Biodegradable hybrid scaffolds with PDRN/BMP2 nanocomplex using human fetal derived mesenchymal stem cells for bone regeneration

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Statement of Purpose: Poly(lactic-co-glycolic) acid (PLGA) has been widely used as a biomedical material due to its great biocompatibility and biodegradability. However, there are considerable obstacles because the degradation byproducts of PLGA result in an acidic environment of implanting site. And, the synthetic polymer has a weak mechanical property for medical applications, especially for bone regeneration. To overcome these limitations, the PLGA was combined with ricinoleic acid-modified magnesium hydroxide (mMH) and bone-extracellular matrix (bECM). The magnesium hydroxide has been used as a pH neutralizing agent. In addition, the bECM not only improves mechanical properties of synthetic polymer with its inorganic composition, but also acts as an osteoconductive materials for the proliferation of bone. We have identified that human fetal-derived mesenchymal stem cells (hfMSCs) showed a superior potential for osteogenic differentiation than other MSCs. Moreover, bone morphogenetic protein-2 (BMP2), known to induce osteogenic differentiation of MSCs, formed nanocomplex (NC) with bioactive polydeoxyribonucleotide (PDRN) to enable the sustained release of BMP2. Taken together, the hybrid scaffold immobilized with NC has high potential effects on bone tissue regeneration. With these results, our approach would have great bone regeneration capacity in comparison with other biomaterials.

Methods: The PLGA/MH/bECM hybrid scaffolds were prepared using the freeze-drying method with micro-ice particles as a porogen. The mechanical property of scaffolds was measured using a universal testing machine (UTM). The size and zeta potential of NC were obtained using dynamic light scattering (DLS) instrument. The surface morphology of the scaffold was visualized using a field emission scanning electron microscope (FE-SEM) and the loading efficiency of BMP2 on the scaffold was evaluated by human BMP2 ELISA kit. The quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR) was utilized to evaluate osteogenesis-related factors after seeding hfMSCs on the scaffold. *In vivo* angiogenesis property was monitored using microfil. Furthermore, various analyses, including computed tomography (micro-CT), histological analysis, and immunohistochemistry (IHC), were performed to evaluate new bone formation in *in vivo* system.

Results: The mean size of NC was ranged from 90 to 140 nm depending on the ratio of PDRN. And, the zeta potential, an indicator of ionic interactions between BMP2 and PDRN, was also changed with the amount of PDRN. The zeta potential of BMP2 showed positive charge, but changed to negative after combining with PDRN. At the optimized condition for NC formation, the mean size and zeta potential were 92.9±8.48 nm and 31.4±2.46 mV, respectively. The ionic strength between BMP2 (+) and PDRN (-) enabled sustained release of BMP2 for 60 days. We have identified that the PLGA/mMH/bECM/NC hybrid scaffold prevented inflammatory response and improved osteoconductive and osteoinductive capacity with hfMSCs. Moreover, the results for *in vitro* analyses showed that the PLGA/mMH/bECM/NC hybrid scaffold is a good candidate to facilitate osteogenesis, angiogenesis, and anti-inflammatory responses.

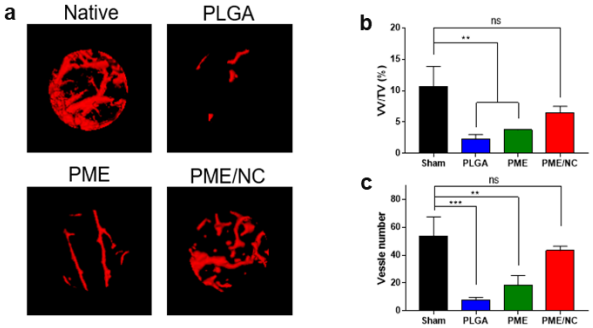

Figure 1. Angiogenic and osteogenic effects of the hybrid scaffolds *in vivo*. (a) Microfil images and quantification of (b) vessel volume density, and (c) vessel number.

Finally, the synergistic effects of bone repair and vascularization were demonstrated in a critical-sized rat calvarial defect model. Based on these results, an advanced hybrid scaffold, PLGA/mMH/bECM/NC, can be suggested as an integrated bone graft substitute for bone regeneration.

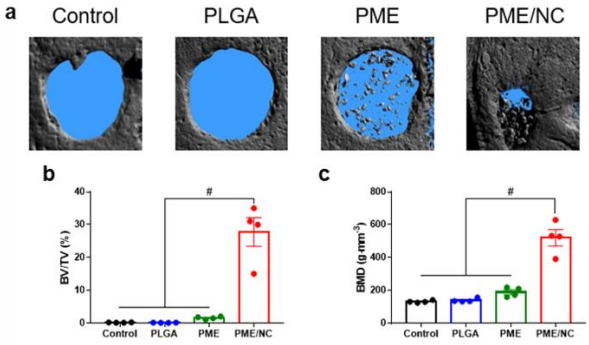

Figure 2. (a) Representative 3D constructed images of calvarial defect using micro-CT and quantification of (b) bone volume density and (c) bone mineral density.

References: Bae, Soon Eon, et al. J Control Release 2012;160:676-684.