Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Arylboronate esters mediated self-healable and biocompatible dynamic G-quadruplex hydrogels as promising 3D-bioink

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General methods:

All the chemicals and reagents were purchased from Sigma-Aldrich and Alfa Aesar and used without further purification. Milli-Q water was used for all the experiments and gel preparation.

Preparation of hydrogels:

Desired amount of boronic acids (concentration = 50 mM in 2 mL) and guanosine (28.3 mg, concentration = 50 mM in 2 mL) were taken into a fresh vial. 2 mL of freshly prepared aqueous KOH solution (25 mM) was added into the vial. The mixture was kept under sonication for 1 min for well mixing of components. After that, the vial was heated at 80 ^{o}C for 15 minutes to solubilize the mixture. Then, the solution was kept at room temperature. After 5 minutes, the clear solutions turned into hydrogels. Molar ratio of 1:1:1 (guanosine: boronic acids: KOH; 50 mM each) was used to form hydrogels. However, weak gel was formed in this condition. In place of KOH, KCl was also used. At lower concentration (25 mM) of KCl, gel was not formed. However, at higher concentration (100 mM) gel was formed but precipitated out within 2 h. NaOH and LiOH were also used instead of KOH. Weak gel was formed in NaOH. However, in LiOH, free flowing liquid was formed.

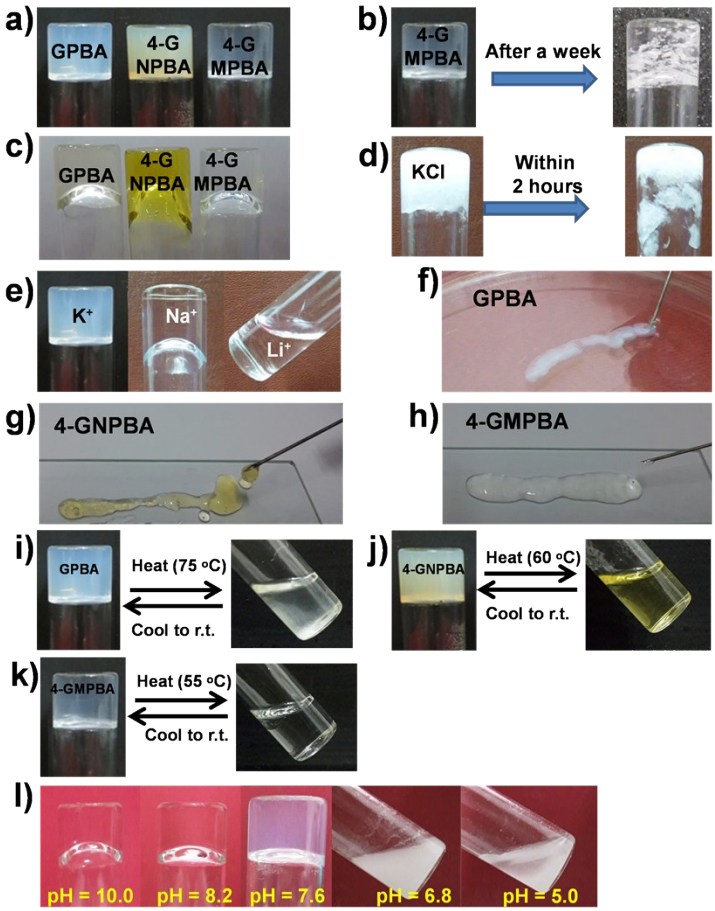

Figure S1. (a) **GPBA**, **4-GNPBA** and **4-GMPBA** at the molar ratio (concentration) of G: aryl boronic acid: KOH = 1:1:0.5 (50 mM: 50 mM: 25 mM). (b) Formation of precipitation of **4-GMPBA** after a week. (c) Weak gels of **GPBA**, **4-GNPBA** and **4-GMPBA** at the molar ratio (concentration) of G: aryl boronic acid: KOH = 1:1:1 (50 mM: 50 mM: 50 mM). (d) **GPBA** gel was precipitated within 2 hours in KCl solution (100 mM). (e) **GPBA** in presence of KOH, NaOH and LiOH was showing strong gel, weak gel and liquid respectively. (f) After injection, **GPBA** was showing stable nature, (g) water part of **4-GNPBA** was separated out from gel, (h) 4-**GMPBA** was injectable but weak for 3D printing. (i) – (k) Thermo-reversibility of the hydrogels. (l) **GPBA** at different pHs.

Transmission Electron Microscopic experiments:

Transmission electron microscopic (TEM) images were taken using a PHILIPS electron microscope (model: CM 200), operated at an accelerating voltage of 200 kV. 5% (v/v) of dilute gel solution was dried on carbon-coated copper grids (300 mesh) by slow evaporation in air, then allowed to dry separately under vacuum at room temperature.

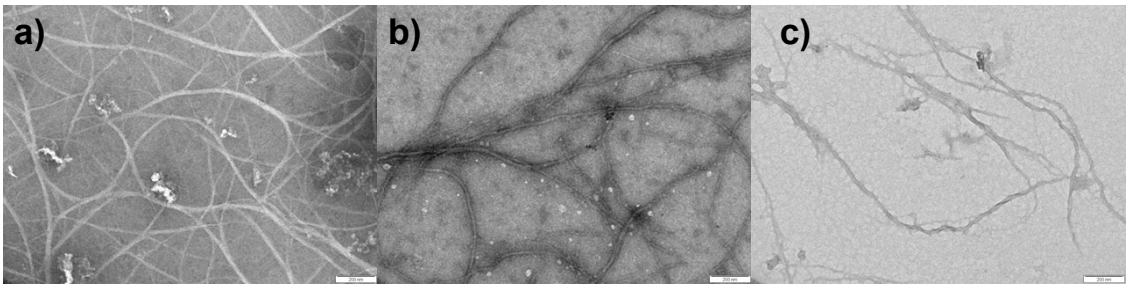

Figure S2. TEM images of hydrogels (scale bar = 200 nm) of **GPBA** (a), **4-GNPBA** (b) and **4-GMPBA** (c).

NMR experimental procedure:

All NMR spectra were recorded at 400 MHz Bruker Advance III 400 NMR. To study ^{11}B NMR experiments, desired amount of aryl boronic acids (concentration = 50 mM in 500 µL) and guanosine (7.08 mg, concentration = 50 mM in 500 µL) were taken into NMR tube. After that, 250 µL of freshly prepared 50 mM KOH in H_2O, 200 µL Mili-Q H_2O and 50 µL of D_2O (Final KOH concentration = 25 mM in 500 µL , D_2O = 10% v/v) were added into it. After adding all the components and solvents, NMR tube was heated to solubilize the suspension. After solubilizing all the components, a clear solution was appeared. The tube was kept at room temperature to form gel. After formation of gel, NMR spectra were recorded. NMR samples for only boronic acids were prepared by same conditions without adding guanosine.

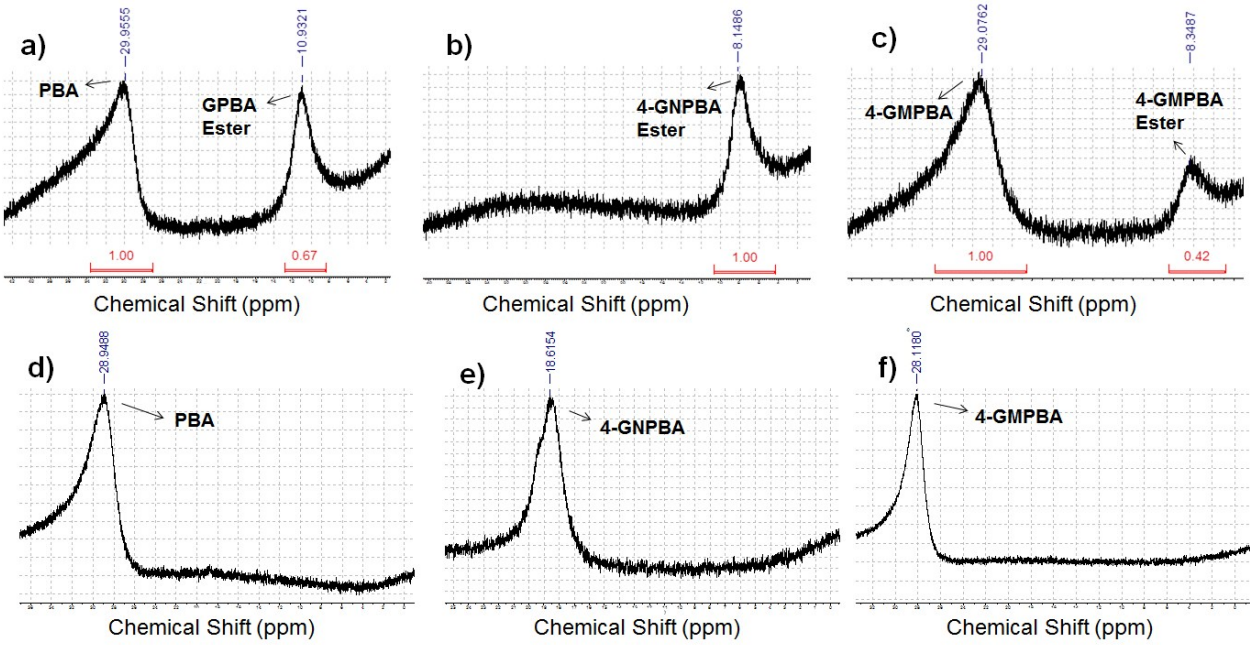

Figure S3. ^{11}B NMR (128 MHz, 10% D_2O) spectra of **GPBA** (a), **4-GNPBA** (b) and **4-GMPBA** (c), **PBA** (d), **4-NPBA** (e), **4-MPBA** (f).

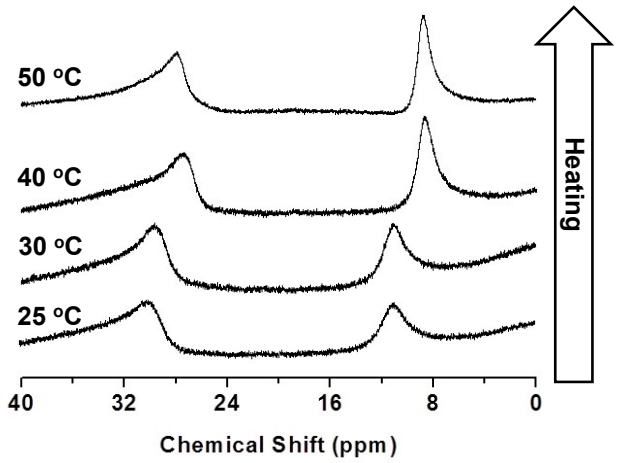

Figure S4. Temperature dependent ^{11}B NMR spectra of **GPBA** hydrogel.

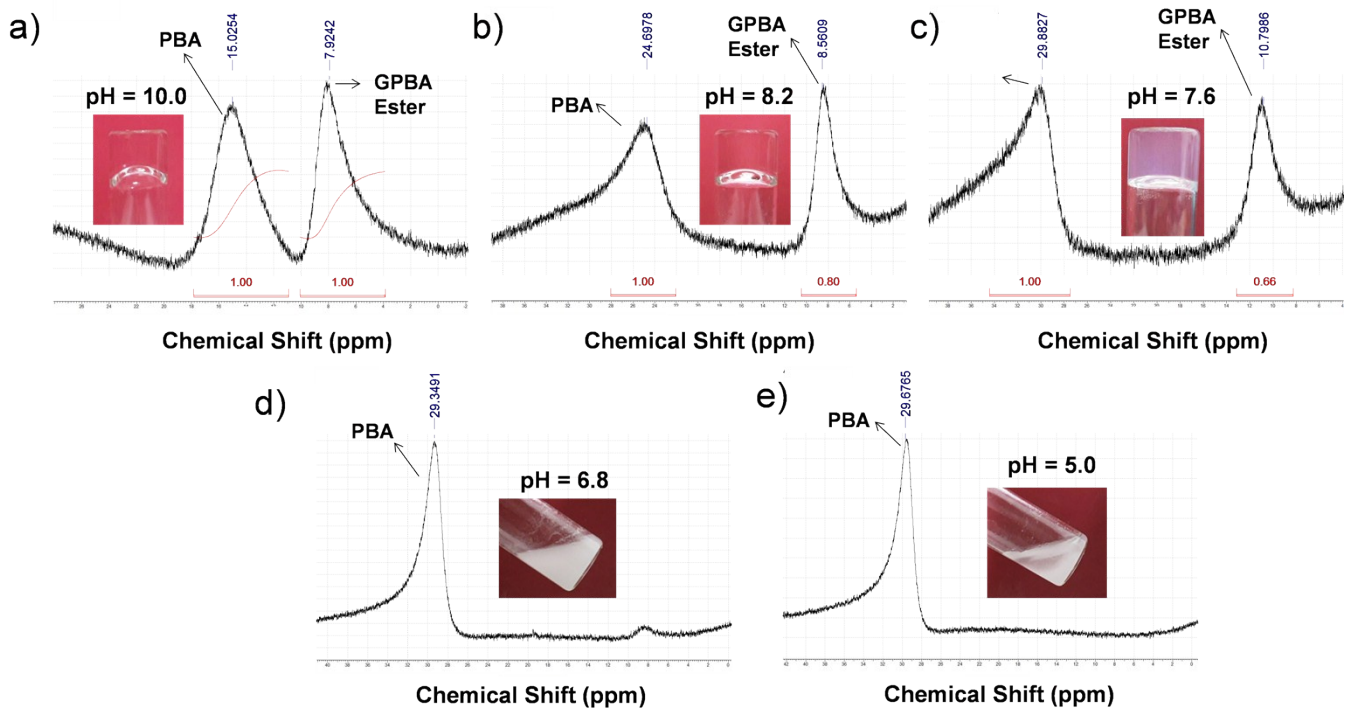

Figure S5. ^{11}B NMR spectra and images (inserted) of **GPBA** at different pH, (a) pH = 10.0, (b) pH = 8.2, (c) pH = 7.0, (d) pH = 6.8 and (e) pH = 5.0

Table S1. Molar ratio of PBA and GPBA ester at different pH.

pH	PBA : GPBA ester (molar ratio)
10.0	1.0 : 1.0
8.2	1.0 : 0.80
7.6	1.0 : 0.66
6.8	Trace amount of ester
5.0	No ester formation

Circular Dichroism (CD) study:

Circular dichroism (CD) spectra were recorded at 25 °C on a Jasco J-815 spectropolarimeter. Spectra were measured from 350 nm to 200 nm with a data pitch of 0.1 nm. The bandwidth was set to 1 nm with a scanning speed of 20 nm min^{-1} and a response time of 1 s. The path length was 1 mm quartz cell. All the experiments were repeated for three times.

Powder X-ray Diffraction study:

Powder X-ray Diffraction (PXRD) of gels was carried out by Brucker (D2 Phaser) with a wavelength of 1.5406 A˚ at 25 °C. PXRD studies of corresponding xerogels were performed by placing the samples on the glass plate. X-rays were produced using a sealed tube and X-ray was detected using a linear counting detector based on silicon strip technology (Scintillator NaI photomultiplier detector).

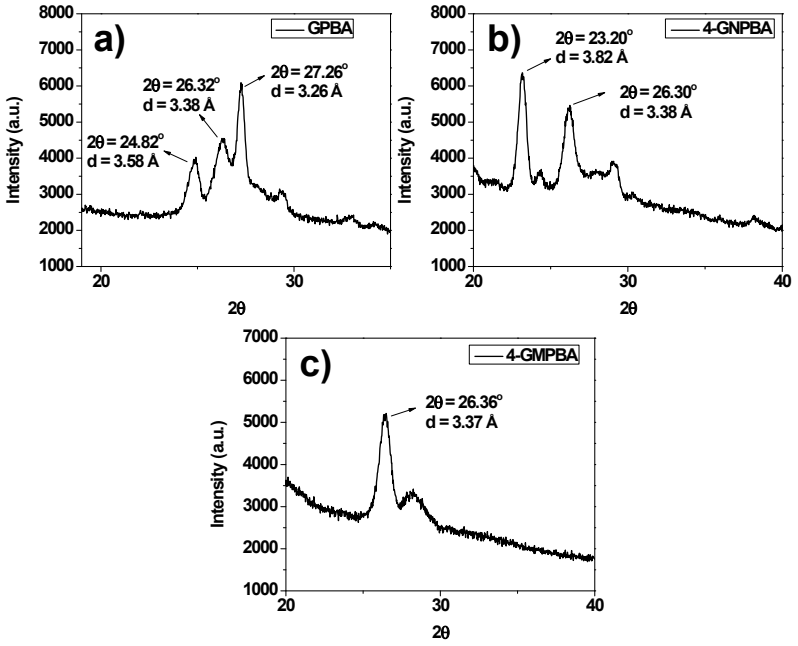

Figure S6. PXRD spectra of **GPBA** (a), **4-GNPBA** (b) and **4-GMPBA** (c).

Rheological Measurements:

The rheological study was performed to determine the mechanical properties of the hydrogels by using an Anton Paar Physica Rheometer (MCR 301, Austria) with parallel plate geometry (25 mm in diameter) and the temperature was at 25 °C. The dynamic moduli of the hydrogel were measured as a function of frequency in the range of 0.05-100 rad/s with a constant strain value 0.1%. For thixotropic test, alternative higher and lower strain was set at constant angular frequency of 10 rad/s. The higher strain was 100% and lower strain was 0.1%. All the experiments were repeated at least three times. For all the experiments, around 200 µL of gel was placed to the geometry plate by using spatula.

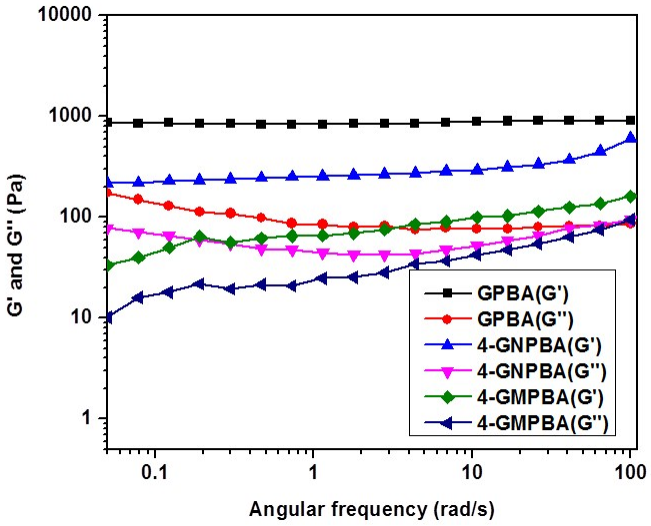

Figure S7. Frequency sweep experiments of the hydrogels (at constant 0.1% strain) showing **GPBA** is strongest and most stable and **4-GMPA** is the weakest gel.

3D printing process:

Printing of hydrogels was carried using INKREDIBLE^{+} bioprinter (CELLINK). Printing speed of 10m/s, nozzle diameter of 0.25 mm and pressure of 13KPa were maintained during the printing process.

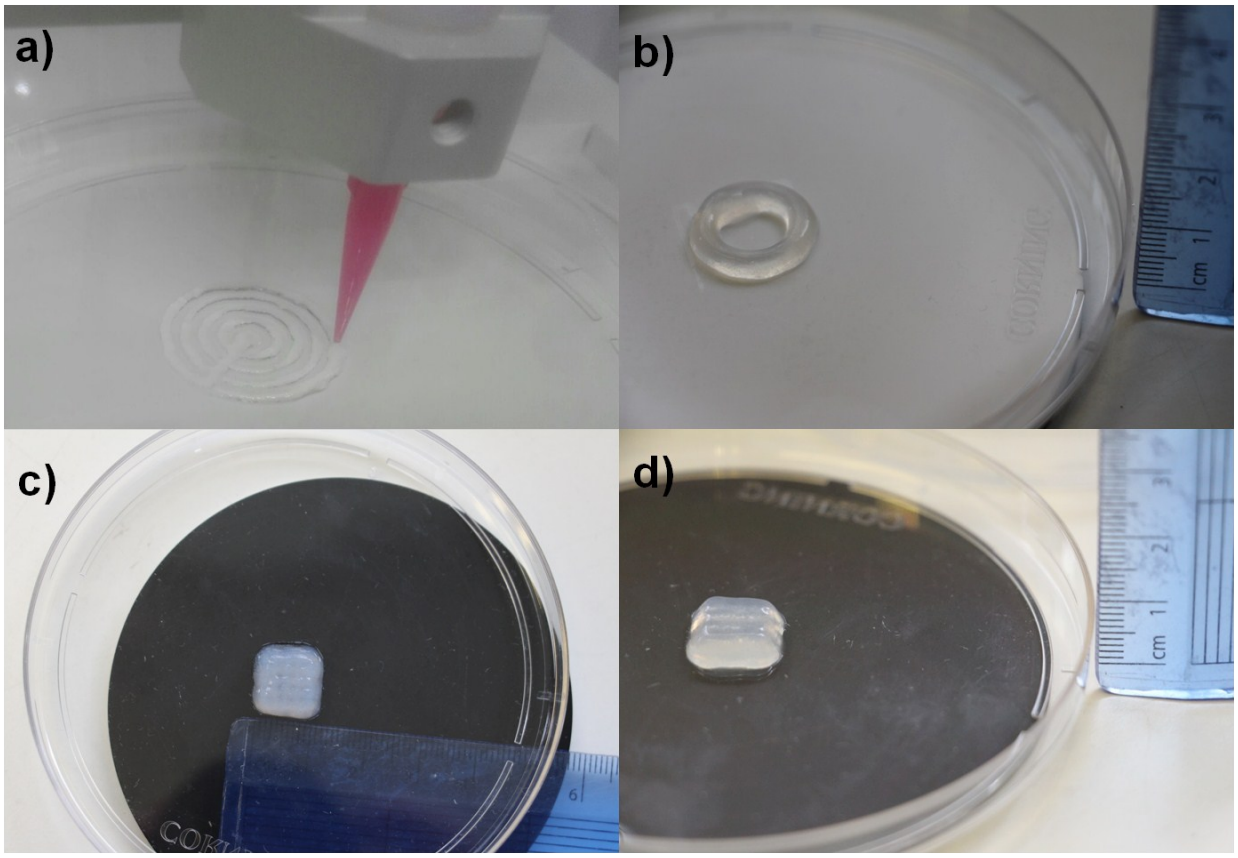

Figure S8. Different 3D printed patterns of **GPBA**; (a) spiral, (b) circle, (c) & (d) upper and side view of 3D printed block by using **GPBA** as ink.

Cell culture procedure:

Adult human dermal fibroblast (HDFs) was procured from Promocell. Cells were cultured using high-glucose media supplemented with 10% FBS, 1% penicillin-streptomycin. Cells were expanded when they reached 70-80 confluency. All experiments were performed using cells at passage 10.

Cytotoxicity test of GPBA hydrogel:

Cytotoxicity of **GPBA** hydrogel was evaluated using two methods described in ISO 10993-5 (2009) Standard Test for Cytotoxicity: extract method and direct contact method. Briefly, direct contact method consists of the addition of the tested hydrogel covering 10% of the surface of the cultured cells in a well plate. Elution method is performed by conditioning cell culture media with the hydrogel for 24 h at 37°C. Successively, by exposing this media to pre-seeded cells for further 24 h Standard culture on tissue culture plate (TCP) was used as control sample. For both methods and control, 20,000 cells were seeded in each well of the 24 well plates.

Method for cell encapsulation into GPBA hydrogel:

A solution containing 10^6 HDFs in 200 µL of media was added every mL of gel. First, 100 µL of the solution were added to the gel and mixed with the positive displacement pipette tip for 30 seconds. The step was repeated with the remaining 100 µL. 150 µL of cell-laden gel were transferred to well inserts, supplemented with culture media and incubated for evaluating their viability with live/dead assay.

Live/dead assay:

Live/dead assay was performed to determine cell viability. Live/Dead cell viability assay kit was purchased from Thermofisher Scientific. The staining solution was made using 2 µM Calcein AM (green, excitation/emission 494/517nm) and 4 µM Ethidium Bromide homodimer-1 (red, excitation/emission 517/617nm) in PBS. After 24 h of incubation with the test gels, the cell media was removed and samples were washed with PBS gently for 5 minutes. The staining solution was then added to the gels, and incubated for 10 minutes at 37°C in an incubator. The samples were then washed with PBS and analyzed under the ZEISS ApoTome.2 fluorescence sectioning microscope. Images were captured from 4 random fields of view for each sample.

Fluorescently labeled cells in green (live cells) and red (dead cells) were counted using Image analysis software, ImageJ. The percentage of cell viability was calculated using the following formula, as a measure to evaluate the toxicity of the test gel.

$$\frac{cells\ alive}{cells\ alive + \ cells\ dead} \times 100$$

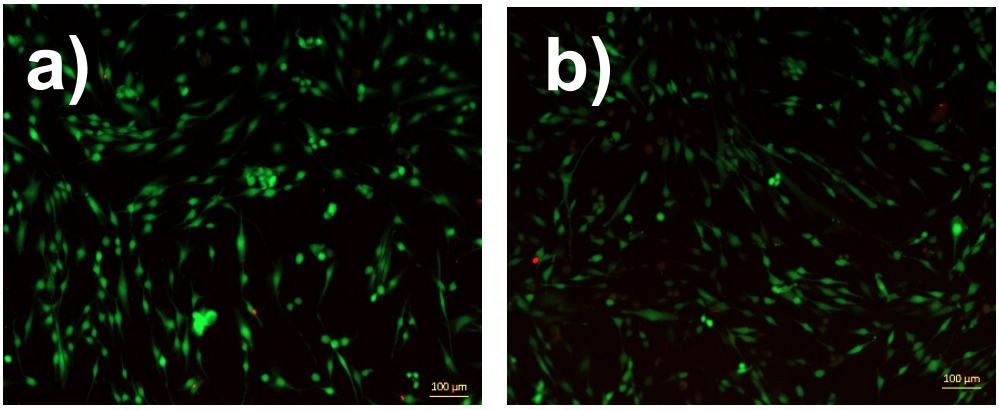

Figure S9. Fluorescence microscopy images of cells (a) in culture media (Control test) and (b) in **GPBA** after 24 hrs incubation.