From gristle to chondrocyte transplantation: treatment of cartilage injuries

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This review addresses the progress in cartilage repair technology over the decades with an emphasis on cartilage regeneration with cell therapy. The most abundant cartilage is the hyaline cartilage that covers the surface of our joints and, due to avascularity, this tissue is unable to repair itself. The cartilage degeneration seen in osteoarthritis causes patient suffering and is a huge burden to society. The surgical approach to cartilage repair was non-existing until the 1950s when new surgical techniques emerged. The use of cultured cells for cell therapy started as experimental studies in the 1970s that developed over the years to a clinical application in 1994 with the introduction of the autologous chondrocyte transplantation technique (ACT). The technology is now spread worldwide and has been further refined by combining arthroscopic techniques with cells cultured on matrix (MACI technology). The non-regenerating hypothesis of cartilage has been revisited and we are now able to demonstrate cell divisions and presence of stem-cell niches in the joint. Furthermore, cartilage derived from human embryonic stem cells and induced pluripotent stem cells could be the base for new broader cell treatments for cartilage injuries and the future technology base for prevention and cure of osteoarthritis.

1. Introduction

Cartilage can be found of three different phenotypes in the body—hyaline, elastic and fibrous, and the dominating cell in cartilage is the chondrocyte which comprise only a fraction of the cartilage volume. The most abundant cartilage is hyaline cartilage that covers the surfaces in our joints and comprises the rib cartilage and part of the airways structure (trachea, larynx and nose). Hyaline cartilage is the precursor of bone during fetal development and the epiphyseal cartilage present in the ends of long bones is a specialized form of hyaline cartilage that is the cellular structure for longitudinal bone growth. Elastic cartilage is found in the external ears and part of the airways (epiglottis and larynx). Fibrous cartilage is found in the intervertebral discs, joint capsule and ligaments. The different phenotypes of cartilage are the result of the extracellular matrix composition with large proteoglycan molecules combined with fibrillar components.

Cartilage on bone was described in old rhymes as gristle (AD 700) according to the Oxford Dictionary Thesaurus and in the sixteenth century the word cartilage emerged in written texts: ‘A structure or formation consisting of cartilage, a gristly part; as the cartilages of the ribs’ (AD 1540). Chemical analysis of cartilage in the 1870s describes a structure with gelating properties and that it contains sugar in part. The concept of repairing damaged cartilage in humans has been considered futile, as elegantly stated by the Scottish surgeon William Hunter 1718–1783: ‘If we consult the standard Chirurgical Writers from Hippocrates down to the present age, we shall find that an ulcerated Cartilage is universally allowed to be a troublesome Disease; that it admits of a Cure with more difficulty than carious Bone; and that, when destroyed, it is never recovered’ [1, p. 520].
2. The chondrocyte and hyaline cartilage

The word chondrocyte emerged in *Dorland's Illustrated Dictionary* in 1903. The chondrocyte is the main cell in cartilage and is the producer of the matrix components of cartilage [2]. The chondrocyte is surrounded by a territorial and interterritorial matrix where the molecular constituents of the cartilage matrix are laid down into large multimolecular assemblies (for review see [3]; figure 1).

The most abundant proteins in extracellular matrix of hyaline cartilage are the fibril forming collagens where type II collagen is the main component, while type I is prominent in most other tissues, with elastin abundant in elastic cartilage. The fibrillar assembly of collagen is fine-tuned by other fibrillar collagens, e.g. type XI with type II collagen and molecules from the small leucin rich repeat protein family, i.e. decorine, fibomoduline, as well as the matrilins and thrombospondins. The major non-protein components of hyaline cartilage are the proteoglycans that are also present in high loading structures like the aorta. The major proteoglycan in cartilage is aggrecan that is aided in its assembly into large molecules by the polysaccaride hyaluronan [4]. It is generally appreciated that the cartilage matrix proteoglycan is constantly turned over with a long half-life for the collagen fibres (decades) and for a long time it was an established fact in the textbooks (e.g. in the 1937 *Textbook of Histology* by E. E. Hewer) that in the mature state the chondrocytes do not divide and thus are unable to regenerate themselves and, even more unlikely, repair a damaged tissue. Furthermore, the chondrocytes have a very low metabolism and cartilage is unable to respond by the usual inflammatory responses as the tissue is not vascularized and not innervated. These facts laid the foundation of the wear and tear hypothesis of cartilage, which has dominated the view of the arthritis disease for decades, but progress in cell and molecular biology has now challenged the hypothesis [5].

3. The treatment of osteoarthritis and cartilage defects

Osteoarthritis (OA) is an increasingly common degenerative joint condition, estimated to affect several hundred million patients worldwide and more than 40% of people over 70 years of age [6]. *Primary osteoarthritis* is generally associated with ageing and the ‘wear and tear’ of life but not everyone gets it—not even the very old—which means that OA is a disease and not a result of ageing. The disease is characterized by cartilage degradation, formation of osteophytes and subchondral sclerosis that leads to joint destruction and severe impairment of mobility. The OA disease causes a burden to society and the healthcare system (30% of healthcare costs in the USA), but despite the increase in knowledge in medical science no drug-based disease modifying therapy exists [7], basically because of the facts that (i) the disease process stretches over decades, (ii) no biological markers able to monitor the early stages and the progression of OA exist and (iii) no specific drug target and disease mechanism have been identified.

*Secondary osteoarthritis*, caused by joint trauma, tends to develop relatively early in life, typically 10 or more years after a specific cause and approximately 5% of the population...
between 35 and 54 years have radiographic signs of OA [8,9] probably due to trauma.

The concept regarding the chondrocyte as a non-dividing cell has underpinned the view of the degenerating cartilage in arthritis as a consequence of wear and tear of the tissue. The clinical paradigm in the early- and mid-1900s was that cartilage damage was to be left unattended as no repair was possible, although single cases were reported where complete debridement of cartilage or transplantation of skin flaps with adipose tissue were used to operate on the cartilage defect. In the 1950s, the tibia osteotomy technique was introduced by Jackson where the weight bearing on the afflicted condyle—most commonly the medial condyle—was released by a correction of the load axis [10]. The introduction of the Pridie drilling technique was a later improvement based on the thought that by introducing bleeding and subsequent scar tissue into the joint the cartilage would repair [11]. Partial-thickness cartilage defects, even very small ones, do not heal spontaneously whereas full-thickness osteochondral lesions below a critical size do, although with fibrous cartilage tissue of inferior functional quality. When cartilage is wounded, the tissue at the wound edge is damaged and cells die, resulting in debris similar to wounds in other tissues. However, this damaged and avital cartilage tissue will not be removed. Wound healing is prevented by avascularity in combination with an impaired migration capacity of cartilage cells through the dense extracellular matrix. If a full-thickness defect is penetrating the subchondral bone, bleeding from the underlying bone marrow will trigger a wound healing reaction although the resulting fibrocartilaginous repair tissue will gradually degenerate over time. Besides the Pridie drilling or the more gentle marrow stimulation techniques, there was no other regenerative treatment option for symptomatic joint surface defects available.

(a) The cell-therapy approach

Cell therapy for treatment of cartilage defects emerged in the 1960s with the pioneering work of the British Cryobiologist Audrey Smith (1915–1981) who pioneered the freezing technique for blood cells and was also the first to demonstrate successful freezing and thawing of chondrocytes [12]. Furthermore, frozen cartilage and chondrocytes for treatment of cartilage defects were tried in rabbit models [13] as well as endochondral allografts and chondrocytes [14]. A cellular approach to cartilage repair was re-addressed in rabbits by Grande et al. in the early 1980s [15]. In 1984, a collaborative work started between this author, involved as a PhD student in a research programme studying growth hormone effects on chondrocytes [16], and the orthopaedic surgeon Lars Peterson with the aim to establish a human treatment model based on autologous chondrocyte transplantation. Mats Brittberg also joined the group as a PhD student and the first human autologous chondrocyte transplantation (ACT) took place in 1987 based on the earlier rabbit studies and with a culture technology adapted to human chondrocytes. The culture technique is based on autologous chondrocyte culture using autologous serum; the first pilot trial was published in 1994 and the technology subsequently found its way into the clinic ([17]; figure 2).

(b) Cell therapy and cartilage regeneration: state of the art

The ACT technology [17] has in the last decades emerged as the first disease modifying treatment with long-term excellent clinical result in patients with isolated cartilage injuries [18–22] and osteochondral lesions in the knee and ankle [23,24]. The ACT technique for the treatment of cartilage injuries has been spread worldwide with over 45 000 patients treated (estimation 2012); among those were 1800 treated by our group over the last 20 years and the treatment has been approved by the Food and Drug Administration since 1997 and by the European Medicines Agency since 2010. Four randomized controlled clinical trials have demonstrated treatment superiority for ACT over conventional treatment.
[25–27] while one showed no difference, although ACT produced a better hyaline repair tissue [28]. However, the latter study does not take into account that inclusion criteria for ACT treatment in our hands are repeated failed previous conventional treatments as one-third of patients with cartilage injuries diagnosed for the first time improve spontaneously after debridement [29]. Recent reports from our group with 15-year follow-up gives further support for the hypothesis that the ACT technology is a local disease modifying treatment where the chondrocyte graft has contributed to local cartilage regeneration [20]. Furthermore, long-term follow-up using delayed gadolinium-enhanced magnetic resonance imaging demonstrated a hyaline repair tissue similar to surrounding cartilage [21].

(c) Improvements in autologous chondrocyte transplantation cell technology: scaffolds and alternative cell sources

The OA patients and younger patients with small injuries are not subjected to ACT treatments due to the limited source of 'normal' cartilage in an OA joint and the high costs of good manufacturing practice-produced cells or grafts. Chondrocytes isolated from human articular cartilage are pluripotent with a unique differentiation capacity towards cartilage, bone and fat cells while mesenchymal stem cells (MSCs) have a default differentiation towards bone and not hyaline cartilage in vivo [30] making them less suitable for ACT. Furthermore, MSCs and articular chondrocytes also differ in several bHLH genes [31] partly explaining the different cartilage phenotypes.

Articular cartilage is generally thought to have poor self-renewal capacity but articular cartilage lesions undergo perfect regeneration in fetal life [32]. In articular cartilage, there are cells with progenitor phenotype distinguishable from resident chondrocytes by their migratory behaviour, multidifferentiation capacity and clonogenicity [33,34] that could potentially be used for cartilage regeneration. Furthermore, OA cells could potentially be used for ACT as proteoglycan synthesis in OA cells was found in comparable amounts to normal cartilage from ACT donors although collagen synthesis was significantly lower [35]. When OA cells were cultured in a hyaluronan scaffold only a few genes were differentially expressed between OA and normal hyaline cartilage, and the risk of differentiation into hypertrophic cartilage was not increased. OA chondrocytes could thus fulfill the requirements for matrix-associated cartilage ACT and OA patients could likely benefit from the treatment [36]. However, OA cells are not used clinically since knees with damaged cartilage are not healthy and the cartilage harvest for ACT is thus affected by the joint pathology.

The ultimate goal for human cartilage cell-based repair would be an off-the-shelf arthroscopic product based on a universal donor cell line combined with a suitable scaffold. For proof of concept, we derived chondroprogenitor cells from human embryonic stem cells (hES cells) [37] and hES cells cocultured with irradiated human chondrocytes induced the best chondrogenic differentiation so far in hES cells [38].

An alternative to hES cells is to induce patient-specific induced pluripotent stem (iPS) cells. The cellular reprogramming technique for adult cells was first demonstrated in mouse fibroblasts by Yamanaka and co-worker [39] followed by human fibroblasts using four pivotal genes: Oct3/4, Sox2, Klf4 and c-Myc with a retroviral system [40]. Although the cells have similar properties as hES cells with potential to differentiate to most adult tissue cells, c-Myc is an oncogene and 10% of the cell lines developed tumours in SCID mice. Furthermore, as the virus stably integrates into the iPS cell genome cells would be unsuitable for human treatment. Recent protocols using mRNA, proteins or drugs have overcome part of the problem of transformed cells. We have established an iPS cell line from human cartilage with a chondrogenic differentiation capacity using mRNA reprogramming thus resulting in a genetic footprint-free cell line [41].

Other cell sources for cartilage regeneration are using bone marrow stromal cells or MSCs where several papers have been published (for an extensive review see [42]). However, the bone marrow stimulating techniques or drilling all at aiming at getting more bleeding and thus stem cells into the injured area and randomized trials using cultured MSCs are limited. Other cell sources that have been considered are fat stromal cells and umbilical cord cells although no clinical trial has been published so far. An interesting approach using nasal septal cartilage that is derived from the neural ridge has been demonstrated to have good cartilage regenerating capacity in vivo and there are ongoing clinical trials with promising results [43].

The original two-step technology of ACT has been gradually modified and the peristeum has been replaced by synthetic collagen membranes and further developed into an arthroscopic procedure using Matrix-Assisted Chondrocyte Implantation (MACI) techniques. In the latter case, the chondrocytes are cultured expanded and subsequently cultured on a three-dimensional matrix thus forming a primitive cartilage that could be manipulated and implanted arthroscopically—for an extensive review see [44].

(d) Stem-cell niches and molecular control mechanisms in the knee joint: implications for cartilage repair and osteoarthritis

The basic concept of the non-dividing chondrocyte has been challenged over the last decades and the culture of chondrocytes and subsequent treatment with ACT has demonstrated that cell cultures of chondrocytes and transplantation is an effective treatment for cartilage with a long-term result and even combined defect of bone and cartilage has been successfully treated with a long-term clinical result [45]. The treatment concept has been improved but also basic research on chondrocyte biology with translational implication has emerged as a result of our publication in 1994 [17]. Interestingly, the concept of cartilage stem cells was almost non-existent in the literature before 1994, but today there are several hundred publications each year (nine publications in 1993 and 533 in 2014, PubMed search term: cartilage stem cells).

Cartilage stem cells and growth was poorly understood until the work of Archer where appositional growth was demonstrated in articular cartilage in the knee joints of the marsupial Monodelphis [46]. Interestingly, the surface layer of the synovial joint, the lamina splendens, expresses the Notch receptor and thus could be the location of stem cells of the cartilage [47]. We have studied the Notch signalling pathway due to its role in regulation of joint development and growth [48] and demonstrated that the Notch receptor is expressed in the surface layer of human articular cartilage—laminae splendens [49]. We were also able to demonstrate that Notch1, its ligand...
Genome-wide association scans have uncovered novel genetic associations OA is a highly polygenic disease with susceptibility alleles of only moderate-to-low individual effect. The general belief before large-scale sequencing techniques and the publication of the human genome was that the genes connected to the OA disease are found in the extracellular matrix, as monogenetic mutations in the collagen type II gene will cause osteoarthritis in early adolescence. But recent reviews of mega data has revealed that several of the loci associated with OA contain genes encoding key regulators of skeletal growth and endochondral ossification instead of genes encoding structural proteins of the cartilage extracellular matrix. Furthermore, direct and indirect regulation of gene transcription is highlighted as an important factor in this disease. This paradigm shift from the regulatory rather than a structural level is important and could be the base for new therapies in the future.

We have focused our interest on GDF5 which participates in the development, maintenance and repair of bone, cartilage and other tissues of the synovial joint and demonstrated that GDF5 is downregulating one of the main degrading enzymes in OA—MMP13—via the WNT inhibitor DKK1 [61]. The known polymorphism site SNP rs143383, a T-to-C transition in the untranslated region of the GDF5 gene, is associated with osteoarthritis at genome-wide significance level. The T-allele seems to result in a reduced level of GDF5 because of the elimination of a methylation site compared to the C-allele, and it supports the hypothesis that normal joint maintenance requires an adequate GDF5 level.

4. Future perspectives

The future of cartilage regeneration therapy in humans will be based on a deeper knowledge of cartilage developmental biology and an understanding of the regenerative potential of normal hyaline cartilage. The cell-therapy approach to cartilage regeneration although the functional mechanisms need to be elucidated.

Jagged1 and downstream suppressor gene HES5 were only expressed in a few cells localized within the uppermost cell layers of the surface zone lamina splendens of normal cartilage, in sharp contrast to the abundant expression of these markers detected in OA biopsies [50]. The presence of Notch receptors in this area suggest a more important role of the surface layer in cartilage regeneration although the functional mechanisms need to be elucidated.

An additional important contribution to the understanding of cartilage regeneration and repair has been the work on cartilage MSCs. Cartilage MSC niches reside in the synovial tissue and based on double labelling techniques the cells were demonstrated to undergo proliferation and chondrogenic differentiation following injury in vivo [31]. The basic understanding of the MSCs was founded on the pioneering work of Friedenstein and co-workers in 1988 by the presentation of the concept that bone, cartilage, marrow adipocytes and haematopoiesis-supporting stroma could originate from a common progenitor and potential stem cell [52]. However, the definite proof of the concept has not been fully explored until recently when the combined technology of rigorous single-cell analysis and lineage tracing revealed the riddle of the skeletal stem cell. A skeletal stem cell was identified and downstream clones were linked to several phenotypes in the skeletal tissue. Interestingly, adipose cells were not connected with the skeletal stem cell [53,54].

We have demonstrated the presence of a stem-cell niche in the zone of Ranvier close to the knee joint (figure 3) as well as in the intervertebrate disc region by means of long-term labelling and co-expression of stem-cell niche markers, i.e. β-catenin, Notch1, Noggin and c-kit [55,56]. With specific labelling of cells in the niche we were also able to demonstrate that cells from the niche migrate and support joint structures, e.g. meniscus, ligament and cartilage [57]. The niche cells could thus potentially be involved in human cartilage regeneration—a finding that would mean a paradigm shift for the concept of cellular treatment for cartilage defects and the OA disease mechanism. Furthermore, the niche area could potentially be the source for osteophyte formation in late OA.

Primary OA has an estimated heritability of 40% for the knee, 60% for the hip and 65% for the hand [6]. Although genome-wide association scans have uncovered novel genetic

Figure 3. Illustration and visualization of a stem cell niche in the rabbit knee. A, perichondrial groove of Ranvier; B, growth plate; C, articular cartilage. Adapted with permission from [55].

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injuries pioneered by our group has been spread worldwide and has repaired dysfunctional knees in thousands of patients. The further development of ACT to MACI technology has improved the quality of life further by reducing the rehabilitation period following surgery due to the replacement of open knee surgery with arthroscopic techniques. The autologous technique for cartilage regeneration will remain for the foreseeable future although alternative cell sources and modification of the methodology will find its way into clinical trials, i.e. nasal cartilage source. However, a broader access to the therapy will need a universal donor cell line suitable for many patients. This will probably be achieved with iPS technology and/or decellularized cultured scaffolds.

A more functional understanding of the genes involved in OA disease is needed in the future as well as better diagnostic markers for early OA in order to find a more precise pharmacological treatment. This will probably be achieved by combining iPS technology with modern gene editing technology where the complicated interaction between the cellular regulatory system of regeneration and inflammation could be studied in detail.

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