

RESEARCH ARTICLE

CURRENT CONCEPTS AND TECHNIQUES IN ENHANCEMENT OF FRACTURE HEALING IN CANINES: A REVIEW.

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Abstract

Traditionally used metallic implants for fracture stabilisation even though have potential capability for fracture healing have been associated with significant disadvantages like patient incompliance and implant loosening leading to mal union, non-union and dis-union. Veterinary orthopaedics during the last decade has visualised a significant development with respect to use of biomaterials particularly in the field of fracture treatment and stabilisation. A large variety of biomaterials are available in the form of bone grafts and bone substitutes for commercial use to treat significant bone defects due to trauma and to enhance the rate of fracture healing. The present article aims to give an insight into the various bone grafts and bone substitutes available, their potential uses, applications, advantages and limitations in veterinary field.

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Introduction:-

Fractures are defined as the break in the continuity of the bone and is characterised by pain, immobility and loss of function. Fractures in animals invariably causes pain and suffering to them apart from loss of function of the affected limb (Vardhan et al., 2017). Various internal and external skeletal fixation devices have been used in veterinary practice for stabilisation of fracture with variable results. Thus enhancement of fracture healing and achieving complete fracture union as fast and early as possible has become the goal of modern veterinary orthopaedics. The main goal of fracture treatment is to achieve the normal anatomic alignment of the bones and to maintain the bone segments in apposition until complete healing occurs. Open reduction remains the standard technique of fracture treatment in veterinary practice. Traditionally many bioinert metallic implants in the form of intramedullary pins and nails, plates and screws and external skeletal fixators have been used. But these have been associated with delayed union, mal union and non-union mainly due to excessive movement of the patient and resultant implant loosening. Modern methods of fracture treatment involves the use of natural, semi-synthetic or synthetic bone grafts or bone substitutes to fill the gap between the defect and to hold the implant against the bone thereby enhancing bone healing, hastening the fracture union and avoiding complications like implant loosening, migration, non-union, mal-union and delayed union. Many such bone filling agents are available for use by practicing veterinarian that enhances the fracture healing and holds the implant against the bone thereby keeping the fracture segments stable which helps in enhanced osteogenesis.

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Current Concepts in Enhancement of Fracture Healing

Bone regeneration is comprised of a well-orchestrated series of biological events of bone induction and conduction, involving a number of cell types and intracellular and extracellular molecular signalling pathways with a definable temporal and spatial sequence in an effort to optimise skeletal repair and restore skeletal function (Cho et al., 2002). Repair of fracture involves a sequence of dynamic events which ultimately restores the integrity of bone and its biomechanical properties (Einhorn, 1998). Bone repair requires four critical elements: osteogenic cells, osteoinductive signals provided by growth factors, an osteoconductive matrix, adequate blood and nutrient supply (Vardhan et al., 2017). All these required elements are provided by biomaterials. Biomaterials are biocompatible materials used inside the body to treat or augment disease conditions. In veterinary practice, biomaterials are used mainly to treat bone fractures. Such biomaterials are capable of providing an environment that simulates the natural conditions of the body. In addition it also provides key components in bone fracture repair. Traditionally the biomaterials used for fracture treatment include bioinert intramedullary pins, screws and plates made of stainless steel and titanium. But these have been associated with mal union, non-union and delayed union mainly because of the considerable degree of movement between the fracture segments. Modern fracture fixation system involves the use of natural, semi-synthetic and synthetic bone grafts and bone substitutes that gets degraded over a period of time and enhances bone healing and promotes clinical union with lesser complications by stabilising the fracture segments in apposition. The ideal material used for fracture fixation must be biocompatible, resorbable and porous to facilitate rapid vascularization and progressive replacement by newly formed bone tissue (Hannouche et al., 2001).

Fracture healing can be enhanced by the use of various growth factors, bone grafts and bone substitutes. The most commonly used materials to promote bone healing are bone grafts (autografts and allografts), bioceramics (Hydroxyappatite, Tricalcium phosphate, Dicalcium phosphate, Calcium sulphate, Bioactive glass), biopolymers (Polylactic acid, Polyglycolic acid, PMMA) and metals (Stainless steel, titanium and its alloys) (Vardhan *et al.*, 2017). Use of bone grafts and bone substitutes like bioceramics and biopolymers either alone or in combination with traditionally used fixation implants to enhance fracture healing have been the latest development in the field of veterinary orthopaedics. These biomaterial scaffolds serve primarily as osteoconductive moieties on which newly formed bone deposits through creeping substitution from adjacent living bone (Groeneveld *et al.*, 1999). They provide structural support to fractures and should be able to withstand certain levels of loading (Huang *et al.*, 2012).

Growth Factors

Growth factors play a vital role in healing of any damaged tissue whether it's soft tissue or bone. Bone releases several growth factors at the site of the fracture including bone morphogenic proteins (BMP), TGF-beta, PDGF, IGF-I, IGF-II and basic and acidic Fibroblast Growth Factor (FGF). Bone Morphogenic Proteins are the only factor known to induce bone formation heterotopically by inducing undifferentiated mesenchymal cells to differentiate into osteoblasts (Hannouche *et al.*, 2001). The release of fibroblast growth factor play an important role in the initial phase of healing process, since they have shown angiogenic properties and mitogenic activity on the osteoblast lineage (Bostrom *et al.*, 1999). Bone marrow is a rich source of all these growth factors and hence use of bone marrow at the fracture site can enhance fracture healing by the release of these growth factors.

Bone Morphogenic Proteins are soluble, bioactive signalling proteins that include many factors implicated in osteoinduction (Huang *et al.*, 2012). BMPs are the only factors known to induce bone formation heterotopically by inducing undifferentiated mesenchymal cells to differentiate into osteoblasts (Hannouche et al., 2001). They induce the mitogenesis of mesenchymal stem cells and other osteoprogenitor and their differentiation towards osteoblasts (Dimitriou *et al.*, 2011). These BMPs have been used in variety of clinical conditions including non-union, open fractures, joint fusions, aseptic bone necrosis and critical bone defects (Giannoudis and Einhorn, 2009).

Bone Grafts and Bone Substitutes

Present medical and veterinary surgical practice involves the use of bone grafts and bone substitutes to replace or regenerate the bone defects. The principle of bone tissue engineering is based on using natural or synthetic scaffolds that are biocompatible and similar to the bone matrix (Salamasi *et al.*, 2016). The graft materials required to fill the fracture gap should promote healing by osteoinduction, osteogenesis and osteoconduction and minimize complications like non-unions or delayed unions (Bishnoi, 2013). Bone grafting is a commonly performed surgical procedure to augment bone regeneration in a variety of orthopaedic and maxillofacial procedures with autologous bone being considered as the gold standard bone grafting material as it combines all properties required in a bone graft material like osteoinduction (BMPs and other growth factors), osteogenesis (osteoprogenitor cells) and

osteoconduction (scaffold) (Bauer and Muschler, 2000). Significant bone defects or post traumatic complications such as delayed union, non-union or mal union may require bone grafting in order to fill the defect.

Bone grafts

Cellular events in fracture repair are controlled to a large part by growth factors and low molecular weight glycoproteins which induce migration, proliferation and differentiation of an appropriate subset of cells in the site of fracture (Hannouche *et al.*, 2001). These elements involved in fracture healing are naturally present in bone grafts and hence can be implanted at the site of fracture to enhance bone healing. Bone grafts functions as a source for osteogenesis, osteoinduction and even mechanical support (Basset, 1972). Natural bone grafts include autogenous bone grafts, allogeneic bone grafts and bone marrow.

Autogenous Bone Grafts

Autogenous bone grafts are considered as the gold standard for bone replacement mainly because they offer minimum immunologic reaction, complete histocompatibility and provide best osteoconductive, osteogenic and osteoinductive properties (Samartzis et al., 2005). Autogenous bone grafts are of two types: Autogenous Cancellous bone grafts and Autogenous Cortical Bone Grafts. Autogenous cancellous bone is the most commonly used bone graft which is readily available and easily harvestable but it lacks mechanical strength (Beaumont, 1970). Autogenous cancellous bone grafts has been considered more osteogenic as compared to cortical grafts because of the presence of spaces within their structure that allows diffusion of nutrients and limited revascularisation by microanastomosis of its circulating vessels (Khan et al., 2005). Autogenous cancellous grafts mainly acts mainly as an osteoconductive substrate which efficiently supports the ingrowth of new blood vessels and infiltration of new osteoblasts and osteoblasts precursors (Marx and Wong, 1987). Autogenous cancellous bone grafting is indicated when early production of bone and rapid healing is desired (Vaccaro, 2002). A variety of sites can be used for bone graft harvesting with the anterior and posterior iliac crests of pelvis being the most common donor sites (Dimitriou et al., 2011). Intramedullary canal of long bone has been used as an alternative harvesting site providing a large volume of autologous bone graft (Giannoudis et al., 2009). Johnson (1986) and Penwick et al (1991) stated that fresh autogenous cancellous grafts is more osteogenic than other bone grafts and the most common harvest sites in dogs and cats are the proximal humerus and proximal tibia (Bishnoi, 2013). These offer structural support to implanted devices and ultimately become mechanically efficient structures as they are incorporated into surrounding bone through creeping substitution (Greenwald et al., 2001). Autologous bone grafts even though considered as gold standard graft material are associated with a number of disadvantages like donor site morbidity, pain, haematoma or infection (Damien and Passons, 1991; Meister et al., 1990). Harvesting requires an additional surgical procedure with well documented complications and discomfort for the patient and has additional disadvantage of quantity restriction and substantial costs (Younger and Chapman, 1989). But autologous bone is biocompatible and nonimmunogenic, reducing the immunoreactivity and transmission of infections (Dimitriou et al., 2011).

Allogenic Bone Grafts

Allogenic bone grafts are obtained from cadavers and such grafts has both osteoinductive and osteoconductive properties but lack osteogenic properties because of absence of viable cells (Habibovic and de Groot, 2007). Allogenic bone grafts are available as Demineralized Bone Matrix (DBM), morselized and cancellous chips, corticocancellous and cortical grafts and osteochondral and whole bone segments (Dimitriou *et al.*, 2011). The advantages of allogenic bone grafts is their easy availability, can be obtained as per required size and shape, avoids the use of host structures thereby reducing morbidity and has reduced chances of immunoreactivity. But such grafts are associated with increased risk of disease transmission. Harvesting and storage of such grafts provides additional problems in the practical use of these grafts.

The use of grafts either autogenous or allogenous bone graft alone do not ensure optimum healing. A number of other factors also tends to play a role in healing process with the use of grafts. Time interval between procurement and transplantation of graft is an important factor (Bohatyrewicz *et al.*, 2006). It is said that an autogenous bone graft retains its viability for upto 2 hours when kept in normal saline (Laursen *et al.*, 2003). In addition an accurate contact must be ensured between the graft and the graft bed for optimum healing. Vascular supply, lack of infections and surrounding microenvironment also influences fracture healing.

Bone Marrow

Bone marrow has been used to stimulate bone formation in skeletal defects and non-union through cytokines and growth factors secreted by the transplanted cells (Connolly, 1995). Lindholm *et al.* (1982) stated that bone marrow

contains osteoprogenitor stem cells that are able to form bone when combined with various elements of an osseous matrix. Bone marrow is a rich source of growth factors and other osteogenic cells. The delivery of bone marrow aspirates from the recommended sites to the fracture region can enhance fracture healing to a significant extent since these bone marrow aspirates are rich in bone morphogenic proteins and other growth factors.

Bone Substitutes

Bone graft substitutes are an alternative to autogenous or allogenic bone grafts. They consist of scaffolds made of synthetic or natural biomaterials that promote the migration, proliferation and differentiation of bone cells for regeneration (Dimitriou et al., 2011). The concerns over the use and availability of autogenous or allogenic bone graft materials have prompted studies aimed at developing suitable synthetic bone substitutes (Pilliar et al., 2001). An ideal bone graft substitute material must be osteoconductive in order to allow as rapid as possible integration with the host bone, biodegradable at a perfect rate in order to eventually be replaced by newly formed natural bone and strong enough to fulfil required load bearing functions at least during the early post implantation period (Pilliar et al., 2001). Bone substitutes should be highly efficient in terms of bone ingrowth to allow a faster regeneration and a better functional recovery (Mastrogiacomo et al., 2006). The bone graft substitutes include naturally occurring materials like bovine collagen mineral composites, Hydroxyappatite and Synthetic materials like calcium sulphate, calcium phosphate and bioactive glass. The efficacy of these materials in the fracture healing depends on stability of fracture fixation (Bishnoi, 2013). Among the bone substitutes, bioceramics (Calcium phosphates and Hydroxyappatite) are very promising candidate in bone substitutes because of their bone like chemical composition and mechanical properties (Mastrogiacomo, 2006). The advantages of bone graft substitutes are their availability in large quantities, shape and size but such materials lack osteogenic and osteoinductive potential and hence an osteogenic or osteoinductive material should be added to make a composite graft to promote osteogenic potentials (Zamprogno, 2004).

Natural Bone Graft Substitutes

Collagen

The main component of the bone are collagen and hydroxyappatite matrix which is osteoconductive in behaviour. Collagen acts as a matrix over which the bone cells gets attached and stimulate bone formation. Collagen can be used alone or in combination with other bone graft substitutes to promote bone healing. In many research purposes, collagen in combination with hydroxyappatite have been used as bone substitutes and reliable results have been obtained.

Lignin

Lignin is a complex natural polymer that is biocompatible and has great potential in association with hydroxyappatite as a biomaterial for bone repair (Martinez et al., 2009). Lignin is also known to have antimicrobial and antioxidant properties and hence can be used even in infected and contaminated sites also. Addition of lignin with other bone cements is known to increase the strength 4-6 times and toughness by an order of magnitude (Sun *et al.*, 2007). Composites can also be made flexible with addition of chitosan in higher contents (Chow, 2009) and this is advantageous in grafting at irregular and non-uniform sites.

Chitosan

Chitosan, a natural product derived from the polysaccharide chitin, an abundantly available natural biopolymer found in the exoskeleton of crustaceans like shrimps, crabs, lobster and other shell fish would be an effective material to repair bon defects due to its biocompatibility (Mukherjee *et al.*, 2003). Chitosan is a naturally occurring polymer based composite biomaterial that have attracted considerable attention for bone tissue engineering purposes owing to their pore forming ability, binding capacity with anionic molecules, antibacterial activity and biodegradation (Venkatesan and Kim, 2014). The main disadvantage associated with use of chitosan is that these materials require close proximity with the host bone to achieve optimum healing by osteoconduction. The composite of chitosan and hydroxyappatite has shown good biocompatibility and osteoconduction in reconstruction of bone defects (Yuan *et al.*, 2008). Chitosan fibers which has excellent properties such as biocompatibility, biodegradability and non-toxicity can significantly improve the mechanical properties of calcium phosphate cement (Huang et al., 2012).

Synthetic Bone Graft Substitutes

The advent of synthetic materials for bone fixation is of paramount importance in orthopaedic surgery (Bostman, 1991). Many synthetic materials as bone substitutes are easily available including metals, polymers, ceramics and

glasses (Mastrogiacomo *et al.*, 2006). The first study concerning biodegradable materials used for implantation was presented in 1966 by Kukri et al who studied the biocompatibility of poly-L-lactic acid (PLLA) in animals (Kontakis *et al.*, 2007). The most bioactive materials include calcium phosphate ceramics and silicon based bioglasses characterised by the formation of a very tight chemical bond with the bone (Popkov *et al.*, 2016).

Hydroxyappatite

Hydroxyappatite is a biocompatible ceramic produced through a high temperature reaction and is highly crystalline form of calcium phosphate (Nandi *et al.*, 2010). Hydroxyappatite was first introduced because of its similarity with mineral phase of the bone (Hannouch *et al.*, 2001). The most unique property of this material is chemical similarity with the mineralized phase of bone. This similarity accounts for their osteoconductive potential and excellent biocompatibility (Ghosh *et al.*, 2008). Hydroxyappatite has been established to be an excellent carrier of osteoinductive growth factor and osteogenic cell populations which greatly add to their utility as bioactive delivery vehicles in the fracture (Noshi *et al.*, 2000). Porosity of hydroxyappatite material also tends to influence the healing properties. Microporosity allows body fluid circulation whereas macroporosity provides scaffold for bone cell colonization (Daculsi, 1988). Synthetic hydroxyappatite has been long known as one of the best coating materials for metallic implant due to its biocompatible, osteoconductive and osteoinductive properties (Fidancevska et al., 2007).

Calcium Sulphate and Calcium Phosphate Bioceramics

Calcium sulphate and calcium phosphate bioceramics are considered as promising synthetic bone graft substitutes particularly because of their comparable chemical and mechanical properties to that of natural bone. These materials mimic the mineral phase of the bone and are resorbed at a rate similar to the rate of new bone formation. Hence these are able to provide some structural support and prevent ingrowth of fibrous tissue while facilitating creeping substitution by host bone (Bishnoi, 2013). Bioceramics have excellent bone-bonding capacities but they are brittle and have poor resistance to compressive stress (Hannouche *et al.*, 2001). Bioceramics have been proven efficient as bone substitutes as these are available in large amounts, are biocompatible, bioactive and osteoconductive (Nicalazo *et al.*, 2003)

Calcium phosphates are osteoconductive and undergo gradual remodelling over time in a pattern similar to that of normal bone (Refai et al., 2004). Within few minutes after injection, calcium phosphate cement hardens due to crystallization. As the injected calcium phosphate interdigitates with the adjacent bone, a structure is formed that is more stable than either cancellous bone grafts or the pellets or blocks of hydroxyappatite, calcium phosphate, calcium sulphate often used to fill spaces (Larsson and Hannink, 2011). Fully cured calcium phosphate has a compressive strength between that of cancellous bone and cortical bone but tensile and shear strength are much lower than cancellous bone. Osteoclastic resorption of the cement, vascular penetration and bone formation occurs in a pattern that suggests remodelling similar to that of normal bone (Larsson, 2006). A proposed mechanism suggests that osteoinductive biomolecules such as bone morphogenetic protein 2 (BMP-2) are adsorbed onto the surface of calcium phosphate bioceramics after their implantation and these adsorbed osteoinductive biomolecules then initiate bone formation which appears as osteoinduction (Cheng et al., 2007). Calcium phosphates are available in two forms: Tricalcium phosphate and Dicalcium phosphate. The rate of resorption of calcium phosphate ceramics vary inversely with the calcium: phosphate ratio and also depends on the density, the size and the porosity. Tricalcium phosphate with a ratio of 1.5 is highly resorbable compared with hydroxyappatite having ratio of 1.67. Porous calcium phosphate implants prepared by sintering at lower temperature showed a higher osteoinductivity than those sintered at higher temperature (Ouhayoun et al., 1992). Sintering at lower temperature produced higher specific surface area that allowed adsorption of more bone morphogenetic proteins (Cheng et al., 2010).

Calcium sulphate is one of the first biomaterials used in bone reconstruction in medicine for over 100 years as a resorbable biomaterial for bone grafting (Szponder *et al.*, 2013). Calcium sulphate bioceramics immediately starts to degrade after implantation in a bone defect by passive dissolution caused by an ion exchange with the body fluids and these are brittle and weak in nature (Larsson and Hannink, 2011). The main advantages of calcium sulphate are biocompatibility, complete resorption in a relatively short period, conferred osteoconductive properties as well as relatively low cost and easy application (Szponder et al., 2013). Thus by combining fast resorbing calcium sulphate and slow resorbing calcium phosphate cements ,composite materials have been developed that might enhance vascular infiltration and replacement of the graft by new bone while providing osteoconductive and mechanical support (Johnson *et al.*, 1996). Calcium sulphate is a material with high surgical flexibility and its physical

properties like hygroscopicity and plasticity allows its easy application in the filling of the bony defect in small animals without the risk of biomaterial displacement to surrounding soft tissue (Szponder et al., 2013).

Bioactive Glass

Bioactive glass ceramics also known as bioglass composed of silica, sodium oxide, calcium oxide and phosphates are biocompatible and osteoconductive in nature and binds to bone without an intervening fibrous connective tissue interface (Zhang *et al.*, 2009). This material is used either alone or in combination with other bone grafts or substitutes in the form of composites for bone defects. It acts as a scaffold on which newly formed bone can be deposited after vascular in growth and osteoblast differentiation (Nandi *et al.*, 2010). Bioglass was found to trigger new bone formation by allogenic demineralized bone matrix and the biocompatibility of the glass was verified by the absence of adverse cellular reactions (Pajamaki et al., 1993).

Biodegradable/Bioabsorbable Polymers

Biodegradable organic based porous scaffolds like polylactide, polyglycolide or co-polymers formed from these materials (Laurencin and Lane, 1994) are used as bone graft substitutes. Traditionally such materials have been used to design various screws, plates, pins and rods. Modern practice involves the use of such synthetic polymers for the purpose of fabricating tissue engineered scaffolds with potential to promote in vivo bone ingrowth and subsequently repair or regenerate bone to replace missing tissue (Asti and Gioglio, 2014). These materials provide a scaffold over which the osteoblasts are able to lay down the matrix and enhance healing by creeping osteogenesis. Their relatively low modulus and strength properties make them unsuitable for load bearing bone substitute applications (Pilliar *et al.*, 2001). Biopolymers have also been used as carriers of growth factors mainly bone morphogenetic proteins. Although synthetic polymers can offer wide advantage including controlled degradation, biocompatibility, mechanical stability and many more, they lacks osteoconductivity (Salmasi *et al.*, 2016).

Polymethylmethacrylate (PMMA)

Polymethylmethacrylate commercially known as Bone Cement was introduced by Charnley and Smith in the late 1960s. PMMA have been considered as the gold standard in joint arthroplasty (Huang *et al.*, 2012). PMMA can be moulded into different shapes and sizes (Vardhan *et al.*, 2017), confirms to the shape of the surroundings, allows even distribution of implant loads and forms a strong mechanical bond with the implants (Kim *et al.*, 2004). PMMA are available as two component systems. It consist of a powder and a liquid monomer which when mixed result in a polymerization reaction forming polymethylmethacrylate. PMMA has been used since long time for hip arthroplasty procedures in human medicine and stabilisation of vertebral fractures and luxations in veterinary. PMMA acts as a space filler that creates a tight space which holds the implant against the bone (Vaishya *et al.*, 2013) and therefore finds significant use in fracture sites where implant loosening is a problem. The use of PMMA has several potential disadvantages including complications associated with exothermic polymerization and potential to serve as nidus of infection should the PMMA be contaminated (Lanz *et al.*, 1999).

Summary

Non-union is a common complication following long bone fracture with defects and its incidence depends on the location and severity of the injury to the bone, soft tissue and vascular structure (Sen and Miclau, 2007). Various biological bone grafts have been used for osteogenesis, osteoconduction and even osteoinduction, but due to systemic influences and local factors the outcome of successful incorporation of a bone graft has not been successful (Sharifi *et al.*, 2002). One of the most attractive features of injectable bone substitutes, besides providing mechanical support is their potential use for controlled release of therapeutic or bioactive agents (Sun *et al.*, 2007). Calcium phosphate and hydroxyappatite ceramics are considered among the most promising bone substitutes because of their bone like chemical composition and mechanical properties (Mastrogiacomo *et al.*, 2006). In the future, control of bone regeneration with strategies that mimic the normal cascade of bone formation will offer successful management of conditions requiring enhancement of bone regeneration and reduce their morbidity and cost in the long term (Dimitriou *et al.*, 2011). As an alternative to local augmentation of the bone regeneration process, the use of systemic agents including growth hormone and parathyroid hormone is also under extensive research (Dimitriou *et al.*, 2011).

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