Titanium Dioxide Nanotube Arrays Coated with Laminin Enhance C2C12 Skeletal Myoblast Adhesion and Differentiation

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Figure S1. X-ray photoelectron spectroscopy of a representative array of TiO_2 nanotubes (50 nm diameter). Deconvolution of the C1s peak after anodization and cleaning (a), and after annealing (b).



Figure S2. Scanning electron microscopy images of annealed titanium foil before and after laminin coating.



Vinculin

Figure S3. Confocal fluorescence microscopy image of immunostained C2C12 cells on arrays of 10 nm diameter (a, b). Crosses evidence fluorescence maxima as identified by ImageJ software (b). The same process was repeated on the blue channel for automated counting of nuclei (not shown).

Vinculin clusters/cell: substrate comparison		Statistically significant difference
uncoated control	uncoated 10 nm	-
uncoated control	uncoated 50 nm	_
uncoated control	uncoated 100 nm	_
uncoated control	coated control	**
uncoated control	coated 10 nm	***
uncoated control	coated 50 nm	**
uncoated control	coated 100 nm	-
uncoated 10 nm	uncoated 50 nm	-
uncoated 10 nm	uncoated 100 nm	-
uncoated 10 nm	coated control	**
uncoated 10 nm	coated 10 nm	***
uncoated 10 nm	coated 50 nm	**
uncoated 10 nm	coated 100 nm	-
uncoated 50 nm	uncoated 100 nm	-
uncoated 50 nm	coated control	***
uncoated 50 nm	coated 10 nm	***
uncoated 50 nm	coated 50 nm	***
uncoated 50 nm	coated 100 nm	*
uncoated 100 nm	coated control	***
uncoated 100 nm	coated 10 nm	***
uncoated 100 nm	coated 50 nm	***
uncoated 100 nm	coated 100 nm	-
coated control	coated 10 nm	*
coated control	coated 50 nm	-
coated control	coated 100 nm	-
coated 10 nm	coated 50 nm	*
coated 10 nm	coated 100 nm	***
coated 50 nm	coated 100 nm	-

Table S1. Complete statistical analysis of the number of vinculin clusters *per* cell, as quantifiedwith ImageJ analysis on fluorescence microscopy images of immunostained C2C12 cells.*: p < 0.05; **: p < 0.005; ***: p < 0.001.

% area covered by cells:		Statistically significant
substrate comparison		amerence
uncoated control	uncoated 10 nm	-
uncoated control	uncoated 50 nm	*
uncoated control	uncoated 100 nm	-
uncoated control	coated control	**
uncoated control	coated 10 nm	***
uncoated control	coated 50 nm	-
uncoated control	coated 100 nm	***
uncoated 10 nm	uncoated 50 nm	**
uncoated 10 nm	uncoated 100 nm	-
uncoated 10 nm	coated control	**
uncoated 10 nm	coated 10 nm	***
uncoated 10 nm	coated 50 nm	-
uncoated 10 nm	coated 100 nm	***
uncoated 50 nm	uncoated 100 nm	-
uncoated 50 nm	coated control	* * *
uncoated 50 nm	coated 10 nm	***
uncoated 50 nm	coated 50 nm	***
uncoated 50 nm	coated 100 nm	***
uncoated 100 nm	coated control	***
uncoated 100 nm	coated 10 nm	***
uncoated 100 nm	coated 50 nm	**
uncoated 100 nm	coated 100 nm	***
coated control	coated 10 nm	**
coated control	coated 50 nm	-
coated control	coated 100 nm	-
coated 10 nm	coated 50 nm	**
coated 10 nm	coated 100 nm	-
coated 50 nm	coated 100 nm	*

Table S2. Complete statistical analysis of the percentage area covered by cells, as quantified withImageJ analysis on fluorescence microscopy images of Live/Dead® stained C2C12 cells.*: p < 0.05; **: p < 0.005; ***: p < 0.001.

WST-1 assay:		Statistically significant difference	
substrate comparison		24 h	72 h
uncoated control	uncoated 10 nm	-	***
uncoated control	uncoated 50 nm	**	-
uncoated control	uncoated 100 nm	*	*
uncoated control	coated control	-	***
uncoated control	coated 10 nm	-	***
uncoated control	coated 50 nm	-	***
uncoated control	coated 100 nm	-	***
uncoated 10 nm	uncoated 50 nm	-	***
uncoated 10 nm	uncoated 100 nm	-	-
uncoated 10 nm	coated control	*	***
uncoated 10 nm	coated 10 nm	-	***
uncoated 10 nm	coated 50 nm	-	*
uncoated 10 nm	coated 100 nm	-	**
uncoated 50 nm	uncoated 100 nm	-	-
uncoated 50 nm	coated control	***	***
uncoated 50 nm	coated 10 nm	-	***
uncoated 50 nm	coated 50 nm	*	***
uncoated 50 nm	coated 100 nm	-	***
uncoated 100 nm	coated control	***	***
uncoated 100 nm	coated 10 nm	-	***
uncoated 100 nm	coated 50 nm	*	***
uncoated 100 nm	coated 100 nm	-	***
coated control	coated 10 nm	*	-
coated control	coated 50 nm	-	-
coated control	coated 100 nm	*	-
coated 10 nm	coated 50 nm	-	**
coated 10 nm	coated 100 nm	-	*
coated 50 nm	coated 100 nm	-	-

Table S3. Complete statistical analysis of the metabolic activity by cells, as quantified with WST-1assay on C2C12 cells. *: p < 0.05; **: p < 0.005; ***: p < 0.001.

PicoGreen assay:		Statistically significant difference	
substrate comparison		24 h	72 h
uncoated control	uncoated 10 nm	*	-
uncoated control	uncoated 50 nm	-	-
uncoated control	uncoated 100 nm	-	***
uncoated control	coated control	-	***
uncoated control	coated 10 nm	* * *	***
uncoated control	coated 50 nm	-	***
uncoated control	coated 100 nm	***	-
uncoated 10 nm	uncoated 50 nm	-	-
uncoated 10 nm	uncoated 100 nm	*	-
uncoated 10 nm	coated control	-	***
uncoated 10 nm	coated 10 nm	-	***
uncoated 10 nm	coated 50 nm	*	*
uncoated 10 nm	coated 100 nm	***	-
uncoated 50 nm	uncoated 100 nm	-	***
uncoated 50 nm	coated control	-	***
uncoated 50 nm	coated 10 nm	**	***
uncoated 50 nm	coated 50 nm	-	* * *
uncoated 50 nm	coated 100 nm	***	***
uncoated 100 nm	coated control	-	-
uncoated 100 nm	coated 10 nm	* * *	**
uncoated 100 nm	coated 50 nm	-	-
uncoated 100 nm	coated 100 nm	***	**
coated control	coated 10 nm	* * *	-
coated control	coated 50 nm	-	-
coated control	coated 100 nm	* * *	* * *
coated 10 nm	coated 50 nm	* * *	*
coated 10 nm	coated 100 nm	* * *	* * *
coated 50 nm	coated 100 nm	* * *	**

Table S4. Complete statistical analysis of the fluorescence from ds-DNA quantity of C2C12 cells,as quantified with PicoGreen assay. *: p < 0.05; **: p < 0.005; ***: p < 0.001.

Myotube width:		Statistically significant
substrate comparison		difference
uncoated control	uncoated 10 nm	*
uncoated control	uncoated 50 nm	-
uncoated control	uncoated 100 nm	**
uncoated control	coated control	-
uncoated control	coated 10 nm	***
uncoated control	coated 50 nm	**
uncoated control	coated 100 nm	**
uncoated 10 nm	uncoated 50 nm	-
uncoated 10 nm	uncoated 100 nm	-
uncoated 10 nm	coated control	*
uncoated 10 nm	coated 10 nm	***
uncoated 10 nm	coated 50 nm	-
uncoated 10 nm	coated 100 nm	-
uncoated 50 nm	uncoated 100 nm	-
uncoated 50 nm	coated control	-
uncoated 50 nm	coated 10 nm	***
uncoated 50 nm	coated 50 nm	-
uncoated 50 nm	coated 100 nm	-
uncoated 100 nm	coated control	**
uncoated 100 nm	coated 10 nm	***
uncoated 100 nm	coated 50 nm	-
uncoated 100 nm	coated 100 nm	-
coated control	coated 10 nm	***
coated control	coated 50 nm	**
coated control	coated 100 nm	**
coated 10 nm	coated 50 nm	***
coated 10 nm	coated 100 nm	***
coated 50 nm	coated 100 nm	-

Table S5. Complete statistical analysis of the myotube widths, as quantified with ImageJ analysison fluorescence microscopy images of TRITC-phalloidin and DAPI stained C2C12 cells. *:p < 0.05;**: p < 0.005; ***: p < 0.001.