Supporting Information

Cell derived extracellular matrix-rich biomimetic substrate supports podocyte proliferation, differentiation and maintenance of native phenotype

Abhigyan Satyam, Maria G. Tsokos, Jason S. Tresback, Dimitrios I. Zeugolis, George C. Tsokos**

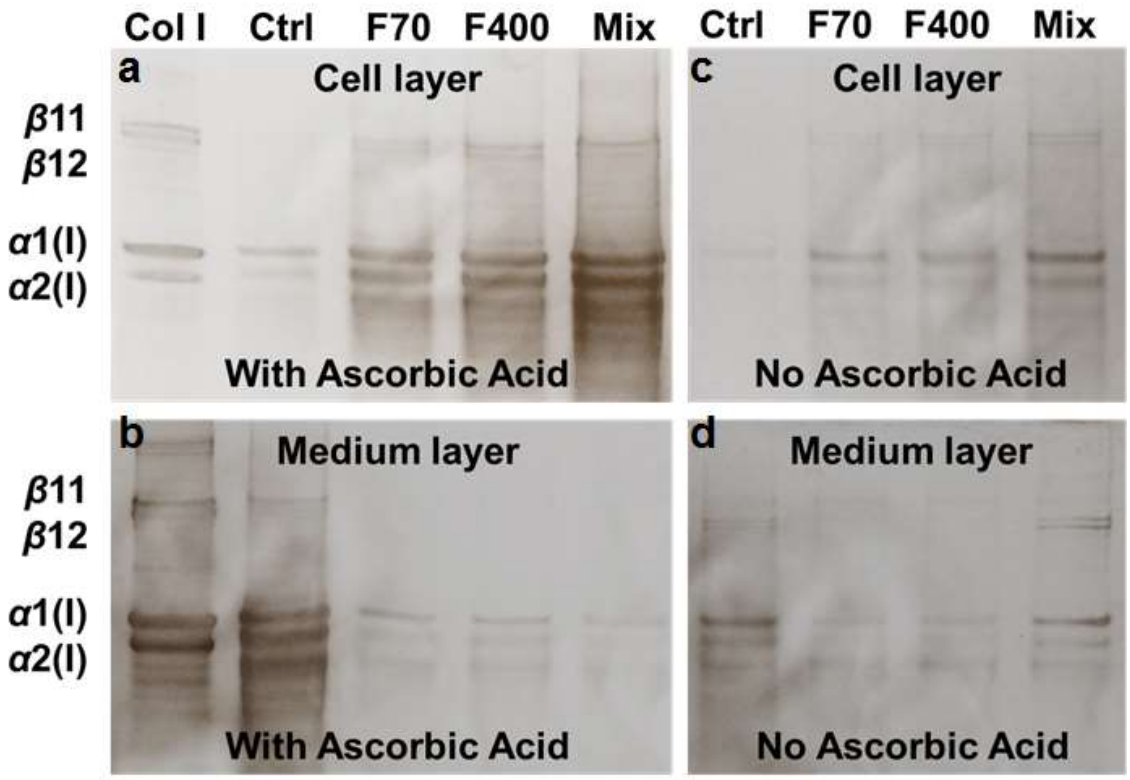

Figure S1: SDS-PAGE analysis confirmed that ascorbic acid enhances the production of collagen type I (a and b) as compare to culture conditions without ascorbic acid (c and d). Moreover, SDS-PAGE revealed that in the absence of crowding molecules (F70 or F400 or mix) collagen remained in the medium layer (b and d), while in the presence of crowding molecules collagen was deposited in the cell layer (a and c). Ctrl – Control (no crowder added). Ficoll (F), mix [F 70 + F400] MMC conditions. Col I – 100 µg/ml collagen type I from rat tail. Ascorbic acid – 100 µM of L-ascorbic acid 2-phosphate.

The fibrillar collagen type I synthesize by mesenchymal cells and exported into the extracellular space as water-soluble procollagen. The subsequent proteolytic removal of the C- and N-terminal propeptide converts procollagen to collagen. A low procollagen conversion rate is the default state of in vitro cell culture systems, which means an excess of procollagen in the culture medium and poor collagen matrix formation resulting in low productivity for tissue building. Here we demonstrate, by applying the biophysical principle of MMC that this cell culture-intrinsic problem can be overcome. Our result and previously published data suggest that crowding of the culture medium confined the space of molecules of comparable

size and thus increased the interaction of the substrate (procollagen) with respective enzymes for trimming (C and N proteinase, respectively), resulting in an increase of collagen deposited in the matrix and a massive decrease of procollagen in culture medium. [1]

Collagen type I consists of alpha-1 (α1I, 139 kDa) and alpha-2 chains (α2I, 129kDa). Since collagen type I is a triple helix consisting of one alpha-2 chain, two alpha-1 chains and crosslinked collagen alpha -chain dimers (β11 and β 12).

[1] a) D. Cigognini, D. Gaspar, P. Kumar, A. Satyam, S. Alagesan, C. Sanz-Nogués, M. Griffin, T. O'Brien, A. Pandit, D. I. Zeugolis, *Scientific reports* **2016**, 6, 30746; b) J.-Y. Dewavrin, M. Abdurrahiem, A. Blocki, M. Musib, F. Piazza, M. Raghunath, *The Journal of Physical Chemistry B* **2015**, 119, 4350; c) V. Magno, J. Friedrichs, H. M. Weber, M. C. Prewitz, M. V. Tsurkan, C. Werner, *Acta Biomaterialia* **2017**, 55, 109; d) A. Satyam, P. Kumar, D. Cigognini, A. Pandit, D. I. Zeugolis, *Acta Biomaterialia* **2016**, 44, 221; e) A. Satyam, P. Kumar, X. Fan, A. Gorelov, Y. Rochev, L. Joshi, H. Peinado, D. Lyden, B. Thomas, B. Rodriguez, M. Raghunath, A. Pandit, D. Zeugolis, *Advanced Materials* **2014**, 26, 3024; f) I. M. Kuznetsova, K. K. Turoverov, V. N. Uversky, *Int J Mol Sci* **2014**, 15, 23090; g) I. M. Kuznetsova, B. Y. Zaslavsky, L. Breydo, K. K. Turoverov, V. N. Uversky, *Molecules* **2015**, 20, 1377.

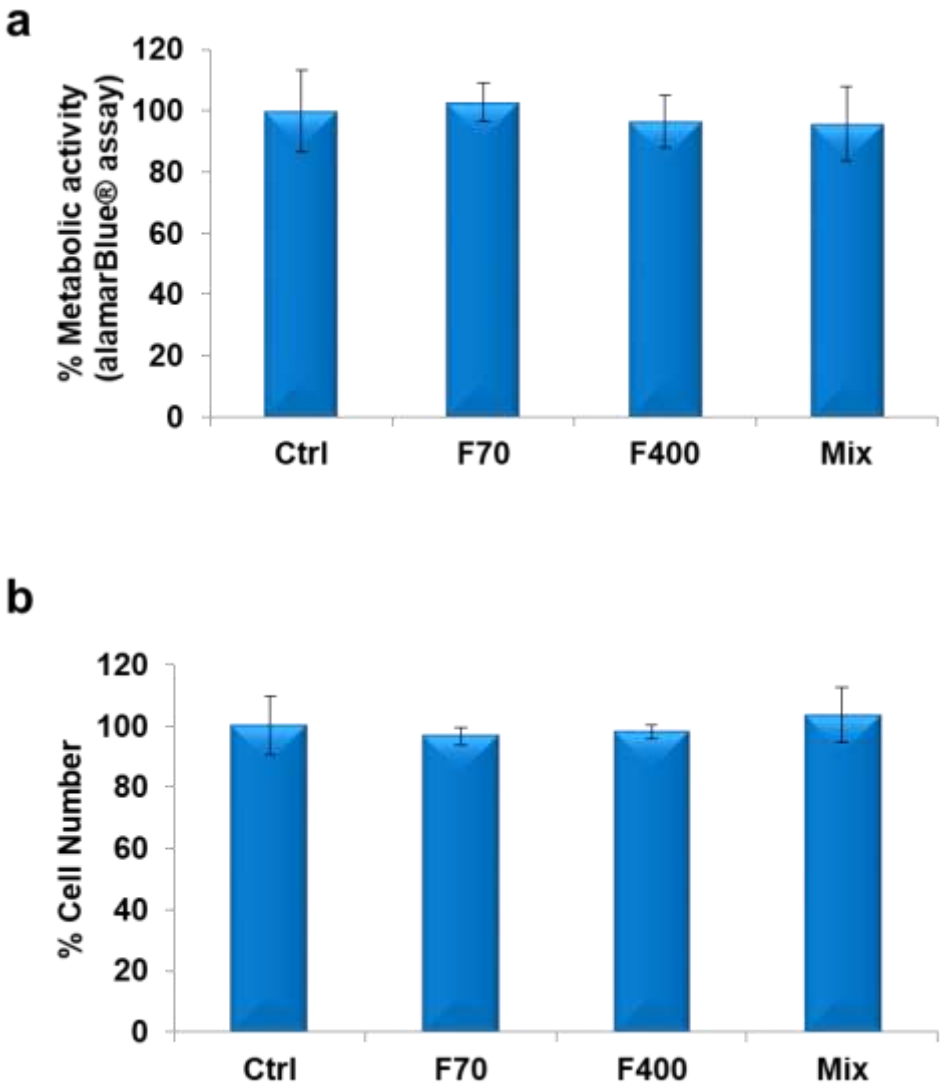

Figure S2: (a) alamarBlue® analysis revealed that fibroblasts maintained their cellular metabolic activity after macromolecular crowding. **(b)** DAPI stained nuclei counting for cell viability demonstrated that the used macromolecules did not affect cellular viability after macromolecular crowding. F70 – 37.5 mg/mL Ficoll 70. F400 – 25 mg/mL Ficoll 400. Mix - mixture of 37.5 mg/mL Ficoll 70 and 25 mg/mL Ficoll 400. Ctrl – Control (no crowder added).

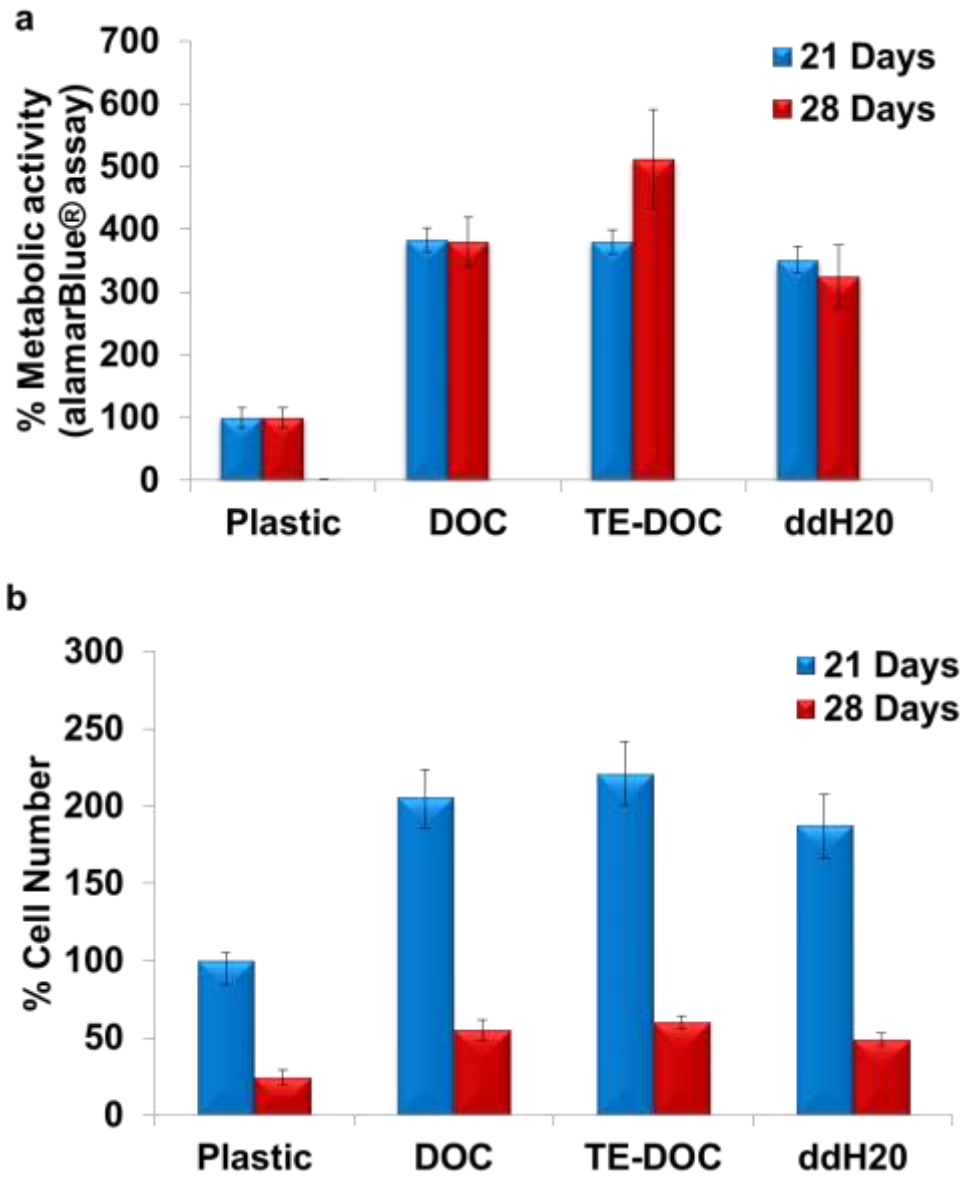

Figure S3 : (a) alamarBlue® analysis revealed higher cellular metabolic activity of podocytes cultured for 28 days on DCM plates those were decellularized by TE-DOC method. **(b)** DAPI stained nuclei counting for cell viability demonstrated that the podocytes cultured on DCM plates with various decelularization method increases cellular viability compared to plastic well plates. The decrease in cell cell number was observed in all conditions. DOC - 0.5% Sodium deoxycholate. TE-DOC - 0.5% Sodium deoxycholate with Triton X-100 and EDTA and (iii) double distilled water. Podocytes were cultured for 21 and 28 days at 37°C.

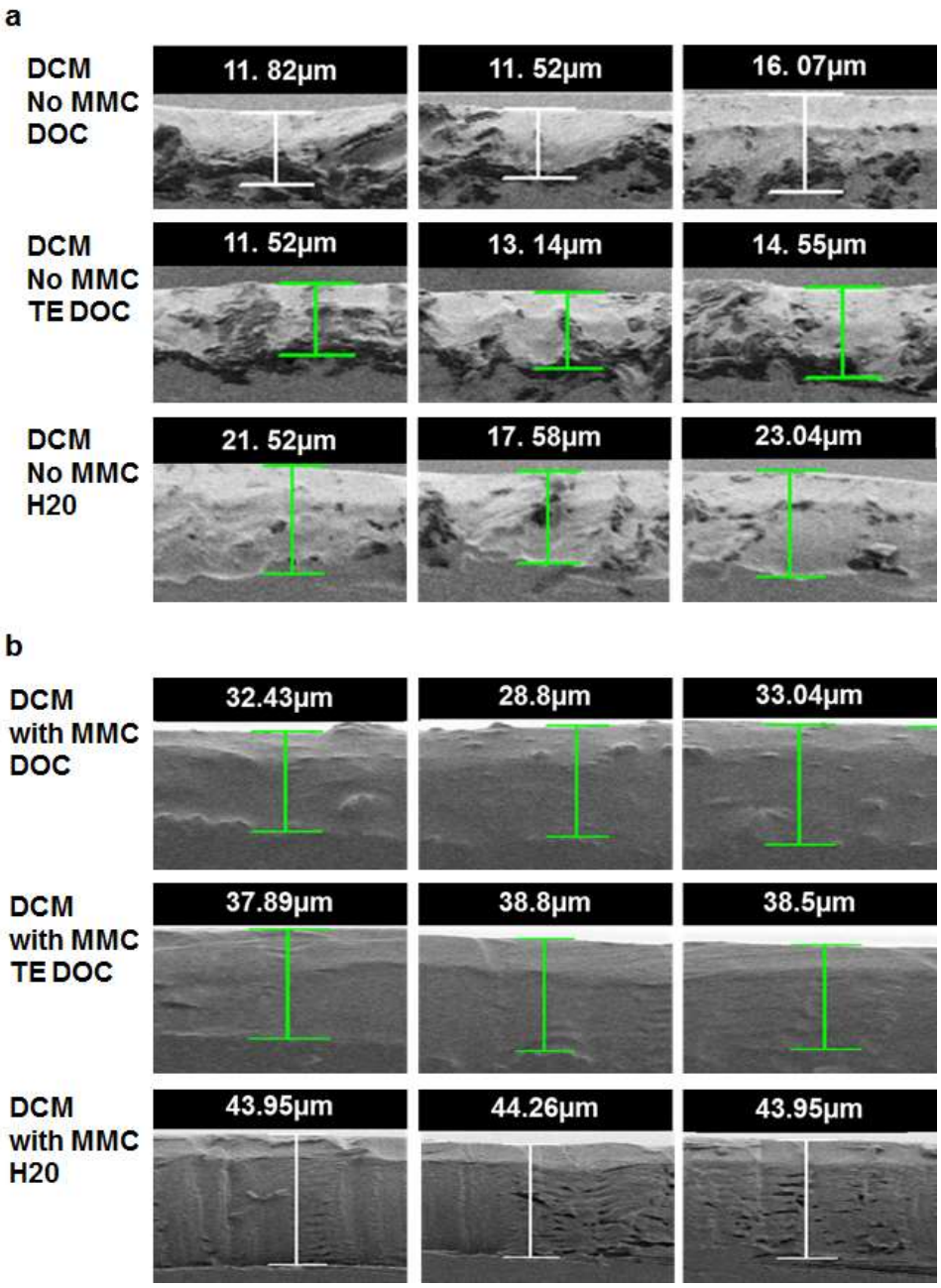

Figure S4: Scanning electron microscopic images of collagen type I coated and cell derived decellularized matrix (DCM, with and without MMC) sections of coverslips. (3 images/section).

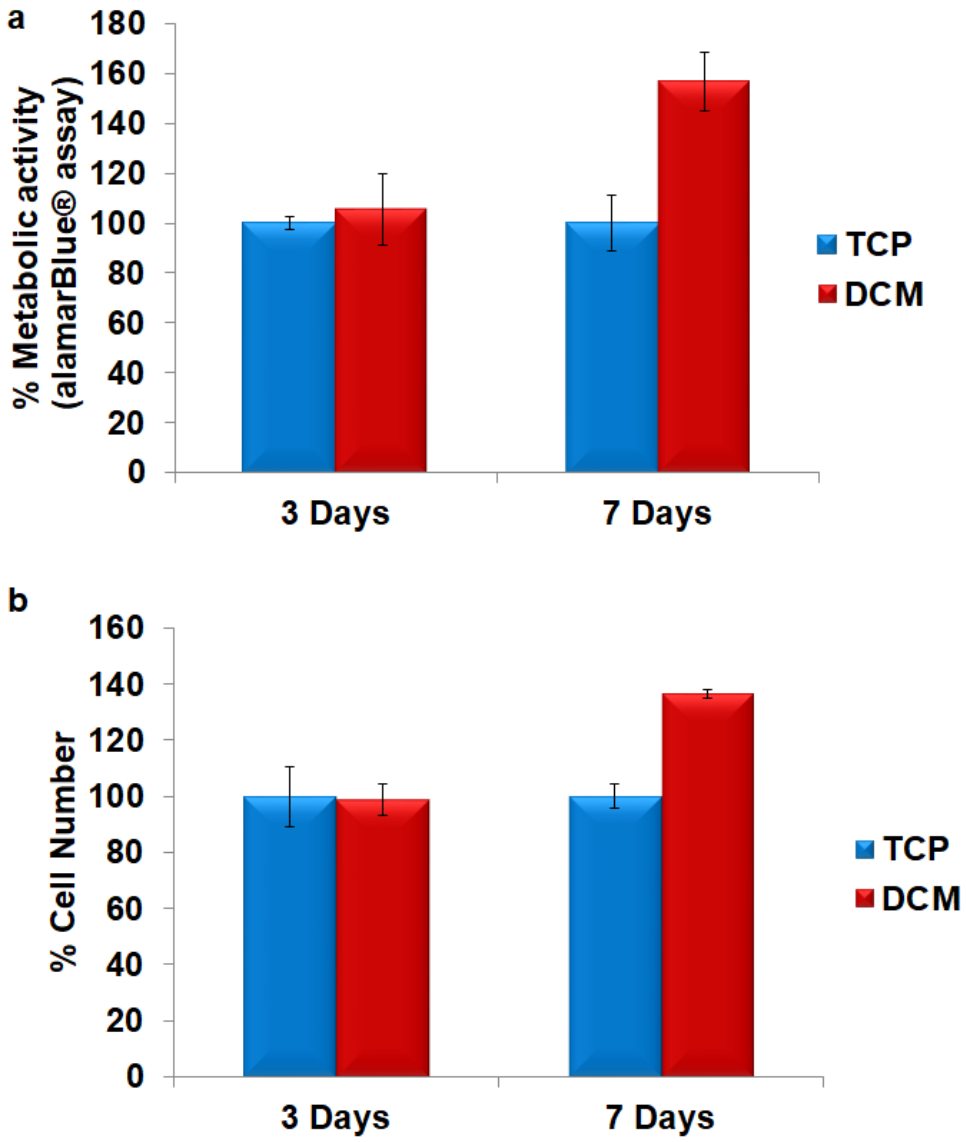

Figure S5: (a) alamarBlue® analysis confirmed the higher cellular metabolic activity of podocytes cultured at 33 °C (proliferation condition) for 7 days on DCM plates. **(b)** DAPI stained nuclei counting for cell viability demonstrated the higher viability of podocytes cultured at 33 °C (proliferation condition) for 7 days on DCM plates. TCP – Tissue culture plastic paltes. DCM – Cell derived decellularized matrix well plates.

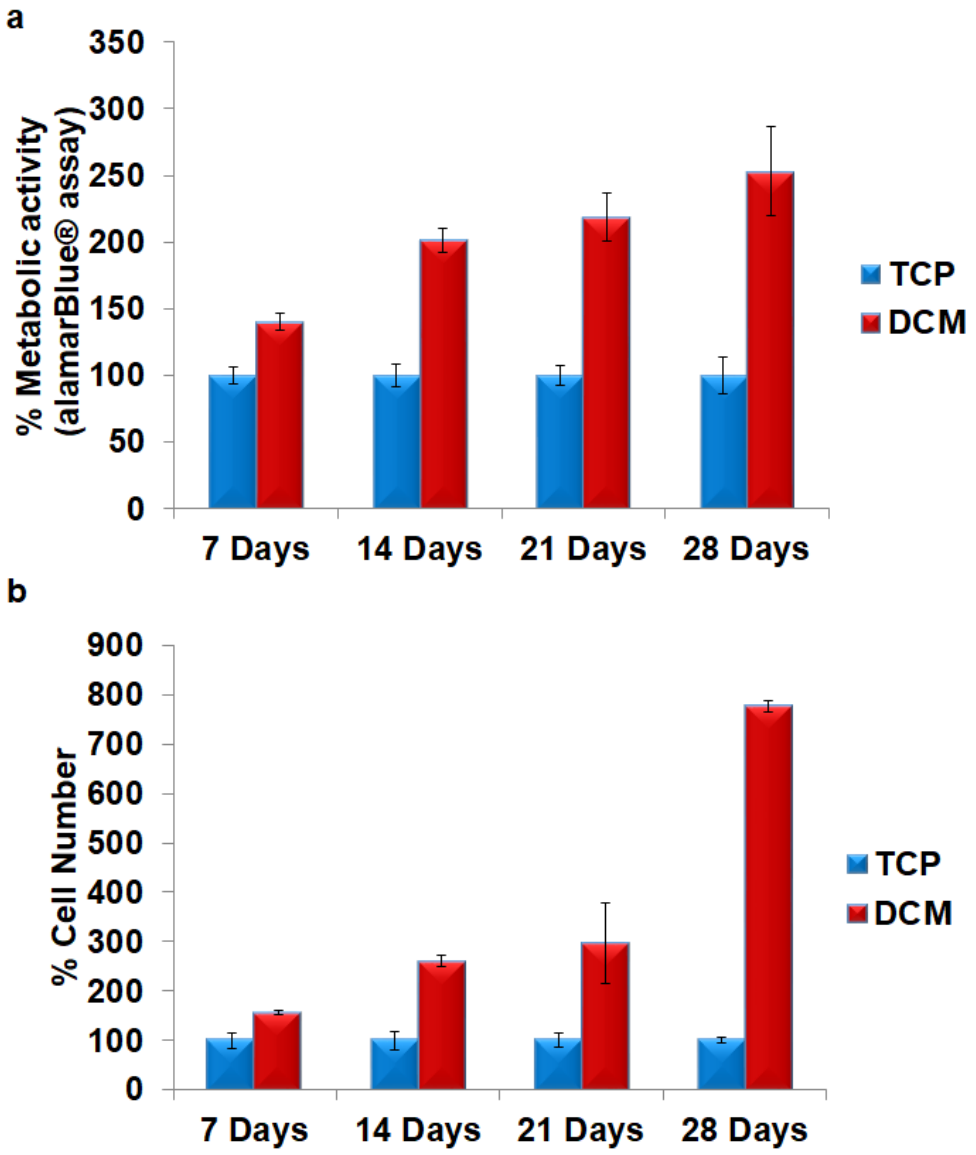

Figure S6: (a) alamarBlue® analysis confirmed the higher cellular metabolic activity of podocytes cultured at 37 °C (differentiation condition) for 7, 14, 21, and 28 days on DCM plates compared to TCP well plates. **(b)** DAPI stained nuclei counting for cell viability demonstrated the higher viability of podocytes cultured at 37 °C (differentiation condition) for for 7, 14, 21, and 28 days on DCM plates compared to TCP well plates. TCP – Tissue culture plastic paltes. DCM – Cell derived decellularized matrix well plates. All the values were compared between TCP and DCM at respective time points.

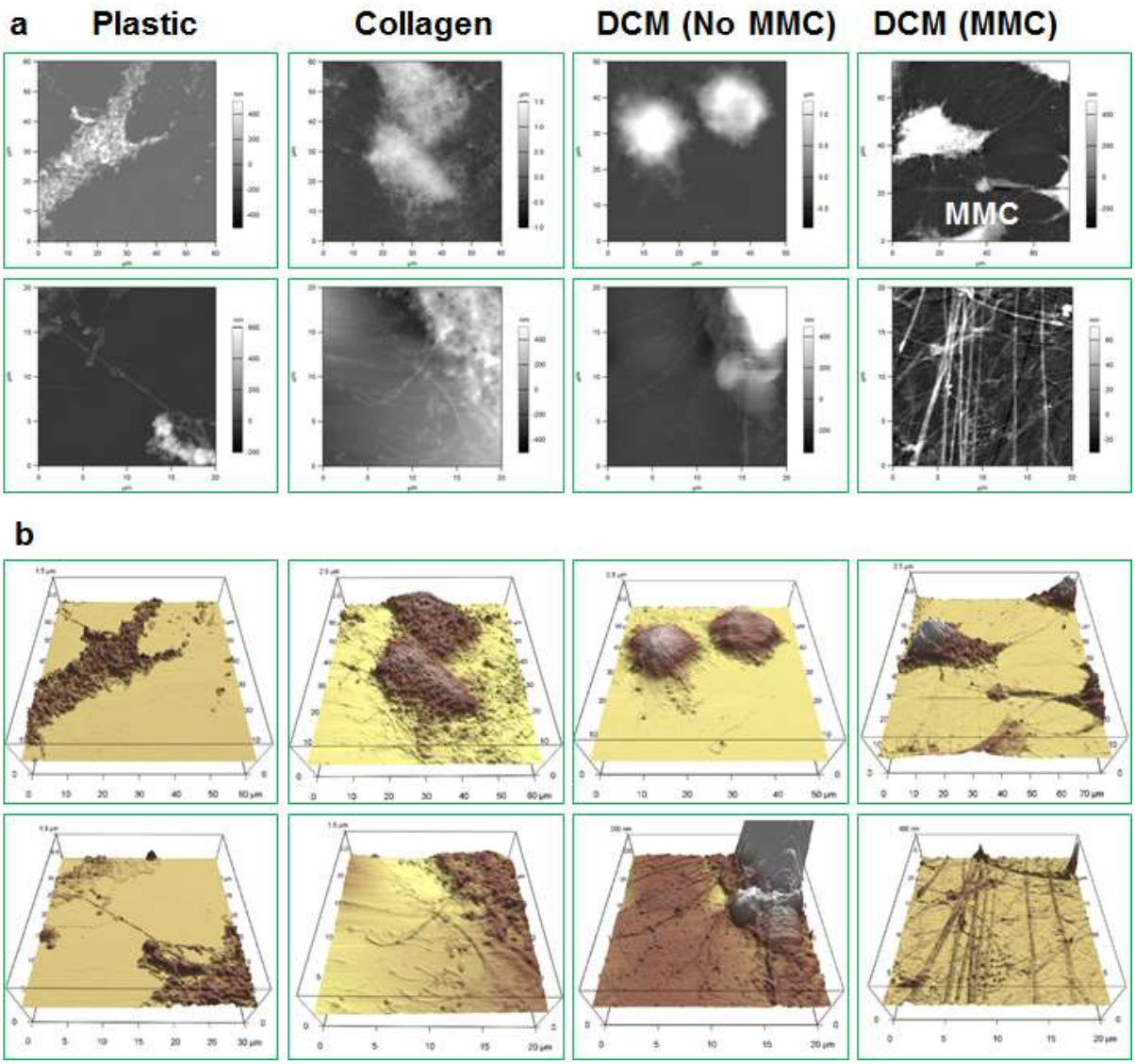

Figure S7: 2-dimentional (a) and 3-dimentional (b) Atomic force microscopy images of plastic, collagen coated, DCM (without MMC) and DCM (with MMC) surfaces with podocytes. The lower panel of (a) and (b) shows the image of intercellular space.

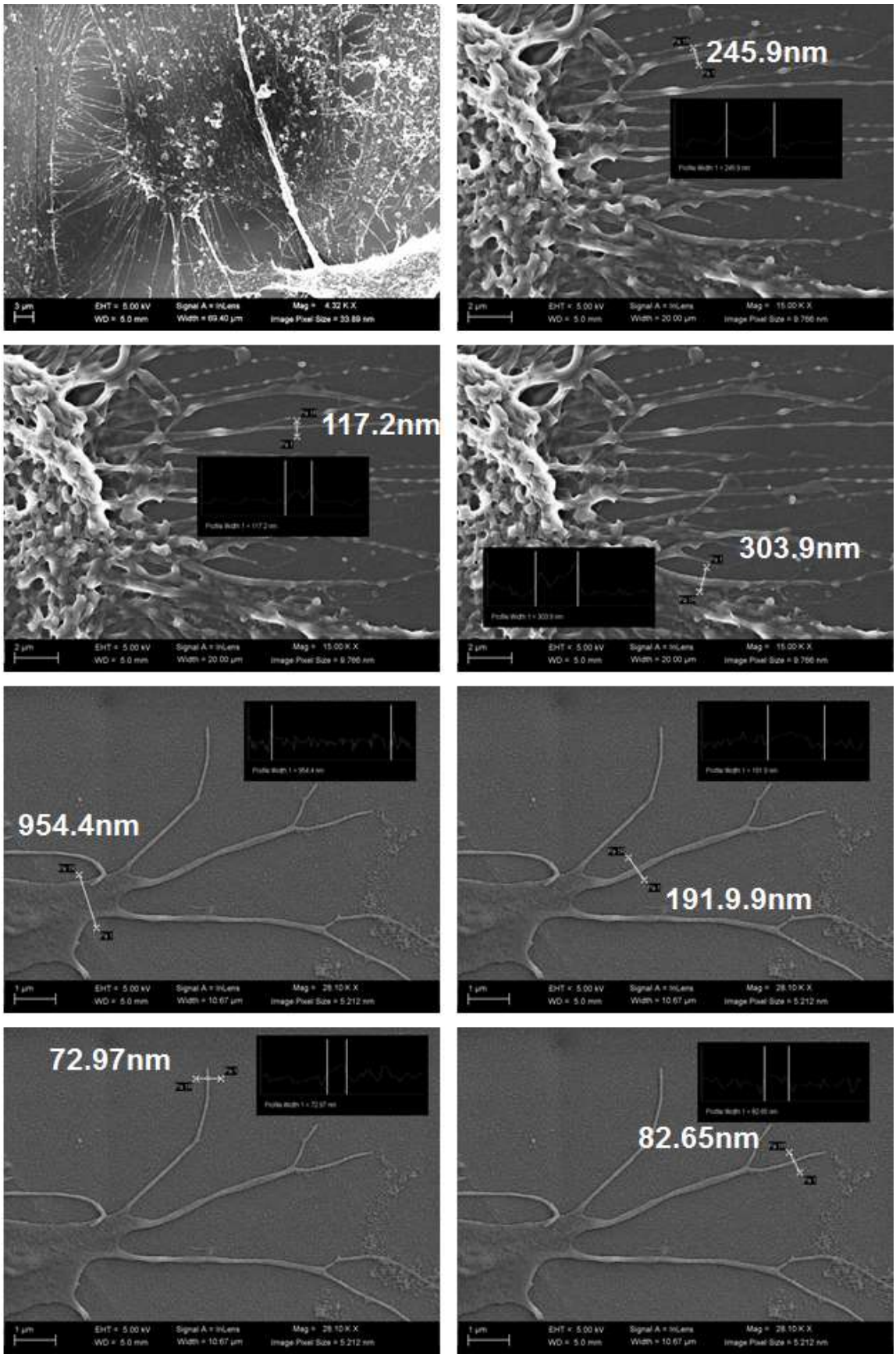

Figure S8: Scanning electron microscopic images of cell derived decellularized matrix (DCM, with MMC) sections show the orientation and thickness of foot process.

a

	A	B	C	D	E	F	G	H	I	J	K	L
1	Pos	Pos	Neg	Neg	AR	bFGF	b-NGF	EGF	EGF R	FGF-4	FGF-6	FGF-7
2	Pos	Pos	Neg	Neg	AR	bFGF	b-NGF	EGF	EGF R	FGF-4	FGF-6	FGF-7
3	GCSF	GDNF	GM-CSF	HB-EGF	HGF	IGFBP-1	IGFBP-2	IGFBP-3	IGFBP-4	IGFBP-6	IGF-I	IGF-ISR
4	GCSF	GDNF	GM-CSF	HB-EGF	HGF	IGFBP-1	IGFBP-2	IGFBP-3	IGFBP-4	IGFBP-6	IGF-I	IGF-ISR
5	IGF-II	M-CSF	M-CSFR	NT-3	NT-4	PDGFRα	PDGFRβ	PDGF-AA	PDGF-AB	PDGF-BB	PIGF	SCF
6	IGF-II	M-CSF	M-CSFR	NT-3	NT-4	PDGFRα	PDGFRβ	PDGF-AA	PDGF-AB	PDGF-BB	PIGF	SCF
7	SCFR	TGF-α	TGF-β	TGF-β2	TGF-β3	VEGF	VEGFR2	VEGFR3	VEGF-D	BLANK	BLANK	Pos
8	SCFR	TGF-α	TGF-β	TGF-β2	TGF-β3	VEGF	VEGFR2	VEGFR3	VEGF-D	BLANK	BLANK	Pos

b

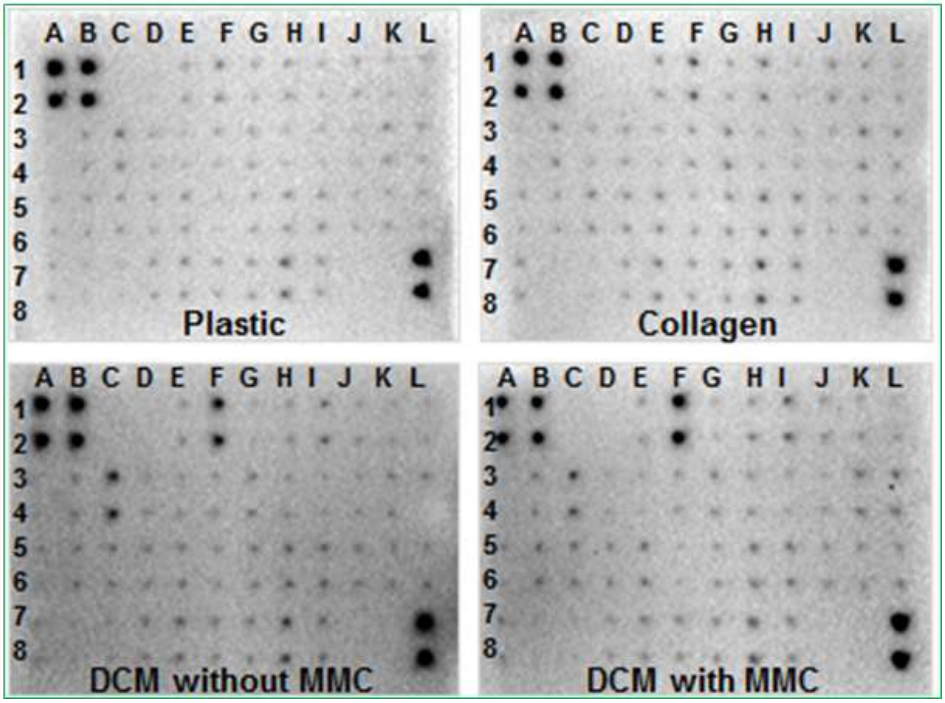

Figure S9: Multiplex growth factors membrane antibody array analysis. (a) Design of membrane for positive control (Pos), negative control (Neg), blanks and growth factors and associated molecules (see figure S10 for abbriviation). (b) Multiplex growth factors membranes after exposure for densitometry analysis.

Basic Fibroblast growth factor	bFGF
Fibroblast growth factor	FGF-4
Fibroblast growth factor	FGF-6
Fibroblast growth factor	FGF-7
Transforming growth factor alpha	TGF-α
Transforming growth factor beta	TGF-β
Transforming growth factor beta-2	TGF-β2
Transforming Growth Factor-β3	TGF-β3
Insulin-like growth factor I	IGF-I
Insulin-like growth factor 2	IGF-II
member of the epidermal growth factor	AR
Epidermal growth factor	EGF
Heparin-binding EGF-like growth factor	HBEGF
Vascular endothelial growth factor A	VEGF-A
Vascular endothelial growth factor D	VEGF-D
Stem cell factor	SCF
Placental growth factor	PIGF
Insulin-like growth factor-binding protein 1	IGFBP-1
Insulin-like growth factor-binding protein 2	IGFBP-2
Insulin-like growth factor-binding protein 3	IGFBP-3
Insulin-like growth factor-binding protein 4	IGFBP-4
Insulin-like growth factor-binding protein 6	IGFBP-6
Epidermal growth factor receptor	EGFR
Insulin like growth factor 1 receptor	IGF-I sR
Macrophage colony-stimulating factor receptor	MCSF-R
Platelet-derived growth factor receptor-alpha	PDGFRα
Platelet-derived growth factor receptor-beta	PDGFRβ
Stem cell factor receptor	SCFR
Vascular endothelial growth factor receptor 2	VEGFR2
Vascular endothelial growth factor receptor 3	VEGFR3
Glial cell line-derived neurotrophic factor	GDNF
beta-Nerve Growth Factor	bNGF
Neurotrophin-3	NT-3
Neurotrophin-4	NT-4
Platelet-derived growth factor-AA	PGDF-AA
Platelet-derived growth factor-AB	PGDF-AB
Platelet-derived growth factor-BB	PGDF-BB
Granulocyte-colony stimulating factor	GCSF
Granulocyte-macrophage colony-stimulating factor	GM-CSF
Macrophage colony-stimulating factor	M-CSF
Hepatocyte growth factor	HGF

Figure S10: Multiplex growth factors membrane antibody array analysis abbriviation.

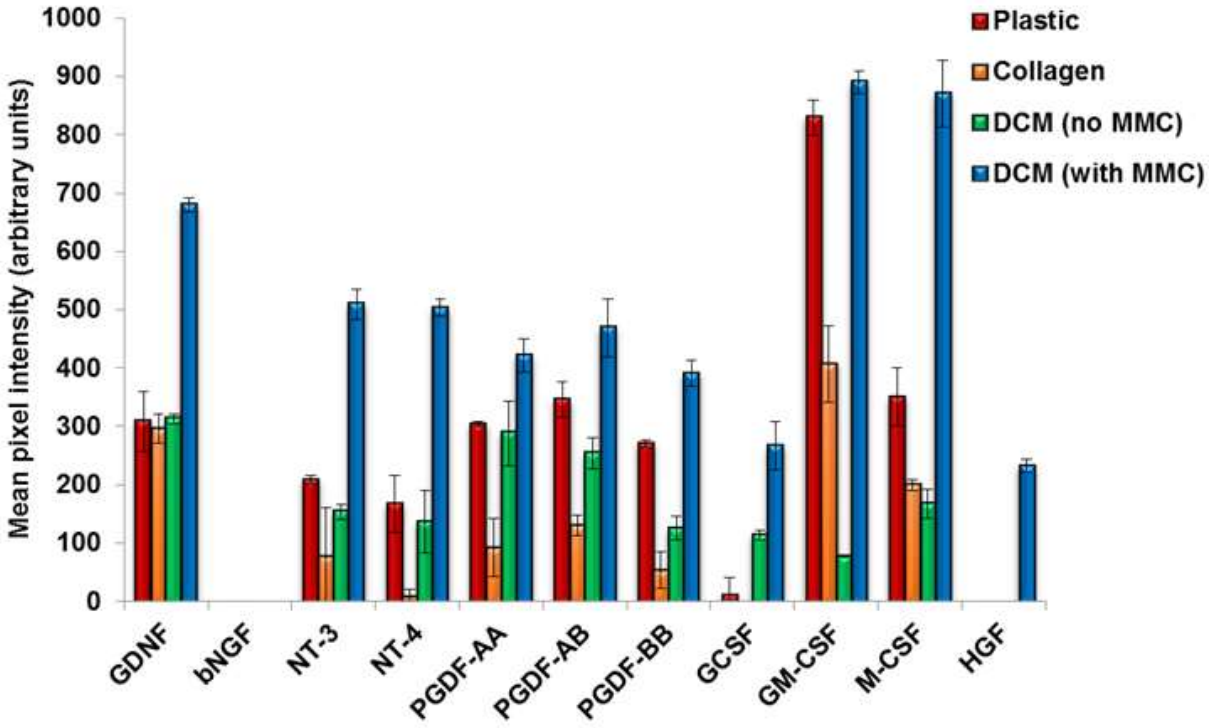

Figure S11: The densitometry analysis of multiplex growth factors membrane antibody array confirmed higher expression of many growth factors (GDNF, NT-3, NT-4, PGDF-AA, PGDF-BB, GCSF, M-CSF and HGF) in podocytes cultured on decellularized matrix with MMC in comparison with other culture conditions (plastic, collagen coated, DCM without MMC).

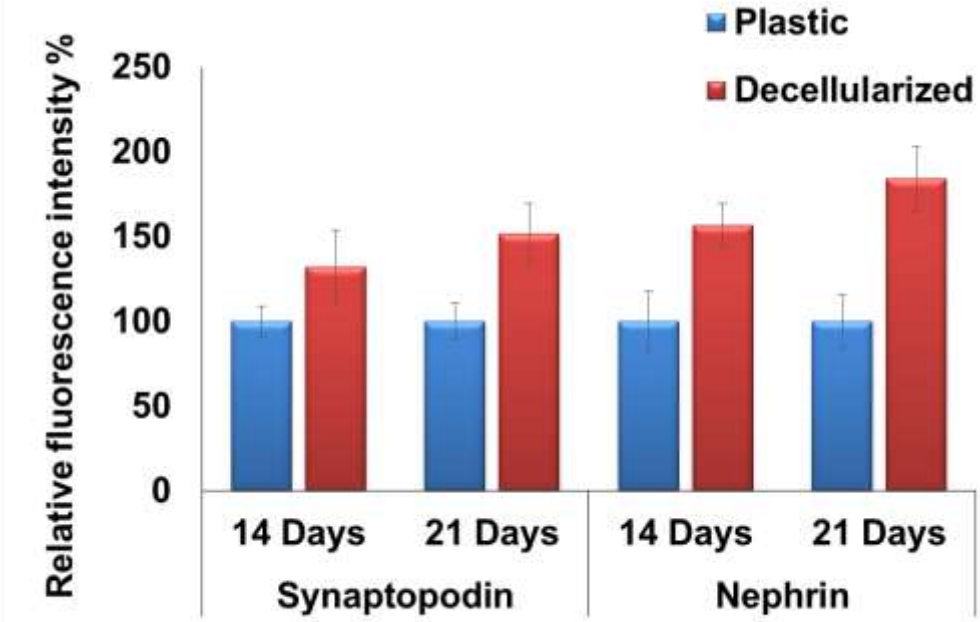

Figure S12: The relative fluorescent intensity calculation of immunofluorescence stained podocytes confirmed higher expression of synaptopodin and nephrin at 14 and 21 days cultured on decellularized ECM-rich substrate under differentiation conditions.

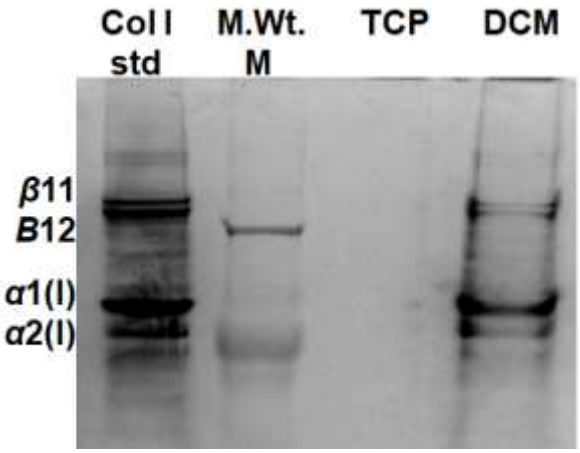

Figure S13: Coomassie brilliant blue stained SDS-PAGE gels confirmed the absence of collagen type I in samples isolated for podocyte cultures on TCP. Higher deposition of collagen type I was observed in DCM well plates. TCP – Tissue culture plastic plates. DCM – Cell derived decellularized matrix well plates. Col I std – 100 µg/ml collagen type I from rat tail (Sigma). M. Wt. M – SeeBlue® molecular weight marker.