Chapter 10
The Genesis of Autologous Chondrocyte Transplantation/Implantation: From a Hypothesis via an Animal Model to a Clinical Reality

Lars Peterson

Abstract For more then 2000 years articular cartilage lesions have been recognized as a clinical problem and with no intrinsic healing or optimal treatment. In 1987 the first autologous cell transplantation was performed using isolated and cultured chondrocytes which were injected into an articular cartilage lesion covered with autologous periosteum. This first transplantation was based on solid information from *in vitro* chondrocyte research with animal and human cell culture techniques as well as extensive animal model studies. These animal studies included the first results using a semisynthesized collagen type I sponge injected with chondrocytes and implanted in defects. This research was the beginning for future use of artificial resorbable scaffolds and arthroscopic surgical technique. Since 1994 the results of ACT/ACI have been repeatedly reported. The result of 10–20 years follow up was reported in 2010 showing durable outcomes. The subjective results are supported by objective evaluations of histology of biopsies, immunohistochemistry, mechanical stiffness tests, gadolinium enhanced MRI after 8–20 years showing functional hyaline –like tissue. Even with wider indications towards early posttraumatic osteoarthritis such as large, uncontained, unshouldered lesions, multiple and bipolar lesions, the results have been acceptable. However, this would not have been possible without addressing and correcting concomitant background factors creating an optimal environment for the repair tissue to survive over time. Further improvement and simplifications in the treatment could be expected with optimal cell sources and development of biodegradable scaffolds/membranes and gels allowing arthroscopic technique, early and safe weightbearing but complex cases still may need extensive open surgery for success.

Keywords Autologous • Chondrocyte • Transplantation • Long term results • Background factors

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Key Points

- ACT/ACI can be successful when choosing the right indication, using adequate surgical techniques, addressing background factors, and understanding the biologic healing process.
- Large, uncontained, unshouldered lesions, multiple lesions, and bipolar lesions can be tried with acceptable results. Concomitant procedures such as stabilizing and unloading procedures must be considered and performed carefully, as well as meniscus deficiencies, bony defects, or other bone pathology which should be corrected.
- The rehabilitation program has to be adjusted to the individual situation; small well contained lesions full WB can be reached at 6–8 weeks, more complex cases usually need a longer period of progressively increased weight bearing, for bipolar and multiple lesions full WB at 16 weeks- is recommended.
- Return to impact sports and heavy labor depends on the maturation time of the repair tissue and the individual injuries and their severity. Concomitant procedures may also play a role and return to football takes between 12 and 18 months.
- The lack of objective noninvasive evaluation methods is a problem, but in our experience gadolinium enhanced MRI is a promising technique but still to expensive for routine evaluations. It takes between 9 and 18 months after cartilage repair before glycosaminoglycan concentrations have returned to 80 % or normal.

10.1 Introduction

In the coming decades we will be seeing an intensive research work to prevent tissue degeneration and promote regeneration of the human body for preserving the health and wellbeing of the aging population. In this research regenerative medicine and preventive medicine will be two important disciplines to overcome and prevent degenerative and other diseases as well as sequelae after trauma. In the regeneration of complete organs e.g. liver, heart, kidney, lungs, as well as regeneration of specific tissues (e.g. in the musculoskeletal system) the fundamental key to success is the choice of the potential stemcell, from embryonic or mesenchymal origin or from tissue specific progenitor cell sources etc. The understanding of the normal and pathological function of the different cell types on a molecular level must be the platform for progress in regeneration as well as repair of organs and specific tissues. The collaboration of multidisciplinary researchers in cell biology at micro- and macromolecular levels, biomaterials, biomechanics and clinical specialities is a necessity for a successful outcome. The great possibility for the future treatment of many diseases and injuries lies in regenerative medicine with multidisciplinary approach. In this work the potential in gene therapy will be of outmost importance.

In the musculoskeletal system it seems that the regeneration of a limb with all tissues involved like bone, muscle, tendon, ligament, periosteum, cartilage, synovium, meniscus, vessels and nerves, is complicated and far away. The separate tissues, however, offer possibilities for local regeneration or repair in case of injury. In that aspect the nerve and cartilage have a minimal capacity of intrinsic healing and in the case of cartilage, an injury may, over time, progress into posttraumatic osteoarthritis by the combined effect of enzymatic autodigestion and mechanical wear accelerated by high activity levels e.g. in sports [1].

More than two thousand four hundred (2400) years ago Hippocrates (460-377b. Chr.), the leading physician at that time, was the first to recognize and treat articular cartilage injuries and in 1743 Hunter stated: "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" [2]. This insight has over time created a nihilistic approach to the treatment of cartilage injuries by most physicians even up till the present time. In spite of the enormous progress and great development in medicine and related technology in the last centuries, the improvement in treating cartilage injuries has been very slow and not so successful. In osteoarthritis the introduction and improvement of total joint replacement techniques has made a great solution and difference for this patient group. For the traumatic articular cartilage injuries in the young and middle aged patients no optimal treatments have been present. However, during the last decades new treatment techniques for articular cartilage injuries have evolved and opened up for better short and long term results and hope for the future such as autologous chondrocyte transplantation/implantation, microfracture, and osteochondral grafting [3–5]. An exciting development is the fast growing number of centers for cartilage research and repair being established in many universities around the world with multidisciplinary teams ready to work for better understanding and treatment options of articular cartilage injuries and diseases in the future.

10.2 Background

In 1970, after 5 years of general surgery, I was offered a residency at the Department of Orthopaedic Surgery, Sahlgrenska University Hospital, Gothenburg University under the directorship of the late professor Bertil Stener and got involved in an intense period of sports medicine-traumatology, knee surgery, and the introduction of arthroscopy. Professor Stener, my esteemed chief and teacher, encouraged and allowed me to establish a section for reconstructive surgery and arthroscopy with focus on athletic injuries. In the following years we gradually turned from open to arthroscopic surgery and during this period of intense surgical work I noticed a high number of articular cartilage injuries when treating acute and chronic knee and other joint injuries. As the results were improving on meniscus and cruciate ligament surgery, there was no really good treatment for cartilage injuries at the time. This is how I got interested in cartilage injuries.

Going through the literature of previous and actual treatments of cartilage injuries such as debridement (Magnuson), spongialization (Ficat), multiple drilling (Pridie), high tibial osteotomy (Coventry) there were no durable results and no durable repair tissue [6–9].

In other attempts to repair cartilage lesions, perichondrium or periosteum were sutured to the debrided defects [10–13] with initially good short term results but deteriorating with longer follow-up as the repair tissue was fibrous in character and did not resist the wear and tear over time. For some years I tested all these procedures including single bone-cartilage autografts with mostly disappointing results.

In the late 60's Salter and O'Driscoll showed in a rabbit model with periosteum sutured to an osteochondral defect and treated with continuous passive motion a chondrogenic repair potential from the cells in the cambium layer [14]. In 1968 Chesterman and Smith performed homotransplantations of isolated chondrocytes to a tibial defect in the rabbit knee for which they showed that there was no repair of the defect [15].

10.3 Articular Cartilage Structure and Function

Articular cartilage is composed of chondrocytes and matrix built up by collagen type II, proteoglycans and water, is organized in four zones with different appearances and functions from the subchondral bone plate to the superficial layer of artricular cartilage allowing extremely low friction of the surface and diffusion of synovial fluid in and out of the matrix (Fig. 10.1). Articular cartilage is a unique tissue compared to other tissues in the musculoskeletal system by lacking vascular, nerve and lymph supply. This means that there is no inflammatory reparative response to injury and no pain elicited from the cartilage itself. However cartilage degradation products (e.g. after trauma) may cause an inflammatory response of the synovial membrane of the joint. The nutrition of the chondrocytes, which are less than 10 % of the total tissue volume, is maintained via diffusion of synovial fluid passing through the lamina splendens with inflow during non weight-bearing and outflow during weight-bearing. The oxygen tension is low and the metabolism almost anaerobic. The chondrocyte is synthesizing the matrix and maintaining the matrix by a slow turnover of mainly collagen type II, which takes up 10–20 % of the wet weight and proteoglycans (aggrecans), 4–7 % of the wet weight. The collagen type II fibers are anchored in the subchondral plate running up to the surface forming the Benninghoff's arcades and are reinforcing the matrix and adding tensile and compressional strength to the matrix. Together the chondrocytes, collagen type II, the proteoglycans and their content of water, the subchondral bone plate, and the trabecular bone form the osteochondral functional unit. This unit stands for most of the mechanical function such as shockabsorbtion (Fig. 10.1). The water content adds to the shockabsorbtion capacity and stands for between 65 and 80 % of the total cartilage volume and is maintained by the hydrophilic negatively charged proteoglycans [16].

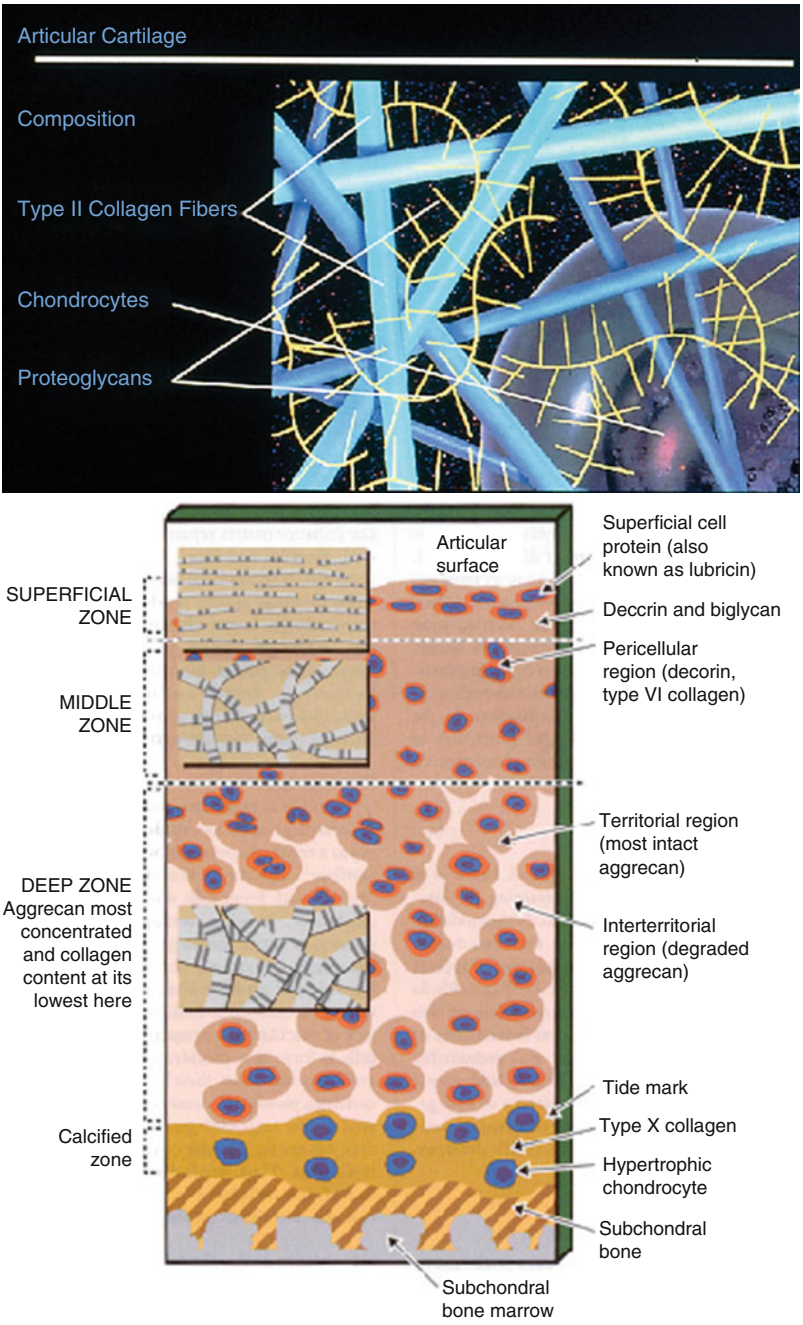

Fig. 10.1 Schematic drawings of articular cartilage structure. *Upper figure*: Close up of main components with the chondrocyte, the collagen type II and the proteoglycans. *Lower figure*: Cellular and matrix structure in different zones, from the lamina splendens to the trabecular bone; the ostechondral functional unit

10.4 Articular Cartilage Healing Capacity

It is claimed that articular cartilage has limited or no intrinsic repair capacity. Injury to the tissue will not cause a reparative inflammatory response to the cartilage itself due to the lack of vascularization, unless the subchondral bone is involved as in an osteochondral fracture [16]. It seems that there is an initial cell mitotic activity immediately after trauma in the area of the injured cartilage, but for unknown reasons this activity ends at about 14 days and leaves no repair. This may be caused by the matrix and cell damage with apoptosis that occurs when the damaged cell membrane starts to leak collagenases and proteases which degrade the newly produced collagens and proteoglycans. Healthy cells which may migrate out from the borders, where about 20 % of the cells are apoptotic, will meet a barrier of necrotic, degrading tissue and released enzymes from damaged or apoptotic cells and will not survive. The lack of sufficient inflammatory response to trauma will not start adequate macrophage or phagocyte activity to remove the necrotic tissue, so the barrier remains and the enzymatic and mechanic breakdown may progress into posttraumatic osteoarthritis over time, as there is no intrinsic cartilage repair or regenerative healing capacity [1] (Fig. 10.3). Cartilage debris and fragments –loose bodies as well as leakage of enzymes through the damaged cell membrane are causing an inflammatory response from the synovial membrane but has no healing effect on the cartilage but will disturb the joint homeostasis.

10.5 How to Address the Situation and Create Healing Conditions?

All damaged, necrotic tissue in the area must be removed down to the subchondral bone and excised 2 mm into healthy surrounding cartilage to minimize the number of apoptotic cells in the excised side (ref. Lindahl A, 2009). The defect has to be repopulated with cells with the capacity to regenerate hyaline cartilage, i.e. articular chondrocyte progenitor cells in increased numbers committed to produce hyaline cartilage. We therefore need to find adequate cell sources from a biopsy harvested from minor weight-bearing areas with minimal donor site morbidity, and develop a safe and optimal cell culture technique to achieve mitotic active and viable cells for implantation and repopulation of defects. We must find adequate autologous tissue or create biocompatible, degradable materials for keeping the cells in the defect. Then develop an animal model to study and evaluate the hypothesis: "*It is possible to heal a full thickness* (*down to the subchondral bone*) *articular defect using enzymatically isolated autologous chondrocytes grown in culture and implanted under an autologous tissue membrane or synthetic degradable biomaterials sutured or fixed to the defect*."

10.6 Cell Culture Technique and Design of a Rabbit Experimental Model

In 1982 I was invited for a year as a visiting professor at the Hospital for Joint Diseases, Orthopaedic Institute, New York City University, in Manhattan, New York City. The director was professor Victor Frankel and he allowed me to use all facilities of the hospital including research laboratories and animal operating resources with the aim to design an animal experimental model in the rabbit to test the hypothesis. In the laboratory there was a small section for cell biology with some experience of growing cells in culture. Dr David Menche and the chief of the Sports Medicine Department, dr Mark Pitman together with a young Ph.D. student Daniel Grande were running a research project and immediately we joined in a team to work on the chondrocyte cell culture and the rabbit model.

Step 1 was to establish a safe, sterile and efficient cell culture technique. Daniel Grande was the key person involved in this first step. Rabbits were operated on both knees and biopsies of articular cartilage were taken from a small area on the upper medial trochlea and from a 3 mm diameter punch defect down to the subchondral bone plate of the central medial femoral condyle or the central patella. The biopsies were brought to the laboratory, prepared and minced in small pieces, and then undergoing enzymatic digestion of the matrix according to the technique described by Audie Smith using collagenase [15]. The cells were then separated from matrix and isolated and grown for 3 weeks in standardized culture media and fetal calf serum added. After some failures with cell death, too small numbers of cells, infections etc. the whole procedure was optimized using a strict and controlled culture technique in which we were able to repeatedly grow sufficient cellnumbers for implantation [17].

Step 2 was to design the optimal experimental model in the rabbit knee. During this initial period we worked on selection of the optimal defect area, the optimal autologous cover to keep the cells in the defects and autologous versus allogenic chondrocytes. After several pre-studies we found that the cartilage thickness for suturing a cover was best on the patella, that the periosteal membrane according to Salter had a chondroid potential in the cambium layer cells and in our tests was superior to synovial membrane, tendon sheath, muscle fascia. We also found the periosteal membrane to be optimal when facing the cambium layer into the defect [18]. For safety reasons we chose the autologous chondrocytes for possible future use of autologous cells in humans. They also seemed superior to allogenic cells from the pre-studies. During this time we also built two continuous passive motion machines for rabbits according to Salter to use in the postoperative care. They were however not used later because the two first transplanted rabbits which were put into the machines, fell out of the machines during the first night and stressed themselves to death. We later found out that Salter did not use the machines during nights.

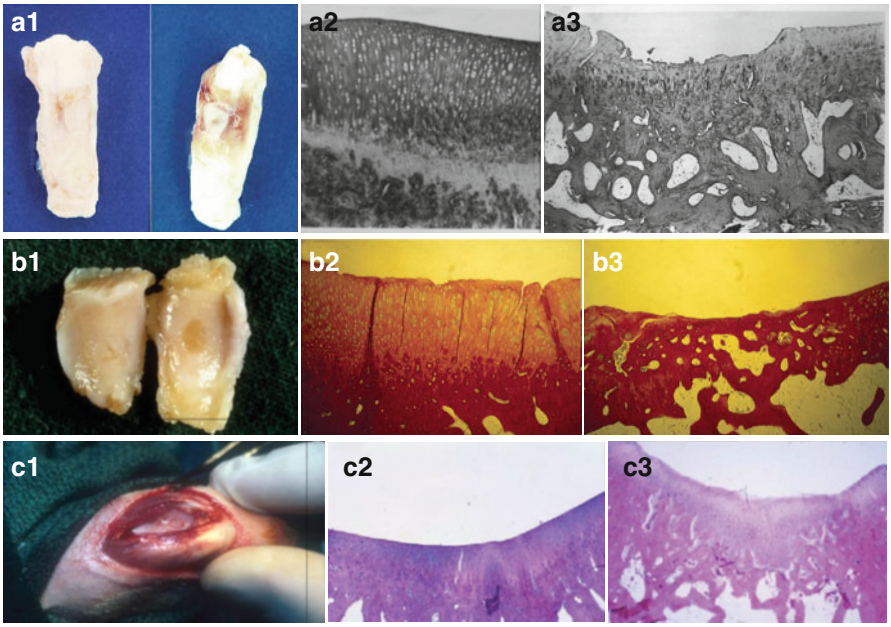

Fig. 10.2 Series of experimental work using a rabbit knee model. A 3 mm diameter articular cartilage defect down to subchondral bone in the rabbit patella. *Upper row* (**a**) showing macroscopic and microscopic results 12 weeks after autologous chondrocyte transplantation; picture (*A1*) showing filling of experimental side to the *left* and control side to the *right*. (*A2*) Histology experimental side showing hyaline cartilage, *A3* the control side showing limited filling of the defect. *Middle row* (**b**) same model with 1 year result. (*B1*) Experimental side showing good filling to the *left* and control side showing no filling to the *right*. (*B2*) Histology experimental side showing hyaline cartilage. (*B3*) The control side showing no filling of the defect. *Lower row* (**c**) same model showing results after a 12 week follow-up in 3 mm defect, (*C1*) surgical picture: the chondrocytes injected into a semi-synthesised collagen type I resorbable sponge from Indian rat tail, implanted with press-fit technique into the defect. (*C2*) Experimental side showing good filling with hyaline appearance. (*C3*) Control side showing no filling of the defect

The first results of the rabbit model were presented at the Orthopaedic Research Society meeting in Atlanta, 1984, showing an 80 % fill in the defects operated with chondrocytes and periosteal cover compared to 20 % fill in the defects without chondrocytes but with periost alone after 12 weeks [17, 19]. Microscopy showed the same staining characteristics as normal cartilage in the experimental group (Fig. 10.2).

In September 1983 I left New York to work in a chief position at the Department of Orthopaedic Surgery at the East Hospital, University of Gothenburg. In 1984 I met Anders Lindahl, who was an MD working on his thesis on epiphyseal cartilage at the Department of Physiology and has been my most important coworker to transfer ACT/ACI into human clinical practice and to continue basic and clinical

research in cartilage regeneration-repair. Together with Anders Nilsson another MD and Ph.D. student we continued the animal experiments and repeated the study from New York with a 1 year follow up. The results at 1 year after transplantation were presented at the yearly meeting of the Swedish Society of Physicians in Stockholm 1986, showing excellent filling and microscopy in the experimental group versus no filling in the control group [20] (Fig. 10.2).

At the same time I continued the collaboration with the New York group. During the early literature review I came over a paper by Shwapil, who was the first to semisynthesize collagen type I resorbable sponges from Indian rat tails. This fitted well into my idea to use scaffolds as a vehicle to support the cells in the early postoperative period but also make it possible to use arthroscopic surgical technique for implantation. Dr. Shwapil allowed us to use the sponches and we injected them with autologous chondrocytes. Using the rabbit model we compared a group with autologous chondrocytes injected into the sponge with a group with the sponge only, fixed by press fit technique into the cartilage defects. The results were presented at the ESSKA meeting in Salzburg, Austria, 1985 and showed excellent filling and histology in the experimental group compared to minimal filling in the control group (Fig. 10.2) This was the first experimental study using a degradable semisynthesized scaffold as a carrier of cells opening up to autologous chondrocyte implantation, second generation and arthroscopic technique.

10.7 Transfer of the Animal Model into Human Clinical Practice

My strategy was to transfer this animal treatment technique as far as possible into human surgical treatment and provide the highest safety for the patients by avoiding any problems like rejections, immunologic reactions, contamination with serious infections etc. The first condition was to use autologous cells, tissues, serum to minimize serious complications.

The transfer of the rabbit articular chondrocyte culture technique into human articular chondrocyte culture technique started in 1984 by Anders Lindahl and myself. Articular cartilage from anterior cruciate ligament reconstructions were harvested from the intended drillhole in the tibia and from the notch plasties under sterile conditions and were, together with the patient's own serum, transported to the laboratory. The standardized culture medium containing Ham's F 12 medium with supplements and 15 % of the patient's own (autologous) serum was added instead of fetal bovine serum [3]. The culture medium was tested for bacteria and fungi, before the cultured chondrocytes were released to use, as well as check of the cell number, character, viability etc. After about 3 years of optimizing and standardizing a safe and optimal cartilage harvesting technique arthroscopically, the work in

the laboratory, studying consequences of cell transportations, freezing of the cells for cell viability or contaminations with repeated tests, we had achieved a safe and reproducible technique for biopsies and optimal cell culture of human chondrocytes in the laboratory using autologous human serum. In 1987 we got the approval by the Ethical Committee of the Medical Faculty of the University of Gothenburg to use autologous chondrocytes cultured in laboratory for the treatment of chondral injuries in the human knee. The first patient was transplanted with autologous chondrocytes in October 1987 at the Department of Orthopaedic Surgery, East Hospital, University of Gothenburg, Sweden. Mats Brittberg at that time a young resident and my Ph.D student assisted me at this "historic surgery" and later defended his thesis on cartilage repair in 1996.

10.8 Indications, Surgical Technique, Classification, and Rehabilitation

10.8.1 Indications

ACT/ACI is indicated in symptomatic full thickness cartilage lesions or osteochondral lesions according to ICRS or Outerbridge classifications III-IV. Age of the patient should be between 15 and 55 years but there is no definite limit. Size of the defect is between 2 and 16 cm^2. Gradually with increased experience the indications have widened and larger (over 16 cm^2) uncontained, multiple defects or bone to bone compartmental lesions could be tried as a relative indication (salvage procedure) in young and active middle age patients (Fig. 10.3).

Contraindications are generalized osteoarthritis, rheumatoid arthritis and other systemic diseases. Background factors such as instability, varus-valgus deformities, patella malalignment or instability, meniscus deficiency, bone pathology or defects must be addressed [21].

10.8.2 Surgical Technique

ACT/ACI is a 2 step procedure. The preoperative arthroscopic evaluation is to decide the indication, to plan the surgical approach and if concomitant procedures like ACL-reconstruction, varus or valgus osteotomies, patellar realignment procedures, meniscus allograft transplantation, bone grafts are needed etc. One can decide if the procedures should be staged or done in a single operation. Then a biopsy is

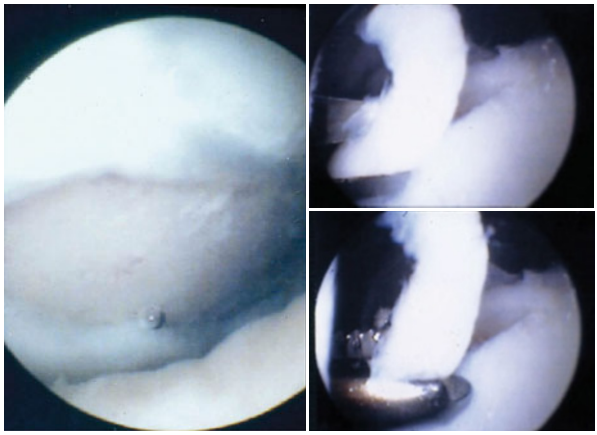

Fig. 10.3 Arthroscopic preoperative assessment; *left image* showing a contained lesion down to subchondral bone. *Right images* showing biopsies taken from upper, medial trochlea

harvested from one of following locations; (1) the upper medial trochlea, (2) the upper lateral trochlea, and (3) the lateral intercondylar notch. Consider possible meniscus surgery at this time (Fig. 10.3).

For implantation of the chondrocytes, adjust the arthrotomy to the location, size and numbers of defect. Radical excision of the defect to healthy cartilage. Debride carefully down to subchondral bone plate. Do not leave any damaged cartilage. Make a template of the prepared defect. A small incision is made medial proximal tibia below the pes anserinus insertion, dissect carefully down to the periosteum, remove fat, fibrous tissue and passing vessels, incise the periosteum around the template. Then dissect the flap from the cortical bone using an elevator (raspartorium), keep the flap moist and go directly to the defect and use 6:o vicryl to suture the flap to the vertical edges of the defect. Seal the intervals between the sutures with fibrin glue (Tisseal), check for tightness by gentle injection of saline, if ok aspirate the fluid and inject the cells, close the injection site and close the incision. If a resorbable membrane is used suture and fix as above [21] (Fig. 10.4). Postoperatively prophylactic antibiotics for 24 h and antithrombotic treatment. CPM 8 h after surgery (cell adhesion time) for 6–8 h/24 h.

10.8.3 Classification of ACT/ACI Cartilage Repair Techniques

The introduction of new materials and techniques has been followed by new classifications regarding the differences between them. The following classification has been proposed:

First generation ACT/ACI: ACT/ACI as first described in 1994 with the use of autologous chondrocytes grown in culture and injected in suspension under a periosteal cover [3].

Second generation ACI: Autologous chondrocytes grown in culture and injected under or into and delivered with tissue engineered matrix support (TEMS) of animal tissue origin (bovine, porcine origin or others) or chemically synthesized matrix support (polyglycolic-polylactic acids), or others. (MACI, Chondrogide) [22, 23].

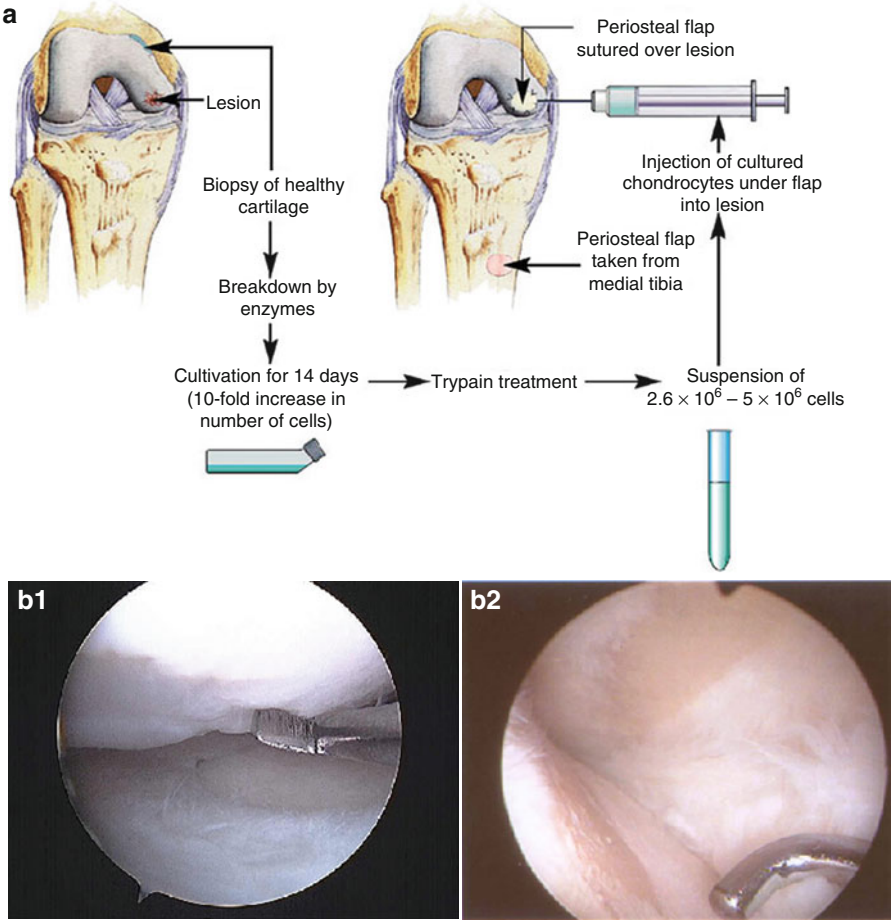

Fig. 10.4 Schematic diagram of ACT/ACI *upper row* (*A*). Case 1, *left row* (*B1-D1*). (*B1*) Isolated lesion treated with microfracture 1 year before, still symptomatic. Arthroscopic assessment. (*C1*) ACT/ACI treatment lateral femoral condyle defect using artrotomy for chondrocyte implantation. (*D1*) Second look arthroscopy at 12 months with excellent healing. Returned to professional football (*soccer*) at 15 months. Case 2, *right row* (*B2-D2*) (*B2*) Preoperative arthroscopy showing bipolar medial femoral and tibial condyle down to bone lesions in 37 year old soccerplayer after total medial meniscectomy at age 16. (*C2*) Bipolar ACT/ACI of large uncontained lesions. Compartment unloaded with a concomitant closing wedge proximal tibial osteotomy. (*D2*) Second look arthroscopy at 4 years showing complete healing. Still asymptomatic 12 years after surgery

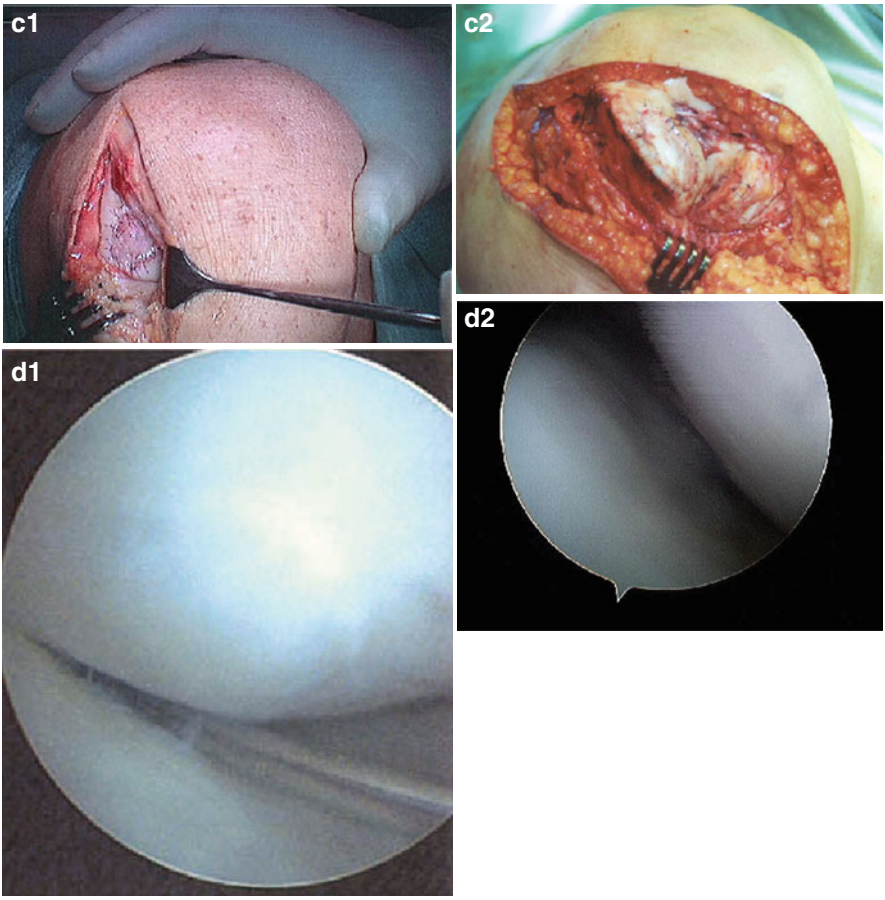

Fig. 10.4 (continued)

Third generation ACI: ACI with three- dimensional TEMS of animal or chemical origin used as scaffolds for growing and delivering the chondrocytes to the joint. (Hyalograft C) [24, 25].

10.8.4 Rehabilitation

The great challenge in the rehabilitation after ACI is to regain full weight-bearing (WB) as early as possible without jeopardizing the new delicate tissue formed by the implanted chondrocytes. Progressive increase of WB is essential to stimulate the matrix production, the remodeling and the maturation of the cartilage. In general the WB could increase after the initial 3 weeks to full WB with the limitation of pain and swelling in contained, sized 4–6 cm^2, isolated lesions. More complex

cases as uncontained, unshouldered, bipolar or multiple lesions are recommended 6–8 weeks of 20–40 kg WB and full WB to be reached at 12–16 weeks. Cases with concomitant procedures will affect the rehabilitation and adjusted accordingly (Fig. 10.6).

The immediate postoperative training includes continuous passive motion, (after 6–8 h to allow the cells to adhere to the subchondral bone and surrounding edges of normal cartilage), during 6–8 h/24 h. Activation of the quadriceps muscle and assisted active flexion from the first postoperative day and mobilization with crutches and partial WB. The early partial loading and unloading is essential for the exchange of fluid for the nutrition of the cartilage as well as a stimulation for the implanted chondrocytes to produce adequate matrix. Non WB is not recommended for this reason.

It is helpful to the patient, physiotherapist and physician to divide the rehabilitation after ACI in four different phases and to understand the healing process, and adjust the training to ensure a short and long term success of the treatment. Each phase has one key word for the actual healing process and repair tissue and one key word for the main focus during this time period. For detailed training activities and instructions [21, 26].

10.8.4.1 Phase I: Proliferation and Protection (1–6 Weeks)

During the first hours the cells are still in suspension and active in mitosis. Within 6–8 h the cells will adhere to the subchondral bone and the edges of surrounding cartilage and start matrix production in the early proliferation phase (Lindahl A, Peterson L, 1996). During this phase the proliferation activity and the newly formed repair tissue are vulnerable to overload, and still the chondrocytes need mechanical stimuli for optimal matrix proliferation by-loading – unloading and by motion for water exchange and nutrition. During this phase the tissue is soft and like a gel under the periosteal flap and has to be protected from overload, too high compression and shear forces. The main goal during phase I is the protection of the fragile tissue proliferation and this needs a progressive increase in partial weightbearing (WB) from 20 to 40 kg for this phase. For patellar and trochlear lesions we allow full WB after 3 weeks but not in up and downstairs climbing not until 10–12 weeks. when loaded full WB in kneeflexion is allowed.

10.8.4.2 Phase II: Transition and Progression (7–12 Weeks)

During this period the repair tissue increases filling the defect and getting more resistant to WB with a transition from partial to full WB and will allow a progressive increase in therapeutic and functional exercises. The main goal is a safe progress to full WB, ROM (full extension and almost full flexion) and increasing quadriceps and hamstring strength preparing for next phase.

10.8.4.3 Phase III: Remodeling and Function (3–6 Months)

During this period there is an ongoing matrix production leading to a continuous remodeling and functional adaptation into a more organized structure. The formation of cells arranged in columns, collagen type II building up the Benninghoff's arcades anchored to the subchondral bone, and filling the interspace with proteoglycans and water, creates a functional, firm, structured tissue with increasing biomechanical properties over time. The training is focused on optimizing in muscle strength, endurance, flexibility and neuromuscular function and gradually increasing functional activities become more important, preparing for going back to low-impact sports.

10.8.4.4 Phase IV: Maturation and Optimizing (7–12–18 Months)

The maturation of the repair tissue is an ongoing process starting in Phase I- III and continues into the normal cartilage tissue turnover which is the ultimate goal and there is no defined endpoint in time. The goal is to gradually return to full preinjury activity level including low-impact sports and hard labour as individually tolerated. The training in high- impact loading sports are gradually started for the definite maturation and tissue healing allowing return to sport specific training and competition which may be possible at an average of 15 months after surgery.

10.9 Results of Autologous Transplantation/Implantation

The results of the first 23 patients operated with autologous chondrocyte transplantation/ implantation were published in The New England Journal of Medicine in October, 1994 [3]. At an average follow up of 36 months, 14 of 16 patients had a good/excellent results on femoral condyle lesions but only 2 out of 7 patients with patellar lesions reported good/excellent results. Biopsies showed hyaline appearance in 11 of 15 patients on the femoral condyle (Fig. 10.5).

Continuously we have published our results. In 2000 we reported 2–9 years outcome after ACT/ACI in Clinical Orthopaedics and Related Research showing an average of 85 % Good/Excellent results in isolated femoral condyle lesions, in multiple (2 or more) lesions, in osteochondritis dissecans, in patella and in isolated femoral condyle lesions combined with ACL reconstructions with a double bundle vascularized graft [27]. The results were supported by objective evaluations such as arthroscopic, macroscopic assessments of repair tissue, showing good filling, good integration to surrounding borders and acceptable surface tissue and biopsies of 2 mm diameter from the center of the repaired defects showed histology with hyaline appearance in over 80 % [27].

In 2002 clinical results, biomechanics of repair tissue were published in the American Journal of Sports Medicine [28]. A 5–11 years outcome of 61 patients showed at 2 years good to excellent (G/E) result in 50 patients, and the same results

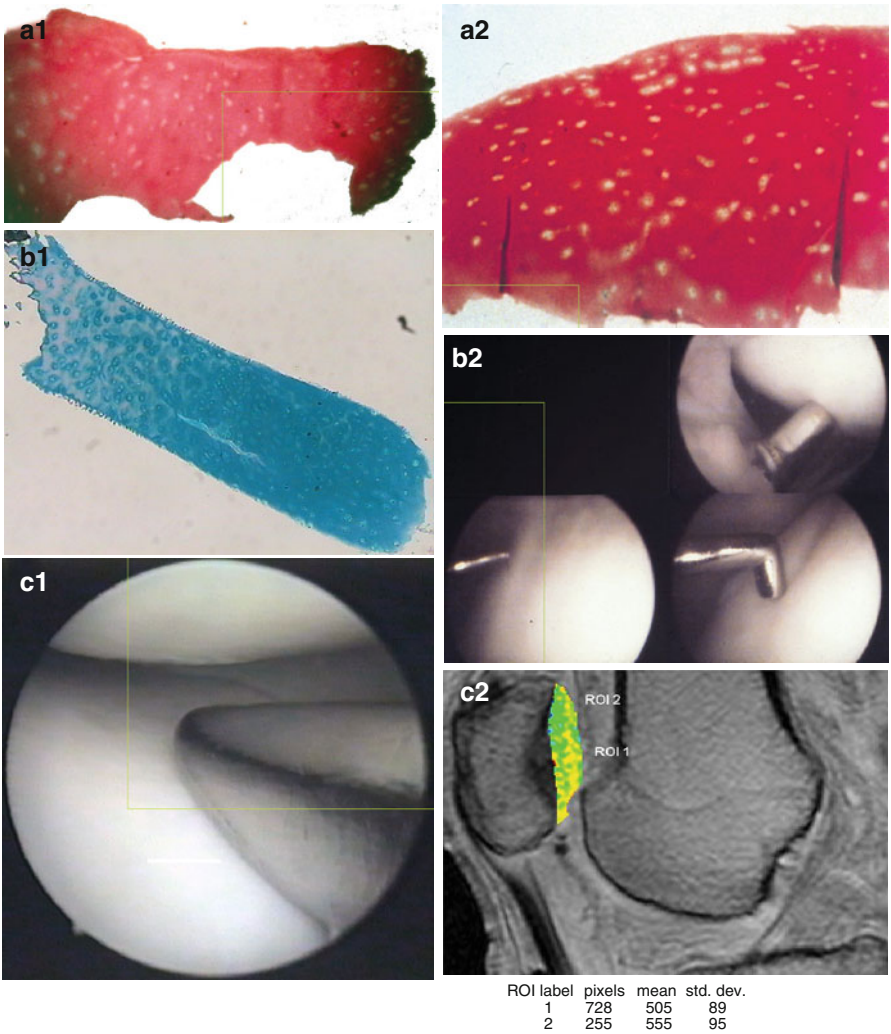

Fig. 10.5 Objective evaluations. (*A1*), (*A2*) and (*B1*): Biopsies 2 mm diameter taken 2–4 years after surgery. Histology showing articular cartilage. (*B2*) Showing artroscopic assessment and probing. (*C1*) Showing arthroscopic indentation test of stiffness of repair tissue. (*C2*) Showing normal glycosaminoglycan concentration in the patella 11 years after surgery

after 5–11 years. The results were supported by biopsies in 12 patients showing hyaline appearance and homogenous structure in polarized light microscopy. Arthroscopic indentation tests of the stiffness of repair tissue with hyaline appearance in biopsies, were equal to the stiffness of normal cartilage tissue. Those with fibrohyaline biopsies had a significant lower stiffness [28] (Fig. 10.5).

In 2003 the long time follow up of osteochondritis dissecans of the knee treated with ACT/ACI was published in Journal of Bone and Joint Surgery, supplement, showing G/E results in 91 % of the patients between 2 and 10 years [29] (Fig. 10.6).

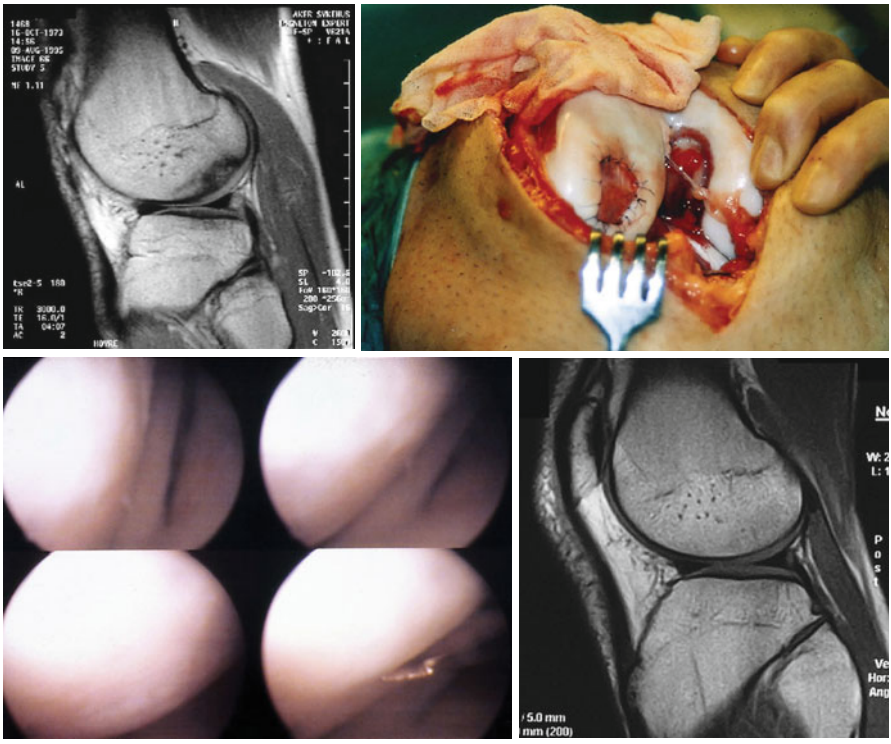

Fig. 10.6 Osteochondritis dissecans treated with ACT/ACI is one of the best treatments reported. *Upper row, left*: preoperative MRI showing large and deep OCD of lateral femoral condyle in 24 year old soccerplayer. *Upper row right*: showing the defect at surgery. *Lower row, left*: second look arthroscopy at 1 year, returned to professional football (*soccer*) at 15 months and are still playing at age 38. *Lower row, right*: MRI 9 years after surgery showing complete healing of both bone and cartilage

The latest and longest follow up reported on 10–20 years in American Journal of Sports Medicine (2010) showed in comparison to previous follow ups no significant differences in results. Ninety-two percent of the patients would have the surgery again [30]. In the same journal May, 2010 we reported on the result of delayed gadolinium enhanced magnetic resonance imaging dGEMRIC in 35 knees in 31 patients treated with ACI 9–18 years ago. In 28 knees the proteoglycan concentration was the same in the operated area as in the normal cartilage and in 7 knees the concentration was somewhat lower both in the operated area as well as in the surrounding cartilage. It shows that the implanted chondrocytes have the capacity to reach and maintain homeostasis in the proteoglycan metabolism in relation to the surrounding cartilage either it is normal or somewhat lowered concentrations [31] (Fig. 10.5).

The initial results of ACI in the patellofemoral joint were not promising. In the patella lesions only 28 % had G/E results in the first publication. Later ACI in trochlear lesions reached over 80 % G/E results and even the patella results improved to 68 %. The improvement was due to concomitant realignment procedures. In a separate follow up of cartilage injuries in the patellofemoral joint with 10–20

years the improvement varied from 44.4 % in kissing lesions, in isolated patellar lesions 79.5–100 % in the isolated trochlear lesions. However, 92 % of the patients would have the operation again [3, 31].

10.9.1 Summary of Objective Evaluations to Support the Clinical Outcome

Arthroscopic macroscopic assessment of repair area according to ICRS showed out of maximal 12 points in isolated femoral condyles in average 10.3 points, in isolated lesions with ACL reconstruction 10.9 points, and in OCD 10.5 points meaning a good filling, a good integration and a good surface. In over 120 biopsies with microscopic, histologic assessments from repair area compared to normal cartilage in the same joint 80 % showing hyaline like appearance. Immunohistochemical analysis of the biopsies showing collagen type II, cartilage oligomeric matrix protein (COMP) and aggrecan similar to normal articular cartilage.

Arthroscopic indentation tests of stiffness in the repair area with hyaline like appearance compared to normal articular cartilage showed no significant difference in stiffness.

With dGEMRIC technique the glycosaminoglycans uptakes were normal in 28 knees and at the same concentration as in the surrounding cartilage in 7 knees in patients 9–18 years after ACI [3, 27, 28, 31] (Fig. 10.5).

10.9.2 What Have We Learned in the Last 25–30 Years with ACT/ACI?

The hypothesis was proven right: It is possible to use isolated and cultured autologous chondrocytes to repair articular cartilage injuries in the human knee. Long term follow up outcome studies show subjectively good results in about 85 % of all diagnoses. The indications have widened from small isolated, contained lesions to large, uncontained, multiple lesions (2 or more) in the same knee, bipolar –kissing lesions in any compartment (medial and lateral tibiofemoral or patellofemoral) or posttraumatic osteoarthritis.

It is necessary for the short and long term success of ACT/ACI that background factors like instability, varus or valgus malalignment, functional meniscus deficiency after subtotal or total meniscectomies as well as bone defects or pathology are addressed adequately [21].

Common concomitant procedures in the tibiofemoral joints are varus and valgus osteotomies to unload the affected compartment. X-rays in standing position with hip – knee – ankle included are valuable to assess the degree of correction-unloading needed. Anterior, posterior, or collateral ligament instability should be reconstructed. Meniscus allograft transplantation to restore the joint mechanics should be performed at the same

time as ACI or 7–8 months later depending on access to the graft and experience. Bony defects after OCD or bone cysts or other bone pathology should be bone grafted with spongious autologous bone from the iliac crest, tibial or femoral condyles depending of the amount of bone needed. Preoperative MRI could help to plan the surgery.

In the patellofemoral joint the background factors have been shown to play an important role for the short and long term results. Patella alta and malalignment, increased q-angle, patellotrochlear dysplasia, patellar instability including patellar lateral tracking, tilt, subluxation and dislocation are important findings to recognize and address properly. That may need tibial tuberosity transfer to correct the q-angle, by medialization, unloading by ventralization and distalization when patella alta is present. The instability also need medial soft tissue stabilization and reinforcement including reconstruction of the medial patellofemoral ligament (medial transverse retinaculum) and vastus medialis obliquus shortening. To achieve this, a lateral release is necessary. If trochlear dysplasia is a part of the instability a proximal trochlea plasty should be done [32, 33]. The unloading by ventralization is important in large uncontained patella or trochlear lesions and in kissing lesions. For a successful ACI all the background factors should be treated. Adequate physiotherapy, mainly with closed chain technique the first 3–4 months and early motion is an important part of the initial treatment. Computerized tomography including quadriceps relaxation and contraction with the knee in extension is a good technique to diagnose instability and trochlear dysplasia (Fig. 10.7a).

10.10 Future of Cartilage Repair/Regeneration

10.10.1 Optimal Cell Sources

The search for other cell sources than autologous articular cartilage has been ongoing for a long time allowing a one step procedure with cells as an on the shelf product. Among new cells explored are cells of allogenic or xenogenic origin, from fetal, juvenile and adult donors. Direct isolation in the operating room by mincing cartilage biopsies, seeding it on resorbable membranes and implant them arthroscopically, as well as directly aspirated and concentrated autologous bone marrow mesenchymal stem cells are under investigational studies.

10.10.2 Tissue Engineered Matrix Support (TEMS)

TEMS include membranes, gels, scaffolds in ACT/ACI and other cartilage repair or regeneration techniques. The ideal TEMS has to be safe for the patient, be compatible, noncarcinogenic, not causing inflammatory or immune reactions, not cytotoxic for the implanted cells or surrounding tissues. However still the mechanical properties and the resorbtion time have to be evaluated and adapted to specific situations

and demands. The development and use of degradable chemically synthesized membranes-gels-scaffolds, such as hyaluronic acid (Hyalograft) [24] or polyglycolic-polylactic acids as well as semisynthesized materials of porcine, bovine or other animal origin such as MACI (Verigen) [22] and Chondro-Gide (Geistlich) [23] have reported medium-term results and are undergoing intense research and clinical trials [25, 34]. It is, however, of outmost importance that the intended functions of different types of TEMS are defined and established, that the resorbtion time is studied and decided, that the mechanical properties are specified, regulated, and tested regarding surface friction coefficient, mechanical stiffness in relation to resorbtion

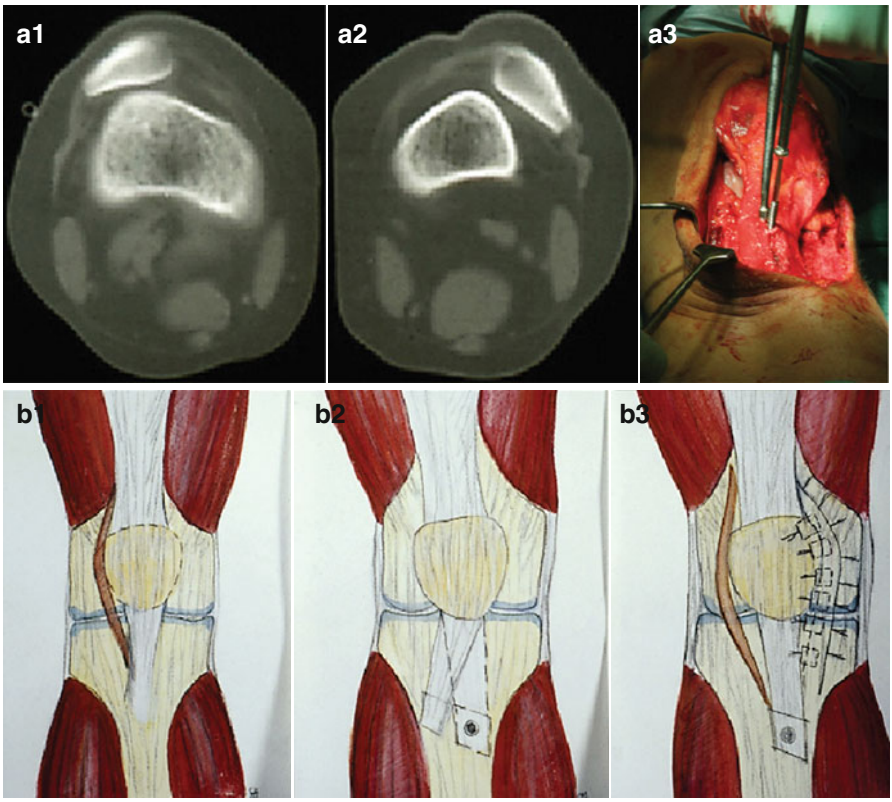

Fig. 10.7 Surgical corrections of background factors to patellofemoral articular cartilage lesions. (*A1-2*) Computerized tomography with the knee in extension and quadriceps contraction. Both patellas dislocated laterally. Note the trochlear dysplasia! (*B1-2-3-A3*) Distal and proximal realignment procedures to correct increased Q-angle as a part of instability or lateral tracking. (*B1*) Lateral release to allow (*B2*) tibial tuberosity transfer (medial or anterior, or distal directions). (*B3*) Medial soft tissue shortening and reinforcement of the medial patellofemoral ligament (medial transverse patellar retinculum) and vastus medialis obliquus. (*A3*) Antero-medial-distalisation of tibial tuberosity and screw fixation. (*C-D-1-2-3*) Schematic and surgical steps in proximal trochleaplasty for correction of trochlear dysplasia. (*C-D1*) Showing trochlear dysplasia and release of the synovial lining from the cartilage. (*C-D2*) Using a curved osteotome or a burr and make 10 × 30 groove in the cartilage and bone. (*C-D3*) suturing the synovial membrane back to the cartilage edge. (*E*) Large kissing lesions need unloading by anteriorisation (ventralisation) to protect the repair

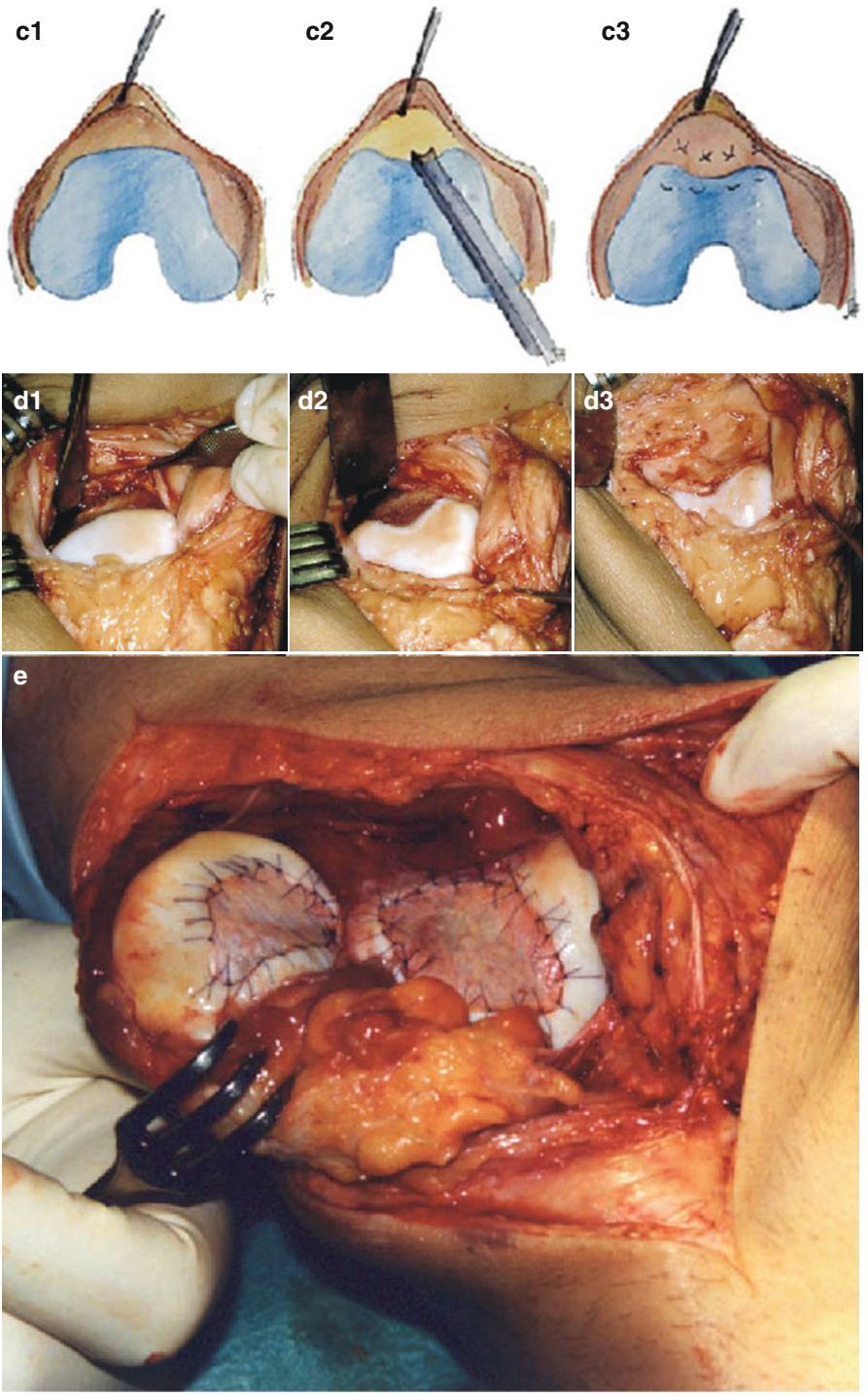

Fig. 10.7 (continued)

time, have the mechanical strength to allow adequate fixation for early and safe weight-bearing etc. Membranes could be used to replace the periosteal membrane, or used as a carrier to be injected with cells and implanted, or to grow the cells in for direct implantation. Should the time to complete resorbtion be short, 6–12 weeks, or medium 3–6 months or long 7 to 12–15 months, to gradually be replaced by regenerated tissue and give mechanical support during part of or whole the maturation process of the regenerating cartilage?

Membranes are now used as cover of defects prepared by microfracturing to host invading mesenchymal stemcells as well as fibroblasts from the subchondral bone and sometimes filled with aspirated and concentrated mesenchymal stemcells. Only short term result have been presented.

Resorbable gels mainly from animal collagen has been used to grow the cells in and used as a carrier to deliver the cells into the defects. Comparable results to ACI has been reported from Japan [35].

Scaffolds of three dimension such as Hyalograft from esterified hyaluronic acid with a resorbtion time of about 4 months, has been used to grow the chondrocytes in for 3 weeks and then used as a carrier to be implanted in small, contained defects using arthroscopic technique or miniarthrotomy [25, 34].

10.11 Summary

It all started with a great interest for sports medicine, traumatology, reconstructive and arthroscopic surgery in a very sports friendly environment under the leadership of professor Bertil Stener, director at the Department of Orthopaedic Surgery II, Sahlgrenska Hospital, University of Gothenburg. During an intense period of acute and reconstructive surgery and the introduction of arthroscopy in the department we established a sportsmedicine section handling most of the athletes in the region. We could diagnose and treat most of the athletic injuries of traumatic or overuse type in an acceptable way but when it came to chondral injuries there was no spontaneous healing or acceptable treatment to use. I tried debridement, periosteum transplantation, spongialization, multiple drilling, osteochondral grafting, most of them with just short relief of symptoms. The review of actual literature was very disappointing.

Injury to articular cartilage does not heal with inflammation as there is no vascular supply and no phagocytic cell activity to remove the necrotic tissue in the injured area. The chondrocytes are not capable of repopulating the area and regenerate new cartilage.

Furthermore no actual treatments at that time showed good long term results. In England Audie Smith was able to isolate rabbit chondrocyte by collagenase degradation. Injections of isolated chondrocytes to an experimental defect in the tibial articular surface of a rabbit knee did not show any healing. Salter showed a chondrogenic repair potential after transplantation of periost to an osteochondral defect in the rabbit knee. Human studies did not show any good long term results.

With the hypothesis: "*It is possible to heal a full thickness articular cartilage defect using enzymatically isolated autologous chondrocytes grown in culture and implanted under an autologous tissue membrane sutured to the defect*", the work started. The hypothesis was proven after 4 years using an experimental rabbit model. Two studies were performed and the first study showed at 3 months follow-up results with over 80 % filling of the defects and hyaline cartilage appearance on microscopy. The second study with 1 year follow up showed over 80 % filling and hyaline cartilage on microscopy. No filling in the control.

From 1984 Anders Lindahl and myself worked on transferring the cell culture technique from the rabbit to the human chondrocyte using autologous serum instead of fetal calf serum. It took us 3 years to reach a safe, efficient and sterile technique and create criteria for an approved cell culture, suitable for autologous transplantation in humans. In 1987 the Ethical Committee of the Medical Faculty of the Gothenburg University approved the technique for clinical use in the human knee. In the autumn, 1987 the first patient was operated with her own isolated and cultured cells implanted in a cartilage defect and still after 25 years she is happy with her knee function.

Since then more than 2,000 defects have been operated with autologous chondrocyte transplantation in Gothenburg and worldwide over 35,000 patients treated.

The results have been reported from medium to long term follow ups showing over 90 % good to excellent results in isolated femoral and trochlear lesions and in osteochondritis dissecans of the knee. The overall results on isolated lesions was 84 % G/E at the 10–20 years latest follow-up and 92 % of the patients would have the surgery again.

The ACT/ACI has the longest follow up, has the most objective data to support the good long-term clinical results, such as arthroscopic macroscopic assessment, indentation test of mechanical stiffness, biopsies showing hyaline cartilage appearance, immunohistochemistry showing collagen type II, aggrecan and COMP concentrations close to normal, and gadolinium enhanced MRI (dGEMRIC) showing normal concentrations of proteoglycans in the repair area 9–18 years after surgery.

For the first time in orthopaedics autologous cells have been isolated, grown in culture and reimplanted into articular cartilage lesions with regeneration and healing with hyaline cartilage appearance and long durable results.

The new emerging techniques using different cell sources and degradable scaffolds, membranes and gels etc., will make cell treatment easier, improve the results, widen the indications, shorten the rehabilitation time and (at least) make some lesions possible to treat with arthroscopic technique and reduce the surgical trauma and the morbidity. However long term randomized studies need to be carried out and the great interest from the young generation of orthopaedic surgeons gives great promises for the future.

The development of cell transplantation is opening up for cell therapy in other tissues in the musculoskeletal system and maybe in the future also for organ regeneration for transplantations.

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