6.1 INTRODUCTION

Orthotopic liver transplantation is the only treatment clinically available for patients suffering from end stage liver failure caused by chronic disorders. However, poor donor availability of healthy liver tissue, immune rejection and lifelong immuno suppressive treatment restricts the potential of transplantation. Alternative approach like hepatic lineage (e.g., hepatocytes) transplantation in the injured liver has been found to overcome present limitations [1, 2]. But availability of fresh and healthy cells for restoration of functional hepatocytes is extremely challenging as the primary hepatocytes rapidly lose its viability and functionality due to the trans-differentiation to other type of cells [3, 4].

Major source of cells for hepatic tissue engineering includes primary hepatocytes isolated either from animal or human and hepatocellular carcinoma (HepG2) cell lines [5]. Though the liver is populated with heterogeneous cells, primary hepatocytes dissociated from liver tissue has gained much attention as the mature adult hepatocytes are solely responsible for maintaining liver functions such as metabolic homeostasis, detoxification and albumin and urea synthesis [5]. It was also observed that the hepatocyte injection (1000 hepatocytes) into the hepactomised animals creates hepatocyte nodules and restore the liver functions due to its extraordinary proliferation rate [6]. On the contrary, use of HepG2 cells are limited for tissue engineering applications for development of bioartificial liver device (extracorporeal
liver assisted device) as it carries potential risk for metastases progression [6]. However, *in vitro* expansion and phenotype retention of hepatocytes are the two major prevailing challenges in the therapeutic utility of primary hepatocytes [6]. Hence, it is essential to identify a key element to overcome existing constraints of establishing functional primary hepatocyte culture.

This challenge has motivated tissue engineers to identify a suitable microenvironment to make the hepatocytes functionally active. Collagen coating as well as sandwich gels provide an *in vivo* microenvironment since collagen is the major component of the extracellular matrix [6-12]. However, immunogenicity, batch to batch variation, rapid degradation rate and poor mechanical property of collagen restricts its prolonged use in tissue regeneration [13].

Hepatocyte growth factor (HGF) loaded heparin based hydrogel has been found to maintain the hepatocyte functions for 21 days [8]. Subcutaneous transplantation of hepatocyte spheroid embedded polyurethane foam filled with HGF loaded heparin-collagen gel into the 70 % partial hepatectomized rat has been proved to promote angiogenesis. This also aids in the reconstruction of tissue like structure with respect to single hepatocyte embedded polyurethane foam filled with HGF loaded heparin-collagen gel [14]. Nakamura *et al.*, identified the synergistic effect of HGF and components from decellularized rat liver matrix (L-ECM) on native biological activity and liver specific functions such as albumin secretion, urea synthesis and action of ethoxyresorufin-o-deethylase in primary rat hepatocytes [15]. Liver extracellular matrix (LEM) coated hydrogels has been found to promote higher albumin and urea secretion of primary hepatocytes due to its elastic and gelation properties when compared to collagen coated hydrogel [4]. Hence, search for the appropriate material
and suitable scaffold design with stimulatory cues are required for the restoration of functional primary hepatocytes for prolonged period in culture [12].

The present study involves the *in vitro* evaluation of HGF loaded nanospheres embedded in galactose containing 8:2 and 9:1 P/G hydrogel scaffolds using primary rat hepatocytes. These scaffolds were chosen for this study since they possess ideal properties required for liver regeneration (characterized in chapter 4) and found to exhibit sustained release of hepatocyte growth factors (characterized in chapter 5). Four different groups of scaffolds namely 9:1 P/G-galactose, 8:2 P/G-galactose, HGF loaded PLGA nanospheres in 9:1 P/G-galactose and HGF loaded PLGA nanospheres in 8:2 P/G-galactose were evaluated in the present study and collagen coated plates were used as control. All scaffolds were seeded with primary hepatocytes and characterized for cell-material interaction, hepatocyte viability, spheroidal morphology and metabolic functionality (albumin and urea secretion) up to 28 days.

### 6.2 MATERIALS & METHODS

#### 6.2.1 Materials

PVA ($M_w$: 186 kDa), PVA ($M_w$: 80 kDa), BSA ($M_w$: 66 kDa) and Gelatin from Bovine skin, Type B ($M_w$: 50 kDa) were purchased from Sigma Aldrich, India. PLGA (50:50) ($M_w$: 118 kDa and intrinsic viscosity: 0.79 dL/g) was procured from Lakeshore Biomaterials, USA. D-Galactose and dichloromethane (DCM) were procured from Himedia, India and Merck, India respectively. Recombinant human HGF ($\alpha$-chain: 463 amino acid residues and $\beta$-chain: 234 amino acid residues) were obtained from PeproTech, USA. Cryopreserved primary rat hepatocytes, thawing and
maintenance media and collagen coated plates were purchased from Invitrogen, India. Live/dead assay kit (Molecular probes, USA), CellTiter\textsuperscript{96} aqueous MTS solution (Promega, USA), Rat albumin ELISA kit (Immunology Consultants laboratory, Portland) and Quantichrom\textsuperscript{TM} Urea Assay kit (BioAssay systems, USA) were acquired.

### 6.2.2 Cell Seeding

Prior to cell seeding, both sides of the scaffolds were sterilized by UV radiation for 2 hours. Cryopreserved primary rat hepatocytes were carefully thawed using thawing and plating media following the manufacturer protocol. Briefly, the cryopreserved vials were thawed at 37 °C and transferred to 48 mL thawing and plating media under aseptic condition, centrifuged at 50 g for 3 minutes at 25 °C. The media was discarded and the pellets were gently re-suspended in 5 mL of fresh thawing and plating media. Around 96 % viable primary rat hepatocytes of density $1 \times 10^6$ were seeded onto collagen coated plates and scaffolds placed in 48 well plates with thawing and plating media and were incubated at 5 % CO\textsubscript{2} in 37 °C for 4 hours. Thawing and plating media was then replaced with maintenance media, which was changed every 24 hours.

### 6.2.3 Cell-Material interaction

Primary rat hepatocyte interactions on all four scaffolds were determined at various time intervals (1 hour, 6 hours, 1 day and 3 days) using field emission scanning electron microscope (FE-SEM, JSM 6701F, JEOL, Japan). Briefly, the media was aspirated from the hydrogel scaffolds at pre-determined time intervals and scaffolds
were washed twice with phosphate buffered saline (PBS) followed by fixation in 4 % glutaraldehyde overnight at 4 °C. The hydrogel scaffolds were then subjected for dehydration using graded concentrations of ethanol (50 %, 60 %, 70 %, 80 %, 90 % and 100 %) for an hour in each concentration. Following dehydration, the scaffolds were air-dried overnight and stored in vacuum dessicator for a week. After gold sputter coating, the samples were analyzed using FE-SEM at an accelerating voltage of 3 kV.

6.2.4 Cell Viability

Viability of primary rat hepatocytes on all scaffolds were evaluated using a live/dead cell viability kit under laser scanning confocal microscope (FV1000, Olympus, Tokyo, Japan) [9]. In brief, about 1 µL of calcein and 2 µL of ethidium bromide were mixed with 1 mL PBS. About 200 µL of the mixture was added to each scaffold and incubated at 37 °C in 5 % CO₂ for 30 minutes and viewed under confocal microscope.

6.2.5 Intracellular Activity

Intracellular activity of primary rat hepatocytes cultured on various scaffolds was quantified and compared with control using colorimetric MTS assay at different time points (1, 3, 7, 14, 21 and 28 days). In short, hepatocytes cultured on hydrogel scaffolds at every time points were rinsed with PBS and transferred to new well plate. About 100 µL of MTS with 1 mL of serum-free maintenance media were added to hydrogel scaffolds and incubated for 2 hours at 37 °C. After incubation, sodium
dodecylsulphate (100 µL of 10 %) was added to stop the reaction and absorbance was read at 490 nm in a multimode reader (Tecan, Infinite M100, Australia) (n=4).

6.2.6 Albumin Assay

Albumin secretion was evaluated by Rat Albumin ELISA kit (Immunology Consultants Laboratory Inc, Portland) for a period of 28 days in order to determine the albumin secreting function of primary rat hepatocytes cultured on all scaffolds. At pre-determined time points, media was collected from the P/G hydrogel scaffold and centrifuged at 5000 rpm for 10 minutes and supernatant was stored at -70 °C until further analysis according to manufacturers’ protocol. Shortly, samples were added to pre-coated well plates and incubated for 30 minutes followed by the washing of well plates using wash solution. Following the incubation of well plates with enzyme-antibody conjugate for 30 minutes, TMB substrate was added to the well plates and incubated for 10 minutes under dark conditions. The reaction was stopped using stop solution and the absorbance was recorded at 450 nm using multimode plate reader (n=3).

6.2.7 Urea Secretion

Quantichrom urea Assay kit (Bioassay systems) was used to enumerate the urea synthesis by primary hepatocytes on all scaffolds at programmed time points (1, 3, 7, 21 and 28 days). Hydrogel scaffolds were incubated with 1 mM ammonia containing fresh maintenance media for 24 hours and the media was stored at -70 °C until analysis. The assay was carried out as per the manufacturer protocol. Briefly, equal
volume of working reagents was incubated with the sample for 20 minutes at room temperature and the absorbance was measured at 520 nm (n=3).

6.2.8 Statistical Analysis

All values are expressed as mean±SD obtained from at least three independent experiments. Two-way analysis of variance (p<0.05) followed by Tukey post-hoc test at 95% confidence limits (p<0.05) was used to evaluate the significance between hydrogel scaffolds and time points for intercellular activity, albumin synthesis and urea secretion.

6.3 RESULTS & DISCUSSION

Success in functional restoration of damaged liver is mainly based on the development of ECM analogue, which provide temporary framework for establishing cell-scaffold and cell-cell communications along with the ability to deliver appropriate growth factors to retain viable functional hepatocytes. Developed three-dimensional HGF loaded nanosphere embedded P/G hydrogel scaffolds could support strong cell-cell communication for hepatocyte spheroid formation while preventing the de-differentiation of hepatocytes through the sustained delivery of HGF.

6.3.1 Cell-Material Interaction

The viability and metabolic functions of hepatocytes on the hydrogel scaffolds were studied to understand the interaction of hepatocytes since it influences three-dimensional organization. Hepatocyte-hydrogel interaction at pre-determined time
points (1 hour, 6 hours, 1 day and 3 days) were evaluated using scanning electron microscope (Figure 6.1). Scanning electron micrographs of various scaffolds after one–hour post seeding of primary hepatocytes is shown in Figure 6.1 A1-D1. Primary hepatocytes were found to adhere on all the scaffolds, suggesting that the galactose and HGF supported cell-scaffold interaction even after an hour.

After four hours of culture, HGF loaded nanosphere embedded galactose containing hydrogels showed better cell-cell interaction than galactose containing hydrogels which was evident from the presence of lesser pores on the surface of the scaffolds (Figure 6.1 A2-D2). At the end of day 1, galactose containing hydrogel scaffolds demonstrated superior cell adhesion when compared to 4 hours by the reduction in the size of surface pores (Figure 6.1 A3-B3). On the contrary, primary hepatocytes cultured on HGF encapsulated nanospheres embedded galactose P/G hydrogels stretched across the porous structures, which indicated the stronger primary hepatocyte-scaffold communication (Figure 6.1 C3-D3). Primary hepatocytes cultured on galactose containing hydrogels after 3 days have shown the transversal extension of cells along the pores (Figure 6.1 A4-B4). There was no marked difference on the surface morphology of primary hepatocytes on HGF encapsulated nanospheres embedded galactose P/G hydrogel scaffolds after 3 days when compared to 1 day suggesting that the combination of HGF and galactose in the HGF encapsulated nanospheres embedded galactose P/G hydrogels have influenced the cell-cell interaction at the earliest as compared to galactose containing hydrogels (Figure 6.1 C4-D4).
Figure 6.1: Primary rat hepatocyte adhesion on [A1] 9:1 P/G hydrogel scaffolds with galactose after 1 hour of culture; [B1] 8:2 P/G hydrogel scaffolds with galactose after 1 hour of culture; [C1] HGF loaded nanospheres incorporated on 9:1 P/G hydrogel scaffolds with galactose after 1 hour of culture; [D1] HGF loaded nanospheres incorporated on 9:1 P/G hydrogel scaffolds with galactose after 1 hour of culture; [A2] 9:1 P/G hydrogel scaffolds with galactose after 4 hours of culture; [B2] 8:2 P/G hydrogel scaffolds with galactose after 4 hours of culture; [C2] HGF loaded nanospheres incorporated on 9:1 P/G hydrogel scaffolds with galactose after 4 hours of culture; [D2] HGF loaded nanospheres incorporated on 9:1 P/G hydrogel scaffolds with galactose after 4 hours of culture; [A3] 9:1 P/G hydrogel scaffolds with galactose after 1 day of culture; [B3] 8:2 P/G hydrogel scaffolds with galactose after 1 day of culture; [C3] HGF loaded nanospheres incorporated on 9:1 P/G hydrogel scaffolds with galactose after 1 day of culture; [D3] HGF loaded nanospheres incorporated on 9:1 P/G hydrogel scaffolds with galactose after 1 day of culture; [A4] 9:1 P/G hydrogel scaffolds with galactose after 3 days of culture; [B4] 8:2 P/G hydrogel scaffolds with galactose after 3 days of culture; [C4] HGF loaded nanospheres incorporated on 9:1 P/G hydrogel scaffolds with galactose after 3 days of culture; [D4] HGF loaded nanospheres incorporated on 9:1 P/G hydrogel scaffolds with galactose after 3 days of culture. Magnification – 2000 X. Scale bar – 10 µm
6.3.2 Cell Viability

Live/dead assay was used to evaluate the performance of various P/G hydrogel scaffolds on viability of primary hepatocytes. Lee et al., have reported presence of the live hepatocytes on three-dimensional hydrogel fabricated from decellularized liver ECM for 7 day [4]. Cuboidal morphology of the primary hepatocytes was clearly observed on hydrogel P/G hydrogel scaffolds after 3 and 7 days of culture confirming the ability of the scaffolds to retain the cell viability (Figure 6.2 A1-D2). In addition, presence of higher cuboidal hepatocytes all over the HGF encapsulated galactose-P/G hydrogel scaffolds than galactose containing P/G hydrogels confirms the synergistic effect of galactose and HGF on cell retention. Though the number of hepatocytes was found to be lesser in subsequent time interval (28 days), cells were found to be aggregated on all scaffolds in which HGF nanosphere embedded galactose containing hydrogel scaffolds demonstrated better aggregation than galactose containing P/G hydrogels (Figure 6.2 A3-D3).

The presence of viable cells on all scaffolds up to 28 days confirmed that the scaffolds supported the retention of viable primary hepatocytes for extended culture points, which in turn corroborate with the results of the intracellular activity studies. After 28 days of hepatocyte culture on all scaffolds, galactose containing P/G hydrogels embedded with HGF loaded nanospheres demonstrated hepatocyte spheroids and 8:2 scaffolds showed stronger aggregation of cells as compared to 9:1 scaffold (Figure 6.2 A3-D3). Further, prominent spheroid formation on galactose containing 8:2 hydrogels-HGF than other scaffolds explained the role of both sustained release of mitogen (HGF) and galactose on stronger cell-cell interaction.
Figure 6.2: Live/dead staining on [A1] 9:1 P/G hydrogel scaffold with galactose after 3 days of culture; [B1] 8:2 P/G hydrogel scaffold with galactose after 3 days of culture; [C1] HGF loaded nanospheres incorporated on 9:1 P/G hydrogel scaffolds with galactose after 3 days of culture; [D1] HGF loaded nanospheres incorporated on 8:2 P/G hydrogel scaffolds with galactose after 3 days of culture; [A2] 9:1 P/G hydrogel scaffold with galactose after 7 days of culture; [B2] 8:2 P/G hydrogel scaffold with galactose after 7 days of culture; [C2] HGF loaded nanospheres incorporated on 9:1 P/G hydrogel scaffolds with galactose after 7 days of culture; [D2] HGF loaded nanospheres incorporated on 8:2 P/G hydrogel scaffolds with galactose after 7 days of culture; [A3] 9:1 P/G hydrogel scaffold with galactose after 28 days of culture; [B3] 8:2 P/G hydrogel scaffold with galactose after 28 days of culture; [C3] HGF loaded nanospheres incorporated on 9:1 P/G hydrogel scaffolds with galactose after 28 days of culture; [D3] HGF loaded nanospheres incorporated on 8:2 P/G hydrogel scaffolds with galactose after 28 days of culture.

Magnification - 40 X. Scale bar - 20 µm
Figure 6.3 shows the three-dimensional Z-stacking of primary hepatocytes on galactose containing 8:2 and 9:1 P/G HGF hydrogel scaffolds. The presence of cuboidal hepatocytes all through the scaffold after 3 days of culture confirmed the infiltration of viable primary hepatocytes through the interconnected pores (Figure 6.2 A1–B1). Three-dimensional analysis of Z-stacking after 28 days of culture confirmed the presence of higher and larger spheroid hepatocytes on 8:2 galactose containing P/G-HGF hydrogels in deeper planes than 9:1 galactose containing P/G HGF hydrogels (Figure 6.3 A-D).

Figure 6.3: Three-dimensional Z- stacking after 28 days of culture on [A] 9:1 P/G hydrogel scaffold with galactose; [B] 8:2 P/G hydrogel scaffold with galactose; [C] HGF loaded nanospheres incorporated on 9:1 P/G hydrogel scaffolds with galactose and [D] HGF loaded nanospheres incorporated on 8:2 P/G hydrogel scaffolds with galactose. Magnification - 40 X. Scale bar - 20 μm
6.3.3 Intracellular Activity

Metabolic activity of primary hepatocytes cultured on various scaffolds at different time points (1, 3, 7, 14, 21 and 28 days) was determined using MTS assay in which collagen coated plates were used as control (Figure 6.4). After 1 day of culture, intracellular activity of primary hepatocytes was found to be significantly higher on control when compared to three-dimensional scaffolds \( (p<0.05) \). Primary hepatocytes cultured on HGF encapsulated nanospheres embedded galactose P/G (8:2) hydrogels showed significantly higher metabolic activity when compared to galactose P/G (8:2) hydrogels after 1 day \( (p<0.05) \). It was also observed that the primary hepatocytes on galactose containing 8:2 & 9:1 P/G hydrogel scaffolds showed significantly lesser intracellular activity when compared to cells cultured on respective HGF nanosphere embedded galactose containing hydrogels \( (p<0.05) \). It was also noted that the metabolic activity of primary hepatocytes on control was found to be significantly reduced when compared to three-dimensional scaffolds suggesting that the two-dimensional environment does not support the viability of primary hepatocytes after 3 days \( (p<0.05) \). Feng et al., showed that the rat hepatocytes cultured on TCPS was found to retain 3.13 % of initial seeding density after 15 days of culture due to the death and shedding of the cells [16]. However, all three-dimensional scaffolds demonstrated better cell retention compared to control. After 7 days of culture, the intracellular activity was significantly higher when compared to cells cultured on HGF loaded nanosphere embedded galactose containing hydrogels of 8:2 and 9:1 with respect to galactose containing hydrogels \( (p<0.05) \). In addition, prominent increase in the metabolic activity of primary cells cultured on HGF loaded nanosphere embedded galactose containing 8:2 P/G hydrogels than galactose containing 9:1 P/G hydrogels loaded HGF-nanosphere \( (p<0.05) \).
After 14 days of culture, 8:2 P/G HGF scaffolds demonstrated significantly higher intracellular activity when compared to other scaffolds, which may be attributed to better cell retention on 8:2 P/G-HGF scaffold ($p<0.05$). Shedding of cells due to cell death on control was comparable for 3, 7 and 14 days of post seeding of primary hepatocytes. Though the number of cells seeded was found to be lesser on all scaffolds after 21 days when compared to 7 days, the number of metabolically active cells was found to be significantly higher on three-dimensional hydrogel scaffolds compared to two-dimensional monolayer culture ($p<0.05$).

By 28 days, HGF incorporated 8:2 P/G scaffolds showed better retention of hepatocytes than other scaffolds and maintained higher intracellular activity compared to two-dimensional collagen coated control and other scaffolds. The increase in retention of primary hepatocytes cultured on HGF incorporated 8:2 P/G scaffolds may be due to the presence of galactose and higher content of gelatin resulting in stronger hepatocyte-hydrogel communication.
Figure 6.4: Intracellular activity on all the scaffolds determined by MTS at different time points [* indicates statistical significance between scaffolds and collagen coated plates, # indicates statistical significance between scaffolds (p<0.05)]

6.3.4 Albumin Assay

Functional activity of primary hepatocytes cultured on various P/G hydrogels was determined by evaluating the production of albumin. Figure 6.5 shows the albumin secretion level on all P/G hydrogel scaffolds for a period of 30 days. After day 1, albumin secretion of primary hepatocytes cultured on galactose containing 9:1 P/G hydrogel scaffold was comparable to control (p>0.05). On the contrary, HGF embedded galactose containing scaffolds demonstrated significantly elevated level of albumin at the end of day 1 and day 3 when compared to their galactose containing P/G hydrogels (p<0.05). It was also noted that the albumin secretion was found to be reduced drastically in the control group suggesting the obliteration of hepatocyte
functional activity. Likewise, reduction in albumin secretion was observed on control continuously throughout the culture period, which was evident from the negligible presence of albumin after day 28. After day 21, statistical significance of albumin level was observed on HGF nanosphere embedded galactose containing 8:2 P/G hydrogels with respect to other scaffolds \((p<0.05)\), which clearly indicated the role of HGF and higher content of gelatin on conservation of albumin secreting function of hepatocytes. Though the albumin level was found to be lesser with respect to previous time point, similar pattern (higher in 8:2 P/G-HGF) was observed when compared to other scaffolds. The results highlight the sustained release of HGF and the presence of galactose along with the higher percentage of gelatin in HGF incorporated galactose containing 8:2 P/G hydrogel scaffolds collectively support the functional retention of primary hepatocytes up to 28 days. Chua et al., had maintained the albumin secreting function of rat primary hepatocytes on galactosylated poly(ε-caprolactone-co-ethyl ethylene phosphate) (PCLEEP) nanofibers till 5 days [17]. Three-dimensional scaffolds made of chitosan-hyaluronic acid prepared via layer-by-layer assembly sandwiched between primary hepatocytes and liver sinusoidal endothelial cells exhibited native liver sinusoidal structure, thereby maintaining albumin secretion till 7 days [18]. Kim et al., maintained the albumin secretion of primary hepatocytes up to 20 days on heparin hydrogel through the incorporation of HGF [19].
6.3.5 Urea Secretion

Catabolism of ammonia into urea is the chief function of healthy primary hepatocytes. Hence, urea production of primary hepatocytes cultured on various scaffolds was quantified to determine the functional retention of primary hepatocytes (Figure 6.6). After day 1, HGF encapsulated nanospheres in galactose containing 8:2 P/G hydrogel scaffolds showed significantly higher urea content than other scaffolds and control ($p<0.05$) and this correlates with albumin production. It was observed that the HGF embedded galactose containing hydrogel scaffolds was found to show significantly higher urea secretion than its respective galactose containing hydrogel scaffolds all through the culture period ($p<0.05$). Interestingly it was also noted that all the
scaffolds maintained urea level to greater extent with respect to control till 28 days. In addition, though there was a significant difference observed in the amount of urea secretion between 8:2 and 9:1 P/G–HGF hydrogel scaffolds at all time points, there was no significance difference between the galactose containing P/G hydrogels till 21 days. This has clearly indicated that the controlled release of HGF and higher proportion of gelatin play a dominating role on the retention of functional hepatocytes than the combination of gelatin and galactose alone. Urea secretion of hepatocytes on heparin gel was retained up to 7 days than on PEG gel [20]. HGF loaded heparin based hydrogel was found to show elevated secretion of urea than on unloaded hydrogel for 20 days [19].
Figure 6.6: Urea secretion on P/G hydrogel scaffolds at different time points [* indicates statistical significance between scaffolds and collagen coated plates, # indicates statistical significance between scaffolds (p<0.05)]

6.4 CONCLUSIONS

The primary rat hepatocytes cultured on galactose containing P/G hydrogel scaffolds and HGF loaded nanospheres embedded in galactose P/G hydrogel scaffolds were evaluated to identify the potential material for the retention of functional primary hepatocytes for 28 days. As the culture period increased, primary hepatocytes seeded on two-dimensional collagen coated plates showed drastic reduction in the retention of cells, albumin and urea secretion signifying the importance of three-dimensional scaffolds on the retention of cells. In addition, significantly higher urea and albumin content was observed on HGF embedded galactose containing 8:2 P/G hydrogel
scaffolds when compared to other scaffolds demonstrating the function of HGF, galactose and gelatin on the conservation of functional primary hepatocytes till 28 days. Hence, the present study have demonstrated that the sustained release of mitogen and presence of a recognition motif exhibits stronger cell-matrix communication; swell-ability and wettability mimics the native swollen network of ECM; presence of appropriate pore size and higher porosity with interconnectivity provides effective diffusion of nutrients and cellular infiltrations. In addition, appropriate tensile strength similar to native liver; biodegradability and biocompatibility helps in the spheroid forming ability of hepatocytes, which further aid in the functional regeneration.

6.5 REFERENCES


