Update distraction osteogenesis/histogenesis

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Abstract : Distraction osteogenesis (DO) techniques have been developed in orthopaedics with outstanding clinical outcomes. This review discusses recent advances in understanding the basic biological mechanisms of DO, methods of assessing and promoting bone consolidation in DO and new clinical applications of DO. Mechanical stimulation is known to regulate many genes in skeletal tissues. The changing patterns of BMPs expression, cell proliferation, angiogenesis and apoptosis regulate bone regeneration in DO. High magnitude strain promotes bone remodelling, whereas low magnitude stain stimulates angiogenesis and bone formation. For clinical assessment, plain radiography is the most common imaging technique, followed by ultrasound, mechanical testing, DEXA and QCT. For promoting bone consolidation during DO, minimally invasive interventions are preferable, such as weight-bearing exercise, ultrasound, and electromagnetic stimulation; whereas systemic administration of anabolic agents and hormones may also be employed and local application of growth factors such as BMPs and other growth factors, peptides remains the last resort. New applications of DO have been extended into treating difficult vascular diseases, cosmetic lengthening, spinal deformities. Through studying DO technique we realise body's selfrepair and self-regeneration potentials and its principles and new clinical applications are to be extended to functional tissue engineering in many different systems and disciplines.

Introduction

Distraction osteogenesis (DO) techniques have been developed over the last 50 years and is now widely accepted and practiced in orthopaedics, traumatology, and craniofacial surgery. Using DO methods, many previously untreatable conditions have been successfully managed with outstanding clinical outcomes^{1)~8)}. Although the biological mechanisms of DO are still not yet fully defined, it is generally accepted that mechanical stimulation is the key in promoting and maintaining tissues' regenerating capacities. This review discusses recent advances in understanding the basic biological mechanisms of DO, new methods of assessing and promoting bone consolidation during DO treatments and its potential new clinical applications.

Biological mechanisms of DO

Many genes have been found being up- or down-regulated in the bone cells responding to mechanical stimulation⁹⁾. Mechanical stimulation is the single, most important factor that can

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trigger changes in gene expression and lead to cell proliferation, angiogenesis, cell death and tissue formation and remodelling. For instance, the nuclear proto-oncogene c-fos and c-jun were found to be up-regulated at early stages of DO^{10} , Fos- and Jun-related genes were related to mechanotransduction and embryonic bone development, their strong expressions during DO provide further evidence to support Ilizarov's hypothesis that DO resembles many aspects of embryonic development. Slow dividing tissues such as Schwann cells retain ability to synthesize myelin during gradual nerve elongation¹¹⁾, suggesting that DO process is indeed a process of histogenesis, where soft tissues such as muscle, ligament, tendon, blood vessels as well as nerve will regenerate in response to mechanical traction. Mechanical signals play an integral role in bone homeostasis. Low magnitude of tensile stain (2-8% equibiaxial strain) in the tissues has antiinflammatory effects and inhibits proinflammatory gene expression (such as IL-1 β , interleukin 1 beta and COX-2, cyclooxygenase-2), whereas tensile stain of high magnitude (15% equibiaxial strain) induces proinflammatory gene expression, rapidly up-regulated COX-2 mRNA expression and PGE₂ (prostaglandin E2) synthesis¹²⁾. Several studies have suggested that growth factor signalling is also involved in the transduction of mechanical stimuli, for example, epidermal growth factor receptor expression is up-regulated in osteoblastic cells under fluid flow9). Taken together, these observations reveal an important mechanism that bone resorption may occur in a field experiencing high magnitudes of strain and bone formation results in fields exposed to physiologic or low magnitude of strain. This may also explain the stimulatory effects on bone regeneration/consolidation by weight bearing exercise. pulsed electromagnetic field stimulation, ultrasound and short-wave treatment.

For bone regeneration during DO, bone morphogenic protein (BMP)-2, 4, 5, 6 and 7 are all expressed continuously from the beginning of DO till 2 weeks after the completion of DO^{13} suggesting BMP genes are responsible for controlling the balance of bone formation and remodelling during DO. During DO, new bone forms and undergoes rapid remodelling, the localization of apoptotic cells at the different regions of the regenerate, accompanied by the osteoclast activities, suggest that apoptosis is closely related to bone formation and remodelling during DO¹⁴⁾. It is well documented that DO is a vascular-dependent process. As to the source of bone-forming progenitors during DO, many believe that the periosteum and bone marrow are the main contributors. Poorly preserved periosteum at surgery may indicate slow bone regeneration or poor regenerate quality¹⁵⁾. With appropriate soft tissue preservation and mechanical stimulations, good quality of bone regeneration usually occurs, even in the bone following chemotherapy¹⁶⁾. DO stimulate expression of angiogenic factors, such as VEGF (vascular endothelial growth factor) and bFGF (basic fibroblast growth factor) in the newly formed bones¹⁷. DO results not only in increased local expression of VEGF and its receptors at the site of distraction gaps, but also leads to increased expressions of VEGF and its receptors levels in distant muscle sides¹⁸⁾, suggesting that DO induces systemic responses, such as releasing of growth factors, cytokines, hormones, stem cells that promote healing¹⁹⁾.

Assess bone quality in DO

DO can be a lengthy procedure and the healing

index, the time needed for each centimetre of new bone to form and mature and to maintain its structure after fixator removal, ranges from 20 days to 4–5 months depending on patient age, bone location, total lengthening and surgical managements³⁾. Past research has also suggested that long duration of DO treatment can have negative impact upon the physical and psychological well-being of patients, particularly the young person. Recent study in young people with DO treatment has suggested that with proper support and education, (young) patients can tolerate DO treatment without sustained adverse psychological impact²⁰.

Non-invasive imaging such as plain radiography remains the most cost effective imaging technique to monitor the regenerate³⁾, but plain x-ray is not reliable to predict bony union or the quality or quantity of the regenerating bone, since an estimated 40% increase in radio-density is needed to visualize a radiological change, and radiographic changes did not correlate to the changes of mechanical stiffness²¹⁾. Supplemental techniques including mechanical testing for bone strength and stiffness, DEXA (dual-energy x-ray absorptiometry) for bone mineral density, QCT (quantitative computerized tomography) for density and cortical continuity, ultrasound for cyst detection and Doppler or angiography for local blood flow and vascularity have all been used clinically. Among them, ultrasound is a useful and accurate method to evaluate bone cyst in DO²²⁾²³⁾. Ultrasound examination does not produce metal artefact and radiation exposure and the cost is low, but the facilities for ultrasound follow-up must be developed with an experienced radiologist and it is only recommended where this possibility exists²³⁾. The mechanical stiffness does not always correlate with the plain radiographic

and ultrasound data²¹⁾, even when radiographic consolidation of the distraction regenerate is observed, the literature recommends waiting for 2 extra months before removing the external fixation²⁴⁾, hence the clinical decision of fixator removal has to be made on case by case basis by experienced clinicians.

Promote bone consolidation in DO

Although DO has revolutionized the treatment of many orthopaedic disorders, one of the problems of this technique is the long waiting period for newly formed bone to consolidate, which can cause considerable morbidity to the patients, such as pin-track infection, delayed consolidation and discomfort caused by the bulky frame²⁴⁾. Various approaches have been tested to enhance bone formation during DO, these are summarised in Table 1.

Mechanical stimulation by controlled weightbearing exercises promotes bone consolidation by stimulating angiogenesis, the newly formed vessels in the periosteal region are more sensitive to mechanical stimulation than the endosteal vessels²⁵⁾. This again suggests the importance of periosteum preservation and postoperative physiotherapy managements. Pulsed electromagnetic field stimulation may be a safe and cost-effective way of promoting bone consolidation in DO, since electromagnetic field stimulation increases callus formation but does not affect the callus remodelling phase²⁶⁾, and electromagnetic stimulation can reduce the latency period, from 7-10 days to 1 day, following osteotomy without compromising overall bone regeneration of DO²⁷⁾.

Some studies suggest that early conversion from external to internal fixation may be an alternative for reducing complications of DO²⁴⁾ or using lengthening over the nail technique²⁸⁾.

Table I. Factors Promote Bone Consolidation in Distraction Osteogenesis

Mechanical

- Weight-bearing(mechanical compression)
- Ultrasound(low velocity)
- Electromagnetic field stimulation
- Electrical currents stimulation
- Short-waves treatment

Biomaterials/Cells

- Calcium sulfate
- Tri-calcium phosphates
- Autologous bone grafts and allografts
- Chitosan and other biopolymers
- Osteoblastic cells
- Bone marrow extracts
- Platelets

Hormones/Anabolic and Antiresorptive Agents

- Growth Hormone
- PTH
- PGE₂ receptor modulator
- Bisphosphonates/Zoledronic acid

Biomolecules/Growth Factors

- BMP-2/BMP-4
- BMP-7/OP-1
- VEGF
- FGF-2
- TGF- β
- Thrombin-related peptide
- Others

After the lengthening is achieved by external fixator, fixator can be removed and the limbs supported with interlocked intramedullary nails, this will reduced the complications of re-fracture and infection following DO treatment.

Systemic administration of anabolic agents and hormones to promote bone regeneration is an appealing strategy. Growth hormone has shown to promote early bone consolidation when given a daily subcutaneous injection of 1 IU/kg in a dog DO model²⁹⁾, and bone mechanical strength increased 3 times in the growth hormone group than in the control group. Prostaglandins are anabolic agents *in vitro*, but they can not be used *in vivo* due to its gut-intestinal side effects³⁰⁾. Recent study into the PGE₂ receptor, such as EP2 receptor-selective agonist, may lead to a new class of anabolic agents that can be administrated locally and systemically to stimulate osteogenesis. fracture healing and DO³¹⁾. Antiresorptive agents such as bisphosphonate have been reported to have positive effect on fracture healing. A recent study showed that in a rabbit model of leglengthening, systemic administration of zoledronic acid (0.1 mg/kg) once or twice increased distraction regenerate volume, mineralization and strength³²⁾, suggesting bisphosphonate may have anabolic effect in addition to its antiresorptive effects. However, a dose-related negative effect of zoledronic acid on the longitudinal growth of young rabbits has been noted³²; therefore, it may not be safe to give bisphosphonates such as zoledronic acid to children undergoing DO treatment.

Local application of growth factors such as FGF-2, BMP-2 and other biological agents to promote fracture healing and spine fusion has become an accepted clinical alternative. In a rabbit DO model with rapid rate of lengthening(2) mm/day), single application of rhBMP-2(75 μ g) by injection or implantation at the end of distraction period has significantly enhanced bone maturation and bone consolidation³³⁾. In the similar rabbit model of DO, a single injection of $300 \,\mu g$ thrombin-related peptide (TP508) has also shown to promote bone consolidation and the effects were more potent when the TP508 was delivered in slow-released form³⁴⁾³⁵⁾. Since DO is a vascular-dependent process, the angiogenic factors may also have positive effects on bone regeneration during DO. It has been reported that infusion of FGF-2 into the distraction gap in rabbit has significantly promoted bone consolidation³⁶⁾. In summary, additional growth factors may enhance bone regeneration and consolidation in conditions where bone argumentation is clearly needed.

New clinical applications of DO

In addition to its well-known applications in orthopaedics and traumatology, DO techniques have also been widely accepted and performed in craniofacial surgery^{$2(4)\sim 8$}). Mandibular DO is becoming the first choice of treatment for patients with hypoplastic mandibles⁵⁾⁸⁾. Micrognathia accompanying obstructive sleep apnea syndrome, which is a difficult clinical problem with mandible deformities (shortening) and temporomandibular joint ankylosis, usually results in airway space narrowing⁶⁾. Mandibular DO has achieved excellent clinical outcome for treating this special syndrome with no age limit of the patients⁶⁾. DO in craniofacial surgery has been developed rapidly and will advance the management of complex craniofacial anomalies, but at the present, its procedures have not been fully established in comparison to its applications in orthopaedics, and more basic and clinical studies are needed to perfect DO techniques in craniofacial surgery⁴⁾.

Another exciting new development in DO application is to treat vascular conditions such as peripheral chronic artery obliterations³⁷⁾. Qu et al have reported treating patients with thromboar-teritis thromboangiitis obliterans successfully by transverse tibial cortex transport. In that, a longitudinal cortical osteotomy was performed splitting a piece of cortex, which was fixed to an external fixator and the split cortex was length-ened at 1 mm/day for 20 days transversely. During the bone transport, the symptoms of cool sensation of leg, the tingling of feet, and the intermittent claudication disappeared gradually in all patients. On angiography, 25 days after the bone transport completed, a richer vascular

network has developed in the treated leg compared to before the treatment. The increased blood flow and vasculature after transverse tibial lengthening (thickening) by Ilizarov method has also been reported in a dog DO model³⁸⁾, where changes in blood flow and vasculature were most pronounced in the distraction leg and sustained a long-lasting stimulatory effect of promoting circulation in the treated extremity³⁸⁾. The positive effects of DO on angiogenesis, vasculature formation and blood flow of the treated extremity suggest that DO may be an invaluable tool to treat vascular or circulation related disorders, such as diabetic ulcers, chronic artery obliterations, avascular necrosis of femoral heads and chronic soft tissue infections. Recently, the first author has paid a visit to Russian Ilizarov Centre, Kurgan Russia and found that Russian doctors are applying the DO technique on the skull to treat patients suffering from strokeassociated complications, such as paralysis and loss of speech, with outstanding outcome. The principle is similar to the tibial cortex transport, where a small window was created on the skull above the areas of blocked blood vessels (guided by angiography) and a small piece of skull is then transported, during which process it promoted rapid angiogenesis and new vessel formation underneath the skull, and hence enhanced blood supply to the stroked area and improved symptoms of the patients significantly. However, reports on this aspect are lacking and more research is needed in this area of new DO application.

Summary

Under normal circumstances with correct surgical management and adequate support of postoperative physiotherapy, DO treatment has good clinical outcome and does not need any form of additional intervention (s). However, if bone consolidation becomes a clinical concern, minimal non-invasive means of intervention for promoting bone consolidation are preferable, such as weight-bearing exercise, ultrasound, electromagnetic stimulation. Systemic administration of anabolic agents and hormones may also be useful and local application of growth factors such as BMPs remains the last option, as their working mechanisms in DO are still not clearly known and are expensive.

Being a great surgical technique for tissue repair and regeneration. DO also has a wider implication in understanding body's self-repair and self-regeneration potentials, and its new clinical applications are to be extended to functional tissue engineering, management of soft-tissue repair, treatment of vascular diseases and others.

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延長による骨新生と組織新生:最新の話題

要 旨 仮骨延長法は整形外科分野ですばらしい臨床的成果をあげながら発展し てきた この論文では基礎的な仮骨延長における生体反応、仮骨形成の促進法や評 価手段、そして仮骨延長法の新しい臨床応用などに関し、最近の研究を概説する 機 | 械的負荷は骨組織において多くの遺伝子の発現を調節することが知られている ま た延長仮骨において BMP の発現パターンの変化、細胞増殖、血管新生、そして細胞 アポトーシスなど骨の形成を調整する因子も知られている 骨への負荷が強大であ ると骨のリモデリングが促進され、小さければ血管新生と骨増殖が促進される 臨 床評価手段としては単純X線所見はもっとも普遍的な画像評価法であるが、その後、 超音波、力学的テスト、DEXA、そして QCT などが導入されている。 仮骨の成熟を 促進する方法としては低侵襲の方法がいくつか推奨される。たとえば体重負荷訓練, 超音波刺激、電磁場刺激などである。しかしタンパク合成薬やタンパク合成ホルモ ンの全身投与もあり得るし、BMP やそのほかの成長因子、あるいはペプチドなどの 局所投与などは最終手段として残されている。仮骨延長は新しく難治性の血行障害, 美容外科、脊柱変形などに適応が拡大されてきた、仮骨延長法を研究しているうち にわれわれは生体の自己治癒能力、自己再生能力というものに目を開かれた そし て仮骨延長原理と新しい応用技術により、いくつもの異なった系統や分野で、生体組 織工学はより機能的なものへと拡大してゆくはずである

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