Chapter-5

Chapter 5

Blood and Cellular Biocompatibility of Functionalized Poly(vinyl chloride)/Layered double hydroxides Nanocomposites

5.1 Introduction

Synthetic polymer as a biomaterial extensively used in biomedical area due to their good elasticity, favourable mechanical and chemical properties [Ramakrishna et al (2001)]. Simultaneously, these polymers used as an inert material to surrounding tissue for saving a life of patient but it may not always are desirable. After the implantation of any material a living tissues in growth difficulty may occur as a effect of interaction of synthetic material with adjacent cellular environment. A material behave a biocompatibility, several approaches has been carried out in this regarding [Wang et al. (2004)]. It is find out that the behaviour of the adhesion and proliferation of different types of cells on polymeric materials depend on the surface characteristics such as wettability, chemistry, charge, roughness, and rigidity.

A large number of research groups have studied the interactions of different types of cultured cells with various polymers with different properties to correlate the relationship between surface of material and blood- or tissue-compatibility [Wang et al. (2004);] . Different methods are applied for increasing the biocompatibility of polymeric material such as addition of charged moiety of functional group [Ito et al. (2003)]; Fischer et al. (2003)].

Several reports are available to improve the properties of material by reinforcement of nano material into polymer matrix due to its structure resemble with

natural clay and has compatibility with biological environment. LDHs are positive charge layer and have the capability to exchange their interlayer anions with another. Various research studies have been reported on the basis of anion exchange Changwen et al. (2001) reported that the sheets of LDHs are the host species, and the interlayer anions and water molecules are the guest species. The host and the guest are combined into a two-dimensional super molecular layered system via electrostatic and hydrogen bonding interactions, and can be assigned to the research field of supermolecule chemistry. Therefore, we name this kind of LDHs as the supermolecular layered double hydroxides. Choy et al. (1999), (2000), (2001) focused on synthesis of bio-LDH, in which DNA, nucleotide, flouresceine 5-isothicynate, antisense oligonucleotide as anion exchanger. Particularly, the single or double stranded DNAs have huge applications in different biological areas [Choy et al. (2007)] such as gene therapy; biosensing etc., Choy et al. (2000), (2007) also developed vectors for delivery of antisense oligonucleotides.

A promising potential advantage of using LDH over other molecule is that it could be also recovered very easily by exposing DNA‑LDH hybrids to an acidic condition due to the solubility of LDHs in acidic medium found in cytoplasm of cell, where LDH separate with their host molecule and degrade there. LDHs have other possible applications in pharmaceuticals over biology and medicine field. In addition, Hwang et al. (2001) prepared two type of inorganic molecule, hydrozincite and layered double hydroxide for the intercalation of functionalized organic molecule such as retinoic acid, ascorbic acid, indole acetic acid, citric acid, salicylic acid, acidic dye by co-precipitation method for the pharmaceutical, cosmeceutical and nutraceutical functions. Pharmaceuticals technology requires formulation to be able to maintain pharmacologically active drug levels for long periods avoiding repeated administration and to localize the drug release at its pharmaceutical target. The interlayer region of LDHs may be considered as microvessel in which an anionic drug may be intercalated and released via deintercalation process [Evans et al. (2006)].

LDHs have the general formula $M^{II}_{1-x}M^{III}_{x}(OH)_2(A^{n-})_{x/n}.m\ H2O$, where M^{II} is a divalent cation (Mg^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+} Ni^{2+}, Cu^{2+} Zn^{2+},or Ca^{2+}); M^{III} is a trivalent ion ($A1^{3+}$, Cr^{3+}, Mn^{3+}, Fe^{3+}, Co^{3+}, Ni^{3+}, or La^{3+}) and A^{n-} is the gallery anion such as Cl^{-}, CO_3

$^{2-}$, NO_3^-, etc. [Costa et al. (2008)]. Because of the low price, availability, high aspect ratio as well as desirable nanostructure and interfacial interactions, clays can provide dramatic and adjustable improved properties at very lower loadings along with retaining the original useful properties of the polymer. The nature and properties of material components as well as preparation methodology and conditions affect the final properties of polymer/clay nanocomposite [Olad et al. (2011)].

5.2 Result and Discussion

5.2.1 Determination of Hemolysis activity of functionalized PVC/LDH composites

Release of haemoglobin is primary and quantify test to diagnosis the material biocompatibility, when any material comes in contact with blood [Kamal et al. (2013)]. We have compared the same amount of different ionomers polymeric material for this experiment and reported in Figure 5.1. Phosphate buffered treated RBCs and double distilled water used as negative and positive control. Under these circumstances and according to Autin (1975), the maximum range of haemolysis is 5% and these parameter fulfilled by PVC-TS, PVC-TU and PVC-S [Monika et al. (2015)]. However, all the polymer composites exhibit within the normal limit (below5 %) except these PVC, PVC-1% and PVC-1.5 % composites. Figure 5.1 (a) describe that PVC-2% show 4.4% only, besides this PVC-TS composites demonstrate 2.7, 3.7 for 4.1% haemolysis with PVC-TS-1%, PVC-TS-1.5% and PVC-TS-2% (Figure 5.1 (b)). Next with these result, PVC-TU composites prove better haemolysis for PVC-TU-1%, PVC-TU-1% and PVC-TU-1% such as 1.2, 0.9 and 0.7% respectively (Figure 5.1 (c)). Last all ionomers composites of PVC-S illustrate excellent haemolytic properties than all other (Figure 5.1 (d)). The following results signifying that these different PVC ionomers composites are advanced biomaterials and could be used as alternatives to the pure form of PVC. However, effort is in development to further get better the polymers.

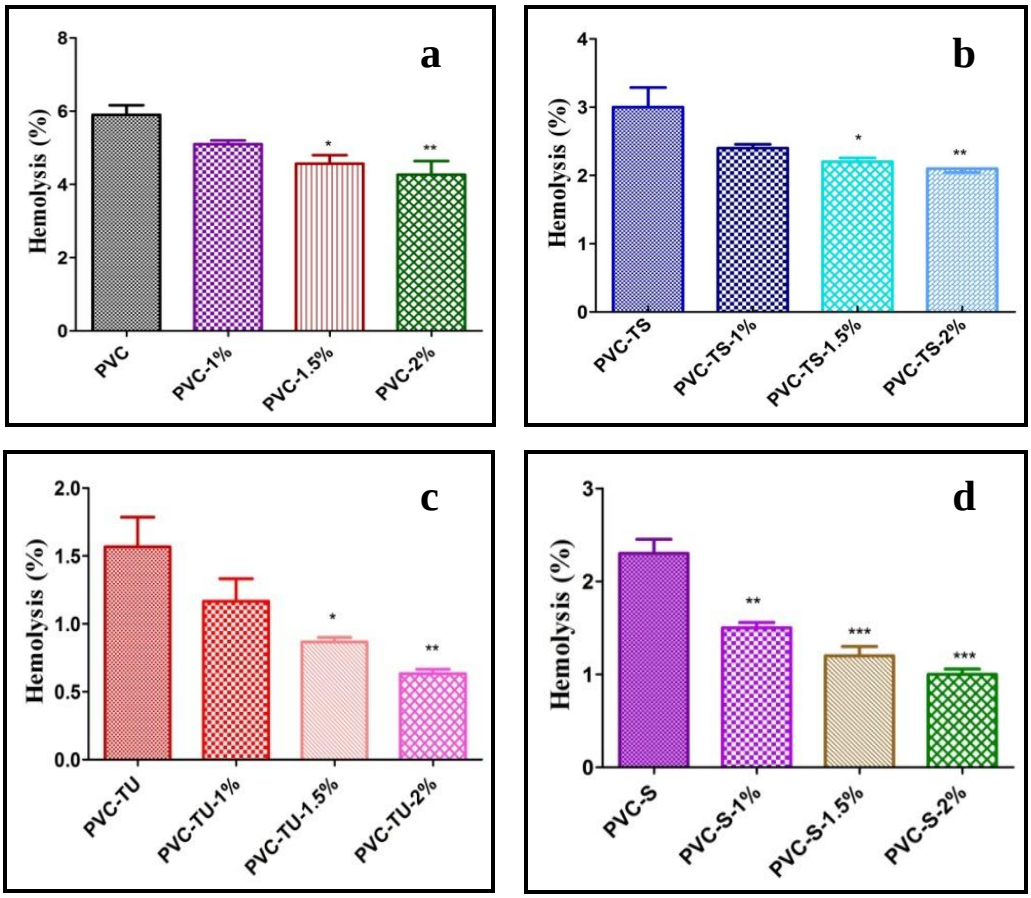

Figure 5.1 Hemolysis activities of different polymer functionalized PVC composites, (a) PVC and its composites with different wt% of LDH; (b) PVC-TS and its composites with different wt% of LDH; (c) PVC-TU and its composites with different wt% of LDH; (d) PVC-S and its composites with different wt% of LDH.

*P < 0.05
** P<0.01
*** P < 0.001

5.2.2 Determination of clot formation on functionalized PVC/LDH composites

The weight of blood clots obtained after incubation of blood with PVC, PVC-TS, PVC-TU and PVC-S composites for 30 min was summarized in Table 5.1 – 5.4. These results are consistent with the previous studies of functionalized PVC [Monika et al. (2015)]. It was observed that these

Table 5.1: Weight (mg) of clot formation on the surface of PVC and its different composites with LDH.

Samples	Clot wt. (mg)
PVC	1.9
PVC-1%	1
PVC-1.5%	0.9
PVC-2%	0.8

Table 5.2: Weight (mg) of clot formation on the surface of PVC-TS and its different composite with LDH.

Samples	Clot wt. (mg)
PVC	1.3
PVC-1%	1
PVC-1.5%	0.9
PVC-2%	0.8

Table 5.3: Weight (mg) of clot formation on the surface of PVC-TU and its different composites with LDH.

Samples	Clot wt. (mg)
PVC	1.6
PVC-1%	1
PVC-1.5%	0.9
PVC-2%	0.8

Table 5.4: Weight (mg) of clot formation on the surface of PVC-S and its different composite with LDH.

Samples	Clot wt. (mg)
PVC	1.1
PVC-1%	0.9
PVC-1.5%	0.8
PVC-2%	0.7

The surface properties play a vital function at a molecular level in governing surface-induced haemolysis [Kazuhika et al. (2000)]. Notably, hydrophilicity nature of the material directly corresponds to their improved biocompatibility. In addition, several studies suggest that a biomaterial with the positively charged surface promotes thrombogenesis when exposed to blood, while negative charged biomaterials tend to suppress the thrombogenesis process [Black (2005)], most likely due to the fact that blood cells and platelets have net negative charge on their surface.

5.2.3 Cell adhesion on functionalized PVC/LDH composites

All forms of polymers supported cellular adhesion under the standard conditions. Figure 5.2 shows the percentage of mesenchymal stem cells adhered to composites of PVC, PVC-TS, PVC-TU and PVC-S polymers after 4 h treatment. Polystyrene tissue cultured Petri dish (without sample) used as a control in all cases. The total set of polymer ionomers composites shows appreciably superior level of adhesion percentage compared

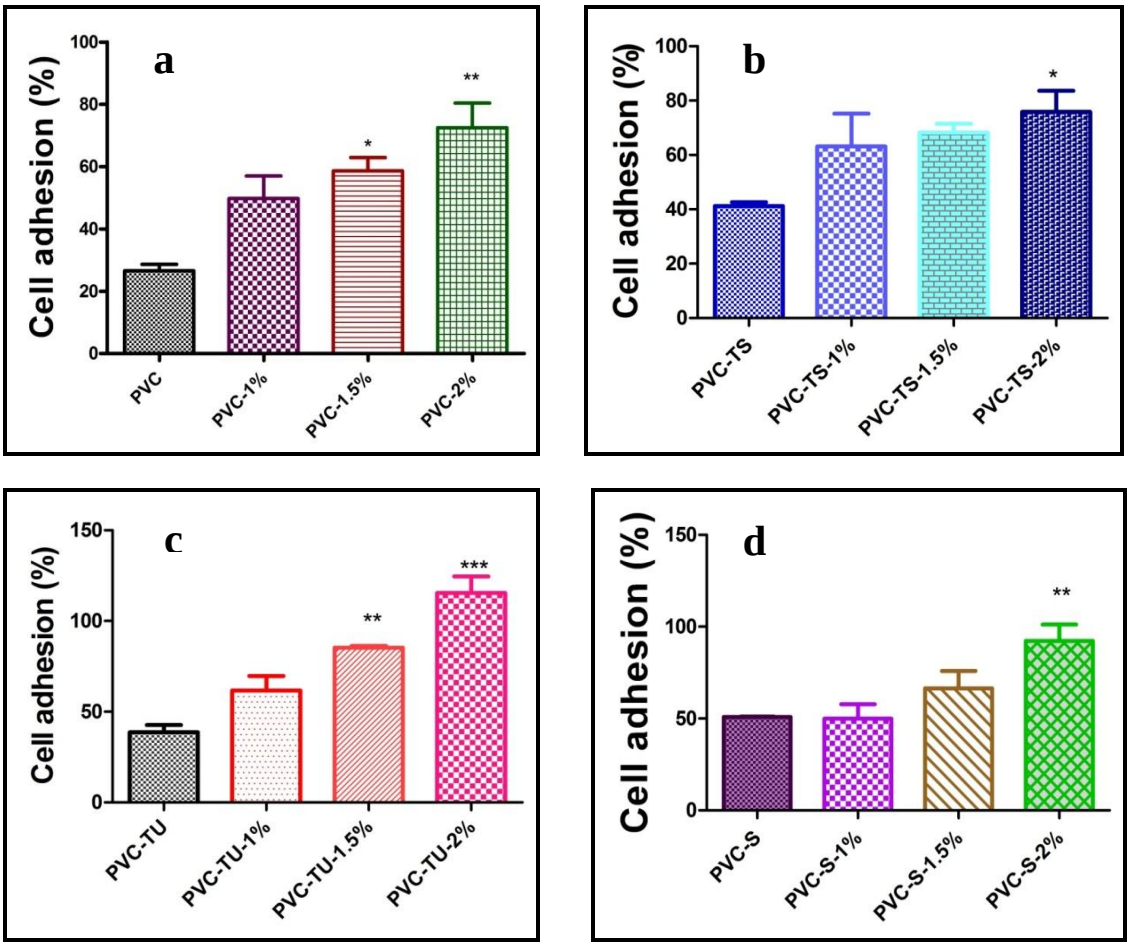

Figure 5.2 Cell adhesions of different polymer ionomers composites on mMSCs, (a) PVC and its composites with different wt% of LDH; (b) PVC-TS and its composites with different wt% of LDH; (c) PVC-TU and its composites with different wt% of LDH; (d) PVC-S and its composites with different wt% of LDH.

*P < 0.05
** P<0.01
*** P < 0.001

to the pure form of PVC. The level of cellular adhesion was found notably increased in all polymer composites samples as the increase level of wt% of LDH in polymer sample. Cell adhesion was particularly more favorable at PVC-TU-1.5 and PVC-TU-2% because it thiourea has amino moiety beside this PVC-2%, PVC-TS-2% and PVC-S-2% showed relatively similar range of cellular adhesion on their surface.

5.2.4 Cell proliferation on functionalized PVC/LDH composites

To determine effects of the functional polymers on metabolic activity, the MTT test was performed. Cytotoxicity of polymeric materials after their incubation with cells for 1, 3 and 5 day was observed in a culture medium. The cytotoxicity was measured by determining the cellular viability using a MTT assay. Figure 5.3 – 5.6 represents the plot for the viability percentage of mMSCs and shows significantly lower levels of cytotoxicity in case of functionalized polymer composites materials. Viability of the cells seeded on a bare tissue culture grade polystyrene petri dish was considered as a control**.** It was observed that in spite of the decrease in the percentage of viable cells with an increase in the extract concentration, none of the tested materials showed complete cytotoxicity for mMSCs.

Figure 5.3 describes **t**he cell viability of PVC and its various composites, it was found to be ~ 43% for PVC after 1 day of culture while it increased significantly by another ~8%, ~13%, 33% for PVC-1%, PVC-1.5%, and PVC-2%, respectively. But, after 3 days of culture, some changes was observed in result, viability on PVC-1 & 1.5% polymer composites did not grow so much comparatively PVC-2% and its viability was noted to be around 41% in PVC and 1%, -2%, 34% for PVC-1%, PVC-1.5%, and PVC-2%, respectively. While culture maintained their growth following 5 days of culture as, PVC was 1% and increased by 4%, 5%, 30% in PVC-1%, PVC-1.5% and PVC-2% respectively.

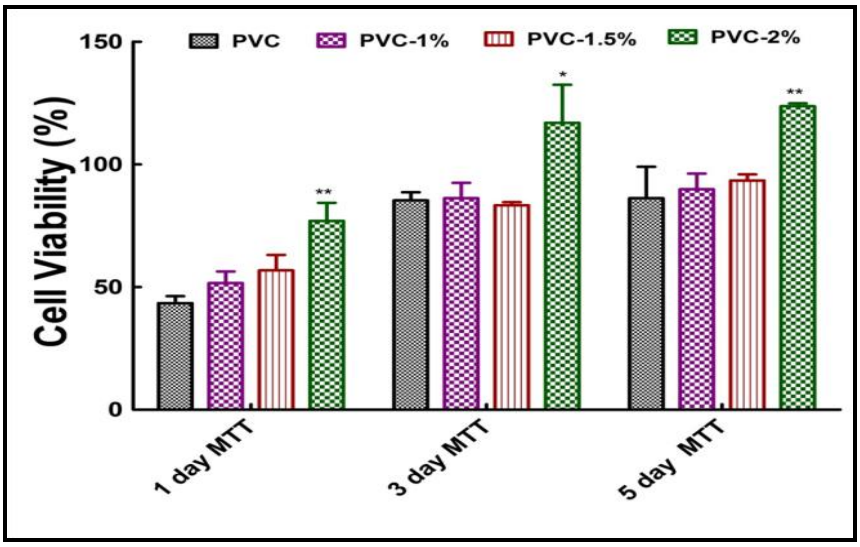

Figure 5.3 Cell Proliferation of PVC and its composites with different wt% of LDH on mMSCs

PVC-TS shows better cell viability than PVC [Monika et al. (2015)], Figure 5.3 explain graphically that first PVC-TS show 123% cell viability and increased by 6%, 7% and 9%(near about) in PVC-TS-1%, PVC-TS -1.5%, and PVC-TS -2%, respectively. Cell viability increases continuously at third day also by 11 % in PVC-TS compare than its first day, while other materials growth greater than before by16%, 20 %, 22%. On the other hand, viability disturbed slightly at fifth day, firstly increased by 24 % in PVC-TS compare than its first day, but slow down in PVC-TS-1 and 1.5% after that increased in case of PVC-TS-2% by 21%.

Behavior of mMSCs on PVC-TU materials always different because it contain amino group that favors the growth of cells. PVC-TU and its composites maintain mMSCs growth continuous increasingly at its surface, shown in figure 5.4. PVC-TU starts with 130 % cell viability and get better by 29%, 42%, 68% at first day. Third day observation showed 18%, 15%, 27%, 74% in PVC-TU, PVC-TU-1%, PVC-TU-1.5% PVC-TU and PVC-TU-2% respectively. Same like above pattern, at fifth day cell growth increased by 10%, 14%, 46%, 94% in PVC-TU, PVC-TU-1%, PVC-TU-1.5% PVC-TU and PVC-TU-2% respectively.

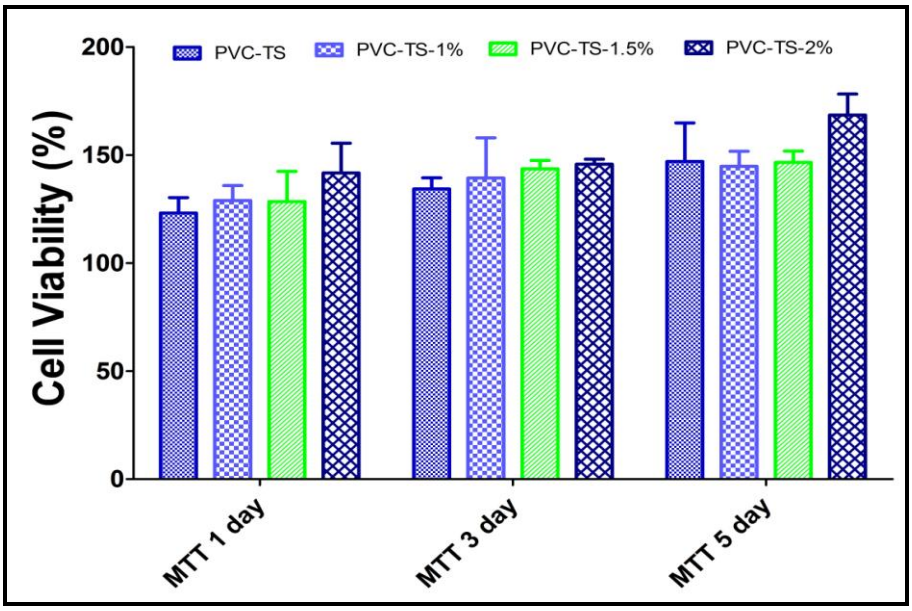

Figure 5.4 Cell Proliferation of PVC-TS and its composites with different wt% of LDH on mMSCs.

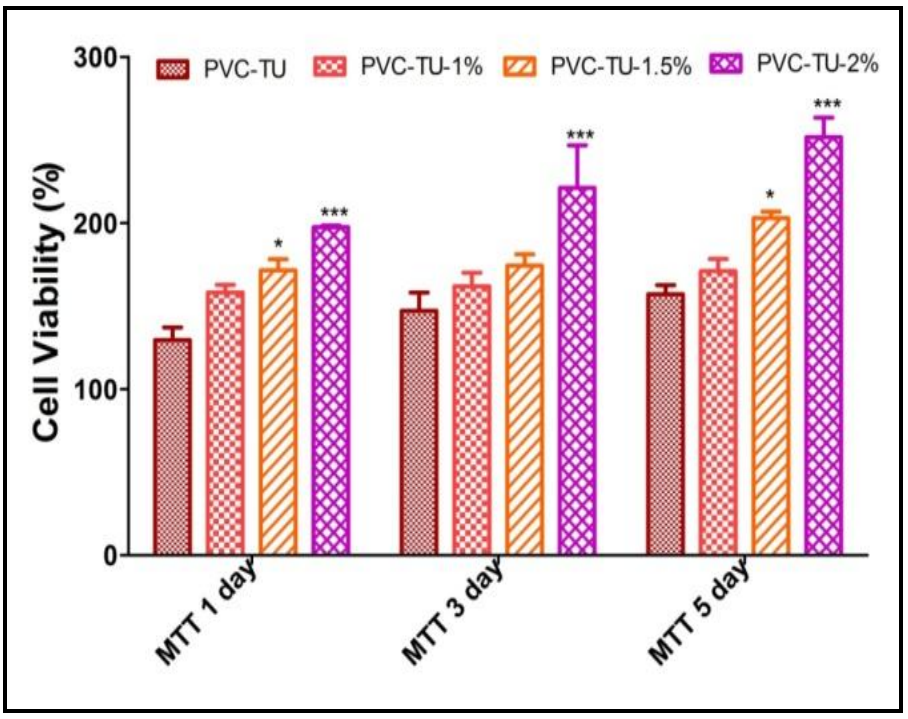

Figure 5. 5 Cell Proliferation of PVC-TU and its composites with different wt% of LDH on mMSCs

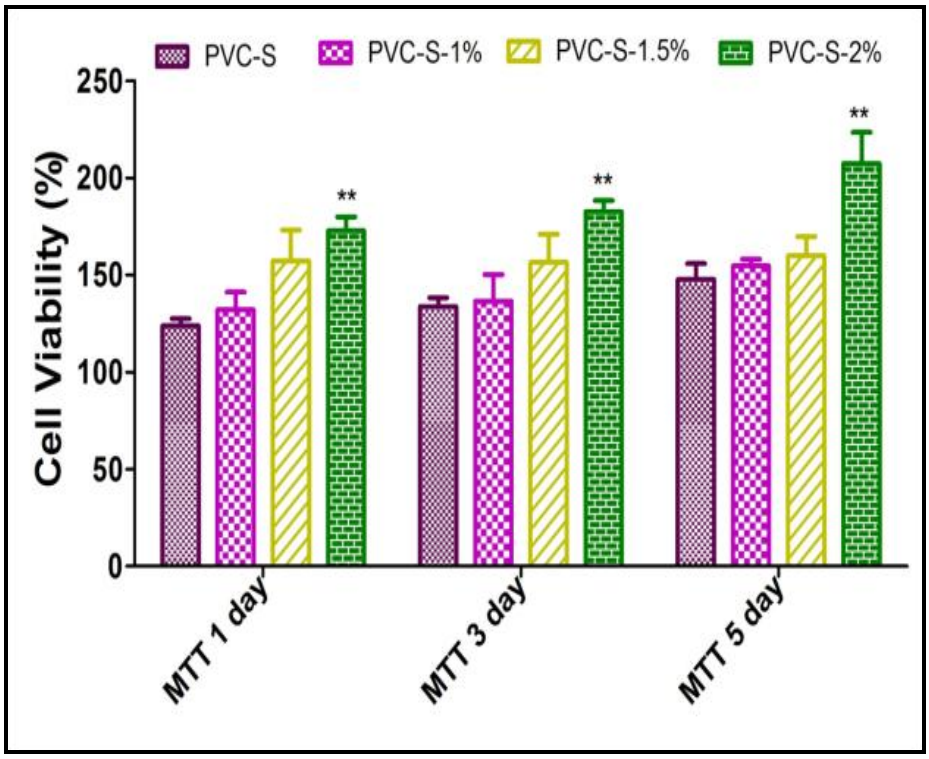

Figure 5.6 Cell Proliferation of PVC-S and its composites with different wt% of LDH on mMSCs.

The cell viability was found to be 124% in PVC-S which was more than PVC at first day [Monika et al.(2015)]. It increases by 8 %, 33%, 49% in PVC-1%, PVC-1.5% and PVC-2% respectively [Figure 5.5]. The third day observation described 10%, 3%, 23% and 49% increased in PVC-S, PVC-S-1%, PVC-S -1.5% and PVC-S -2%. Similarly, same pattern was follows at fifth day, growth increased by 14%, 7%, 12% and 59% in PVC-S, PVC-S -1%, PVC-S -1.5% and PVC-S -2%. Fischer et al. (2003) have investigated cell viability of different polycations and magnitudes of the cytotoxic effects of all polymers were found to be time- and concentration dependent. However, Govinda et al. (2013) also used trivalent aluminium in place of bivalent magnesium in different molar ratio with bone cement proves the better biomaterial.

5.2.5 Nuclear Staining on functionalized PVC/LDH composites

All forms of polymers supported cellular adhesion under the standard conditions. Figure 5.2 shows the percentage of mesenchymal stem cells adhered to composites of PVC, PVC-TS, PVC-TU and PVC-S polymers after 4 h treatment. Polystyrene tissue cultured Petri dish (without sample) used as a control in all cases. The total set of polymer ionomers composites shows appreciably superior level of adhesion percentage compared

Figure 5.7-5.10 shows the nuclei of adhered mesenchymal stem cells adhered on PVC and its functionalized PVC composites. Nuclear staining indicates that the cells adhered on modified forms of PVC were significantly higher in comparison to that of control PVC. Microscopic images further reveal that pure PVC does not support cellular adhesion at all while PVC-TS, PVS-TU and PVC-S assist adherence of cells to a significant extent compared to the pure material. Thus, these results suggest that modification of the PVC resins with different functional groups leads to enhancement in their biocompatibility properties.

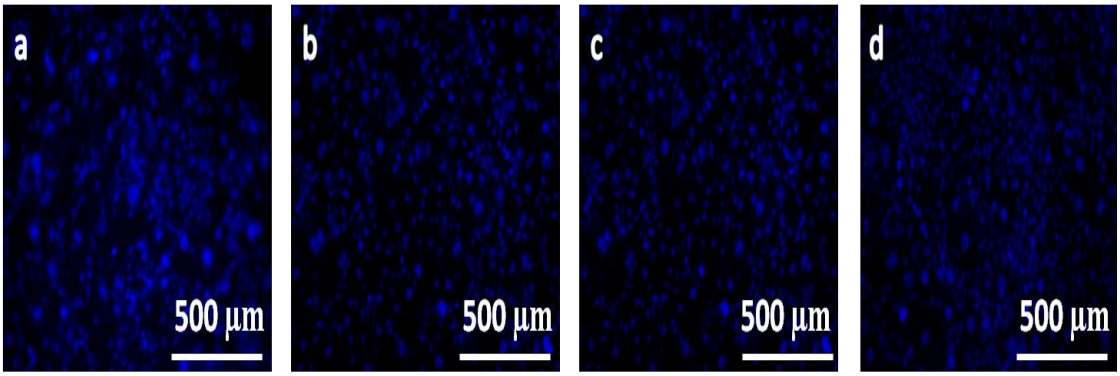

Figure 5.7: Morphological observation of mMSCs grown on different functionalized polymer nanocomposites surface for 24h. Cells were cultured direct in contact with various samples and analyzed with fluorescence microscope. (a) PVC; (b) PVC-1%; (c) PVC-1.5%; (d) PVC-2%.

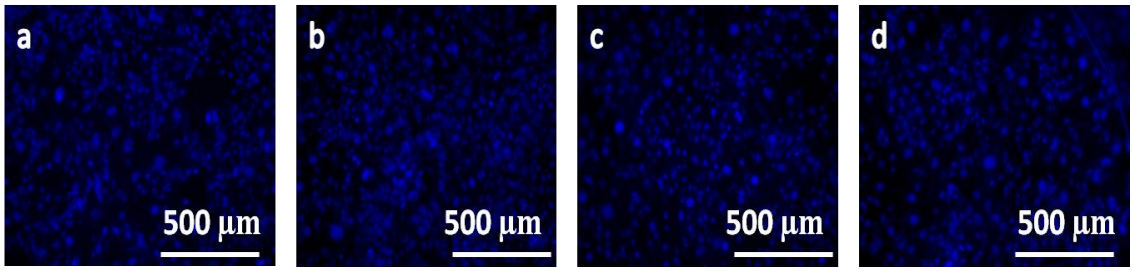

Figure 5.8: Morphological observation of mMSCs grown on different functionalized polymer nanocomposites surface for 24h. Cells were cultured direct in contact with various samples and analyzed with fluorescence microscope. (a) PVC-TS; (b) PVC-TS-1%; (c) PVC-TS-1.5%; (d) PVC-TS-2%.

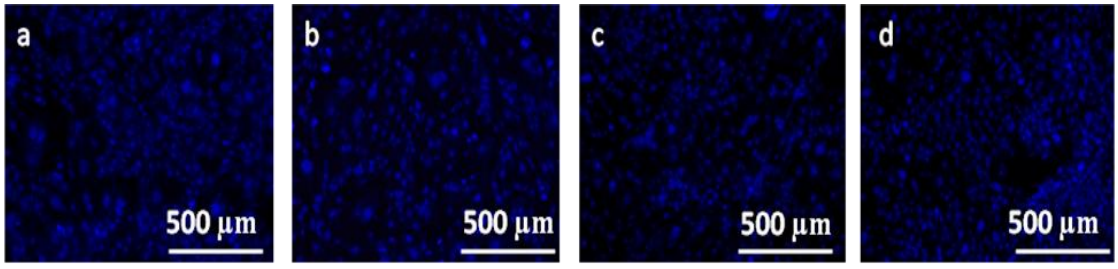

Figure 5.9: Morphological observation of mMSCs grown on different functionalized polymer nanocomposites surface for 24h. Cells were cultured direct in contact with various samples and analyzed with fluorescence microscope. (a) PVC-TU; (b) PVC-TU-1%; (c) PVC-TU-1.5%; (d) PVC-TU-2%.

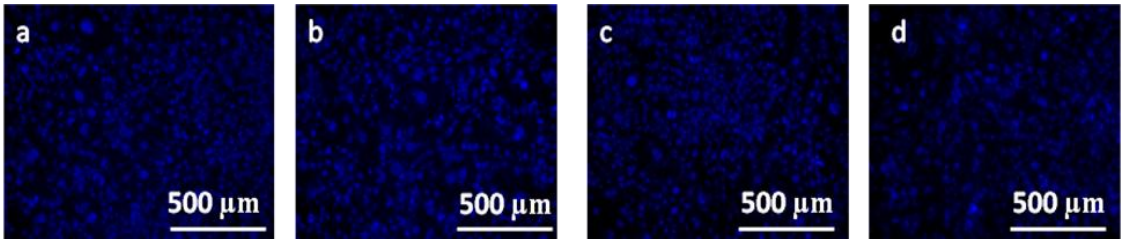

Figure 5.10: Morphological observation of mMSCs grown on different functionalized polymer nanocomposites surface for 24h. Cells were cultured direct in contact with various samples and analyzed with fluorescence microscope. (a) PVC-S; (b) PVC-S-1%; (c) PVC-S-1.5%; (d) PVC-S-2%.

5.2 Summary

This work demonstrates the influence of different functional groups with nanoclay on the characteristics of PVC surface and the resulting biocompatibility property. For this purpose, functionalized forms of PVC using thiosulphate, thiourea and sulphate have been fabricated by a nucleophilic substitution reaction using a phase transfer catalyst along with synthesis of LDH by co-precipitation method. The outcome reveals that functionalized polymers are hydrophilic in nature, shows reduced hemolytic activity, and supports bacterial and cellular adhesion significantly. While adding on LDH in these functionalized PVC become more hydrophilic which stimulate the biocompatibility. Further research including in vivo testing for improving the biocompatiblity of the surface modified PVC polymers and their composites are considered necessary to fully authenticate their possible uses in biomedical-related applications.