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**Superparamagnetic Anisotropic Nano-assemblies With Longer Blood Circulation in vivo: A High Efficient Drug Delivery Carrier for Leukemia Therapy**

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**Experimental section**

*Materials.* $Fe_3O_4$@OA stored in hexane was obtained from Jiangsu Laboratory for Biomaterials and Devices, Southeast University (Nanjing, China); Poloxamer 188 was purchased from BASF (Shanghai, China); vincristine was purchased form Hubei Kangbaotai Fine-Chemicals Co., Ltd (Wuhan, China). vincristine sulfate injection was purchased form Resources Saike Pharmaceutical Co., Ltd. (Beijing, China). Other chemical reagents were either analytically pure or chromatographically pure.

Animals were purchased from Qinglongshan Animal Center (Nanjing, China), and the experiment was conducted in accordance with the guideline issued by the China Food and Drug Administration (CFDA). Animals were housed and cared for in accordance with the guidelines established by the National Science Council of the Republic of China.

*Preparation of VCR-SANs.* Took precisely $Fe_3O_4$@OA (3 mL) stored in hexane, added ethanol to destabilize the dispersion, separated under the magnetic adsorption, the supernatant was discarded, then added tetrahydrofuran (THF) (1.5 mL), and dispersed under ultrasound to form an uniform systems. Subsequently, VCR (2 mg) and poloxamer 188 (10mg) were added into the dispersion with stirring to be completely dissolved. Drop the obtained dispersion into distilled water (7.5 mL) rapidly and followed by processed with probe ultrasound. Next, set the dispersion under magnetic field with the strength of 0.52T while leave standing to volatilize the THF. After that, filtered through a polycarbonate membrane ($\Phi$ = 100nm), and collected the filtrate. The VCR-SANs was hereto obtained.

*Characterization.* The samples were dispersed on amorphous carbon-coated copper grids with negative staining by phosphotungstic acid (2%) for morphology analysis, respectively. And The morphology was observed using transmission electron microscopy (TEM, Tokyo JEOL). The

hydrodynamic diameter, size distribution and zeta potential of samples were examined using TEM and Zetasizer Nano-ZS90 (Brookhaven Instrument Corporation) by dynamic light scattering (DLS) and electrophoretic mobility at 25 °C. The magnetism and M−H curve of VCR-SANs was obtained by a vibrating sample magnetometer (VSM, Lakeshore VSM 7407).

*Drug release.* To distinguish the shape effect on drug release, VCR was also loaded into superparamagnetic isotropic nano-assemblies (termed VCR-SINs), which were prepared by similar method of VCR-SANs, only magnetic field was neglected. Vincristine sulfate injection, which is a solution of vincristine sulfate (abbreviated as VCR), and VCR-SINs were both as the comparison groups.

Ratio of drug to iron of VCR-SINs was regulated to the same level with VCR-SANs. Three milliliter aliquots of each ultimate preparation were placed in dialysis bag (3000 molecular weight cutoff). The dialysis bag was immersed in phosphate buffer (100 mL, pH 7.4) and shaked continuously at 37 °C. One milliliter aliquot of release medium at 10, 30, 60,120 min, and 4, 6, 8, 24 h, was removed and the same volume of fresh medium was added periodically. Twenty microliter aliquots of the released drug were quantified at 297 nm by HPLC (Shimadzu 2020 LC system, Shimadzu Corp., Japan) using Lichrosphere C18 column (150 mm × 4.6 mm, 5 μm particle size; Hanbon Sci. & Tech., Huaian, China), with potassium dihydrogen phosphate–methanol (30:70, v/v) as mobile phase at 30 °C at a flow-rate of 1.0 mL $min^{-1}$.

*Anti-leukemia effects in vivo.* NOD/SCID mice, irradiated 270cGy on body by 137Cs, were randomly divided into four groups. K562 cells were injected into mice through tail vein, each mouse got about $1\times10^{6}$ cells. After three weeks, animals were received intravenous administration of VCR-SINs and VCR-SANs, respectively, at day 0, 3, 6 and 9 at equal dose of VCR of 1 mg $kg^{-1}$. K562 cells in peripheral blood were identified by Wright's stain. Leukocyte count and positive

expression of CD 13 in peripheral blood were monitored and recorded by crystal violet stain and flow cytometry, respectively.

*Pharmacokinetics and tissue distribution in vivo.* 9 male Sprague-Dawley rats were divided randomly into three groups after fasting for 16 h. VCR-SINs and VCR-SANs were injected into tail vein at identical dose of 6 mg $kg^{-1}$, respectively. After injection, blood samples were taken from the retro-orbital plexus at 0.083, 0.25, 0.5, 1, 2, 4, 8 h, and subsequently centrifuged at 2000 rpm for 10 min. Then, took plasma (200 μL) precisely, added methanol (400 μL), shook and centrifuged at 15000 rpm for 10 min, determined the supernatant by HPLC as mentioned above. Plasma concentration-time curves were fitted by Pksolver pharmacokinetics software.[10] The pharmacokinetics parameters obtained using the principle of statistical moments were analysed by the t-test.

45 male mice were divided into 3 groups randomly, which were injected VCR-SINs and VCR-SANs at the identical VCR dose of 10mg $kg^{-1}$, through tail vein. And the tissues including heart, liver, spleen, lung, and kidney, were excised, subsequently emerged into 30% saccharose solution and then cut into slices to stain with prussian blue for 30 min, washed using PBS for 3 times, and next was stained using nuclear fast red for 5 min, washed using PBS, then observed under the microscope.

*Safety assay.* Took the excised tissues including heart, liver, spleen, lung, and kidney, which was obtained from the experiment of tissue distribution using prussian blue staining, emerged into saccharose solution (30%) and then cut into slices to stain with hematoxylin for 30 min, washed using PBS for 3 times, and next was stained using eosin for 3 min, washed using PBS, and then processed orderly with ethanol and dimethylbenzene, subsequently observed under the microscope.

All the animal experiments were performed in compliance with the Guidelines of the Animal Research Ethics Board of Southeast University. Full details of approval of the study can be found in the approval ID: 20080925.

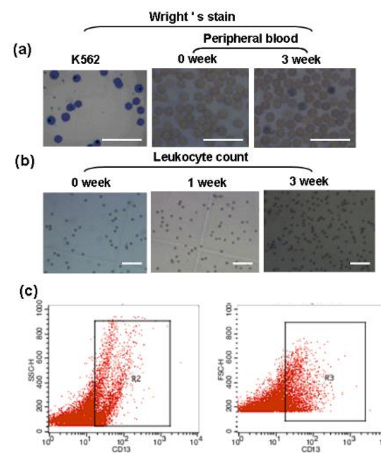


**Figure S1.** Changes in numbers of leukocytes in peripheral blood after the administration via tail vein for one week and three weeks. (a) The leukemia cells were found on peripheral blood smear by Wright's stain, (b) the count of peripheral blood leukocyte was 5 times as much as that before inoculation, (c) CD 13 in peripheral blood was detected and positive expression ratio of SSC (side scatter) and FSC (forward scatter) were 31.48% and 29.56%. (Scale bar =100 μm)

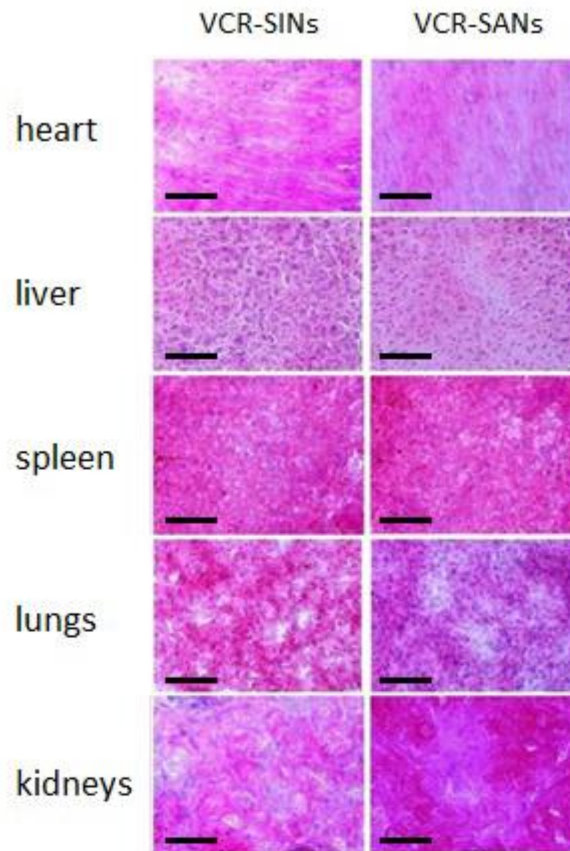


**Figure S2.** Histopathological evaluation of heart, liver, spleen, lungs and kidneys of mice after intravenous administration (10 $mgkg^{-1}$) of VCR-SINs, VCR-SANs. No abnormal histopathological findings were observed. Samples were stained with hematoxilin and eosin (HE) and observed at ×400. (Scale bar = 100 μm)