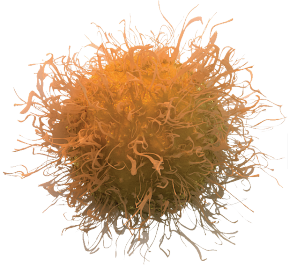

It's Time to Kick Out the Conventional View of Bone Healing — There's a New Kid On the Block.

LUKAS VAN DIJK, FLORENCE DE GROOT AND CHARLIE CAMPION

Conventional wisdom teaches that there are three cell types involved in normal bone homeostasis: osteoblasts – the builders – who lay down osteoid; osteocytes – the supervisors – who direct bone formation and remodeling through mechanotransduction; and osteoclasts – the demolition crew – who resorb bone tissue according to Wolff's law. The reality, as we now know from countless years of research by world-renowned scientific experts such as David Hume (UK); Allison Petit (Australia), Stephen Badylak (USA), Richard Miron (Switzerland), and Huipin Yuan (Netherlands), is that the cells of our immune system play a far greater role in bone maintenance and regeneration than we had previously given them credit for.

Macrophages are pivotal in bone regeneration, as they are key modulators of a normal wound healing cascade.[1,2] Macrophages are highly plastic—they can adopt a pro-inflammatory phenotype (known as "classically activated" or "M1" macrophages), an anti-inflammatory phenotype (known as "alternatively activated" or "M2" macrophages), or exist in an intermediate state in the spectrum between these phenotypes.[2,3] Zhang et al. have clinically demonstrated the contribution of M2 macrophages to bone regeneration; they found a correlation between accelerated bone healing and a greater M2 population in patients with clavicle fracture and concomitant traumatic brain injury.[4]

Research in this emerging field of osteoimmunology has reached a tipping point: not only are we able to observe the effect of our immune system on bone healing, but we are also able to harness the immune response to stimulate the formation of bone instead of scar tissue. The implications of this research in clinical application are far-reaching, not least in spine surgery where surgeons constantly battle against the risk of pseudarthrosis. How can immunomodulation be utilized to target pseudoarthrosis incidence and treatment? Using bone grafts as an example, the theory can be explained in three simple steps: Polarize; Regenerate; Propagate.

(1) Polarize: naïve human-derived immune cells (monocytes) are differentiated to macrophages that are subsequently polarized, by needle-shaped features that are submicron in size and located on the graft surface, into the pro-healing and anti-inflammatory M2 macrophage phenotype (Figure 1).

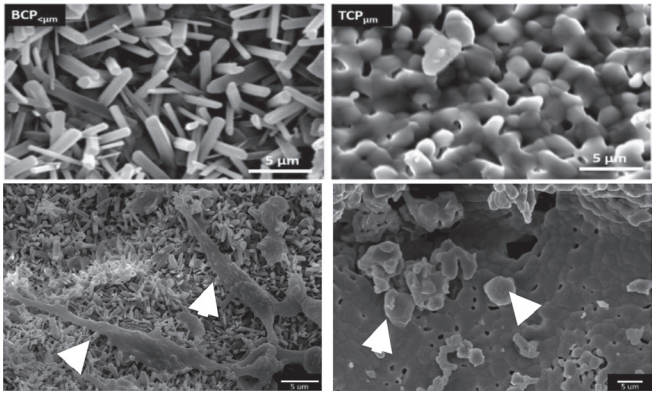

Figure 1. Biphasic calcium phosphate (BCP<µm) with needle-shaped submicron topography (top left; MagnetOs™; Kuros Biosciences); Tricalcium phosphate (TCPµm) with a micron-sized grain-shaped surface (top right; Vitoss®; Stryker Corp.); macrophages (arrows) that have differentiated from human-derived monocytes (isolated from donated buffy coats) after 24 hours culture on BCP<µm (bottom left) or TCPµm (bottom right). Elongated, connected cells indicate the pro-healing phenotype, while smaller, spherical cells indicate the pro-inflammatory phenotype. Data on file at Kuros Biosciences.

(2) Regenerate: anti-inflammatory M2 macrophages liberate mesenchymal stem cells from the tissue matrix and upregulate osteogenic cells via the prostaglandin pathway, causing them to differentiate into osteoblasts (Figure 2 & Figure 4), which begin laying down osteoid. Endothelial cells are stimulated to form angiogenic tube networks (Figure 3), that deliver nutrients and yet more osteogenic cells to the site of repair.

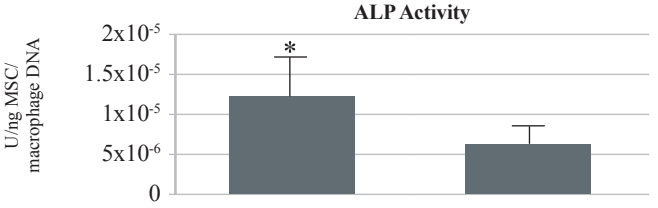

Figure 2. Alkaline phosphatase (ALP) expression by human-derived mesenchymal stem cells following 10 days' culture in media conditioned by macrophages cultured on either BCP<µm (left) or TCPµm (right). ALP expression is an early-stage marker for osteogenic differentiation. (*= $p<0.05$). Data on file at Kuros Biosciences.

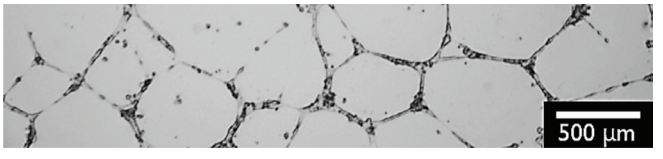

Figure 3. Angiogenic tube networks formed by human-derived endothelial cells following 10 days' culture in media conditioned by macrophages cultured on BCP<µm.

(3) Propagate: the interaction between the surface of the material and circulating osteogenic cells triggers bone formation, meaning that bone propagates in the core as well as throughout the graft (Figure 4), rather than only from the outside-in via creeping edge repair (osteoconduction from the host bone, which is the primary mode of action for conventional grafts—Figure 4C).

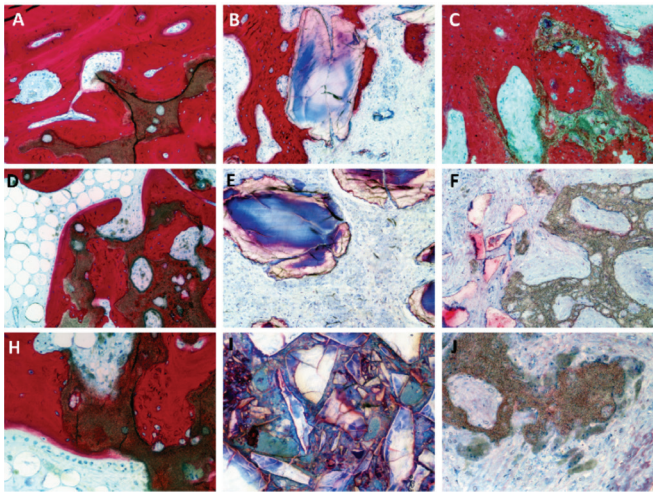

Figure 4. Representative, high-magnification micrographs from histological sections of spinal levels treated with BCP<µm (A,D,H), Bioglass (Novabone®; B,E,I), and TCP combined with Bioglass (Vitoss BA2X; C,F,J). Micrographs were obtained from regions near the host transverse process (A-C) and the core of the graft within the central region of the intertransverse fusion (D-F). Images H-J show cellular processes observed near the graft materials, including osteoblasts depositing osteoid against BCP (H), cell-mediated resorption of BCP and TCP (H,J), and large, foreign body giant cells in regions with fragmented Bioglass (I): Images from Van Dijk L, et al. Clin Spin Surg 2020;33(6): E276-E287.

Bone propagation in the core of the graft reduces the risk of a zone of fibrous tissue forming between two opposing fronts of bone, as might be the case in critical-sized defects such as those found in the posterolateral gutters of the spine.

In challenging and clinically relevant ovine models of spine fusion, in which graft was laid between transverse processes and not over the facets, either pro-healing or pro-inflammatory tissue responses were observed for grafts with or without the specific submicron-sized needle-shaped surface features, respectively (Figure 4). In these preclinical models, there was an improvement in fusion from 33% for the grafts without an augmented surface to 100% for grafts with the submicron-sized needle-shaped surface.

In human clinical cases, we observe the development of mature bridging bone after application in the posterolateral spine (Figure 5). Computed tomography (CT) analysis demonstrates a transition from a granular nature of the graft to a trabeculated bony structure that is encased in an outer pseudo-cortex.

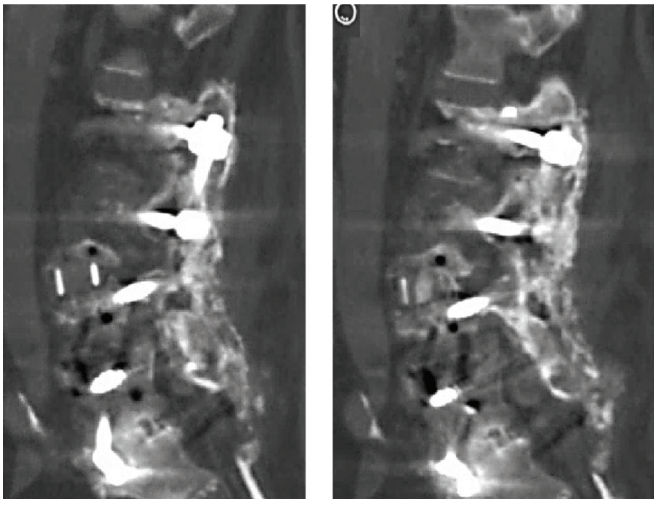

Figure 5. Clinical CT's of a patient 10 months postoperative who underwent a posterior spinal fusion with two-level ALIF at L4-L5 and L5-S1 and two-level XLIF at L2-L3 and L3-L4. Images courtesy of Dr. R. Todd Allen, M.D., Ph.D., University of California San Diego, San Diego, CA, US.

Conclusion: Research in the field of osteoimmunology is exploding, and researchers across clinical specialties are evaluating the application of immunomodulation in biomaterials science for multiple medical applications. The clinical utility of these research findings in spinal surgery is clear—an estimated 150,000 patients will experience a pseudarthrosis in the spine each year in the US, of which over one half will require a second operation to adequately resolve their clinical symptoms.

References: 1. Italiani P, Boraschi D. Front. Immunol. 2014 Oct 17;5: 514. doi: 10.3389/fimmu.2014.00514; 2. Klopfleisch R. Acta Biomater. 2016 Oct 1;43: 3–13. doi: 10.1016/j.actbio.2016.07.003; 3. Murray PJ, et al. 2014 Jul 17;41(1): 14–20. doi: 10.1016/j.immuni.2014.06.008; 4. Zhang R et al. J Orthop Surg Res. 2018 Aug 29;13(1): 213. doi: 10.1186/s13018-018-0926-7.

Disclosures and COI: the authors are full-time employees of Kuros Biosciences and hold stock options in the parent company. Disclaimers: results from in vitro or in vivo laboratory testing may not be predictive of clinical experience in humans. MagnetOs is cleared for stand-alone use in the extremities and as an autograft extender in posterolateral spine.

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