Enhancing Bone Regeneration by Functional Microenvironment of Bone-Like Scaffold

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Statement of Purpose: Although advances have been made in tissue engineering regarding the use of cells, required growth factors, and various scaffolds with- or without bioreactors, the technical challenge of achieving bone regeneration in large segmental defects remains unmet. The absence of a functional microenvironment in most grafts has hampered the potential for clinical applications and the success of bone tissue engineering, especially, for large synthetic bone grafts. A functional microenvironment that allows cells infiltration and habitation is required for successful bone regeneration. Moreover, the constructs should provide an adequate vascular-like structure to supply oxygen and nutrients to the new tissue until vasculogenesis occurs. Osteogenesis and vasculogenesis have been reported to be highly dependent upon the local oxygen tension which plays a critical role in the differentiation of mesenchymal stem cells (MSCs). To this end, we investigated the functional outcomes of a novel trabecular-like scaffold in terms of the physicochemical microenvironment for enhancing bone regeneration via osteogenic and vasculogenic differentiation of MSCs.

Methods: In order to fabricate a scaffold with fluid/gas-communicating channels that are observed in natural bones, we endeavored to make micro-channels and nano-pores (MCNP-scaffold) within the trabecular septa. We used a polyurethane template coating technique with nano-sized hydroxyapatite (HA) powders in a distilled water-based slurry. To investigate the bone regeneration competence of the novel MCNP-scaffold, we created a 2 cm-long segmental defect in a canine tibia and repaired the defect with the MCNP-scaffold. The incorporation, new bone formation, and vascularization properties of the MCNP-scaffold were investigated by radiology, micro-CT, dynamic histomorphometry, and histology.

Results: SEM images showed 1) fully interconnected macro-pores (300~400 um) trabecular structure, 2) intra-septal micro-channels (25~70 um), and 3) nano-pores (100~400 nm) on its surface. These three components are intended to mimic human trabecular bone networks and to provide body fluid access, diffusion, nutritional supply, gas exchange, communication around the bone, and cell anchorage. By nature, channels with diameters on the micron scale exhibit highly effective fluid absorption. Our novel MCNP-scaffold demonstrated terrific absorption and retention capacity of bone marrow compared to any other conventional scaffold or bone. This capillary motion via the micro-channel structure may be the primary reason for the even distribution of cells throughout the MCNP-scaffold. A 2cm-long segmental bone defect in a beagle tibia showed detailed microstructures with micro-channels and nano-pores, which demonstrated an even distribution of cellular mobilization, settlement, proliferation, differentiation throughout the MCNP-scaffold, and successful incorporation with host bone. Strikingly, we confirmed the presence of cells and mineralization of new bone formation in the micro-channels, suggesting that the micro-channels not only enhance fluid ingress but also provide additional space for cell habitation. Furthermore, strong HIF1 and VEGF expression throughout the microenvironment of the MCNP-scaffold was clearly identified *in vitro* as well as *in vivo*.

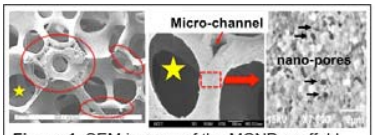

Figure 1. SEM images of the MCNP-scaffold strut resembling trabecular bone composed of macro-pores (star), micro-channels (red circle: formed into the trabecular septa), and nano-pores (black arrow: exist on the surface of the trabecular)

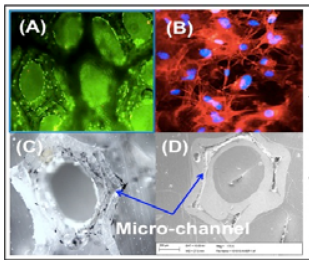

Figure 2. Attachment and migration onto MCNP-scaffold surface and micro-channel: (A) live-dead cell staining; green fluorescent dye to stain live cells and red to stain dead cells (x20), (B) fluorescence image showing cell nuclei (blue) and actin filaments (red) (x100), (C) infiltration into micro-channel (x40), and (D) SEM of micro-channel.

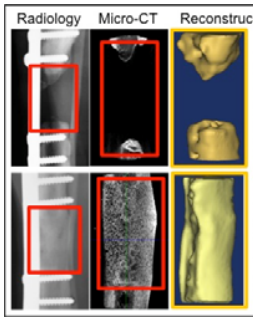

Figure 3. Top images represent no treatment (defect only; negative control) and bottom images represent MCNP-scaffold treatment (experimental) at 8 weeks post-surgery in 2cm-long canine tibia defect, respectively. Red rectangle denotes region of interest.

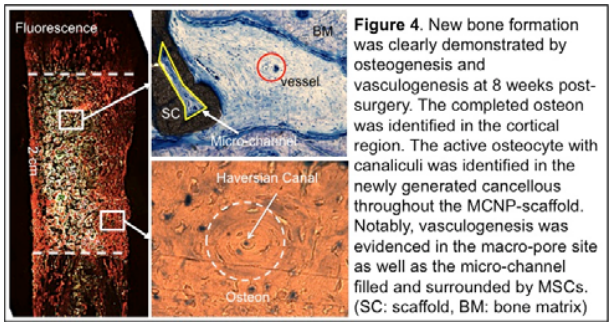

Figure 4. New bone formation was clearly demonstrated by osteogenesis and vasculogenesis at 8 weeks post-surgery. The completed osteon was identified in the cortical region. The active osteocyte with canaliculi was identified in the newly generated cancellous throughout the MCNP-scaffold. Notably, vasculogenesis was evidenced in the macro-pore site as well as the micro-channel filled and surrounded by MSCs. (SC: scaffold, BM: bone matrix)

Conclusions: Highly efficient in cell ingress and habitation, the MCNP-scaffold was successfully developed using a polyurethane template coating method to fertilize tissue engineering. Combinatorial effects of internal macro-, micro-, and nano- structures result in a host-adapting construct that enhances cell migration and habitation from the host bone marrow throughout the entire scaffold. Thereafter, completed bone regeneration was accomplished by the MCNP-scaffold in a canine tibia segmental defect. Given these findings, we posit that our novel MCNP-scaffold has incredible potential for bone repair in massive skeletal defects.