**Introduction**

The aim of the present study was to compare the clinical outcome and radiographic bone changes in augmented ridges utilizing a synthetic NanoBone block versus an allograft bone block, and to investigate histologically the success of a synthetic NanoBone block versus an allograft bone block for ridge augmentation. In the previous issues of *implants: international magazine of oral implantology*, the authors gave a detailed introduction to their topic and explained the materials and methods used in their study (*implants* 1/2013) and the results of clinical outcomes & radiographic bone changes (*implants* 2/2013). In this issue, their report is completed by the histological results of their investigations and an extensive discussion.

**Bone regeneration process**

*Phase 1: Bone regeneration*¹

Within the graft, whether for a mandibular continuity defect, a sinus augmentation surgery or dental implant is placed in a dead space filled with clotted blood. The platelets entrapped in the clot degranulate within hours of graft placement, releasing PDGF, TGF-b1 and TGF-b2. Both of these factors initiate the bone regenerative process. PDGF binds to endothelial cells to initiate the growth of capillaries, while TGF-b1 and TGF-b2 bind to the endosteal osteoblasts and marrow mesenchymal stem cells to initiate mitosis to increase their numbers as well as stimulate their production of osteoid. This continues during the first 3 days of the graft, at which time capillaries are already seen to be entraping the graft. However, by this time, the platelets have degranulated and are no longer a primary source of growth factors to drive the bone regenerative process. At these times, macrophages take over this role. Macrophages were initially attracted to the graft as circulating monocytes of free tissue cells by inherent oxygen gradient in the graft. Thus, the inherent properties of the wound, particularly the oxygen gradient, PDGF and TGF-b initiate early angiogenesis from surrounding capillaries and mitogenesis of the transferred osteocomponent cells. The complete revascularization of the graft is seen by day 14. By this time, the endosteal osteoblast have already laid down osteoid on the original bone trabeculae and the marrow stem cells have dramatically increased in number and have begun differentiating into osteoblasts. The stem cell population and endosteal osteoblasts produce small amount of osteoid.

During the first 3 to 4 weeks, the biochemical and cellular phase of bone regeneration proceeds to clinically consolidate the graft by coalescing individual osteoid islands, surface osteoid on cancellous trabeculae and host bone. This process is essentially transplanted osteogenesis. However, it uses the fibrin network of the grafts as a framework. This is referred to as osteoinduction, which provides a scaffold for what has been called (creeping substitution). That is, the normally non motile
osteoblasts can be somewhat motile via the process of endocytosis along a scaffold like fibrin.

The process of endocytosis is merely the transfer of cell membrane from the retreating edge of the cell, through the cytoplasm as a vesicle, to the advancing edge to reform a cell membrane and thus increase the cellular surface area at the advancing edge. This mechanism slowly advances the cell and allows it to secrete its product in the process. In this case, the product is osteoid on the fibrin network. This cellular regeneration is often referred as phase 1 bone regeneration or woven bone phase. By the time it is nearly complete (4 to 6 weeks), sufficient osteoid production and mineralization have occurred to permit graft function. At this stage, the bone has formed without going through a chondroblastic phase and histologically appears as random cellular bone that a pathologist would refer to as woven bone.

Phase 2: Bone regeneration

The cellular bone regeneration that has occurred in phase 1 produces this disorganized woven bone that is structurally sound but not the degree of mature bone. The random organization and hypercellular nature of this bone is similar to that seen in fracture callus. This bone will undergo an obligatory resorption and replacement type of remodeling. Eventually, it is replaced by phase 2 bones, which is less cellular, more mineralized and structurally more organized into lamellar bone.

The replacement of phase 1 by phase 2 bone (woven bone by lamellar bone), like all bone remodeling, is initiated by osteoclast. Osteoclasts are fused monocellular cells that arrive at the graft site through the newly de-
Fig. 3 & b. X400, H&E stained section showing NanoBone granules undergoing degeneration by osteoclast cells, surrounded by dense connective tissue with plentiful osteoblast cells.

Figs. 4a–c. X100, H&E stained section showing numerous blood vessels in NanoBone graft.

Figs. 5a & b. X100, H&E stained section showing numerous inflammatory cells, few blood vessels and few new bone formation in the Fisiograft group.

Fig. 6. X400, H&E stained section showing less vascular vessels and large remnants of Fisiograft.

Fig. 7. X100, H&E stained section showing small remnants of NanoBone graft.

Figs. 8a & b. Showing NanoBone block immediately after augmentation (a) and six month after augmentation which was firm and strongly attached to natural bone (b).

-Histological results-

During processing of NanoBone and Fisiograft samples, we noticed that the samples of augmented bone do not need more than ten days to be decalcified in EDTA while the remaining part (normal bone) of the bone core still calcified. A microscopic analysis at x100 magnification allowed the author to observe numerous mineralised areas of newly formed bone of various sizes, which were scattered in all the NanoBone group (Figs. 1–3), and limited in the Fisiograft group (Fig. 5). These bone areas were surrounded by an osteoid layer composed of osteoblasts, which synthesize the organic component of the extracellular matrix (the osteoid substance) and control its mineralization. Microscopic observation of the sample at higher magnification showed some osteoclast cells were found near the remaining spicules of the bone graft, multiple osteoblasts and numerous osteocytes situated within well-defined lacunae (Fig. 3). In some areas, new bone contained small islands of residual bone graft; these could be distinguished from live bone by empty osteolytic lacunae (Figs. 5–7). They were showing signs of continuous resorption by osteoclasts and simultaneous deposition of bone. The presence of large amount Fisiograft remnants was seen in all group sections (Fig. 6), while in NanoBone group specimens the NanoBone graft remnants were few and at the periphery of the specimens (Fig. 6). The presence of blood capillaries, defined by endothelial
cells, demonstrated well differentiated capillary vascularization was numerous in all NanoBone group specimens (Fig. 4), while it was few or absent in the Fisiograft group specimens. There was no evidence of acute or chronic inflammatory infiltrate in all sections of NanoBone group. Inflammatory cell infiltration within the new bone, primarily mononuclear cells such as lymphocytes and macrophages, was seen in all samples of the Fisiograft group (Fig. 5). The inflammatory cells were considered indicative of a significant inflammatory or immune response. Histologically, NanoBone granules exhibited adequate vascularization and high biocompatibility comparable to Fisiograft bone, as indicated by the fact that in NanoBone groups, signs of angiogenesis and large vessels and cells could be observed in the center of the tissue (Fig. 4), while Fisiograft bone was invaded by small vessels and cells (Fig. 5).

**Discussion**

Bone substitutes act as space maintainers by providing a scaffold that allows them to colonize by bone-promoting cells and to replace by newly formed bone.\(^2\) One of the major challenges in the application of bone substitutes is adequate vascularization and biocompatibility\(^3\) and rapid vascularization of the block graft is paramount for successful neo-osteogenesis.\(^4\) Our histological results showed that NanoBone architecture allows better vascularization (Fig. 4), as well as colonisation of the bone graft by the host progenitor cells and promotes the osteoconductive properties of the material. On the other hand, the Fisiograft samples showed less vascularization (small vessels and cells, Figs. 5 & 6). Our histological results showed furthermore that inflammatory cells infiltration within the new bone, primarily mononuclear cells such as lymphocytes and macrophages, was seen in all samples of Fisiograft group (Fig. 6). The inflammatory cells considered indicative of a significant inflammatory or immune response. This finding is not consistent with the finding of Scarano et al., who stated that Fisiograft is free from inflammation effect.\(^5\) Our histological results showed that, in the NanoBone group specimens, the NanoBone graft remnants were few and at the periphery of the specimens, ongoing resorption and surrounded by osteoclasts. This is consistent with Heinemann et al., who postulate that nanocrystalline HA has osteoconductive and biomimetic properties and is integrated into the host’s physiological bone turn over at a very early stage.\(^6,7\) And it is also consistent with Cannolo et al., who proposed that the newly formed bone in NanoBone was already found at three months of healing and new trabecular bone was found at six months of healing.\(^8,9\) During implant placement, the quality of grafted bone was evaluated clinically, especially during drilling and implant placement. In all NanoBone group patients, the grafted bone was firm and strongly in contact to the natural bone (Fig. 8). Copious bleeding occurred during drilling in the grafted bone (Fig. 9). This is consistent with the histological results which showed better vascularisation, as well as colonisation of the bone graft by the host progenitor cells and promotes the osteoconductive properties. We also noticed that, during crestal incision and flap reflection, it was difficult to dissect the mucosa over the augmented NanoBone block and we used a scalpel to dissect it. We refer that to an absence of the peristeum that covers the grafted NanoBone block. On the other hand, in Fisiograft group patients, the grafted bone had no clear contact to natural bone and little bleeding occurred during drilling in the grafted site.

**Conclusions**

NanoBone block (NanoBone, ARTOSS) showed faster bone formation, better vascularisation, as well as colonisation of the bone and less inflammatory cells, while Fisiograft showed less vascularisation and numerous inflammatory cells. NanoBone graft degraded earlier than Fisiograft.