Supporting information

**Glucose Microenvironment Sensitive Degradation of Calcium Sulfate Reinforced Poly(lactide-co-glycolide) Composite Scaffolds stimulated by Arginine**

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**2.2 Synthesis and Characterization of CSH and ArgCSH particles**

A mild chemical homogeneous precipitation method was used to synthesize CSH, in which L-arginine (Arg), amino acid with maximum isoelectric point acts as a precipitant during the synthesis process[13]. The reactant was prepared by mixing 0.1mole (NH4)2SO4 and 0.1 mole Ca(NO3)24H2O in a flask equipped with a reflux condenser on top of it, containing 100 ml of 3.5 M CaCl2 solutions with and without 2 mg/mL Arg as crystal modifier at 105 ℃ by oil bath for 4 hours. The temperature was monitored by a thermometer and maintained within ± 0.5 °C. Then the precipitates were harvested by filtration, washed with boiling deionized water twice and ethanol, dried for 80 ℃ in an oven for 24 h, and the products were denoted as CSH and ArgCSH respectively.

SEM studies of inorganic particles were carried out on a Philips XL30 field Emission Scanning Electron Microscope. The average lengths, diameters and aspect ratios of the products for each sample were counted using around 100 complete particles from the SEM images. Particle data were obtained from nine positions evenly distributed in the sample. The length, diameter and aspect ratio of each position in SEM images were measured with 10 - 12 complete particles, and the magniﬁcation was 500 - 1000. IR spectra were recorded from KBr pellets on a Perkin d Elmer 580B IR spectrophotometer. The molecular information of samples was obtained by X-ray photoelectron spectroscopy (XPS, Thermo ESCALAB 250). Crystalline phase of inorganic samples were identified by means of X-ray diffraction (XRD: D8 ADVANCE, BRUKER, Germany) using CuKα radiation. X-ray diffraction patterns were collected over the 2θ range 10-60° at a step size of 0.03° and counting times of 4s per step in order to determine the phase composition. Phase identification was achieved by comparing the sample diffraction patterns with ICDD (JCPDS) standards (Bassanite, syn. card No. 41-0024). To perform TGA (TA Instruments TGA500, USA) and DSC (TA Instruments DSC100, USA) analysis for inorganic phase identification and crystal water content determination, 20 mg dry sample was sealed in an Al2O3 crucible with a lid and scanned at a rate of 10℃/min under N2 atmosphere.

In order to further study the influence of Arg addition on the physical and chemical properties of CSH, the compression mechanical properties of inorganic cements were also analyzed. In this section, the solid phase composition of calcium sulfate cement was CSH (ArgCSH), and the liquid component is deionized water, and the sample preparation process was as follows. Briefly, deionized water was added into a proper amount of solid phase with a liquid-solid ratio of 0.7 mL/g, blended it into slurry, and poured into a PTFE mold and solidified it into a cylindrical sample with a height of 20mm and a diameter of 8mm.

**2.3 Fabrication and Characterization of CSH(ArgCSH)/PLGA composite Scaffolds**

CSH(ArgCSH)/PLGA composite scaffolds were fabricated by a combining method of thermally induced phase separation according to our previous method[5]. In brief, the CSH (ArgCSH) particles were evenly dispersed in N-Methyl-2-pyrrolidone (NMP). Afterward, the suspension was transferred into a 0.2g/L PLGA/NMP homogeneous solution to achieve blends with a certain weight ratios of PLGA to the inorganic ceramics. The dope of CSH(ArgCSH)/PLGA was put into a customized cylindrical Teflon mold and frozen at - 80 ℃ for 2 h in a refrigerator to induce phase separation. Then the mixture were immersed in pre-cooled 50% ethanol aqueous solution at -20 ℃, and the NMP was extracted and CSH phase solidification for at least 7 days with ethanol aqueous solution was changed every 6 h to completely remove NMP. After drying in a vacuumed freeze dryer, porous cylindrical composite scaffolds were obtained and stored in a desiccator for further uses.

The mechanical (compression and bending) properties of the scaffolds the composites were measured by a mechanical testing machine (Instron 1121, UK). The cylindrical samples with 8 mm diameter 20 mm length, and 5 mm diameter 40 mm length were employed in compression test and bending test, respectively. The test was performed at a crosshead speed of 0.5 mm/min at ambient temperature. The corresponding modulus was determined by the first linear slope (5-10%) of the stress strain curve. All results were the averages of 6 parallel measurements.

**2.4 In vitro degradation and Characterization**

The porous scaffolds specimens for in vitro degradation were made into cylindrical bars with a diameter of 8 mm and a height of 10 mm. The samples were immersed in phosphate buffered saline (PBS) with glucose concentration of 0, 1 and 2 g/L at pH 7.4 and incubated at 37℃, respectively. A ratio of 6 mg sample to 1 ml medium was used for all samples. Each of the scaffolds is assembled into a 50 mL centrifuge tube. The buffer solution was replaced with fresh PBS weekly. At selected time points (1, 3, 5, 7 and 9 weeks), five specimens of each samples were taken out, washed with deionized water and dried over 1 week to further evaluate the degradation. For the convenience of statement, the CSH/PLGA and ArgCSH/PLGA scaffolds degraded in PBS with glucose concentration of 0, 1 and 2 g/L were denoted as glucose free group, low glucose group and high glucose group, respectively.

To further explain the influence of glucose on the degradation law of composites, the degradations of pure PLGA scaffolds were performed in PBS with glucose concentration. To accelerate the degradation proceed, the lipase and high temperature were also employed during the degradation. The PLGA scaffold samples were immersed in PBS with lipase (40 mg/mL) and glucose (0, 1 and 2 g/L) at pH 7.4 and incubated at 37 ℃ and 45 ℃ for 10 days, respectively. A ratio of 6 mg sample to 1 ml medium was used for all samples. Each of the scaffolds is assembled into a 50 mL centrifuge tube. At selected time points (0, 3, 7 and 10 days) the pH values of degradation media were tested and after 10 days incubations the figures of samples were recorded and the mass loss were measured.

**2.4.1. Mass loss**

All the average values were reported from five specimens. W0 is the original mass of the specimen before degradation; W1 is the residual mass of a completely dry sample after degradation for a certain time. The percentage of mass loss was determined using the following equation.

**2.4.2. pH Value and Ca2+ concentration Changes of degradation medium**

The initial pH of PBS was 7.4. The pH of the buffer solution during degradation was monitored by an electronic bench-top pH meter (SevenEasy, Mettler Toledo, Switzerland). The pH meter is calibrated with standard solutions before each measurement. The changes in Ca2+ concentration of the buffer solution during degradation were detected by inductively coupled plasma atomic emission spectrometry (ICP-MS, X2, Thermo, USA).

**2.4.3. Morphology and microstructure analysis by micro-CT**

Micro-computed tomography (micro-CT, Bruker, SkyScan1172, Germany) was carried out on the scaffolds before and after degradation. The bottoms of scaffolds were coated with silicone grease to fix them during collection. Data were collected at 80 kV and 100 µA without filter. Transmission images were obtained by rotating the samples for 180 within an integration time of 400 ms. Cone Beam Reconstruction software (Skyscan, Belgium) was used to reconstruct the images with an isotropic voxel resolution of 16 µm, where the density range was set as 0.05-0.18. Then the obtained cross-sectional images were analyzed using CTAn software (Skyscan, Belgium) to measure the volume of open pore and number of closed pores. A pixel threshold was selected from 60 to 255 in CTan, and white speckles smaller than 10 voxels were removed. Corresponding mapping images were constructed by Data Viewer software (Skyscan, Belgium).

**2.5 Biological characterization**

Murine calvarial preosteoblasts (MC3T3-E1, ATCC CRL-2594) were employed to examine the cytocompatibility of the composites scaffold. They were cultured in the Dulbecco's modified Eagle's medium (DMEM, GIBCO), with 10% fetal bovine serum (FBS, Corning, USA) at 37 °C in a humidied atmosphere of 5% CO2. The culture medium was changed every other day. The details of the cell functional assay are available in the Supporting Data.

**2.5.1 Cell Functional Assay**

For the in vitro cell functional tests, CSH(ArgCSH)/PLGA composite samples were sterilized using electron beam irradiation (9 kGray 30s). The cytotoxicity tests were carried out by indirect assay. Extraction medium was prepared using DMEM serum free medium as the extraction medium for 72 h incubation in a humidiﬁed atmosphere with 5% CO2 at 37 °C. DMEM high glucose (DMEM HG with the glucose concentration 4.5 g/L) was employed to simulate the degradation environment rich in glucose, and DMEM low glucose (DMEM LG with the glucose concentration 1 g/L) corresponding to the degradation environment with glucose absence. The corresponding control groups involved the use of DMEM HG and DMEM LG as respective negative control.

**2.5.2 Cytotoxicity and Proliferation.**

Cells were seeded on 96-well cell culture plate。s at a density of 5 × 103 cells/well and incubated for 24 h to allow cell attachment. The medium was then replaced with 100 μL of extracts. After incubated for 1, 3 and 7 days, respectively, cell morphology was observed by light microscope and the relative cell proliferation rate was studied via Cell Counting Kit-8 (CCK-8, Dojindo) according to the literature method. Brieﬂy, CCK-8 solution with a 10% volume of culture medium was then added into the wells, and the samples were incubated at 37 °C for 2 h. Then, 100 µL of the reaction solution was transferred into a new 96-well plate, and the optical density was measured at 450 nm by a full wavelength microplate reader (Inﬁnite M200, TECAN).

**2.5.3 Alkaline Phosphate (ALP) Activity Test.**

Alkaline phosphatase (ALP) staining was applied to assess the activity of ALP in MC3T3-E1 cells treated with different composite scaffolds. MC3T3-E1 (2×104 cells/well) were seeded onto microspheres in 24 well plates and incubated in a humidified atmosphere with 5% CO2 at 37 ℃. When cultured on different extracts for 7 days, the cells were washed three times with PBS, fixed with 4% PFA for 15 min, and washed with PBS again. Thereafter, the cells were immersed in 500 µL ALP dye (Beyotime, China) for at least 12 h at room temperature under dark conditions. The extra ALP dye was washed away with PBS and then the purple color intensity was observed by stereoscopic microscope. The ALP relative activity was tested by Alkaline phosphatase detection kit (Beyotime, China). After cultured for 7 days, MC3T3-E1 cells were washed with PBS three times and split by adding 200 mL of Cell lysis buffer for Western and IP (Beyotime, China), freezing at -80 oC for 25 min and thawing at 37 oC. Then, p-nitrophenol phosphate substrate and BCA solution were added, followed by incubation in the dark for 30 min at 37 oC. The absorbances at 405 nm (OD405) and 562 nm (OD562) were read using a multifunction microplate scanner. The corresponding ALP quantitative evaluation was calculated according to the equation OD405/OD562.

**2.5.4 Calcium Deposition**

The alizarin red staining was used to evaluate calcium-rich deposits in MC3T3-E1 cells treated with different composite scaffolds. MC3T3-E1 (2×104 cells/well) were seeded onto microspheres in 24 well plates and incubated in a humidified atmosphere with 5% CO2 at 37 ℃. After 14d of culture, calcium deposition by MC3T3-E1 was analyzed by Alizarin Red S staining. The cells were fixed as described in the above section and then washed with deionized water for three times. The cells were then incubated in 2% (w/v) Alizarin Red S (Sigma) solution at 37°C for 20min. After the removal of Alizarin Red S solution, the stained cells were rinsed with deionized water three times and observed under a light microscope. Calcium quantification was measured via Cetylpyridinium chloride (CPC) treatment. ARS-stained membrane substrates were washed with distilled water and treated with 1 mL of 10% CPC solution for 1 h to desorb the calcium ions.

**2.5.5 AGEs adsorption on CSH and ArgCSH**

50 mg samples of CSH and ArgCSH particles were evenly dispersed in 1ml of PBS (pH 7.4) which contents 1 mg/mL AGE, and incubated at 37°C for a specific period of time, and the supernatant obtained by high-speed centrifugal was then collected at various time intervals and subjected to UV−vis analysis.

**2.5.6 Cell Viability affected by AGE and CSH/ArgCSH**

The cell counting kit-8 kit was used to detect cell proliferation. Brieﬂy, the MC3T3-E1 were seeded in 96-well plates at a density of 5 × 103 cells/well. After 24 h cultures, cells were incubated with 80 μg/ml CSH or ArgCSH particles and 80 μg/ml AGE for 48h and 96h. Cells incubated with 80 μg/ml BSA were recorded as BSA-control. Then the medium was replaced by fresh culture medium with 10% CCK-8. The cells were incubated for another 2 h. Subsequently, 200 µL solution was transferred to 96-well culture plate, and the optical density (OD) values were read at the 450nm wavelength by a multifunction microplate scanner.

**2.5.7 CSH/ArgCSH rescued AGE-inhibited osteogenic differentiation**

The alkaline phosphatase staining and quantitative analysis were used to detect cell osteogenic differentiation. Brieﬂy, the MC3T3-E1 were seeded in 96-well plates at a density of 5 × 103 cells/well. After 24h culture, cells were incubated CSH or ArgCSH particles with 80 μg/ml AGE for 7 days, respectively. Cells incubated with 80 μg/ml BSA were recorded as BSA-control. The ALP relative activity was tested by Alkaline phosphatase detection kit (Beyotime, China). After cultured for 7 days, MC3T3-E1 cells were washed with PBS three times and split by adding 200 μL of Cell lysis buffer for Western and IP (Beyotime, China), freezing at -80 ℃ for 25 min and thawing at 37 ℃. Then, p-nitrophenol phosphate substrate and BCA solution were added, followed by incubation in the dark for 30 min at 37 ℃. The absorbances at 405 nm (OD405) and 562 nm (OD562) were read using a multifunction microplate scanner. The corresponding ALP quantitative evaluation was calculated according to the equation OD405/OD562.

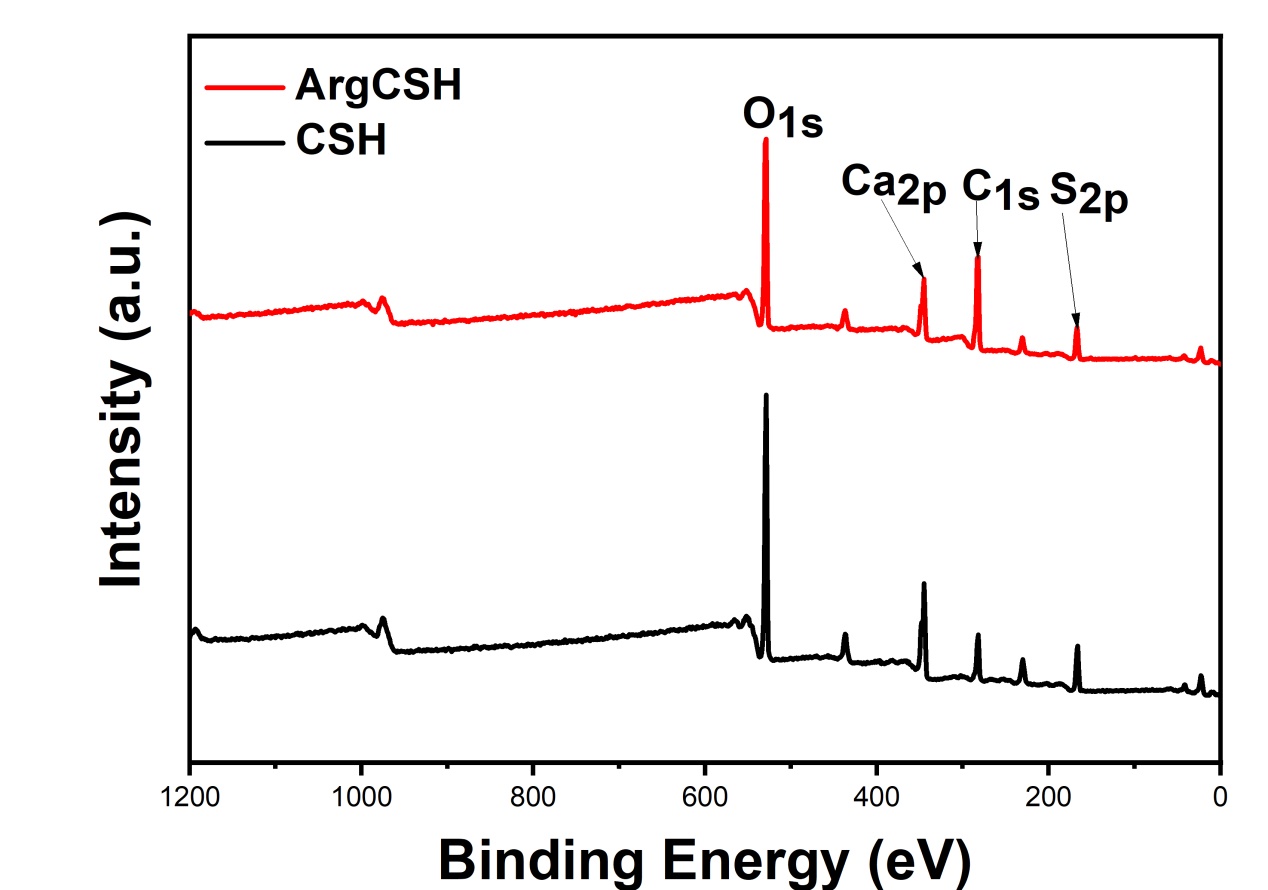


Fig S1. XPS survey scan spectra of CSH and ArgCSH samples.

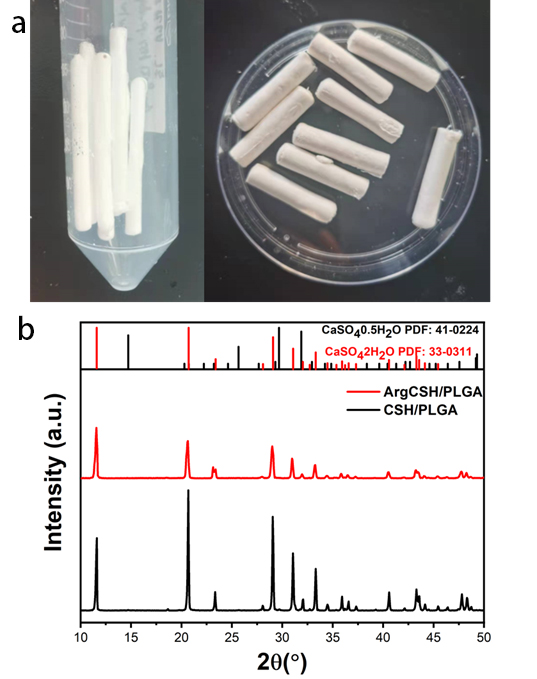


Fig S2 (a) CSH(ArgCSH)/PLGA composite scaffolds prepared using phase separation method. (b) XRD patterns of CSH(ArgCSH)/PLGA composite scaffolds.

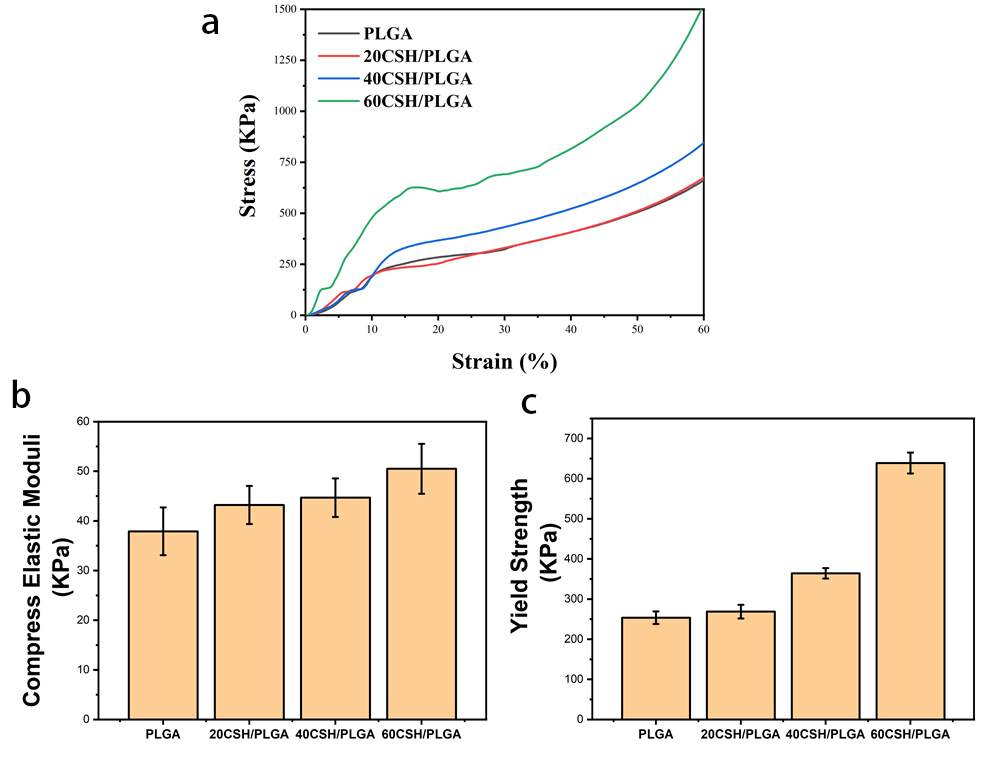


Fig S3 Stress strain curve (a), elastic moduli (b) and yield strength (c) of compression test of PLGA scaffold and CSH/PLGA composite scaffolds with CSH amounts of 20, 40, 60 wt % (20CSH/PLGA, 40CSH/PLGA, 60CSH/PLGA), respectively.

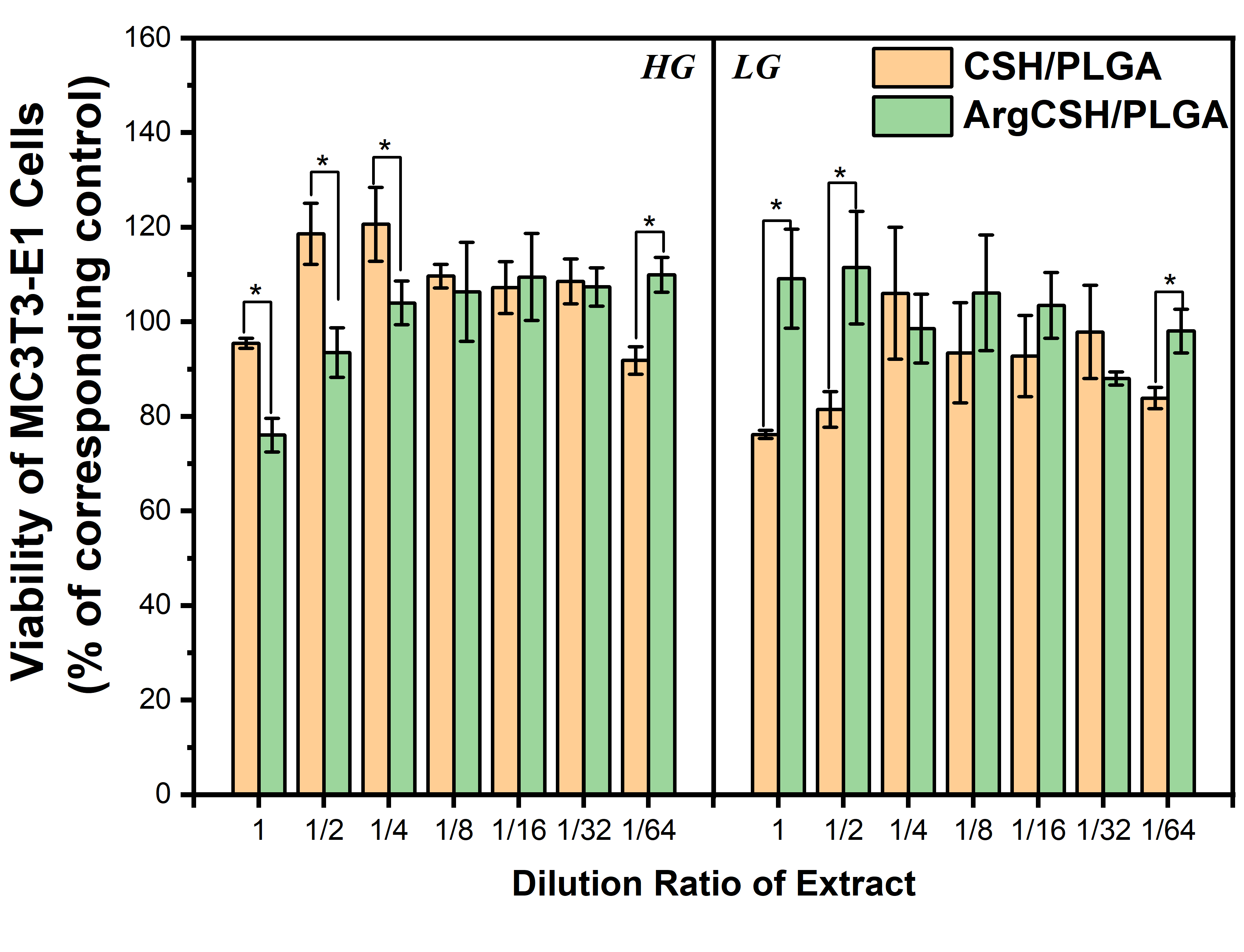


Fig S4 Cytotoxicity of MC3T3 cells treated with extracts of CSH(ArgCSH)/PLGA scaffolds with different dilution ratios.

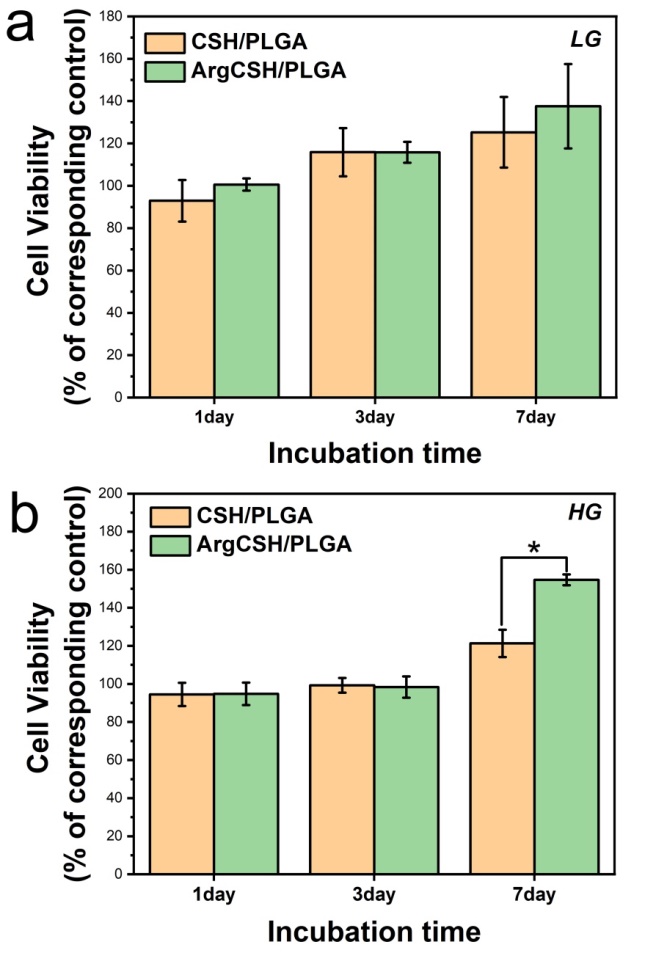
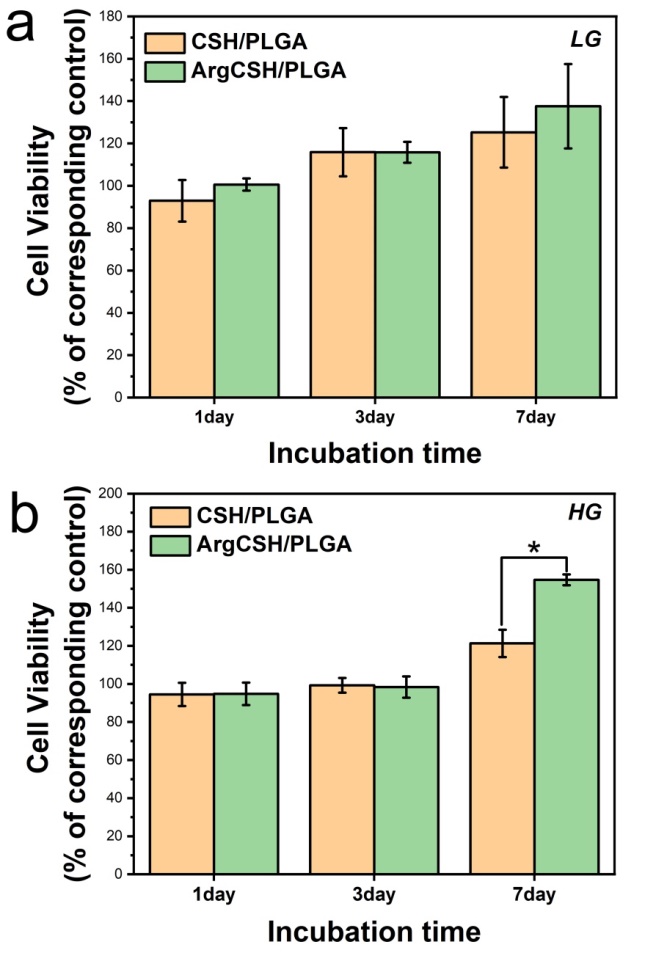


Fig S5 Quantitative analysis of MC3T3-E1 cell proliferation treated with extracts of CSH(ArgCSH)/PLGA scaffolds at 1, 3 and 7 days. \* denote significant differences (p < 0.05).