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**Structural, mechanical, imaging and *in vitro* evaluation of the combined effect
of Gd³⁺ and Dy³⁺ in the ZrO₂-SiO₂ binary system**

S. Vasanthavel^{a,b}, Sharad Awasthi^b, Arunkumar Dhayalan^b, Brian Derby^c and S. Kannan^{a*}

*^aCentre for Nanoscience and Technology,
Pondicherry University, Puducherry-605 014, INDIA*

*^bDepartment of Biotechnology,
Pondicherry University, Puducherry-605 014, INDIA*

*^cSchool of Materials, University of Manchester
Manchester, M13 9PL, UNITED KINGDOM*

Corresponding Author's Address

Dr. S. Kannan

Centre for Nanoscience and Technology,

Pondicherry University, Puducherry-605014, INDIA

E-mail: para_kanna@yahoo.com

Phone: 0091-413-2654973

ABSTRACT

Mechanical strength and biocompatibility are considered the main prerequisites for materials in total hip replacement (THR) or joint prosthesis. Non-invasive surgical procedures are necessary to monitor the performance of a medical device *in vivo* after implantation. To this aim, simultaneous Gd^{3+} and Dy^{3+} additions to the $\text{ZrO}_2\text{-SiO}_2$ binary system have been investigated. The results demonstrate the effective role of Gd^{3+} and Dy^{3+} to maintain the structural and mechanical stability of cubic zirconia ($c\text{-ZrO}_2$) up to 1400 °C, through their occupancy of ZrO_2 lattice sites. A gradual tetragonal to cubic zirconia ($t\text{-ZrO}_2 \rightarrow c\text{-ZrO}_2$) phase transition is also observed that is dependent on the Gd^{3+} and Dy^{3+} content in the $\text{ZrO}_2\text{-SiO}_2$. The crystallization of either ZrSiO_4 or SiO_2 at elevated temperatures is delayed by the enhanced thermal energy consumed by the excess inclusion of Gd^{3+} and Dy^{3+} at $c\text{-ZrO}_2$ lattice. The addition of Gd^{3+} and Dy^{3+} leads to an increase in the density, elastic modulus, hardness, and toughness above that of unmodified $\text{ZrO}_2\text{-SiO}_2$. The multi-modal imaging contrast enhancement of the Gd^{3+} and Dy^{3+} combinations were revealed through MRI and CT contrast imaging tests. Biocompatibility of the Gd^{3+} and Dy^{3+} dual doped $\text{ZrO}_2\text{-SiO}_2$ systems was verified through *in vitro* biological studies.

Key words: $\text{ZrO}_2\text{-SiO}_2$; Gadolinium; Dysprosium; Co-substitution; Structure; Properties.

1. INTRODUCTION

The $\text{ZrO}_2\text{-SiO}_2$ binary oxide system has been proposed as a new class of synthetic substitute material suitable for hard tissue replacements because of its appropriate mechanical strength and biocompatibility.¹ The rationale for choosing this specific combination is based on the following reasoning. ZrO_2 is a well-established bio-inert ceramic that has many years of commercial application because of its superior mechanical properties. However, the failure of ZrO_2 implants caused by the slow degradation of the toughening tetragonal zirconia ($t\text{-ZrO}_2$) phase to monoclinic zirconia ($m\text{-ZrO}_2$) *in vivo* after implantation has been reported.² SiO_2 as one of the prime component in bioactive glasses has been known to elicit a biocompatible response that promotes host-implant interaction by forming a hydroxyl carbonate apatite layer during *in vivo* exposure.³ SiO_2 is also employed to stabilize $t\text{-ZrO}_2$, in preference to the other two polymorphs namely $m\text{-ZrO}_2$ and cubic ($c\text{-ZrO}_2$).⁴ It has also been reported that the presence of SiO_2 as an amorphous matrix in $\text{ZrO}_2\text{-SiO}_2$ systems may help prevent the unwanted $t \rightarrow m\text{-ZrO}_2$ transition during accelerated leach tests.⁵ Nevertheless, the partial transformation of $t \rightarrow m\text{-ZrO}_2$ in the $\text{ZrO}_2\text{-SiO}_2$ system beyond 1100 °C limits the densification of sintered specimens and leads to mechanical properties inferior to yttria stabilized $t\text{-ZrO}_2$ polycrystals (Y-TZP).

There is an interest in developing biomaterials for bone implant applications that can be monitored non-invasively *in vivo* through advanced imaging methods, while retaining adequate levels of mechanical strength and biocompatibility. This has led to large numbers of studies that have reported on the introduction of rare earth oxides into biomaterials.⁶⁻⁹ Moreover, the ability of rare earth metal oxides to stabilise the $t\text{-ZrO}_2$ polymorph at high temperatures might improve the sinterability of the $\text{ZrO}_2\text{-SiO}_2$ system. This has been investigated in previous works by the authors in which the influence of individual additions of La^{3+} (LZS), Gd^{3+} (GZS), Dy^{3+} (DZS) and Ce^{4+} (CZS) to the $\text{ZrO}_2\text{-SiO}_2$ system have been reported.¹⁰⁻¹³ La^{3+} additions stabilizes the $t\text{-ZrO}_2$

polymorph up to 1000 °C, whereas Gd^{3+} induces a phase transition from t - $\rightarrow$ c - ZrO_2 that is dependent on Gd^{3+} content and moreover the structural stability of c - ZrO_2 is retained beyond 1100 °C. The addition of Ce^{4+} up to a certain concentration preserves t - ZrO_2 to 1300 °C by occupying the lattice sites of ZrO_2 , while its further addition yields c - CeO_2 alongside t - ZrO_2 .¹² In contrast, Dy^{3+} additions stabilizes t - ZrO_2 to 1400 °C with additions up to 50 wt. % concentration without formation of additional phases.¹³

The current study investigates the simultaneous addition of both Gd^{3+} and Dy^{3+} to the ZrO_2 - SiO_2 system using an *in-situ* sol-gel synthesis method. Among the lanthanides, Gd^{3+} based complexes are commercially employed as positive (T_1) MRI contrast agents owing to the unique magnetic properties conferred by its seven unpaired $4f$ electrons.¹⁴ Dy^{3+} , with a high magnetic moment of 10.2 μB and asymmetric electronic ground state of $^6\text{H}_{15/2}$, enunciates enhanced transverse relaxivity at high magnetic fields, which makes them suitable for use as negative (T_2) MRI contrast agents.¹⁵ The contrast nature of Dy^{3+} doped ZrO_2 - SiO_2 system has been previously reported by the authors.¹³ Furthermore, the high atomic number and K-edge energy values of $Z = 66$ and 53.8 keV respective of Dy^{3+} ensures a high X-ray attenuation coefficient of 3.36 $\text{cm}^2.\text{g}^{-1}$ at 100 keV and hence it is also employed as a X-Ray computed tomography (CT) contrast agent.¹⁶ Thus this study explores the simultaneous additions of Gd^{3+} and Dy^{3+} to the ZrO_2 - SiO_2 system followed by a systematic characterisation of the structural behaviour, resulting mechanical properties and multi-modal imaging features.

2. MATERIALS AND METHODS

2.1 Powder synthesis

The sol-gel technique was used to prepare Gd^{3+} and Dy^{3+} doped ZrO_2 - SiO_2 (GDZS) binary oxides. Pure ZrO_2 - SiO_2 (PZS) without any additions of Gd^{3+} and Dy^{3+} content was also prepared by using the same technique for comparative purposes. The precursors used for the synthesis were

Gd(NO₃)₃, Dy(NO₃)₃, ZrOCl₂ and (C₂H₅O)₄Si, purchased from Sigma-Aldrich, Bangalore, India. An equal molar ratio of ZrOCl₂ and (C₂H₅)₄OSi was maintained for all the synthesis whereas varied molar concentrations of Gd(NO₃)₃ and Dy(NO₃)₃ were added with respect to the concentrations of ZrOCl₂ and (C₂H₅O)₄Si. **Table 1** presents the concentrations of the compositions synthesized and their respective sample codes.

The synthetic procedure is briefly explained as follows. Initially (C₂H₅)₄OSi was added to the required amount of ethanol and stirred vigorously at room temperature. Separately prepared individual stock solutions of Gd(NO₃)₃, Dy(NO₃)₃ and ZrOCl₂ were added to this mixture and stirred at 80 °C for 30 minutes; then, 0.1 M of HNO₃ was added as a catalyst. The resultant homogeneous solution was stirred to yield a wet gel and dried at 120 °C for 24 hours to remove the excess water content. The dried gel was ground to a fine powder and subsequently characterized.

2.2 Powder characterization

The synthesized powders were heat treated at different temperatures in a muffle furnace for 4 hours and their phase behaviour was analysed by X-ray diffraction (XRD) (Ultima IV, Rigaku, Tokyo, Japan) with Cu K α radiation (λ = 0.15406 nm) produced at 40 kV and 30 mA, scanning the diffraction angles (2 θ) between 10 and 70° with a step size of 0.02° at 2 θ s⁻¹. Phases were identified using Standard ICDD (International Centre for Diffraction Data) Card Nos. 01-083-0944 for *m*-ZrO₂, 01-078-1765 for *t*-ZrO₂, 01-071-4810 for *c*-ZrO₂, 01-083-1378 for ZrSiO₄ and 00-039-1425 for cristobalite (*c*-SiO₂). The vibrational modes of the functional groups in the powders were evaluated using Raman spectra recorded from a Confocal Raman microscope (inVia Raman microscope, Renishaw, Wootton-under-Edge, UK). Vibrating sample magnetometer (VSM 7404 Series, Lake Shore Cryotronics, Westerville, OH, USA) was utilized to analyse the magnetic behaviour of the powders through magnetic hysteresis measurements at

ambient temperature with a maximum applied magnetic field of 15 kGauss that varied from -10 kOe to 10 kOe. Morphological features and selected area electron diffraction (SAED) patterns were recorded using Transmission Electron Microscopy (TEM) and Scanning Transmission Electron Microscopy (STEM) using a Tecnai F30, 300 kV, field emission gun (FEG) microscope (FEI, Eindhoven, Netherlands) equipped with a high angle annular dark field detector for STEM imaging and a silicon drift detector for energy dispersive X-ray spectroscopy (EDX) analysis (Oxford Instruments, High Wycombe, UK). Rietveld method was used for the quantitative phase estimation of the recorded XRD patterns using GSAS-EXPGUI software package.¹⁷ For this purpose, standard Crystallographic Information Files (CIF) corresponding to the recorded phases such as *m*-ZrO₂, *t*-ZrO₂, *c*-ZrO₂, ZrSiO₄ and *c*-SiO₂ were obtained from Smith *et.al.*,¹⁸ Howard *et.al.*,¹⁹ Wyckoff,²⁰ Hazen *et.al.*,²¹ and Dera *et al.*,²² (American Mineralogist crystal structure database) respectively. The protocol for the structure refinement was performed in accordance with the authors' previous report.¹

2.3 Mechanical evaluation

The hardness and elastic modulus of the PZS and GDZS samples were determined at room temperature by nanoindentation using a CETR Apex indenter (Bruker, Billerica, USA). For this purpose, the volatile impurities of the as-dried powders were removed by calcining at 700 °C and then milled for 2 hours in a planetary ball mill (Retsch, Haan, Germany). The resultant powders were pressed into circular pellets with 13 mm diameter and 1 mm thickness using a semi-automatic hydraulic press machine (Kimaya Engineers, Thane, India) under an applied load of 10 kN for 60 s. The resulting pellets were heat treated at specific temperatures for 4 hours followed by diamond polishing prior to mechanical testing. The pellets were analysed for their surface morphological features prior to diamond polishing using FE-SEM (QuantaTM FE 250, FEI),. Nanoindentation was performed using a Berkovich indenter tip with a radius of 50 nm at an applied load of 100 mN. A conventional depth-sensing procedure was carried out to acquire

mechanical data with a cycle consisting of a loading segment, dwell time at maximum load and an unloading segment. Data was analysed in accordance with the procedures described in ISO 14577.²³ A single indent mode with 10 indents per pellet at random locations was systematically performed for all the pellets. Density measurements were carried out using Archimedes method and fracture toughness tests were performed using micro-indentation (microtest, MTR3/50-50/Ni, Madrid, Spain). Indentation was performed at five different locations on the specimen surface with an applied load of 40 N using a Vickers indenter tip. Fracture toughness was calculated using the following relation as given below.^{24,25}

$$K_{IC} = 0.0824(P/c^{3/2}) \quad (1)$$

where K_{IC} is the fracture toughness, P is the applied load and c is the indentation induced crack length.

2.4 *In vitro* MR imaging

Siemens Magnetom Avanto 1.5 Tesla MRI scanner was used to determine the T_1 and T_2 relaxivity of the GDZS-50 sample. The relaxivity values were quantified from the *in vitro* phantom images obtained from GDZS-50 at different Gd^{3+} and Dy^{3+} concentrations. Matlab, (The Mathworks Inc., MA, USA) software was used to calculate the relaxation rate maps by fitting all the corresponding curves. T_1 mapping was obtained by setting the following parameters: a fast spin echo (FSE) sequence with an echo train length = 16, repetition time (TR) = 3000 ms, matrix size = 512×512 , slice thickness = 5 mm, and number of slices = 1. Similarly T_2 mapping was carried out using the following parameters: echo time (TE) = 22 to 352 ms (difference = 22 ms) and images = 16. The resultant images were used to determine the T_1 and T_2 relaxation times.

2.5 *In vitro* CT imaging

To analyse CT contrast effectiveness, various concentrations of GDZS-50 samples (0, 1.25, 2.5, 5, 10 mg/mL) were evenly dispersed in aqueous solution in 5 mL tubes and images were acquired using a multislice spiral CT system (GE HISPEED CT/e, GE Healthcare, Chicago, IL, USA). Imaging parameters were set as follows: thickness of 0.9 mm at 120 KVp and 160 mA, Field of view (FOV) of 54.07 mm 9146.00 mm and an exposure time of 800 ms/rotation. Kodak Molecular Imaging Software (Eastman KODAK Company, Rochester, NY, USA) was used for the analysis of Phantom CT images and the GE HISPEED CT software was used to measure the Hounsfield Unit (HU) values.

2.6 *In vitro* cellular studies

The methodology of MTT assay, live/dead cell analysis, ALP activity tests and RT-PCR are presented in Supplementary information.

3. RESULTS

3.1 XRD analysis

The experimental results from our previous studies confirm that the PZS system contains *t*-ZrO₂ as a unique component at 1100 °C and this undergoes partial degradation to *m*-ZrO₂ at 1200 °C (supplementary information **Figure S1**). The XRD patterns obtained from the GDZS samples recorded after heat treatment at 1200 °C are illustrated in **Figure 1a**. The compositions containing lower concentrations of Gd³⁺ and Dy³⁺ (GDZS-5 and GDZS-10) show *t*-ZrO₂ as a solitary crystalline phase. A partial phase transition with *t*- → *c*-ZrO₂ is apparent for GDZS-20 (**Figure 1a inset**) and for all the compositions that contained higher Gd³⁺ and Dy³⁺ concentrations (GDZS-30 and GDZS-50) only *c*-ZrO₂ was present as a solitary phase. The phase behaviour at 1300 °C (**Figure 1b**) is similar to that found at 1200 °C for all the GDZS compositions. Although, very small amounts of *m*-ZrO₂, *c*-SiO₂ and ZrSiO₄ are seen for compositions with Gd³⁺ and Dy³⁺

additions in the range of 5 - 20 wt. % (GDZS-5, GDZS-10 and GDZS-20). However, *c*-ZrO₂ as a distinct phase is retained for GDZS compositions with high Gd³⁺ and Dy³⁺ content (GDZS-30 and GDZS-50) at 1300 °C. The XRD patterns of the powders heat treated at 1400 °C (**Figure 1c**) indicate ZrSiO₄ as a major component alongside minor amounts of *t*-ZrO₂ and *c*-SiO₂ for all the compositions barring GDZS-50 that still displayed solitary *c*-ZrO₂.

3.2 Raman spectra

Figure 2 presents the Raman spectra for all the powders recorded after heat treatment at 1200 °C and the GDZS-50 at 1400 °C. The 148, 268, 320, 465, 650 cm⁻¹ bands pertaining to *t*-ZrO₂²⁸ are clearly observed for GDZS-5 and at a slightly reduced intensity for GDZS-10, however with GDZS-20 the bands are only slightly more intense than the background. The bands characteristic of *c*-ZrO₂ are at 618, 305 and 140 cm⁻¹.^{13,29} For GDZS-30 only the band at 618 cm⁻¹ is evident and shifted towards a lower wave number. This shift, although less marked is also seen with GDZS-50 with the 618 and 305 cm⁻¹ bands. GDZS-50 displayed enhancement in the intensity of the bands that correspond to *c*-ZrO₂ at 1400 °C with a reduced shift and this infers the formation of highly crystalline *c*-ZrO₂. Thus the observations from Raman spectra are consistent with the XRD results.

3.3 Transmission electron microscopy

TEM micrographs for GDZS-50 at 1400 °C are displayed in **Figure 3**. A clustered dispersion of larger sized ZrO₂ grains in the glassy SiO₂ matrix is evident in **Figure 3a**. **Figures 3b** and **3c** display a homogeneous distribution of multi-grain crystalline ZrO₂ clusters embedded in the SiO₂ matrix. The electron diffraction patterns (**Figure 3d**) affirmed the existence of diffraction planes that can be indexed to *c*-ZrO₂. The glassy nature of the SiO₂ is revealed through **Figure 3e** in which diffraction spots were negligible. Elemental mapping obtained via EDX microanalysis for the GDZS-50 sample is displayed in **Figure 4**. The elemental maps indicate the widespread

distribution of ZrO₂ grains throughout the SiO₂ matrix. The correlation of Gd³⁺, Dy³⁺ and Zr⁴⁺ presence at the same positions affirms the Gd³⁺ and Dy³⁺ are accommodated on the lattice sites of ZrO₂.

3.4 Quantitative analysis

The phase fractions and lattice parameters determined from Rietveld refinement are presented in **Table 2**. The refined diffraction pattern of GDZS-5 (**Figure 5a**) at 1200 °C corresponds to the structure of *t*-ZrO₂ with a tetragonal unit cell space group 137, *P42/nmc*. The pattern of GDZS-50 (**Figure 5b**) at 1400 °C shows the *c*-ZrO₂ structure space group 225, *Fm-3m*(225). The refined data (**Table 2**) at 1200 °C confirms the formation of *t*-ZrO₂ as a sole phase for GDZS-5 and GDZS-10, whereas its complete conversion to the *c*-ZrO₂ phase is found for GDZS-20, GDZS-30 and GDZS-50. The refined lattice data for *t*-ZrO₂ reveals steady increase in the *a* = *b*-axis values and a simultaneous reduction in the *c*-axis with increasing Gd³⁺ and Dy³⁺ additions. A similar trend of steady increment in lattice parameters with increasing Gd³⁺ and Dy³⁺ additions is witnessed for *c*-ZrO₂ stabilized systems. This is a reasonable observation, which is justified by comparing the lattice parameters of standard *t*-ZrO₂ (*a* = *b* = 3.5957 Å and *c* = 5.1844 Å for ICDD card No. 01-079-1765) and standard *c*-ZrO₂ (*a* = *b* = *c* = 5.100 Å for ICDD card No. 01-071-4810). The *a* = *b*-axis value of standard *t*-ZrO₂ < *c*-ZrO₂, while *c*-axis value of standard *t*-ZrO₂ > *c*-ZrO₂. Therefore, the results that highlights the corresponding enhancement and reduction of *a* = *b*-axis and *c*-axis of *t*-ZrO₂ infers the critical role of Gd³⁺ and Dy³⁺ combination to impart *t* → *c*-ZrO₂ transition through their occupancy at the Zr⁴⁺ lattice sites. This mechanism is supported by the combined accommodation of Gd³⁺ and Dy³⁺ along the *a* = *b*-axis of *t*-ZrO₂ resulting in its lattice expansion and simultaneous reduction along the *c*-axis that led to the *t* → *c*-ZrO₂ transition. It is also apparent that a critical amount of 20 wt. % Gd³⁺ and Dy³⁺ combination is sufficient to ensure *a* = *b*-axis expansion to accomplish the *t* → *c*-ZrO₂ transition.

The presence of c -SiO₂ and m -ZrO₂ alongside dominant t -ZrO₂ was determined from the refined data at 1300 °C for GDZS-5, GDZS-10 and GDZS 20. This indicates that heat treatment is an important factor for the crystallization of c -SiO₂ and the t -ZrO₂ $\rightarrow$ m -ZrO₂ transition, which may be associated with the lattice expansion of t -ZrO₂ and transformation on cooling.

However, the absence of m -ZrO₂ in GDZS-30 and GDZS-50 at 1300 °C confirms that the crystallization of c -ZrO₂ in a ZrO₂-SiO₂ system is highly stable and does not undergo transformation to t -ZrO₂ or m -ZrO₂ on cooling. Further thermal treatment to higher temperatures triggers the reaction amongst ZrO₂ and amorphous SiO₂ to yield ZrSiO₄ and this is confirmed from the refined data at 1400 °C. The enhanced ZrSiO₄ crystallization is found in t -ZrO₂ stabilized GDZS systems whereas the delayed ZrSiO₄ crystallization is detected in c -ZrO₂ stabilized GDZS systems. Moreover, there is a decline in ZrSiO₄ formation with increasing Gd³⁺ and Dy³⁺ additions and there is a complete absence of ZrSiO₄ in GDZS-50. However, the absence of any significant trend for the phase content of c -SiO₂ in the GDZS systems suggests that c -SiO₂ crystallization is an independent phenomenon that is not influenced by the formation of ZrO₂ polymorphs present in the ZrO₂-SiO₂ system.

3.5 Morphological analysis

Secondary electron (SE) micrographs (**Figures 6a, 6b and 6c**) indicate the widespread distribution of crystalline ZrO₂ particles throughout the intermittently aligned non-crystalline phase and these particles are found vary in size and shape. However, close observation shows that the particles are embedded in a matrix like structure. The EDX spectrum obtained from spot analysis for the grain indicated by a cross in **Figure 6c** shows the presence of Gd, Dy, Zr and O elements in significant quantity, which confirms that the embedded grains are ZrO₂ and the matrix is amorphous SiO₂. Moreover, the presence of Gd and Dy elements in equal proportion affirms their respective

addition as precursors during the synthesis. These observations exhibit good corroboration with the elemental analysis determined from TEM.

3.6 Mechanical behaviour

Table 3 illustrates the density and mechanical data of PZS system at 1100 °C and GDZS-50 at 1400 °C. Five different measurements were performed to obtain the mean value and standard deviation of density and fracture toughness. The elastic modulus and hardness data were derived from ten different indents at random locations. The load vs. depth nanoindentation profiles of GDZS-50 (**Figure 7**) were found to be highly uniform and consistent. The mechanical data recorded for the PZS is found lower than the reported values available in the literature^{30, 31} and this is believed to be a consequence of the high SiO₂ content, inadequate densification due to the low temperature sintering (1100 °C) and the employed synthetic conditions. The presence of radial cracks (indicated by the arrow marks) due to indentation on the polished surface of GDZS-50 are shown in **Figure 8**, fracture toughness was calculated from the length of the se cracks (equation 1). The enhanced thermal stability of GDZS-50 is demonstrated by its highly dense nature. The density and mechanical data of GDZS-50 are superior to the measured values of PZS. This suggests that Gd³⁺ and Dy³⁺ additions induce improved phase stability of GDZS-50 at elevated heat treatment that results in better densification and superior mechanical properties.

3.7 MRI and CT imaging characteristics

The room temperature magnetization curves of the GDZS system are shown in **Figure 9** and **Table 4** presents the calculated magnetic parameters obtained from the plotted curves. The results indicate the enhanced saturation magnetization (Ms) with simultaneous increase in Gd³⁺ and Dy³⁺ additions. The presence of poor hysteresis loops confirms the paramagnetic behaviour of Gd³⁺ and Dy³⁺ in ZrO₂-SiO₂ systems. Nonetheless, the *in-vitro* MRI and CT data displays positive contrast behaviour for the GDZS-50 sample that are presented in **Figure 10, 11** and **12**. T₁

weighted MR images (**Figure 10**) show enhanced brightness for the increasing GDZS-50 concentrations and an ascending trend is observed for the relaxivity values. The continuous rise in $1/T_2$ values and diminution in brightness of T_2 weighted MR images with respect to the increasing GDZS-50 concentrations are evident from **Figure 11**. Also, CT phantom images (**Figure 12**) display the enhanced brightness for the increasing GDZS-50 concentration and the same trend is reflected for the recorded Hounsfield unit values.

3.8 Cytotoxicity Tests

The results from the cytotoxicity tests (**Figure 13**) revealed the absence of significant toxicity upon the treatment of PZS and GDZS-5 samples up to 200 $\mu\text{g/ml}$ of concentration. We observed a marginal reduction in the cell viability upon the treatment of GDZS-10 and GDZS-20 samples. In case of higher Gd^{3+} and Dy^{3+} containing samples (GDZS-30 and GDZS-50), a significant reduction in the cell viability is observed with higher concentrations. The MG-63 cells incubated with maximal concentrations (200 $\mu\text{g/ml}$) of PZS or GDZS-10 or GDZS-30 or GDZS-50 compositions were subjected to morphological analysis using optical microscope. No significant morphological changes are observed in the MG-63 cells upon treatment with the PZS and GDZS samples (**Figure 14**).

On further investigation of biocompatibility of the investigated materials, a live/dead staining assay was used with the MG-63 cells after incubating with PZS and GDZS samples. The fluorescence micrographs of MG-63 cells (**Figure 15**) depict live cells stained as green while the dead cells stained as red. These fluorescence micrographs demonstrate the presence of live cells in green colour with devoid of dead cells in PZS and GDZS-10 whereas the presence of dead cells (exemplary cells are indicated by arrow marks) coloured in red are obvious for GDZS-30 and GDZS-50.

3.9 ALP (Alkaline phosphatase) gene expression analysis

During the differentiation of osteoblast cells, ALP is expressed as a key enzyme and it is also considered as a marker enzyme for osteoblast proliferation. The ALP gene expression in MG-63 cell line were determined using qRT-PCR upon the treatment of PZS, GDZS-10, GDZS-30 and GDZS-50 samples. The ALP expression was not altered in the cells which were treated with PZS and GDZS-10 samples indicating the unperturbed proliferation capacity of the MG-63 cells. However, the ALP expression is reduced by 10 % and 24 % in the cells which were treated with GDZS-30 and GDZS-50 samples respectively (Figure 16).

4. DISCUSSION

The results from XRD and Raman data establish the combined influence of Gd^{3+} and Dy^{3+} to induce $t \rightarrow c$ - ZrO_2 transition. The compositions > 10 wt. % Gd^{3+} and Dy^{3+} result in a comprehensive transformation to c - ZrO_2 at 1200 °C. Further heat treatment activated the simultaneous occurrence of c - SiO_2 crystallization and $t \rightarrow m$ - ZrO_2 transition in a minor level for t - ZrO_2 stabilized systems. Moreover, heat treatment beyond 1300 °C results in the formation of $ZrSiO_4$ for all compositions, with the exception of GDZS-50 that preserve c - ZrO_2 in amorphous SiO_2 matrix till 1400 °C. The distribution of crystalline c - ZrO_2 grain clusters throughout the SiO_2 matrix is observed from TEM studies. The Gd^{3+} and Dy^{3+} accommodation in the ZrO_2 lattice is confirmed from the EDX spectra and elemental mapping.

The Rietveld refinement results revealed a $t \rightarrow c$ - ZrO_2 transition due to the accommodation of Gd^{3+} and Dy^{3+} along the $a = b$ -axis lattice sites of ZrO_2 and this is established from the obvious enhancement in $a = b$ -axis values and decline in c -axis values of t - ZrO_2 . The observation of enhanced $ZrSiO_4$ crystallization in t - ZrO_2 stabilized systems and its formation in minor level in case of c - ZrO_2 stabilized GDZS systems infers that t - ZrO_2 is more susceptible to react with glassy SiO_2 in comparison with c - ZrO_2 . This inference corroborates with the report of Itoh²⁸ that reported

enhanced kinetics between t -ZrO₂ and amorphous SiO₂ rather than the slow kinetics that occurs between amorphous ZrO₂ and amorphous SiO₂ in the ZrO₂-SiO₂ system.

The observation of minor ZrSiO₄ formation even with c -ZrO₂ stabilized compositions and its absence for the maximum Gd³⁺ and Dy³⁺ doped composition (GDZS-50) is explained by the observations based on our previous investigations reported on La³⁺ (LZS), Gd³⁺ (GZS), Ce⁴⁺ (CZS) and Dy³⁺ (DZS) additions in ZrO₂-SiO₂ binary systems.¹⁰⁻¹³ The considerable size difference between La³⁺ (1.06 Å) and Zr⁴⁺ (0.79 Å) restricts the entry of La³⁺ into the ZrO₂ lattice and as a consequence the quantitative analysis implies negligible changes in the lattice data of t -ZrO₂ in LZS systems. The smaller ionic size of Ce⁴⁺ (0.97 Å) than La³⁺ permits the entry of Ce⁴⁺ at the t -ZrO₂ lattice until 20 wt. % additions in CZS system. Beyond this limit, the excess Ce⁴⁺ is rejected from the ZrO₂ lattice and individually precipitates as c -CeO₂ at ambient conditions. Gd³⁺ (0.94 Å) has a smaller ionic size than Ce⁴⁺ and this favours the additional accommodation at the ZrO₂ lattice till 25 wt. %. Due to the accommodation of excess Gd³⁺, a gradual increase in the lattice parameter of t -ZrO₂ and subsequent t → c -ZrO₂ transition occurs in GZS system. Nonetheless, the predominant presence of ZrSiO₄ at 1200 °C for low Gd³⁺ additions and its reduced existence for higher Gd³⁺ additions infers that c -ZrO₂ is less reactive with SiO₂ in comparison with t -ZrO₂. In the case of the DZS system, the ZrO₂ lattice prefers to host a maximum of 50 wt. % Dy³⁺ ions, which is mainly due to the lower ionic radius of Dy³⁺ (0.91 Å) compared with other investigated rare earth elements. This hypothesis is confirmed from the quantitative analysis results in which a continuous expansion of the t -ZrO₂ lattice and its associated t → c -ZrO₂ transition is witnessed in DZS systems. It is worth noting that c -ZrO₂ experiences higher lattice expansion in GZS system rather than DZS systems.

In the present GDZS system, the combined size effect of Gd³⁺ and Dy³⁺ $[(0.94+0.91)/2 = 0.925$ Å] is less than Gd³⁺ and this facilitates their excess accommodation in the ZrO₂ lattice and as a

consequence the maximum lattice expansion of c -ZrO₂ is determined for GDZS-50, which remains stable until 1400 °C devoid of ZrSiO₄ formation that was encountered previously in the GZS system. Therefore, the ionic radii of dopants, quantity of the dopant concentration and associated lattice expansion are all factors that determine the t - $\rightarrow$ c -ZrO₂ transition and the resultant structural stability of c -ZrO₂. Consequently, the enhanced reaction kinetics with respect to temperature created by the Gd³⁺ and Dy³⁺ combinations that induce t - $\rightarrow$ c -ZrO₂ transitions in GDZS system suppresses the crystallization of amorphous SiO₂ even at 1400 °C. Moreover, the preservation of SiO₂ in amorphous state is highly beneficial for bone implant applications.²⁹

The SEM studies also reveal the embedding of crystalline ZrO₂ grains throughout the glassy SiO₂ matrix that yields enhanced mechanical properties. The mechanical features of GDZS-50 are thus superior to the DZS and CZS systems. Also, the elastic modulus of GDZS-50 is 23 % higher than Y-TZP and exhibit good matches with the zirconia toughened alumina (ZTA) system whose elastic modulus is reported in the range of 280-300 GPa.³²⁻³⁴ The fracture toughness of GDZS-50 is higher than alumina ceramics and marginally lower than Y-TZP.³ It is worth noting that GDZS-50 yields superior mechanical properties even in the presence of amorphous SiO₂ whose concentration is equivalent to the molecular ratio of ZrO₂. The mechanism behind the enhancement in density and mechanical data is attributed to the improved densification of amorphous SiO₂ matrix and its reinforcement by the highly crystalline ZrO₂ grains.

The presence of Gd³⁺ in GDZS-50 enhances the T₁ weighted MRI contrast ability and in addition the presence of Dy³⁺ in GDZS-50 yields both significant T₂ MRI and CT contrast ability that is confirmed by *in-vitro* imaging studies. Maximal Gd³⁺ and Dy³⁺ containing samples (GDZS-50) are toxic to the MG-63 cells. The level of toxicity was higher when the cells are treated with higher concentration (200 µg/ml) of GDZS-50 samples. However, the level of cytotoxicity is less than 20 % when the cells are treated with the lower concentration of GDZS-50 samples. Overall studies

enlighten the superior mechanical features of GDZS-50 allied with multi-modal imaging contrast features and biocompatibility.

5. CONCLUSIONS

The $t \rightarrow c$ -ZrO₂ transition in ZrO₂-SiO₂ system with respect to increased Gd³⁺ and Dy³⁺ additions is accompanied by their incorporation at the ZrO₂ lattice sites. The phase stability of c -ZrO₂ is retained till 1400 °C for the maximum Gd³⁺ and Dy³⁺ co-substitutions in ZrO₂-SiO₂ systems and this effect is reflected in the resulting highly crystalline and dense material. The Gd³⁺ and Dy³⁺ content and its concomitant positive effects in lattice volume of c -ZrO₂ plays a critical role in delaying ZrSiO₄ crystallization at higher temperatures. The mechanical strength of GDZS-50 is determined to be superior to the commercially available synthetic bone implants. Multi-modal imaging contrast features of GDZS-50 are documented. *In vitro* analysis suggests the low cell toxicity and confirms the biocompatibility of the materials.

Supporting Information

The following Supporting Information is available free of charge on the ACS Publications website at DOI <https://pubs.acs.org/doi/suppl/10.1021/acs.inorgchem.8b00337>

Cytotoxicity Tests, Live/Dead staining, Quantitative RT-PCR, Primers used in qRT-PCR and XRD patterns of pure ZrO₂-SiO₂ system at two different temperatures.

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Table 1 Precursor concentrations used in the synthesis procedure

Sample code	Precursor concentrations (M)				% of Gd ³⁺ and Dy ³⁺ with respect to Zr ⁴⁺
	Dy(NO ₃) ₃	Gd(NO ₃) ₃	ZrOCl ₂	(C ₂ H ₅ O) ₄ Si	
PZS	-	-	0.5 M	0.5 M	-
GDZS-5	0.0125 M	0.0125 M	0.5 M	0.5 M	5
GDZS-10	0.0250 M	0.0250 M	0.5 M	0.5 M	10
GDZS-20	0.0500 M	0.0500 M	0.5 M	0.5 M	20
GDZS-30	0.0750 M	0.0750 M	0.5 M	0.5 M	30
GDZS-50	0.1250 M	0.1250 M	0.5 M	0.5 M	50

Table 2 Refined structural parameters for the different GDZS compositions at specific temperatures

1200 °C										
Sample code	χ^2	R_p (%)	R_{Bragg}	Phase fraction (%)					Lattice data for t -ZrO ₂ and c -ZrO ₂	
				t -ZrO ₂	m -ZrO ₂	c -ZrO ₂	$a = b$ axis	c - axis		
							$a = b = c$ axis			
GDZS-5	1.2	05.60	06.10	100	-	-	-	3.6048(3)	5.1941(2)	
GDZS-10	1.4	06.90	07.50	100	-	-	-	3.6139(4)	5.1884(4)	
GDZS-20	2.1	08.33	07.10	-	-	-	100	*5.1543(2)		
GDZS-30	1.9	08.17	06.20	-	-	-	100	*5.1603(5)		
GDZS-50	1.8	08.77	09.60	-	-	-	100	*5.1880(3)		
1300 °C										
Sample code	χ^2	R_p (%)	R_{Bragg}	Phase fraction (%)					Lattice data for t -ZrO ₂ and c -ZrO ₂	
				t -ZrO ₂	m -ZrO ₂	c -SiO ₂	ZrSiO ₄	c -ZrO ₂	$a = b$ axis	c - axis
									$a = b = c$ axis	
GDZS-5	1.6	07.45	11.00	80.70	5.40	13.90	-	-	3.6048(5)	5.1844(5)
GDZS-10	3.7	10.69	11.20	69.70	5.30	25.00	-	-	3.6095(4)	5.1737(4)
GDZS-20	2.2	08.54	10.50	-	-	20.20	-	79.80	*5.1512(6)	
GDZS-30	1.9	05.31	13.30	-	-	-	-	100	*5.1602(2)	
GDZS-50	1.9	09.11	10.30	-	-	-	-	100	*5.1869(3)	
1400 °C										
Sample code	χ^2	R_p (%)	R_{Bragg}	Phase fraction (%)					Lattice data for t -ZrO ₂ and c -ZrO ₂	
				t -ZrO ₂	m -ZrO ₂	c -SiO ₂	ZrSiO ₄	c -ZrO ₂	$a = b$ axis	c - axis
									$a = b = c$ axis	
GDZS-5	2.5	12.01	08.80	24.90	3.70	12.20	59.20	-	3.6079(6)	5.1857(2)
GDZS-10	2.1	09.44	10.30	22.00	-	3.80	74.20	-	3.6460(4)	5.1685(3)
GDZS-20	2.8	09.69	12.90	-	-	6.30	58.20	35.50	*5.1635(6)	
GDZS-30	1.9	03.58	13.44	-	-	-	05.90	94.10	*5.1686(5)	

Table 3 Physical and mechanical properties of PZS and GDZS-50 compositions.

Sample	Density (gm.cm⁻³)	Fracture Toughness (MPa.m^{1/2})	Hardness (GPa)	Elastic Modulus (GPa)
PZS	3.79 (0.03)	1.31 (0.58)	0.45 (0.50)	6.81 (1.00)
GDZS-50	4.99 (0.32)	9.89 (1.15)	15.80 (0.57)	266.69 (2.41)

Table 4 Magnetic properties of Gd³⁺ and Dy³⁺ doped ZrO₂-SiO₂ binary oxides.

Sample code	Coercivity (G)	Magnetization (emu/g)	Retentivity (emu/g)
GDZS-5	33.40	1.97×10^{-3}	10.90×10^{-6}
GDZS-10	20.93	3.09×10^{-3}	10.53×10^{-6}
GDZS-20	17.79	6.96×10^{-3}	13.97×10^{-6}
GDZS-30	13.53	12.34×10^{-3}	17.32×10^{-6}
GDZS-50	10.97	17.17×10^{-3}	22.27×10^{-6}

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Gd³⁺ and Dy³⁺ combination in ZrO₂-SiO₂ binary system delays the crystallization of ZrSiO₄ and stabilizes *c*-ZrO₂ in amorphous SiO₂ matrix