

PLGA BASED DESIGNING OF SCAFFOLD AND IT'S EFFICIENCY ON CANCER CELL

Submitted in partial fulfillment of the requirements for the award of
Bachelor of Technology Degree
in
Biomedical Engineering

by

ANUPRIYA SONI (37240008)



**DEPARTMENT OF BIOMEDICAL ENGINEERING
SCHOOL OF BIO AND CHEMICAL ENGINEERING**

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

Accredited with Grade "A" by NAAC

JEPPIAAR NAGAR, RAJIV GANDHI SALAI, CHENNAI - 600 119

MARCH 2021



SATHYABAMA
INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)

Accredited with Grade "A" by NAAC

JEPPIAAR NAGAR, RAJIV GANDHI SALAI, CHENNAI- 600 119

www.sathyabama.ac.in

DEPARTMENT OF BIOMEDICAL ENGINEERING

BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **ANUPRIYA SONI**. who carried out the project entitled "**PLGA BASED DESIGNING OF SCAFFOLD AND IT'S EFFICIENCY ON CANCER CELL**" under our supervision from **JANUARY2021 to March 2021**.

Dr.ANIMA NANDA, Ph.D.,FNRS.,FIAS,FAB

Guide and Supervisor

Dr. T. SUDHAKAR, M.Sc., Ph.D.,

Head of the Department

Submitted for Viva voce Examination held on_____

Internal Examiner

External Examiner

DECLARATION

I **ANUPRIYA SONI (37240008)** hereby declare that the Project Report entitled **“PLGA BASED DESIGNING OF SCAFFOLD ANT IT’S EFFICIENCY ON CANCER CELL”** done by us under the guidance of **Dr. ANIMA NANDA, Director, IQAC, Department of Biomedical Engineering** is submitted in partial fulfilment of the requirements for the award of **Bachelor of Technology** Degree in **Biomedical Engineering**.

DATE:

PLACE:

SIGNATURE OF THE CANDIDATE

ACKNOWLEDGEMENT

I am pleased to acknowledge my sincere thanks to **Board of Management of SATHYABAMA** for their kind encouragement in doing this project and for completing it successfully. I am grateful to them.

I convey my thanks to **Dr. Anima Nanda, PhD., FNRS., FIAS., FAB.,** Director, IQAC, **Department of Biomedical Engineering** and **Dr. V.KARTHICK(Scientist B), Mr. Vineeth Kumar(Junior Research Fellowship)** for her valuable guidance, suggestions and constant encouragement paved way for the successful completion of my project work.

I would convey my sincere deep thanks to **Dr. T. Sudhakar, M.Sc., PhD,** Head of the department. I wish to express my thanks to all Teachers, **Dr. J. Premkumar, Ms. Bethanny Janney, Ms. Sindu Divakaran, Dr. Krishna Kumar and Mr. G. Umashankar** of **Department of the Biomedical Engineering** and the **and Non-teaching** staff members for providing me necessary support and details at the right time during a progressive reviews in the entire duration of my project tenure.

Last but not least , I am express my heartfelt gratitude to my beloved parents , family members and friends, who have played a instrumental role in finishing my project to receive the degree.

(Anupriya Soni)

ABSTRACT

Recently biodegradable polymers have been used to fabricate porous scaffolds for three-dimensional (3-D) cell or tissue culture to regenerate tissue-based artificial organs. Poly(DL-lactic-co-glycolic acid) (PLGA) is one of the most widely used biodegradable polymers since it is biocompatible and its degradation rate can be easily controlled by varying the copolymer ratio of lactic to glycolic acid. The ideal scaffold must meet several requirements to promote cellular interactions and tissue development, and have adequate mechanical and physical properties. Scaffolds of various materials have been tested for bone tissue engineering, including metals, ceramics, polymers (natural and synthetic), and their combinations. Ceramics such as hydroxyapatite (HAP) and tricalcium phosphate (TCP) are biomaterials with good osteoconductive and bone-bonding ability, but have limited application because of brittleness and a slow degradation rate. Using natural materials to coat the scaffolds used for tissue-engineered bone-repair techniques is expected to increase osteoblast adhesion to the scaffold and to express normal physiological function. To test this hypothesis, we modified poly(DL-lactic-co-glycolic acid) (PLGA) substrate by coating it with natural biomaterial and then evaluate their effects on osteoblast /cancer cells attachment, proliferation, and differentiation.

The adherence of cancerous cells to skeletal tissues which mainly consist of hydroxy apatite (HAp), it shows that correlation between tumors cells and hydroxy apatite. Therefore, it is well accepted that an efficient experimental model (in-vitro) which imitate the microenvironment in-vivo is of great importance for oncogenic research. Herein, for project we incorporate the hydroxy apatite nanoparticles into poly(lactic-co-glycolic acid) PLGA nanofibers to design biomimetic nanofibrous scaffold as an in-vitro cancer model. The surface morphologies, structure, thermal and mechanical properties of the incorporated PLGA/ HAp scaffold were studied or characterized.

TABLE OF CONTENTS

CHAPTER NO.	TITLE	PAGE NO.
	ABSTRACT	I
	LIST OF ABBREVIATIONS	iv
	LIST OF FIGURES	v
1.	INTRODUCTION	1
1.1	INTRODUCTION OF POLYMERS	1
1.2	PLGA (POLY LACTIC GLYCOLIC ACID)	3
1.3	PVA (POLY VINYL ACID)	4
1.4	CHLORAMBUCIL	5
2.	LITERATURE SURVEY	7
3.	MATERIALS AND METHODS	8
3.1	SYNTHESIS OF THE MICROSPHERE (BARE)	8
3.1.1	Field Emission Scanning Electron Microscopy (FESEM)	9
3.1.2	Nuclear magnetic resonance (NMR):	
3.2	IN-VITRO ANTI CANCER STUDIES	
3.2.1	Cytotoxicity assay using THP-1	
4.	RESULTS AND DISCUSSION	11
4.1	FESEM	12
4.2	NMR	
4.3	CYTOTOXICITY ASSAY USING IN THP-1(MTT)	13
4.4	CYTOTOXICITY ASSAY USING MCF-7(MTT)	15
5.	SUMMARY AND CONCLUSION	15
6.	REFERENCES	19

LIST OF ABBREVIATIONS

PLGA	POLY LACTIC CO-GLYCOLIC ACID
HAP	HYDROXY APATITE
SEM	SCANNING ELECTRON MICROSCOPE
FTIR	FOURIER TRANSFORM INFRARED SPECTROMETRY
MTT	3-(4,5-DIMETHYLTHIAZOL-2-Y)-2,5-DIPHENYL
PVA	POLY VINYL ALCOHOL
MCF-7	MICHIGAN CANCER FOUNDATION -7
PGA	POLY GLYCOLIC ACID

LIST OF FIGURES

FIGURE. NO	FIGURE NAME
4.1	FE-SEM
4.2	NMR
4.3	CYTOTOXICITY ASSAY USING IN THP-1(MTT)
4.4	CYTOTOXICITY ASSAY USING MCF-7(MTT)

CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION TO POLYMERS

In the past three decades, biodegradable polymers play a critical role in pharmaceutical and biomedical fields (Huang et al., 2010). Despite many major developments in biodegradable materials, some drug delivery vehicles were found to induce acute inflammation (Kawazoe et al., 2009). Controlled release technology is an effective methodology and approach to optimize the dosage of the drug released to a particular site for particular duration of time (Liu et al., 2011). Tissue engineering has been widely studied as an attractive approach in treatment of malfunction or lost tissues or organs. Injectable scaffold especially hydrogels (Taguchi et al., 2005), collagen (Nam et al., 1999), (Spencer, et al., 2008), gelatin (A.G.Mikos et al., 1993), chitosan (T.W.King., et al 2000), poly(ethylene glycol) (PEG) (King et al, 2000) are generally used to improve the structural continuity as well as stability.

1.2 PLGA (POLY LACTIC GLYCOLIC ACID)

Poly (lactic-co-glycolic acid (PLGA) is a polymer used for the preparation of scaffold which could be used for developing the bone structure as it is a biodegradable material polymer (Go et al., 2012). Many biomaterials used in tissue engineering caused immune reaction in *in vivo* which reduces the effectivity for generation of tissue. But PLGA was found to be effective as it physically interacted with melanocyte stimulating hormone reducing the immune response to greater extent. The effect of the delivery of Rifamycin by PLGA nanoparticle was also studied on maturation of dendritic

cells (A.Haddadi et al., 2008) and successful results have been obtained.

Transferrin conjugate magnesium silica PLGA nanoparticles facilitate the transport of drug through blood brain barrier for effective treatment of brain glioma (Cui et al., 2013). Similar study was done to develop a poly(ether)urethane–Polydimethylsiloxane/fibrin-based scaffold containing poly(lactic-co-glycolic acid) (PLGA) nanoparticles loaded with vascular endothelial growth factor (VEGF) to evaluate its wound healing properties in diabetic mice (db/db) (P.Losi et al., 2013). It is also found out that the novel fabricated porous PLGA/chitosan PEC microspheres could be used as injectable cell carriers for cartilage (J.Fang et al., 2014).

Wound healing is a complex process that encompasses three phases: inflammation, proliferation and tissue remodeling. These events are driven by growth factors and cytokines, which are secreted at the wound site by inflammatory cells and other stromal cells in response to tissue injury, development of controlled-release systems that protect the recombinant growth factors from enzymatic degradation and provide sustained delivery at the wound site during healing to enhance tissue regeneration (J.Fang et al., 2014). PLGA is a biocompatible, biodegradable polymer with a history of over forty years use in medical resorbable sutures (Zolnik et al., 2007). Besides these examples, encapsulation of curcumin in PLGA nanoparticles could also increase bioavailability (U.Milano-bicocca et al., 2013). Degradation of PLGA can be employed for sustained drug release at desirable doses by implantation without surgical procedures. PLGA was selected since poly esters including poly lactic acid and their copolymers are the most widely used and studied class of biodegradable polymers for the pharmaceutical use due to their biocompatibility and biodegradability (L.Brannon et al., 1995). Study has been done to investigate the encapsulation of the growth factor, BMP-2 (bone morphogenetic protein) within micro particles. Morphology, size distribution, encapsulation efficiency and release kinetics were investigated and

have demonstrated a sustained release of bioactive BMP-2 for tissue regeneration at site of damage. Several techniques have been developed for loading of drug into PLGA including water–oil-water(w/o/w) (Romero et al., 2013), phase separation (Kumar et al., 2012), Nano precipitation (Govender et al., 1999) and spray drying (Nikitczuk et al., 2013). The PLGA are injectable scaffolds and are biodegradable in nature (Hong et al., 2005). They can simultaneously act as cell micro carrier (Prior et al., 2000), (Ki et al., 2004), delivery vehicles for drug (Xue et al., 2006), growth factors (King et al., 2000) and 3D scaffold piling by spheres piling (D.H.R Kempene et al., 2006). Fast cell proliferation can be achieved since these microspheres have large surface area (Y.Hong et al., 2005). The micro carriers having cells on their surfaces can be directly injected into the defect, piling into a 3D scaffold *in vivo* to induce cell infiltration and tissue regeneration. The transplanted cells proliferate and differentiate *in-situ* inside the micro carrier in a normal histological condition and these micro carriers are not easy to handle. For these context compounding the microspheres with a binding agent as fibrin gel may overcome these shortcoming (Zhao et al., 2009). PLGA has good biocompatibility and biodegradability and is approved by the US Food And Drug Administration (Y.Qui et al., 2013). The specific physical and chemical characteristic of PLGA can be controlled by varying its composition and molecular weight (T.G.Park et al., 1995). Rat mesenchymal stem cell were cultured on the scaffold, live/dead assay, water soluble tetrazolium-8 assay and alkaline phosphatase (ALP) activity was used to evaluate cell viability, proliferation and differentiation on the scaffold constructs. The scaffolds were implanted in the defects of distal end of femora's of New Zealand white rabbits to observe the bone formation in PLGA/CPC scaffold. Study has been also done where PLGA scaffold embedded spatially with hydroxyapatite particles on pore walls were designed and fabricated by a porogen leaching method using hydroxyapatite coated paraffin spheres as the porogen for

suitable drug permeability and drug delivery. The manufacturing of 3D scaffolds cultivated with osteoblast for reconstructive and bone surgery will develop the field of bone engineering (M.Wanger et al., 2007).

1.3 PVA (POLY VINYL ALCOHOL)

Polyvinyl alcohols (PVOH) are a group of products that have a similar empirical formula but different properties and applications depending upon their molecular weight and the degree of removal of the acetate groups from the polyvinyl acetate raw material. Polyvinyl alcohol attracted particular attention due to its plenty advantageous properties such as water solubility, hydrophobicity, chemical and thermal stability, and biocompatibility (W. Liu et al., 2012).

1.4 CHLORAMBUCIL

Chlorambucil 4-[p-[bis [2-chloroethyl]amino] phenyl]-butanoic acid (CHB) is a nitrogen mustard alkylating agent used as antineoplastic for chronic lymphocytic leukaemia, Hodgkin's disease, and others. Although it is less toxic than most other nitrogen mustards, it has been listed as a known carcinogen in the Fourth Annual Report on Carcinogens (NTP 85-002, 1985). (Merck Index, 11th ed). An alkylating drug is nothing but which the substance replaces the hydrogen group with alkyl group. It is used in the treatment of Chronic lymphocytic leukemia (CLL), Hodgkin's disease, non-Hodgkin's lymphoma, breast cancer, ovarian cancer, testicular cancer, Waldenstrom's Macroglobulinemia, thrombocythemia, choriocarcinoma [3]. CHB is usually taken orally. The direct forms of CHB causes many side effects which includes several allergic reaction. Person taking more than 30% of chlorambucil suffers from low blood counts, the RBC, WBC and platelets count may decrease temporarily,

resulting in risk for infection, anaemia and bleeding. Bone marrow suppression is the most common side effect on using chlorambucil.

To overcome the harmful side effects and to increase the bioavailability of the drug, it is encapsulated in PLGA matrices from which the release of drug is at slow rate at prolonged time period, thereby increasing the effectiveness. Successful encapsulation with PLGA offers compliance and reducing discomfort, protection of the therapeutic compound within the body, and avoidance of peak-related side effects by maintaining more-constant blood levels of the drug. Here we investigate the possible use of PLGA and CHB composite against the Cancer cells and testing the optimum concentration of PLGA+CHB for the applications.

CHAPTER 2

LITERATURE SURVEY

2. LITERATURE SURVEY

The most commonly used biodegradable synthetic polymers for three-dimensional (3D) scaffolds in tissue engineering are saturated poly(α -hydroxy esters), including poly(lactic acid) (PLA) and poly(glycolic acid) (PGA), as well as poly(lactic-co-glycolide) (PLGA) copolymers (Mano et al.2004) (Lin et al.2002) Once degraded, the monomeric components of each polymer are removed by natural pathways. PGA is converted to metabolites or eliminated by other mechanisms, and PLA can be cleared through the tricarboxylic acid cycle. Due to these properties PLA and PGA have been used in biomedical products and devices, such as degradable sutures which have been approved by the US Food and Drug Administration as reported (Jagur et al.1999) clinical application of pure PLGA for bone regeneration is hampered by poor osteoconductive and exhibits suboptimal mechanical properties for use as load-bearing applications. Therefore, PLGA is often used in combination with other materials, such as ceramics, bioactive glass, or it is opportunely modified in order to render PLGA more biomimetic and able to enhance bone regeneration (Pan et al.2012). Ajioka *et al.* reported a simple one-step method, a direct polycondensation from LA and GA, carried out in the azeotropic solvent diphenyl ether, characterized by a high boiling point. However, this solvent introduces higher levels of complexity of both process control and purification of the end product, and thus the resultant polymers are very expensive to produce. As known from literature, the PLGA sequence influences dramatically the degradation rate, because random PLGA degrades quicker than analog-sequenced PLGAs, prepared by ring-opening polymerization. Recently, a new method to obtain repeating-sequence PLGA

copolymers with different toxicity has been proposed by Li *et al.* These results may be considered promising for the use of sequenced PLGA in biomedical applications, *i.e.*, in drug delivery applications, where the drug/biomolecule kinetic release is dramatically influenced by the polymeric degradation rate. The combination of enhanced mechanical properties, biocompatibility, and fibrous formability of the scaffolds is believed to have great potential for bone tissue regeneration. Different fibre-forming techniques were proposed in literature to fabricate micro- and nano-fibrous composite scaffolds. Amorphous PLGA copolymers are suitable for biomedical applications, as provides a more homogeneous dispersion of the active species in the polymer matrix (Habraken *et al.* 2006). PLGA is widely used in bone tissue engineering due to its suitable physico-chemical properties and biodegradability rate; however, compared to natural polymers such as collagen and chitosan which have numerous ionic molecular groups, the synthetic polymers have relatively few of these groups, making it very difficult to induce mineralization (Kawasaki *et al.* 1998). Wan *et al.* demonstrated that appropriate oxygen plasma treatment could not only incorporate functional groups on PLGA surface, increasing its negative charge, but also could change surface topography due to etching effects. Qu *et al.* treated PLGA films and scaffolds with oxygen plasma and then incubated them in a modified SBF solution to obtain a bone-like apatite layer. The formation of bone-like apatite was enhanced by oxygen plasma treatment, allowing an increased number of negative charge and surface roughness. The formed apatite was similar in composition and structure to the natural bone mineral and enhanced the adhesion and proliferation of OCT-1 osteoblast-like cell when compared with neat PLGA.

CHAPTER 3

MATERIALS AND METHODS

3.1 SYNTHESIS OF THE MICROSPHERE (BARE)

PLGA (SIGMA) Microspheres was prepared by double emulsification method. Briefly, 500 mg PLGA in the ratio 50:50 was added to 8 mL of dichloromethane (CH_2Cl_2) and 1 mL of heparin. The mixed solution was emulsified by vortexing for 40 sec. During mixing heparin was not mixed properly hence proper care was taken to ensure mixing. The primary solution was added drop wise using a 2G syringe into 100 mL of 1 % of PVA aqueous solution, which was mixed for 2 minutes at 9500 rpm with a turbine homogenizer resulting in a secondary emulsion. To this emulsion was further added 100 mL of 2 % isopropanol aqueous solution which was magnetically stirred at for 4 hours at 300 rpm. The beads were magnetically stirred for 4 hours and centrifuged 3 times at 2000 rpm. After complete removal of solvent by decanting the supernatant the beads were freeze dried for 3 hours and stored for further use.

Similarly, 500 mg PLGA in the ratio 50:50 was added to 8 mL of dichloromethane (CH_2Cl_2) and 1 mL of heparin along with 10 mg chlorambucil. The mixed solution was emulsified by vortexing for 40 sec. Then the solution is added drop by drop to PVA Aqueous solution. The solution was mixed for two minutes at 9500 rpm. The emulsion was added to 2 % isopropanol aqueous solution which was magnetically stirred at for 4 hours at 300 rpm. The beads were magnetically stirred for 4 hours and centrifuged 3 times at 2000 rpm. After complete removal of solvent by decanting the supernatant the beads were freeze dried for 3 hours and stored for further use.

3.1.1 Field Emission Scanning Electron Microscopy (FESEM)

The lyophilized samples of bare and loaded microsphere were used for SEM analysis. For analysis the bare and loaded microsphere were placed on carbon tube mounted onto a carbon tape for SEM imaging. The SEM was taken using Field Emission Scanning Electron Microscope (FESEM-SUPRA 55) - CARL ZEISS, GERMANY.

3.1.2 Nuclear magnetic resonance (NMR):

The lyophilized powder was dissolved in solvent DMSO-d6 at 300 K analyzed with C13 and H1 NMR ULTRASHEILD 600 WB PLUS (BRUKER) for the confirmation of the compound.

3.2 IN-VITRO ANTI CANCER STUDIES

3.2.1 Cytotoxicity assay using THP-1

- THP-1 cells (5×10^3 cells/well) were incubated with various concentrations (0.6-10 $\mu\text{g/mL}$) of PLGA + Heparin.
- The culture media were removed and the cells were incubated with 5 mg/mLMTT in fresh medium at 37 °C for an additional 4 hrs.
- After this period, the supernatants were removed and 100 μL DMSO was added to each well to dissolve the formazan crystals.
- The plates were read on a micro plate reader at a test wavelength of 540 nm and a reference wavelength of 650 nm.

3.2.2 Cytotoxicity assay using MCF-7

- Michigan cancer foundation (MCF-7) cells (5×10^3 cells/well) were incubated with various concentrations (0.6-5 $\mu\text{g/mL}$) of PLGA + Heparin and PLGA + Heparin + Quercetin.

- The culture media were removed and the cells were incubated with 5 mg/mL MTT in fresh medium at 37 °C for an additional 4 hrs.
- After this period, the supernatants were removed and 100 µL DMSO was added to each well to dissolve the formazan crystals.
- The plates were read on a micro plate reader at a test wavelength of 540 nm and a reference wavelength of 650 nm.

CHAPTER 4

RESULTS AND DISCUSSION

4.RESULTS AND DISCUSSION

4.1 FE-SEM (FIELD EMISSION SCANNING ELECTRON MICROSCOPY)

SEM images of both the samples showed a regular spherical shape and the drug loaded PLGA showed high amount of porosity and the spheres were mono dispersed. The size of the spheres of bare PLGA was found to be 36.20 μm and drug(dceitin) PLGA was 90.16 μm . This result showed that it is feasible to load the drug for optimum drug delivery to the system. This study shows the SEM images of Chlorambucil/ PLGA micro sphere which were also found to be spherical and mono dispersed and was prepared by the four fluid nozzle spray. Here the Rifamycin loaded microsphere was used for the anti-tuberculosis activity (Katsu et al., 2009). Another study revealed the SEM size of the perilyl alcohol — bearing PLGA micro particles of were 768 ± 215 nm. These particles were prepared by oil-in-water-based emulsion solvent evaporation technique (Farazuddin et al., 2012). Porous and non-porous PLGA microspheres prepared by oil / water emulsion technique, showed that the porous microspheres were bigger and size had high density of pores where as the non- porogen microspheres were found to be smaller in size with less pores (T.Zhang et al., 2014). Studies on SEM images have also revealed that novel controlled release formulation for the anticancer drug paclitaxel in PLGA nanoparticles containing vitaminE showed the spherical shape with various degree of smoothness in the microsphere (Mu et al., 2003).

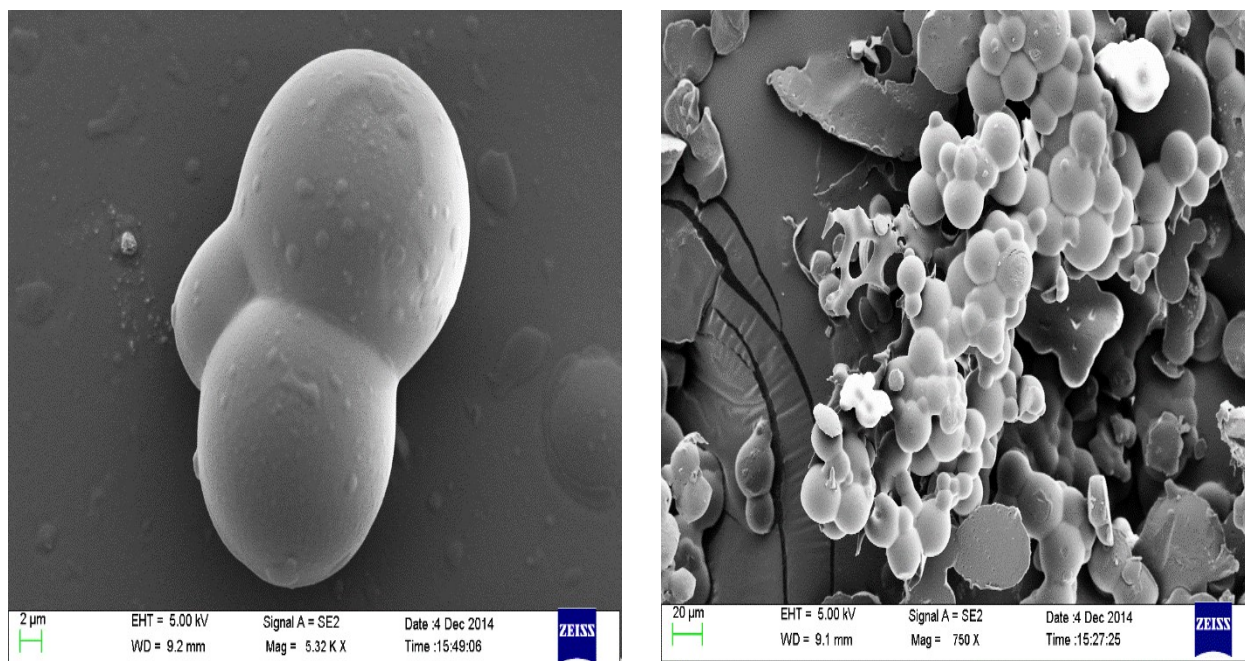


Figure 4.1: Scanning Electron Microscope Images of PLGA Microspheres

4.2 CYTOTOXICITY ASSAY IN THP-1(MTT)

The cytotoxicity assay was performed in THP -1(Human monocytic cell) lines for evaluating the toxicity of PLGA carrier the normal cell lines and to evaluate the dosage beyond which the carrier is toxic to the cell lines. It was found that the 80 % cells are viable in the range of 0.625 $\mu\text{g/mL}$ to 5 $\mu\text{g/mL}$. From these range the dosage of carrier was decided. This study shows the cytotoxicity assay done curcumin loaded PLGA nanoparticles prepared by single emulsion–solvent evaporation method showed destruction of amyloid aggregates and no cytotoxic effect was found in the formulation as 70 % of cells were found to be viable (A.Mathew et al., 2012).

4.3 CYTOTOXICITY ASSAY (MCF-7) MTT

The bare carrier and the drug loaded carrier were tested for their anti- cancer activities against the Michigan cancer foundation (MCF-7) cancer lines. In the study it was found out that the carrier has very less or negligible amount of anti-cancer activity but in comparison the drug loaded carrier displayed excellent anti-cancer activity by decreasing the cell viability to 40 % in the range of concentration of 0-2 $\mu\text{g/mL}$.

The inhibition concentration (IC 50) value was found in the concentration of 3.648 μg . So, comparing the above, cytotoxicity of carrier in normal cell lines and cytotoxicity of the carrier + drug in the cancer cell lines the range of 1.5 μg and 3.0 μg concentrations was found to be optimum for anti-cancer activity. Studies revealed that cell viability assay in pancreatic cell lines treated in various concentrations of free curcumin and nanocurcumin. It was found that the nanocurcumin showed high cytotoxicity to IN pancreatic cell lines in higher concentration of 15 μM (S.Bisht et al., 2007). It is also known that the quercetin enhances anti-cancer activity of trichostatin. A through up regulation of p53 protein as Chan and co-worker have studied both in *invivo* and *in vitro* (S.T.Chan et al., 2013). The PLGA- curcumin Nano formulation was targeted on to MCF-7 breast cancer cell lines by Verderio and group and they observed high toxicity to cancer cells showing potential anti-cancer activity (Verderio et al., 2013). Other studies have also indicated that cell proliferation has decreased significantly in PLGA-curcumin formulation which was subjected to human colon cancer cell lines 116 in comparison to native curcumin within 6-24 hours (Waghela et al., 2015).

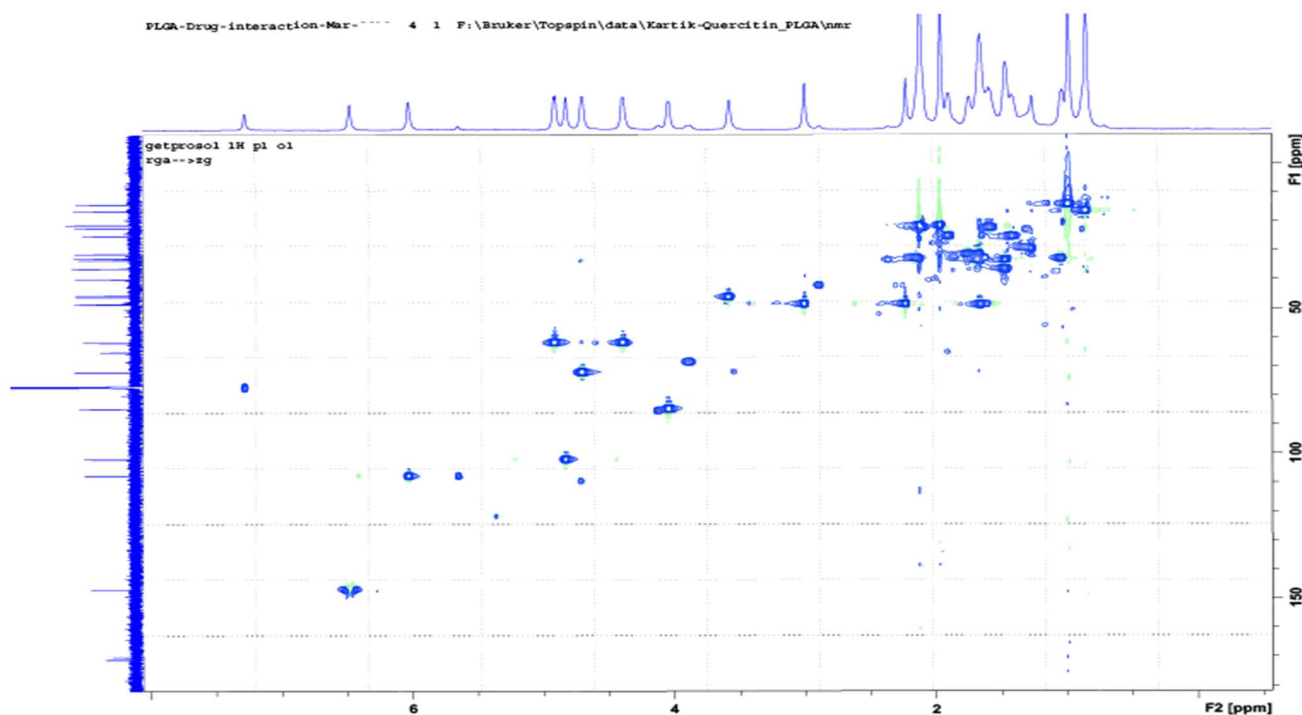


Figure 4.2: NMR Spectra of PLGA+ CHB

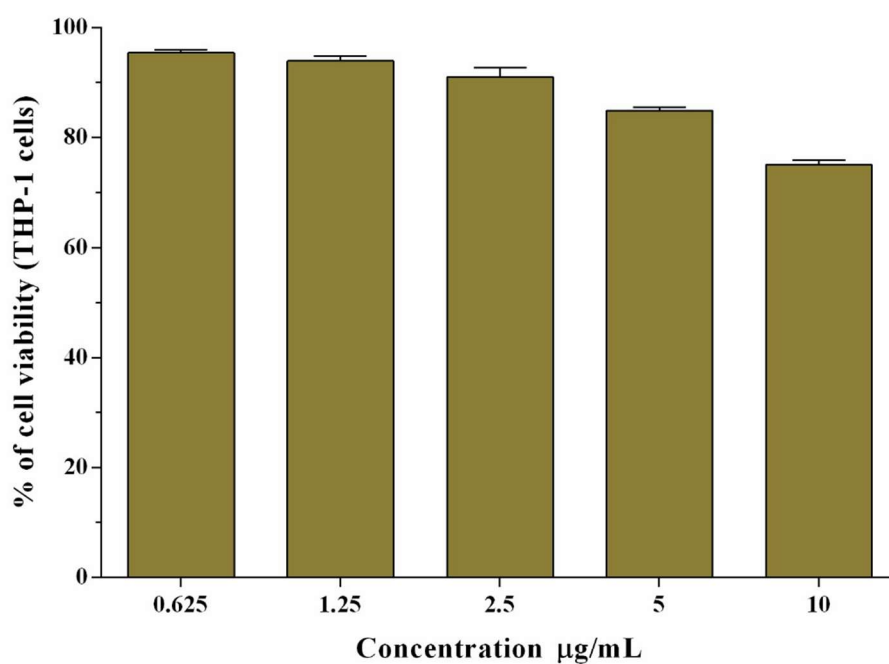


Figure 4.3: CYTOTOXICITY ASSAY IN THP-1 (MTT)

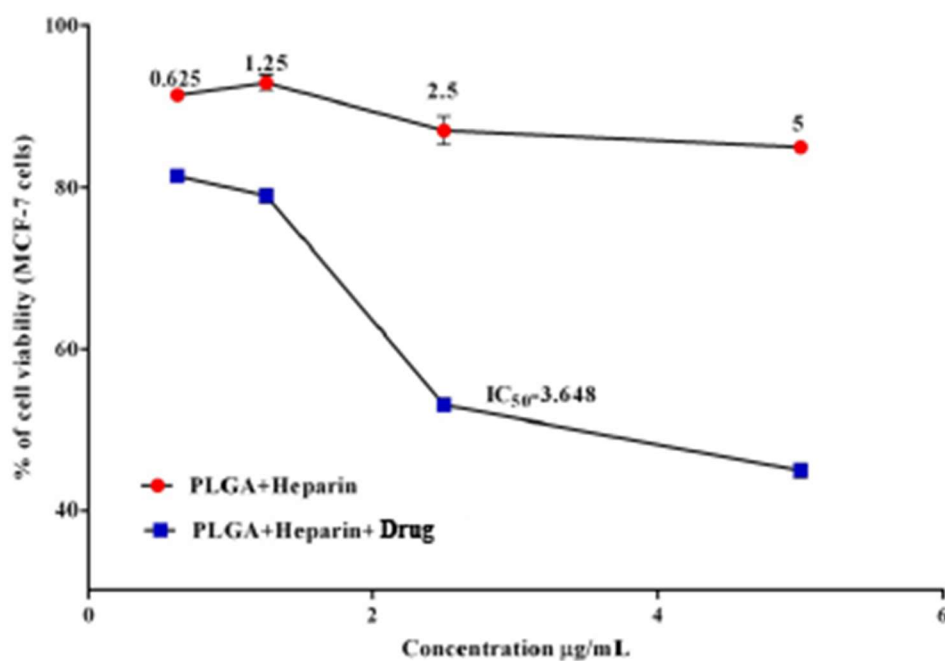


Figure 4.4: CYTOXIXCITY ASSAY USING MCF-7(MTT)

CHAPTER 5

SUMMARY AND CONCLUSION

5. SUMMARY AND CONCLUSION

In this study, the effect of chlorambucil loaded PLGA microspheres on cancer cell lines has been evaluated. The electron micrographs of bare and loaded microspheres suggest that the particles are found to have spherical shaped structures for bare microspheres. On the other hand, loaded PLGA microspheres shows pores on the surface suitable for drug delivery studies. Bare and Chlorambucil loaded microspheres were prepared using double emulsification and its cytotoxicity against MCF-7 cell lines were tested. The chlorambucil loaded PLGA showed excellent anti-cancer activities tested using Annexin-V FITC/PI staining. These results suggest that chlorambucil loaded microspheres can be potent anticancer agent and tested further on other cell lines for development of novel anticancer agents.

REFERENCES

1. A. Ashkenazi, V.M. Dixit, *Science*, 281, (1998), 1305-1308.
2. A. Haddadi, P. Elamanchili, A. Lavasanifar, S. Das, J. Shapiro, J. Samuel, *J. Biomed. Mater. Res. A*, 84, (2008), 885-898.
3. A. Mathew, T. Fukuda, Y. Nagaoka, T. Hasumura, H. Morimoto, Y. Oshida, T. Maekawa, K. Venugopal, D.S. Kumar, *PLoS One* 7, (2012), 23-28.
4. A. Zachowski, *Biochem. J*, 294, (1993), 1-14.
5. A.G. Mikos, Y. Bao, L.G. Cima, D.E. Ingber, J.P. Vacanti, R. Langer, *J. Biomed. Mater. Res*, 27, (1993), 183-189.
6. B. Piątosza, 32, (2007), 247-257.
7. B.N. Waghela, A. Sharma, S. Dhumale, S.M. Pandey, C. Pathak, *PLoS One*, 10 (2015), 1175-1181.
8. B.S. Zolnik, D.J. Burgess, *J. Control. Release*, 122, (2007) 338-344.
9. D. Kumar, D. Sharma, G. Singh, M. Singh, M.S. Rathore, *ISRN Pharm*, 12, (2012), 474-478.
10. D. Staedler, E. Idrizi, B.H. Kenzaoui, L. Juillerat-Jeanneret, *Cancer Chemother. Pharmacol*, 68, (2011,) 1161-1130.
11. D.H.R. Kempen, L. Lu, C. Kim, X. Zhu, W.J.A Dhert, B.L Currier, M.J. Yaszemski, *J. Biomed. Mater. Res. A*, 77, (2006), 103-111.
12. D.P. Go, J.A. Palmer, S.L. Gras, A.J. O'Connor, *J. Biomed. Mater. Res A*, 100, (2012), 507-517.
13. F. Shen, M. Herenyiova, G. Weber, *Life Sci*, 64, (1999), 1869-1876.
14. G. Koopman, C.P. Reutelingsperger, G.A. Kuijten, R.M. Keehnen, S.T. Pals, M.H. Van Oers, *Blood*, 84, (1994), 1415-1420.
15. G. Liu, C. Wu, W. Fan, X. Miao, D.C. Sin, R. Crawford, Y. Xiao, *J. Biomed. Mater. Res. B. Appl. Biomater*, 96, (2011), 360-368.
16. G. Niu, S.Yin, S. Xie, Y. Li, D. Nie, L. Ma, X. Wang, Y. Wu, *Acta Biochim. Biophys. Sin*, 43, (2011), 30 -37.
17. G. Romero, Y. Qiu, R.A. Murray, S.E. Moya, *Macromol. Biosci*, 13, (2013), 234-241.
18. G. Weber, F. Shen, H. Yang, N. Prajda, W. Li, *Anticancer Res*, 19, (1999), 3703-3709.
19. G. Weber, F. Shen, N. Prajda, Y.A. Yeh, H. Yang, M. Herenyiova, K.Y. Look, *Anticancer Res*, 16, (1996), 3271-3282.
20. H. Zhao, L. Ma, C. Gao, J. Shen, *J. Biomed. Mater. Res. B. Appl. Biomater*, 88, (2009), 240-249.

21. H.O. Katsuya Ohashi, T. Kabasawa, T. Ozeki, J. Control. Release, (2009), 19-24.
22. I. Schmid, W.J. Krall, C.H. Uittenbogaart, J. Braun, J.V. Giorgi, Cytometry, 13, (1992), 204-208.
23. J. Chen, J.H. Kang, Pharmazie, 60, (2005), 856-826.
24. J. Fang, Y. Zhang, S. Yan, Z. Liu, S. He, L. Cui, J. Yin, Acta Biomater, 10, (2014), 276-288.
25. J.B. Lee, S. Miyake, R. Umetsu, K. Hayashi, T. Chijimatsu, T. Hayashi, Food Chem, 134, (2012), 2164-2168.
26. J.M. Xue, C.H. Tan, D. Lukito, J. Biomed. Mater. Res. B. Appl. Biomater, 78, (2006), 417-422.
27. K. Vasanth, K. Ilango, R. Mohankumar, Colloids Surf. B Biointerfaces, 117, (2014), 354-359.
28. K.A. Mauritz, C.J. Hora, A.J. Hopfinger, ACS Adv. Chem, 42,s1980,124–154.
29. K.P. Nikitczuk, R.S.Schloss, M.L.Yarmush, E.C.Lattime, J. Cancer Ther, 20, (2013), 280-290.
30. L. Brannon-Peppas, Int. J. Pharm,116, (1995), 1-9.
31. L. Mu, S.S. Feng, J Control Release, 86, (2003), 33-48.
32. M. Farazuddinm, B. Sharma, A.A. Khan, B. Joshi, M. Owais, Int. J. Nanomedicine, 7, (2012), 35-47.
33. M. Richter, R. Ebermann, B. Marian, Nutr. Cancer, 34, (1999), 88-99.
34. M. Van Engeland, L.J. Nieland, F.C. Ramaekers, B. Schutte, C.P. Reutelingsperger, Cytometry, 31, (1998), 1-9.
35. M. Wagner, N. Kiapur, M. Wiedmann-Al-Ahmad, U.H ubner, A. Al-Ahmad, R. Schon, R. Schmelzeisen, R. Mulhaupt, N.C. Gellrich, J. Biomed. Mater. Res. A, 83, (2007), 1154-1130.
36. M.K. Gadhwal, S. Patil, P. D'Mello, U. Joshi, R. Sinha, G. Govil, Indian J. Pharm. Sci, 75, (2013), 233-237.
37. N. Kawazoe, C. Inoue, T. Tateishi, G.Chen, Biotechnol. Prog, 26, (2009), 819-826.
38. N. Phay, T. Higashiyama, M. Tsuji, H. Matsuura, Y. Fukushima, A. Yokota, F. Tomita, Phytochemistry, 52,(1999), 271-274.
39. N.J.Spencer, D.A.Cotanche, C.M.Klapperich, Biomater, 29, (2008), 1028-1042.
40. P. Losi, E. Briganti, C. Errico, A. Lisella, E. Sanguinetti, F. Chiellini, G. Soldani, Acta Biomater, 9, (2013), 7814-7821.

41. P. Verderio, P. Bonetti, M. Colombo, L. Pandolfi, D. Prosperi, *Biomacromolecules*, 14, (2013), 672-682.
42. P.F. Devaux, *Biochemistry*, 30, (1991), 1163-1173.
43. R. Drewa, G. Wozqak, A. Palgan, K. Schachtschabel, D.O Grzanka, A. Sujkowska, *Neoplasma*, 41, (2001), 12-18.
44. R.S.Y. Wong, *J. Exp. Clin. Cancer Res*, 30, (2011), 87-91.
45. R.VidyaPriyadarsini, R. SenthilMurugan, S. Maitreyi, K. Ramalingam, D. Karunagaran, S. Nagini, *Eur. J. Pharmacol*, 649, (2010), 84-91.
46. S. Bisht, G. Feldmann, S. Soni, R. Ravi, C. Karikar, A. Maitra, A. Maitra, *J. Nanobiotechnology*, 5, (2007), 3 -10.
47. S. Krishna, S. Renu, *J. Drug Deliv. Ther*, 3, (2013), 109-113.
48. S. Prior, C. Gamazo, J.M. Irache, H.P. Merkle, B. Gander, *Int. J. Pharm*, 196, (2000), 115-125.
49. S.K. Koester, J.U. Maenpaa, V.J. Wiebe, W.J. Baker, G.T. Wurcz, R.C. Seymour, R.E. Koehler, M.W. DeGregorio, *Breast Cancer Res. Treat*, 32, (1994), 57-65.
50. S.T. Chan, N.C. Yang, C.S. Huang, J.W. Liao, S.L. Yeh, *PLoS One*, 8, (2013) 542-555.
51. S.Y. Zheng, *Mol. Med. Rep*, 5, (2012), 822-826.
52. T. Govender, S. Stolnik, M.C. Garnett, L. Illum, S.S. Davis, *J. Control. Release*, 57, (1999), 171-185.
53. T. Taguchi, L. Xu, H. Kobayashi, A. Taniguchi, K. Kataoka, J. Tanaka, *Biomaterials*, 26, (2005), 1247-1252.
54. T. Zhang, Q. Zhang, J. Chen, K. Fang, J. Dou, N. Gu, *Colloids Surfaces A Physicochem. Eng. Asp*, 452, (2014), 115-124.
55. T.G. Park, *Biomaterials*, 16, (1995), 1123-1130.
56. T.W. King, C.W. Patrick, *J. Biomed. Mater. Res*, 51, (2000), 383-390.
57. U. Milano-bicocca, S. Biomediche, L. Sacco, U. Milano, O.L. Sacco, G.B. Grassi, *BIO Macromol*, 14, (2013), 672-682.
58. W. Lai, Z. Wu, H. Lin, T. Li, L. Sun, Y. Chai, W. Chen, *J. Nat. Prod*, 73, (2010), 1053-1057.
59. W. Liu, S. Thomopoulos, Y. Xia, *Adv. Healthc. Mater*, 1, (2012), 10-25.
60. W.C. Ki, S.Y. Hyuk, J.Y. Jun, G.P. Tae, *Biotechnol. Prog*, 20, (2004), 1797-1801.
61. Y. Cui, Q. Xu, P.K.H. Chow, D. Wang, C.H. Wang, *Biomaterials*, 34, (2013), 8511-8520.
62. Y. Hong, C. Gao, Y. Xie, Y. Gong, J. Shen, *Biomaterials*, 26, (2005), 6305-6313.
63. Y. Huang, J. Ren, T. Ren, S. Gu, Q. Tan, L. Zhang, K. Lv, K. Pan, X. Jiang, *J. Biomed. Mater. Res A*, 95, (2010), 993-1003.

64. Y. Qiu, R. Palankar, M. Echeverría, N. Medvedev, S.E. Moya, M. Delcea, *Nanoscale*, 5, (2013), 12624-12632.
65. Y.S. Nam, T.G. Park, *J. Biomed. Mater. Res*, 47, (1999), 8-17.
66. Z. Darzynkiewicz, G. Juan, X. Li, W. Gorczyca, T. Murakami, F. Traganos, *Cytometry*, 27. (1997). 1-20.