Scientific Evidence

HETEROLOGOUS BIOMATERIALS CONTAINING PRESERVED OR HYDROLYZED COLLAGEN VERSUS ß-TCP: AN *IN VITRO* STUDY

Evaluation of the cellular interaction of heterologous biomaterials containing preserved or hydrolyzed collagen, compared with ß-TCP, a synthetic material.

From the Bioteck Academy Editorial Office

The use of bone grafts in regenerative surgery aims to promote tissue regeneration and accelerate healing. In recent years, research into biomaterials has led to the development of increasingly effective solutions to support bone regeneration, reducing the risk of disease transmission and accelerating the healing process. Among the various solutions available, heterologous biomaterials of animal origin represent a promising option due to their structural similarity to human bone. In particular, heterologous biomaterials containing collagen appear to offer additional advantages over substitutes that do not contain it.
Type I collagen is one of the main components of the bone tissue matrix, to which it confers structure and elasticity, and is fundamental to bone regeneration processes.
Its presence in biomaterials helps to maintain good mechanical properties and promote the formation of new bone tissue, as demonstrated by preclinical[1] and clinical[2,3] studies.
Based on the available evidence, it is important to assess how the presence or absence of collagen within biomaterials – whether in its native or hydrolyzed form – may affect the cells at the surgical site, thereby influencing the regenerative process. The characterization of such materials can, in fact, provide valuable support in guiding clinical decisions and identifying the most suitable solution depending on the surgical site and the type of procedure.

1. Perrotti V. et al., 2009, DOI: 10.1111/j.1600-0501.2008.01608.x
2. Di Stefano D.A. et al., 2015, DOI: 10.11607/jomi.4057
3. Di Stefano D.A. et al., 2019, DOI: 10.3390/dj7030070

Materials

This sheet summarizes the results of the *in vitro* study published in *Minerva Dental and Oral Science*[4] in 2025 and carried out in collaboration with the IRCCS San Raffaele in Milan.
The biological properties of ß-tricalcium phosphate (ß-TCP) granules – a synthetic, collagen-free material used as a negative control – were compared with various granules of equine origin (Bioteck SpA): those with preserved collagen (Osteoplant/Osteoxenon) and those with hydrolyzed collagen (Bio-Gen).

The latter products are manufactured using Zymo-Teck (Bioteck SpA), a patented enzymatic process for antigen removal capable of preserving the extracellular bone matrix (mineral and collagen). The three different types of biomaterials were then brought into contact with viable cells in order to test their biocompatibility, their interaction with the cells and their ability to promote the production of new tissue matrix, at both the microscopic and molecular levels.

4. Di Stefano D.A. et al., 2025, DOI: 10.23736/S2724-6329.25.05209-X

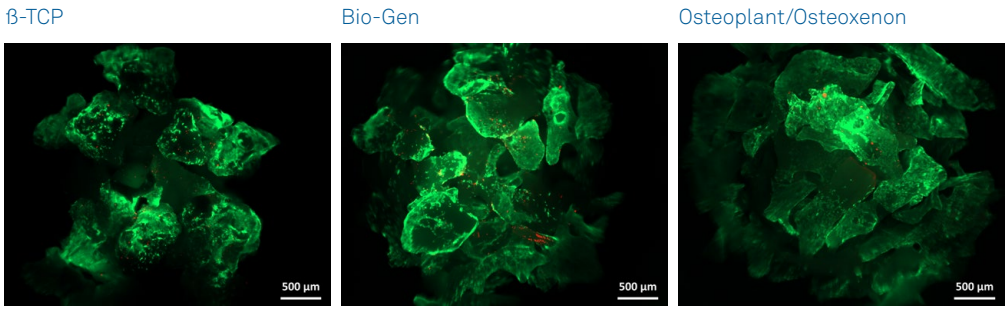

Fig. 1 *- Assessment of biocompatibility in cell cultures using cell viability assays. Live cells, which are prevalent in all biomaterials, are shown in green; dead cells are shown in red.*

Fig. 2 *- The images show cells adhering (darker dots/clusters) to the surfaces of the three biomaterials. In the ß-TCP, individual cells can be seen, while on Bio-Gen, tissue fibers are visible between the granules (yellow arrow). In the case of Osteoplant/Osteoxenon, however, a more compact layer of cells is observed (red arrow).*

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Results

The results show good biocompatibility for the three types of biomaterials, as assessed by cell viability assays (which distinguish between live and dead cells) (Figure 1).

Cell adhesion was then assessed (Figure 2), i.e. the ability of cells to adhere to the surface of the biomaterial, a crucial step in initiating bone regeneration.
The results show that ß-TCP is the biomaterial least colonized by cells. Bio-Gen demonstrated an intermediate capacity, with the formation of bridging structures between the granules. Osteoplant/Osteoxenon, on the other hand, demonstrated the best colonization capacity, with the formation of a compact cellular layer on its surface.

As regards the assessment of the three biomaterials' contribution to the processes of new tissue formation, they were placed in contact with cells cultured under different conditions to verify the extent to which the biomaterial was able to support their activation (Figure 3).
This aspect is important because it represents a key phase in bone regeneration, during which cells begin to produce the matrix that will provide structure to the new bone. Cultures with ß-TCP showed the most limited response, unlike those with Bio-Gen, which revealed the presence of connecting structures between the cells and the biomaterial, but poor matrix production.
Cultures treated with Osteoplant/Osteoxenon, on the other hand, showed both cells and matrix, suggesting a greater capacity to promote bone regeneration processes among the three biomaterials.

This finding is also confirmed by molecular analyses, which revealed substantial differences in the presence of ALPL: a growth factor and early indicator of the activation of the regeneration process (Figure 4).

In conclusion, although all the biomaterials showed comparable biocompatibility, they led to different biological responses. The collagen-containing biomaterials performed best, with Osteoplant/Osteoxenon (preserved collagen) ranking first and Bio-Gen (hydrolyzed collagen) showing intermediate results.

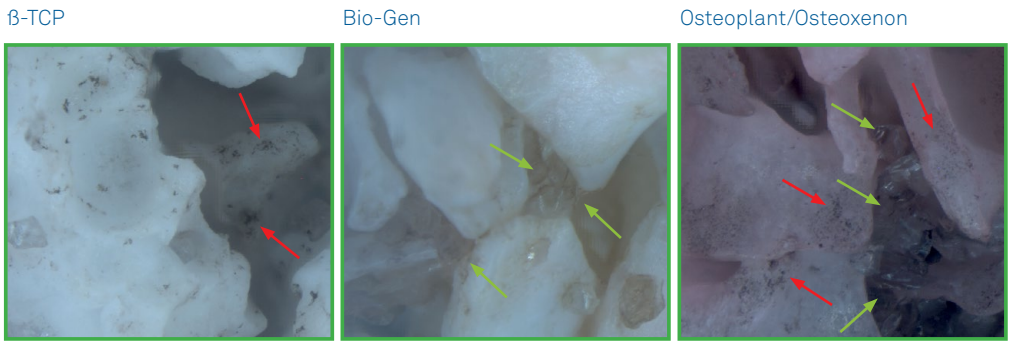

Fig. 3 *- Comparison of the ability of biomaterials to support the formation of new bone tissue. On ß-TCP, only minimal matrix deposition is observed (red arrows). On Bio-Gen, a predominance of tissue fibers is observed (green arrows), but no matrix deposition. On Osteoplant/Osteoxenon, both tissue fibers and matrix are detected.*

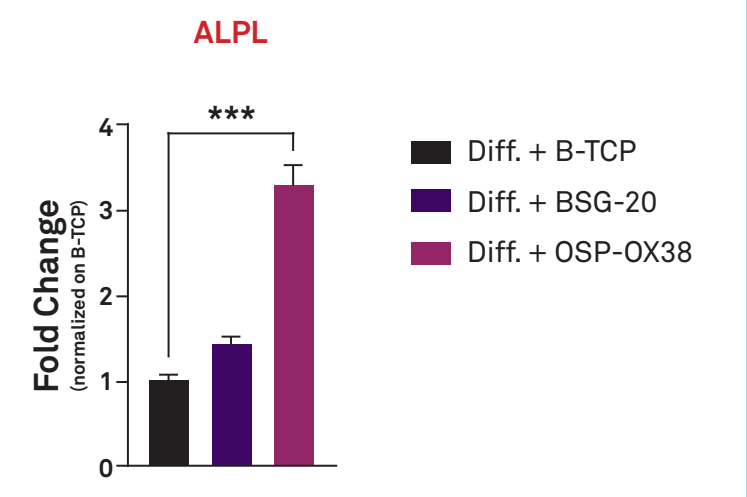

Fig. 4 *- The molecular analyses carried out show that cells in contact with Osteoplant/Osteoxenon are able to produce statistically higher levels of ALPL (alkaline phosphatase), a growth factor involved in bone formation processes.*

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