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## Support Information

### Immunological Electrospun Scaffold for Tumor Imprison Killing and Healthy Tissue Regeneration

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## **Materials and methods**

Poly (L-lactide) (PLLA,  $M_w = 100$  kDa,  $M_w/M_n = 1.83$ ) was purchased from Jinan Daigang Co. (Jinan, China). Dichloromethane (DCM) was supplied from Jiangsu Qiangsheng Functional Chemistry Co. Ltd. N,N-Dimethylformamide (DMF) was provided by Shanghai Qiangshun Chemical Reagent Co.Ltd (Shanghai, China). Dopamine hydrochloride (98%) was purchased by Sigma-Aldrich (Saint Louis, USA). Lymphocyte Separation Medium (Human, Tianjin, China). Rat anti CD40 antibody (Clone No. 3/23, IgG, specific to human and mouse) and Rat antibody (IgG) was supplied from Abcam (Cambs, Britain). Commercial IgG-FITC and commercial CD40mAb-FITC was obtained from eBioscience (California, USA). MDA-MB-231 and MC3T3-E1 cells were obtained from Shanghai Institute of Biochemistry and Cell Biology (Shanghai, China).

## **Electrospinning**

PLLA solution was prepared as following: 1 g PLLA was added into 8g DCM, the mixture was stirred severely for 20 minutes, then 4g DMF was added into the mixture to obtain the control electrospinning solution. The electrospinning processes were performed as described previously.<sup>1</sup> Briefly, the following parameters for electrospinning were applied: voltage, 12 kV; solution feed rate, 0.2 ml/min; distance between the needle and the collector, 12 cm; syringe needle diameter, 0.9 mm. All electrospun fibrous scaffolds were dried with vacuum at room temperature for 2 days before using.

## **PDA coating and CD40mAb grafting onto the electrospun fibers**

All solutions used were filtrated for sterilization. PLLA electrospun fibrous scaffolds

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(PLLA) were immersed in 75% ethanol at room temperature for 5h. The PLLA electrospun fibrous scaffolds were precoated with PDA through incubating with PDA tris-buffer solution (10 mM Tris, pH 8.5) at room temperature for 24 hand rinsed with Phosphate Buffered Saline (PBS, pH 7.4). Then the PDA-coated PLLA electrospun fibrous scaffolds (PLLA-PDA) were immersed into CD40mAb solution (12  $\mu$ g/ml, PLLA-PDA-CD40mAb) and IgG (12 $\mu$ g/ml, PLLA-PDA-IgG) at 4°C for 24h. The antibody-binding electrospun fibrous scaffolds were rinsed 5 times with PBS for future use.

### **Characterizations of electrospun microfibers**

The morphology of the fibrous scaffolds was observed by scanning electron microscopy (SEM, FEI Quanta 200, Netherlands) with an acceleration voltage of 10 kV. The surface of the fibers was sputter-coated with platinum before SEM observation. A contact angle analyzer (DSA25S, Data Physics Corporation) was applied to measure the static water contact angles (WCA) of the fibrous scaffolds. X-ray photoelectron spectroscopy (XPS, XSAM800, Britain) was used to determine the chemical compositions of the fibrous scaffolds surface.

The dry fibrous scaffolds were punched into dumbbell shaped specimens (15.0 $\times$ 3.0 $\times$ 0.13 mm<sup>3</sup>) before testing. The uniaxial tensile test was administrated by a mechanical testing machine (Hengyi, China).

### **Binding assay of CD40mAb**

PLLA-PDA electrospun fibrous scaffolds (2 $\times$ 3cm<sup>2</sup>) were immersed in a 1 ml of CD40mAb solution (12  $\mu$ g/ml) at predetermined time intervals. 20  $\mu$ l of the released buffer was collected for analysis, and 20 $\mu$ l of fresh PBS was added back for

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continuing incubation. Subsequently, electrospun fibrous scaffolds were washed with 100  $\mu$ l PBS to remove unbounded CD40mAb. The amount of unbounded CD40mAb in the washing buffer was determined by BCA Protein Assay Kit (Beyotime, China) according to manufacturer's instructions.

### ***In vitro* CD40mAb release**

To evaluate the released profile of CD40mAb, the PLLA-PDA-CD40mAb electrospun fibrous scaffolds were first punched into small squares ( $2\times 3$  cm<sup>2</sup>), which were then immersed in 500  $\mu$ l of PBS (pH 7.4) and incubated at 4°C. At predetermined time intervals, 100  $\mu$ l of the released buffer was collected for analysis, and 100  $\mu$ l of fresh PBS was added back for continuous incubation. The released CD40mAb in the PBS was determined by BCA Protein Assay Kit (Beyotime, China) according to manufacturer's instructions.

### **Detection of CD40mAb grafting onto the electrospun fibrous scaffolds**

The grafting of CD40mAb on the PLLA-PDA electrospun fibrous scaffolds was further analyzed by immunofluorescence staining. The electrospun fibrous scaffolds ( $2\times 3$  cm<sup>2</sup>) were immersed in CD40mAb-FITC (12  $\mu$ g/ml) at 4°C overnight, then washed 3 times and images were collected by fluorescence microscopy (Carl Zeiss, Germany).

### **Scanning Electron Microscope (SEM)**

MDA-MB-231 cells ( $1\times 10^4$ /ml) were cultured on PLLA-PDA, PLLA-PDA-IgG, and PLLA-PDA-CD40mAb scaffolds for 24h, and MC3T3-E1 cells ( $1\times 10^4$ /ml) were cultured on PLLA-PDA, PLLA-PDA-IgG, and PLLA-PDA-CD40mAb scaffolds for 3 days. In briefs, scaffolds were harvested, washed with PBS and fixed with 4%

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glutaraldehyde for 30 min at room temperature. Then the samples were dehydrated using graded ethanol series followed by three rinsing steps with distilled water. Dry specimens were sputter coated with gold and scanned by SEM.

### **Live/dead staining**

Live/dead staining assay were performed using Live/Dead viability/cytotoxicity kit (Molecular Probes, USA). Briefly, MDA-MB-231 cells ( $1 \times 10^4$ /ml) were cultured on scaffolds for 24h and MC3T3-E1 cells ( $1 \times 10^4$ /ml) were cultured on scaffolds for 3d.  $5 \mu\text{l}$  3',6'-Di(O-acetyl)-4',5'-bis[N,N-bis (carboxymethyl) aminomethyl] fluorescein, tetraacetoxymethyl ester (calcein-AM) (Component A) and  $20 \mu\text{l}$  ethidium homodimer<sup>1</sup> (Component B) were diluted into 10 ml PBS to create staining solution. The cell medium was removed and  $200 \mu\text{l}$  of the staining solution was added directly to cells. After 30 min incubation at  $25^\circ\text{C}$  in the dark, the cells were imaged under fluorescence microscope (Carl Zeiss, Germany).

### ***In vitro* cytotoxicity assay**

The proliferation of MDA-MB-231 and MC3T3-E1 cells were measured by Cell Counting Kit-8 (CCK-8, Dojindo, Japan). Briefly, PLLA-PDA, PLLA-PDA-IgG, PLLA-PDA-CD40mAb were incubated in cell medium and the released buffer were collected at 12, 24, 48h. The cells ( $1 \times 10^4$ /ml) were subsequently incubated with those extracted buffer at  $37^\circ\text{C}$  with 5%  $\text{CO}_2$  on the 96-well plates ( $200 \mu\text{l}$ /well). After incubation, the cell medium was removed and replaced with  $100 \mu\text{l}$  culture medium and  $10 \mu\text{l}$  CCK-8 reagent for another 2h for color development. The Optical density (OD) value was collected by the microplate reader (BioTek, USA) at 450 nm.

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### **Identification of cell apoptosis**

Cellular apoptosis of MDA-MB-231 cells were measured by Annexin V Apoptosis Detection Kit FITC (eBioscience, USA). Briefly, MDA-MB-231 cells were cultured in the released buffer of the scaffolds (24h released, final concentration, 1  $\mu\text{g/ml}$ ) for 24 h, cells were collected, and then resuspended. Subsequently, 5  $\mu\text{l}$  Annexin V-FITC was added to 195  $\mu\text{l}$  cell suspension and incubated for 10 min at room temperature in the dark. Then, cells were washed and resuspended in 200  $\mu\text{l}$  Binding buffer with 5 $\mu\text{l}$  propidium iodide (1  $\mu\text{g/ml}$ ).

Last, the fluorescent signal was quantified with a Guava Easycyte HT system (Merk Millipore, Germany) and data was analyzed by Flowjo 7.6.1 software (TreeStar, USA).

### **qRT-PCR for gene expression**

To assess the apoptosis-related gene expression, MDA-MB-231 cells ( $5 \times 10^4$  per ml) were seeded in the released buffer of the different grafted fibrous scaffolds (24 h released, final concentration, 1  $\mu\text{g/ml}$ ) for 1d. The total RNA was extracted using the TRIzolkit (Invitrogen, USA) according to the manufacturer's instructions, and the RNA concentration was determined by NanoDrop (Thermo Scientific, USA). Reverse-transcribed into complementary DNA (cDNA) with the M-MLV reverse transcriptase kit (TaKaRa, Japan). By using a Brilliant SYBR Green QPCR Master Mix (TakaRa, Japan), the cDNA templates were subjected to qRT-PCR to semi-quantify the gene expression. The primers (**Table S1**) were all obtained from Sangon (Shanghai, China). The relative gene expression data was analyzed using the  $2^{-\Delta\Delta\text{Ct}}$  method. All gene expression values were normalized to the GAPDH level.

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## **Induction of DC maturation**

Under the approval of the Ethics Committee of the First Affiliated Hospital of Soochow University, periphere blood was collected after healthy donors gave written informed consent. Periphere blood mononuclear cells (PBMCs) were resuspended in RPMI1640 at cell density of  $3 \times 10^6/\text{ml}$  and seeded in 24-well culture plate (0.5 ml/each), then incubated at 37 °C for 2h in a humidified incubator at 37°C with 5% CO<sub>2</sub>. Then the suspended cell were removed, and the adhesive cells from PBMC were replace with cell culture medium containing GM-CSF (100 ng/ml) and IL-4 (20 ng/ml), purified CD40mAb (1 μg/ml) or the released buffer (24 h, final concentration, 1 μg/ml) of the different grafted fibrous scaffolds, respectively. Cells were incubated in a humidified incubator at 37 °C with 5% CO<sub>2</sub>, the culture medium was changed each 3d. At d7, TNF-α (10 ng/ml) was added to the plates and cells were incubated for another 3d. The antibodies specific to human CD11c-APC, HLA-DR-PE (eBioscience, USA) were applied to stain the cells.<sup>2</sup> Cell phenotype was analyzed by FCA.

## ***In vivo* tumor therapy**

The experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health and the protocol was approved by the Animal Care and Use Committee of Soochow University.

6-week-old female SCIDs were used to bear human breast cancer as previous description.<sup>3</sup> MDA-MB-231 cells ( $2 \times 10^6$ ) are implanted into axillary region by injection to develop humanized breast cancer ectopically. 3 weeks later, after developed-tumors were confirmed by palpation, breast cancer-bearing mice were generally anesthetized by inhalation. Part of the tumor mass was removed surgically



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and the volumes ( $3 \times 3 \times 3 \text{ mm}^3$ ) of developed tumor mass were left *in situ*. The surgical mice were divided into 4 groups randomly. The residual tumors of one group were treated with  $2 \text{ }\mu\text{g}$  CD40mAb in  $20 \text{ }\mu\text{l}$  normal saline around the residual tumors. The ones in the other three groups are wrapped by the PLLA-PDA, PLLA-PDA-IgG, and PLLA-PDA-CD40mAb scaffolds ( $1 \text{ cm}^2$ ,  $1.49 \text{ }\mu\text{g}$ ), respectively. All mice were euthanatized after 2 weeks and tumor mass are collected, scaled, and photographed. The tumors were then fixed in 4% (v/v) paraformaldehyde solution, embedded in paraffin, sectioned into  $5 \text{ }\mu\text{m}$  thickness, and stained with hematoxylin and eosin (H&E) (Beyotime, China) for histological analysis. Immunohistochemical staining was performed using Ki67 antibodies as the markers of cell proliferation. The tumor slides were subjected to epitope retrieval in sodium citrate solution, washed in phosphate buffer solution-Tween (PBST), and incubated with specific antibodies at  $4 \text{ }^\circ\text{C}$  overnight. Following staining, sections were visualized by microscopy (Zessi, Germany). Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) (Beyotime, China) assay was performed to evaluate the apoptosis of tumor cells. Typically, the tumor slices were incubated with deparaffin and protease K, stained with TUNEL solutions and DAPI, and finally observed using microscopy (Zessi, Germany). The expression of Ki67 and the intensity of TUNEL positive staining were quantitated by Bioquant image analysis corporation (Bioquant, USA).

### **Statistical Analysis**

One way ANOVA with the Tukey's post-hoc test were used to discern the statistical difference between groups. Data were presented as mean  $\pm$  standard deviation (SD).

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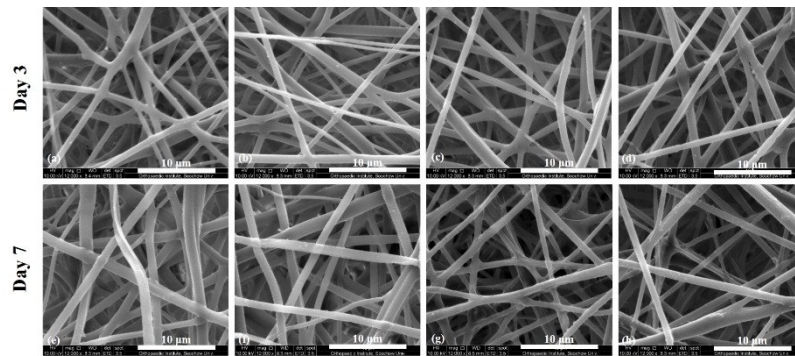
All the data were processed using IBM SPSS Statistics 22 for Windows. A probability value of \*:  $p < 0.05$  was considered to be statistically significant.

#### Reference

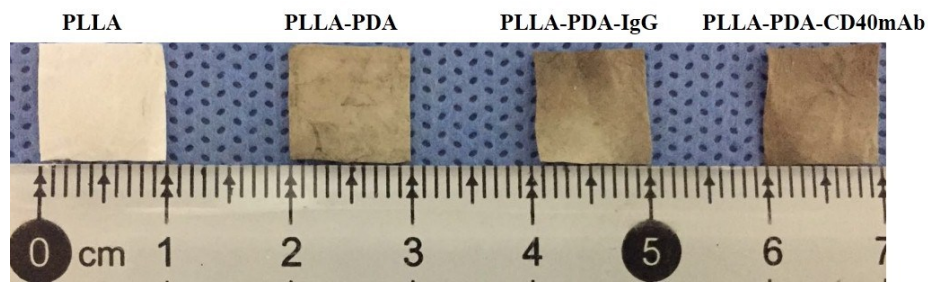
[1] K. L. Ou, C. S. Chen, L. H. Lin, J. C. Lu, Y. C. Shu, W. C. Tseng, J. C. Yang, S. Y. Lee, C. C. Chen, *Eur.Polym.J.* 2011, **47**, 882.

[2] U. Johansson, M. Londei. *Scand. J. Immuno.* 2004, **59**, 50.

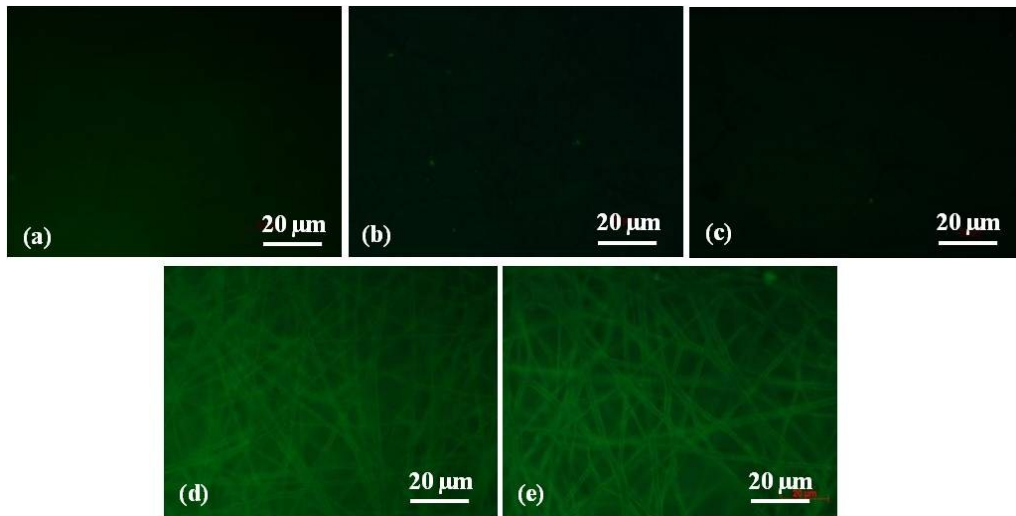
[3] J. Zhang, G. Li, C. Yuan, F. Lei, G. Chen, F. Bai, M. Ma, J. Lyu, Q. H. Meng, *J. Cancer* 2017, **8**, 1849.



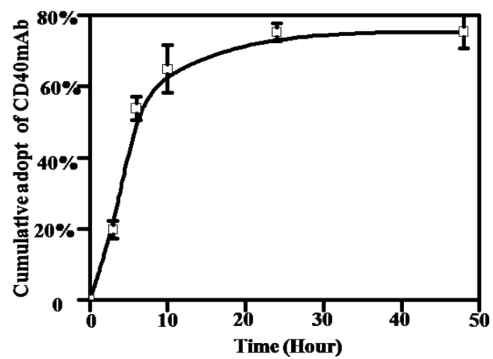
**Figure S1.** SEM images of PLLA (a, e), PLLA-PDA (b, f), PLLA-PDA-IgG (c, g) and PLLA-PDA-CD40mAb (d, h) scaffolds immersed into cell culture medium at 37°C for 3 and 7d.



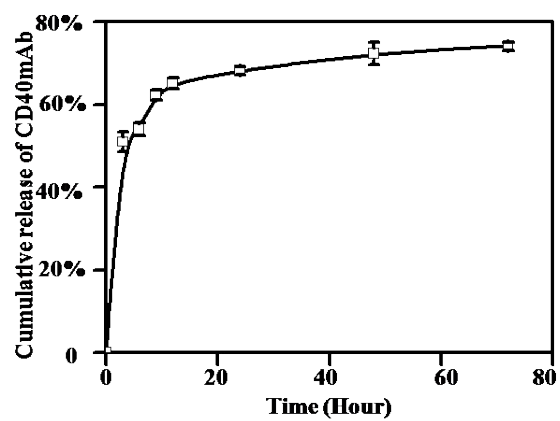
**Figure S2.** The images of PLLA and PLLA modified by PDA or antibodies.



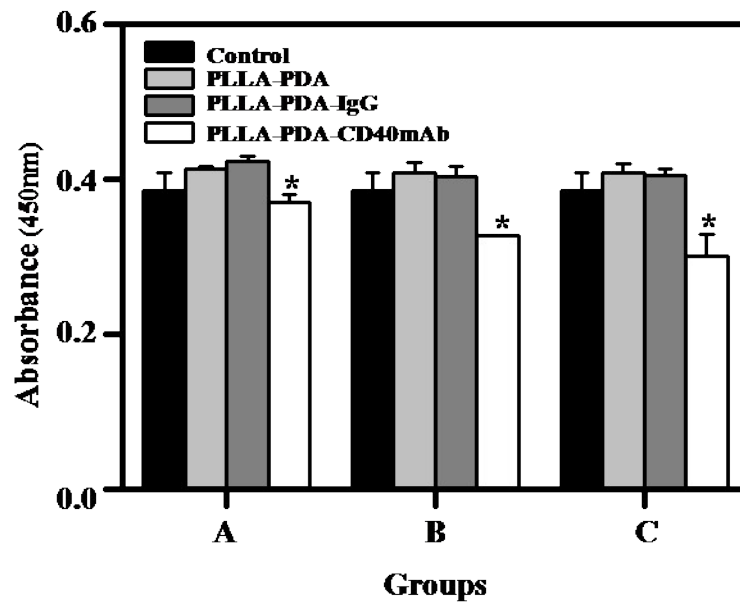
**Figure S3.** Images of the different scaffold by fluorescent microscope.(a) PLLA, (b) PLLA-PDA, (c) PLLA-PDA-CD40mAb, (d) PLLA-PDA-IgG-FITC and (e) PLLA-PDA-CD40mAb-FITC.



**Figure S4.** The adsorb profile of CD40mAb to electrospun fibrous scaffolds at different time points.

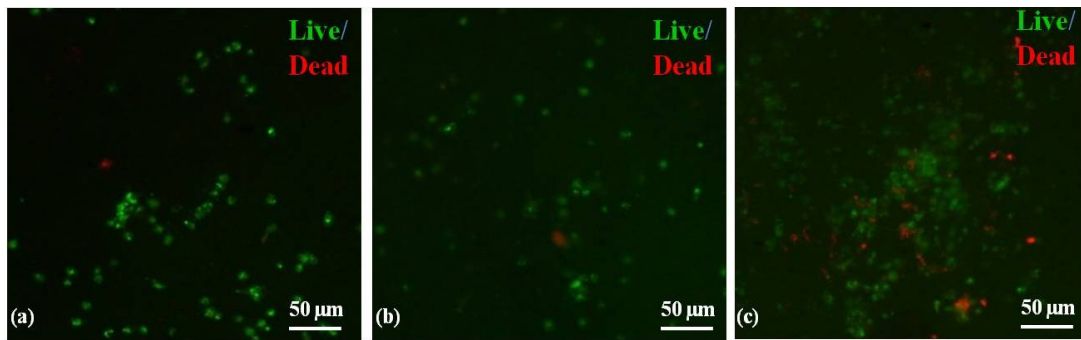


**Figure S5.** Released profile of CD40mAb from electrospun fibrous scaffolds at different time points.

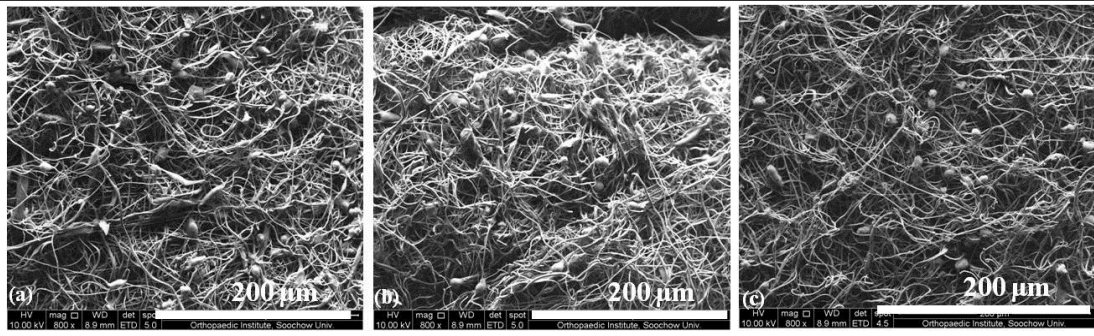


**Figure S6.** CCK-8 assay of MDA-MB-231 cells cultured in the released buffer (12, 24, 48h) for 3d A: 12h, B: 24h, C: 48h. Control: MDA-MB-231 cells seeded on the plate with cell culture medium. \*:  $p < 0.05$

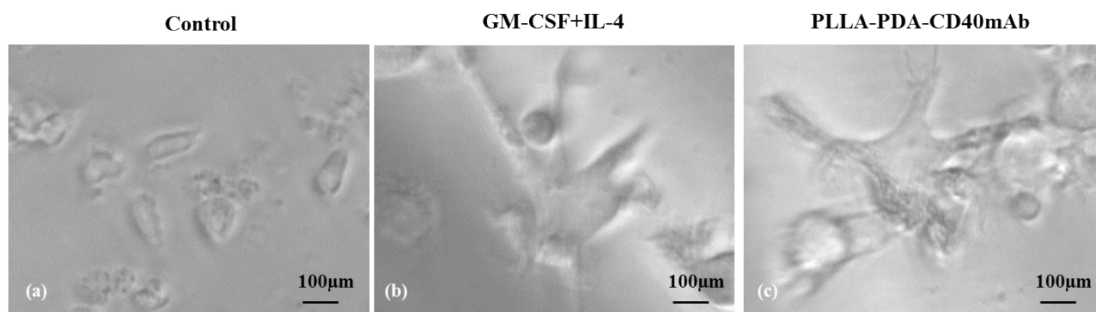




**Figure S7.** Live/dead staining of MDA-MB-231 cells seeded on electrospun for 24 h (a) PLLA-PDA, (b) PLLA-PDA-IgG and (c) PLLA-PDA- CD40mAb.



**Figure S8.** SEM images of MDA-MB-231 cells seeded on electrospun for 24 h (a) PLLA-PDA, (b) PLLA-PDA-IgG and (c) PLLA-PDA-CD40mAb.



**Figure S9.** The morphological images of DC. (a) Control, (b) GM-CSF and IL-4, (c) PLLA-PDA-CD40mAb.

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**Table S1.** Primer sequences specific to human for qRT-PCR.

Target	Primer sequences
BCL-2	GTCTGGGAATCGATCTGGA (5'-3')
	A
BAX	GCAACGATCCCATCAATCT (5'-3')
	T
GAPDH	AGCGACTGATGTCCCTGTCT (5'-3')
	CTCAGCCCATCTTCTTCCAG (5'-3')
	GAGTCAACGGATTTGGTCG (5'-3')
	T
	TTGATTTTGGAGGGATCTCG (5'-3')

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**Table S2.** The Expression of CD11c and HLA-DR.

	CD11c(%)	HLA-DR(%)
Control	28.0±2.21	14.38±4.21
GM-CSF+IL-4	63.5±2.15*	41.7±1.39*
CD40mAb	53.6±0.29*	40.1±1.26*
PLLA-PDA	32.7±2.34	12.28±1.02
PLLA-PDA-IgG	25.6±1.89	8.3±1.25
PLLA-PDA-CD40mAb	42.9±5.25#	29.8±3.07#

\* $p < 0.05$ , compared with the group of the control group; # $p < 0.05$ , compared with the group of PLLA-PDA-IgG

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**Table S3.** The rate of apoptotic cells.

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Control	PLLA-PDA	PLLA-PDA-IgG	PLLA-PDA-CD40mAb
4.25%±0.36%	5.15%±2.19%	4.66%±0.8%	13.03%±1.55%*

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\* $p < 0.05$ , compared with the group of PLLA-PDA-IgG