4.1 SUMMARY

This thesis work focussed mainly on the surface modification strategy on metallic Ti to enhance the early osteointegration potential of Ti based implants. Considering the shortcomings of the current surface modification techniques in eliciting adequate bone-implant integration, an alternative approach was proposed in the present study, wherein a 3D biopolymeric scaffolding of fibrin/alginate was generated on metallic Ti. The efficacy of such a scaffolding approach in favouring hMSC attachment, proliferation and its subsequent differentiation into functional osteoblasts was investigated in comparison to a conventional Ti. Considering the clinical application of the implants in joint arthroplasties mainly hip arthroplasties, the interactions with blood components such as platelets and RBCs were analyzed in detail. Further, to understand the ability of the modification strategy in enhancing the bone-bonding and new bone formation, in vivo implantation studies were carried out in NewZealand White rabbits. A comparative analysis was made on the osteointegration potential of modified Ti, modified Ti with osteogenically induced ADSCs and conventional commercially utilized Ti rods. The summary of the major conclusions observed in this research work is as below:

- A novel method for fabricating a 3D microporous nanofibrous scaffold structure on metallic Ti has been developed using biodegradable polymers such as fibrin and alginate in combination with dopamine as a crosslinker. The lyophilization step used further to these chemical modifications helped to generate a porous architecture.
SEM analysis revealed microporous nanofibrous architecture of the bio-polymeric scaffolding, which mimics the ECM architecture of native bone.

AFM analysis demonstrated an enhanced surface roughness values for modified Ti compared to control polished Ti. After fibrin/alginate deposition, the surface roughness increased considerably (~600 nm) indicating the successful adhesion of the bio-polymeric layer on Ti surface.

The XPS analysis revealed that the surface scaffolding over Ti is uniform and is masking the Ti substrate. An enhanced N1s and C1s peak was noticed on modified Ti indicating the presence of proteinaceous fibrin and alginate matrix over the Ti. The dominant Ti peak was not found on the modified Ti, whereas it was clearly visible on control Ti demonstrative of a complete coverage of scaffolding on top of Ti.

The water contact angle measurements were found to be lowered on modified Ti compared to control Ti, indicating the enhanced hydrophilicity of the coating applied. Increased hydrophilicity is always associated with improved protein adsorption. The serum protein adsorption was found to be consistently high on modified surfaces compared to control polished surface, highlighting the role of surface modification approaches in modulating the protein adsorption.

The inclusion of a crosslinker dopamine along with the fabrication process was found to enhance the adhesion of fibrin/alginate over the Ti surface. The microscratch analysis revealed the improved scratch resistance behaviour of dopamine treated samples. In dopamine treated samples the LC2, where the delamination occurs have been substantially
enhanced, signifying the influence of dopamine in scaffolding approach.

*In vitro* cell-material interactions using hMSCs collectively demonstrated that the modified Ti plates with fibrin/alginate scaffolding exhibited better cell adhesion, proliferation and subsequent differentiation into osteoblasts compared to control Ti. Actin cytoskeletal arrangements and focal adhesion organization was found to be better on MTi than CTi. The scaffolding also favoured the deposition of calcium phosphate, similar to bony apatite.

Gene expression analysis by qRT-PCR and immunocytochemistry revealed an up-regulated expression of both early and late stage osteoblastic markers, such as, Alkaline Phosphatase (ALP), Runt related transcription factor (RUNX2), Collagen Type I (COL I), Osteopontin (OPN) and Osteocalcin (OC) on modified Ti (MTi) compared to control Ti (CTi) surfaces. Nearly 20 to 70 fold increase in the expression levels of osteo-specific genes were observed when hMSCs were grown on MTi compared to CTi.

The blood interaction studies indicated that a mild activation of platelets occurred on interaction with MTi surfaces with an enhanced expression of secreted platelet protein p-selectin (CD62P), which could be beneficial in promoting the osteointegration of endosseous implants. Apart from this, the surface scaffolding approach did not induce hemolysis or other alterations to the normal coagulation pathway.

The bio-polymeric scaffolding approach also favoured the adhesion, proliferation and differentiation of rabbit ADSCs. The ALP activity and biomineralization was found to be enhanced on MTi compared to CTi suggesting the efficacy of
fibrin/alginate in enhancing the osteogenic differentiation of stem cells into functional osteoblasts.

*In vivo* osteointegration studies demonstrated minimal fibrous tissue encapsulation around modified Ti rods compared to control Ti rods. The presence of new deposited bone with minimal fibrous tissue encapsulation at 12 weeks post implantation around surface scaffolded Ti indicates the potential of the coating process in inducing acceptable *in vivo* response.

The surface scaffolding approach also favoured the osteointegration within a short span of 12 weeks. The interfacial shear strength values were higher for modified Ti rods compared to control rods, indicating the efficacy of the surface scaffolding approach in aiding early bone-bonding around Ti implants.

Our investigations collectively demonstrated that the modified Ti with fibrin/alginate scaffolding exhibited better stem cell adhesion, proliferation and subsequent differentiation into osteoblasts. It offered excellent biocompatibility, enhanced bone apposition and mechanical stability *in vivo*. A significant achievement of our study is the development of a novel yet simple 3D scaffolding process which could be easily translated to modify and improve the biofunctionality of the existing clinical metallic implants. We envision that this method could be a better solution for improving the osteointegration of current orthopaedic implants.
4.2 FUTURE PROSPECTS

In the present thesis, a biopolymeric scaffolding approach on metallic Ti was demonstrated that in turn favoured stem cell adhesion and its differentiation into functional osteoblasts. Though, a detailed *in vitro* evaluation was carried out to study the cell behaviour on modified Ti, a comprehensive molecular investigation on the downstream signalling pathways pertaining to osteoblasts is lacking in our study. Hence the future work might focus on the different signalling pathways influencing the differential cellular response.

Another aspect that might require attention is on the *in vivo* implantation studies. In the present study, a short term implantation of 12 weeks was carried out to study the osteointegration. However, considering the clinical applications, long term implantation studies are needed to validate the efficacy of the implanted material. Hence, long term implantation studies utilizing large animals such as dogs, sheep, goats or porcine models will be carried out in future.

Translation of this surface modification approach to modify other widely utilized orthopaedic implant materials such as titanium alloys, stainless steel, nitinol, cobalt-chromium alloy etc are also under consideration.