ABSTRACT

One major weakness that all orthopaedic implants have in common is the limited osteointegration which results in post-implant loosening. Though biomimetic and biospecific molecules coated on materials were shown to enhance the function of cells, the 3D organization and spatial distribution of these molecules play a critical role in controlling adhesion, growth, viability, differentiation, and function of cells. Here, we report an approach of surface scaffolding on metallic titanium (Ti) using bioactive natural polymers to enhance osteointegration and bone regeneration as an alternative to surface modification. Instead of just depositing the biomimetic molecules, we generated a 3D scaffolding of fibrin and alginate on metallic Ti by a method involving chemical crosslinking with dopamine followed by lyophilization. The developed microporous-nanofibrous scaffold was well characterized using Scanning electron microscopy (SEM), X-ray photo electron spectroscopy (XPS) and Atomic Force Microscopy (AFM). SEM analysis of modified bio-polymeric Ti showed a close resemblance to the native bone extracellular matrix (ECM) in terms of the 3D architecture. Uniform coverage of the scaffolding over metallic Ti was observed when the material was subjected to XPS analysis. The elemental composition analysis confirmed the presence of proteinaceous fibrin and alginate over the Ti, marked by the presence of high intensity carbon and nitrogen peaks. Mechanical stability of the scaffold over Ti substrate analyzed by microscratch testing revealed good adhesion strength of the scaffolding and found to be firmly attached to the Ti surfaces. Further, the effectiveness of the obtained scaffold to act as a better matrix for the attachment and proliferation of stem cells and their differentiation into osteogenic lineage was studied using human Mesenchymal Stem Cells (hMSCs). The alkaline phosphatase (ALP) activity and subsequent mineralization of the scaffolds by the differentiated osteoblasts were enhanced greatly on the modified Ti than control Ti. 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 carried out on MTi and CTi indicated that a mild activation of platelets occurred on interaction with MTi plates, whereas no significant activation was observed on CTi. The enhanced expression of secreted platelet protein p-selectin (CD62P) can aid in promoting bone regeneration via the recruitment of osteoblast progenitors to the fracture site. Moreover, the surface scaffolding approach did not induce any sort of hemolysis or other alterations to the normal coagulation pathway.

Further, to analyze the potential of the surface scaffolding approach in terms of early bone-bonding and new bone formation in vivo implantation studies were carried out in New Zealand White rabbits. Here, we wanted to analyze how the surface scaffolding of metallic Ti alone and in combination with osteogenically induced ADSCs would favour the osteointegration compared to conventional Ti implants. Three experimental groups were introduced (i) Polished Ti rods (CTi), (ii) Ti rods after fibrin/alginate scaffolding (MTi) and (iii) in vitro developed bony layer on fibrin/alginate Ti (MTi + cells - Fibrin/alginate Ti rods subjected to osteogenic induction with rabbit ADSCs for a period of 21 days to generate bony layer on surface). After the 12 week study period, significantly enhanced bone apposition was found around MTi + cells and MTi implants. No necrosis or inflammation noticed around any of the implanted Ti rods. The quantitative evaluation of the interfacial shear strength at the implant-host bone also revealed a high shear strength value for modified Ti rods compared to CTi. Thus, we found out that along with the surface modification, a pre-integrated surface bony layer over the metallic Ti can further advance the osteogenesis and early bone-bonding. Hence, the generation of biomimetic 3D scaffolding on Ti surface seems to be a potential approach for improving osteointegration of orthopaedic implants in lieu of or in combination with surface modification.