chapter **ONE**

GENERAL INTRODUCTION

BACKGROUND

Musculoskeletal disorders are the most common cause of severe long-term pain and physical disability affecting hundreds of millions of people around the world. To stress the importance of these diseases, the WHO has declared the first decade of the 21st century the bone and joint decade (visit www.bjdonline.org). Osteoarthritis, which is an important disease in this group of musculoskeletal diseases, constitutes 50% of all chronic conditions in people aged over 60 years, thereby placing a huge burden on societies and health care systems (http://whqlibdoc.who.int/trs/WHO_TRS_919.pdf). Next to osteoarthritic degeneration, articular cartilage damage can arise from inherited disorders or traumatic injuries. In studies of knee arthroscopies, articular cartilage lesions and focal chondral defects were observed in up to 66% of knees[1-3]. These disorders have estimated yearly costs to the U.S. economy of more than 60 billion dollars as a result of 20 million disabled citizens[4,5]. Similar figures are reflected in most Western nations, with arthritis representing an economic burden of 1–2.5% of gross domestic product, signifying a definite global epidemic[6]. This epidemic will even increase further, as it is estimated that by 2020 around 60 million people will be affected in the USA alone, resulting in nearly 600,000 hip replacements and 1,4 million knee replacements[7]. Patients with symptomatic cartilage lesions often report pain, swelling, joint locking, stiffness, and clicking. Symptoms may cause significant functional impairment, often limiting one's ability to work, play sports, and perform activities of daily living[8]. Eventually it can result in severe osteoarthritis with loss of the affected joint.

Until recently, no regenerative therapeutic option was available to prevent, delay, halt or heal the osteochondral damage[9]. Accordingly, therapies aimed at the replacement of the diseased joint with a prosthesis. Unfortunately, these prosthetic materials only have a limited lifespan and are often associated with side effects like infection, stiffness or instability of the operated joint, dislocation or even failure[10-12]. Consequently, also bearing in mind the aging and increased life-expectancy of the population, regeneration of damaged cartilage is of eminent clinical importance. To design appropriate regenerative therapies, a more thorough understanding of cartilage characteristics is mandatory.

1. CARTILAGE TISSUE: STRUCTURE, FUNCTION AND DYSFUNCTION

Articular cartilage is a narrow layer of highly elastic connective tissue lining the diarthrodial joints. It is composed of a relatively small number of cells embedded in an abundant extracellular matrix, consisting mainly of collagen type II, proteoglycans and non-collagenous proteins, along with several other matrix components which are not unique to cartilage, but have important roles in cartilage structure and function (Table I)[13].

Together, these substances maintain the proper amount of water within the matrix which confers to its unique mechanical properties. More specifically, 60-80% of the wet weight of cartilage consists of water, 90-95% of the collagen in articular cartilage is type II, the predominant proteoglycan is aggrecan and only 1.5-2.0% of the volume is comprised

Table I. *Cartilage matrix proteins**

Collagens
Type II
Type IX
TypeXI
Type VI
Type X (growth plate)
Types XII, XIV
Proteoglycans
Aggrecan
Biglycan
Decorin
Fibromodulin
Lumican
Noncollagenous proteins
COMP
Cartilage matrix protein (matrilin 1)
Anchorin II
Tenascin
Thrombomodulin
PRELP
Chondroadherin
Fibronectin
Membrane proteins
Syndecan
CD44
Integrins (α1, 2, 3, 5, 6, 10; β1, 3, 5)

** COMP = cartilage oligomeric matrix protein; PRELP = proline/ arginine-rich end leucine-rich repeat protein.*
Copied from Goldring[16]

of the chondrocytes[14,15]. There is a very real symbiotic relationship between these chondrocytes and the extracellular matrix material. The chondrocytes are responsible for matrix synthesis and turnover, while the state of the matrix has a direct influence on chondrocyte function[14-17]. Chemical and mechanical changes can either signal a necessary alteration in cellular synthetic function, or may directly damage chondrocytes by impairing nutrition[15-17]. Although appearing homogeneous, articular cartilage has a highly ordered structure. It is organized at two levels: the structure and composition of articular cartilage varies according to its distance from the surface and also in relation to the distance from the cells[14,15,18]. Typically, articular cartilage is divided into four zones (*Figure*. 1): superficial, middle (or transitional), deep (or radial), and the zone of calcified cartilage.

Chondrocytes from the different zones differ in size, shape, and metabolic activity[19]. The superficial zone is the thinnest, and forms the gliding surface of the joint. It is composed of thin collagen fibrils aligned parallel to the joint surface, with elongated, inactive chondrocytes directly subjacent. The middle zone is thicker than the superficial zone, with more spherical cells and with larger collagen fibrils that are not oriented in a parallel fashion. In the deep zone, the cells are spheroidal, arranged in a columnar orientation. The collagen fibrils here are oriented in a parallel fashion, similar to the cells, perpendicular to the joint surface. In the zone of calcified cartilage, collagen fibrils insert into the calcified cartilage, providing both a mechanical transition from the cartilage to bone, as well as fixation between the two tissues[16]. This highly organized zonal structure is related to the functions it has to fulfil: chondrocytes in the superficial layer have the capacity to provide a low friction surface due to production of lubricin, and chondrocytes in the deeper layers distribute loads to the subchondral bone[15,20].

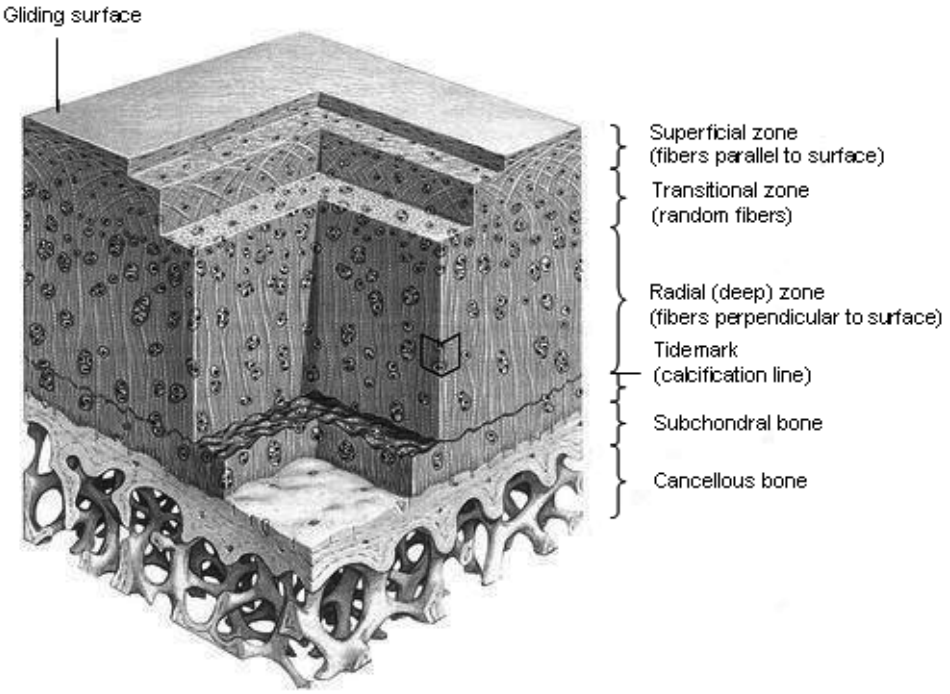

Figure 1. *Structure of articular cartilage. Schematic diagram of the cellular organization and collagen fiber architecture in the zones of articular cartilage. (Copyright 1995. Reprinted with permission from James*[1]

It is well-known that due to its avascular and aneural origin the self-regenerative capacity of cartilage is limited. This was observed as early as 1743 by Hunter[21]. Moreover, those injuries of the articular cartilage that do not penetrate the subchondral bone do not heal, and usually progress to the degeneration of the total articulating surface[22]. These lesions are often associated with disability and with symptoms such as joint pain, locking phenomena and reduced or disturbed function. If not treated, these lesions generally progress to severe forms of osteoarthritis (OA)[14,23-25].

The process of osteoarthritis is initiated by the loss of proteoglycans form the extracellular matrix concomitant with the disruption of the collagenous fibrillar network, finally leading to cell metaplasia and cell loss. Although lesions start in the superficial zone of the cartilage tissue, ultimately the entire thickness of the articular cartilage layer will be destroyed[13,26,27]. Once the lesion reaches the subchondral bone, and penetrates the bone marrow space, local bleeding may occur and the resulting blood clot serves as basis for localized spontaneous repair. Various types of stem cells are implicated, originating from the bone marrow space, adipose tissue, vascular and perivascular tissues and bone itself, as well as from the synovium[28-32]. However, such a blood clot can only fill a defect void with a lateral diameter between 1 and 2 mm, whereas smaller or larger lesions may not become fully occupied, and in these cases healing is compromised[28]. Furthermore,

remodeling of this blood clot can form new subchondral bone, but remodeling of the overlying cartilage results in a fibrous like tissue without arcade-like organization of its fibers nor a well defined zonal stratification of its chondrocytes as in hyaline like cartilage. This fibrous cartilage has suboptimal functional characteristics, and will degenerate over time[33-35].

2. REGENERATIVE THERAPIES

As mentioned above, the spontaneous repair response forms the basis for and rationale behind a number of surgical interventions aimed to induce repair or regeneration of articular cartilage lesions. Current therapies vary from lavage, debridement and shaving for small cartilage lesions to alleviate pain to more invasive procedures used to correct articular cartilage defects in joints, including marrow stimulation techniques (i.e. subchondral drilling, abrasion arthroplasty or microfracture technique), mosaicplasty or osteochondral allograft/autograft transplantation. Since these procedures are cost effective and clinically useful as patients often have reduced pain and improved joint function, they are generally considered first-line treatment for focal cartilage defects[36-38]. However, these surgical procedures have important shortcomings such as suboptimal long-term outcome and implantation, long-term presence of alloplastic material, donor site and joint morbidity, invasive surgical approach, risk of infection, and structural failure[39-41]. Allografting and autografting strategies have other shortcomings such as the possibility of disease transmission, rejection of allograft tissue, lack of available tissue, contour irregularities, and potential need for immunosuppression[42]. Therefore alternative treatment modalities were sought to overcome the disadvantages of these therapeutic tools. Tissue engineering is such an alternative treatment modality.

3. TISSUE ENGINEERING

Tissue engineering is the interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function or a whole organ[43]. It is a relatively young treatment modality, which currently is under high attention and investigation witnessed by the amount of articles (> 20,000) published so far. Tissue engineering strategies are usually composed of three key elements: *cells*, which are seeded on a supportive carrier material (*scaffold*) and stimulated by specific inductive biochemical or biophysical *signals*.

It is stated that the tissue engineering approach recapitulates embryonic or fetal cell/tissue differentiation processes[44]. In cartilage tissue, this process of chondrogenesis is composed of recruitment of mesenchymal cells and aggregation/condensation to form chondroblasts, underlining the importance of cell-cell contact in this early phase[45-47]. In a later phase a shifting occurs from cell-cell to cell-matrix interaction due to the production of abundant extracellular matrix[48,49]. However, the microenvironment within mature tissues, as well as the adult precursor cells available, preclude this recapitulation process.

On the other side, the fetal and adult organisms share the same signaling substances, but reactivity and target cells change during the course of the ontogenic process. Accordingly, basic modifications have to be instituted for successful tissue engineering in adults. These endeavors will be faced later after discussing the basic elements of these tissue engineering strategies: cells, scaffolds and inductive signals.

3.1 Cells

Cells used for tissue engineering purposes can be divided in several categories, based on their origin (autologous versus allogenous) or their differentiation state (pluripotent embryonic stem cells, multipotent adult mesenchymal stem cells or unipotent fully differentiated chondrocytes). Although embryonic stem cells are the most potent cells, capable of differentiation into every type of cell of our body, their use is limited due to ethical considerations and the risk of teratoma formation[50]. On the other edge of the scale, fully differentiated cells like chondrocytes of both autogenic and allogeneic[51-54] origin have been widely tested *in vivo*[39,55-58] and are even nowadays used clinically combining autologous chondrocytes with a collagenous or hyaluronic acid scaffold material for the treatment of cartilage defects (e.g. Chondrogide®, Carticel®, Hyaff-11®)[59-63]. Up to now results are promising, although long term results are not yet clear. A more detailed description of cellular therapies for cartilage regeneration is presented in the next section.

3.2 Scaffolds

Ideally scaffold materials used for cartilage tissue engineering should meet at least the requirements as specified in Table II. These include biocompatibility, bioactivity (chondroconductivity or even chondroinductivity), controllable biodegradability to allow ECM formation of the tissue, and sufficient structural characteristics and mechanical properties to facilitate correct joint function. Furthermore, other criteria are important for the application of the candidate material: it should be readily available, not too expensive, easy to manufacture and finally officially approved[64].

Numerous materials have been tested in the passed years varying from natural materials like collagen, fibrin, alginate, agarose, hyaluronan, chitosan and chondroitin sulfate, to synthetic materials such as polymers, and their composites[65] (Table III). Unfortunately so far no ideal candidate has emerged from these materials. Natural materials score high points on their enhanced biological interaction with the host, but the immunogenic and purification issues associated with their clinical use are still difficult to manage with current technology. Further, the significant variability of material properties, complex processing activities, and demanding regulatory requirements do limit their clinical relevance up till now[66]. Synthetic materials on the other hand allow for the accurate control of chemical, mechanical, and structural properties of the scaffold[67]. In addition, laboratory fabrication can be relatively easy scaled up to meet clinical demand. However, the limited bioactivity of these synthetic materials has prompted investigations into semi-synthetic or biomimetic material alternatives that aim to enhance biomaterial-cell interactions and consequently direct superior biological signaling for enhanced tissue growth or regeneration[64,68].

Table II *Matrix requirements*

Matrix properties	Biological basis
Porosity	Cell migration
Carrier	Lodgement and release of signaling substances
Adhesion	Cell attachment
Biodegradability	Physiological remodeling
Volume stability	Smooth surface contour of repair tissue flush with that of native articular
	cartilage
Biocompatibility	Good contact with the native tissue compartment
Bonding	Enhances interfacial integration between collagen fibrils in repair and
	native tissue compartments
Internal cohesiveness	Prevention of matrix outflow
Elasticity	Resilience during and following dynamic or static deformation
Structural anisotropy	Promotion of native anisotropic tissue organization
Matrix properties specific to the mode of surgical application	
Fluid state during application with subsequent solidification in situ	Arthroscopic implantation
Stiff and amenable to press-fitting	Arthrotomy (open surgery of a joint)

Adapted from Hunziker et al[67]

3.3 Inductive factors

To induce cells into the chondrogenic lineage biochemical cues like (combinations of) growth factors have been extensively used, in particular members of the tissue growth factor β superfamily (TGFβ1 and BMPs)[69-81], insulin growth factor[82,83], or fibroblast growth factor[84-86] (Table IV). In addition biophysical cues like hypoxia[87-93], hyperosmolarity[94-98] and ultrasound[99,100] have been found to induce chondrogenesis. The role of mechanical stimulation in determining cell differentiation has been recognized for some time[35,101-104], and is even mentioned to be of utmost importance in tissue engineering. In this regard, experiments using intermittent active or continuous active/passive motion have shown improved chondrogenesis in chondrocytes and mesenchymal stem cells of various origin[105-109].

3.4 Cellular therapy approaches

The first-generation cell therapy, autologous chondrocyte implantation (ACI), was developed because current techniques, at the time, could not treat the entire spectrum of lesions observed, particularly larger defects. ACI was first introduced in 1987 and published in 1994[110] and involved the implantation of a suspension of healthy autologous cultured chondrocytes into a chondral defect under an autologous periosteal patch[10-12,39,111-119].

Table III *Chemical classes of matrix*

Protein-based polymers
Fibrin
Collagen
Gelatin
Carbohydrate-based polymers
Polylactic acid
Polygycolic acid
Hyaluronan
Agarose
Alginate
Chitosan
Artificial polymers
Dacron (polyethylene terephthalates)
Teflon (polytetrafluoroethylene)
Carbon fibers
Polyesterurethane
Polybutyric acid
Polyethylmethacrylate
Within/between classes
Crosslinkage
Chemical modifications
Geometrical modifications (to produce fibrillar forms or foams)
Matrix combinations

Copied from Hunziker et al [67]

However, although significant improvement in function, reduction in symptoms, and the generation of hyaline-like cartilage were observed on the short term[10-12,39,111-119], this therapeutic option on the long term often resulted in a fibrocartilaginous tissue with suboptimal functional characteristics[24,120-122] and widespread adoption of ACI was limited owing to technical challenges of the surgery, the invasiveness of the procedure, cost, and postoperative complications such as hypertrophy associated with the periosteal patch[116,123]. Second-generation ACI using a bioabsorbable collagen membrane cover instead of an autologous periosteal cover, known as collagen-covered ACI (CACI)[60,124-128] had to address these issues. Although initial reports of this second-generation cell therapy showed clinical improvement similar to that of ACI with fewer complications such as hypertrophy, an open surgical technique with sutures was still required. Today's demand for transarthroscopic procedures has resulted in the development of third-generation cell therapies that deliver autologous cultured chondrocytes using cell carriers or cell-seeded scaffolds (i.e. matrix-induced ACI [MACI], Genzyme Biosurgery, Cambridge, Massachusetts) or the penetration of cultured autologous chondrocytes within a 3-dimensional scaffold or fleece (eg, Hyalograft-C, Fidia Advanced Biopolymers, Abano Terme, Italy; Novocart 3D, Tissue Engineering Technologies AG, Reutlingen, Germany; BioSeed-C, Biotissue Technologies, Freiburg, Germany)[56,61]. This MACI procedure is minimally invasive, requires less surgical time compared with first- and second-generation ACI, has a low incidence of postoperative complications and subsequent surgical procedures, and can be used to access difficult-to-reach defect sites. Clinical studies evaluating the efficacy and safety of the MACI procedure for the treatment of articular cartilage defects, in general, suggest an overall improvement in clinical outcomes and induction of hyaline-like repair tissue, although results are preliminary. These findings suggest that matrix-induced autologous chondrocyte implantation is a promising third-generation cell therapy for the repair of symptomatic, full-thickness articular cartilage defects. Although rare, postoperative complications and/or adverse events associated with the MACI procedure have been reported in clinical studies, including tissue hypertrophy, infections, subsequent surgical

Table IV *Growth factors for cartilage repair*

Inductive signal	Reference	Function
Transforming growth factorβ (TGF-β 1, 2, 3)	72,74-77, 79,80	Stimulation of chondrogenic differentiation and cartilage matrix synthesis and/or cell proliferation
Bone morphogenetic protein (BMP-2, -4, -6, 7)	73,78,81-84	Stimulation of chondrogenic differentiation and cartilage matrix synthesis and/or cell proliferation
Insulin growth factor (IGF-1)	85,86,113	Stimulation cartilage matrix synthesis and/or cell proliferation
Fibroblast growth factor (FGF1,2,18)	114-116	Stimulation cartilage matrix synthesis and/or cell proliferation
Smad 4,5	117-119	Stimulation of chondrogenic differentiation and cartilage matrix synthesis and/or cell proliferation
Cartilage derived morphogenic proteins (CDMP-1, -2, -3 /GDF-5, -6, -7)	85,86,120-122	Stimulation of chondrogenic differentiation and cartilage matrix synthesis and/or cell proliferation
Wnts	123-125	Stimulation of chondrogenic differentiation
Platelet derived growth factor (PDGF) Endothelial growth factor (EGF) Hepatic growth factor (HGF)	126-128	Stimulation of cartilage matrix synthesis and/or cell proliferation
Noggin, chordin	129,13	Inhibition of osteogenesis/ Hypertrophy by TGFβ/BMP action
Parathyroid hormone related peptide (PTHrP)	131-133	Inhibition chondrocytic hypertrophy
Indian/Sonic/ Hedgehog (IHH, SHH)	134-137	Inhibition of osteogenesis/ hypertrophy

procedures, and treatment failure[124,127,129,130]. Reported incidence rates of postoperative complications in knees treated with the MACI procedure are low (0%-6.3%),However, further long-term studies are required before this technique can be widely adopted[124].

Although the use of fully differentiated cells like chondrocytes might sound promising, it also has some major drawbacks, e.g. 1) harvesting of chondrocytes gives rise to additional articular cartilage damage[131-134], 2) chondrocytes are only limited available, thus only applicable in small defects, or have to be culture expanded, 3) upon culturing chondrocytes show dedifferentiation[135-137], 4) chondrocytes from patients suffering from osteoarthritis show decreased proliferative activity and matrix synthesis[135,138-141], and moreover 5) articular cartilage aggrecan released by chondrocytes is of inferior quality compared to both embryonic and MSC secreted counterparts[142].

An alternative pool of cells are the (adult) mesenchymal stem cells. Mesenchymal stem cells can be obtained from the adult and are widely used because of their multidifferentiation potential, including differentiation into the chondrogenic lineage. In addition to bone marrow, periosteum[143], muscle[144], adipose tissue[145] and skin[146] also appear abundant sources of mesenchymal stem cells. Subcutaneous adipose tissue and

dermis are particularly attractive reservoirs of progenitor cells, because they are easily accessible during surgery, rather abundant, and self-replenishing. Both tissues are derived from the mesodermal germ layer and contain a supportive stromal vascular fraction (SVF) that can be easily isolated[147,148]. The dermis is a less well studied source of stem cells but recently increasing studies are emerging on the use of this cellular source for tissue engineering therapies[146,149-153].

The SVF of adipose tissue and dermis consists of a heterogeneous mixture of cells, including endothelial cells, smooth muscle cells, pericytes, leucocytes, mast cells, and pre-adipocytes[154-157]. In addition to these cells the SVF contains an abundant population of multipotent adipose-tissue or dermal derived stem cells (ASCs or DSCs respectively) that possess the capacity to differentiate into cells of mesodermal origin *in vitro*, e.g. adipocytes, chondrocytes, osteoblasts and (cardio)myocytes[148,158-164] (*Figure 2*). Moreover these cells express phenotypical markers of mesenchymal stem cells, display an embryonic stem cell marker expression profile, possess immunomodulatory features and have the capacity to migrate to the site of injury (homing)[153,157,165,166] .Because of these favourable

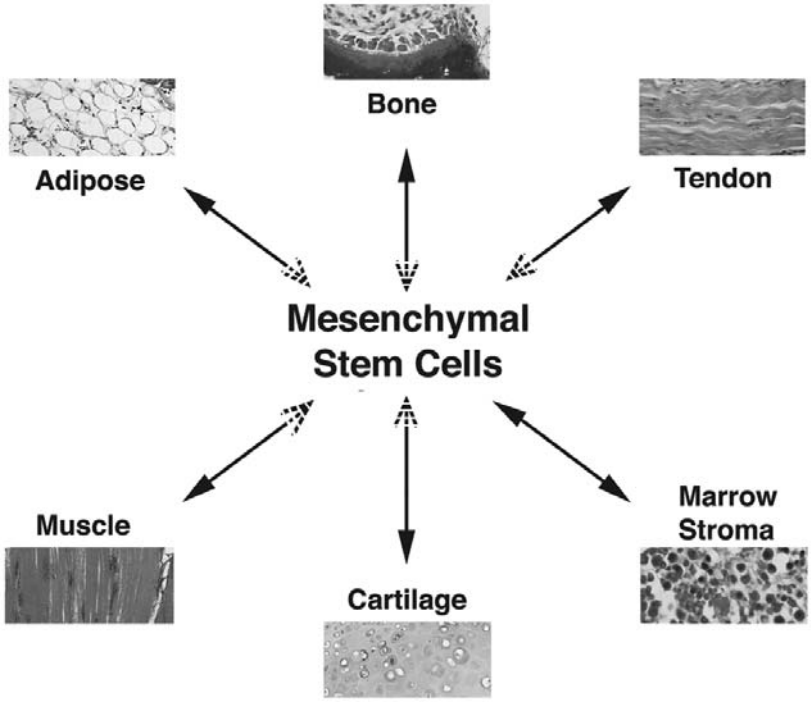

Figure 2. *Multilineage differentiation potential of mesenchymal stem cells (MSCs). Under appropriate conditions, MSCs are able to differentiate into cell types of different lineages, including bone, cartilage, adipose, muscle, tendon, and stroma. The arrows are presented as bidirectional, indicating that differentiated MSCs are capable of dedifferentiation and trans-differentiation. Adapted from Chen and Tuan*[2]

characteristics there has been growing interest in the application of ASC and (to a lesser extent) DSC for cell-based therapies such as tissue engineering.

3.4.1 Cellular therapies using stem cells

The use of this MACI technique is not only limited to the use of chondrocytes as cellular component, which is associated with certain drawbacks as stated above. Due to their multipotentiality and proliferative activity, MSC from various sources are being investigated for application in a MACI procedure. To this end the use of adipose derived stem cells and dermal derived stem cells has shown promising results in regenerating cartilage tissue both *in vitro*[73,146,150-153,167-173] and *in vivo*[72,149,174]. As stated earlier, the use of fully differentiated chondrocytes or bone marrow derived mesenchymal stem cells requires ex vivo expansion of these cells, which is not only time-consuming, but moreover costly, strictly regulated by official organs (FDA, CE mark) and unfriendly to the patient necessitating a second intervention, altogether making it an intricate procedure. Besides, although sophisticated bioreactors mimicking the intra-articular environment are frequently used to optimize the tissue engineering process, the cartilage defect environment as a "bioactive chamber" provides a more natural setting[175]. To this background and the reported high yield of adipose derived stem cells (0.2-5% of isolated stromal cells versus 0.001-0.01% in bone marrow) we formulated an innovative concept for a one-step surgical procedure for osteochondral bone regeneration, in line with a previously described one-step surgical

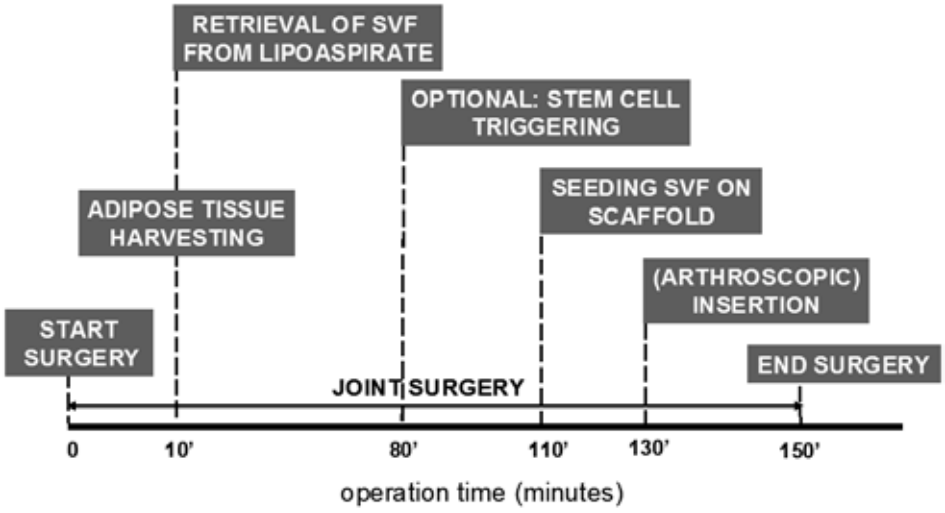

Figure 3. *Concept of a one-step surgical procedure. The surgery starts with harvesting of the adipose tissue followed by a split procedure. The surgeon continues the surgery, whereas the tissue engineer isolates the stem cell–containing cell population from the adipose tissue, treats the cells to induce differentiation into the proper phenotype, and seeds the stimulated cells on the scaffold. The surgeon then implants the scaffold containing the stem cells and finishes the surgery. The whole procedure takes approximately 2.5 hours.*

procedure for spinal fusion[176]. *Figure 3* describes this concept, which forms the backbone of the thesis described in the next chapters.

AIMS OF THIS THESIS

The ultimate goal of research in this area would be the regeneration of hyaline articular cartilage tissue. This thesis discusses issues regarding the development of a one-step surgical procedure for the treatment of cartilage defects. First of all the cellular modality was investigated. We hypothezided that mesenchymal stem cells from adipose tissue and dermis would be suitable for application in the OSP. Whether these fibroblast-like cells harvested form adipose tissue and dermis are similar to mesenchymal stem cells from bone marrow depends on minimal criteria defined for mesenchymal stem cells, as proposed by the International Society for Cellular Therapy[177]. These include i) plastic adherence, ii) a mesenchymal surface marker profile, and iii) multilineage differentiation towards at least the osteogenic, chondrogenic and adipogenic lineage. In **Chapter 2** we investigated these characteristics for adipose derived stem cells and compared these characteristics with those of the dermal derived stem cells. Moreover, **Chapter 2** deals with the homing potential of both the adipose-derived and dermal stem cells by comprehensively investigating chemokine receptor (CKR) profiles and its ligands.

As stated earlier, the earliest morphogenetic events during natural chondrogenesis are the reduction in intercellular space and the formation of extensive cell to cell contacts between mesenchymal prechondrogenic cells[45-47,178]. It is believed that during tissue engineering these embryonical processes are recapitulated[44], and hence, for cartilage regeneration high density cellular therapy is required. Several papers have addressed this issue, investigating the role of various densities on the amount and quality of cartilage formation[57,174,179-184]. Overall, they found that increasing cell density leads to better cartilage regeneration. Taking this into account, a prerequisite for the development of the one-step surgical procedure is the availability of high numbers of adipose derived stem cells to regenerate qualitatively good hyaline cartilage. Accordingly, we investigated two different adipose tissue harvesting sites, most frequently used for adipose tissue harvesting using resection or liposuction procedures, i.e. the abdomen and hip/thigh region, on yield, proliferation and osteo- and chondrogenic differentiation capacity of adipose derived stem cells in **Chapter 3.**

In the one-step surgical procedure (OSP) cells are harvested and given back to the patient within the same procedure. Next to overcoming lengthy and costly *in vitro* culturing steps, this procedure is thus highly beneficial to the patient, by making a second intervention unnecessary**.** Various tissue harvesting sites are identified as sources for the procurement of adipose derived stem cells, the abdomen and hip/thigh region being the most prevalent. Besides these well-known harvesting sites, other anatomical sites might also contain adipose tissue depots which are valuable sources of stem cells for application during regenerative therapies. In this regard, the knee also contains a fat pad, known as the infrapatellar or Hoffa's fat pad[185-189]. The choice of the infrapatellar fat pad (IFP) as source for SVF cells would be beneficial to the patient during regenerative knee

surgeries, as with a single incision both harvesting of regenerative cells and treatment of the chondral defect could be realized by application of a cell-loaded scaffold. In **Chapter 4** we determined whether stromal vascular fraction cells (SVF) from the infrapatellar fat pad are a suitable cell source for future application in a one-step surgical procedure for the regeneration of focal cartilage defects. Several requirements for the development of this one-step procedure were addressed. First the rapid cell procurement, including the need for a high yield for regenerative therapy. Then whether the procured cells contained stem cell characteristics, and finally if these cells were able to differentiate into the chondrogenic lineage when seeded in a 3D scaffold material.

During the second phase of the one-step surgical procedure, harvested cells are triggered with a specific biophysical or biochemical cue to induce these cells into the desired lineage. However, the cells might also be triggered by the natural biophysical environment the cells are transplanted into, this being the joint environment without previous induction by a biophysicochemical cue. In **Chapter 5** we therefore investigated the influence of hyperosmolarity and hypoxia, two biophysicochemical cues prevalent under physiological conditions in the joint environment, on the chondrogenic differentiation of adipose derived stem cells, when seeded in a collagen type II gel.

The third phase of the one-step surgical procedure comprises seeding the cells onto a scaffold material. Next to the multiple characteristics the scaffold material should possess, one of the characteristics for application in the one-step procedure must be the rapid attachment of the cells to the material, to limit the time-frame of this procedure (ideally lasting ≤ 2.5 hours).To this end, the suitability of both a macroporous poly(L-lactide-co-caprolactone) and a porous collagen type I/III scaffold for rapid cell attachment was evaluated in **Chapter 6**.

In **Chapter 7** the unique concept of the one-step surgical procedure was investigated *in vivo* in a cartilage defect model in the knee of the goat. Adipose tissue was harvested from the thoracolumbar region, stromal cells residing in the adipose tissue were isolated and seeded onto a collagen type I/III sponge, and subsequently this cell loaded construct was implanted into the right knee of skeletal mature goats. At the same time, cultured cells which were harvested during an earlier surgery were also implanted in the same knee to compare the regenerative potential of the freshly isolated heterogeneous stromal vascular fraction cells (SVF) with a more or less homogeneous population of cultured adipose derived stem cells (ASC).

Chapter 8 encompasses the general discussion on the development of this one-step surgical procedure to treat cartilage defects, the use of adipose derived stem cells and appropriate scaffold materials for this concept, as well as methods and cues used for induction of these cells. It also includes future perspectives on topics like gene therapy, development of new smart scaffold materials and economical aspects of these tissue engineering strategies. **Chapter 9** summarizes the preceding chapters and concludes this thesis.

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