Electronic Supplementary Material (ESI) for RSC Advances. This journal is © The Royal Society of Chemistry 2015

Supplementary Information: Dendrimer templated bioactive glass ceramic nanovehicle for gene delivery applications

Nidhi Gupta¹, Deenan Santhiya^{1*}, Anusha Aditya² and Kishore Badra¹

¹Delhi Technological University, Department of Applied Chemistry and Polymer Technology, Bawana Road, Delhi-110 042, India

²Institute of Genomics and Integrative Biology (CSIR), Mathura Road, Delhi-110025, India

*Corresponding author Tel: +91 9958580295

E-mail addresses: deenan.santhiya@dce.ac.in

Contents:

- **1. S1.** Discussion on methodology adopted for bioactivity test and cell culture studies.
- 2. S2. Effect on PAMAM dendrimer concentration on the textural tunability of the bioglass.
- **3. Fig.S1:** Represent TEM images for different concentration of PAMAM generation 3 dendrimer templated bioglass (a) less than 10 mM (b) 15 mM (c) greater than 20 mM.
- **4.** Fig. S2: Nitrogen adsorption–desorption isotherm and pore size distribution (inset) of pre-heated BIS-nBG-DG3 glass-ceramic sample through normal method of analysis.
- **5. Fig. S3.** TGA images representing the % weight loss for the as prepared, Heat treated and lyophilized sample.
- 6. Fig. S4. TEM images recorded after pre-heating treatment of 200 °C of the bioglass samples.
- **7. S3.** Discussion on the differential spectrum of the PAMAM interaction with the precursors.
- **8. Fig. S5:** Represent Differential FTIR spectrum of PAMAM dendrimer templated Precursor ((a): TEOS and (b): TEP) reaction.
- **9. Fig. S6:** Represent Differential FTIR spectrum of PAMAM dendrimer templated Precursor ((a): Sodium acetate (NaAc) and (b): Calcium acetate (CaAc)) reaction.
- **10. Fig. S7:** FTIR spectra for the aminated bioglass nanoparticle.
- 11. Fig S8: TEM microimage for the aminated bioglass nanoparticle.
- 12. S4: Electronic Supplementary Information References.

S1. Discussion on methodology adopted for bioactivity test, cell culture and transfection studies

S1.1 Bioactivity Test

In brief, the powder sample were pelleted of equal shape and size of 10mm diameter and dipped in SBF. The ratio of sample to SBF was maintained at 1 mg into 1 ml SBF solution for 1, 15 and 30 days in an ORBITEK incubator (Scigenics Biotech) at 37°C under sterile conditions. The SBF solution was replaced twice a week because the cations concentration decreased during the course of the experiment, as a result of the changes in the chemistry of the sample. Once removed from the incubation solution, first the samples were rinsed gently with Milli-Q water and then in acetone. Subsequently, the samples were dried in air at room temperature and stored in a desiccator for further characterizations by XRD, FTIR and FESEM. After incubation of sample in SBF, the specific ion concentration was evaluated by Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES).

S1.2 Cell studies

The U2OS cell lines were maintained in DMEM high glucose medium with 10% heatinactivated fetal bovine serum and 0.1% streptomycin at 37° C with 5% CO₂. The cells were detached from the culture plate at 80–85% confluence and further used for evaluating the cytocompatibility of the biomaterial as follows.

In method I, elution test (ISO 10993-5), extracts from the pellets were prepared by incubating the pre-sterilized pellets (under UV light for one hour) in culture medium for 24 h at 37°C with agitation. The medium with glass-ceramic extract was collected in a falcon tube and the culture medium of the seeded cells was replaced after 24 h by the culture medium containing the biomaterial extract. Cells were incubated on the extract for 24 h and 48 h and subjected to MTT assay. In MTT assay, the viability of cells grown in the presence of the BIS-nBG-DG3 extract was determined using the colorimetric MTT assay. MTT assay measures the reduction of the tetrazolium component (MTT) into formazan crystals by viable cells reflecting the level of cell metabolism. For the assay, cells were then seeded onto 96-well plates with a density of 10⁴ cells per well and were incubated under standard culturing conditions. In order to carry out the assay, initially, 100mL of 10% MTT reagent was added and the plate was covered with foil leaving some free space to allow air in, and incubated for 2 h at 37°C under cell culture conditions. After

2 h the plate was taken out and then 100mL of dimethyl sulfoxide was added by removing the cell culture medium and incubated for 20 min at cell culture conditions to dissolve formazan crystals. After incubation, the plate was taken and read with 570 nm and 630 nm wavelengths using a TECAN multimode plate reader. In addition to this, cells were also observed under an optical microscope Floid Cell Imaging Station for visible signs of toxicity in response to the test. The cells cultured in DMEM medium were used as positive control, while 10% SDS treated DMEM medium was used as negative control. All experiments were run with five samples and the data was represented as means standard deviation. The statistical difference was analyzed using Students' GraphPad and a P value of <0.05 was obtained.

In method II, prior to cell seeding, the glass pellets were sterilized using UV treatment for 1 h and incubated with culture medium for 24 h at 37°C with 5% CO₂ and 85%humidity. Then the pellets were removed from the culture medium. Cells were seeded dropwise onto the top of the pellets (105, 80,000 and 20,000cells per sample for 1, 2 and 6 days respectively), which fully adsorbed the medium, allowing the cells to distribute throughout the pellets. Subsequently, cell-seeded pellets were kept at 37°C in a humidity incubator under standard culturing conditions for 4 h in order to allow the cells to attach to the pellets. After each 4 h, the pellets were fed additional culture medium and cells on the pellets were grown for 1, 2 and 6 days. The grown cells were fixed in 2% paraformaldehyde at 48°C overnight followed by gradient ethanol drying and placed in a desiccator overnight. Samples were then gold coated and subjected to FESEM analysis to examine the morphology and spreading pattern of the cells against negative control (bioglass sample and media).

S1.3 Transfection studies

The transfection studies were carried out as described elsewhere.¹ CHO-K1 cell line were maintained in Ham's F-12K media supplemented with 10% (v/v) fetal bovine serum at 37 °C and 5% CO₂ in humidified incubator. Cells were seeded 24 h before transfection in 24-well plates. For the luciferase assay, complex were prepared with final DNA concentration of 4 μ g/well (pMIR-ReportTM Luciferase) and incubated for 30 min at room temperature. 100 μ l of the pDNA/ABG complex was added to cells (~70% confluency) in serum free media (OptiMEM, Invitrogen). After 4 h of incubation at 37 °C, the media was drawn out; cells were washed with phosphate buffered saline (PBS, pH 7.4) and appended with 500 μ l complete growth medium.

After 24 h of treatment, cells were washed with PBS and lysed with 100 μ l of cell culture lysis buffer (1× CCLR, Promega). 50 μ l of cell lysate supernatant was evaluated for luciferase expression using the luciferase assay substrate (Promega) as a function of Light emission by integration over 10s in Orion microplate luminometer (Berthold Detection System, Germany). Luciferase activity was normalized with total protein content of the cells calculated using BCA protein assay (Pierce). All experiments were performed thrice in triplicates.

S2. Effect on PAMAM dendrimer concentration on the textural tunability of the bioglass:

In bioinspired synthesis, the synthetic methodology has been initiated with the dendrimer generation 3 aiming to get BG nanoparticles for gene delivery application. The pH was chosen to be 8 on the grounds of higher positive charge density at the polymer surface above pK_a values of PAMAM dendrimer's outer shell primary amine i.e. 7-9.² During the synthesis, we could observe the role of dendrimer concentration on the particle size distribution. We initiated our experiment with various concentration of dendrimer. Herein, we observed for lower concentration of below 10 mM, the irregularity in the particle morphology and size which could be attributed to the inconsistency in the heterogenous nucleation of the BG nanoparticle on the surface of the dendrimer followed by the homogenous nucleation in the solution phase. As expected, at higher concentration of greater than 30 mM,³ miceller aggregation of dendrimer takes place resulting in the aggregated lumps of the particles. However, 15 mM concentration resulted in the synthesis of homogenous monodispersed nanoparticles.



Fig.S1. Represent TEM images for different concentration of PAMAM generation 3 dendrimer templated bioglass (a) less than 10 mM (b) 15 mM (c) greater than 20 mM.

For the intermediate concentration we could get nBG particles of our interest, so we further proceeded the in detail characterization and experiments with the same. With these results certainly, particle size could be tuned in accordance with the PAMAM dendrimer concentration used as a template in the bio-inspired synthesis route of bioglass nanoparticle.



Fig. S2. Nitrogen adsorption–desorption isotherm and pore size distribution (inset) of pre-heated BIS-nBG-DG3 glass-ceramic sample through normal method of analysis.



Fig. S3. TGA graph representing the % weight loss for the as prepared, Heat treated and lyophilized sample.



Fig. S4. TEM images recorded after pre-heating treatment of 200 °C of the bioglass samples

S3. Discussion on the differential spectrum of the PAMAM interaction with the precursors.

The differential spectrum of PAMAM-TEOS (ESI Fig. S5(a)) indicates that the N-H band of pure PAMAM dendrimer observed at 1630, 1563 cm⁻¹ (Fig. 2) were shifted to 1625, 1540 cm⁻¹ suggesting the electrostatic interaction of the dendrimer template with TEOS. The new band appearance at 1410 cm⁻¹ was indicative of OH-bending and CH-wagging resulted from the hydrolysis of TEOS. More interestingly, the appearance of new peak at 959 cm⁻¹ corresponding to characteristic Si-O-Si bond indicates the nucleation of network formation onto the template. In addition, the emergence of small peaks at 782, 749 and 710 cm⁻¹ further confirmed Si-O-Si bond formation. After the addition of TEP to the PAMAM-TEOS mixture (ESI Fig.S5(b)), disappearance of characteristic P=O and P-OC peaks in the differential spectrum was observed. Overlapping of peaks in the region 1074-1041 cm⁻¹ could be attributed to Si-O-P and Si-O-Si. Remarkably, the characteristics peaks (N-H, Si-O-Si, O-H bending and C-H wagging) were retained by the reaction mixture even after the addition of calcium acetate i.e. till the end, with the negligible shift (ESI Fig. S5 (b), ESI Fig. S6 (a,b)). Although the peaks (namely Si-O-Si) were intensified with the addition of precursors and time, thus suggestive of growth of glass (SiO₂) network after interaction with different sequentially added precursors. It can also be attributed to the simultaneous distortion of silica network by Na⁺ and Ca²⁺ ions indicated by the increase in the intensity of Si-O stretch at 1071 cm⁻¹ in the differential spectrum obtained after the addition of calcium acetate (ESI Fig. S6(b)). On the other hand, decrease in the intensity of the band corresponding to the template has been observed suggesting that the template has been concealed into the network.



Fig. S5. Represent Differential FTIR spectrum of PAMAM dendrimer templated Precursor ((a): TEOS and (b): TEP) reaction.



Fig. S6. Represent Differential FTIR spectrum of PAMAM dendrimer templated Precursor ((a): Sodium acetate (NaAc) and (b): Calcium acetate (CaAc)) reaction.



Fig. S7. FTIR spectra for the aminated bioglass nanoparticle.



Fig. S8. TEM for the aminated bioglass nanoparticle.

S4. References:

- A. Mann, V. Shukla, R. Khanduri, S. Dabral, H. Singh and M. Ganguli, Mol. Pharmaceutics 2014, 11, 683–696
- 2. D. A. Tomalia, H. Baker, J. Dewald, M. Hall, G. Kallos, S. Martin, J. Roeck, J. Ryder and P. Smith, Polymer Journal 1985, **17**,117-132.
- 3. H. K. Alajangi and D. Santhiya, Phys. Chem. Chem. Phys., 2015, 17, 8680.