Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary material

pH-responsive and porous vancomycin-loaded PLGA microspheres: Evidence of controlled and sustained release for localized inflammation inhibition *in vitro*

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#: Yu and Pan contributed equally to this work.

Quantification of Van

The concentration of Van was determined by the absorbance method at 280 nm using UV-vis spectrum. The absorbance measurement of the solution of blank PLGA microsphere in the CH_3CN/water co-solvent system was also carried out to ensure the exclusive absorbance of Van at 280 nm.

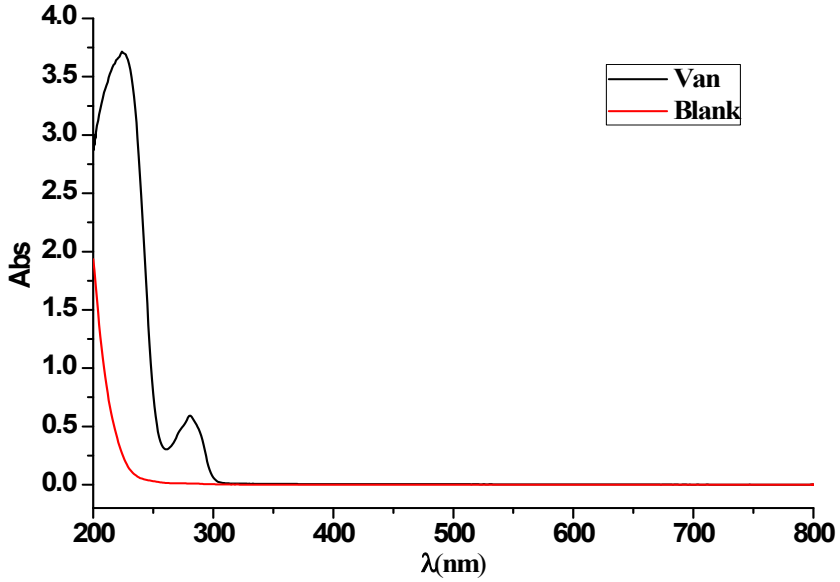


Fig.S1.The UV-vis spectra of blank PLGA microspheres and Van in the acetonitrile/water (5:5, v/v)

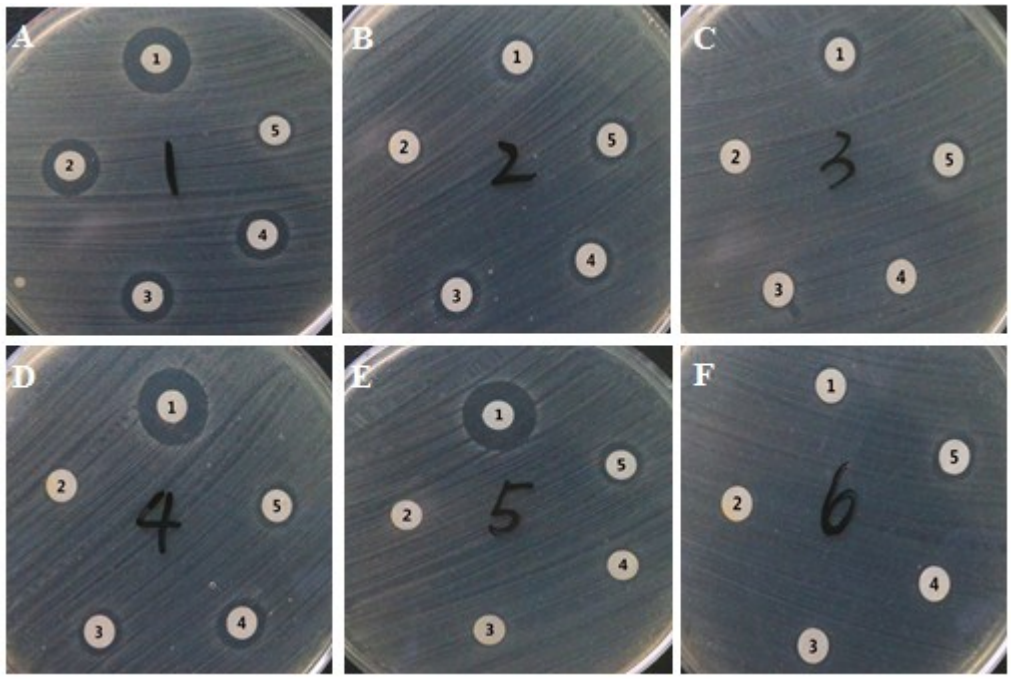
Preparation of PLGA–Van microspheres

PLGA-Van microspheres were prepared with $W_1/O/W_2$ double emulsion-solvent evaporation method. A certain amount of Van was dissolved in gelatin solution to form an internal aqueous phase. Then, 1.0 mL of CH_2Cl_2 containing a certain amount of PLGA was added to the solution. The solution was mixed well and then emulsified using an ultrasonic cell disintegrator for 1 min (1 s on and 1 s off) at 200 W in an ice bath. The primary emulsion (W_1/O) was transferred quickly to a breaker containing 20 mL aqueous PVA solution containing NaCl (previously cooled to 4 °C) under stirring. The PVA solution with a certain content of NaCl was used as external aqueous solution. And then, emulsification was continued at 1400 rpm for 3 min to form a $W_1/O/W_2$ emulsion. Later, The double emulsion was further stirred at 300 rpm for 4 h. Temperature was maintained at 40 °C to allow the evaporation of the organic solvent. And then, PLGA–Van microspheres were precipitated and harvested through centrifugation at 8000 rpm for 10 min. PLGA–Van microspheres were then washed five times with ultrapure water and lyophilized using a freeze dryer after refrigerating under -20℃ overnight. The final product was stored in a desiccator at room temperature. The condition of the preparation, including the concentration of PLGA, the content of PVA, different drug-loading, the volume of internal aqueous phase, the concentration of NaCl in external aqueous phase, the ultrasonic time in the preparation of W/O emulsion, the stirring rate for the formation of $W_1/O/W_2$ emulsion, the original content of gelatine for the internal aqueous and so on, were optimized to obtain the best procedure for the preparing of PLGA–Van microspheres. As shown in Table S1, the optimized conditions was as follows: the concentration of PLGA in CH_2Cl_2 was 75 mg/mL, 2% PVA aqueous solution with 50 mg/mL NaCl was used as outer solution, 10 mg Van was used as drug-loading, the volume of internal aqueous phase was 300μL, the ultrasonic time using in the preparation of W/O emulsion was 1 min, the stirring speed for the formation of $W_1/O/W_2$ emulsion was 1400 rpm, the original content of gelatine for the internal aqueous was 2.5%. In the optimized procedure, PLGA-Van microspheres were smooth with loading efficiency of 6.14% and encapsulation efficiency of 51.85%.

Table S1.Influence of the preparing factors on the properties of PLGA-Van microsphere

Parameters		LE±SD (%)	EE±SD (%)	Particle sizes (μm)
the concentration of PLGA(mg/mL)	30	4.77±0.59	18.87±2.35	29.12±1.37
	50	4.83±0.14	29.86±0.84	30.84±1.42
	75	4.24±0.10	36.31±0.89	33.44±0.82
	100	3.38±0.37	36.33±4.17	35.08±0.61
The content of PVA(%)	1	3.60±0.26	29.80±2.19	37.00±1.21
	2	4.24±0.10	36.31±0.89	33.44±0.82
	3	3.72±0.22	30.91±1.80	26.72±0.62
different drug-loading (mg)	7.5	2.28±0.14	25.4±1.52	39.28±1.26
	10	4.24±0.10	36.31±0.89	33.44±0.82
	15	4.92±0.01	29.28±0.03	32.6±0.83
	25	6.04±0.08	24.49±0.34	32.92±1.57
the volume of internal aqueous phase phase (μL)	100	1.87±0.05	15.47±0.44	30.66±0.95
	300	4.24±0.10	36.31±0.89	33.44±0.82
	500	2.78±0.08	23.01±0.68	39.90±0.87
	1000	1.62±0.19	13.43±1.57	29.44±2.35
NaCl concentration in external aqueous phase (mg/mL)	0	1.48±0.06	12.10±0.50	26.16±1.18
	25	2.83±0.22	23.55±1.82	30.20±2.03
	50	4.24±0.10	36.31±0.89	33.44±0.82
the ultrasonic time (s)	20	3.50±0.16	30.00±1.37	27.08±2.26
	40	3.82±0.16	24.29±1.35	35.61±0.92
	60	4.24±0.10	36.31±0.89	33.44±0.82
the stirring rate (rpm)	950	3.32±0.26	27.77±2.20	39.66±1.54
	1400	4.24±0.10	36.31±0.89	33.44±0.82
	2050	4.13±0.32	35.63±2.72	30.20±0.24
The original content of gelatine for the internal aqueous phase (%)	0	1.71±0.33	10.34±2.02	28.40±1.81
	2.5	6.23±0.62	53.75±5.12	24.56±1.95
	5	4.24±0.10	36.31±0.89	33.44±0.82
	8	3.38±0.20	22.28±1.64	36.47±0.89

a



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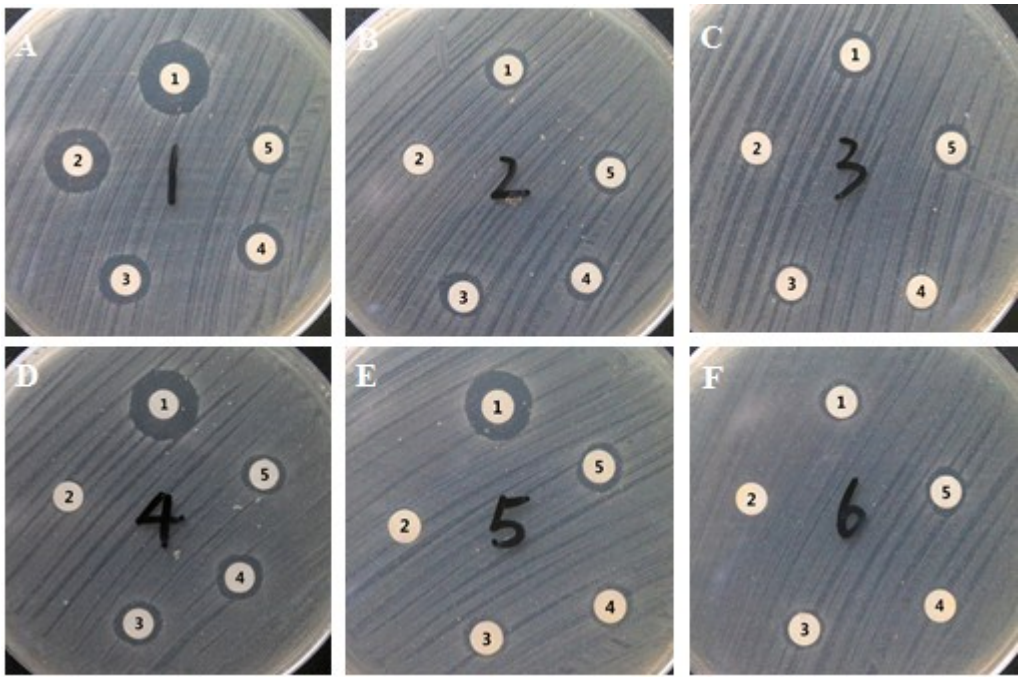

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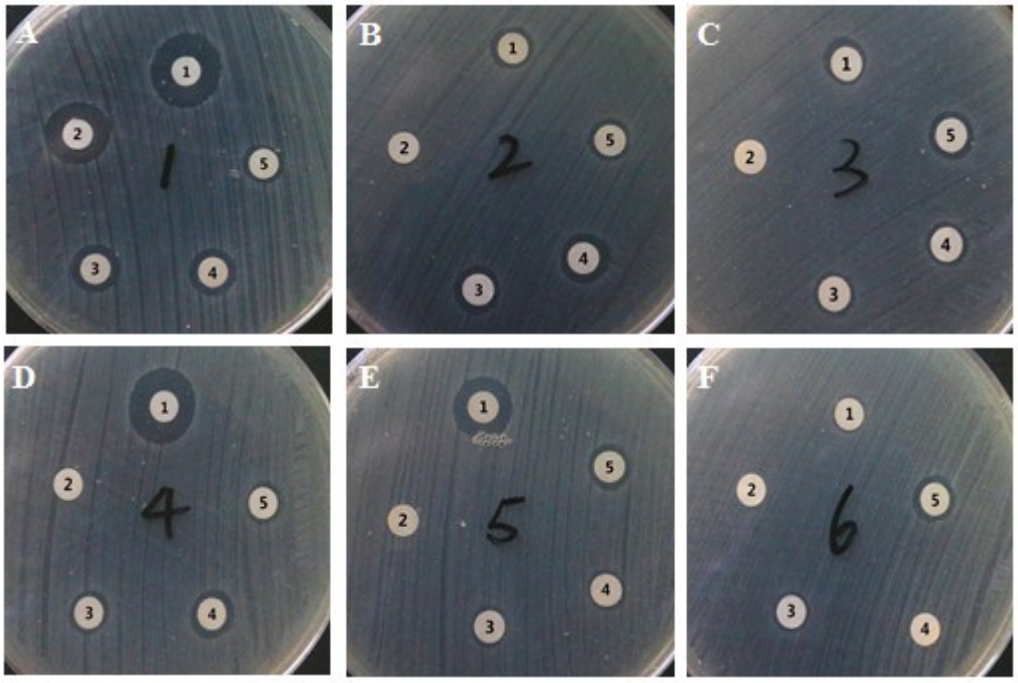
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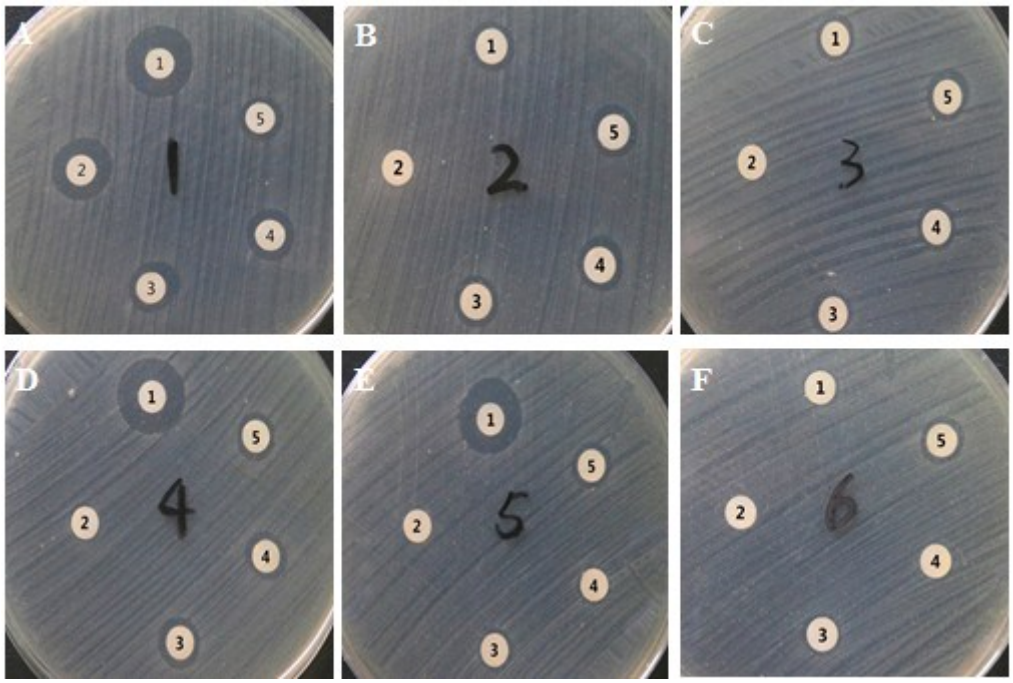
d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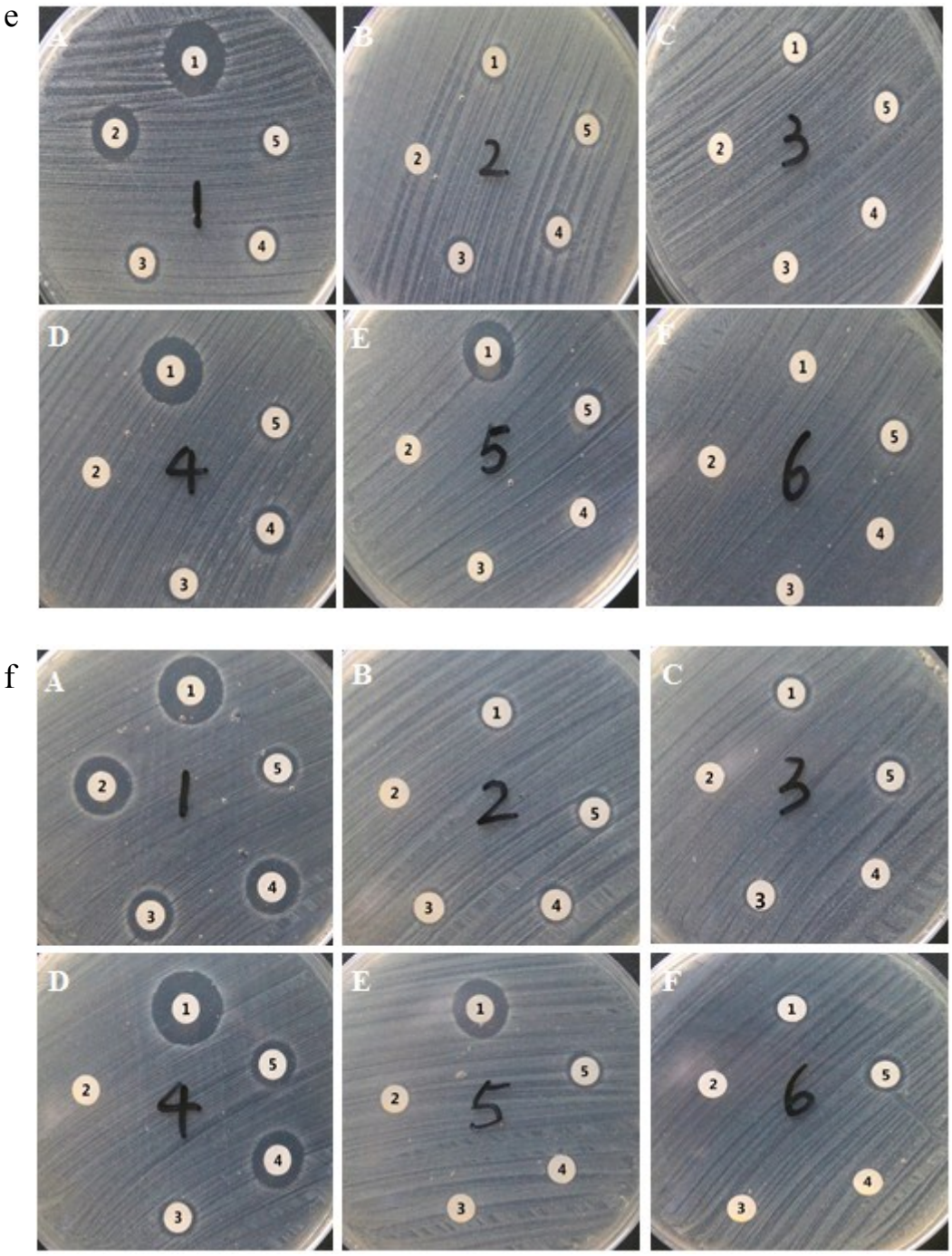
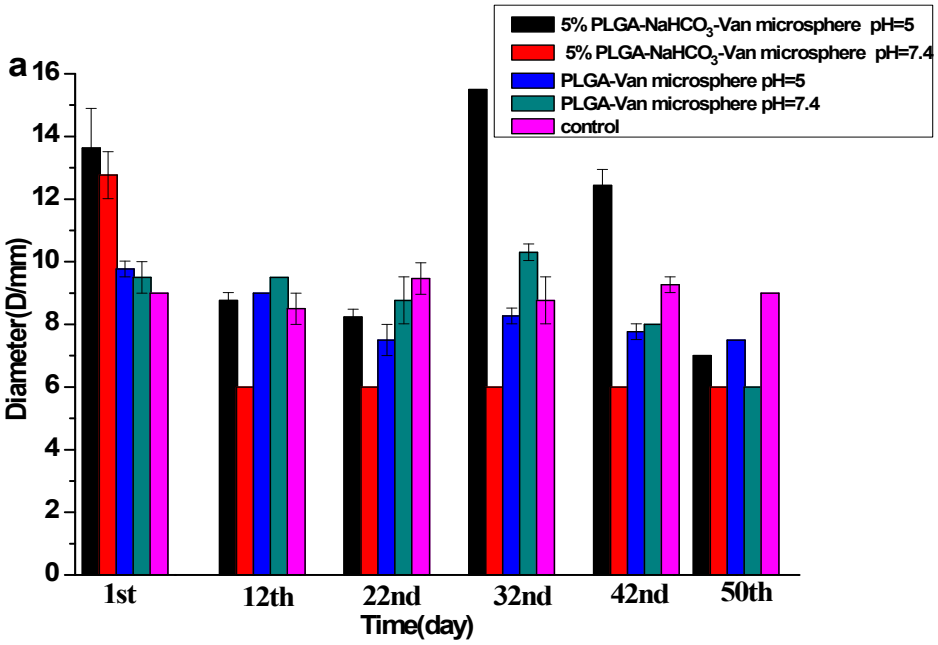
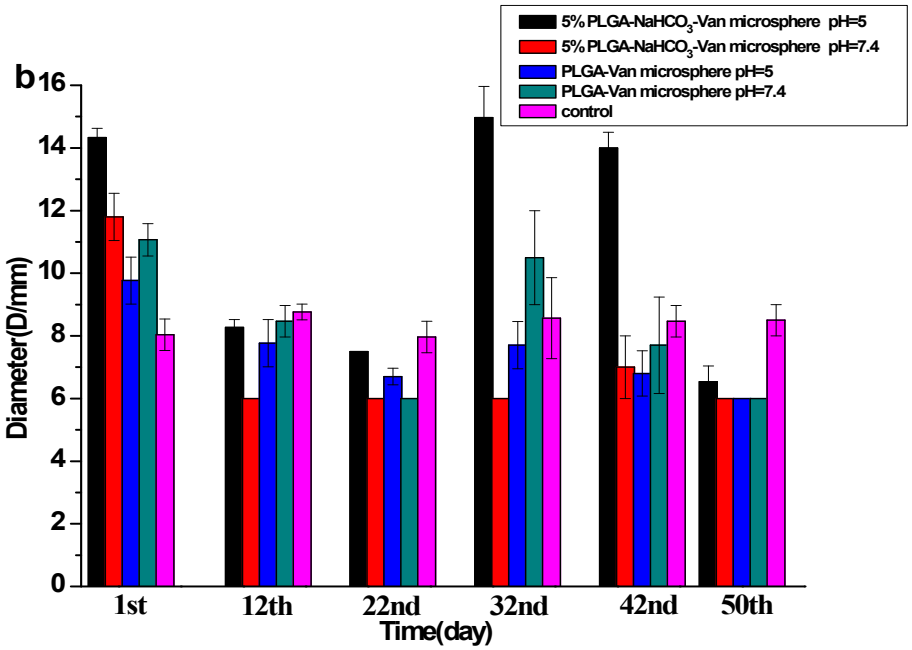
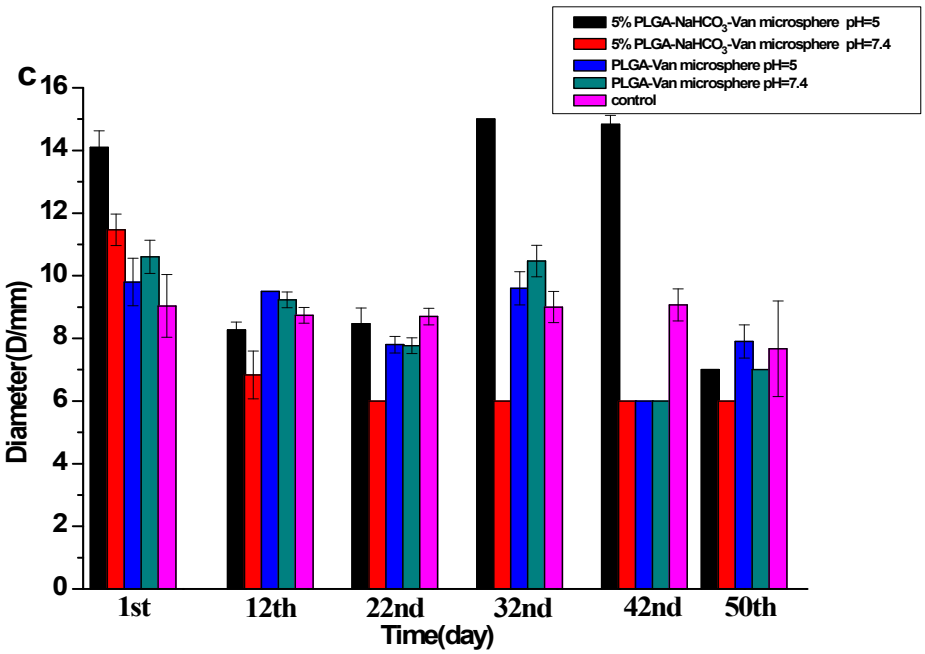
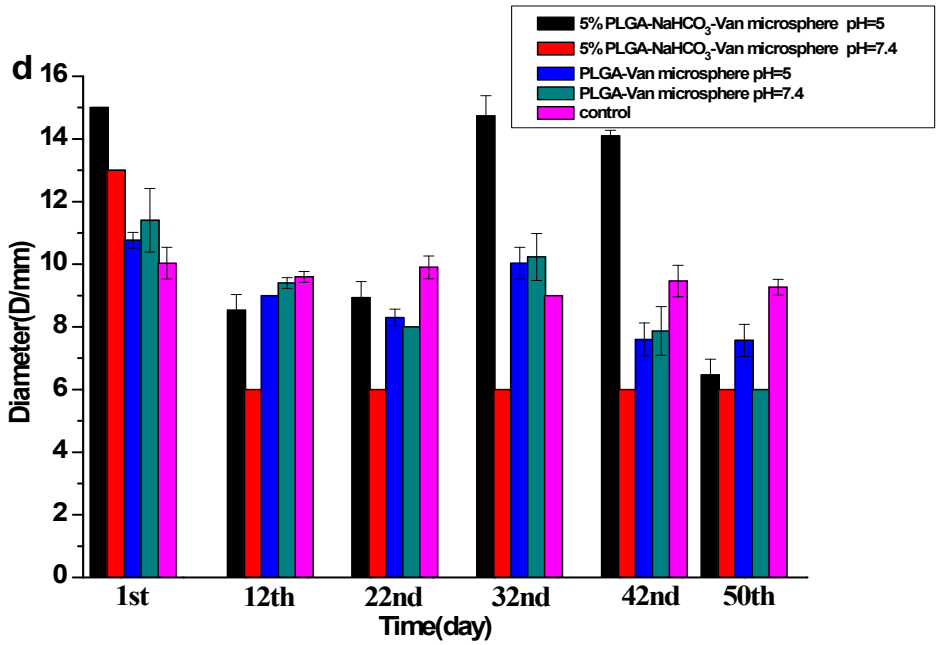


Fig.S2. Bacteriostatic rings of MRSA infecting clinical samples (a-f) cultures on agar treated with 1) 5% PLGA–$NaHCO_3$–Van microspheres under pH 5.0, 2) 5% PLGA–$NaHCO_3$–Van microspheres under pH 7.4, 3) PLGA–Van microspheres under pH 5.0, 4) PLGA–Van microspheres under pH 7.4, and 5) the positive control of 0.2 mg/mL Van. Letters A–F represent the treatment with released Van collected on the 1st, 12th, 22nd, 32nd, 42nd, and 50th days of the releasing experiment.


a
5% PLGA-NaHCO3-Van microsphere pH=5
5% PLGA-NaHCO3-Van microsphere pH=7.4
PLGA-Van microsphere pH=5
PLGA-Van microsphere pH=7.4
control
Diameter(D/mm)
0
2
4
6
8
10
12
14
16
1st
12th
22nd
32nd
42nd
50th
Time(day)


b
5% PLGA-NaHCO3-Van microsphere pH=5
5% PLGA-NaHCO3-Van microsphere pH=7.4
PLGA-Van microsphere pH=5
PLGA-Van microsphere pH=7.4
control
Diameter(D/mm)
0
2
4
6
8
10
12
14
16
1st
12th
22nd
32nd
42nd
50th
Time(day)


c
5% PLGA-NaHCO3-Van microsphere pH=5
5% PLGA-NaHCO3-Van microsphere pH=7.4
PLGA-Van microsphere pH=5
PLGA-Van microsphere pH=7.4
control
Diameter(D/mm)
0
2
4
6
8
10
12
14
16
1st
12th
22nd
32nd
42nd
50th
Time(day)


d
5% PLGA-NaHCO3-Van microsphere pH=5
5% PLGA-NaHCO3-Van microsphere pH=7.4
PLGA-Van microsphere pH=5
PLGA-Van microsphere pH=7.4
control
Diameter(D/mm)
0
2
4
6
8
10
12
14
16
1st
12th
22nd
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42nd
50th
Time(day)

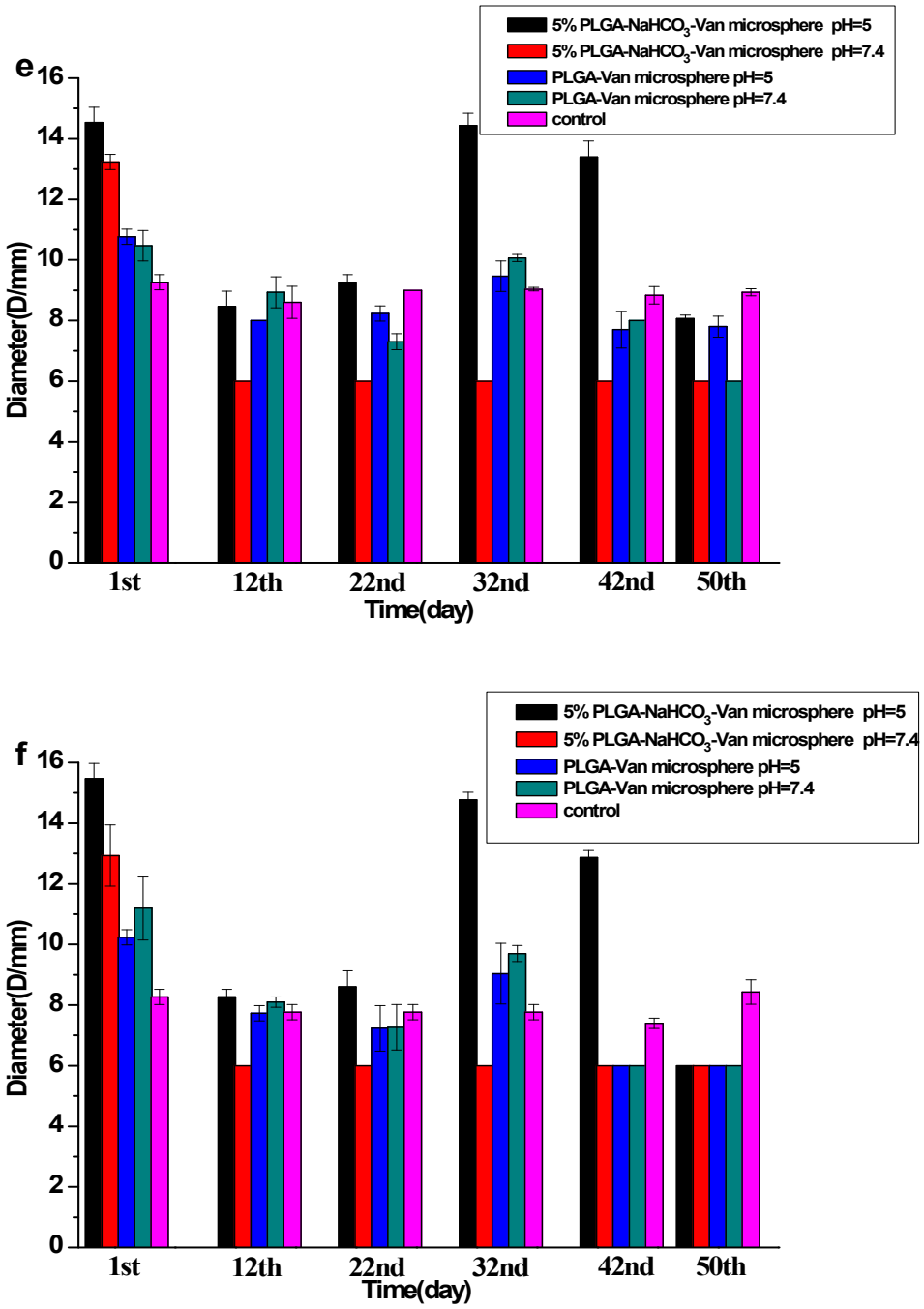

Fig.S3. Change trends of ZOI diameters for MRSA cultures (a-f) at different time (error bars represent the SDs of three independent testing) of different agar treated with Van released from 5% PLGA–$NaHCO_3$–Van microspheres under pH 5.0, 5% PLGA–$NaHCO_3$–Van microspheres under pH 7.4, PLGA–Van microspheres under pH 5.0, PLGA–Van microspheres under pH 7.4, and the positive control of 0.2 mg/mL Van, respectively.