Introduction

Articular cartilage is a specific tissue, which can endure a repeatedly high loading for a long time. It is hyaline cartilage and is 2 to 4 mm thick. Unlike most tissues, articular cartilage does not have blood vessels, nerves, or lymphatics. It is composed of a dense extracellular matrix (ECM) with a sparse distrib ution of highly specialized cells called *chondrocytes* ***(Mauck, 2003 and Ramallal et al., 2004).***

Collagen is the most abundant structural macromolecule in ECM, and it makes up about 60% of the dry weight of cartilage. Type II collagen represents up to 95% of the collagen in ECM. Collagen types IV, V, VI, IX, XI, and I are also present but contribute only a minor proportion ***(Burks et al., 2006)***.

Although the reparative tissue may produce Type *II* collagen, the new tissue consists predominantly of Type *I* collagen, resulting in fibrocartilage formation. This new tissue lacks the similar biomechanical properties to articular cartilage ***(Furukawa T et al., 1980; Buckwalter, 2002).***

Numerous strategies have been employed to repair cartilage defects with an end goal of filling the defect with tissue having biochemical and biomechanical properties approximating surrounding native tissue, to improve joint function. Such clinical and experimental efforts include subchondral drilling or microfracture technique ***(Blevins et al., 1998),*** perichondrial grafts ***(Homminga et al., 1990),*** periosteal grafts ***(Poussa et al., 1980),*** osteochondral grafts ***(Burks et al., 2006),*** and cell transplantation therapy either terminally differentiated chondrocytes ***(Brittberg et al., 2003)*** or undifferentiated bone marrow–derived mesenchymal stem cells (MSCs***) (Wakitani et al., 1994).***

Subchondral drilling or microfracture technique used to stimulate articular cartilage healing ***(Johnson et al., 1989)***. By breaching the subchondral bone to form fibrocartilage that fails to withstand the mechanical demands of articular cartilage, so its usage had been very limited ***(Steadman et al., 1997).***

However, none succeeded in restoring the hyaline cartilage. Although transplantation of periosteum or perichondrium has shown good short-term results, but do not generate hyaline cartilage, only fibrocartilage. It often results

in bone formation due to endochondral ossification ***(Johnson et al., 1989).***

The early work by **Tizzoni** in **1878** documented that the chondrogenic potential of the perichondrium of the ear cartilage; auricular cartilage defect was rapidly filled with new cartilage. Therefor restoring cartilaginous articular surface had been suggested through auricular perichondrial graft. However, since perichondrium of the ear contains elastic fibers, so it is not ideal for joint arthroplasty.

The use of rib perichondrium for articular cartilage reconstruction; was introduced and investigated, Compared with ear perichondrium, it was found that the perichondrium of the rib produced a repair tissue that closely resembles hyaline cartilage ***(Kwan et al, 1989 and Homminga et al., 1990; Coutts et al.,1992).***

Katsoros et al., 1995 suggested that a better quality of cartilage; could be produced and maintained with incorporation of a thin layer of cartilage with the perichondrium ***(Aboualwafa et al., 2002; El Badawi et al., 2004).***

Abo-Elwafa et al., 2002 reported that the histological picture of the neocartilage revealed a cellular pattern closely

resembling normal hyaline cartilage in 50%, while the other 50% exhibited fibro-cartilaginous tissue.

Lashin et al., 2012 documented that mesenchymal stem cells (MSCs*)* usage shows better results, as mixed pattern of hyaline and fibrocartilage in 66.7% and fibrocartilage in 33.3% only.

In the past decade, platelet-rich plasma (PRP) has emerged as a non-operative treatment modality for cartilage injuries**;** the rationale for its use is largely dependent on its functional components. While there are significant variations in its makeup, the initial PRP consistently contains highly concentrated platelets and a number of plasma proteins associated with platelets during its preparation ***(Andia I et al., 2012).***

Platelets are produced by megakaryocytes (nucleated cells***) (Macaulay et al, 2005).*** A variety of growth factors, coagulation factors, adhesion molecules, cytokines, chemokines and integrins are stored in platelets ***(Watson et al., 2011).*** After activation, the platelets in PRP can release a multitude of growth factors at concentrations significantly higher than the baseline blood levels, including transforming *growth factor-β, platelet-derived growth factor (PDGF),*

insulin like growth factor (IGF), basic fibroblast growth factors, vascular endothelial growth factor (VEGF), epidermal growth factors, and many others ***(Nurden et al.,2008*)**. These are all considered chondro-promoting and chondro-protective **(*Fortier LA et al., 2010; Lee CH et al., 2010; Woodell-May J et al., 2011).***

PRP also contains a variety of plasma proteins, which are known to be critical components in the healing mechanism of connective tissues**.** Different from serum plasma which contains fibrinogen and other clotting factors, which can be activated to form a provisional fibrin scaffold for cells to adhere, migrate and proliferate ***(Xie et al., 2012)***.

The clinical benefits of the PRP fibrin matrix (platelet gel) have been well known in maxillofacial surgery and chronic wound repair, PRP fibrin enhances the viability of diced cartilage grafts and should be considered an appropriate biological wrapping material for cartilage grafting ***(Manafi et al.,2016)***.

Platelet Gel is a substance that is created by separating platelet-rich plasma (PRP) from whole blood and combining it with thrombin and calcium to form a coagulum, which can

be used as a gel. ***(Stevenson et al., 2015) (Güler I et al., 2016)***

A number of studies have investigated all these treatment options, in an attempt to bypass articular cartilage's limited ability to self-repair, and to achieve tissue similar to native surrounding tissue. Nevertheless, controversy and uncertainty remain with respect to the best available treatment option. However, no previous studies investigated using perichondrial grafts with platelet gel in reconstruction of full thickness articular cartilage defects.

Aim of the Work

To evaluate and compare the effect of using Platelet-Rich Fibrin Glue ***(Platelet Gel)*** preparation on perichondrial grafts healing as a method of repair of full thickness articular cartilage defects in rabbits.

Physiology of Articular Cartilage

Adult articular cartilage is composed of a specialized matrix of type II collagen and the large aggregating proteoglycan, aggrecan, along with several "minor" collagens and small proteoglycans. Its unique structural organization provides tensile strength via the collagen network and compressive resistance via the proteoglycans, which contribute to the capacity of the matrix to accommodate more than 70 % water ***(Heinegard and Saxne, 2011; Onnerfjord et al., 2012; Hunziker et al., 2014)***.

Cartilage is relatively hypo cellular compared with other tissues, with the chondrocytes constituting only 1–2 % of the total cartilage volume, and it lacks a vascular supply and innervation. In normal adult articular cartilage, the chondrocyte has limited proliferative capacity and its ability to perform low-turnover repair declines with age. The chondrocyte is involved mostly in replacing the glycosaminoglycans on the aggrecan and other small proteoglycan core proteins. The importance of these matrix proteins in determining the structural and functional properties of the articular cartilage can be observed in chondro-dysplasia and other heritable disorders where mutations or deficiencies in cartilage matrix genes result in

altered skeletal development often associated with the premature development of OA ***(Sandell, 2012)***

Chondrocytes in articular cartilage exist in lacunae as single cells encased in a peri-cellular matrix (PCM) consisting of collagen VI micro fibrils, hyaluronan, perlecan, biglycan, aggrecan as monomers or small aggregates, and type IX collagen but virtually no type II collagen ***(Wilusz et al., 2014).***

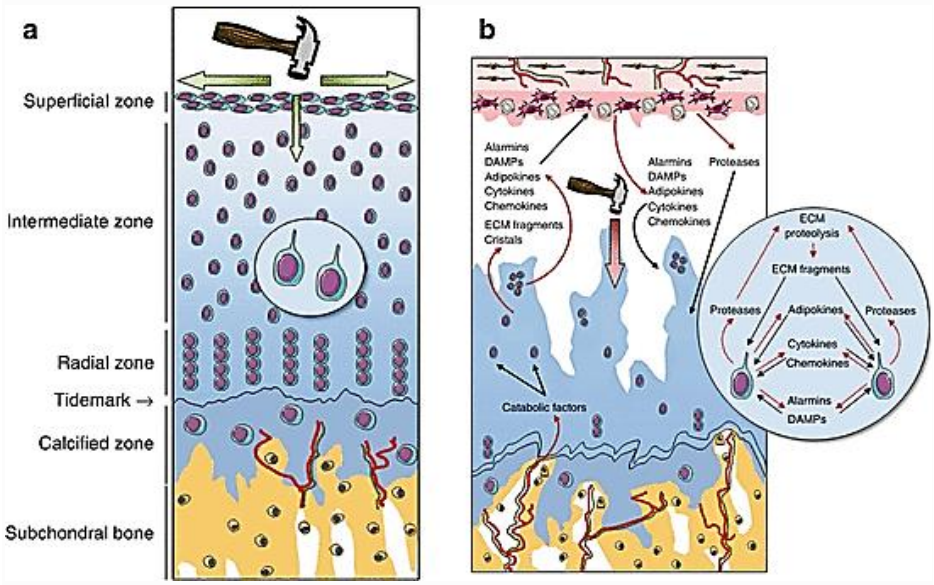

Figure (1): Diagram illustrate different zones of adult articular cartilage Heinegard and Saxne, 2011

The PCM helps to maintain the chondrocyte in a low-turnover, survival state by protecting it from interacting with molecules in the interterritorial cartilage matrix via cell-surface

receptors such as integrins, cell determinant 44 (CD44), annexins, syndecans, and discoidin domain receptor 2 (DDR2). Chondrocytes exist in a low-oxygen tension environment and intracellular survival factors such as HIF-1α are required for maintenance of homeostasis and adaptation to the mechanical environment ***(Maes et al., 2012; Pap and Bertrand 2013; Loeser 2014; Xu et al., 2014).***

Primary cilia located on the chondrocyte surface are required for chondrocytes to respond to mechanical forces and to maintain hedgehog signaling ***(Wann et al., 2012; Ho et al., 2013; Ruhlen and Marberry 2014; Thompson et al., 2016).*** These organelles contain mechanosensitive receptors, including the transient receptor potential vanilloid 4 (TRPV4), piezo channels, and connexin 43, as well as integrins, which permit the chondrocytes to sense and adapt their metabolic activity in response to physical forces ***(Knight et al., 2009; Loeser 2014; O'Conor et al., 2014; Mayan et al., 2015).***

Cartilage also provides a unique articulating surface with a very low coefficient of friction, facilitated by a boundary layer of lubricants, including lubricin, encoded by the PRG4 gene, and hyaluronic acid that are produced by

chondrocytes and synovial cells ***(Waller et al., 2013; Jay and Waller 2014).***

Joint motion and mechanical loading induce fluid movement between the cartilage and the synovial fluid, facilitating the diffusion of molecules across cartilage and thus providing nutrition. Soluble products transferred from the underlying subchondral bone could also be an important source of nutrients, especially for the deeper layers of the articular cartilage ***(Knight et al., 2009; Mayan et al., 2015).***

Types of cartilage nutrition: Synovial Nutrition (SyN) vs. Subchondral Nutrition (SuN)

Healthy articular cartilage does not contain blood vessels. It consists only of chondrocytes embedded in extracellular matrix produced by these chondrocytes. Consequently, nutrition and oxygen for the metabolism of the cells inside the cartilage must be maintained by diffusion, and the diffusing nutrients have to reach the cartilage somehow. Possible routes for this traffic of nutrients would be from the synovia, supplied by the vessels into the joint capsule (synovial nutrition: SyN), or through the subchondral bone supplied by the epiphyseal plexus (subchondral nutrition SuN) or both ***(Hoenig et al., 2013)***.

Hodge and McKibbin 1969 reported that the notion that cartilage may be supplied by the synovial fluid alone (SyN) is mainly based on experiments with young and adult rabbit joint**.** These experiments clearly show that in young rabbits, a route through the subchondral bone exists which is however completely closed in the adult animals. This leads the authors to the conclusion that nutrition of the cartilage in adult animals can only be mediated through the synovial fluid ***(Hoenig et al., 2013)***.

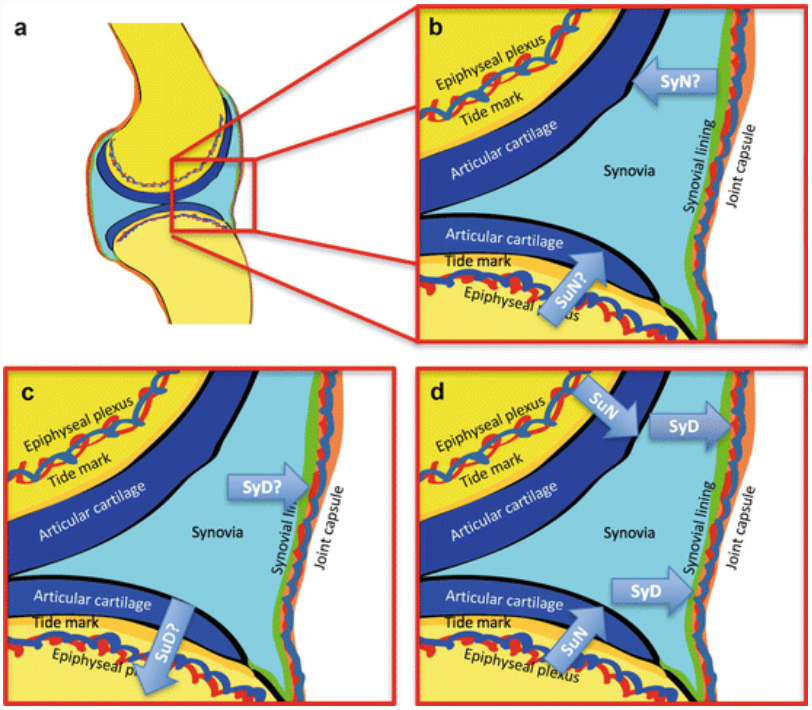

Figure (2): (a) Schematic representation of possible routes of nutrition (b) and disposal (c) for the cartilage; SyN synovial nutrition, SuN subchondral nutrition, SuD subchondral disposal, SyD synovial disposal; (d) SuN-SyD Hypothesis ***Hoenig et al., 2013***.

The density of cells in the cartilage however increases with distance from the subchondral bone. If the cells are viewed separately, this could be interpreted as an indication that the nutrition at the surface is higher and therefore there are more cells in this area. The cell density of a tissue is determined by relative ratios of cells and ECM assuming that either a high cell density means that there are many cells or that there is little ECM ***(Grimshaw and Mason 2001)***.

In humans however, there is intra osseous and extra osseous blood supply to the distal femoral condyles**. Holmdahl and Ingelmark** described two types of channels connecting the medullary cavity through the subchondral bone with the cartilage: (1) "ampulla-like" with a diameter of 40 μm and (2) "canal-like" or "dendritic" with a diameter of around 15 μm ***(Zhou et al., 2004).***

For human joints, it was demonstrated that a fluorescent dye instilled into the marrow space can be found in the cartilage after 16 h and completely penetrates the cartilage after 48 *h*. In a modeling study, ***Zhou et al., 2004*****,** showed that a favorable oxygen concentration around 5% throughout the cartilage can only be maintained by diffusion, if the lower part of cartilage is supplied by the bone route Indeed, gadolinium

(Gd (DTPA) 2) penetrated into cartilage from the articular surface, when injected intra-articularly, and from both sides when injected intravenously. Furthermore, intravenous injection showed a shorter overall penetration time ***(Zhou et al., 2008).***

However, in taking the production of the extracellular matrix (ECM) by the cells into account, a different picture emerges: the expression of collagen II by chondrocytes rises with rising oxygen tension, nearly doubling from 0% oxygen to 20% oxygen**.** If we assume a SuN route, the oxygen tension would be high close to the subchondral bone; thus, the cells would produce more collagen II in this area and therefore stand further apart leading to lower cell density at the subchondral bone border. With rising distance from the subchondral bone, oxygen tension would drop, eventually leading to hypoxia and decrease of collagen II production, which would lead to down regulation of matrix synthesis and therefore indirectly increase the cell density at the joint surface. This is also in accordance with a higher collagen II content in smaller vertebral disks ***(Boubriak et al., 2013).***

Interestingly, while in different species the thickness of articular cartilage in the knee ranges over a factor of nearly 50×

(0.05–2.3 mm), the number of cells over a square millimeter of subchondral bone is surprisingly constant (25,500 ± 8800 cells/mm2), suggesting that a given surface area of subchondral bone only has the ability to supply a given amount of cells. If O2 would be transported by the synovial fluid (SyN), there would be no obvious reason for such a relationship. Furthermore, more synovial fluid should increase the oxygen tension in the joint. Surprisingly, however, the amount of synovial fluid in the joint is inversely related with its oxygen tension ***(Richman et al., 1981; Quinn et al., 2001*)**.

This observation of lower levels of oxygen in joints with more synovial fluid can hardly be explained with changes in influx. Increased influx of freely diffusing fluid from the circulation should bring in more oxygen, not less. There are two possible ways to explain these findings. First, patients with higher volumes of synovial fluid may have an even higher metabolism of the cells in the joint (e.g., due to inflammatory processes), with higher counts of leucocytes in the joint. There was however no correlation between white blood cell counts and oxygen consumption in these experiments Second, a diminished disposal of synovial fluid may be responsible ***(Richman et al., 1981; Quinn et al., 2001).***