Electronic Supplementary Material (ESI) for RSC Advances

Supporting Information

Actuation Based on Thermo/Photosalient Effect: Biogenic Smart Hybrid Driven by Light and Heat

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Contents:

Figure S1. Effect of aging films of NaCas doped with PHA during the preparation.

Figure S2. Marking of the film tip used in the procedure for tracking of film motility.

Figure S3. Effect of heating on doped (orange) and non-doped (colorless) films of NaCas.

Figure S4. Individual tracked motion of the film tip by excitation with light of different wavelength.

Figure S5. SEM micrograph of the surface of the doped film after exposure to strong UV light.

Figure S6. Results of the cytotoxicity tests on cancer cell lines.

Figure S7. Thermogravimetric curves of the dopant, non-doped film and doped film.

Figure S8. Differential scanning calorimetry results of the dopant and doped film.

Table S1. Mechanical properties of non-doped and doped films.

Movie S1. Bending of a PHA-doped film induced by lateral heating with a pointy heater.

Movie S2. Bending of a PHA-doped film induced by heating with a plate heater from the bottom.

Movie S3. Bending of a PHA-doped film by alternative heating and excitation with UV light.

Movies S4 – S31. Bending of PHA-doped films by excitation with LED light at different wavelengths and straightening by excitation with cold white light. Note that these recordings are presented at 3.3 times their original rate (30 frames per second) to facilitate the observation of the motion.

Experimental Section

Preparation of the films: PHA was prepared according to a previously published procedure[39] and recrystallized by slow evaporation, at room temperature, from toluene or butyronitrile. PHA-doped films were prepared similar to published procedure.[41-42] For inclusion in the films, 0.05 g PHA was dissolved in 2 mL DMF with heating. Two types of films with different dopant–matrix ratios were prepared; the more diluted film exhibited better elastic properties and was used for optical and mechanical characterization. 0.5 g (or 0.25 g) of sodium caseinate (NaCas) were mixed with 9 mL (or 4 mL) water, and 10 (or 5) drops of glycerol were added. The mixture was stirred and heated in the water bath at ~70 °C until dissolution, and the solution of PHA was added. At occasions when PHA precipitated, the heating was continued until dissolution, transferred to a polystyrene Petri dish, and dried in a desiccator at 23 °C over silica gel (RH ~ 22%) during a couple of days, whereupon PHA crystallized within the gel.

Thermal analysis: Thermal properties (DSC) were analyzed with differential scanning calorimeter (DSC Q2000, TA) instrument, at a heating rate of 10 °C/min in the temperature range of 20–200 °C. Tzero alumina pans with an auto sampler were used for each DSC measurement. Similarly, thermogravimetric analyses were measured using (TGA SDT Q600, TA) at a heating rate of 10 °C/min using alumina pans. The temperature range for the TGA analysis was 20–700 °C. Dry N_2 gas was used as carrier gas for each measurement.
Spectroscopy: The UV–visible spectra were recorded at room temperature in absorption mode with a Shimadzu UV–3600 UV–Vis–NIR spectrometer. The spectrum of the PHA-doped NaCas was recorded directly by inserting the film in the beam path, followed by scaling. To record the spectra of the pure phases of the two polymorphs of PHA, which were prepared by recrystallization, the crystals were thinly spread on self-adhesive tape using the clean tape to record the background. For recording of the solution-state spectra, PHA was dissolved in spectral-grade toluene and the concentration was adjusted to maximum absorbance of 0.26. The nuclear magnetic resonance (NMR) spectra of the non–doped samples were recorded on a Bruker Avance 400 MHz spectrometer with D_2O as solvent.

Scanning Electron Microscopy: Scanning electron microscopy (SEM) micrographs were taken with a QUANTA FEG 450 electron microscope with primary electron energy of 3–5 kV. The films were directly attached to adhesive carbon tapes. The micrographs were recorded at room temperature at pressure of 100 Pa. As shown in Figure 1, the dopant is uniformly distributed over the polymeric matrixes as rod-like crystals.

Tensile testing: All specimens were tested at 25 °C. In the tensile tests, a sample with an oblong shape (4 × 0.5 cm) was placed in the grips of movable and stationary fixtures in a screw-driven device using KSU 05M tensile testing equipment (Universal Testing Machine Co., Ltd), which pulls the sample until breaking, while recording the applied load versus elongation. The load cell and extensometer were calibrated prior to use. Load was applied at a crosshead speed 10 mm min–1, and the load range was 0–500 N. The tests complied with rules specified by the international standard norms. For proper calculation, the average value and standard deviations were calculated out of a minimum of three specimens.

Photomechanical actuation: Strips of the PHA-doped NaCas typically measuring ca. 2 × 11 × 0.2 mm were cut out and affixed with glue to glass rods. The orientation and position of the hanging samples were controlled with an XYZ micromanipulator, and the movies were recorded with a digital camera (30 fps) coupled to a horizontal microscope. The photomechanical properties were studied by a setup consisting of six light emitting diodes (LEDs) which were attached to the sockets of a metal ring distributed radially at identical distance around the sample. The timing and synchronization of the LEDs was controlled with a custom external controlling unit connected to a PC. The excitation wavelength was tuned by replacing the LEDs with LEDs that emit between 365 nm and 595 nm (the films were not photoresponsive above 595 nm and thus longer wavelengths were not used for excitation).The recovery of the films in the dark was studied on a sample which was bent by a 60 s exposure to 405 nm (5 LEDs). To monitor the shape recovery, at 2 s intervals the bent sample was flashed during 100 μs at 630 nm, where the film is optically transparent and remains unbleached. The tip of the film used was monitored over time. To study the thermomechanical response, the hanging films were heated either with a thermoelectric element from the lower side, or with an axially disposed electrically conducting wire heater mounted on an XYZ–micromanipulator.

Kinematics analysis: Kinematic analysis was carried out by using the computer program Hot Shot Link (ver. 1.2) which allows the user to determine (*x,y*) coordinates of a point with respect to the origin (0,0) on the program window. Inclination of the polymer film tip at different times were extracted as (*x,y*) by selecting a point on the polymer film tip (Figure S3). The first frame (inclination = 0, time = 0) corresponds to the first frame immediately after the UV light was turned on. The (*x,y*) coordinates were extracted at different times. The deflection of the film relative to the position in the first frame was calculated in pixels as $d = [(x_2–x_1)^2 + (y_2–y_1)^2]^{1/2}$, where $x_{1,2}$ and $y_{1,2}$ are the *x* and *y* coordinates of two consecutive points.

Cell lines and culture conditions: Two human cancer cell lines were used in this study, A549 (lung cancer cell line) and melanoma (M8), and were maintained in Dulbecco's Modified Eagle's Medium (DMEM: Gibco, USA) supplemented with 10% fetal bovine serum (Gibco) and penicillin/streptomycin (Gibco). All incubations were done at 37 °C in a humidified atmosphere of 5% CO_2. Mycoplasma was tested at 3 month intervals.

Chemosensitivity assay and data analysis: Stock solutions of all compounds were prepared by dissolution in dimethyl sulfoxide (DMSO) and kept frozen at –80 °C. The appropriate concentrations used in the experiments were prepared by serial dilution in DMEM medium from the stock solution just before the experiment. The highest DMSO concentration did not exceed 2% in all chemosensitivity experiments. Cytotoxicity of the compounds was determined using sulphorhodamine–B (SRB) method. Exponentially growing cells were seeded into 96-well microtiter plates at a concentration of 5 × 103 cells/well. After 24 hours, the cells were incubated with either drug-free medium, medium-containing DMSO, or the compound(s) at different concentrations (0.1–100 µg/mL). At least, triplicate wells were prepared for each concentration. Following 48 hours incubation at 37 °C in a humidified atmosphere of 5% CO_2, cells were fixed for 1 hour at 4 °C after adding 50 µL 50% trichloroacetic acid (TCA) to each well containing 200 µL. Plates were washed several times with tap water, air dried and stained with 0.4% SRB for 30 min and then washed several times with 1% acetic acid to remove unbound stain. Plates were air-dried and the dye was solubilized with 10 mM Tris base (pH 10.5). The optical density was measured spectrophotometrically at 564 nm with an ELISA microplate reader (Meter tech. S960, USA). Fractions of viable cells remaining for each concentration was calculated by dividing the reading at the respective concentration over the control untreated cells after subtracting the absorbance of cells incubated with DMSO alone (to account for the DMSO effect). Survival curves were constructed by drawing the fraction of viable remaining cells against the concentrations used. The IC50 values were calculated using sigmoidal concentration–response curve fitting models (Graph Pad, Prizm software). Statistical analysis, data fitting and graphics were performed by the Prism 3.1 computer program (GraphPad Software, USA). Data are given as mean ±SEM.

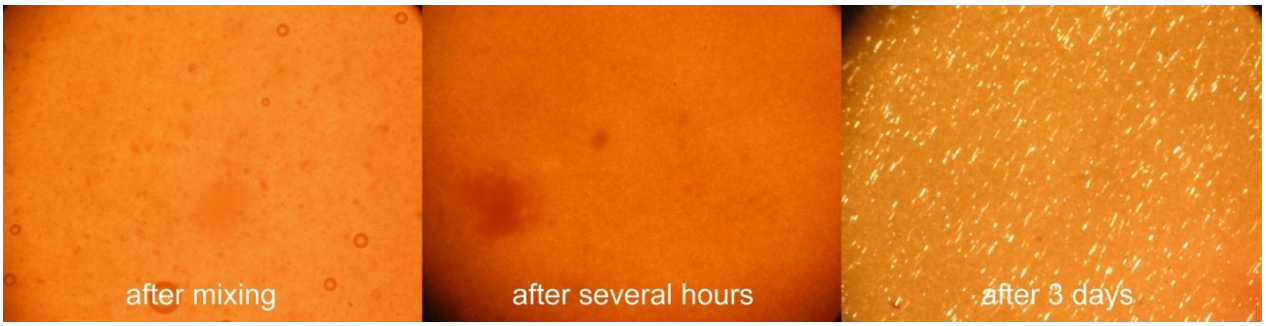

Figure S1. Effect of aging films of NaCas doped with PHA during the preparation. Left: the appearance of the solution immediately after mixing of the NaCas and PHA solutions. Middle: The same solution after several hours. Right: The film obtained after 3 days. Crystals reflect white light from their surface.

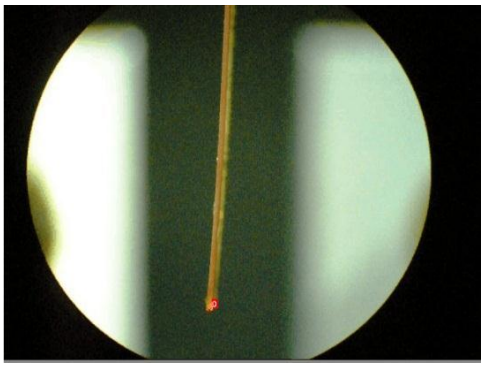

Figure S2. Example of the procedure for tracking of film motility. The red reticle on the bottom shows the point selected for tracking of the polymer film to determine the (*x*,*y*) coordinate.

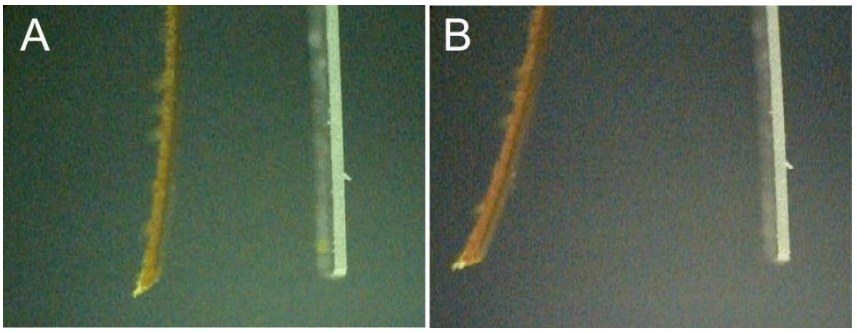

Figure S3. Effect of heating on doped (orange) and non-doped (colorless) films of sodium caseinate. The film was affixed at the top end and uniformly heated from the bottom.

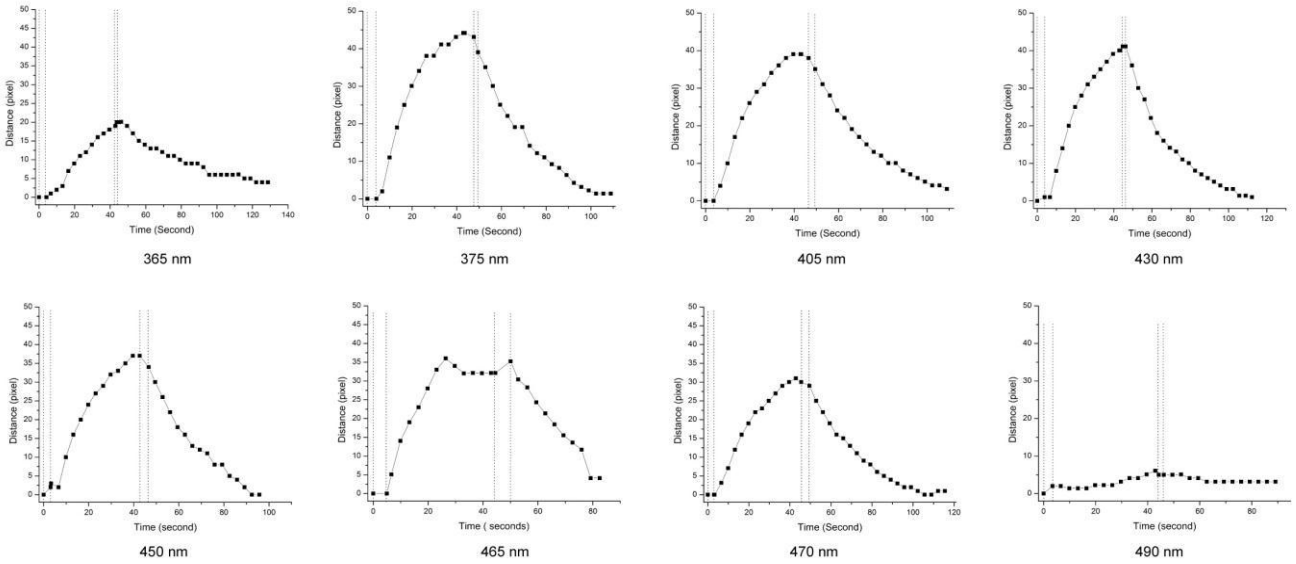

Figure S4. Individual tracked motion of the film tip by excitation with light of different wavelength.

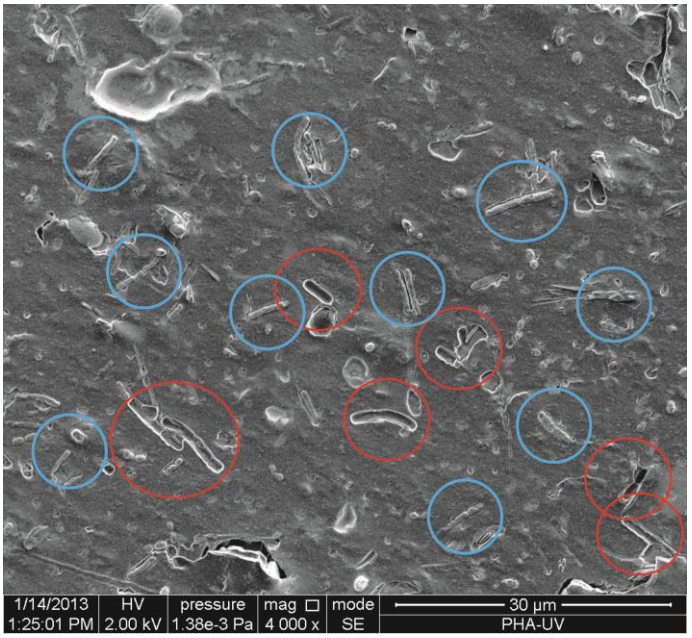

Figure S5. SEM micrograph of the surface of the doped film after 5-seconds exposure to strong UV light from a medium-pressure Hg lamp (SP-7, Ushio). The image shows footprints of crystals that jumped from the film (red circles) as well as from crystals that remained fixed in the film (blue circles). The former confirm that the crystals remain photoactive, while the latter evidence the stabilization effect of the film on the integrity of the crystals that are partially or completely fixed by the matrix.

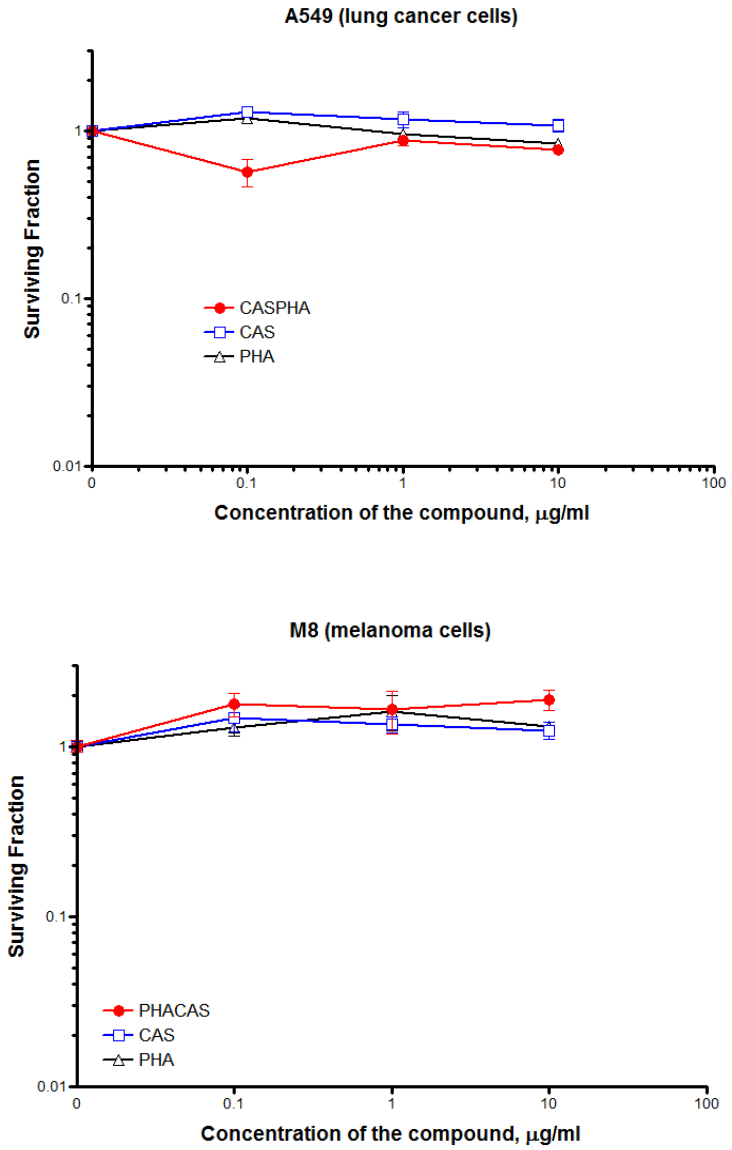

Figure S6. Results of the cytotoxicity tests on cancer cell lines.

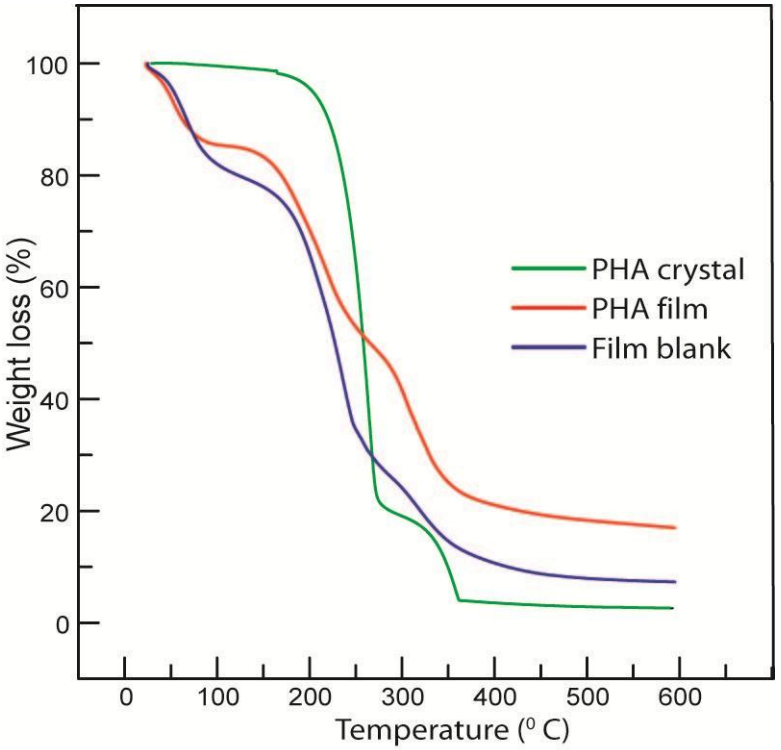

Figure S7. Thermogravimetric curves of NaCas and PHA-doped NaCas.

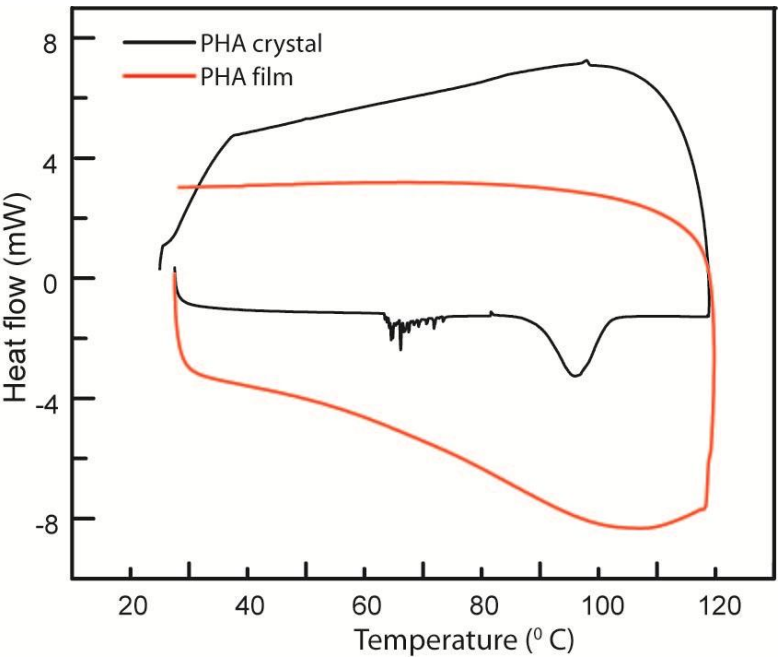

Figure S8. DSC curves of PHA and PHA-doped NaCas.

Table S1. Mechanical properties of non-doped and PHA-doped sodium caseinate films[a]

Samples	Young's modulus / MPa	Elongation / %
A1	0.0557	49.75
A2	0.0695	8.96
C1	0.0299	87.15
C2	0.0220	72.15
D1	0.1069	21.15
D2	0.0386	47.65

[a]Conditions for preparation: sample A1 was prepared from 20 mL NaCas and 0.2 mL PHA; A2 was prepared from 20 mL NaCas and 0.7 mL PHA; C1 was prepared from 20 mL NaCas and 0.2 mL PHA wand had thickness 0.15 mm; C2 was prepared from 20 mL NaCas and 0.2 mL PHA and had thickness 0.10 mm; D1 prepared from 20 mL NaCas with film thickness 0.15 mm; D2 was prepared from 20 mL NaCas and had thickness 0.10 mm.