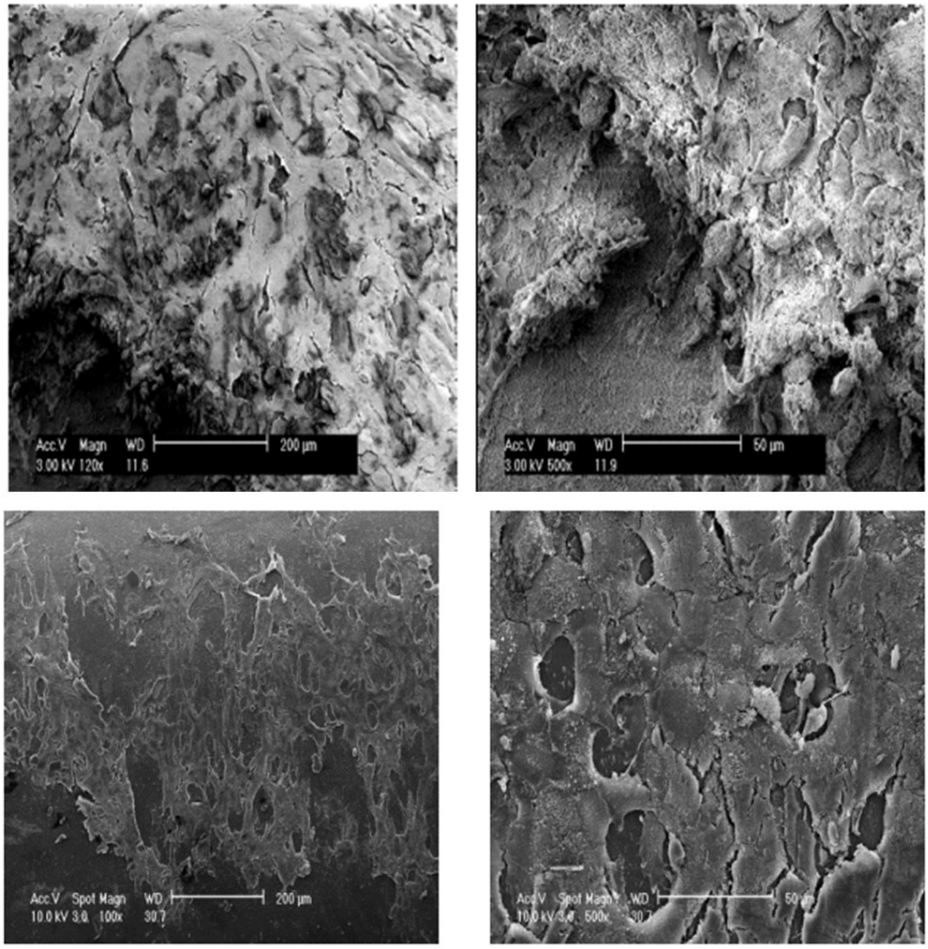
Supplementary Figure 4. Loading capacity of collagen at pH 3 loaded in Ar-, Ox30- and Ox100-surface-treated rods with $(COL/DS)_4$. Ar rods showed the most loading of COL.



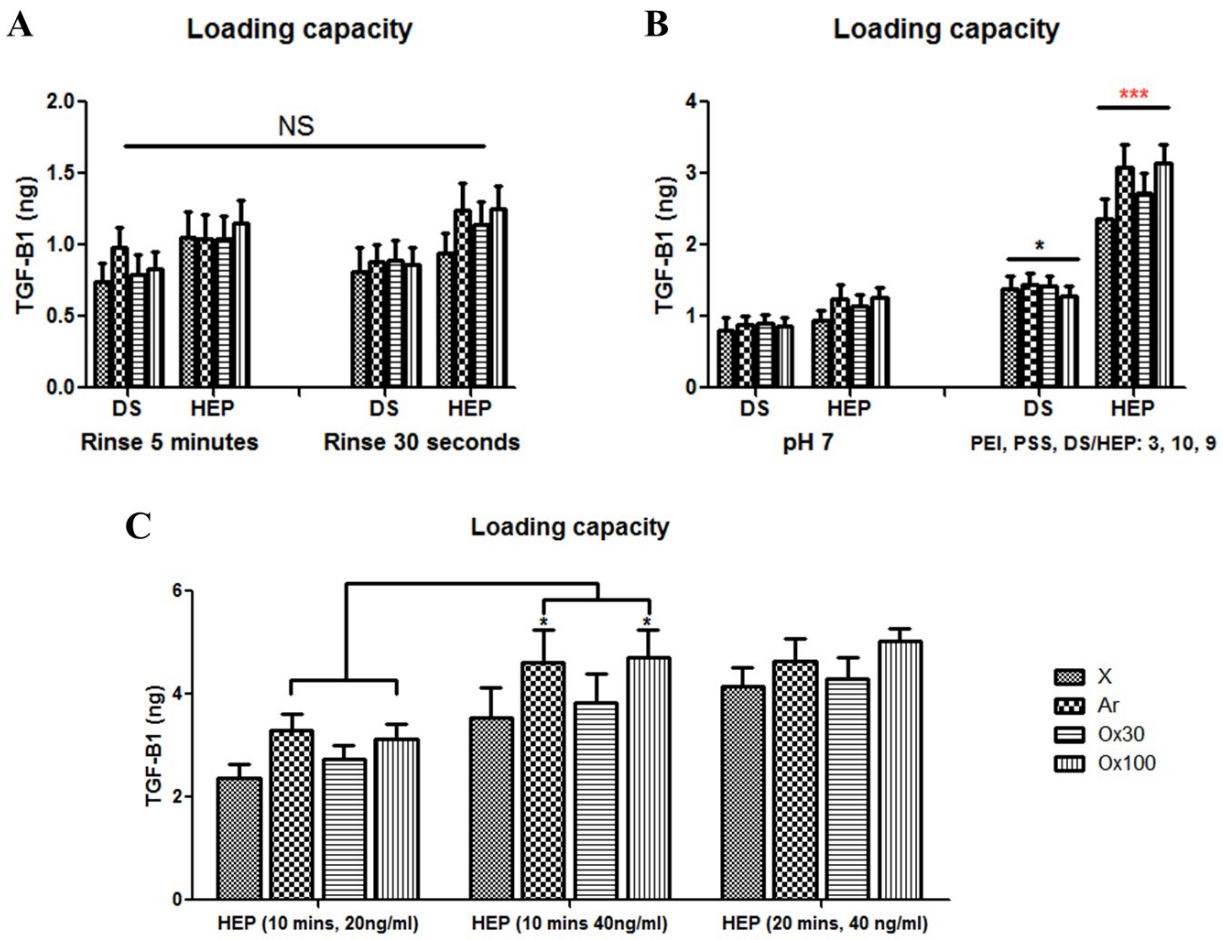
Supplementary Figure 5. Release rate profile at 4 hours, 1, 4 and 7 days on Ar, Ox30, Ox100 LBL implants. (A-B) $(PEI/PSS)_2(TGF\text{-}\beta1/DS)_4$ implants showed a burst release of 0.9 or 1.2 ng/ml for PBS and 1.1 ng/ml for culture medium incubation at 4 hours. At day 1 an increase to 1.3 ng/ml was seen in PBS incubation, while in culture medium the release slightly dropped below 1.1 ng/ml. In culture medium, the concentration was kept at day 4 but decreased at day 7 to 0.8, 0.7 and 0.5 ng/ml for Ar, Ox30 and Ox100 LBL implants respectively. PBS incubation at day 4 resulted in a decreased release to 1.2, 0.9 and 0.7 ng/ml for Ar, Ox30 and Ox100 LBL respectively, but remained constant at day 7. (C-D) $(PEI/PSS)_2(TGF\text{-}\beta1/HEP)_4$ implants displayed a release at 4 hours between 0.55-0.6 ng/ml in PBS incubation which remained constant until day 4. Ar and Ox30 LBL implants diminished to a concentration of 0.45 ng/ml at day 7, while Ox100 LBL implants remained constant at above 0.55 ng/ml. In culture medium incubation, a concentration between 0.8-0.6 ng/ml was seen at 4 hours and day 1 until day 4, when Ar and Ox30 LBL implants had a decrease of about 0.1 ng/ml, while Ox100 showed an increase of 0.05 ng/nl between day 1 and day 4. Concentration at day 7 was 0.45 ng/nl for Ar and 0.55 ng/ml for Ox30 and Ox100 LBL implants.



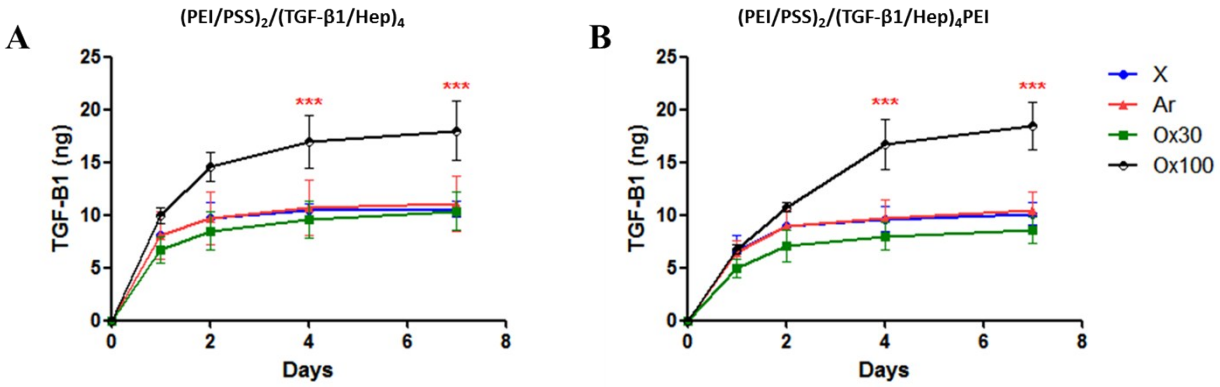
Supplementary Figure 6. DNA assay on non-deposited and deposited TGF- β1 rods. (A) Cell quantification on surface treated rods with or without LBL TGF- β1/DS sequence at day 1 (left) and 4 (right). At day 1 DNA analysis showed higher cell attachment on LBL rods, with deposition on Ox100 rods providing the highest proliferation. (B) DNA assay of non-treated rods (X), argon plasma treated rods (Ar) and argon plasma treated rods with TGF-β1/HEP sequence, (TGF-β1/Hep)4, at 4 hours, day 1 and 4. Analysis showed higher proliferation on (TGF-β1/Hep)4 rods compared to X and Ar rods.



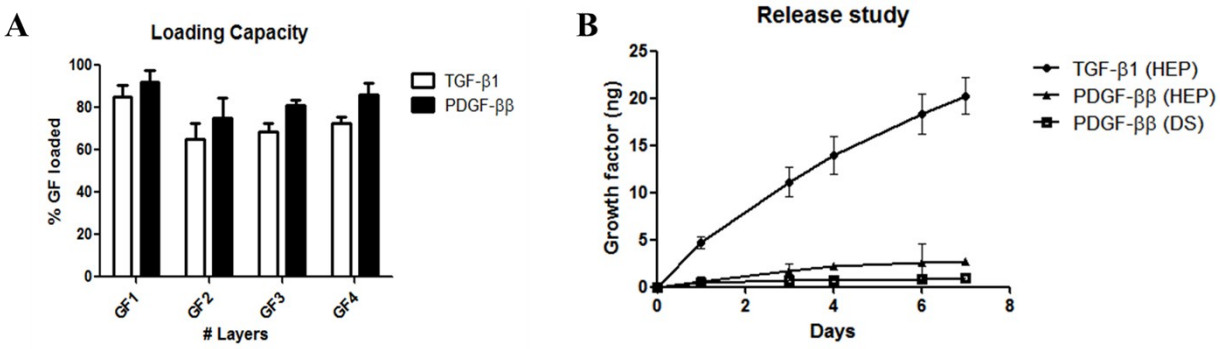
Supplementary Figure 7. SEM images of Ox100 surface treated rods with (top) and without (bottom) LBL TGF-β1, seeded with 500,000 TK173 cells for a period of 4 days. Ox100 surface treated rods with LBL TGF-β1 rods provided higher proliferation showing a thicker cell layer compared to the Ox100 surface treated rods without the LBL coating. Scale bar: 200 µm (left) and 50 µm (right).



Supplementary Figure 8. Loading capacity of TGF-β1 with different parameter modification. (A) No difference in TGF-β1 loading capacity was seen when reducing MilliQ water rinsing time. (B) Changing pH to 3, 10 and 9 of PEI, PSS and DS or HEP, respectively, significantly increased TGF-β1 loading capacity. (C) Increasing TGF-β1 concentration significantly increased the loading capacity in Ar and Ox100 surface treated implants. Star (*P < 0.05, ** P < 0.01, *** P < 0.001) indicate statistically significant values compared to TGF-β1 loading with PEI, PSS DS or Hep at pH 7 or TGF-β1 loaded at 10 ng/ml for 10 minutes. Furthermore, increasing the TGF-β1 deposition and rinsing with PBS instead of MilliQ water increased loading capacity on all surface treated implants with lower variability.



Supplementary Figure 9. Release rate of TGF-β1 before and after additional layer of PEI. TGF-β1 was loaded for 20 minutes (per GF layer (40 ng/ml, pH 5) (A) A burst release was seen, in which more than half of the TGF-β1 loaded was release after 1 day. (B) The burst release decreased after the additional layer of PEI was deposited. In all sequence Ox100 LBL implants showed the highest release in TGF-β1.



Supplementary Figure 10. Loading capacity and release profile of PDGF-ββ and TGF-β1 LBL implants. Both GFs were loaded at RT for 20 minutes per GF layer (40 ng/ml, pH 5)**.** (A) Loading capacity of PDGF-ββ was seen to be higher than TGF-β1. (B) Release rate of PDGF-ββ on both HEP and DS sequence (1 mg/ml) showed hardly any signal of viable PDGF-ββ release compared to TGF-β1.