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*CURRENT CONCEPTS REVIEW*

# The Pentaconcept in skeletal tissue engineering
## *A combined approach for the repair of bone defects*

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**Tissue engineering has become a hot topic in modern medicine. Its application in a surgical setting, such as for the treatment of skeletal defects, still has to tackle some problems that might look simple at first sight, but need a well-structured handling combining surgery and science, with in a central position the patient, who is both cell donor and receptor of the tissue engineered end product. To achieve this goal in a clinical setting, a five steps pathway is described and designated as the Pentaconcept, integrating all ingredients for successful reconstructive procedures.**

**Keywords** : tissue engineering ; skeletal defect ; advanced tissue medicinal product (ATMP).

## INTRODUCTION

Bone healing in general requires a good molecular orchestration of growth factors that promote cell differentiation and proliferation to restore the continuity of a fractured bone (*24*). It is well known from the surgical point of view that a good stabilisation and atraumatic interventions to minimise damage to the blood supply are very important to improve the healing capacity. In case of bone defects where restoration is a real challenge, even more parameters have to be taken into account. Especially in large defects there is insufficient biological potential to fill the gap, as there might not be enough conducting scaffold nor cells to overcome the volume defect. Moreover, under these conditions the biological potential of the surrounding tissues is usually hampered, increasing the difficulty to obtain a good healing.

Autologous versus non-autologous treatments can be used, but autologous grafts from the iliac crest are still considered as the gold standard (*13*). Other techniques using the patient's own bone are free vascularised transplants or bone transport techniques for very large defects (*7,8,10,11,22*). Furthermore the relatively novel method of Reamer Irrigator Aspirator technique allows the filling of bony cavities ; to some extent even less invasive methods such as the Gravitational Platelet System using platelet rich plasma from the patient's own

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blood or a simple bone marrow aspiration with or without concentration of the progenitor cells by centrifugation, have been recommended for some indications (*16,18,29*).

Due to concomitant morbidity of these procedures and on occasions also the unavailability of enough grafting material, non-autologous treatments have found their access to the clinical practice. Allografts, demineralised bone matrix (DBM), ceramics, bioglasses, polymers and metals such as Titanium, Tantalum or alloys of Titanium-Aluminium-Vanadium can act as conductive scaffolds but, among these, only cancellous allografts and DBM have a limited inductive capacity (*15, 23,27,28*).

Other options are the use of purely inductive growth factors of which only the bone morphogenetic proteins BMP-2 and BMP-7 are commercially available but they present three major drawbacks : the need for supraphysiological doses with unknown potential long-term side effects, the absence of a conductive substrate and their high cost. Combinations of non-autologous techniques are found in the literature in numerous variations but none of them are able to mimic bone in a way that they can be considered as a living implant matching autologous grafts.

**New strategies at the horizon**

With the advances made in stem cell technology, Advanced Therapeutic Medicinal Products (ATMPs) form a new generation of therapeutic strategies that will become available in the near future and open new possibilities for tissue engineering approaches in skeletal defects (*4,30*). ATMPs can be divided in somatic cell therapies, gene therapies and the tissue engineered implants, which are the most appropriate for bone defect repair.

For their use five important parameters should be taken into account, all interdependent and essential to obtain the regeneration of a new segment of bone and therefore designated as the Pentaconcept.

1. **Osteogenicity :** without living cells it is impossible to kick start a biological process. In a 'no defect' situation enough mesenchymal stem cells can be recruited to initiate the process, and orchestrated by growth factors these cells will further differentiate and proliferate in a way that they will be able to restore the bone tissue. However once the absolute volume has reached certain dimensions, known in literature as 'critical size defect' and defined as a length of at least two and a half times the radius of the bone shaft (five quarters of the diameter), this can not be overcome anymore. At this point a 'mathematical mismatch' arises between cells and the volumetric dimensions of the cavity to be filled (*28*). This might explain why healing in small rodents is always reported to be easier than in larger animal models. Although these small animals can have similar 'large' defects if calculated in relation to the dimensions of their bones, it is a small defect in absolute figures still leaving a match between the amount of cells available and the calculated volume to be filled. To overcome this 'cell deficit' in larger animals and humans it is generally agreed that cells have to be added to enhance the osteogenicity, which is in fact the base of all tissue engineering strategies (*2,6*). In contrast to direct local administration of cells, ATMPs allow cell distribution throughout appropriate carriers of which different types are under investigation (*9,12*).
2. **Osteoconduction :** in the healing of defects new skeletal tissue has to be formed in a well defined shape and according to the geometry of the original bone. A framework on which the new bone can grow is essential. It guides the bone along the desired dimensions and acts as an anchoring network for the tissue during its development. Types of scaffolds are numerous but based on literature and own previous experiences a calcium-phosphate-collagen combination is found to be most appropriate, both in terms of safety and efficacy (*5*). Another advantage of this type of scaffold is its complete resorption, avoiding the continuous presence of a foreign body.
3. **Osteoinduction :** the stimulation for the formation of new bone is a complex interaction of molecular signalling pathways and is certainly not completely elucidated. It is driven biologi-

cally and to some extent also mechanobiologically by the transmission of mechanical and electrical stimuli into molecular signals (*3,21*). Different pathways act and interact, such as hedgehog, Wnt signalling pathways, RAS-MAPK pathway, and most widely studied and clinically applied, the BMPs, signalling through SMAD proteins. So far only BMP-2 and BMP-7 have been used in human applications and always in a supraphysiological dosis to see sufficient response, either in 'isolated' applications, this is by only adding the osteoinductive factor, or in combination with a scaffold or cells (*17,25*). So far adverse effects due to high dose BMP application have only been reported sporadically but both with regard to safety and cost, lower doses are preferable and in a combined biomimetic implant feasible, according to ongoing research.

4. **Mechanical stability :** biological processes are mandatory for tissue repair, but particularly in the skeleton stabilisation and alignment are important both for a good healing capacity and for an optimal functional outcome. Throughout history different methods of stabilisation have been developed, both surgical and nonsurgical. Aggressive surgical techniques are less biological and should therefore be avoided as to not interfere with the local blood supply. In particular the combination of surgery with implantation of biomimetic structures should be done very carefully to avoid interference with the living implant. Implants carefully wrapped around intramedullary nails are a possibility but external ring fixators are a good alternative which provides a very good stability allowing full weight bearing on the limb, even in the presence of a large defect, while preventing a negative mechanical influence on the reconstructive process (*5*).
5. **Biological chamber :** the previously described parameters mainly rely on external influences to heal a defect. The quality of the ATMP with its added cells and growth factors, a scaffold as a carrier for these cells and molecules, and an optimal mechanical situation form a combination designated as the 'diamond concept' in literature as an expansion of the original triangular concept of interacting cells, growth factors and scaffolds (*14*). The biological situation of the host should however not be underestimated and can be divided into his general health condition and the local situation around the bone defect to be reconstructed. The latter can be considered as an area of high biological activity that should have optimal conditions to allow bone repair. In mathematically small defects this area can be left open allowing 'cross-talking' with a healthy environment. In larger defects, the surrounding tissues are usually disturbed over a large distance and it is preferable to isolate this area and consider it as a closed biological chamber, provided it has its own biological potential (*20*). There is still an entrance point from the intramedullary canal integrating this small local bioreactor. The ideal way to close the chamber seems to be by a natural created membrane, which has its own vascularity and allows some extra secretion of growth factors. Such a bioactive membrane guarantees a containment of the bio mimetic structure put inside and provides a physiological surrounding. The optimal way so far to create such a membrane is by the induction of a foreign body reaction, as occurs by primary insertion of bone cement into a defect. It is known as the Masquelet technique and widely described in literature. The insertion of polymethylmetacrylate cement creates a pseudosynovial membrane that is formed as an envelope around the spacer within 6 to 8 weeks and which contributes to the vascularisation and ingrowth of the implant, according to studies proving the secretion of growth factors by this membrane (*19,26*). Newer developments in tissue engineering might even enable us to engineer a membrane from donor cells and integrate it in the ATMP (*31*).

The implementation of the Pentaconcept in practice is a complex process of cell sampling and expansion, preparation of the implant in bioreactors, storage, transportation and finally careful implantation to avoid cell loss or inactivation, in a meanwhile well prepared host with safety issues on each level of the procedure. A direct interaction

between the surgeon and the ATMP producing laboratory is mandatory and strict time frames have to be respected. Large scale multicentre applications are not possible yet due to the complexity in preparing and handling living tissues and the absolute coordination between the manufacturing process and the surgery. The orchestration for the consecutive steps is a prerequisite for the use of ATMPs which have nowadays reached the preclinical stage of large animal models and are expected to reach the clinic in one or two years under close supervision of new European regulatory guidelines.

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