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# Shear flow induced long-range ordering of rod-like viral nanoparticles within hydrogel

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ABSTRACT

Controlling the alignment of anisotropic nanoparticles within a three-dimensional (3D) environment over large-scale is still a challenge. In this paper, a facile method to align rod-like nanoparticles in hydrogel via shear flow to afford a long-range order is reported. The rod-like tobacco mosaic virus (TMV) was employed as a prototypical anisotropic particles in the study, and the shear force provided by the unidirectional flow of the precursor solution direct the alignment of TMVs, which could be quickly fixed through the fast sol-gel transition *in situ*. The degree of orientation and the distance between the TMV particles could be regulated by adjusting the concentration of hydrogels and TMVs. While the introduction of TMVs could reduce the degree of swelling of the hydrogel and help maintaining the mechanical strength of resultant hydrogels, both repulsion interaction and shear flow contributed synergistically to the assembly. This method does not require the usage of strong magnetic or electric fields, nor does it require the use of specialized lithography, thus offers a facile way to the fabrication of hydrogel materials with control of anisotropic structural features.



## 1. Introduction

Anisotropic nanoparticles have been broadly used in developing novel materials for electronics [1] photonics [2] and tissue engineering [3] due to their high aspect ratios and shape related physical/chemical properties. In particular, orientation and alignment of these nanoparticles afford special properties, such as enhanced electrical conductivity, surface-enhanced Raman scattering compared with their disordered counterparts [4–6], etc. Therefore, various aligned techniques have been applied to organize anisotropic nanoparticles on the two-dimensional substrates, such as solvent evaporation [7], Langmuir-Blodgett technique [8], as well as external forces based on magnetic [9], electric [10], or fluid flow [11]. However, the abilities of these techniques to control the alignment of anisotropic nanoparticles within a three-dimensional (3D) environment over a large-scale are still limited. Especially in the field of tissue engineering, it is still a challenge to fabricate 3D well-organized structures to mimic native functional tissues. For example, many functional tissues, e.g., muscle, blood vessels, and nerve pathway, exhibit hierarchical anisotropy morphologies and well-defined spatial arrangement of cells, which is essential for maintaining the physiological function for these tissues [12].

In our previous studies, highly aligned anisotropic nanoparticles inside capillary tube was fabricated using fluid flow assembly technique, and these organized structures were used to direct cells orientation and differentiation [13,14]. The orientation of aligned structures was dominated by the direction of shearing force, and both the interaction between nanoparticles and substrate and the subsequent drying process contributed to locating the nanoparticles on the substrate. Based on this result, we hypothesize that anisotropic nanoparticles can be aligned in a 3D hydrogel system. The hydrogel precursor solution flow is able to provide unidirectional shear force to control the direction of alignment of nanoparticles, while the orientated structures of nanoparticles can be quickly fixed through the fast transition of sol-gel of hydrogel.

The rod-like tobacco mosaic virus (TMV), was employed as a model anisotropic nanoparticle due to its monodispersity in size and shape, well-defined chemical reactivities [15,16], and

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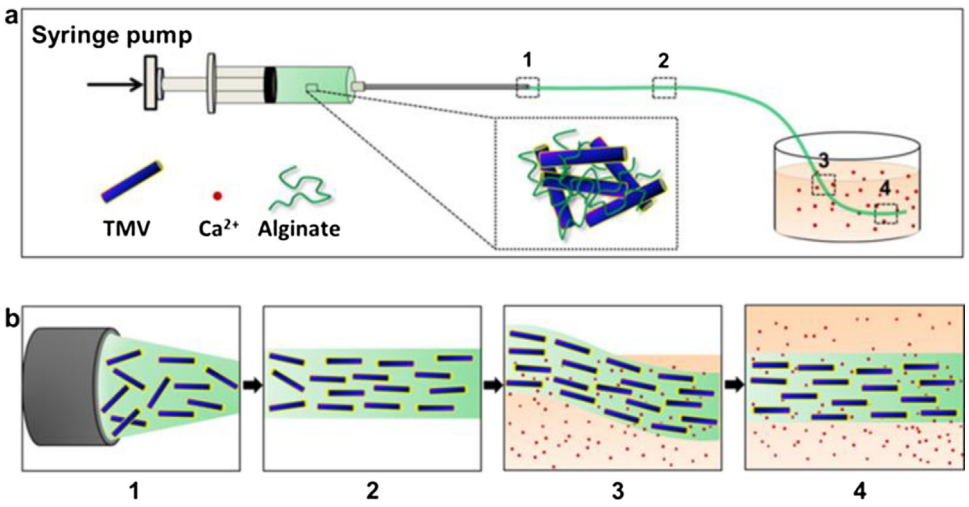


**Scheme 1.** (a) Schematic illustration of orientation of TMVs in the alginate hydrogel induced by shear force. 1, 2, 3, 4 represent TMVs in the alginate solution located at different positions during the flow process. (1) In the initial position of the tube, majority of TMVs were randomly oriented. (2) In the intermediate position of the tube, most of TMVs aligned along the alginate flow direction following the shear flow. (3) Upon TMVs entering the calcium chloride solution, calcium immediately diffused into TMVs and alginate mixtures. (4) TMVs and alginate mixtures were completely immersed in solution of calcium chloride, then the alginate gelation happened and the orientation and position of TMVs were quickly fixed (less than 1 s). (b) Enlarged images of 1, 2, 3, 4 of a.

potential in genetic modification to tailor its surface properties [17,18]. TMV is 300 nm in length and 18 nm in diameter, consisting of 2130 identical protein subunits helically arranged around single strand RNA. On the other hand, alginate hydrogel was chosen because of its good biocompatibility [19–22] and fast gelation process. As shown in Scheme 1, when TMV nanoparticles were mixed with alginate solution, the unidirectional flow of the fluid forced TMV particles to align along the flow direction. Upon the mixed TMV and alginate solution entering calcium chloride ($CaCl_2$) solution, calcium immediately diffused into the mixtures and crosslinked the alginate. It was reported that the alginate gelation could be immediately formed (less than 1 s) when blocks of G monomers cooperatively interact with divalent cations (i.e., $Ca^{2+}$) to form ionic bridges between polymers [23,24], then the ordered and oriented TMV assemblies were instantly preserved in the resulting hydrogels. Considering that a variety of targeting ligands, diagnostic probes and therapeutic cargos could be anchored on the surface of TMVs [15,16,25]. We believe such 3D well-organized oriented structures can be potentially applied in biomedical, sensing, electronic and optical fields.

## 2. Method

### 2.1. Material

Alginate powder, $2H_2O{\cdot}CaCl_2$ were purchased from Aladdin Chemistry Co., Ltd. All water used was deionized and further purified with an ultrapure water purification system (Research Scientific Instruments Co., Ltd). Dulbecco's modified Eagle's medium (DMEM) was purchased from Coring. All other reagents were purchased from Beijing Chemical Works without any further purification.

### 2.2. Preparation of alginate hydrogels loading TMVs

The purification of TMV nanoparticles was carried out according to our previous report [15]. The TMV solution was diluted to 15 mg/mL and alginate powder was added to TMV solution (w/w 2%), mixed and placed into syringe (needle size, length: 15 cm, inner diameter: 1.3 mm). Extrusion speed was controlled at a constant 0.2 mL/s via the syringe pump and injected the solution directly into cross-linked solvent (0.25 M $CaCl_2$) for 10 min. The formation of hydrogel microfiber was washed thoroughly with ultrapure water for three times, and stored in ultrapure water before test. The other samples were prepared in the same manner by varying the concentrations of alginate and TMV. For preparation of the control hydrogel samples without shearing force, hydrogel precursor solutions were mixed in a round mould under slightly stirring, then added cross-linked solvent for 10 min.

### 2.3. Synchrotron small angle X-ray scattering (SAXS) measurements

SAXS experiment was performed at the beamline 1W2A at Beijing Synchrotron Radiation Facility, Beijing, China with the wavelength of X-ray radiation being 0.154 nm. Samples were mounted onto a two-dimensional translational stage at the beamline with a sample to detector distance of 2969 mm. The size of the X-ray beam at the sample position was 1.4 mm horizontal × 0.2 mm vertical. The incident X-ray beam was illuminated at the center of the sample, and SAXS pattern was collected within 60 s by a two-dimensional CCD detector with 2048 × 2048 pixels (resolution: 79.1 × 79.1 μm). The SAXS data were calibrated for background scattering and normalized with respect to the primary beam intensity using the computer program "Fit2D". Changes in scattering intensities due to varying sample thicknesses were corrected by measuring sample adsorption using ionization chambers before and after the sample and by performing the corresponding data correction. For the anisotropic scattering patterns, rectangular slice cuts (with a width of 10 pixels) passing through the beam center were taken to obtain the scattering intensity profiles parallel to the shear flow direction. In order to evaluate the degree of orientation of the sample qualitatively, circumferential slice cuts covering the scattering peaks were taken to obtain the azimuthal scattering intensity distribution curves.

### 2.4. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM)

The surface of hydrogels was observed using a scanning electron microscopy (Phenom System IDs). The morphology of TMV, TMV-alginate mixtures and hydrogel were observed by TEM per-