Advanced Materials Science Research

Extended Abstract Open Access

Effect of Offset/ Gradient Melt Electrowritten (MEW) PCL Scaffolds in Bone Regeneration

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Biomaterial scaffolds engineered to promote osteogenesis which can subsequently remodel in an identical fashion to that of natural tissue, is an excellent clinical strategy for treating the defects of bone in the maxillofacial region. This study has developed, using a novel melt electrowriting (MEW) technique, various graded porous polycaprolactone (PCL) scaffolds able to imitate the bimodal structure of cortical and cancellous bone tissue in terms of their morphological pore structure. These scaffolds were subsequently shown to facilitate the proliferation of osteoblasts with significant alkaline phosphatase (ALP) activity. Scaffolds with a staggered architecture i.e. those where the corresponding fibers in different layers are offset horizontally during the printing process, increases the number of contact points, enables larger pores, facilitates cell attachment and creates a highly porous structure with interconnected networks favourable for improved cell migration and vascularization. MEW is a relatively new technology that fills a gap between conventional fused deposition modelling 3D printing and solution electrospinning by affording significantly better control over the fabrication of the porosity in scaffolds. This is due to the highresolution during printing of the fibers which enables small pore sizes in the desired porosity. PCL polymer with its advantages of less immunoreactivity following implantation and mechanically suitability for the support of bone cells, has flexibility in design and is widely used in bone applications. However poor bioactivity and cellular affinity as well aslong-term degradation issues with PCL have been reported.

A promising solution to overcome these drawbacks is fiber surface modification such as coating with bioactive inorganic components. hydroxyapatite (HAP) is the most stable form of calcium phosphate (CaP) used to improve the hydrophobic characteristics of PCL and enhance the binding affinity to the host tissue and promote new bone growth. Furthermore, CaP coating also accelerates the degradation rate of PCL. While the CaP can directly regulate the bone regeneration process through the release of phosphate and calcium ions, to our knowledge, no study has compared the differing methodologies used to prepare PCL fibers for CaP coating. Similarly, there is limited research evaluating the influence of offset and gradient porosity structured scaffolds during bone healing and vascularization.

Therefore, in the first experimental part of this study, we evaluated the stability of CaP coated MEW PCL scaffolds following pretreatment with either Argon-Oxygen (Ar-O2) plasma or sodium hydroxide (NaOH). In our study, Ar-O2 plasma modification of the PCL prior to immersion in simulated body fluid for 1 hr, resulted in a uniform coating of CaP on the electrowritten fibers. This improved the mechanical properties of the scaffolds by increasing the tensile modulus. Regarding the structure of the coating, halite (NaCl) crystals were found in the coated scaffolds pretreated with NaOH whereas a mixture of HAP and tetracalcium phosphate (TTCP) crystals were found following plasma treatment. This confirmed the stability of the CaP minerals would be higher than NaOH pre-treated coated scaffolds due to the lower solubility of the TTCP and HAP in comparison to the halite structures. This study showed that the plasma modification is more applicable for further study of MEW PCL scaffolds in bone regeneration applications. The second part of the study aimed to first examine the physical and mechanical properties of MEW CaP-coated PCL scaffolds with various homogeneous (250, 500 and 750 µm) and heterogeneous (offset.30.70, offset.50.50 and gradient) pore structures. We also evaluated the scaffold biocompatibility and the effect of these porous architectures on human osteoblast growth and proliferation. Physically, the offset.30.70 scaffold was shown to significantly increase the surface area while 250 µm homogeneous scaffolds were shown to improve the mechanical properties of the MEW scaffolds with the offset.50.50 scaffold having the highest elongation at break result. The scaffold with a 250 µm pore size stimulated cell seeding efficiency however the highest levels of cell infiltration and proliferation were observed in the gradient scaffold structures following 30 days of culture. This study demonstrated that the architectures of offset and graded porosity scaffolds can be efficient in aiding the migration and proliferation of osteoblasts on MEW PCL scaffolds.

For the next step, we assessed the impact of heterogeneous and homogeneous porous scaffolds on osteoblast mineralization and the expression of bone-associated markers. The gradient porous architecture significantly increased ALP activity in osteoblasts cells. In addition, significant expression of osteocalcin was observed by immunostaining. Moreover, the human osteoblast cells were shown to enhance matrix mineralization in offset.50.50 scaffolds. In particular, the expression of associated genes linked to mineralized-tissue formation stage, including osteocalcin and osteopontin were elevated in offset and gradient scaffold structures, thereby able to support the maturation of osteoblast cells essential for initial osteogenesis and mineralization. Based on these findings, the final section of this research was designed to study the capability of these coated scaffolds to promote osteogenesis in vivo. Offset.50.50, 250 µm, 500 µm and two gradient (250top-500middle-750bottom and 750top-500middle-250bottom) scaffold structures were implanted into 5 mm calvarial defects created in Wistar rats to evaluate the neovascularization and bone formation after 4 and 8 weeks of healing. Micro-CT andhistological analysis revealed the highest bone volume in the grad.250top scaffold. Having the larger pores facing the dura mater lead to better permeability of O2 and nutrients in the that resulted in better cell migration, vascularization and bone growth compared to the 500 µm scaffolds and the grad.750top scaffolds. More soft tissue and incomplete newly formed bone was seen in the scaffolds with smaller pore sizes (250 µm and offset.50.50) 8 weeks post-implantation. Although subsequent immunohistochemical investigation showed the expression of all bone markers examined (ALP, Col I, BMP-2, OCN, OPN) in all the scaffold groups, intense staining for osteocalcin was particularly observed in grad.250top and offset.50.50 scaffolds. The expression of vascular endothelial markers (VEGF, vWF, CD34) in all the scaffold groups along with low intensity CD105 expression confirmed angiogenesis and the remodeling phase of bone repair in the defect site.

In conclusion, our research has demonstrated that both homogeneous and heterogeneous porosity within MEW CaP-coated PCL scaffolds improved the mechanical properties of the scaffold structures and stimulated greater interaction and proliferation of osteoblast cells. The offset.50.50 scaffold was the most suitable candidate to increase mineralization in-vitro. Bone healing studies in the rat model showed bone regeneration also among the other porous scaffolds with the most bone formation and angiogenic action observed in the gradient.250top architecture scaffolds. Future studies are now required in large animal models to evaluate the osteogenic capacity ofthis gradient architecture in both load and non-load bearing applications.