

New horizons in fracture treatment

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Abstract

Mechanical stabilization has been the principal method of bone fracture treatment for many years. New approaches with biological substances are introducing and they have been increasingly used. Fracture reparation is a complex physiological mechanism. Special signal molecules play an important role. These new substances are used more and more in treatment of fracture non-unions and they are giving promising results.

Introduction

During the past four decades important progress regarding fracture treatment has been achieved based on the theoretical achievements of the AO School. Careful dealing with soft tissues, development of closed intramedullary fracture fixation and new approach with biological enhancement substances facilitate and speed up the bone reparation process with the help of electricity, electromagnetic field and ultrasound (1).

Despite of all that complications arise in 5 to 10 % of all treated fractures during the healing process. Therefore the technological progress in medicine seeks new, better and faster ways of fracture treatment with the desire to successfully treat every fracture. What is today a historical theory of micro movement, necessary for successful fracture reparation, has been complemented by new knowledge in the field of cell and molecular biology. The approach to fracture treatment has reached a whole new level, focusing also on cellular cycle, molecules and the influence of genes on the healing process. New substances have been developed to allow and even enhance the reparation process (1).

Today, fractures are no longer treated simply with mechanical stabilization; instead it is complemented by those biological substances to encourage the bone tissue to heal better and faster. Tissue engineering, that is implantation of biological or artificial material into the bone tissue, presents a huge step forward to accelerating natural growth and healing of the fractured bone (1, 2).

The basics of cell and molecular biology of fracture reparation

Fracture reparation is a complex physiological process. The healed fractured bone must cope with mechanical burdening. Unlike other tissues that heal through a scar, bone tissue creates new bony tissue, which has to have the same physiological characteristics as before. The classical histological typology divides fracture reparation into primary and secondary (3).

Primary cortical intramembranous reparation requires firm contact between two cortical sides of the fractured bone particles, stabilized by one of the forms of rigid osteosynthesis. Formation of new bony tissue begins immediately after the fracture. Tight contact between the fractured surfaces allows resorption on the edges and the formation of new Havers canals. The perivascular tissue inside those canals consists of osteoprogenitory cells that become osteobla-

sts. Then a spongy bridge is formed, which is later remodelled into lamellar, mechanically firm bone (3).

In **secondary endochondral repair** the main role is probably played by the periosteum because it consists of osteoprogenitory cells and undifferentiated mesenchymal cells, which help the fractured bone to heal through endochondral ossification. The process of endochondral ossification is carried out in four stages: hematoma and inflammation, the formation of new vessels, the formation of chondral "callus" and finally the transformation into bony tissues through remodelling. Rigid osteosynthesis disables this process as the secondary endochondral repair requires micro movement (3).

Intramedullary fracture fixation allows secondary fracture repair with fractured particles in a suitable position. Although the fractured particles are in contact, no actual mechanical load is involved. Load can be later increased in certain types of intramedullary nails with dynamization (i.e. removal of distal locking screws). Studies show that the combination of mechanical stabilization and secondary repair can be very effective in long bone fracture treatment (3-6).

Regulation of the fracture repair process

The main role of phase one in secondary healing is probably the formation of inflammatory agents - **cytokines** and signal **molecules/growth factors**, produced by neutrophils, thrombocytes and other cells. The main cytokines are *interleukins 1 and 6*, and the main growth factors are *platelet derived growth factor* (PDGF) and *tissue growth factor β* (TGF β). Those molecules are important for regulation of proliferation and differentiation of pluripotent mesenchymal cells. They also take part in chemo taxis and encourage cells for the synthesis of bone cytoplasm, and they are also present in the phase of chondrocytes production and in angiogenesis. The complex process of transformation of the progenitory cell into osteoblasts and the controlling mechanisms are presented in picture 1. Cellular protooncogenes such as *c-fos* are also important in this process. They control the expressions of other genes, act as growth factors and receptors or function as intercellular signal molecules (3, 7, 8).

The most important among numerous growth factors is the **bone morphogenetic protein** (BMP) from the group of TGF β . **BMP** is a low-molecular protein which causes new bony tissues to form by causing and enhancing the formation of osteoblasts from progenitory cells and the synthesis of collagen. There are many types of BMP, the most explored among which are BMP-2 and BMP-4. The use of these proteins has already given some promising results (3, 9).

Biological enhancement to fracture repair

The operative fracture treatment affects the healing process gravely. Additional damage on soft tissues, removal of growth factors and progenitory cells, damage on the vessels significantly slow down the healing process.

Today there are many natural and artificial substances, biological and mechanical implants that enhance growth and fracture repair. Various substances and materials that are in use today are presented in table 1. The main characteristics of those substances are *osteogenesis*, *osteochondduction* and *osteoiduction* (10).

Osteogenesis is the ability of the cells to produce new bony tissue, for example osteoblasts. Sources of the cells in the fracture area are bone marrow, periosteum and perivascular tissue, usually

in the form of pluripotent cells or the fore comers of bone cells. The osteogenetic abilities have been discovered in autologue bone transplants and bone marrow (10).

Osteoconduction is a characteristic of the substances that allow fixation, migration and differentiation of bone cells in 3-D space. They are mainly structures into which osteogenetic cells grow. Those features were found in artificial materials, for example in ceramic materials (hydroxyl-apatite, tri-calcium phosphate) as well as in natural materials, e.g. autogene transplant of spongiuous bone, de-proteinized bone matrix, collagen (10).

Osteoinduction is a feature of bioactive molecules that stimulate the formation and development of bone cells and bone cytoplasm. Inductive molecules are growth factors that are specific polypeptides and act as local regulators of bone cells activity. The most important in the process of fracture reparation are the above mentioned BMP, TGFβ, PDGF, *fibroblast growth factor* (FGF), and *insulin-like growth factor* (IGF). Their action can be described as a sequence of the following events:

- Binding to a specific cellular trans-membrane receptor,
- intracellular conformation of the receptor – activation of a subsequence of protein-kinases,
- activation of transcription of the growth genes in mRNA,
- translation and production of specific proteins,
- growth and division of the cell or formation of cytoplasm (10).

Clinical use of bio-active substances

The ideal implant should consist of all three basic elements: osteoinduction, osteoconduction and osteogenesis. The only existing implant that fits the listed criteria today is the "golden standard" among all implants that is the autologous spongeous bone. But there are some disadvantages to the use of this implant:

- sometimes there is not enough transplant (e. g. in children or in large bone defects),
- the removal causes consequences (infection, pain, nerve damage),
- duration of the surgical procedure is extended because of the removal,
- the reparation is insufficient despite the use of autologue transplant,
- removal causes additional costs.

| Implant | osteoconduction | osteoinduction | osteogenesis |
|----------------------------|-----------------|----------------|--------------|
| Autologous spongiuous bone | ++++ | ++ | ++ |
| Bone marrow | - | +/- | +++ |
| Ceramic material | ++++ | - | - |
| De-mineralized bone matrix | + | +++ | - |
| Growth factors | - | ++++ | - |
| Combinations | +* | +* | +* |

Table 1. Currently prevailing materials in bone surgery. Legend: the number of “+” presents the level of influence, “-“ means that there is no influence. *in combinations the level of influence depends on the type of combination (11).

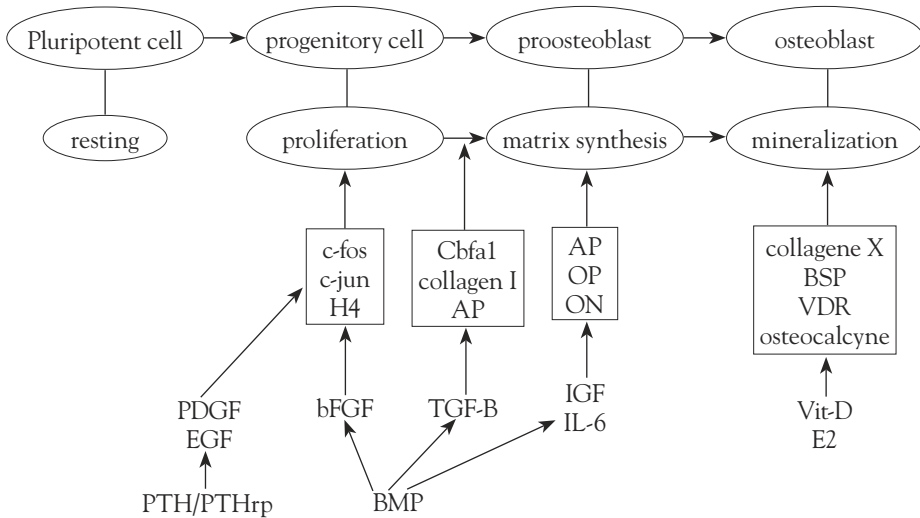
Therefore scientists are looking for new material combinations that would have all the three qualities. In clinical use bone marrow is the main source of osteoprogenitory cells, biologically appropriate ceramic materials are used as osteoconductive material, and key growth factors (BMP) and de-mineralized bone matrix are mainly used as osteoinductive material. The most commonly used substances in clinical practice and their impact on the elements of bone growth are noted in Table 1 (11).

The use of **bone marrow** transplants has given some good results in treatment of insufficiently repaired fractures, in bone defects and arthritis (11).

Ceramic materials (hydroxyl-apatite, calcium phosphate, tri-calcium phosphate) are used a lot mainly for their osteoconductive qualities. They provoke little immunological reactions and are not toxic. They are commonly used in various cement-free prosthetics, in tibial and femoral fractures, and in operations on the spine (11).

De-mineralized bone matrix is obtained through bone modification in an acid medium, where minerals are removed, while growth factors and proteins are preserved. It is assumed that most of the osteoinductive qualities of this implant are present due to growth factors, especially BMP. Use of de-mineralized bone matrix in bone defects, comminuted fractures and non-united fractures is described (11).

Osteoinduction is the key characteristic of **growth factors**, especially **BMP**, which is probably why it is most widely used. It has been used in combination with internal fixation in treat-



Picture 1. Process of osteoblast formation. Levels of differentiation of osteoblast and main activity of the cell at each level, some typical genes that express themselves at each level and the approximate point of activity of some main bone growth factors and hormones. Legend: H4 – histon H4, Cbfa1 – transcribing factor “corebinding factor ±1”, AP – alkalic phospathase, OP – osteopontin, ON – osteonectin, BSP – bone sialoprotein, VDR – vitamine D receptor, PDGF – platelet derived growth factor, EGF – epithelial growth factor, bFGF – base fibroblast growth factor, TGF – transforming growth factor, IGFs – insulin-like growth factors I and II, IL-6 – interleukin 6, E2 – estradiol, Vit D – vitamine D3, PTH – parathyroid hormone, PTHrp – parathyroid hormone related protein, BMP – bone morphogenetic proteins (10).

ment of non-united fractures and in posterior spondylodesis caused by pseudoarthrosis. There are reports of successful use in management of segment bone defects. **Growth factors are today one of the most important fields of research because of their significant influence on the growth of bone tissue and also of other tissues.** Their impact on the growth of cartilage in ligaments and also their use in reparation of meniscuses has also been described (11).

The answer for the future are perhaps **combined implants**. Some studies show the utility of the BMP and artificial bone matrix combination in treatment of femoral fractures in rats. Records exist about the use of beef collagen combined with ceramic material along with autologue bone marrow for treatment of defected long bones (11).

Other biological methods in bone surgery

The use of **bio-degradable nails** is still in the phase of pre-clinical researches. Those nails, first described by Van der Elst et al. (12) who used them for treatment of femoral fractures in sheep, would combine several advantages: only one operation (without removal) possibly combined with osteoinductive substances (growth factors), providing sufficient mechanical stabilization and at the same time with osteoconductive abilities without immunological reaction.

Gene therapy also gives promising results. This usually means a transfer of a gene that codes the growth factor to target cells with the help of a vector (plasmid, virus). Studies of gene therapy for spine fusion with the help of transfected cells of inter-vertebrae plates already exist (13).

Where are we and where are we headed

Some of the mentioned methods are already used in Slovenia, especially autologue transplants and those that are financially the most acceptable, e. g. prosthetics covered in ceramic materials.

Only a few research centers in the world conduct any researches in the field of growth factors, special osteoconductive ceramic materials and bio-degradable substances for fracture treatment. The discovery of growth factors is a large step forward, as their use - especially in combination with other methods - appears promising.

But there are still many questions without answers. Which are the mechanisms in fracture treatment? How to find the precise concentration of the growth factor and then maintain it at a certain level? Which combination of bio-active materials should be used and when? Another drawback is also the price, because the universal preparation of growth factors has not yet been developed. With the help of growth factors, cartilaginous tissue, meniscuses in periphery nerves can be restored. Injecting substances which cause ossification will replace complex surgical procedures in treatment of pseudoarthrosis and spondylodesis (14).

In the future, we can hope for an ideal bio-degradable nail for the use of intramedullary fixation, one that will consist of growth factors and combine osteogenetic, osteoprogenitory and osteoconductive qualities. Such a nail would fixate the fracture, enhance the reparation process and would not require removal (14).

Gene therapy has a lot of potential, although the problem of transinfection of the cell with the gene (potential infection) remains unsolved for the time being. Exact mechanisms of expression and regulation of genes are not clear yet. Gene treatment would add the damaged or missing genes to the bones and also cure other diseases, such as osteopetrosis (14).

This way new possibilities and ideas will arise in bone surgery that we may not even be aware of at this point (14).

Conclusion

The researches of the 21st century will focus on the cell and molecular level and on genetic engineering. Today bone surgery is reaching new dimensions through combining the initial mechanic approaches with new biological methods. This way the bone surgeon of the 21st century is facing new challenges in the field of molecular understanding and should not focus only on the mechanical part of the fracture treatment.

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