

## Applications of Nanostructured Hydroxyapatite for Osteogenesis and Angiogenesis

### Introduction

Hydroxyapatite is a calcium phosphate ceramic which is very similar to the mineral component of bone.

It has excellent biocompatibility and when it is implanted in the body, it has the capacity to form strong and stable chemical bonds with bone tissue. Microscale Hydroxyapatite (HA) has been used extensively in the healthcare industry in prosthetic coatings and bone graft substitutes.

With nanotechnology emerging, nanoscale HA has been studied to improve HA properties. Nanostructured materials can imitate surface characteristics present in natural hard tissues improving their biological performance.

### nanoXIM - Nanocrystalline HA Powders

FLUIDINOVA developed the nanoXIM HAp200 product series, which are high purity, single-phase, nanocrystalline HA powders. These products are well suited for medical applications as they present chemical and structural similarity with natural bone.

The nanoXIM HAp200 series has a high specific surface area greater than 100 m<sup>2</sup>/g and an accurate stoichiometry of calcium or phosphate ions resulting in a Ca/P ratio always near to 1.67.

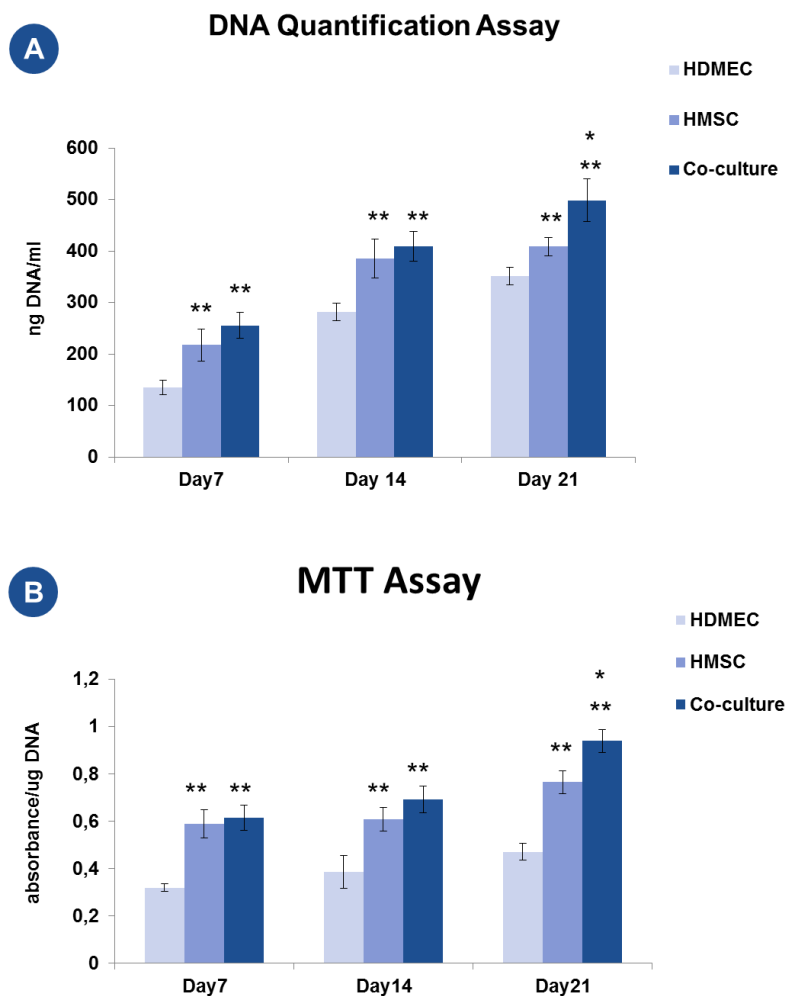
### Impact of nanoXIM HAp202 in a Co-Culture System with Endothelial and Mesenchymal Stem Cells

After implantation of bone graft, the formation of a rapid and stable microvasculature is required to ensure the metabolic requirement of recruited cells to begin the bone regeneration process and support the recently formed tissue.

The process includes a tight communication between endothelial cells, involved in the creation of a vascular network, and mesenchymal stem cells, which can differentiate into osteogenic cells. On implanting of a biomaterial, an adequate environment must be guaranteed for all cell types involved in regeneration of bone.

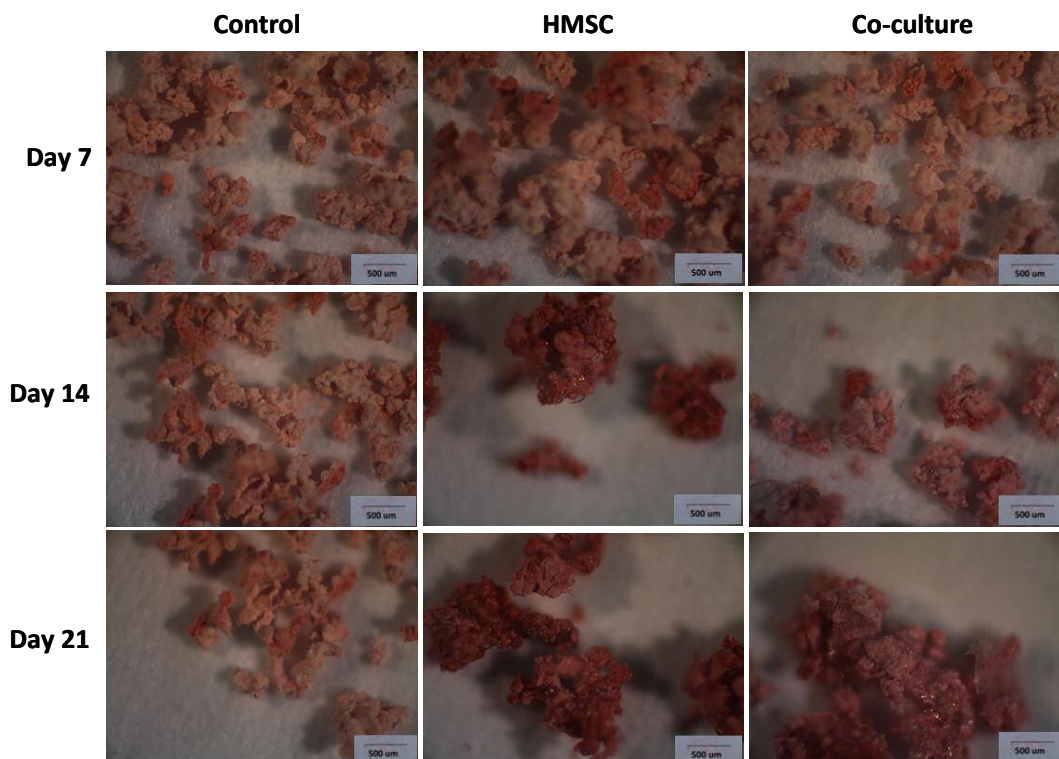
For that reason, it was investigated whether a three-dimensional substrate produced with nanoXIM HAp202 could support a co- culture of human dermal microvascular endothelial cells (HDMECs) and human mesenchymal stem cells (HMSCs) [1].

It is seen that nanoXIM HAp202 substrates provide an adequate microenvironment for cell viability and proliferation for both monocultures and co-cultures as shown in Figure 1.



**Figure 1:** Cell viability and proliferation of monocultured and co-cultured HDMECs and HMSCs assessed by DNA quantification (A) and MTT assay (B) for 7, 14 and 21 days of culture. Data kindly provided by Dr. Marta Laranjeira.

The alizarin red staining showed that both co-cultures and monocultures stained positive for calcium deposits showing induction in mineralized tissue formation as seen in Figure 2.



**Figure 2:** Alizarin red histochemical staining performed in nanoXIM HAp202 substrates without cells (control), with HMSCs and co-culture for 7, 14 and 21 days. Data kindly provided by Marta Laranjeira.

With regards to the genetic profile of HDMECs and HMSCs in monocultures and co-cultures, the expression of angiogenesis and osteogenesis related genes were observed. Furthermore it becomes very essential to note that HDMECs could proliferate in 3D nanostructured surfaces, as against previous reports that cannot adhere and proliferate in microsized HA structures [1].

## Conclusions

It can be concluded that the substrates obtained with nanoXIM HAp200 series could support a co-culture system of endothelial and mesenchymal stem cells and induced important characteristics with regards to angiogenesis and differentiation of HMSCs into the osteoblastic lineage.

HDMECs were capable to proliferate on nanoscale HA, which does not take place in microscale HA structures. These results indicate that nanoXIM HAp200 products are promising materials that can be used in bone regeneration and tissue engineering applications. One approach that the material must be pre-seeded with mesenchymal and endothelial cells before implantation, for a more rapid bone regeneration process.

## References

1. Laranjeira M, Fernandes M, Monteiro F. Response of Monocultured and Co-Cultured Human Microvascular Endothelial Cells and Mesenchymal Stem Cells to Macroporous Granules of Nanostructured-Hydroxyapatite Agglomerates. *Journal of biomedical nanotechnology*. 2013;9(9):1594-606.

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