Contents

Table of contents

List of Publications

List of Figures

List of Tables

Chapter 1	**Introduction**		1-16
1.1	**Background**		1
1.2	**Model bioceramics**		4
1.3.	**Polarization induced cellular and antibacterial response**		6
1.4	**Objectives of the thesis**		7
1.5	**Outlines for the thesis**		8
Chapter 2	**Literature Review**		17-76
2.1	**Introduction**		17
2.2	**Antimicrobial response of tailored biocomposites**		18
2.2.1	Effect of addition of Ag on antibacterial response of biocomposites		18
2.2.2	Effect of addition of TiO_2 on antibacterial response of biocomposites		19
2.2.3	Effect of addition of ZnO on antibacterial response biocomposites		21
2.3	**Effect of nanostructured materials on antibacterial response**		25
2.3.1	Antibacterial response of zinc oxide (ZnO) nanoparticles		25
23.2	Antibacterial response of silver (Ag) nanoparticles		27
2.3.3	Antibacterial response of copper and copper oxide nanoparticles		29

	2.3.4	Antibacterial response of iron oxide nanoparticles	31
	2.3.5	Antibacterial response of magnesium oxide (MgO) nanoparticles	32
	2.3.6	Antibacterial response of gold (Au) nanoparticles	34
2.4		**Effect of external stimuli on antibacterial response**	39
	2.4.1	Effect of magnetic field on antibacterial response	38
	2.4.2	Effect of electrical stimuli on antibacterial response	40
2.5		**Effect of addition of piezoelectric secondary on antibacterial response of bioceramics**	44
2.6		**Cellular response of tailored biocomposites**	44
	2.6.1	Effect of incorporation of piezoelectric material on cellular response	46
	2.6.2	Effect of external stimuli on cellular response	47
2.7		**Closure**	51
Chapter 3		**Materials and Methods**	77-88
3.1		**Synthesis**	77
	3.1.1	Synthesis of hydroxyapatite (HA)	
	3.1.2	Synthesis of HA-xZnO composites	79
	3.1.3	Synthesis of barium titanate ($BaTiO_3$, BT)	79
	3.1.4	Synthesis of calcium titanate ($CaTiO_3$, CT)	79
	3.1.5	Synthesis of sodium potassium niobate ($Na_{0.5}K_{0.5}NbO_3$, NKN)	80
	3.1.6	Synthesis of HA- BT, HA- CT and HA- NKN composites	80
	3.1.7	Synthesis of borate bioglass (1393 B3)	81
	3.1.8	Synthesis of BBG- BT, BBG-NKN composite	82
	3.1.9	Pelletization and sintering of processed samples	82

3.2		**Characterization**	83
	3.2.1	X-ray diffraction and Fourier transform infrared spectroscopic spectroscopy (FTIR)	
	3.2.2	Scanning electron microscopy (SEM) and Energy-dispersive X-ray spectroscopy (EDX)	83
3.3		**Polarization treatment**	84
3.4		**Antibacterial response**	84
	3.4.1	Quantitative analyses (MTT assay)	85
	3.4.2	Qualitative analyses (Live/dead Assay)	85
3.5		**Cellular response**	86
	3.5.1	Quantitative analyses (MTT assay)	86
	3.5.2	Morphological analyses	87
3.6		**Closure**	87
Chapter 4		**Antibacterial and Cellular Response of Hydroxyapatite and HA-x ZnO (x = 3.0, 4.5, and 7.5 wt. %) Composites**	89-126
4.1		**XRD analysis**	89
4.2		**Fourier transform infrared spectroscopic analysis**	90
4.3		**Microstructural analysis**	91
4.4		**Dielectric measurements**	92
	4.4.1	Dielectric behavior	93
	4.4.2	AC conductivity behavior	97
	4.4.3	Impedance analysis	99
4.5		**Antibacterial response of HA-xZnO composites**	101
	4.5.1	MTT assay	101
	4.5.2	Kirby-Bauer disk diffusion method	103
	4.5.3	NBT assay	106

4.5.4	Atomic absorption spectroscopy (AAS) test	108
4.5.5	Live / dead assay	109
4.6	***In-vitro* cellular response**	113
4.6.1	MTT assay	113
4.6.2	Morphological study	116
4.7.	**Closure**	118
Chapter 5	**Antibacterial and Cellular Response of HA, $BaTiO_3$ (BT), $CaTiO_3$ (CT), $Na_{0.5}K_{0.5}NbO_3$ (NKN) and Their Composites**	127-161
5.1	**XRD analysis**	127
5.2	**Fourier transform infrared spectroscopic analysis**	128
5.3	**Surface morphology**	129
5.4	**Contact angle measurements**	131
5.5	**Antibacterial response**	132
5.5.1	MTT assay	132
5.5.2	Colony counting method	133
5.5.3	Antibacterial ratio	137
5.5.4	Live/dead assay	138
5.6	**Enzymatic activity**	142
5.6.1	Superoxide dismutase assay	142
5.6.2	Catalase assay	144
5.6.3	Protein estimation assay	145
5.6.4	Lipid peroxide assay (LPO assay)	147
5.7	**Cellular response**	149
5.7.1	MTT assay	149
5.7.2	Cell adhesion Test	153
5.8.	**Closure**	156

Chapter 6 **Antibacterial and Cellular Response of BBG- based Composites** 162-189

6.1 **Phase evolution** 162

6.2 ***In-vitro* antibacterial response** 163

6.2.1 MTT assay 163

6.2.2 Live/dead ratio 165

6.2.3 Nitro blue tetrazolium (NBT) assay 167

6.2.4 Bacterial adhesion test 169

6.3 **Cell culture Study** 173

6.3.1 MTT assay 173

6.3.2 Morphological analysis 176

6.4 **Closure** 178

Chapter 7 **Conclusions and Future Scope** 183-186

7.1 **Conclusions** 183

7.2 **Scope for the future work** 186

List of Publications 187

List of figures

Fig. 2.1	:	*Schematic illustrating the mechanism of antibacterial response due to silver ions.*	*19*
Fig. 2.2	:	*Schematic diagram of proposed mechanisms for antibacterial response of TiO_2*	*20*
Fig. 2.3	:	*Schematic presentation of mechanisms for antibacterial response of ZnO based biocomposites*	*22*
Fig. 2.4	:	*Schematic illustration represents (a) various modes of antibacterial response due to ZnO nanoparticles and (b) possible mechanisms for ZnO nanoparticles mediated antibacterial response.*	*27*
Fig. 2.5	:	*Schematic illustrating (a) nanoparticles internalization into the cell wall, (b) disruption of cell wall and release of intracellular materials, (c) thickenings of cell wall and release of cytoplasm, and (d) mechanisms of dissolution of nanoparticles in the bacterial membrane, release of metal ions, ROS, and disruption of DNA.*	*29*
Fig. 2.6	:	*Schematic illustration of antibacterial response due to Cu nanoparticles.*	*31*
Fig. 2.7	:	*Schematic diagram representing the (a) interaction of bacterial cells with MgO and generation of ROS and (b) protein disruption with pH variation.*	*34*
Fig. 2.8	:	*Schematic illustrating the possible mechanism for antibacterial response of gold nanoparticle**s**.*	*35*
Fig. 2.9	:	*Schematic representation of mechanism for antibacterial activity due to production of free radicals in magnetic field.*	*39*
Fig. 2.10	:	*Schematic representation for the mechanisms of antibacterial response due to electric field.*	*42*

Fig. 2.11 : *The viability of osteoblast SaOS2 cells, culture on control/ (HA-40 wt. % $CaTiO_3$; H6C4)/ (HA-60 wt. % $CaTiO_3$; H4C6/(HA-80 wt. % $CaTiO_3$; H2C8) samples, after incubation of 3, 5, and 7 days. The symbol (*) represents the statistically significant difference between the samples, at $p < 0.05$. The symbol (**) represents the statistically significant difference between 3, 5, and 7 days of incubation for each composition, at $p < 0.05$.* 46

Fig. 2.12 : *The viability of L929 cells, cultured on control, HA and HA-40 wt. % $BaTiO_3$ composites. The symbol (*) shows the significant difference among the samples, incubated for 5 and 7 days with respect to that incubated for 3 days . The symbols (**) and (***) represent the statistically significant differences among the samples with respect to control disk and HA, respectively, at $P < 0.05$* 48

Fig. 2.13 : *Schematic diagram, representing the effect of surface polarization on adhesion of MC3T3-E1 cells (a) The inorganic ions, amino acids, and proteins are attached on HA surfaces, (b) normal cell adhesion on uncharged surfaces, positively charged ionic groups such as Ca^{2+} are attracted towards in negatively charged surface and promote the formation of the bone-like layer on N-polarized HA surface. The positively charged surface actively adsorbs the anions which is not favorable for apatite layer formation and consequently, the cell adhesion.* 50

Fig. 3.1 : *Flow chart for the synthesis of Hydroxyapatite (HA)* 78

Fig. 3.2 : *Flow chart representing the synthesis of borate bioglass 1393 B3 (BBG)* 81

Fig. 3.3 : *Digital pictographs of compacted samples using uniaxial hydraulic pressing* 82

Fig. 3.4 : *Schematic diagram represents the high voltage corona poling unit to develop the surface charge.* 84

Fig. 4.1 : *The X-ray diffraction patterns for (a) HA, (b) HA-3.0 wt. % ZnO, (c) HA-4.5 wt. % ZnO and (d) HA-7.5 wt. % ZnO composite samples.* 90

Fig. 4.2 : *Fourier transform infra-red (FTIR) spectra for (a) HA, (b) HA-3.0 wt. % ZnO, (c) HA-4.5 wt. % ZnO and (d) HA-7.5 wt. % ZnO composite samples.* 91

Fig. 4.3 : *Scanning electron micrographs of fractured (a) HA, (b) HA-3.0 wt. % ZnO, (c) HA-4.5 wt. % ZnO and (d) HA-7.5 wt. % ZnO composite surfaces.* *92*

Fig. 4.4 : *Variation of dielectric constant and loss with temperature for (a) HA, (b) HA-3.0 wt. % ZnO, (c) HA- 4.5 wt. % ZnO and (d) HA- 7.5 wt. % ZnO at few selected frequencies.* *94*

Fig. 4.5 : *Comparison of experimental values for HA-xZnO (x= 3.0,4.5 and 7.5 wt.%) with those of the values, obtained using theoretical models (Eqs. 4.5-4.7) at frequencies of (a) 10 kHz, (b) 100 kHz and (c) 1 MHz.* *97*

Fig. 4.6 : *Variation of ac conductivity with inverse of temperature for compositions (a) HA, (b) HA-3.0 wt. % ZnO, (c) HA-4.5 wt. % ZnO and (d) HA-7.5 wt. % ZnO.* *99*

Fig 4.7 *Complex plane impedance plots for compositions (a) HA (b) HA-3 wt. % ZnO, (c) and (d) represent the variation of resistances of grain and grain boundaries with the inverse of temperature.* *101*

Fig. 4.8 : *The viability of (a) E. coli and (b) S.aureus bacteria on unpolarized and polarized HA- xZnO composites. Asterisk (*) mark represents the statistically significant difference among all samples with respect to uncharged HA at $p < 0.05$. Asterisk marks (**) and (***) represent the statistically significant difference among all samples with respect to negatively charged and positively charged HA, respectively, at $p < 0.05$.* *103*

Fig. 4.9 : *The Kirby Bauer test performed for unpolarized (U), positively (P) / negatively polarized (N) HA-x ZnO (x = 3.0, 4.5, 7.5 wt. %) composites on agar culture plate, using E. coli bacteria, (a-d) represent the cultured agar plates after incubation of 12 h, (e-h) area under the samples and (i-l) represent the colony formation, after transferring swab from the area under the samples on agar plates.* *105*

Fig. 4.10 : *Kirby Bauer test performed for unpolarized (U) positively (P) / negatively polarized (N) HA-x ZnO (x = 3.0, 4.5, 7.5 wt. %) composites on agar culture plate, using S. aureus bacteria, (a-d) represents the cultured agar plates after incubation of 12 h, (e-h) area under the samples and (i-l) represent the colony formation, after transferring swab from the area under the samples on agar plates.* *106*

Fig. 4.11 : *The superoxide production in (a) E. coli and (b) S. aureus bacteria on unpolarized and polarized HA-x ZnO composites. Asterisk (*) mark represents the statistically significant difference among all samples with respect to uncharged HA, at p < 0.05. Asterisk marks (**) and (***) represent the statistically significant difference among all samples with respect to negatively charged and positively charged HA, respectively, at p < 0.05.* *107*

Fig. 4.12 : *Content of Zn2+ ions, released from HA-x ZnO (x = 3.0, 4.5, and 7.5 wt. %) composites in nutrient broth after incubation of 12 h. Asterisk mark (*) represents the significant difference among all samples with respect to HA -3.0 ZnO, at p < 0.05.* *109*

Fig. 4.13 : *Fluorescent microscopy images of live and dead E. coli bacteria, while cultured on uncharged, positively and negatively charged HA and HA-x ZnO (x= 3.0, 4.5 and 7.5 wt. %) composites. Live bacteria (green) are stained with SYTO 9 dye and dead bacteria (red) are stained with propidium iodide (scale bar corresponds to 100 µm).* *110*

Fig. 4.14 : *Fluorescent microscopy images of live and dead S.aureus bacteria, while cultured on uncharged, positively and negatively charged HA and HA-x ZnO (x = 3.0, 4.5, and 7.5 wt. %) composites. Live bacteria (green) are stained with SYTO 9 dye and dead bacteria (red) are stained with propidium iodide (scale bar corresponds to 100 µm).* *111*

Fig. 4.15 : *Schematic diagram, illustrating the proposed mechanism for antibacterial response of gram positive and gram negative bacteria on polarized HA-x ZnO (x = 3.0, 4.5, 7.5 wt. %) composites* *113*

Fig. 4.16 : *The percentage cell viability of SaOS2 cells on unpolarized and polarized HA- xZnO (x = 3, 4.5 and 7.5 wt. %) composites after (a) 3, (b) 5 and (c) 7 days of incubation, respectively. Asterisk (*) mark represents the significant difference among all the samples with respect to uncharged HA, at p < 0.05. Asterisk marks (**) represent the statistically significant difference between polarized surfaces with respect to unpolarized surfaces for the same sample, at p < 0.05 and Asterisk (***) mark represents the statistically significant difference between negatively charged surfaces with respect to positively charged surfaces for the same sample, at p < 0.05.* 115

Fig. 4.17 : *Fluorescent microscopy images of SaOS2 cells (stained nucleus), adhered on uncharged, positively and negatively charged HA and HA-x ZnO (x = 3.0, 4.5 and 7.5 wt. %) composites (scale bar corresponds to 100 µm).* 117

Fig. 4.18 : *Schematic diagram, illustrating the proliferation of SaOS2 cells on HA-x ZnO (x = 3, 4.5 and 7.5 wt. %) composite; (a) on negatively charged surface, cations interact with proteins and promote cell proliferation, (b) on positively charged surface, adhesion of anionic groups reduces cell proliferation, and (c) on unpolarized surfaces, cations and anions floats and normal cell adhesion take place.* 118

Fig. 5.1 : *The X-ray diffraction patterns for sintered (a) HA, (b) BT, (c) HA-30 vol. % BT, (d) CT, (e) HA-30 vol.% CT, (f) NKN and (g) HA-30 vol. % NKN composites.* 128

Fig. 5.2 : *Fourier transform infra-red (FTIR) spectra of sintered (a) HA, (b) BT, (c) HA-30 vol. % BT, (d) CT, (e) HA-30 vol.% CT, (f) NKN and (g) HA-30 vol. % NKN composites.* 129

Fig. 5.3 : *Scanning electron micrographs of fractured surfaces of (a) HA,(b) NKN, (c) HA-30 vol. % NKN (d) BT, (e) HA-30 vol. % BT, (f) CT and (g) HA-30 vol. % CT (scale bar corresponds to 1 µm).* 130

Fig. 5.4 : *Contact angle values of (a) deionized water and (b) culture media on unpolarized and polarized HA, NKN, BT, CT, and HA-30 BT, HA-30 NKN, HA-30 CT composites. All the samples show the statistically significant difference with unpolarized HA, at p < 0.05 (represented as *). The symbols (**) and (***) reflect the statistically significant difference among the samples while comparing with N-polarized and P-polarized HA, at p < 0.05, respectively.* 131

Fig. 5.5 : *The viability of (a) E. coli and (b) S. aureus bacteria, while cultured on HA, NKN, BT, CT, and HA-30 vol. % BT, HA-30 vol. % NKN, HA-30 vol. % CT composites. All the samples show the statistically significant difference with unpolarized HA, at p < 0.05 (represented as *). The symbols (**) and (***) reflect the statistically significant difference among the samples while comparing with N-polarized and P-polarized HA, at p < 0.05, respectively.* 133

Fig. 5.6 : *Digital camara images, representing the colonies of viable (a) E. coli and (b) S. aureus bacteria, respectively, cultured for 8 h on unpolarized and polarized HA, NKN, BT, CT, HA- vol. % 30 NKN, HA-30 vol. % BT and HA- 30 vol. % CT composites.* 135

Fig. 5.7 : *Log10 CFU/unit area for (a) E. coli and (b) S.aureus bacteria, while cultured on HA, NKN, BT, CT, HA-30 vol. % NKN, HA-30 vol. % BT and HA-30 vol. % CT composites. All the samples show the statistically significant difference with unpolarized HA, at p < 0.05 (represented as *). The symbols (**) and (***) reflect the statistically significant difference among the samples while comparing with N-polarized and P-polarized HA, at p < 0.05, respectively.* 136

Fig. 5.8 : *The antibacterial ratios for (a) E.coli and (b) S.aureus bacteria, cultured on unpolarized and polarized HA, NKN, BT, CT, HA-30 NKN, HA-30 BT and HA- 30 CT composites. The symbol (*) represents the statistically significant difference among all samples with respect to unpolarized HA, at p < 0.05. The symbols (**) and (***) represent the statistically significant difference among all the samples with respect to N-polarized and P-polarized HA, respectively, at p < 0.05.* 138

Fig. 5.9 : *Fluorescent microscopy images of live and dead E. coli bacteria, while cultured on HA, NKN, BT, CT, and HA-30 vol. % BT, HA-30 vol. % NKN, HA-30 vol. % CT composites (scale bar corresponds to 100 µm).* *140*

Fig. 5.10 : *Fluorescent microscopy images of live and dead S.aureus bacteria, while cultured on HA, NKN, BT, CT, and HA-30 vol. % BT, HA-30 vol. % NKN, HA-30 vol. % CT composites (scale bar corresponds to 100 µm).* *141*

Fig. 5.11 *The super oxide production on HA, NKN, BT, CT, and HA-30 vol. % BT, HA-30 vol. % NKN, HA-30 vol. % CT composites, while cultured with (a) E. coli and (b) S. aureus bacteria. All samples show the statistically significant difference with unpolarized HA, at $p < 0.05$ (represented as *). Symbols (**) and (***) reflect the statistically significant difference among samples, while comparing with N-polarized and P-polarized HA, at $p < 0.05$, respectively. .* *143*

Fig. 5.12 : *The catalase activity for (a) E. coli and (b) S. aureus bacteria, while cultured on HA, NKN, BT, CT, and HA-30 vol. % BT, HA-30 vol. % NKN, HA-30 vol. % CT composites. All samples show the statistically significant difference with unpolarized HA, at $p < 0.05$ (represented as *). The symbols (**) and (***) reflect the statistically significant difference among samples, while comparing with N-polarized and P-polarized HA, at $p < 0.05$, respectively.* *145*

Fig. 5.13 : *The concentration of protein in (a) E. coli and (b) S. aureus bacteria, while cultured on HA, NKN, BT, CT, and HA-30 vol. % BT, HA-30 vol. % NKN, HA-30 vol. % CT composites. All samples show the statistically significant difference with unpolarized HA, at $p < 0.05$ (represented as *). The symbols (**) and (***) reflect the statistically significant difference among samples, while comparing with N-polarized and P-polarized HA, at $p < 0.05$, respectively.* *147*

Fig. 5.14 : *The produced oxidative stress in terms of MDA for (a) E. coli and (b) S. aureus bacteria, while cultured on HA, NKN, BT, CT, and HA-30 vol. % BT, HA-30 vol. % NKN, HA-30 vol. % CT composites. All the samples show the statistically significant difference with unpolarized HA at $p < 0.05$ (represented as *). The symbols (**) and (***) reflect the statistically significant difference among samples, while comparing with N-polarized and P-polarized HA at $p < 0.05$, respectively.* *149*

Fig.5.15 : *The Viability of SaOS2 cells, cultured on HA, NKN, BT, CT, and HA- 30 vol. % BT, HA-30 vol. % NKN, HA-30 vol. % CT composites after (a) 3, (b) 5, and (c) 7 days of incubation. All the samples show the statistically significant difference with unpolarized HA, at $p < 0.05$ (represented as *). The symbols (**) and (***) reflect the statistically significant difference among samples while comparing with N-polarized and P-polarized HA at $p < 0.05$, respectively.* *151*

Fig.5.16 : *The Viability of MG-63 cells, cultured on HA, NKN, BT, CT, and HA-30 vol. % BT, HA-30 vol. % NKN, HA-30 vol. % CT composites after (a) 3, (b) 5, and (c) 7 days of incubation. All the samples show the statistically significant difference with unpolarized HA, at $p < 0.05$ (represented as *). The symbols (**) and (***) reflect the statistically significant difference among samples while comparing with N-polarized and P-polarized HA, at $p < 0.05$, respectively.* *152*

Fig.5.17 : *The morphology of MG-63 cells, adhered on unpolarized and polarized HA, NKN, HA- 30 vol. % NKN, BT, HA-30 vol. % BT, CT, and HA-30 vol. % CT, after 3 days of culture (scale bar =100 µm).* *154*

Fig. 5.18 : *Schematic illustrating the (a) interaction of bacteria with polarized surface, (b) mechanisms for antibacterial response of polarized surfaces, and (c) production of Mitogen-activated protein kinases (MAPK) through H_2O_2 which promote the proliferation of mammalian cells.* *155*

Fig. 6.1 : *The XRD patterns for sintered (a) BBG, (b) BBG-30 vol. % NKN and (c) BBG-30 vol. % BT composites.* *163*

Fig. 6.2 : *The antibacterial response (MTT assay) of (a) E. coli and (b) S.aureus bacteria, cultured on unpolarized and polarized BBG-x NKN/BT (x = 30 Vol. %). The symbols (*) and (**) represent the statistically significant difference among all the samples with respect to control sample (glass cover slip) and unpolarized BBG respectively, at $p < 0.05$. The symbols (#) and (##) represent the statistically significant difference among all the samples with respect to N- polarized and P-polarized BBG, respectively, at $p < 0.05$.* *165*

Fig. 6.3 : *Live/dead ratio for (a) E. coli and (b) S.aureus bacteria, while cultured on unpolarized and polarized BBG-x NKN/BT (x = 30 vol. %) composites. The symbol (*) represents the statistically significant difference among all the samples with respect to unpolarized BBG, at p < 0.05. The symbols (**) and (***) represent the statistically significant difference among all the samples with respect to N- polarized and P-polarized BBG, respectively, at p < 0.05.* *167*

Fig. 6.4 : *Super oxide generation on unpolarized and polarized surfaces of BBG-x BT/ NKN (x = 30 vol. %) composites, while cultured with (a) E. coli and (b) S. aureus bacteria. The symbol (*) represents the statistically significant difference among all the samples with respect to unpolarized BBG at p < 0.05. The symbols (**) and (***) represent the statistically significant difference among all the samples with respect to N- polarized and P-polarized BBG, respectively, at p < 0.05.* *169*

Fig. 6.5 : *The scanning electron microscopic images, demonstrating the adhesion of E. coli bacteria on unpolarized and polarized BBG-x BT/ NKN (x = 30 vol. %) composite sample surfaces (scale bar corresponds to 2 µm).* *171*

Fig. 6.6 : *The scanning electron microscopic images, demonstrating the adhesion of S. aureus bacteria on unpolarized and polarized BBG-x BT/ NKN (x = 30 vol. %) composite sample surfaces (scale bar corresponds to 2 µm).* *172*

Fig. 6.7 : *The viability of osteoblast like MG-63 cells, cultured on BBG, and BBG – x BT/NKN (x = 30 vol. %) composites after incubation of (a) 3 (b) 5 and (c) 7 days. The symbols (*) and (**) represent the statistically significant difference among all the samples with respect to control sample (glass cover slip) and unpolarized BBG, respectively, at p < 0.05. The symbols (#) and (##) represent the statistically significant difference among all the samples with respect to P-polarized and N-polarized BBG, respectively, at p < 0.05.* *175*

Fig. 6.8 : *Fluorescence images of MG-63 cells, adhered on unpolarized and polarized surfaces of BBG-x BT/NKN (x = 30 vol. %) composite samples, after incubation of 3 days (scale bars corresponds to 100 µm* 177

List of Tables

Table 2.1	*Effect of addition of secondary phase on antibacterial response of HA.*	*23*
Table 2.2	*Antibacterial response of nanoparticles against various pathogens.*	*36*
Table 2.3	*Effect of incorporation of secondary phase as well as external stimulant on antibacterial response of bioceramic substrates*	*43*
Table 3.1	*Composition and nomenclature used for HA – ZnO composites*	*79*
Table 3.2	*Sintering parameters, optimized for the developed compositions*	*83*