PIG MANDIBULAR RECONSTRUCTION BY ADIPOSE-DERIVED STEM CELLS AND FUNCTIONALIZED LASER-SINTERED POROUS PCL SCAFFOLD WITH PLATELET RICH PLASMA: IN-VITRO AND IN-VIVO STUDY

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Introduction:
Polycaprolactone (PCL) is a biodegradable polymer with potential applications for bone and cartilage repair. In this work, the three-dimensional and porous PCL scaffolds were designed and fabricated via selective laser sintering (SLS). The aim of this study is to evaluate the osteogenic potential of pig adipose-derived stem cells (pASCs) in functionalized laser-sintered PCL scaffold with platelet rich plasma (PRP).

Method:
In the in-vitro study, the laser-sintered PCL(lsPCL) scaffold was seeded with ASCs. It was divided into three groups. Group I: lsPCL/pASCs were cultured in normal medium. Group II: lsPCL/pASCs were cultured in osteogenic medium. Group III: lsPCL/PRP/pASCs were cultured in osteogenic medium. Alkaline phosphatase activity(ALP), RT-PCR of ALP, osteocalcin, Cbfa1 was used to assess the osteogenic ability. SEM and Inverted Fluorescence microscope were used to observe the interaction between scaffold and cell. Energy dispersive spectrum (EDS) was used to analysis the mineralization in each group. In in-vivo study, the 3 cm porcine mandible defect was created and it was reconstructed with either lsPCL only or lsPCL/PRP/pASCs. CT was used to evaluate the bone regeneration 3 months, 6 months after operation. The Young’s modulus of both groups was measured and compared with normal bone. H&E stain and IHC stain of osteocalcin, collagen type I were done for confirmation of bone regeneration.

Results and Discussion:
1) In in-vitro study: The bone formation includes three stages: cell proliferation, extracellular matrix maturation and mineralization. High expression of ALP will expressed first, followed by extracellular matrix maturation and then the ECM mineralizes finally. Thus the ALP activity and calcium deposition were two major markers for bone formation. The expression of non-collagenous proteins is also a strong evidence of bone formation. In this study we use the bone specific marker such as core-binding factor α 1(Cbfa1), ALP, and osteocalcin (OCN).

Fig 1-4 showed alkaline phosphatase activity and RT-PCR all showed the best osteogenic potential in group III (lsPCL/PRP/pASCs) comparing with other groups.

Fig 6A detailed the porous structure of the laser-sintered PCL and the pore size is around 300-400 µm. Fig 6B and 6D showed that the pASCs attached on the lsPCL scaffolds remained in a more or less round shape in NM
and OM respectively. However, ASC attached on the lsPCL/PRP scaffolds showed elongated and well spread morphology. The Fig 6C and Fig 6F provides the elemental analysis of mineralization by EDS. Fig 6C showed the lsPCL/pASCs that were incubated in NM produced no mineralization. In contrast, Fig 5F demonstrated that the lsPCL/pASCs in OM and lsPCL/PRP/pASCs in OM all showed calcium deposits.

The Live /Dead test in Fig 6 also demonstrated well survival of pASCs in all three groups. However, the morphology of pASCs were round shape in Group I (Fig 6A) and II (Fig 6B) which was compatible to the result of SEM. In contrast, the pASCs demonstrated well spreading morphology in group III. (Fig 6C)

(Fig1) ALP/MTS in each group

(Fig2) RT-PCR of Cbfa1 expression

(Fig3) RT-PCR of OCN expression

(Fig4) RT-PCR of ALP expression

(Fig5) SEM and EDS analysis of mineralization

(Fig 6) Live and Dead test

2) In in-vivo study, both groups showed new bone regeneration in lsPCL scaffold. However, the bone density was less and loose in lsPCL group and the Young’s modulus (Fig 7) was only 30% of normal bone. In contrast, the continual and firm bone formation was found in lsPCL/PRP/pASCs group and the Young’s modulus was 90% of normal bone.
The Masson’s trichrome stain showed that the new bone formation in group I was loose among IsPCL scaffold. (Fig 8) The immunohistochemistry of collagen I and OCN all showed positive in new bone formation area with brown stain. In the non-bone formation area on 3D CT, the Masson’s trichrome showed no blue collagen and bone stain and IHC of collagen I and OCN also demonstrated negative stain.(Fig 8)

In contrast, the Masson’s trichrome stain showed tight and dense new bone tissue in group II. The IHC also showed positive collagen type I and OCN stain. This further confirmed the new generation tissue was bone. In the non-bone formation area on 3D CT, we found small scattered blue bone islands in Masson’s trichrome stain. IHC of collagen type I and OCN also showed positive brown stain among PCL fiber. It means the active bone formation was progressing in these non-bone formation areas on 3D-CT. (Fig 9)

(Fig 7) Young’s modulus

(Fig 8) Histology for mandible reconstruction with IsPCL only

(Fig 9) Histology for mandible reconstruction with IsPCL/PRP/ASCs

Conclusions:
In conclusions, modification of the IsPCL scaffold by PRP indeed enhances the affinity and osteogenic potential of pASCs. The pASCs spread well in the PRP/IsPCL scaffold. The ALP activity, mineralization, real-time PCR of Cbfa1, ALP, and OCN were also performed well in this group. The animal study also proved the dense bone formation with stiffness close to normal bone.¹

Reference:²