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# Endochondral Bone Formation as Blueprint for Regenerative Medicine

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## 1. Introduction

During our life moving, walking, sport, etc., are essential for our health and quality of life. Both bones and cartilage enable us to do so. Bones support us, allow muscles to move them, and protect vital internal organs. At the end of most bones articular joints are situated. The side where 2 bones form an articular joint, the ends of these bones are covered with hyaline cartilage. This articular cartilage is able to withstand very high mechanical forces with very low friction and thereby enables easy movement. A large number of bones are formed by a process called endochondral ossification. During this process a cartilage template is replaced by bone, in contrast with the cartilage in newly formed joints which remains cartilage. Both articular cartilage and bone mature and this leads to a well organised architecture and specialisation. The arcade-like architecture of cartilage is capable to withstand an enormous amount of intensive and repetitive forces during life. However, the British surgeon William Hunter made the now famous statement that *"From Hippocrates to the present age it is universally allowed that ulcerated cartilage is a troublesome thing and that once destroyed it is not repaired"* (Hunter 1743). In contrast, bone has a very high regenerative capacity. This difference in self-healing capacity may partially be explained by the access to progenitor cells which contribute to tissue repair. For bone repair, progenitor cells of three different sources have been identified. These sources are: (i) progenitor cells from the blood stream since bone is a highly vascularised tissue, (ii) progenitor cells from the overlying periosteum and (iii) progenitor cells from the bone marrow. Cartilage is not vascularised, is not covered by periosteum, nor has a specialized tissue such as bone marrow and this might be part of the explanation for the limited self-repair capacity of cartilage. Although both tissues start from the same mesenchymal cell condensations, the contrast in self-repair is striking (Hunziker, Kapfinger et al. 2007).

From a clinical point of view there is a need for repair of both bone and cartilage. Bone and cartilage were both identified as tissues for which it was thought to be possible to recreate them in a laboratory setting, using the combination of cell isolation culture techniques and carrier materials. The science of combining cells with carrier materials to reproduce tissues in the laboratory is called Tissue Engineering (TE). The collaboration of scientists of different disciplines such as cell biology, biomaterials, biomechanics, engineering and translational

medicine has already led to fruitful scientific achievements. However, initial expectations of tissue engineering have not been reached completely. Although some treatments which apply to the principles of TE have reached clinical practice, TE-created tissues are not generated on a large scale (Brittberg, Lindahl et al. 1994; Oberpenning, Meng et al. 1999; Macchiarini, Jungebluth et al. 2008). In addition, time consuming and expensive culture procedures and logistics, multiple operations and quality of the repair that is initiated by TE constructs remain important drawbacks.

Upon implantation of a TE-created construct the introduction of cells, biomaterials, growth factors, etc. in the body will have an effect on the local environment and natural repair mechanisms at the implant site. Since it is largely unknown what this local effect is and how these factors contribute to it, a clear shift is observed in the attempts to repair tissue. This shift includes more specific natural stimuli which trigger and enhance the regenerative capacity of the tissue itself. Injection of stem cells or progenitor cells (cell therapies), and the induction of regeneration by biologically active molecules can all be regarded as an example of Regenerative Medicine (RM). For both TE and RM it becomes more and more evident that studying the underlying natural and developmental processes of cartilage and bone can serve as a blueprint to identify important cell sources, biochemical, biomechanical, structural stimuli and timing thereof. It is expected that insight in these biological mechanisms and the process of endochondral ossification will enhance the progress in the field of both TE and RM.

This chapter describes the first phases of endochondral ossification, bone and cartilage (defects) and current approaches in TE and RM. Parallels with RM and endochondral ossification are identified from where endochondral ossification can serve as a blueprint for future RM approaches.

## 2. Endochondral ossification

**Endochondral ossification** is a multistage process that determines the major part of mammalian skeletal development and starts in embryogenesis with condensation of mesenchymal stem cells. The formation of cartilage, a process called chondrogenesis, is a key event in developing limb buds beginning in the center of the condensed mesenchyme. The earliest form of cartilage development is suggested to be 300 million years ago (Urist 1976). In humans, the first skeletal rudiments develop during the 5<sup>th</sup> week of gestation. In the eighth week of the embryological life relatively cell-poor intermediate zones begins to develop, which will form the joint cavities (Gray and Gardner 1950; Anderson 1962; Aydelotte and Kuettner 1992). The diaphyseal cartilage, which is located at the center of the shaft of future long bones, is replaced by bone before birth (primary ossification). However most of the cartilaginous epiphysis at the end of long bones turns into bone after birth (secondary ossification). The remaining cartilage between the primary and secondary ossification centers is called the epiphyseal plate, more commonly known as the growth plate, and it continues to form new cartilage, which is replaced by bone, a process that results in increased length of the bones. Eventually all the cartilage in the growth plate will be converted into bone leaving cartilage only at the articulating surfaces of joints. Although bone and cartilage develop from the same mesenchyme, they have completely different structures, compositions and functions.

Chondrogenesis in both the primary and secondary ossification center and growth plates is characterized by highly proliferative chondrocytes, vectorially dictated to differentiate into hypertrophic chondrocytes before dying from apoptosis. The remaining mineralized extra cellular matrix provides a scaffold for infiltrating blood vessels and for bone cells to adhere to and remodel, setting the stage for *de novo* bone deposition (Kronenberg 2003) (Figure 1). The bone forming cells, osteoblasts, arise from progenitor cells from the overlying periosteal tissue and will form the bone collar (later the cortex) and primary spongiosa (later trabecular bone). In the adult, bone and overlying articular cartilage are attached by an interface of calcified cartilage (Schenk, Eggli et al. 1986). This interface distributes forces and stresses applied during load bearing and acts as a barrier to nutrients. Nutrients for the growing epiphyseal cartilage are supplied by two sources: (i) the synovial cavity and (ii) the vascularized cartilage canals (McKibbin and Maroudas 1979; Kuettner and Pauli 1983). Cartilage and synovium merge at a transitional zone which persists in the adult and is the site of osteophyte formation (Blaney Davidson, Vitters et al. 2007). This osteophyte formation is one of the first examples of endochondral ossification which takes place after growth. Another example is endochondral ossification during fracture healing where a cartilage callus is formed which will be remodelled into new bone. Studying endochondral ossification in normal growth and in healing processes will improve our understanding of both chondrogenesis and osteogenesis and as such may serve as a blueprint for Regenerative Medicine purposes of these tissues.

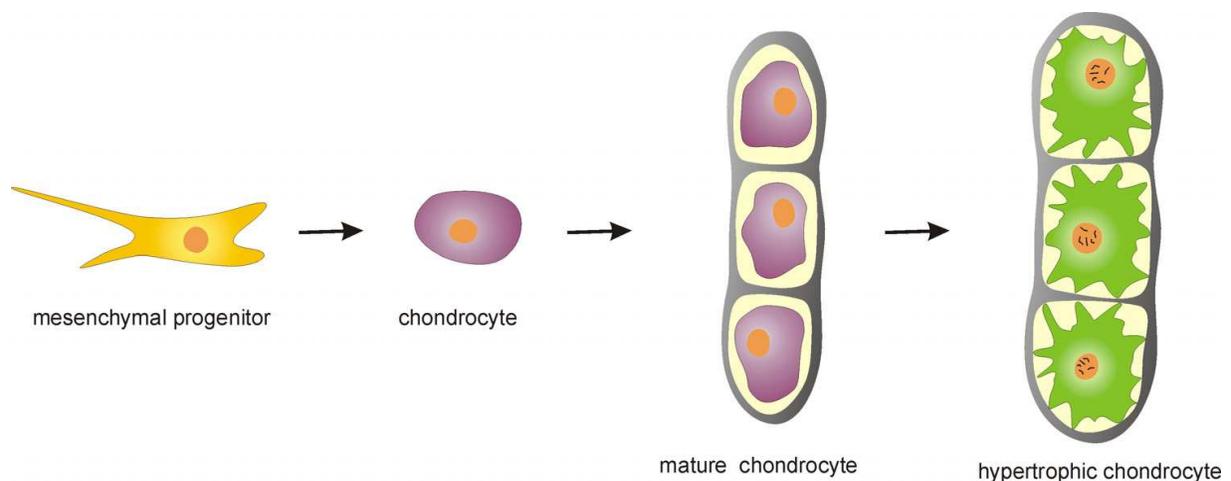


Fig. 1. The different steps of endochondral ossification; mesenchymal progenitor cells condense and undergo chondrogenesis. After maturation these chondrocytes undergo hypertrophy and die by apoptosis leaving a scaffold as a template for bone formation (these last steps are not illustrated nor discussed in this chapter).

## 2.1 Bone and bone defects

**Bone** can be formed by 2 different processes, while endochondral bone formation drives most of the skeletal bone formation, bone can also be formed by another process called intramembranous bone formation. During intramembranous bone formation, no cartilage phase is found and progenitor cells directly differentiate into bone. Intramembranous bone formation is largely responsible for the formation of flat bones as can be found in the skull and pelvis. Endochondral bone formation is largely responsible for the formation of bones

of the axial skeleton. While in cartilage only one type of cell (chondrocyte) can be found, multiple cell types can be found in bone. Generally the bone forming cells are called osteoblasts and the cells which resorb bone are called osteoclasts. Osteoblasts produce the bone matrix (osteoid) which consists mainly of the organic collagen type I which is mineralized by inorganic hydroxyapatite (calcium phosphate). This gives bones a high compressive strength combined with significant elasticity. When osteoblasts become entrapped in their matrix they become osteocytes; the mature bone cells (Harada and Rodan 2003). Osteoclasts, on the other hand, are multinucleated cells that arise from the monocyte stem-cell lineage and are located at bone surfaces in Howship's lacunae. The cells are equipped with phagocytic-like mechanisms and are characterized by high expression of tartrate resistant acid phosphatase (TRAP) and cathepsin K which are able to break down bone matrix (Boyle, Simonet et al. 2003). The process of bone formation and bone resorption is able to adapt to mechanical forces and as such remodel into the desired architecture (Wolff's law). This process is mostly found in trabecular bone and while no evidence has been found that cartilage adapts/remodels after growth, bone is replaced constantly (Hunziker, Kapfinger et al. 2007). Another important function of bone resorption and formation is controlling homeostasis of important minerals such as calcium and phosphate.

**Different specialized structures** can be identified in bone; the bone attached to the joint cartilage is called subchondral bone. The zone directly beneath the subchondral bone is called the metaphysis. The metaphysis is characterized by a thin cortex and a highly vascularised trabecular bone. Within this trabecular bone bone marrow can be found. Bone marrow is also present at the inside of long bones where it enables hematopoiesis. In the center of long bones lies the diaphysial bone. Here, trabeculi become more sparse and the cortex thickens. The outer site of all bones is covered by periosteum. This periosteum is largely responsible for appositional growth of long bones as it contains a lining of osteoprogenitor cells. **Bone defects.** In the field of Orthopaedic and Trauma Surgery a large demand exists for autologous or allogenic bone. Clinical problems which fuel this demand are; large segmental bone defects (after infection, trauma or tumor resection), fracture non-unions (e.g. tibia, femur, humerus, carpal bones, and talus), bone defects in the increasing field of prosthesis related revision surgery, and spinal fusions (e.g. spondylolisthesis, discopathy, etc)(Glowacki 1998; Stevenson 1998; Huitema, van Rhijn et al. 2006). Although bone from the iliac crest is the golden standard, it is limited in source and donor site morbidity is a major concern. Alternatively, allografts are expensive and pose the risk of viral infection. While the inorganic part of bone (e.g. TriCalcium Phosphate (TCP), Hydroxyapatite (HA)) is widely explored as ceramics and combined with cells in the field of TE, this approach is not successful in generating a satisfying bone substitute (Petite, Viateau et al. 2000; Kim, Park et al. 2006; Zhao, Grayson et al. 2006). Main drawbacks are mechanical features and handling properties of these ceramics. Combining ceramic with polymers may overcome this problem, but toxic degradation products often affect healing and remodeling of the bone defect (Martin, Shastri et al. 2001; Kim, Park et al. 2006; Zhao, Grayson et al. 2006). In addition, these materials are often inert for Matrix Metallo-Proteins (MMPs) and often interfere with biomechanical signaling which is essential for repair and remodeling of loaded structures such as bone (Wolff's law). Furthermore, increased infection risk in implanted tissue-engineered devices is recently described (Kuijjer, Jansen et al. 2007) and supply of oxygen and nutrients is the final aspect of concern when treating bone defects (Shastri 2006). Cells in autologous bone are transplanted from a highly vascularized

environment to a hypoxic environment while cells residing in allografts are frozen and stored before transplantation, it is therefore likely that these cells do not contribute to the repair process (Emans, Pieper et al. 2006). Seeding of these materials with bone marrow cells is promising, however also costly, time consuming and infection prone during isolation and expansion (Shastri 2006). Among the disadvantages listed here lies the reason why this topic is currently studied extensively by many groups worldwide.

## 2.2 Cartilage and cartilage defects

**Joint motion** is possible by a both structurally and functionally truly remarkable material called hyaline cartilage (Buckwalter and Mankin 1998; Hasler, Herzog et al. 1999; Poole, Kojima et al. 2001). Hyaline cartilage is predominantly found in articular cartilage. Next to hyaline cartilage, two other types of cartilage can be found in the human body; elastic and fibrocartilage. Elastic cartilage is found in the ear, nose-tip and respiratory tract, whereas the menisci and intervertebral discs contain fibrocartilage.

The only cell type found in articular cartilage is the chondrocyte. In contrast to other tissues, the chondrocyte contributes to a relatively low percentage of the cartilage volume in human (1-5 percent). Articular chondrocytes are formed by chondrogenic differentiation of chondroprogenitor cells as described above and in Figure 1, however these cells arrest in the mature chondrocyte phase and normally do not become hypertrophic cells. Each chondrocyte is a metabolically active unit which expands and maintains the extracellular matrix (ECM) in its immediate vicinity (Aydelotte, Greenhill et al. 1988). In adults chondrocytes lack cell-cell contact; therefore communication between cells has to occur via ECM. Furthermore, cartilage is characterized by the absence of blood vessels, lymphatics and nerve fibers. Due to the lack of vascularisation in cartilage the environment is dominated by low oxygen levels and therefore the chondrocytes have an anaerobic metabolism (Schenk, Eggli et al. 1986). This also implicates that chondrocytes have to obtain their nutrients and oxygen via diffusion from the synovial fluid, through the ECM and from the underlying bone.

**Structure.** In articular cartilage four zones can be distinguished (see Figure 2), based on collagen type II orientation and chondrocyte shape and distribution (Buckwalter and Mankin 1998; Mankin, Mow et al. 2000; Poole, Kojima et al. 2001). In the superficial or tangential zone, chondrocytes are disc shaped and form a layer of several cells thick. The long axis of the cells are parallel to the joint surface and the cells are surrounded by a thin layer of ECM. Thin collagen fibers are oriented parallel with the articular surface. This orientation and the relatively low content of proteoglycans results in high tensile stiffness and the ability to distribute load over the surface. The cells in the transitional or middle zone are more spherical and appear dispersed randomly (Aydelotte and Kuettner 1992; Hunziker 1992), also collagen fibers in this zone are organized randomly. At this zone and at the deep zone, high concentrations of proteoglycans enable the tissue to bear compressive forces. In the radial or deep zone, chondrocytes are ellipsoid, grouped radially in columns of 2-6 cells with their long axes perpendicular to the joint surface. The thicker collagen fibres are also arranged perpendicular to the articular surface. In the calcified zone, chondrocytes are distributed sparsely and remain surrounded by a calcified matrix. The calcified cartilage is less stiff than the subchondral bone. At this calcified zone shear stresses are converted into compressive forces which are in turn transmitted to the subchondral bone (Radin, Martin et

al. 1984). The junction between uncalcified and calcified cartilage is called the “tidemark”, a line which can be seen on histology (Figure 2). Therefore mechanical forces also change at the tidemark which provides a definite boundary for the uncalcified layer (Donohue, Buss et al. 1983; Aydelotte and Kuettner 1992).

**Cartilage defects** can arise due to trauma or cartilage degeneration. Although patient’s history may differentiate between traumatic and degenerative lesions, the exact cause of cartilage defects often remains difficult to diagnose. Since cartilage has no nerve fibers, cartilage lesions often present with only (minor) effusion of the affected joint or without symptoms. Diagnosis of structures likely to be damaged upon trauma (e.g. subchondral bone, ligaments or menisci), may reveal a cartilage lesion. An X-ray indicates a cartilage lesion in the minority of the cases and Magnetic Resonance Imaging (MRI) is the best non-invasive technique available for diagnosis of cartilage lesions. Important developments are new protocols such as delayed Gadolinium Enhanced MRI of Cartilage (dGEMRIC) and sodium MRI which can visualize cartilage on the Collagen and GAG content level (Gold, Burstein et al. 2006). Overall the MRI is expected to diagnose cartilage lesions in an early stage and will become more important in evaluation of progression of cartilage degeneration and cartilage repair techniques.

As early as 1743 it was recognized that articular cartilage, once destroyed, does not heal spontaneously (Hunter 1995; Hunziker 1999). Whereas the progenitor cells of bone marrow and periosteum contribute to bone formation during fracture healing, articular cartilage is deprived of these progenitors. Although it has been shown that the superficial layer of cartilage and the synovium contain progenitor cells (Dowthwaite, Bishop et al. 2004; Park, Sugimoto et al. 2005), cartilage has a limited ability for self repair (Mankin, Mow et al. 2000; Emans, Surtel et al. 2005). Therefore cartilage and tissue engineering approaches are studied in an attempt to overcome the inability of cartilage to repair itself.

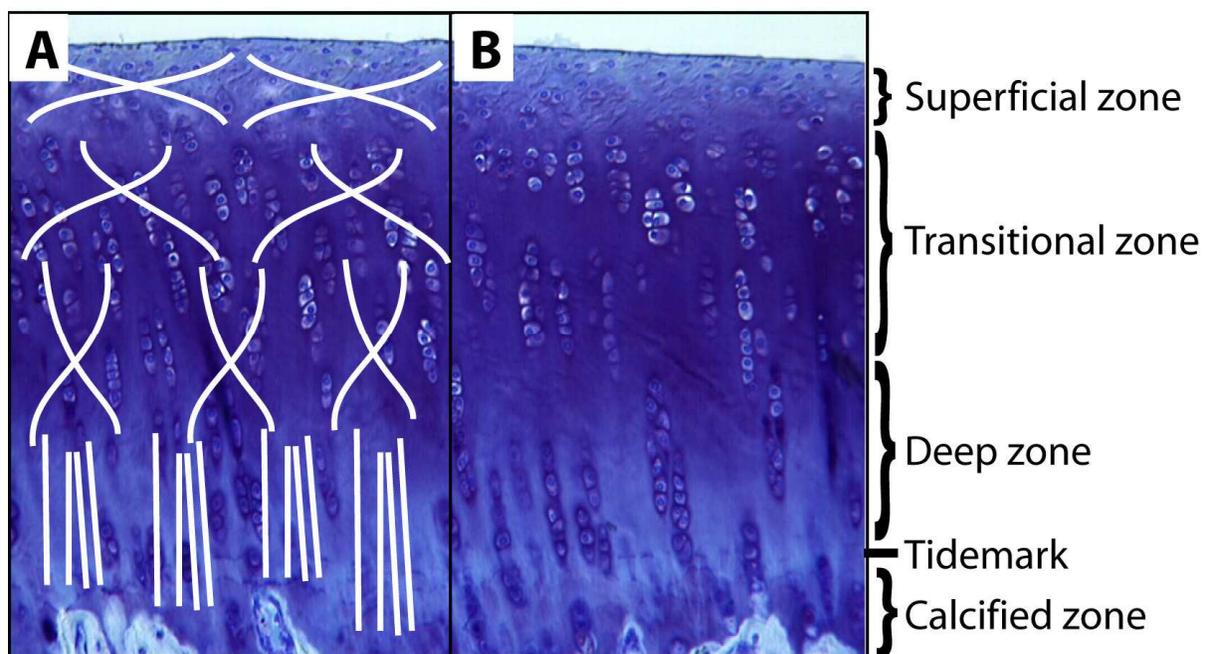


Fig. 2. Architecture of articular cartilage. Four zones can be distinguished with respect to (A) orientation of collagen fibers and (B) cell shape and orientation

### 3. Tissue engineering and regenerative medicine

Combining technologies from material science, cell biology, and clinical needs has led to the rise of the field of TE and RM. In the 1960's researches proposed the idea of creating tissues in a laboratory which may replace damaged or diseased tissues and cell biologists observed that cells could sort themselves *in vitro* to populations with tissue-like characteristics (Steinberg 1962). Adding a structure (material) such as a collagen gel to fibroblast cultures was shown to further resemble structural characteristics of skin. Later the work of Brittberg and co-workers showed that chondrocytes could be cultured and successfully be transplanted for the repair of cartilage defects (Brittberg, Lindahl et al. 1994). This technique is entitled Autologous Chondrocyte Transplantation or Implantation (ACT or ACI). The combination of specific tissue features and the early findings of culturing and transplanting chondrocytes and fibroblasts, skin, cartilage and bone were identified as tissues which potentially could be repaired by engineering these tissues in the laboratory by combining cells and supporting scaffolds. In the beginning of ACT no artificial structures were used to keep the chondrocytes in the cartilage defect. Optimization of ACT has led to the introduction of collagen meshes to support and maintain chondrocytes which were transplanted into the defect. Already earlier, in the mid-1980s, Langer and co-workers proposed that biodegradable polymers could serve as a scaffold for the organisation and maturation of cells into the desired tissues. As such it was proposed that this approach would enable engineering of thicker and hard tissues such as cartilage. Although cell therapies based on TE for skin are commercially available, which apply to the definition of TE such as Carticel® and Epicel® of Genzyme, the initial expectations of TE and RM have not been met. Although some examples of successful treatment by engineered tissues such as bladder and trachea can be found in the clinic, engineering tissues is not performed on a large scale (Oberpenning, Meng et al. 1999; Macchiarini, Jungebluth et al. 2008).

In the approach to engineer tissues in a laboratory setting and subsequently transplanting them into the body lies the key question; *"until what level should we engineer tissue and when should nature take over?"*. It is often the aim of many researchers to engineer a mature tissue which is directly able to take over the function of the diseased tissue or organ. Per example it is often a goal that engineered cartilage and bone should be able to bear mechanical forces directly after implantation. In contrast, in nature a cascade of interactions occur during the process of tissue repair. During this process both the environment as well as the reparative tissue adapt to each other and the biomechanical requirements. In such a manner both integration of repair tissue and tissue remodelling is achieved. The capacity of a mature TE tissue to adapt to the local needs such as integration, remodelling, etc. is lower than a relatively less mature tissue. In addition, in order to create a robust and thicker tissue, the use of scaffolds, growth factors and more differentiated cells may be inevitable. However the question remains whether the local environment is able to adapt in an appropriate manner to all non-physiological stimuli which are introduced. Per example how does the normal tissue remodelling, repair and integration respond to a scaffold which alters local biomechanical stimuli which are known to be essential for tissue remodelling? How do transplanted and environmental cells respond to material properties such as material surface, breakdown products, architecture etc? How does the normal fine-tuned orchestra of tissue repair respond to transplanted cells which are normally not present at a certain phase

of tissue repair? Finally, the use of cells in RM and TE approaches often implies the use of two surgical procedures as well as costly and time consuming culture procedures and logistics.

### 3.1 Bone repair

**Natural bone healing.** As described above, endochondral ossification drives skeletal growth. Similar sequential steps of endochondral ossification are largely responsible for fracture healing of long bones (Bostrom, Lane et al. 1995; Einhorn 2005). Periosteum is the main source of progenitor cells capable of creating large volumes of non-vascularized cartilage surrounding a fracture (Hall and Jacobson 1975). This first phase of endochondral bone formation is called soft callus. During the second phase chondrocytes become hypertrophic, mineralize (hard callus) (Figure 1), secrete pro-angiogenic factors such as VEGF and finally bone is deposited. In the final phase the newly formed bone is vascularized and will remodel under influence of mechanical forces. Bone healing by endochondral ossification is influenced by many regulatory mechanisms. However, while interaction of Indian hedgehog (Ihh) and Parathyroid hormone related protein (PTHrP) is one of the best known regulatory mechanism in the growth plate, such an interplay is yet unknown for fracture healing (Wu, Ishikawa et al. 1995; Vortkamp, Lee et al. 1996; Volk and Leboy 1999). The role of growth factors during bone healing processes is better studied. Chondrocytes at different stages of maturation release cytokines and growth factors such as Fibroblast Growth Factor (FGF), Transforming Growth Factor (TGF)- $\beta$ , Bone Morphogenetic Proteins (BMPs) and Vascular Endothelial Growth Factor (VEGF) (Gibson 1998; Gerber, Vu et al. 1999; Blunk, Sieminski et al. 2002). For instance FGF-2 and TGF- $\beta$  control endochondral ossification by inhibition of chondrocyte proliferation, hypertrophy and apoptosis (Gibson 1998) and in addition from our own findings we know that TGF- $\beta$  is important for osteo- and chondrogenesis both in *ex vivo* and *in vivo* models (Kuijjer, Emans et al. 2003). *In vivo*, in the Osteoarthritic (OA) joint TGF- $\beta$  is produced. Under the influence of this TGF- $\beta$  osteophytes are formed which are derived from periosteum adjacent to the joint via endochondral bone formation (van der Kraan and van den Berg 2007). BMPs are also positively involved in ectopic cartilage and bone formation, partly by opposing the actions of the FGF pathways (Yoon and Lyons 2004; Miyazono, Maeda et al. 2005; Yoon, Pogue et al. 2006). Neo-vascularization under influence of VEGF ensures blood vessel formation which supply oxygen and nutrients to osteoblast and osteoclasts. The latter produce MMP-9 and -13 which degrade the matrix surrounding terminally hypertrophic chondrocytes (Gerber, Vu et al. 1999). Blocking VEGF in the hypertrophic zone of the growth plate prevents degradation of this zone which in turn enlarges (Gerber, Vu et al. 1999).

**Current approaches for bone repair.** Multiple causes may lead to impaired healing of large bone defects. As mentioned before, nature has a good regenerative capacity for fractures, however from a clinical perspective the need for bone is not in fracture repair but mostly for the filling of large bone defects after revision arthroplasty and spondylodesis. These bone defects can be regarded as “non-natural” occurring bone defects and bone healing or filling is impaired at these sites because endochondral ossification does not occur. To deal with this problem a scaffold is introduced as a template for bone ongrowth, ingrowth and remodelling. Currently many bone fillers (scaffolds) and growth factors are available for treatment of bone defects. Taking the scaffold which is formed during endochondral

ossification (also see chapter “scaffolds”) as a blueprint, bone fillers need to be further optimized; next to being expensive, these aids only address one or a few aspects of the cascade of tissue responses which are necessary for bone repair. Most bone fillers are osteoconductive (supportive) and they lack the timing and onset of essential growth factors to be osteoinductive (stimulating bone growth). Growth factors by themselves have been shown to be osteoinductive but addition of one of the essential growth factors does not necessarily recapitulate the physiological, initial tissue response which leads to fracture/bone repair.

Inflammation is the first and essential phase of tissue repair in general and bone repair in particular. Mimicking this inflammatory response may be a method to enhance bone fracture healing. Several clinical examples such as spondylodesis after infection of the intervertebral disc (e.g. after discography) and the method described by Masquelet confirm that inflammatory responses contribute to osteogenesis (Guyer, Collier et al. 1988; Masquelet and Begue 2010). However, in contrast, from an engineering perspective, the aim is often to create bone which has comparable mechanical features as native bone. The initial mechanical properties of currently used bone chip auto or allografts are incapable of withstanding the mechanical forces to which they are exposed. During impaction of these chips the mechanical properties of the impacted bone as a whole are capable to withstand mechanical forces in a non-loadbearing environment. After vascularisation, bone ingrowth and remodelling of the repaired bone defect adapts to finally bear full loading. As such surgical handling properties, osteoconduction, and most important osteoinduction are features one should aim for rather than engineering mature bone with biomechanical properties comparable to native bone. As mentioned before during endochondral ossification large amounts of cartilage are generated. This cartilage does not have the required mechanical features of the bone it should repair, but does have strong osteoconductive and osteoinductive features. Another challenge when aiming for creation of bone is the scale to which should be generated. During fracture healing bone defects can be repaired by deposition of large amounts of bone which is formed by endochondral ossification. In the pre-remodelling phase of endochondral ossification, the generated bone histologically resembles the metaphyseal bone chips which are used on a large scale for bone impaction grafting. In conclusion, regarding endochondral ossification as a blueprint for engineering or regeneration of bone, it has the potential to generate vast amounts of bone, with good handling properties, and is osteoinductive and osteoconductive.

### 3.2 Cartilage repair

**Treatment of damaged cartilage** can be grouped to four concepts of principle: the four R's (O'Driscoll 1998). The joint surface can be: (i) resected, (ii) relieved, (iii) replaced or (iv) restored. A joint prosthesis is an example of joint replacement; joint distraction and osteotomies can induce joint relieve. Osteotomies are used to re-align the axis of loading in patients with a malalignment of the leg. By transferring the load to the less affected cartilage (e.g. previously less loaded/damaged cartilage) the damaged part is relieved. Arthrodesis is an example of joint resection. For TE and RM techniques the focus is on cartilage restoration.

Restoration implies methods to heal or regenerate the joint surface with or without the subchondral bone into healthy hyaline articular cartilage. Three strategies can be considered when attempts are made to heal or restore cartilage.

- i. Subchondral Drilling, Abrasion or Microfracture are techniques to allow penetration of bone marrow through the subchondral bone into the damaged cartilage (Meachim and Roberts 1971; Insall 1974; Mitchell and Shepard 1976; Furukawa, Eyre et al. 1980; Vachon, Bramlage et al. 1986; Bradley and Dandy 1989; Rae and Noble 1989; Kim, Moran et al. 1991; Altman, Kates et al. 1992; Aglietti, Buzzi et al. 1994). These techniques improve the clinical well being of the patient and the joint surface defect may be healed to some extent. However the healing process is inadequate since no functional hyaline cartilage but fibrocartilage is formed (Vachon, Bramlage et al. 1986; Altman, Kates et al. 1992). Nonetheless, these methods are cheap and easy to perform and are therefore seen as the currently best option to relieve the complaints. Other clinical studies have suggested that any beneficial effect is related to the arthroscopic procedure itself. A nonspecific effect might be related to joint lavage rather than the penetration of the subchondral bone (Jackson 1986; Ogilvie-Harris and Fitsialos 1991). In conclusion, these techniques may have some benefit with regard to small defects but no effect has been proven in relation to large defects, osteoarthritic joints or older patients (Kim, Moran et al. 1991).
- ii. Implants vary from non-degradable and degradable, cells, periosteum or perichondrium, Osteochondral Autograft Transfer System (OATS or Mosaicplasty) and Osteochondral Allografts (Elford, Graeber et al. 1992; Freed, Vunjak-Novakovic et al. 1993; Nixon, Sams et al. 1993; Hendrickson, Nixon et al. 1994; Reddi 1994; Chu, Coutts et al. 1995; Grande, Halberstadt et al. 1997). The biomaterials and periosteum can be combined with cells or growth factors. Periosteal Arthroplasty is an interesting way of treating cartilage defects since many have reported the chondrogenic potential of periosteum (O'Driscoll, Keeley et al. 1986; O'Driscoll, Keeley et al. 1988; Zarnett and Salter 1989; Nakahara, Bruder et al. 1990; Nakahara, Dennis et al. 1991; Nakahara, Goldberg et al. 1991; Nakata, Nakahara et al. 1992; Iwasaki, Nakata et al. 1993; Gallay, Miura et al. 1994; Iwasaki, Nakahara et al. 1994; Iwasaki, Nakahara et al. 1995; O'Driscoll, Saris et al. 2001; Emans, Surtel et al. 2005). Over 90 percent of collagen type II in the hyaline cartilage formed in the cartilage defects treated with periosteal grafts has been reported (O'Driscoll, Keeley et al. 1986; O'Driscoll, Keeley et al. 1988). Perichondrial Arthroplasty used for human cartilage repair was first described by Skoog et al. (Skoog and Johansson 1976). This technique has been reported to give an initial cartilage repair (Homminga, Bulstra et al. 1990; Homminga, Bulstra et al. 1991). On the long term poor results related to overgrowth of the graft and calcification are reported by Bouwmeester *et al.* (Bouwmeester, Beckers et al. 1997). These authors concluded that a better fixation of the graft might improve the results. In a study comparing periosteum with perichondrium, chondrogenesis was observed significantly more using periosteal grafts (Vachon, McIlwraith et al. 1989). This finding and the accessibility make periosteum to be preferred over perichondrium.
- iii. Osteochondral Grafts can be divided in autologous and allogenic. Mosaicplasty or OATS involves harvesting one or more osteochondral plugs from a relatively less weight-bearing region of the joint and subsequent implantation of these plugs into an articular defect. Possible donor site morbidity is bypassed if osteochondral allografts are used (Gross, McKee et al. 1983; Garrett 1986; Czitrom, Keating et al. 1990; Convery, Meyers et al. 1991; Garrett 1994; Ghazavi, Pritzker et al. 1997; Garrett 1998; Horas, Schnettler et al. 2000; Gross, Aubin et al. 2002).

**The role of endochondral ossification in cartilage repair.** When using progenitor cells for cartilage repair, ossification of the repaired tissue may impair clinical results. Examples hereof are ossification and formation of interlesional osteophytes when applying techniques such as microfracture and periosteum or perichondrium plasty (Bouwmeester, Beckers et al. 1997; Cole, Farr et al. 2011). These findings illustrate that maintaining differentiated progenitor cells in their chondrogenic state remains challenging in cartilage repair. It appears that in contrast to chondrocytes, progenitor cells have the tendency to follow the different phases of endochondral ossification towards hypertrophy and mineralisation when triggered to differentiate into cartilage. As such, locking cells in their desired differentiation state is of the utmost importance when applying these cells for RM purposes. Findings of Hendriks and co-workers showed that chondrocytes stimulate progenitor cells towards chondrogenesis when both cell types are co-cultured (Hendriks, Riesle et al. 2007). These findings were later bolstered by Fisher and co-workers showing that human articular cartilage-derived soluble factors and direct co-culture are potent means of improving chondrogenesis and suppressing the hypertrophic development of mesenchymal stem cells (Fischer, Dickhut et al. 2010). In this study and other work of the group of Richter the PTHrP is an important candidate soluble factor involved in this effect. PTHrP is primarily known as a key regulator in the process of endochondral ossification. Furthermore, we have recently shown that cyclooxygenase (COX) inhibitors are also able to decrease hypertrophy of chondrocytes (unpublished data). Thus studying the process of endochondral ossification and further unravelling how and why articular chondrocytes maintain their phenotype as well as prevention of hypertrophy may enhance cartilage repair techniques by generating stable cartilage which does not lead to intra-lesional osteophytes. Finally, cartilage defects lead to early OA, also in the process of OA more evidence is found that articular chondrocytes lose their capacity to maintain their phenotype and seem to undergo endochondrogenesis since they become hypertrophic and express collagen type X (Saito, Fukai et al. 2010). As such understanding and controlling the process of endochondrogenesis may be of relevance for future insight and treatment of OA.

### 3.3 Scaffolds

**Scaffold or carrier material** refers to a wide variety of artificial 2D or 3D structures that are designed for the purpose of tissue engineering. Scaffolds may be seeded with cells before implantation or are designed to recruit or retain cells at the desired place. (Bentley and Greer 1971; Wakitani, Kimura et al. 1989). For bone and cartilage regeneration, relevant cells are (mesenchymal) stem cells of different origins (bone marrow, adipose tissue, dental pulp, iPS etc.) as well as differentiated cells like chondrocytes. Different variables are important parameters for scaffold design: pore diameter, shape, kind of material, (bio)degradability, implantation site, functionalization, mechanical stability and others. Several materials have been and are being explored for this purpose. Generally scaffold materials can be divided in natural or synthetic. Examples of natural material-based scaffolds for cartilage and bone regeneration are: fibrin, hyaluronan, alginate, agarose, demineralized bone matrix, collagen etc. Synthetic scaffold materials include ceramics and copolymers PolyGlycolic Lactic acid (PGLA) and PolyethyleneGlycol-terephthalate/PolyButylene Terephthalate (PEGT/PBT) (Figure 3) etc. When applying a collagen as carrier material, some authors find enhanced cartilage healing, while others conclude that collagen scaffolds have a limited usefulness for chondrocyte grafting in large defects (Wakitani, Kimura et al. 1989; Nixon, Sams et al. 1993;

Sams, Minor et al. 1995; Sams and Nixon 1995). The use of fibrin as carrier material was reported to give superior cartilage healing compared to controls (empty defect) (Hendrickson, Nixon et al. 1994). Within time an ideal scaffold should degrade or allow the populated cells to take over functionality of the artificial tissue implant. Breakdown products and biomechanical features of the scaffold should not negatively interfere with differentiation towards this tissue. It is therefore challenging to design a scaffold with all the optimal characteristics; proper initial mechanical stability, timed release of required growth factors, timed degradation which allows biomechanical stimuli to remodel the formed tissue, no release of degradation products which interfere with tissue repair, good handling properties, etc. Next generation scaffolds will be so called “smart scaffolds”. These scaffolds will be loaded with bioactive factors (e.g. TGF- $\beta$ 1 and members of its superfamily such as BMPs) that can directly influence the differentiation pathways (Sellers, Peluso et al. 1997; Sellers, Zhang et al. 2000; Huang, Goh et al. 2002). Effort is being put in e.g. functionalized scaffolds with specific affinity peptides to retain cells (Dong, Wei et al. 2009). Also, the release of e.g. growth factors may be regulated by “on demand” smart systems that depend on incorporated microspheres or proteolytic degradation of linker-peptides. Unfortunately, an ideal material for artificial scaffolds for cartilage and bone regeneration has not been identified yet, as the biological processes involved are far more complex than anticipated.

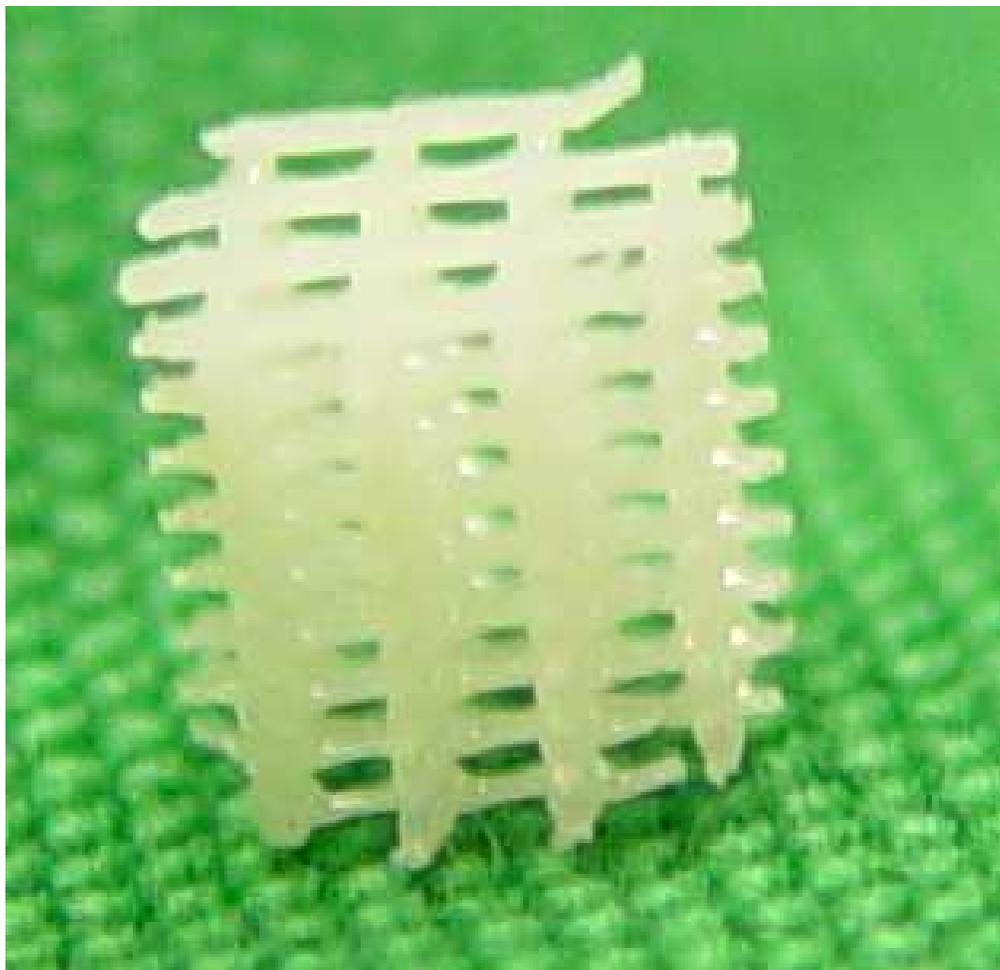


Fig. 3. A PolyethyleneGlycol-terephthalate/PolyButylene Terephthalate (PEGT/PBT) scaffold produced by using a three dimensional rapid prototyping technique.

**The endochondral scaffold.** During endochondral ossification nature creates its own scaffold. Hypertrophic chondrocytes die and leave a large scaffold. During this process multiple growth factors are released in an orchestrated manner. The scaffold itself is used as a template for invading cells to deposit bone and provide vascularisation. The scaffold itself is resorbed by osteoclasts which in turn respond to biomechanical and biochemical stimuli. As such the scaffold degrades and simultaneously the proper factors are released. The repair tissue remodels to the appropriate architecture as defined by Wolff's law. Studying this process in detail reveals the challenge when "artificial" scaffolds are designed from a material science point of view, and so far no scaffolds have been created with the same properties capable to dictate the same processes of endochondral remodelling.

### 3.4 Cells

Cells for "orthopaedic" tissues, such as bone and cartilage, originate from the mesenchymal cell lineage and may be derived from different autologous or allogenic sources. Interestingly, cells used for bone regeneration are almost always progenitor cells, whereas for cartilage regeneration also differentiated cells are used, next to progenitor cells. Some authors prefer the use of chondrocytes for transplantation while others prefer the use of undifferentiated multipotent cells (Skoog and Johansson 1976; O'Driscoll, Keeley et al. 1986; Homminga, Bulstra et al. 1991; Brittberg, Lindahl et al. 1994; Lindahl, Brittberg et al. 2003; Nathan, Das De et al. 2003; Emans, Surtel et al. 2005; Park, Sugimoto et al. 2005). Mature chondrocytes can be released from their cartilaginous matrix, selected and expanded *in vitro*. In this way a relatively small amount of autologous tissue can be used as an appropriate cell source. Both chondrocytes and progenitor cells originating from different cell sources have been studied in combination with various biomaterials (Bentley and Greer 1971; Haynesworth, Baber et al. 1992; Freed, Marquis et al. 1993; Freed, Vunjak-Novakovic et al. 1993; Iwasaki, Nakata et al. 1993; Brittberg, Lindahl et al. 1994; Bruder, Fink et al. 1994; Freed, Grande et al. 1994; Gallay, Miura et al. 1994; Wakitani, Goto et al. 1994; Iwasaki, Nakahara et al. 1995). Bone marrow, adipose tissue, synovium, dental pulp, perichondrium and periosteum can serve as a source for multipotent cells (Skoog and Johansson 1976; Homminga, Bulstra et al. 1991; Bouwmeester, Beckers et al. 1997; Chu, Douchis et al. 1997; O'Driscoll, Saris et al. 2001; Nathan, Das De et al. 2003; Emans, Surtel et al. 2005; Park, Sugimoto et al. 2005). Numerous publications described subpopulations of progenitor cells in these donor tissues that might be more optimal cell sources than e.g. whole cell pool isolates. However, lots of these studies were only performed *in vitro* and one can question whether selection of subtypes based on cell surface markers may bias the outcome of the intervention in a difficult to predict way. The involvement of subchondral bone may play a role in cell source selection as chondrocytes are capable of producing cartilage under the appropriate conditions, but in a situation where simultaneous bone formation is required (involvement of the subchondral bone), multipotent cells might be a better cell source. After selection and expansion, the main challenge is to keep these cells in the damaged area of the joint and this challenge becomes even bigger in larger defects (Bentley and Greer 1971). Grande *et al.* reported that only 8 percent of the total number of cells in the healing tissue originated from transplanted chondrocytes (Grande, Pitman et al. 1989). Chondrocytes can be maintained in the defect by suturing a periosteal flap or a collagen mesh over the defect (Grande, Pitman et al. 1989; Brittberg, Lindahl et al. 1994; Bartlett, Skinner et al. 2005). As discussed above, chondrocytes can also be seeded in a matrix or scaffold. This matrix can be

implanted in a cartilage defect. This Matrix Assisted Chondrocyte Transplantation (MACT) is technically less demanding and has shown identical results compared to Autologous Chondrocyte Transplantation on the short term (Bartlett, Skinner et al. 2005). The use of allogenic chondrocytes has been reported to be successful in rabbits but experiments in horses do not support this finding (Wakitani, Kimura et al. 1989; Freed, Grande et al. 1994; Sams, Minor et al. 1995; Sams and Nixon 1995). Immunological rejection or allogenic chondrocytes upon implantation in rabbits has been reported and this remains a major concern when applying allogenic cells. The use of periosteal tissue or cells has been suggested by several authors and is one of the most clear examples of the use of endochondral ossification as a blueprint for cartilage and bone regenerative medicine. The periosteum is populated with mesenchymal progenitor cells that normally contribute to endochondral bone fracture healing. The differentiation capacity of these cells can also be used to create cartilage or bone for regenerative purposes. This principle is further explained below (see paragraph 3.6).

When using differentiated or undifferentiated cells for cartilage, bone or osteochondral repair it is a challenge to differentiate these cells into their desired state and maintain their desired phenotype. As cells from the mesenchymal lineage, once differentiated into chondrocytes, have a natural tendency for terminal differentiation via the endochondral pathway. This is a big concern for regenerative applications. Much effort is being put in technologies that prevent hypertrophic differentiation of transplanted chondrocytes, while on the other hand for bone regeneration hypertrophic differentiation may be a prerequisite for success (Fischer, Dickhut et al. 2010; Scotti, Tonmarelli et al. 2010).

### 3.5 Biochemical signaling pathways

**Growth factors.** In growth plate development, homeostasis of articular cartilage as well as bone formation and maintenance, several signaling pathways are interacting or shared between the different tissues. Indian hedgehog (Ihh) and Parathyroid hormone related peptide (PTHrP) coordinate chondrocyte proliferation and differentiation in the so-called PTHrP-Ihh feedback loop (Kronenberg 2003). This coordination influences the length of proliferative chondrocyte columns as well as chondrocyte hypertrophy. Next to the Ihh and PTHrP loop, fibroblast growth factor crucially regulates chondrocyte proliferation and differentiation. Many of the 22 distinct FGF genes and their four receptor genes are expressed at every stage of endochondral bone formation (Ornitz and Marie 2002). Also Bone Morphogenic Proteins (BMPs) have multiple roles during bone and cartilage formation, as well as growth plate development. Interestingly, BMPs were discovered because of their remarkable ability to induce endochondral bone formation when injected subcutaneously in mice. In a cartilage context, BMPs are involved in early chondrogenesis, cartilage maintenance and hypertrophic differentiation. In a bone context they drive differentiation of progenitor cells to osteocytes and induce alkaline phosphatase activity in osteocytes. TGF- $\beta$  isoforms are also involved in similar processes and interestingly were found to trigger the formation of osteophytes upon intra-articular injection and during OA (Elford, Graeber et al. 1992; van Beuningen, van der Kraan et al. 1993; van Beuningen, van der Kraan et al. 1994; Hunziker 2001). As osteophyte formation itself is an example of endochondral ossification, the role of TGF- $\beta$  isoforms in endochondral ossification is supported by this finding. Remarkably, some characteristics of OA resemble chondrocyte

differentiation processes during skeletal development by endochondral ossification. Resemblances are: chondrocyte proliferation, chondrocyte hypertrophic marker expression (e.g. Collagen type X and MMP-13), vascularisation and focal calcification of joint cartilage. This suggests that during OA the articular cartilage is terminally differentiating via “normal” endochondral pathways. However, how the mature articular cartilage is kept in its cartilaginous state and why it starts a terminal differentiation program in OA is currently poorly understood. In the final stage of endochondral bone formation secretion of pro-angiogenic factors such as VEGF is essential. Sox9 and RunX2 are important transcription factors. Sox9 is the master regulator of chondrogenesis and acts as a negative regulator for chondrocyte hypertrophy, cartilage vascularisation and bone marrow formation (Hattori, Muller et al. 2010). Amongst others it does this via negatively regulating expression of RunX2 via Nkx3.2 (also known as BapX1) (Yamashita, Andoh 2009). RunX2 is a central regulator for the transition from proliferating to hypertrophic chondrocytes, as it drives the transcription of Collagen type X. Interestingly, RunX2 also drives multiple osteogenic developmental programs. Inflammatory pathways are other key players in endochondral ossification (Einhorn, Majeska et al. 1995; Mountziaris and Mikos 2008). Bone fracture healing by endochondral ossification depends on a haematoma-induced inflammatory environment (Grundnes and Reikeras 1993) and several inflammatory molecules (e.g. IL-6, TNF $\alpha$ , COX-2 and iNOS) are involved in bone fracture repair (Einhorn, Majeska et al. 1995; Mountziaris and Mikos 2008) by influencing chondrocyte maturation and osteogenic development. An important chondrogenic growth factor is Insulin Growth Factor 1 (IGF-1). Together with its receptors and several IGF binding proteins it determines chondrocyte proliferation and differentiation. Importantly IGF-1 appears to play a role in preventing chondrocyte apoptosis. Hence, it determines the pace of hypertrophic differentiation and thus growth plate development and fracture callus maturation. It was shown that IGF-1 exerts its action via NF- $\kappa$ B/p65 signaling (Wu, Gong et al. 2008). Furthermore, IGF-1 also directly influences osteocyte biology. It has been reported that IGF-1 stimulates cancellous bone formation and increases the activity of resident osteoblasts (Zhao, Monier-Faugere et al. 2000). RANK is crucially important for bone homeostasis and remodelling. Activation of RANKL on monocytic cells by RANK on osteoblasts induces osteoclastogenesis of committed monocytic cells. Multinucleation is induced, ultimately leading to the generation of mature bone resorbing osteoclasts (Novack and Faccio 2011). This process is counterbalanced by the soluble factor osteoprotegerin (OPG), thereby preventing bone loss due to osteoclast activation. Activation of the RANKL system is potentiated by prostaglandins. PGE<sub>2</sub>, one of the main cyclooxygenase metabolites is reported to increase bone resorption.

In conclusion, the process of endochondral ossification is dictated by spatiotemporal expression and use of variable interacting growth factors and other molecules. It is clear that mimicking this complex, yet incompletely known, tissue formation in an *in vitro* setting on the same scale as TE was expected to do is quite challenging. Several findings such as endochondral ossification after subcutaneous injection of BMPs show that, *in vivo*, this process may be triggered using stimuli which trigger and enhance the regenerative capacity of the tissue itself. In such an approach the amount of unknown stimuli is expected to be limited and the body's own regenerative capacity is used to generate cartilage or bone, which in turn can be transplanted into the damaged site. As such, this approach applies more to the principles of RM than to the principles of TE. The application of a specific *in vivo*

trigger to stimulate endochondral bone formation has many advantages; no expensive culture procedures, no more harvesting of cells, and no introduction of factors which possibly conflict with the natural tissue repair and integration. Table 1 summarises the differences in tissue features, currently applied (TE) techniques for restoration, and remaining challenges.

### 3.6 Examples of endochondral ossification as blueprint for regenerative medicine

Currently for TE purposes cells are harvested during the first operation and the implantation of the graft/cells is performed during the second procedure. A question that remains is the amount of cells that survives the transplantation. It has been shown that periosteal cells show a much poorer survival compared to chondrocytes after transplantation into the hostile environment of a fresh osteochondral defect (Emans, Pieper et al. 2006). However, the disadvantage of using chondrocytes is the fact that the joint is further damaged. It would be ideal to generate cartilage in an ectopical place which does not further interfere with the joint homeostasis, survives the transplantation and is capable to adapt and repair the defect. In line with this, an interesting variation for cartilage repair is a reported by Takahashi *et al.* who used the early fracture callus, induced at the iliac crest (Takahashi, Oka et al. 1995). The early fracture callus was implanted into osteochondral defects of rabbit knees with excellent results. A paper of our group also reported excellent results after transplantation of periosteum derived cartilage callus into osteochondral defects (Emans, van Rhijn et al. 2010). Stevens *et al.* published an interesting paper on inducing chondrogenesis by subperiosteal injection of a hyaluronan-based gel containing the antiangiogenic factor Suramin. The resulting tissue also resembled cartilage of early fracture callus (Stevens, Marini et al. 2005). The main advantage of this approach is that the body is used as its own “*in situ* incubator”; cells provide their own matrix and complex and costly isolation, selection and culturing procedures are bypassed. After this first report focussing on bone, we aimed to control the local environment by injecting a gel into the space between bone and periosteum which would initiate endochondrogenesis. Both agarose and a gel loaded with TGF- $\beta$ 1 were successful to trigger endochondrogenesis. This tissue was harvested during its first chondrogenic phase and successfully implanted into an osteochondral defect where an excellent lateral integration and no calcification of the cartilage adjacent to the joint was observed (Emans, van Rhijn et al. 2010).

It was recognised by the group of Martin that TE and RM attempts to create bone using the intramembranous pathway (Scotti, Tonnarelli et al. 2010). In contrast, during development most bones are formed by endochondral ossification and the parts that do not ossify forms articular cartilage. In addition, during fracture healing bone gaps and defects are often repaired by endochondral bone formation, during which large amounts of callus can be formed. Depending on the phase in which specific tissue is generated by endochondrogenesis, this tissue can be harvested for different purposes. If tissue in the early chondrogenic phase is harvested this may be ideal to heal both bone and cartilage. If this tissue is harvested at a later stage it resembles trabecular bone which has the potential to be used for bone impaction grafting. Compared to the frequently used TE approach to create bone directly (intramembranous), it seems more logical that endochondral bone formation which is capable to produce large amounts of cartilage and bone, even in an ectopic site, may fuel further research

Challenges in Tissue Engineering					
Tissue characteristics					
	Bone		Cartilage		
Function	Weight bearing		Weight bearing and joint articulation		
Cells	Osteoblasts/osteocytes and osteoclasts		Chondrocytes		
Origin	Mesenchymal and monocyte lineage		Mesenchymal		
ECM	Collagen I and Calcium phosphate		Collagen II, Proteoglycans, GAGs and Hyaluronic acid		
Functional ECM water content	No		Yes, important		
Cell-cell contact	Yes and important		No, 'communication' via ECM		
Vascularisation	Yes		No, hypoxic tissue		
Nutrient/oxygen supply	Via vascularisation		Via diffusion		
Remodelling	Constant (Wolff's law)		Low grade of remodelling?		
Regenerative capacity	High		Low		
Access to progenitor cells	Bloodstream, Periosteum, Bone marrow		Superficial layer cartilage? Synovium?		
Endochondral ossification	Complete		Has to stop at chondrocyte-phase		
Current approaches in TE					
	Bone			Cartilage	
	Advantages	Disadvantages		Advantages	Disadvantages
Auto- and Allografts	Osteoconductive Osteoinductive? Native bone	Expensive Host-vs-graft reaction Infection Freezing cells? Donor site morbidity	Osteochondral grafts	Native cartilage	Expensive Host-vs-graft reaction (allografts) Infection Donor site morbidity Fixation Cell morbidity during storage
Decellularized bone	Osteoconductive Resembles native bone	Not osteoinductive Host-vs-graft reaction Infection	Subchondral drilling, abrasion, microfracture	Activation of bone marrow Very effective Cheap and easy	Fibrocartilage
HA/TCP/Bonefillers	Osteoconductive Resembles native bone properties Can be loaded with cells/growth factors	Not osteoinductive Mechanical features Handling properties Interference with biomechanical signalling	ACI/ACT MACT	Good integration Native cartilage	Infection Time consuming Expansion of cells Fixation Expensive
Growth factors	Osteoinductive	Not osteoconductive Expensive Overload of growth factor Ectopic bone formation	Implants/biomaterials	Can be loaded with cells/growth factors Initial cartilage repair	Calcification of cartilage Fixation Toxic degradation products

Challenges		
Current advantages		Remaining challenges
<b>Scaffolds</b>	Conductive Can be loaded with cells and/or growth factors to recruit, retain and/or differentiate the cells Immediate initial mechanical stability	Scaffold design: pore diameter and shape (bio)Material: natural or synthetic Biodegradability and degradation at right time Breakdown products Integration / fixation Release of growth factors at right time Interference with tissue environment <b>Cartilage:</b> Nutrient supply in scaffold Allow ECM formation, but prevent mineralisation Allow articulation, repetitive mechanical loading and load damping <b>Bone:</b> Nutrient and oxygen supply Stimulate vascularisation Allow bone mineralisation Support high mechanical loading <b>Endochondral:</b> progenitors differentiate to hypertrophic chondrocytes which leave a natural 'scaffold' for bone cells to adhere and remodel and provides in essential growth factors and vascularisation at appropriate time points.
<b>Growth factors</b> (BMPs, TGF- $\beta$ s, PTHrP, VEGF, etc)	Inductive Can regulate differentiation of cells Easy	Does not recapitulate total physiological repair response Keep growth factors at damaged area Effect on tissue <i>in vivo</i> incompletely known Still expensive
<b>Progenitor cells</b>	High potential to differentiate into required tissue Various origins (bone marrow, dental pulp, adipose tissue, periosteum, blood etc.)	Have to differentiate and remain differentiated into required tissue Infection If allografts: host-vs-graft reaction Keep cells at damaged area <b>Cartilage:</b> Nutrient and oxygen supply Have to stop differentiating at chondrocyte phase <b>Bone:</b> Nutrient and oxygen supply Vascularisation Cell isolation and culturing is still time consuming and expensive
<b>Adult cells</b>	Inductive of natural tissue Only for cartilage cells?	Donor site morbidity Cells are out of natural environment, can lead to cell death or dedifferentiation If allografts: allogenic reaction Keep cells at damaged area <b>Cartilage:</b> Nutrient supply Prevent further differentiation towards hypertrophic chondrocytes

Table 1. Differences in: tissue characteristics, currently applied Tissue Engineering, and remaining challenges for bone and cartilage.

for generating both bone and cartilage. Creating cartilage or bone by triggering endochondrogenesis in an ectopic site bypasses expensive and time consuming culture techniques, logistics, and when triggered by injection of a specific stimulus may even limit the total approach to one operation.

#### 4. Conclusion

From nature it is known that vast amounts of cartilage are formed in the process of endochondrogenesis. Chondrocytes in this cartilage tissue are replaced by a matrix deposited by hypertrophic chondrocytes which die by apoptosis. This matrix is used as an active scaffold for cells that contribute to bone formation. Following embryonic joint formation and post natal growth, the adult skeleton maintains the cellularity and phenotype of articular cartilage, whereas growth plate cartilage completely disappears. This process entitled endochondral ossification can be recapitulated in other places than growth plates. Examples hereof are fracture healing, osteophyte formation and peri-articular ossifications. Even in the process of OA endochondrogenesis plays a role. Next to the formation of osteophytes in OA, evidence has been reported that during the process of OA, articular chondrocytes are triggered to follow the final phase of endochondral ossification (Saito, Fukai et al. 2010).

A scaffold which serves as a template for tissue generation has also been introduced in the field of TE. Thusfar TE has not met initial expectations. Materials used as a scaffold to engineer bone are often engineered to be biocompatible and have good initial biomechanical properties. These properties may interfere with biomechanical stimuli needed for tissue organisation and degradation products from these artificial scaffolds may interfere with the natural healing response. In contrast to a natural endochondral scaffold, artificial scaffolds do not orchestrate ingress of progenitor cells, vascularisation etc.

Periosteum seems to play an important role in postnatal endochondrogenesis. However subcutaneous injection of growth factors leads to generation of bone via the endochondral pathway. The first examples of successful generation of bone and cartilage by triggering the progenitor cells of periosteum are found in literature (Emans, Surtel et al. 2005; Emans, van Rhijn et al. 2010). Also repair of cartilage and bone has been reported to be successful in animal studies using this approach. Using the postnatal endochondrogenic capacity for generation of cartilage and bone has many advantages: expensive culture procedures and logistics are bypassed and sufficient amounts of tissue are likely to be generated. Depending on the stage in which endochondral tissue is harvested, different clinical needs could be treated varying from (osteo)chondral defects to bone defects (Scotti, Tonmarelli et al. 2010). Finally, studying the process of endochondrogenesis may not only be a logical direction for tissue generation, but is also expected to provide useful information how to lock progenitors in the desired phase and will contribute to our understanding of diseases like OA.

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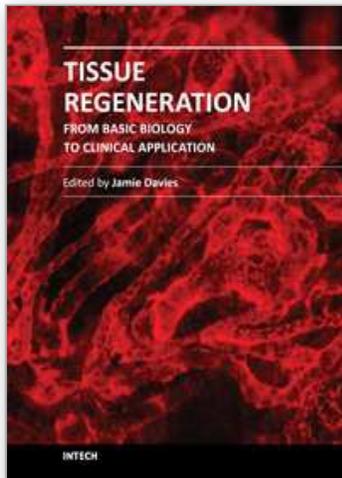
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When most types of human tissue are damaged, they repair themselves by forming a scar - a mechanically strong 'patch' that restores structural integrity to the tissue without restoring physiological function. Much better, for a patient, would be like-for-like replacement of damaged tissue with something functionally equivalent: there is currently an intense international research effort focused on this goal. This timely book addresses key topics in tissue regeneration in a sequence of linked chapters, each written by world experts; understanding normal healing; sources of, and methods of using, stem cells; construction and use of scaffolds; and modelling and assessment of regeneration. The book is intended for an audience consisting of advanced students, and research and medical professionals.

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