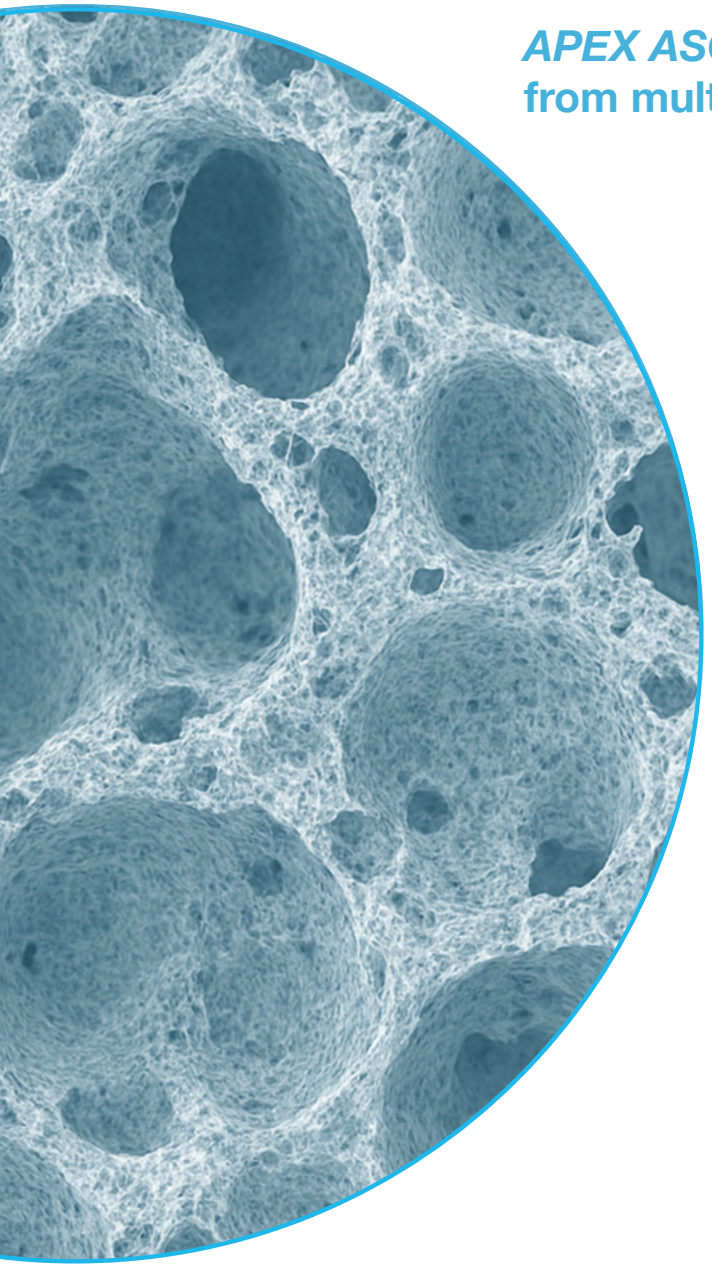
APEX ASG
ADVANCED SYNTHETIC GRAFT



***APEX ASG* is a novel, 4th generation, fully synthetic bone graft made from multi-pore structure ceramic granules, and bio-inert synthetic gel.**

It is the <u>only</u> synthetic bone graft product that can mimic the natural bone remodeling process down to the cellular level.

Exceptional handling characteristics

- Ready to use - no mixing required
- Moldable putty is easy to form
- Excellent cohesion to host bone
- Does not wash-out during irrigation

Provides the ideal environment for rapid osteointegration through natural regulated bone remodeling.

- Novel MultiPore ceramic properties facilitates rapid osteocyte population throughout the porous structure
- Resorption of ceramic is regulated by natural bone remodeling process, and does not rely on its chemical composition.

APEX ASG was developed around the Homogeneous Osteocyte Population concept to achieve robust osteointegration at the implant site.

The Homogeneous Osteocyte Population theory reframes bone fusion from a purely structural problem ("bridging the gap") to a functional cellular integration challenge: the creation of a single, coherent osteocyte syncytium that behaves as one mechanobiological organ.

Successful, durable bone formation and integration depend on the establishment of a continuous, functionally synchronized osteocyte network.

Why a successful bone graft procedure is dependent upon a homogeneous osteocyte population

- The average osteocyte density in a healthy adult is about 25,000 per cubic millimeter[1].
- **Osteocytes trigger coordinated bone healing responses at early phase of fracture healing.** This is followed by the expression of cell growth factors such as BMP-2 and cysteine-rich angiogenic inducers associated with neoangiogenesis, chondrogenesis and callus formation during the intermediate phase[2].
- **Osteocytes exert their effects on bone remodeling via direct cell-to-cell contacts, and by the release of soluble mediators that control the recruitment, differentiation and activity of osteoclasts and osteoblasts.** If osteocyte density is reduced by only five percent however, the amount of the soluble mediators will reduce by fifty percent[3]. Therefore, targeting a robust osteocyte population represents an innovative approach for bone grafting in fusion procedures.

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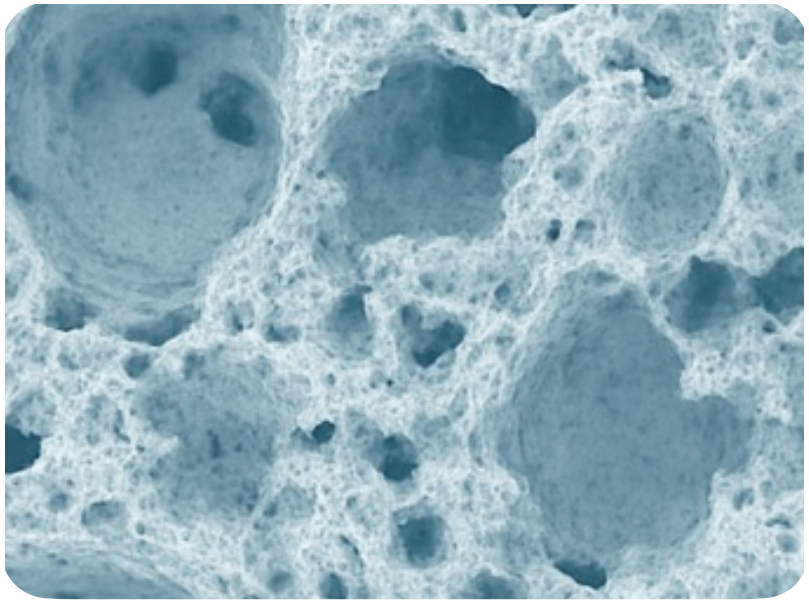
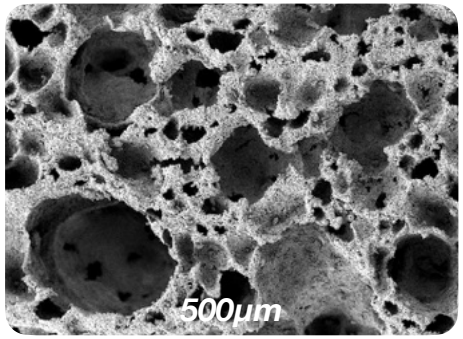
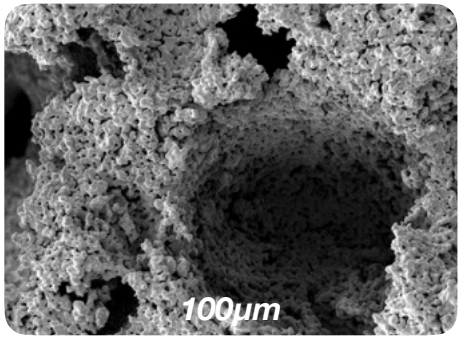


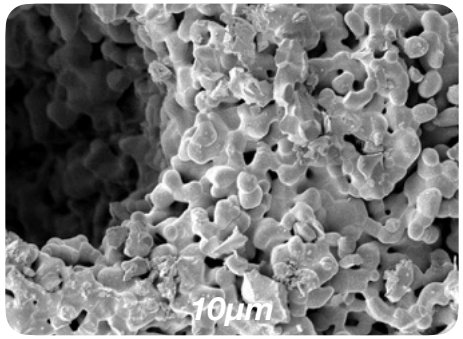
Optimal porous structure and high free surface energy is vital for successful osteointegration.

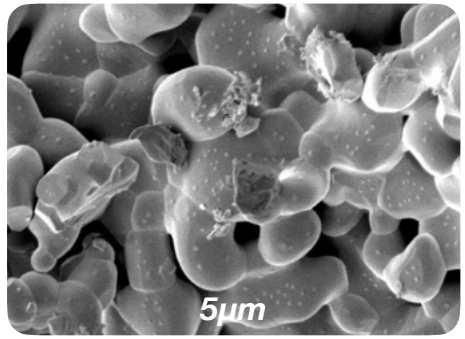
- The composition of the ceramic granules is biphasic hydroxyapatite (80%), β–tricalcium phosphate (TCP), and 0.8% SiO_2. The granules are engineered into a unique, interconnected multi-pore structure.
- The osteoingrowth and the resorption rate of *APEX ASG* is controlled by the natural host bone remodeling process, and not the chemical composition the component materials.

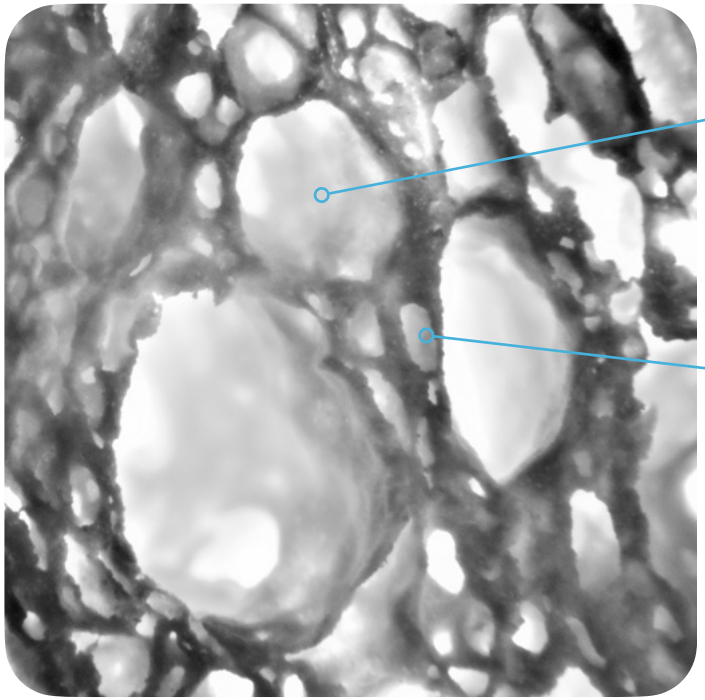
APEX ASG at progressive SEM magnifications









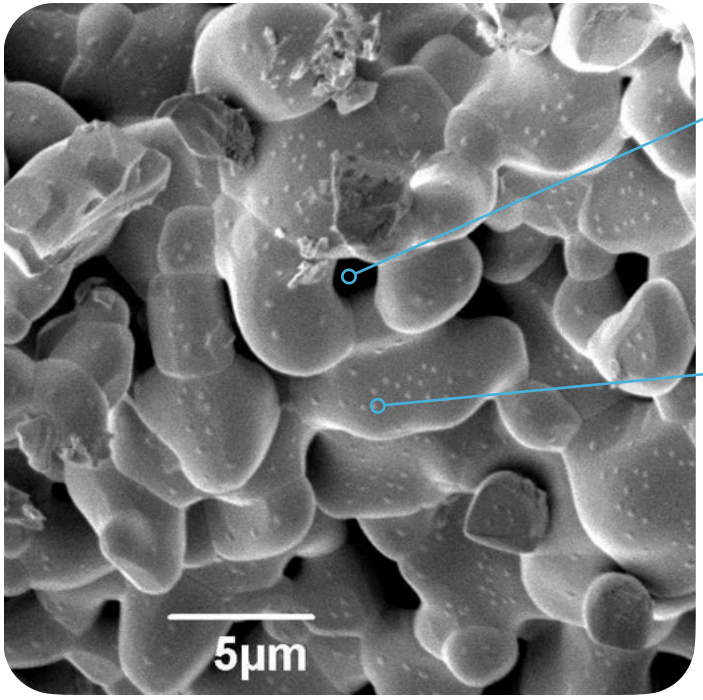


Macropores >100µm

Allows osteogenic cells to easily move through the entire ceramic structure. Controllable macropore orientation aids organization of lamellar bone formation.

Midipores 10~100µm

Midipores between the macropores eliminate ‘thick connecting wall, (increasing porosity without compromising the mechanical strength of the scaffold) and facilitate nutrient flow. The size of the Midipores encouraged facilitate osteoblast migration, and the formation of osteocytes inside the ceramic body.



MicroSpaces 1~10µm

MicroSpaces with high surface free energy point fusion stimulate cell attachment and the release and accumulation of cell growth factors.*

Nano SiO_2 particles

Nano SiO_2 particles create the high surface free energy necessary to stimulate cell attachment and the release and accumulation of cell growth factors.*

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High surface free energy is critically important for promoting bone healing, particularly for orthopedic implants. A high-energy surface promotes osseointegration, the process by which an implant forms a direct, stable bond with the surrounding bone.

High surface free energy on a hydrophilic surface enhances the initial biological reactions that are crucial for successful bone integration. The process unfolds in several key stages:

1. **Rapid protein adsorption:** Immediately upon implantation, the surface is coated with a layer of blood proteins, including fibronectin and vitronectin. **High-energy, hydrophilic surfaces attract these proteins** more readily than low-energy, hydrophobic ones, influencing which specific proteins adsorb and in what conformation.

2. **Enhanced osteoblast attachment and proliferation:** **Osteoblasts attach more effectively and spread more readily on the conditioned protein layer of high-energy surfaces.** In contrast, low-energy surfaces can cause cells to remain rounded and divide at a lower rate.

3. **Increased osteogenic differentiation:** **On high-energy surfaces, the enhanced attachment and spreading of osteoblasts promotes their maturation and differentiation.** This leads to increased production of key markers of bone formation, such as alkaline phosphatase and osteocalcin, and stimulates the secretion of new bone matrix.

4. **Improved mineralization:** **High surface energy increases the quantity and quality of mineral deposition by osteoblasts,** strengthening the new bone matrix and accelerating healing.

5. **Better neovascularization:** **High-energy implant surfaces increase the number of blood vessels that form around the graft site.** A robust vascular network is essential for delivering oxygen, nutrients, and cells to the healing site, preventing fibrous tissue encapsulation that could cause implant failure.

6. **Regulation of inflammatory response:** Research indicates that **hydrophilic, high-energy surfaces help modulate the inflammatory response in the early stages of healing**, creating a more favorable environment for bone regeneration.

What the graft should do (and why HOP helps)

1. **Enable a continuous osteocyte network throughout the graft, as well as the connection to host bone.**
   Interconnected porosity and pore sizes that let osteoblasts infiltrate and mature into evenly distributed, viable osteocytes—and let canaliculi traverse the scaffold—are critical. I**nterconnected pores in the ~150–500 µm range** support vascular/osteogenic ingrowth and uniform tissue formation, which is the substrate for a connected osteocyte syncytium.

2. **Transmit physiologic strain uniformly (mechanotransduction)**
   Osteocytes are the bone's primary mechanosensors; uniform strain fields avoid "hot-spots" of resorption or stress shielding. Recent studies link **lacuna/canaliculi architecture and interstitial fluid flow** to osteocyte stimulation—arguing for scaffold mechanics and micro-architecture that propagate even fluid-shear to cells throughout the fusion mass.

3. **Couple angiogenesis with osteocyte network formation**
   A homogeneous network won't establish without blood supply. Studies on **CYR61/CCN1 and VEGF** in fracture repair indicate that promoting early, spatially distributed angiogenesis correlates with better mineralization and callus maturation.

- Fibronectin is a high-molecular weight glycoprotein of the extracellular matrix that binds to membrane-spanning receptor proteins called integrins. Fibronectin also binds to other extracellular matrix proteins such as collagen, fibrin, and heparan sulfate proteoglycans. Fibronectin exists as a protein dimer, consisting of two nearly identical monomers linked by a pair of disulfide bonds.

- Vitronectin is a glycoprotein of the hemopexin family which is synthesized and excreted by the liver, and abundantly found in serum, the extracellular matrix and bone. In humans it is encoded by the VTN gene. Vitronectin binds to integrin alpha-V beta-3 and thus promotes cell adhesion and spreading. It also inhibits the membrane-damaging effect of the terminal cytolytic complement pathway and binds to several serpins.

- Osteocalcin (also known as bone gamma-carboxyglutamic acid-containing protein) is a small noncollagenous protein hormone found in bone and dentin, first identified as a calcium-binding protein. Its receptors include GPRC6A, GPR158, and possibly a third, yet-to-be-identified receptor. There is evidence that GPR37 might be the third osteocalcin receptor.

- Integrins are transmembrane receptors that help cell–cell and cell–extracellular matrix adhesion. Upon ligand binding, integrins activate signal transduction pathways that mediate cellular signals such as regulation of the cell cycle, organization of the intracellular cytoskeleton, and movement of new receptors to the cell membrane. The presence of integrins allows rapid and flexible responses to events at the cell surface.

Noble B, Chander C, Buckland T, Cameron KR. A novel method for the directed osteogenic differentiation of human mesenchymal stem/precursor cells.
Bone. 2010;47(Supplement):S128.

Green RJ, Davies MC, Roberts CJ, Tendler SJ. Competitive protein adsorption as observed by surface plasmon resonance. Biomaterials. 1999;20:385–391. doi: 10.1016/s0142-9612(98)00201-

Rouahi M, Champion E, Gallet O, Jada A, Anselme K. Physico-chemical characteristics and protein adsorption potential of hydroxyapatite particles: influence on in vitro biocompatibility of ceramics after sintering. Colloids SurfB Biointerfaces. 2006;47:10–19. doi:

Hing KA, Revell PA, Smith N, Buckland T. Effect of silicon level on rate, quality and progression of bone healing within silicate-substituted porous hydroxyapatite scaffolds.
Biomaterials. 2006;27(29):5014–26. doi: 10.1016/j.biomaterials.2006.05.039.

Gentleman, M. M., & Gentleman, E. (2014). The role of surface free energy in osteoblast–biomaterial interactions. International Materials Reviews, 59(8), 417–429. https://doi.org/10.1179/1743280414Y.0000000038

Silicon has been shown in vitro studies to have osteogenic properties.* It has a 35% to 50% porosity, and the silicone allows for a different composition, geometry, and surface charge.

*Patel N, Best SM, Bonfield W, et al. A comparative study on the in vivo behavior of hydroxyapatite and silicon substituted hydroxyapatite granules. J Mater Sci Mater Med. 2002 Dec;13(12):1199-206.

SiCaP and rhBMP-2 were comparable in terms of achieving successful bone growth and fusion.

Licina P, Coughlan M, Johnston E, Pearcy M. *Comparison of Silicate-Substituted Calcium Phosphate (Actifuse) with Recombinant Human Bone Morphogenetic Protein-2 (Infuse) in Posterolateral Instrumented Lumbar Fusion.* Global Spine Journal. 2015;5(6):471–478. doi:10.1055/s-0035-1566230.

Abstract

Porosity and pore size of biomaterial scaffolds play a critical role in bone formation. The effect of these morphological features on osteogenesis in vitro and in vivo, as well as relationships to mechanical properties of the scaffolds, are addressed. Higher porosity and pore size result in greater bone ingrowth, a conclusion that is supported by the absence of reports that show enhanced osteogenic outcomes for scaffolds with low void volumes. However, this trend results in diminished mechanical properties, thereby setting an upper functional limit for pore size and porosity. Thus, a balance must be reached depending on the repair, rate of remodeling and rate of degradation of the scaffold material. Based on early studies, the minimum requirement for pore size is considered to be ~100 μm due to cell size, migration requirements and transport. However, pore sizes >300 μm are recommended, due to enhanced new bone formation and the formation of capillaries. Because of vascularization, pore size has been shown to affect the progression of osteogenesis. Small pores favored hypoxic conditions and induced osteochondral formation before osteogenesis, while large pores, that are well-vascularized, lead to direct osteogenesis (without preceding cartilage formation). Gradients in pore sizes are recommended for future studies focused on the formation of multiple tissues and tissue interfaces. New fabrication techniques, such as solid-free form fabrication, can potentially be used to generate scaffolds with morphological and mechanical properties more selectively designed to meet the specificity of bone-repair needs.

Porosity of 3D biomaterial scaffolds and osteogenesis Vassilis Karageorgiou, David Kaplan
Biomaterials Volume 26, Issue 27, September 2005, Pages 5474-5491

Franz-Odendaal et al. (2006) demonstrated that osteocytogenesis is a regulated, active process involving structural, molecular, and mechanical adaptation.
This transformation is central to the creation of the osteocyte network, which in turn defines the functional integrity and adaptive capacity of bone tissue.

Osteocytes are recognized as the major orchestrator of bone homeostasis, including mechanical sensing and transducing mechanical signals into chemical signals through its lacuna- canalicular system to regulate both bone formation and resorption during bone remodeling.

Schaffler MB, Cheung W-Y, Majeska R, Kennedy O. Osteocytes: master orchestrators of bone. Calcif Tissue Int. 2014;94:5–24.

Abstract
Osteocytes comprise the overwhelming majority of cells in bone and are its only true “permanent” resident cell population. In recent years, conceptual and technological advances on many fronts have helped to clarify the role osteocytes play in skeletal metabolism and the mechanisms they use to perform them. The osteocyte is now recognized as a major orchestrator of skeletal activity, capable of sensing and integrating mechanical and chemical signals from their environment to regulate both bone formation and resorption. Recent studies have established that the mechanisms osteocytes use to sense stimuli and regulate effector cells (e.g., osteoblasts and osteoclasts) are directly coupled to the environment they inhabit—entombed within the mineralized matrix of bone and connected to each other in multicellular networks. Communication within these networks is both direct (via cell–cell contacts at gap junctions) and indirect (via paracrine signaling by secreted signals). Moreover, the movement of paracrine signals is dependent on the movement of both solutes and fluid through the space immediately surrounding the osteocytes (i.e., the lacunar–canalicular system).