7.1 Introduction

From the study done in previous chapters it was observed that the magnesium alloy based composite added with varying amount of 1393 BAG can be used as a potential biodegradable implant. The composite having composition Mg3Al2Zn0.6Ca added with 10 % 1393 BAG was observed to have optimized mechanical and corrosion strength. Any material when developed as an implant can be worthy, if and only if it is biologically non-toxic and should not have any harmful effect on the surrounding tissues. Present chapter studies the effect of addition of 1393 BAG on the biological properties of the base composition Mg3Al2Zn0.6Ca. In the present chapter, the testing was done for different concentrations of 1393 BAG and their effects on cell growth and cytotoxicity were observed by performing tests for biocompatibility and hemo-compatibility. The biological tests were performed for the composites with increased 1393 BAG content, and its effect were observed i.e. Mg3Al2Zn0.6Ca, Mg3Al2Zn0.6Ca10BAG, Mg3Al2Zn0.6Ca15BAG and Mg3Al2Zn0.6Ca20BAG. For testing, the composite developed for its biological properties we have done cell lines and cell culture on K562 cells and DL cells , cell viability assay was done on a colorimetric XTT (sodium 3-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis(4-methoxy-6-nitro) assay (Roche, Indianapolis, IN). The cell growth inhibition assay against tumor cell was studied by MTT assay. Tumor target cells (5×10^3 cells /well) in a 96 well culture dish were treated with serial concentrations of the compounds. Following incubation at 37^0C, 5% CO_2, for 48 hours, the proliferation of tumor cells was assessed by MTT assay using CellTiter 96 kit (Promega, USA).

Cytotoxicity assay was done using the CytoTox 96 Cytotoxicity assay kit from Promega, USA. Tumor target cells (5×10^3) were co-cultured with varying concentrations of the indicated formulations in a 96 well culture dish. Hemolysis assay was done to estimate the concentration dependent kinetics, for this the blood sample was incubated with varying concentrations (10-250 μM) of 0% 1393BAG, 10% 1393BAG, 15% 1393BAG and 20% 1393 BAG for 4 hours. The detailed procedure adopted for performing the biological testing is discussed in chapter 4, section 4.4.4.

7.2 Results & Discussion

7.2.1 Cell viability, growth inhibition & cytotoxicity assay

Effects of the bio-active glass compounds were assessed on the proliferative potential of the dividing cells. Anti-proliferative effects of 0% 1393BAG, 10% 1393BAG, 15% 1393BAG and 20% 1393 BAG against K562 and DL cells were found to be minimum at different concentrations tested. Figure 7.1 shows the highest concentration of the compounds (100 μg/ml) showed tolerance to the proliferation of K562 cells whereas bio-glasses with supplement are more tolerant compared with 0% 1393 BAG. Short-term viability studies showed loss of viability ~30% following treatment with 0% 1393 BAG while treatment with others showed better tolerance with minimum loss in viability as demonstrated by 18 hours XTT assay (Figure 7.1 B). The compounds also showed low levels of cytotoxicity as demonstrated in Figure 7.1 C. Similar results were observed in DL cells (Figure 7.2 A-C).

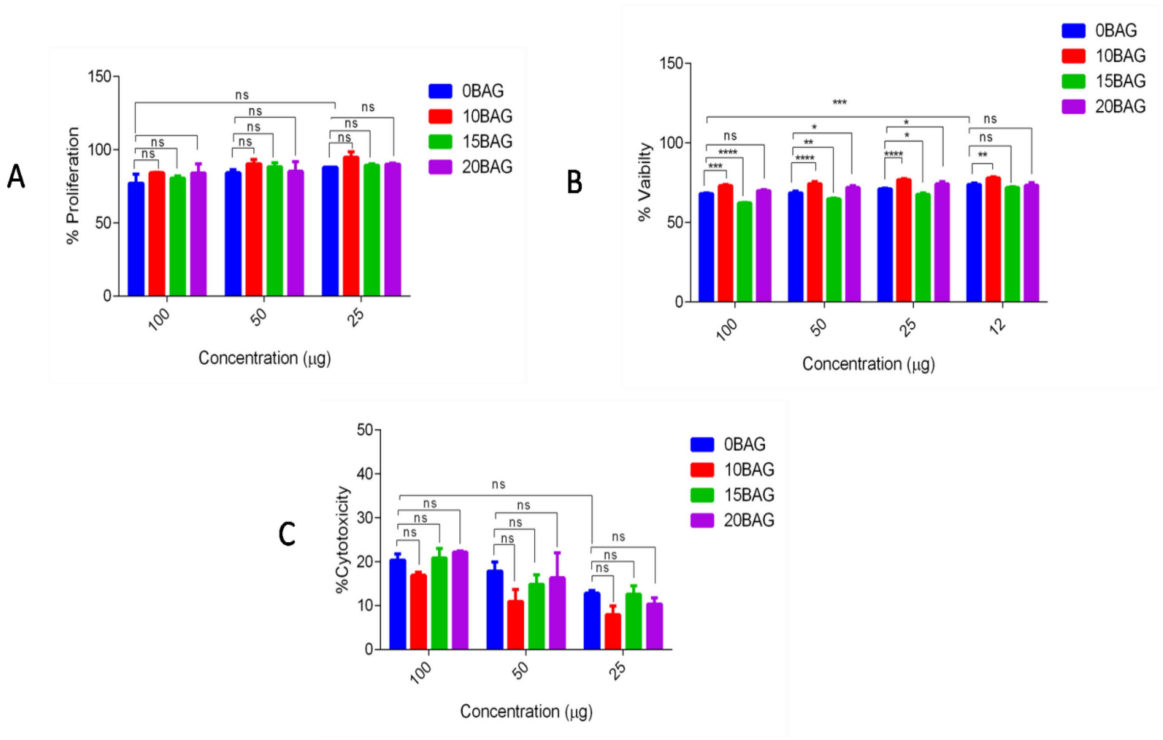

FIGURE: 7.1. Effects of 0% 1393 BAG, 10% 1393BAG, 15% 1393BAG and 20% 1393BAG against K562 cells with respect to proliferative potential (A), viability (B) and cytotoxicity (C) as judged by MTT, XTT and LDH release assay respectively

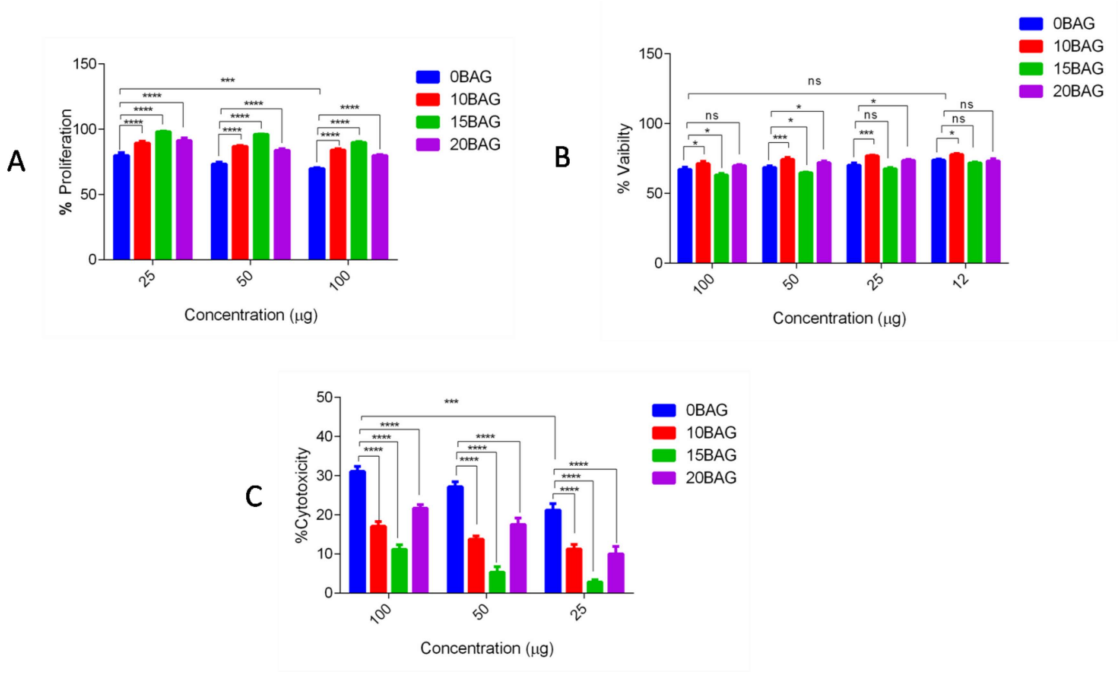

FIGURE 7.2: Effects of 0% 1393BAG, 10% 1393BAG, 15% 1393BAG and 20% 1393BAG against DL cells with respect to proliferative potential (A), viability (B) and cytotoxicity (C) as judged by MTT assay

Blood lymphocyte and monocytes were relatively unaffected following treatment with serial concentration of the constructs. Viability of the lymphocytes remain unaffected except highest concentration of unprotected construct (0BAG) which caused loss in viability ($p<0.01$). Other construct appears to be tolerant to lymphocytes (**Figure 7.3 A**). In contrast, viability of monocytes was significantly compromised following treatment with highest.

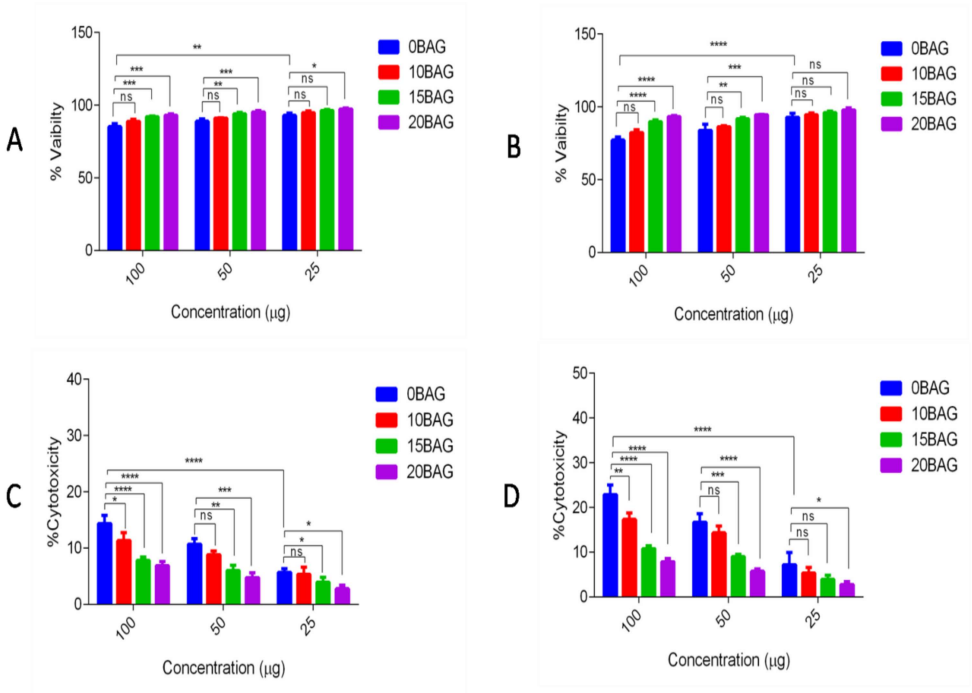

FIGURE 7.3: Analysis of biocompatibity in lymphocyte and monocytes in the presence of 0% 1393BAG, 10% 1393BAG, 15% 1393BAG and 20% 1393BAG with respect to viability (A & B) and cytotoxicity (C & D) as judged by XTT and LDH release assay

Concentration used in the study ($p < 0.01$ &<0.001) (Figure 7.3 B). Lower concentration however, showed tolerant for monocytes (Figure 7.3 B). Lymphocytes showed highest level of cytotoxicity (~15%) in the presence of 0% 1393BAG (100 µg/ml) while bio-protective constructs were tolerant to lymphocyte with <10% cell death at the concentrations tested (Figure 7.3 C). In contrast, monocytes recorded higher percent cytotoxicity compared with lymphocyte following similar treatment ($p<0.01$, <0.001) suggesting cell type specific response towards the bio-active glass materials (Figure 7.3 D).

7.2.2 Hemolysis assay

We also analyzed the RBC hemolysis in the presence of the constructs. Our data suggests that 0BAG induces low levels of hemolysis following concentration dependent treatment compared with the other constructs (Figure 7.4A). Bright field pictorial analysis also supports the observation (Figure 7.4 B-E). From the above experimental observations, we conclude that as synthesized bio-active glass compounds are safe and tolerant to the cells and only induced low levels of loss in viability and cytotoxicity when treated with 0% 1393BAG. The bio-glasses with supplemental protection (10% 1393BAG, 15% 1393BAG and 20% 1393BAG) are much safer for the dividing cells and also for the blood lymphocytes and monocytes. Besides that the bio-glass materials are safe for RBC with minimum lysis of the cells in the presence of 0% 1393BAG.

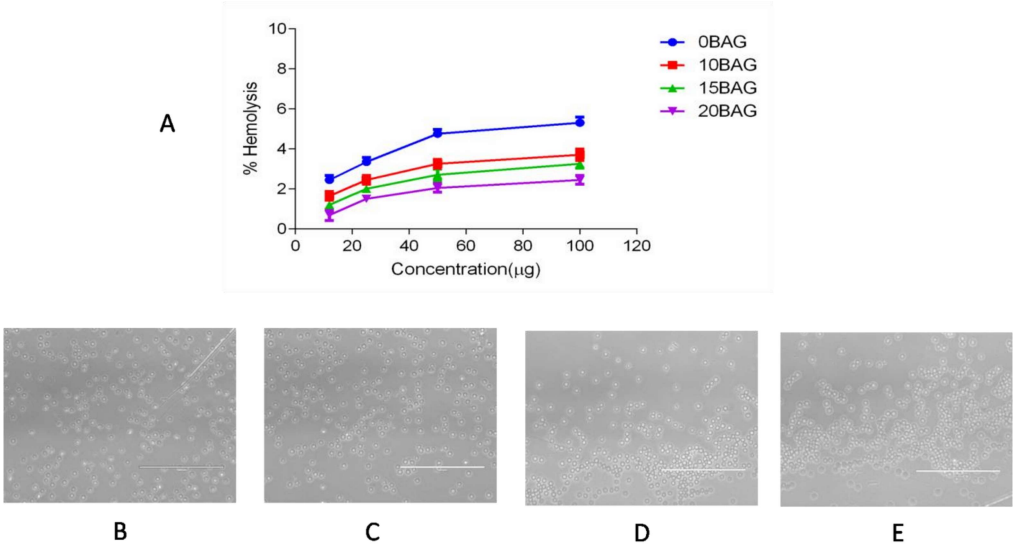

FIGURE 7.4: (A) Concentration dependent haemolysis of RBC in the presence of 0BAG, 10BAG, 15BAG and 20BAG. Bright field images of RBC treated with (B) 0BAG, (C) 10BAG, (D) 15BAG, and (E) 20BAG

It is evident from the Cell viability, growth inhibition, cytotoxicity assay and Hemolysis assay that the 1393 BAG has been observed to form strong bond with the tissues and also the discussion in chapter 5 section 4 has observed that the formation of HA layer was observed to increase with increase in the immersion time in the SBF, it is mainly due to the dissolution of 1393BAG in the simulated body fluid. In fact Hench et al. also investigated the in vitro bonding mechanism with synthetic material because of the chemical reactions taking place over BAG glass surface (L.C. Gerhardt and A. R. Boccaccini, 2010). These chemical reactions powerfully help the implants to bond with the bone tissues (H .Oonishi, et al. 2000).

7.3 Comparative analysis of mechanical properties of bone tissues and materials for orthopedic implants

TABLE 7.1: Mechanical properties of bone tissues and materials for orthopedic implants (M.Geetha, et al. 2009; Q. Chen and G.A. Thouas, 2015; M. Niinomi, et al. 2012))

S.N	Tissue/materials	Density (g/cm^3)	Young's modulus (GPa)	Yield strength (MPa)	Compression strength (MPa)	Tensile strength (MPa)	Flexural strength (MPa)
1.	Cortical bone	1.8–2.0	7–30	NA	100–230	164–240	NA
2.	Cancellous bone	1.0–1.4	0.01–3.0	NA	2–12	NA	NA
3.	Ti6Al4V (casted)	4.43	114	760–880	NA	895–930	NA
4.	Ti6Al4V(wrought)	4.43	114	827–1103	896–1172	860–965	NA
5.	Stainless steel 316 L	8.0	193	170–310	480–620	540–1000	NA
6.	CoCrMo alloy	8.3	240	500–1500	NA	900–1540	NA
8.	Tatalum (wrought)	16.7	186–191	345	NA	480	NA
9.	HA	3.05–3.15	70–120	NA	40–200	100–900	NA
10.	Pure Mg(99.9%, casted)	1.74	41	21	40	87	NA
11.	Pure Mg(99.9%, wrought)	1.74	41	100	100-140	180	NA
12.	**Mg3Al2Zn0.6Ca10 BAG**	**1.72-1.91**	**44.42-48.96**	**NA**	**147.94 –151.14**	**NA**	**126.75–131.03**

7.4 Summary of the chapter

The present chapter is focused on the study of biological properties of the newly developed composite by addition of 1393 BAG in different proportions. Effect of bio-active glass compounds were tested on states of dividing cells and evaluated the biocompatibility and hemo-compatibility properties in mononuclear cells and red blood cells following treatment with the compounds. The concluding remarks of work done are as follows:

1. It was found that the 1393 bioactive glass with increasing content has significant role in providing biocompatibility and hemo-compatibility for the materials and could be used for the implant material. The addition of 1393 BAG appears to have protective functions from potential toxic effects of 0 1393 BAG material. Proliferation of K562 (human immortalized myelogenous leukemia) and DL (Dalton's lymphoma) cells was not significantly affected by the increasing amount of 1393 BAG in a concentration dependent manner as judged by 48 hours MTT assay. Similar to dividing tumor cells, the compounds were tolerant to blood lymphocytes and monocytes with minimum or no effect on viability and cytotoxicity.

2. It can be concluded that when Mg3Al2Zn0.6Ca added with 1393 BAG, the compatibility enhances and hence if mechanical and corrosion characteristics are optimized then the composite with the increasing 1393 BAG content would be an excellent implant material with enhanced biological characteristics.

3. This result opens the scope of application of the composite Mg3Al2Zn0.6Ca10BAG developed, as an implant material. From this, it can be concluded Mg3Al2Zn0.6Ca10BAG can be used as a potential implant, subjected to further working on its in vivo testing.