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Articular cartilage repair: basic science and clinical progress. A review of the current status and prospects

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Summary

Objective: To review the basic scientific status of repair in articular cartilage tissue and to assess the efficiency of current clinical therapies instigated for the treatment of structural lesions generated therein as a result of trauma or during the course of various diseases, notably osteoarthritis (OA). Current scientific trends and possible directions for the future will also be discussed.

Design: A systematic and critical analysis is undertaken, beginning with a description of the spontaneous repair responses in different types of lesion. Surgical interventions aimed at inducing repair without the use of active biologics will then be considered, followed by those involving active biologics and those drawing on autogenic and allogeneic tissue transplantation principles. Cell transplantation approaches, in particular novel tissue engineering concepts, will be critically presented. These will include growth-factor-based biological treatments and gene transfection protocols. A number of technical problems associated with repair interventions, such as tissue integration, tissue retention and the role of mechanical factors, will also be analysed.

Results: A critical analysis of the literature reveals the existence of many novel and very promising biologically-based approaches for the induction of articular cartilage repair, the vast majority of which are still at an experimental phase of development. But prospective, double-blinded clinical trials comparing currently practiced surgical treatments have, unfortunately, not been undertaken.

Conclusion: The existence of many new and encouraging biological approaches to cartilage repair justifies the future investment of time and money in this research area, particularly given the extremely high socio-economic importance of such therapeutic strategies in the prevention and treatment of these common joint diseases and traumas. Clinical epidemiological and prospective trials are, moreover, urgently needed for an objective, scientific appraisal of current therapies and future novel approaches.

Key words: articular, cartilage, repair, review.

Introduction

The numerous experimental and clinical attempts that have been made to induce the healing of histologic and macroscopic lesions within mature articular cartilage aim at re-establishing a structurally and functionally competent repair tissue of an enduring nature. Such lesions are generated during the course of many joint diseases, notably osteoarthritis (OA), in conjunction with a large number of genetic or metabolic conditions, such as acromegaly, Paget's Disease, the Stickler-Syndrome and hemophilia (for review, see Buckwalter and Mankin)[1], or as a result of trauma. Traumatic lesions may occur directly or indirectly in consequence of an intraarticular fracture, a high-intensity impact or following ligament injuries[1,2].

Articular cartilage lesions generally do not heal, or heal only partially under certain biological conditions. They are frequently associated with disability and with symptoms such as joint pain, locking phenomena and reduced or disturbed function. Moreover, such lesions are generally believed to progress to severe forms of OA[1,3,4].

Given the multitude of possible origins, attempts to heal articular cartilage lesions have obviously involved symptomatic measures, which are useful only if patients gain relief, if joint functionality can be significantly restored and if the progression to severe joint destruction can be prevented or at least materially slowed down[4,5]. Simple arthroscopic interventions are currently of great interest in the treatment of such lesions, their attraction being that they can be repeated at low cost whenever necessary. By these means, drastic measures of dealing with joint destruction, such as total joint replacement, which is a risky undertaking in elderly persons—the prime sufferers—could be prevented.

This review article addresses the surgical and biological attempts that have been made to induce a significant and durable repair response[6,7]. It does not deal with the medical treatment of painful joint states, nor with chondro-protective strategies or measures that aim at improving joint lubrication, since these undertakings do not aim at healing structural lesions[8–10].

This review article is organized in such a manner that we initially consider lesion biologies within articular cartilage tissue and specifically analyse the potential and limitations of spontaneous repair responses. We then proceed first to

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surgical interventions undertaken in the absence of active biologics and then to those involving them. Autogenic and allogeneic tissue transplantation procedures are then dealt with, followed by cell transplantation measures involving, for example, autologous chondrocytes or embryonic cells. One of the largest areas covered is the tissue engineering approach to cartilage repair, in which not only cells and matrices[7,11,12] but also signaling substances or genetic measures[13] are employed for the re-establishment of a structurally and functionally competent cartilage repair tissue[14–16]. Finally, some of the specific biological and technical problems commonly encountered with repair-inducing protocols are addressed, for example, chondrocyte loss from the lesion borders, poor tissue integration and mechanical factors[7].

Each section of the review is dealt with in a systematic manner: first the underlying biological concept is discussed and then the available experimental and clinical data analysed. At the close, the author offers the reader his personal critical comments and a brief outlook of the future in this field.

Lesion biology

During the course of OA, structural lesions typically develop within the articular cartilage layer. The process is initiated by the loss of proteoglycans from the extracellular matrix and a disruption of the collagenous fibrillar network therein, these events being followed by cell metaplasia and loss. The initially small focal lesions gradually increase not only in girth but also in length, such that ultimately the entire thickness of the articular cartilage layer may be destroyed (for review see[17–21]). Albeit that the metabolic activity of the surviving resident population of chondrocytes is augmented, this is altered in such a way that degradative processes predominate over anabolic ones[22]. It has been known for decades that such lesions do not heal spontaneously. The invidious process of destruction advances downward until the osseous region is reached. Once the bone and bone marrow spaces are involved in the degradative process, local bleeding may occur, and the ensuing local formation of a blood clot serves as the basis for localized spontaneous repair activity during OA.

The spontaneous repair of articular cartilage is thus associated with defects that penetrate the bone and bone-marrow spaces, being dependent upon the lesioning of blood vessels, bleeding and hematoma formation. Various types of stem cell are implicated, these originating from the bone-marrow spaces, adipose tissue, vascular and perivascular tissues and bone itself, as well as from the synovium[23–27]. Large quantities of growth factors are also released from the bone[28–30], and these play an important role in initiating the repair response. Full-thickness articular cartilage defects created artificially for therapeutic purposes during surgery are generated with a view to initiating an analogous spontaneous repair response.

Since the physiological process of spontaneous repair plays a key role in a number of surgically based strategies, a thorough understanding of this response is of great importance. Moreover, it illustrates the principles that must be followed when endeavouring to modulate and boost healing artificially.

The blood clot that forms the basis of the spontaneous repair response[27,31] consists principally of a fibrin matrix within which are trapped various types of protein, glycoprotein and lipid, as well as, of course, red and white blood cells, platelets containing many different releasable growth factors, and various types of blood-borne cell[32,33]. Such a blot clot can completely fill a defect void with a lateral diameter of between 1 and 2 mm. Smaller or larger lesions may not become fully occupied, and in these cases healing is compromised[23]. The implication of these findings is that an effective natural healing response occurs only within a critical and narrow size range. The spontaneous repair of smaller or larger lesions thus needs to be facilitated by the insertion of a porous matrix that will imbibe blood or be soaked by the hematoma in such a manner that the entire void is permeated.

A spontaneously formed blood clot generally adheres well to the surfaces of bony tissue, but only rarely so to those of articular cartilage and to the defect floor and walls. After a few days, mesenchymal cells begin to penetrate the fibrin matrix, and within a matter of weeks this is completely replaced by a vascularized, scar-like tissue[27]. Thereafter, direct ossification processes, which may be quite extensive, begin upon the bony surfaces. And within the repair tissue itself, enchondral ossification activity is initiated. The neo-ossification front generally proceeds upwards from the sides and floor of the defect towards the joint cavity. Interestingly though, the uppermost portion of the repair tissue, which was originally occupied by the articular cartilage layer, does not ossify but transforms into a fibrous type of cartilage. This repair tissue manifests neither an arcade-like organization of its fibers nor a well-defined zonal stratification of its chondrocytes. Its biochemical composition is indeed more akin to fibrous than to hyaline cartilage[34], and its mechanical competence is significantly inferior to that of the latter[35,36]. It persists for some weeks or even months but then inevitably begins to degenerate, as does the fibrous type of repair cartilage laid down in osteoarthritic individuals.

Spontaneously generated repair tissue integrates but poorly with native cartilage, sites of focal discontinuity being commonly encountered[23]. Not surprisingly therefore, the collagenous fibrillar network of repair cartilage has been found neither to project into native tissue nor to intermingle with its fibrils.

Shapiro *et al.*[27] and other investigators have noted that the native articular cartilage immediately adjacent to an artificially created defect site becomes necrotic after surgery. Some chondrocyte-cluster formation occurs therein, but it is neither remodeled nor resorbed. It appears to remain essentially inert for some considerable time before ultimately degenerating.

The heterogeneous composition and inferior biomechanical properties of spontaneously formed repair cartilage undoubtedly contribute to its functional incompetence and perishability. The compositional heterogeneity is not surprising given the diverse origins of potential stem cells and the uncontrollable influx of signaling substances derived from the bone matrix, blood platelets and other sources. These factors may also contribute to the highly variable results achieved and to the unpredictable outcome. Clearly, for a successful (reproducible and durable) repair result, a more homogeneous repair cell population, which is capable of producing hyaline-like cartilage, is required.

As mentioned above, the spontaneous repair response forms the basis for and the rationale behind a number of surgical interventions that aim to induce the healing of articular cartilage lesions. These therapeutic strategies will now be discussed in detail.

Therapeutic interventions without active biologics

LAVAGE AND ARTHROSCOPY

The irrigation or lavage of a joint with solutions of sodium chloride, Ringer or Ringer and lactate, using the closed-needle-hole procedure[37], has been deemed beneficial in osteoarthritic or trauma patients with painful (mainly knee) joints. Simple exploratory arthroscopic procedures which involve thorough rinsing (unlike gas-based arthroscopy) of the joint, have likewise been reported to alleviate pain in several patient populations[38,39].

Joint lavage was introduced as an empirical approach to treating painful joint conditions involving structural lesions[39,40]. There exists no scientific or biological basis to account for the beneficial effects thereby derived. At best, we can postulate that the extensive rinsing removes some of the intraarticularly active pain-signaling or pain-mediating molecules. It perhaps also extracts proteoglycans and aggrecans from the superficial cartilage matrix compartment, thereby promoting, at least transiently, the adhesion of repair cells[41], which may prompt an antiinflammatory response. But no solid evidence of any biological or repair activity being instigated by lavage is available[42].

Some investigators have reported the beneficial effects of lavage to persist for one year or more[39], whereas others have observed no substantial relief from pain in patients who have undergone this treatment[43]. In yet other studies—principally those in which lavage was conducted in conjunction with arthroscopy—the benefits have been attributed to a placebo effect of the surgical intervention *per se*[38]. The placebo effect of surgery is, indeed, not to be underestimated, and the considerable bias introduced during clinical assessment and by different joint-scoring systems have also to be taken into account[44,45].

Taking the available data as a whole[37], lavage does appear to have a beneficial effect for limited periods of time, irrespective of whether this is performed by the closed-needle-hole technique or in association with arthroscopic intervention[43,46,47]. In their controlled randomized clinical trial, Chang *et al*.[37] observed no difference between the two groups for periods of up to one year, at which time 50% of the treated population had some relief from pain. Moreover, patients who had a history of trauma (such as tearing of the cruciate ligament or meniscus, or traumatic structural lesioning) derived greater benefits than did those suffering from OA[38–40].

SHAVING

Chondral shaving is generally carried out by arthroscopic intervention and aims at the mechanical removal of diseased chondral tissue using appropriate surgical instruments. Nowadays, it is performed infrequently and mainly recommended for chondromalacia patellae or patello-femoral pain[48].

The biological rationale behind this empirical type of intervention is by no means clear. It may represent merely an intuitive move by the surgeon to do the patient 'some good' by removing the fibrillated articular cartilage seen by arthroscopic inspection, and by thus 'smoothing the surface' reduce possible friction. But from a biomechanical point of view[49–51], such an undertaking makes no sense at all.

Experiments with mature rabbits[52] have revealed no evidence whatsoever of repair tissue at defect sites up to 12 weeks after chondral shaving, irrespective of the biomechanical loading protocol applied to the joint. Moreover, the remaining cartilage underwent degeneration. This phenomenon, which is probably attributable to apoptotic cell loss[53], has been observed also by Mitchell and Shepard[54]. It likewise occurs along lesion borders[27] and has been shown quantitatively to involve a substantial cell loss[55].

In one clinical investigation conducted by Ogilvie-Harris and Jackson[48], shaving was found to be advantageous only in a subgroup of patients suffering specifically from post-traumatic joint pain; not in those with femoral pain states. But since no prospective randomized clinical trials have been undertaken, it is impossible to assess the usefulness of this intervention. And on the basis of laboratory data, it is certainly not justified.

DEBRIDEMENT

Debridement is a more drastic version of the shaving procedure, combining this latter undertaking with lavage, meniscectomy, the removal of free bodies from the joint and the limited excision of osteophytes[56].

As in the case of shaving, there exists neither a sound scientific basis for performing debridement nor any evidence from animal experiments of beneficial biological effects derived therefrom. Indeed, if one considers articular cartilage biology and joint biomechanics, one would expect debridement to have deleterious consequences for joint biology. As mentioned in the previous section, shaving (chondrectomy) is associated with cell apoptosis and necrosis[52–54]. In addition to this, meniscectomy will lead to skeletal malalignment and a change in the transarthrodial loading pattern. In the long term, this treatment has been shown both experimentally[57,58] and clinically[59,60] to exacerbate the osteoarthritic condition. Debridement is thus undertaken clinically as a purely palliative measure to relieve patients temporarily of pain in an osteoarthritic joint.

In default of a rational biological concept underlying this procedure and in the complete absence of experimental evidence to justify the intervention, it is not surprising that clinical findings pertaining thereto are highly variable, ranging from good (pain relief in 65% of patients)[56,61,62] through moderate (pain relief in less than 50% of patients)[63,64] to very poor[43,65,66]. Even when the results are reportedly good, the temporary nature of the benefits derived are always stressed by the investigators.

All published reports are based on subjective retrospective studies and are thus of very limited value in yielding meaningful information[5,44,45]. Regrettably, no prospective, double-blinded clinical trials have been carried out. An objective gounding for performing debridement is thus not available.

LASER ABRASION/LASER CHONDROPLASTY

Laser chondroplasty may be used as an alternative to the surgical removal of tissue by mechanical cutting. One of the advantages of employing laser light (of certain wavelengths) as a cutting tool is that it coagulates tissue[67], and, unlike electrothermal cutting instruments, which generate temperatures of up to 250°C, can be used in an aqueous environment[68,69].

Obviously, in the case of articular cartilage, which is avascular, or meniscal tissue, which is only poorly vascularized, blood coagulation is not a prime consideration.

However, since arthroscopic interventions are carried out in an aqueous environment, lasers are certainly preferable to conventional electrothermal instruments in that they avoid water-boiling effects within the rinsing media. Moreover, flexible tubes can be used for the transmission of certain types of laser light, such as neodymium: YAG[68]. Furthermore, laser radiation is also useful for tissue welding and fusion[70,71].

The biological rationale behind the use of lasers in chondroplastic surgery is thus that they serve as gentle cutting instruments[72,73], not that they induce articular cartilage repair, although questionable claims of a biostimulatory effect of laser radiation have been reported[74]. There exists no more justification for performing surgical shaving and debridement with laser light than there does for carrying out these procedures by means of mechanical cutting, no repair of articular cartilage having been reported as a consequence of either undertaking. Indeed, laser chondroplasty has not been subjected to a thorough investigation in experimental animals[75]. Many different sources of laser light have been tested for chondroplastic purposes in a laboratory setting, including carbon dioxide, neodymium: YAG[76], holmium: YAG[77], erbium: YAG[78] and Excimer[79]. The effects produced depend greatly upon wavelength, frequency and energy level, a discussion of which lies beyond the scope of this section. It will suffice here to mention the undesired consequences of such treatment with most, but not all, types of laser; these relate to thermal damage, air-bubble formation[77,78] and tissue necrosis[78]. The main complications associated with the clinical use of lasers in chondroplasty are osteonecrosis[80,81], reactive synovitis, chondrolysis and an acceleration of articular cartilage degeneration[82,83].

The reported biostimulatory effects of laser light alluded to above are based on experiments with dedifferentiated chondrocytes in two-dimensional culturing systems: such cells were found to manifest an increased proliferative activity[74]. These findings are, however, not convincing. In the first place, the behavior of chondrocytes *in vivo* is fundamentally different from that of dedifferentiated ones in two-dimensional cultures. And then, the observed response may have been attributable to a thermogenic effect rather than a direct consequence of laser irradiation, which would be more likely to slow down proliferative activity, as a result of nuclear damage. Indeed, the use of lasers is consistently associated with cell and tissue necrosis.

Tissue welding or fusion effects induced by laser light have not been quantified in the case of cartilage, but the adhesion elicited is unlikely to be sufficiently strong and extensive to withstand the high mechanical demands of joint tissues[84].

Clinical findings pertaining to laser chondroplasty give a falsely over-optimistic view[69,85], good to excellent (65% to 85%) results having been reported. These data are based on retrospective studies, which are generally useful only in addressing safety issues and in appraising certain side-effects. Conclusive information respecting the putative advantages of laser-based over conventional approaches cannot be furnished unless prospective, double-blinded clinical trials are undertaken. One instance of a single- (not double-) blinded prospective study[83] has revealed mechanical meniscectomy to yield better clinical results than the laser-induced approach. Hence, in this instance at least, the high-cost use of lasers is not justified.

Although clinical experience with lavage, shaving, debridement and laser chondroplasty dates back more than 10 or 15 years, the surgical community has failed to undertake the prospective clinical trials that would provide the necessary information to justify the performance of any one of these methodologies or substantiate claimed positive effects.

ABRASION CHONDROPLASTY

Abrasion chondroplasty, as well as Pridie drilling and the microfracture technique (see following sections), involve surgical access to the bone-marrow spaces, which, together with other vicinal compartments (such as the vascular and perivascular spaces, the bone tissue itself and adipose tissue), are consequently stimulated. These three interventions essentially lead to a spontaneous repair response, which is based upon therapeutically-induced bleeding from the subchondral bone spaces and subsequent blood-clot formation. The provocation of such a spontaneous repair response is well known from animal experiments to yield highly variable and non-reproducible healing results, the tissue formed being fibrous in nature[34] and not durable[27]. These techniques were introduced more than 20 years ago, at which time no alternative or better strategies were available, and in the intervening years a considerable body of clinical experience has been gained with them.

Laboratory experiments relating to abrasion chondroplasty of the knee joint have been conducted in both rabbits[52,86] and dogs[31]. Findings confirm that the spontaneous repair response originates from the bone-marrow spaces and results in the formation of a fibrous type of cartilage tissue. Although this contains most of the matrix components found in hyaline cartilage, significant quantities of fibrous constituents are also present[34]. In rabbits subjected to Pridie drilling, repair tissue endured for a longer period than did that in animals treated by abrasion chondroplasty[86]. It has been proposed that if an appropriate biomechanical treatment schedule, such as continuous passive motion[52], is instigated following abrasion chondroplasty, then the hyaline quality and endurance of the repair cartilage may be somewhat improved[87]. On the basis of these animal experiments, a long-term cure cannot be expected from the performance either of abrasion chondroplasty, Pridie drilling or the microfracture technique.

Various retrospective clinical investigations pertaining to abrasion chondroplasty have been conducted[6,38,88,89]. The success rates are variable[90] and depend on many factors, such as the patient's age and activity level, the severity of the arthritic condition and the follow-up period. Only one relevant clinical trial has been undertaken[91]. In this, patients suffering from OA and painful knee conditions were subjected either to concomitant abrasion chondroplasty and osteotomy or to osteotomy alone. Individuals treated by the combined approach manifested a significantly higher proportion of hyaline-like cartilage repair tissue and had a lower incidence of tissue degeneration 12 months after surgery than did those who received only an osteotomy. However, no difference in the clinical outcome was observed after 2 (and up to 9) years.

PRIDIE DRILLING

The idea of drilling therapeutic holes into the subchondral bone-marrow spaces underlying regions of damaged articular cartilage was conceived by Pridie in 1959[92–94]. And, as mentioned in the previous section (ABRASION

CHONDROPLASTY), the rationale behind this concept is to stimulate a spontaneous repair reaction. But, perforce, the tissue formed is variable in composition, structure and durability. The intervention is now performed chiefly in patients with painful osteoarthritic conditions, such as osteochondritis dissecans[92], in an attempt to bring symptomatic relief and to improve joint functionality[95,96] by promoting resurfacing with fibrocartilaginous repair tissue. One study conducted in Germany[97] has revealed Pridie drilling to represent the surgical procedure most frequently adopted in this country for the treatment of osteochondritis dissecans, it being performed much more often than abrasion arthroplasty (chondroplasty).

In laboratory experiments with rabbits[98], Pridie drilling has been demonstrated to result in the formation of fibrocartilaginous repair tissue. Moreover, post-operative joint inflammation was found to be not an exclusively abrasion-related phenomenon but rather, and generally, a surgically-associated one. Removal of debris from the joint by irrigation prior to wound closure is deemed advisable to ameliorate both acute and chronic post-surgical inflammation[98].

Clinical reports suggest that patients suffering from generalized arthroses derive the greatest benefits from Pridie drilling[99]. Although it appears to be a fairly safe procedure[100], the superiority of Pridie drilling over other surgical interventions remains to be established, the appropriate prospective, clinical, epidemiological studies having not, as yet, been undertaken. This procedure appears, however, to confer only short-term benefits; in the long-run (several years after surgery), these are lost[101]. Indeed, in young patients suffering from severe chondral damage in weight-bearing regions of the knee joint, the long-term spontaneous improvement (i.e., that achieved in the absence of surgery) is not inferior to that elicited by surgical intervention[102].

MICROFRACTURE TECHNIQUE

The microfracture technique represents no more than a modificaton of Pridie drilling and thus relies on the same biological principles. Animal experiments confirm that the same type of repair tissue is formed[103]. The microfracture technique was elaborated by Steadman *et al*.[104], who recommended that the very small micro-holes generated be distributed across the entire articular cartilage lesion site, at a distance of 3–4 mm apart and down to a depth of 4 mm, thus yielding about 3–4 holes per cm^2. Since the holes drilled are considerably smaller (approximately 0.5–1.0 mm in diameter) than those created by Pridie drilling (approximately 2.0–2.5 mm in diamter), perforation of the subchondral bone plate is such as to disturb its biomechanics less drastically. The particular advantages of the microfracture technique are that it can be performed by a minimally invasive arthroscopic approach, which requires no costly instrumentation and induces only minimal iatrogenic tissue damage.

The microfracture technique has been applied chiefly in young athletes and in young patients generally. In such individuals, good results (improved joint functionality and relief from pain in 75% of cases) have been reported[105]. Clinical experience gained with osteoarthritic patients has not been published. Indeed, it is questionable whether this technique would be an appropriate one to apply in such individuals, whose spontaneous tissue repair potential is impoverished and in whom the availability of bone-marrow-derived mesenchymal stem cells is reduced[106–108]. The technique has been applied with some success also in veterinary medicine (in horses)[109]. Once again though, no prospective clinical trials have been undertaken that would confirm or refute the claimed advantage of this over other surgical interventions.

SPONGIALIZATION

Spongialization[110], like Pridie drilling, is based upon the stimulation of a spontaneous tissue healing response. It is applied chiefly in patellar surgery to treat very localized defects. It involves complete removal of the subchondral bone plate at the lesion site, thereby exposing the cancellous bone or spongiosa. Bleeding ensues and repair tissue is laid down within the milieu of the resulting blood clot.

Experimental data pertaining to this technique are not available, from which circumstance one may conclude that it has been tested directly in human patients.

Clinical reports[101,110] document the usual good to excellent results (improved joint functionality and relief from pain in 70–80% of patients) which are invariably yielded when a single group of surgeons favors a particular procedure. Although serious drawbacks associated with this technique have not been reported, it has not gained great popularity. Overall, the few potential benefits to be derived from applying this fairly aggressive and destructive approach are of questionable value[111].

Extensive surgical interventions

OSTEOTOMY

High-tibial osteotomy is one of the palliative surgical procedures most frequently adopted for the treatment of painful OA. But it is also undertaken as a corrective measure for large extraarticular deformities, such as those affecting the valgus or varus, which can occur in association with osteo- or rheumatoid arthritis. Severe joint deformities represent complex disturbances in the alignment of ligaments and the underlying muscular apparatus, which are now more frequently treated by combined osteotomy and total knee-joint arthroplasty[112,113]. Osteotomy is thus undertaken with a view not to inducing articular cartilage repair but to relieving pain and to improving alignment as well as biomechanical load transfer in knee joints. It is known empirically that surgical realignment induces a change in contact pressure and contact areal coverage within a knee joint. When such adjustments are made in cases of painful OA, patients are usually relieved of pain for a considerable time. Hence, in these instances, osteotomy serves to alleviate painful symptoms.

When this treatment principle is considered from a biological point of view, it would be expected to have a deleterious effect on cartilage repair and to exacerbate the degeneration of this tissue as a consequence of osteotomy-induced local mechanical overloading. This is perhaps the reason why osteotomy is often combined with other interventions, such as shaving or abrasion chondroplasty[91,95]. Hence, osteotomy is not usually recommended for painful osteoarthritic conditions with no associated deformities. When conducted alone, it tends to be reserved for severe cases of unicompartmental knee-joint OA or for severe knee joint deformities[114–116].

Laboratory experiments with rabbits[117] and dogs[118,119] confirm that osteotomy-induced changes in joint alignment

instigated for valgus or varus deformities precipitate the onset of a slow but progressive osteoarthritic process, which does not occur in sham-operated controls[118]. Typical osteoarthritic changes are wrought within the articular cartilage layer, such as proteoglycan loss, fibrillation, and the formation first of clefts and then of structural defects. Subchondral bone density is also increased[117]. Malalignment of the femoral patella accompanied these pathological changes and affected also the tibial femoral joint line. When the destruction of articular cartilage tissue reaches the bony compartment, focal bleeding occurs, this being followed by a localized spontaneous healing response. Such activity is a usual accompaniment of the late osteoarthritic process and is not a direct consequence of osteotomy. In some studies[120], these issues may have been confused by the investigators. Clearly, though, a distinction between different types of repair activity cannot be made in human patients, since tissue formation cannot be monitored clearly and progressively with time. Not surprisingly therefore, some authors[121] have encountered only a few islands of repair cartilage in patients who have undergone osteotomy, whereas in those who have received this treatment in combination with abrasion chondroplasty or Pridie drilling, a better coverage with fibrocartilaginous tissue was achieved.

Many different combinations of osteotomy with other surgical interventions have been applied, respecting which there exists a fairly sophisticated indication scheme[114,122–125]. Clinical experience with these different combinations has been generally good to very good, success rates of between 70% and 85% having been reported[95,120,126,127]. However, it has not been possible to draw any conclusions as to which couplings for which indications yield the best results[120], since no differences in the success rates between the various therapeutic combinations have been reported. And, once again, the prospective, double-blinded, comparative clinical trials that would furnish the necessary information have not been undertaken.

DISTRACTION OF JOINTS

Joint distraction has been forwarded as a treatment mode for OA with a view to reducing pain and prolonging the time elapsing before it becomes necessary to perform an arthrodesis[128,129]. This measure has also been postulated to promote an articular cartilage repair response within the treated joint, usually the ankle[129].

There exists no rational biological basis for this therapy, since it has been known for decades that joint immobilization leads ultimately to the degeneration of articular cartilage. Indeed, experiments with rabbits have shown that continuous distraction of synovial joints induces changes within the articular cartilage layer[130] that are similar to those observed in osteoarthritic tissue, including loss of proteoglycans, chondrolysis and a diminution in its thickness[131]. Similar effects have been observed after tibial lengthening in the same animal model[132] and after lumbar spine[133] as well as tibial[134] distraction in dogs. In view of these findings, the clinically beneficial effects on immobilized joints documented by van Valburg *et al*.[128] are somewhat surprising. They are attributed by the authors to intermittent changes in fluid pressure of low physiological magnitude. However, this interpretation is inconsistent with current basic intelligence indicating that intermittent changes in fluid pressure have a stimulatory effect on normal articular cartilage only at defined high-frequency levels and amplitudes[135]. Indeed, the experimental set-up and controls described by van Valburg *et al*.[128,136] appear to suffer from conceptual deficiencies.

It is highly questionable whether such a drastic measure as prolonged joint distraction (with all of its associated risks) is really justified as a therapeutic measure in human patients merely to relieve pain, even if the arthritic condition has progressed to such a degree of severity as to render joint replacement unavoidable.

Theraputic interventions with active biologics

AUTOLOGOUS TISSUE TRANSPLANTATION

Perichondrial/periosteal grafts

The chondrogenic potential and repair-promoting properties of perichondrial tissue were first recognized by Haebler *et al*.[137] in 1925, but their findings were not confirmed until a further 30 years had elapsed[138]. Even so, it was not until the 1970s that perichondrial or periosteal tissue was utilized as an autotransplantation material for repair induction in articular cartilage defects[139–141]. Since then, the principle has been exploited in a variety of protocols instigated for the treatment of articular cartilage lesions[142–150].

The biological rationale behind this transplantation principle lies in the observation that the cambial (i.e., germinative) layer of perichondrial (or periosteal) tissue manifests continuous, life-long, chondrogenic (or osteogenic) activity[137,138,151]. A pool of adult-type stem cells must therefore reside in this germinative layer and be capable of reactivation for tissue neoformation. The assumption made in adopting this principle is that when such a tissue graft is laid, cambial layer uppermost[152], on the floor of a full-thickness articular cartilage defect, its proliferative and tissue-differentiation activities will be resumed and result in the formation of repair cartilage within the lesion void.

Various *in-vitro*[153–156] and *in-vivo* studies (mainly with rabbits)[157–162] have been conducted in defence of this principle. The latter mostly reveal some neoformation of cartilage-like tissue[163,164], although authors who have undertaken quantitative analyses have observed spontaneous proliferative activity within the cambial layer to be poor and tissue filling of the lesion void incomplete, albeit occurring to a similar degree for periosteal and perichondrial grafts alike[165]. Consequently, growth and differentiation-promoting factors, such as TGF-β[153,155,156], have been subsequently incorporated into such treatment strategies.

Biomechanical stimulatory protocols, such as continuous passive motion[148,149,166], have also been introduced in an endeavor to boost proliferative and differentiation activities within the cambial layer. Yet another measure has involved overlaying the cambial layer with polylactic acid, to facilitate the spread of repair cells throughout the defect space and, thus, the extent of cartilage neoformation[167]. However, none of these measures has met with very much success.

Clinical experience with human patients has likewise yielded disappointing results, complete restoration of the hyaline articular cartilage layer and long-term stability of the repair tissue formed not having been achieved[146,168,169]. Furthermore, several practical problems, such as graft detachment, are also experienced. Attempts to improve

attachment by suturing or glueing grafts to the defect floor have been hampered by technical difficulties. Moreover, uncontrolled calcification of grafts has been frequently observed and probably contributes to the high rate of loss.

Despite these problems, symptomatic relief from pain and improvements in joint functionality have been reported to be good to very good (50–80%)[146,159,169,170], as is usually the case with surgically-based treatment protocols. But, regrettably, no prospective, double-blinded, comparative clinical trials have been undertaken to assess whether this type of intervention is more effective than simpler surgical measures which yield reportedly similar success rates.

The highly variable outcomes achieved in animal studies as well as in human patients point to the existence of fundamental conceptual problems with this treatment principle. A careful analysis of the biological methodology employed reveals that the procedure involves the surgical creation of a full-thickness defect prior to the insertion of a perichondrial (or periosteal) flap. This step is, of course, associated with an influx of blood from the bone-marrow spaces, which seeps into the defect void around the margins of the graft. Hence, the procedure, when viewed in its entirety, is really a combined approach, involving spontaneous repair activity within a blood-derived fibrinous milieu and a healing response originating from the cambial layer of the graft. Indeed, it is principally as a consequence of the former response that repair tissue is laid down at all. In confirmation of this postulate, and using x- and y-chromosomes as cell markers, Zarnett and Salter[152] have shown about 67% of the repair cells present within rabbit defects that have received a periosteal flap to arise from sources other than the graft itself, most likely from the bone-marrow spaces.

Another factor contributing to the complexity of the perichondrial/periosteal graft principle is that, physiologically, the cambial layer abuts on a solid tissue mass (cartilage or bone), with which it is in mutual signaling contact. It does not operate like a squamous epithelium, which produces cells towards an empty space. Transplanted perichondrial/periosteal grafts thus lack their physiological signaling contacts, a circumstance that may contribute to their poor spontaneous proliferative activity. New systems need to be developed in which the physiological environment is more closely simulated. This might be achieved by introducing an appropriate matrix scaffold containing suitable growth and differentiation factors into the defect void. By adopting such measures, neoformation of tissue by the cambial layer and its upgrowth into the defect void may be achieved, in analogy to postnatal articular cartilage growth. But if the perichondrial/periosteal graft is placed on the floor of a defect, as is invariably the case, the process of chondrocyte maturation will be such that the largest (i.e., hypertrophic) cells are located near the articular surface where they will mineralize the surrounding matrix. A more logical position for the graft would be against the roof of the defect, with the cambial layer facing downward, in which case, tissue would grow down towards its floor (preferably through a porous matrix that would facilitate cell ingrowth and chondrogenic activities). The structure of the repair tissue thus formed would resemble more closely that of native articular cartilage. Another aspect to be considered is that by placing the graft on the floor of a defect, it acts as a structural barrier and thereby impedes the angiogenic activities[171] necessary for osseous upgrowth into the bony compartment. In consequence, the graft will be but poorly anchored. This problem could be readily overcome by perforating it.

Only when the aspects addressed above have been systematically investigated in suitable animal models can we hope to achieve more reliable and reproducible results in human patients. Experimental data thus far gleaned using not only autogenic but also cryopreserved allogeneic grafts[172] are of a sufficiently promising nature to justify continuing efforts with this treatment principle.

Osteochondral transplantation (mosaicplasty)

The idea of implanting chondral or osteochondral tissue itself within articular cartilage defects dates back to the beginning of the last century[173–175]. And this concept still forms the basis of clinical strategies involving both autografts[176–178] and allografts[178–182].

Considering this procedure's long history, it is surprising how little laboratory experience has been gained with autogenic and allogeneic material; especially with autologous osteochondral grafting (mosaicplasty), respecting which, new surgical instruments have been developed[183,184], both for open-joint and arthroscopic interventions. The few studies performed with experimental animals[185,186] have revealed graft material to persist for the short-term duration of the follow-up periods, but the long-term fate of such tissue has not been investigated. However, one veterinary medical report dealing with horses[187] has revealed that the cartilaginous portion of the graft survives for a short time only (approximately 6 months), whereas the osseous one becomes integrated and persists for long periods within the bony compartment.

Despite this situation, there exists a considerable body of literature pertaining to short-term clinical experience with this procedure in human patients[176,177,179,183,184,188]. As with other surgically-based methodologies, retrospective analyses have revealed results to be good to very good (pain relief and improved joint functionality in 60–90% of cases). But, once again, no prospective, double-blinded clinical trials have been undertaken to assess whether this technique is indeed superior to others.

It is a disturbing finding that this intervention has been applied in human patients before having been thoroughly tested in animal investigations, particularly long-term ones which would reveal whether any associated joint pathologies develop. In a recent study using sheep[189], mosaicplasty was found to be associated with the rapid degeneration not only of transplanted cartilage tissue but also of vicinal native chondrocytes. This latter phenomenon was attributed to the lack of lateral mechanical support, since in mosaicplasty, the transplanted tissue cylinders are held in place only by point-to-point contacts with the surrounding cartilage. In order to prevent tissue degeneration and unphysiological deformation during the post-operative phase, lateral support needs to be continuous[189], and achieved using a material that attains at least 80% of the stiffness of normal articular cartilage. Even theoretically, it is possible to identify many additional potential problems with mosaicplasty. In truth, this procedure is doomed from the onset, since in removing osteochondral plugs from normal sites of a joint the surgeon is destroying almost as much healthy tissue as he is attempting to treat that which is diseased. Neither the donor sites nor tissue opposing them on the other side of the joint surface have been subjected to histological analysis, albeit that on the basis of past experience, both regions would be expected to

degenerate[187]. Chondrocytes aligning the surfaces generated after the drilling out of tissue cylinders are indeed known to undergo significant degeneration[52,54]. Moreover, the translocation of tissue from a low-weight-bearing area to a high-weight-bearing one invariably leads to its degeneration, as a consequence of mechanical overloading in the new position. Mosaicplasty is also associated with unphysiological and injurious tissue compression[190], which is induced when osteochondral plugs are hammered into the holes surgically excavated for their reception and better anchorage. But the fate of the diseased tissue intervening between the graft sites has not been followed. Likewise awaiting to be investigated is how the procedure affects congruency between opposing (treated and untreated) joint surfaces: if poor, this feature could exacerbate the osteoarthritic condition.

In summary, mosaicplasty is associated with much potential collateral damage to joint tissue, in view of which circumstance, the short-term benefits derived by osteoarthritic individuals may not justify its performance. With respect to this procedure, the human patient has been used as an experimental subject, which is the more unwarranted in that no prospective clinical trials have been undertaken to identify unfavorable long-term side-effects.

ALLOGENEIC OSTEOCHONDRAL AND CHONDRAL GRAFTING

Allogeneic osteochondral grafts have been used to fill articular cartilage defects for several decades, and much clinical experience has thus been gathered. The approach does not attempt to induce a cartilage repair response, but represents a means of substituting failed or lost tissue with healthy articular cartilage, usually derived from cadavers.

The biological rationale for this approach is based on the knowledge that osteochondral defects do not heal and that it is extremely difficult to elaborate a suitable repair-inducing stimulatory system to promote this process effectively and in a compartment-specific manner within both cartilaginous and osseous carrels. It has thus been deemed simpler to replace the lost tissue volume with allogeneic material retrieved from cadavers[191–193]. Immunological problems are inevitably numbered amongst the drawbacks associated with this technique[194,195]. Nevertheless, patients with large osteochondral defects, such as those generated by tumor resection, osteonecrosis, extensive trauma, broad focal OA or osteochondritis dissecans, have benefited greatly from this treatment strategy.

A large number of relevant investigations have been performed using rats[195–198]. Although cartilage is generally considered to occupy an immunologically privileged position within the joint, these animal experiments reveal that tissue transplants survive longer under immunosuppressive conditions and also if histocompatibility-matching is optimized to reduce cell-mediated cytotoxicity and antibody titre[195,196,198]. It is also evident from these investigations that cryopreservation of tissue leads to the death of chondrocytes, this loss contributing to its deterioration and reduction in mechanical competence[186–201]. Fresh tissue had better structural and mechanical stability as well as a longer life span than did cryopreserved or lyophilized material[200,201].

Clinical experience with this treatment protocol has been surprisingly good, immunological reactions being apparently less extensive in humans than in experimental animals. Human osteochondral transplants also survive for longer periods (some years), even after freezing or lyophilization[180,181,194,202–204]. Success rates range between 65% and 85%, even after follow-up periods of up to 10 years[192,205–207]. Drawbacks associated with this methodology in the clinical situation include the scarcity of fresh donor material and problems connected with the handling and storage of frozen tissue. The small but ever-present risk of disease transmission must also be borne in mind.

The success of this long-established methodology could be further enhanced in the future by improving tissue cryopreservation and by bringing adverse immunological reactions under better control.

The use of purely chondral (as opposed to osteochodral) grafts, such as fetal homografts[208] and adult costal ones[209], has not attracted much attention, either experimentally or in the clinical situation. The reasons for this are two-fold: first, there exist but few sources from which such tissue can be obtained, and second, it is notoriously difficult to adequately secure purely chondral tissue transplants within a defect void, which foredooms them to a high probability of loss. These circumstances may explain why scientists and clinicians have not pursued this avenue more enthusiastically.

Tissue engineering

Tissue engineering can be defined as the art of reconstituting mammalian tissues, both structurally and functionally. Such reconstruction processes can be conducted either entirely *in vitro* or partially *in vitro* and then completed *in vivo*, *in situ*. Success in this technology would obviate the need for tissue transplantation. And if the appropriate precursor cell pools could be obtained from embryonic, fetal or adult allogeneic sources, then the numerous problems associated with the use of donor tissue would be avoided. Autologous stem or precursor cells could, of course, also be employed, but with less facility. Such engineered material could be used at sites where native tissue has been lost or compromised by trauma or pathological processes.

The term tissue engineering is often used quite loosely in conjunction with any component which, either by itself or in combination with others, mediates the formation of repair tissue. Three key constituents usually form the basis of a tissue engineering approach, namely, a matrix scaffold, cells and signaling molecules (such as growth factors or genes). Most of the experience so far gained with this technique has been derived from *in-vitro* studies, although experiments with laboratory animals and humans have also been conducted. Generally speaking, the approaches adopted have been of an empirical nature, as will become apparent in the following subsections.

Many investigators[12,210–214] have stated that the tissue engineering approach is based upon and aims to simulate embryonic or fetal cell/tissue differentiation processes. However, the microenvironment within mature tissue, as well as the adult precursor cell populations available, preclude such a recapitulation process. Although embryonic, fetal and adult organisms share in common the same signaling substances, the target cells and their reactivity change during the course of the ontogenic process. Hence, very basic modifications must be instituted for successful tissue engineering in adult organisms. The highly complex nature of this undertaking, as well as promising directions to be pursued in future endeavors, are discussed under

Table I
Chemical classes of matrix

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

COMMENTARY AND PERSPECTIVES after the important constituents of any tissue engineering system have been dealt with.

MATRICES

Protein-based matrices

Many different types of matrix have been tested *in vitro*, as well as in experimental animals and in human patients, for their efficacy in facilitating or promoting articular cartilage repair. These matrices can be broadly categorized according to their chemical nature into protein-based polymers, carbohydrate-based ones, artificial materials and combinations of these (Table I). Even when a naked matrix (i.e., one that carries no cells or signaling substances) is deposited within an articular cartilage defect, it must be considered as an information-carrying device and thus as an active biologic, irrespective of its chemical composition, physical properties or nature. Such matrices bear biological information and elicit a biological response within bodily tissue compartments, which cannot be prevented unless appropriate modulations are made to guide these in a direction favorable to tissue repair and integration. The basic requirements of a matrix to be used in a biological environment are summarized in Table II (see also Hunziker[215]).

Ideally, a matrix should be porous so as to permit either the migration of loaded cells through its interstices or the infiltration of native ones from the implant surrounds. Its surface properties should be such as to promote cell adhesion, a feature that can be enhanced by coupling the basic matrix material with specific cell adhesion-promoting peptides (such as Arg-Gly-Asp)[216,217]. The material must also be biocompatible, i.e., induce only minimal adverse immunological reactions within its destined tissue compartment. In some instances, the matrix may be required to facilitate or even promote the proliferation of one cell population whilst inhibiting this activity in another. The material must also be biodegradable and be capable of replacement by physiological extracellular components. In this context, it must be borne in mind that the degradation products yielded by many matrices have cytotoxic, nephrotoxic or other undesirable effects. Certain physical characteristics, such as deformability, elasticity and volume stability must also be satisfied by the matrix. Clearly, it is not realistic to expect that any one material will fulfill the entire formidable list of requirements. However, by combining different types of material and by effecting certain chemical modifications, we can go some way towards achieving the ideal.

The value of a naked matrix lies in its ability to enhance or promote a spontaneous repair response within a defect whose dimensions preclude this from occurring unassisted (critical-sized lesions) in a complete and reproducible manner. When a porous matrix is implanted within the void of a full-thickness defect, it will soak up and become

Table II
Matrix requirements

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

(modified after[215])

entirely permeated by blood that is forming a hematoma. By this means, a spontaneously developing hematoma can expand to fill a much larger volume that it would naturally occupy within an empty space. The process is reminiscent of the manner in which a drop of ink spreads on a sheet of blotting paper.

Cells for repair, as well as signaling substances, will migrate into the matrix together with the blood and thus form the basis for a spontaneous healing response, which is supported and enhanced by the implanted scaffold. Moreover, if appropriate measures are taken to attach this material to the defect floor and walls, its secondary shrinkage as a result of blood clot retraction can be prevented, thereby helping to maximize, reproducibly, the lesion volume ultimately filled by repair tissue.

Matrix types deemed suitable for articular cartilage repair purposes will now be dealt with, particular attention being paid to their biocompatibility. This latter term tends to be used in rather too loose a sense and should really be adopted in reference to a specific tissue compartment. An erythrocyte, for example, is generally considered to be a perfectly biocompatible cell. But if autologous erythrocytes are deposited outside the intravascular space, within, for example, the extravascular connective tissue, they are not biocompatible with this, but induce a severe inflammatory response, which is followed by tissue resorption (see COMMENTARY AND PERSPECTIVES for details). The same holds true for an autologous fibrin matrix. Hence, strictly speaking, a biocompatible molecule, cell or matrix is one that elicits neither cytotoxic effects, inflammatory responses nor foreign body giant cell reactions within its destined tissue compartment.

Collagen matrices. Matrices, or sponges, composed of collagen fibrils have been used experimentally for more than 20 years. Although such meshworks have been shown to enhance the spontaneous healing response in rabbit osteochondral defects[218], they have not, as yet, been employed clinically in human patients (except in cosmetic facial surgery). They have generally been utilized not alone, i.e., purely as scaffolds, but as carriers for allogeneic chondrocytes[219–221] or mesenchymal stem cells[222] in rabbits, or embryonic chondrocytes in chickens[223], or for both cells and growth factors[224]. The size of the defect that can be repaired using a chondrocyte-collagen composite in horses has been shown to be much larger than is the case when no such implant is employed[225].

It must be borne in mind that in all of these studies, defects were osteochondral, i.e., full-thickness, ones. Hence, any repair activity elicited by the interventions will have been invariably combined with a spontaneous healing response, based on the influx of blood-borne cells and signaling substances from the subchondral bone space. Indeed, investigations that have addressed the fate of matrix-borne cells have demonstrated these to constitute but a small proportion of the final repair tissue population, those originating from the blood and bone-marrow spaces by far outnumbering them[226].

Unfortunately, biocompatibility issues are only rarely considered seriously in such studies, adverse reactions usually being summarily dismissed as minimal or absent. A more systematic analysis of these aspects undertaken in our own laboratory has revealed these biocompatibility problems to be serious. However, collagen is, after all, a natural bodily constituent whose fibrils furnish a natural adhesion surface for cells and carry the required biological information for their activity. Furthermore, the degradation products of collagen are physiological ones and therefore non-toxic. Hence, endeavors to improve its biocompatibility, perhaps by effecting minor structural modifications (the possibilities for which are numerous), are worthwhile.

Gelatine. Matrices composed of gelatine, which is basically denatured collagen, have not been used purely as scaffolding structures to enhance spontaneous repair in articular cartilage defects. Indeed, even when employed as carriers, such as for mesenchymal stem cells[227], they have not been widely investigated. However, they have attracted some attention as a substrate for the support of chondrocyte growth, both *in vitro*[227,228] and *in vivo*[227]. Gelatine must be expected to elicit significant inflammatory responses and foreign body giant cell reactions when exposed to vascularized tissue compartments, such as bone and bone-marrow spaces, especially if commercial products, e.g., Gelfoam®, are employed. This latter material is indeed primarily indicated for use as a hemostatic surgical sponge, not as a matrix for carrying cells.

Fibrin. Fibrinogen and its polymerized form, fibrin, are natural components of the intravascular space, i.e., the blood, and represent the major extracellular component of this 'fluid' connective tissue. As a polymer unit, fibrinogen exists physiologically in a liquid state within the blood stream, being activated to polymerize only in instances of vascular lesioning or in other pathological situations. It then forms a three-dimensional solid matrix (fibrin) to prevent blood loss from the intra- into the extravascular space. It has no mechanical function but an important inductive one, in that it facilitates and promotes tissue healing activities within the extravascular space[229,230]. Fibrin is proinflammatory and induces its own degradation and substitution by cellular components of the extravascular tissue spaces. Its degradation products, being physiological, are non-toxic.

Fibrin has been employed extensively not only as a naked scaffolding material but also as a carrier for cells and growth factors, owing to its ready availability on both an autologous and an allogeneic basis. Of course, it also plays a key role in regulating spontaneous repair activities within full-thickness articular cartilage defects, this circumstance being put to good effect in surgical strategies employing no active biologics.

As already discussed, spontaneous healing responses are confined to defects that fall within a critical size range. Exogenous fibrin clots, like collagen matrices (see above), can be used to promote the spreading of endogenous blood over a larger defect volume than would otherwise be occupied by the spontaneously developing hematoma, and thus to reproducibly maximize natural repair activities[231,232]. A further improvement has been achieved by incorporating chondrocytes into the exogenous fibrin clot, both *in vitro*[233] and *in vivo*[234,235]. The healing response can also be enhanced by including growth factors[236], as shown in equine and canine models for the repair of articular cartilage[237], and in the latter also for that of the meniscus[232,238]. However, not unexpectedly (see above), some immunological reactions to exogenous fibrin have been observed in several animal studies[239,240].

Some investigators have used fibrin glues as a matrix to enhance articular cartilage repair[235,241–243]. But owing to the exceedingly high (unphysiological) concentrations and

protein densities involved, they impede[244] rather than facilitate cell invasion and thus do not support a healing response[235].

Despite many years of experimental research with fibrin and some clinical experience in veterinary medicine, this type of matrix has not been widely employed in human patients, except for meniscal repair[245]. Clinicians appear to have been satisfied with the results elicited by the spontaneous healing response, the improvement afforded by exogenous fibrin in animal experiments having been deemed insufficiently dramatic or convincing to warrant this more elaborate approach in humans.

Carbohydrate-based polymers

Polylactic/polyglycolic acids. Polylactic and polyglycolic acids, both individually and in combination, are manufactured from alpha-hydroxypolyesters[246]. These polymers were originally elaborated as surgical suturing materials (Dexon®)[247], but their potential value in cartilage repair processes has now been under investigation for more than two decades[247–251]. Structural modifications to these polymers have yielded fine fibrillar meshworks and foams[252], which have been exploited during the past 10 years for tissue engineering purposes. These polymers have not been utilized in their naked form to enhance spontaneous articular cartilage repair *in situ*, probably owing to their relatively poor cell-adhesion and tissue-integration properties and their potentially poor biocompatibility.

However, many *in-vitro* studies have employed these polymers with a view to engineering cartilage tissue[253–256], perichondrial cells[254,257], chondrocytes[249,251] or mesenchymal stem cells[252] having been used for such purposes. In most of these studies, the influence either of the polymer's properties, such as its geometry, or of various cell parameters, such as numerical density, on the quality of the cartilage tissue engineered were investigated. Although such engineered constructs have also been tested in animal models, chiefly in rabbits[254,257–260], they have not been applied in human patients for articular cartilage repair. There are several possible reasons for this. Firstly, such constructs are known to induce foreign body giant cell reactions[246,261]. And secondly, the polymer substrate undergoes hydrolytic activity, which yields toxic—partially cytotoxic—degradation products[246]. These potentially deleterious effects have, as yet, not been thoroughly investigated and thus not excluded.

Agarose. Agarose (sepharose) is a polysaccharide containing L- and D-galactose residues, which is isolated and purified from certain Asian seaweeds. Its importance as a matrix for the culturing of chondrocytes, wherein they express all of their typical gene activities and secrete their key extracellular components, was first demonstrated 20 years ago by Benja and Shaffer[262]. Since then, it has been used in many basic *in-vitro* studies relating, for example, to chondrocyte autoregulatory signaling mechanisms[263–265] and differentiation from mesenchymal stem cells[266], as well as to extracellular matrix biomechanics[267,268] and calcification[269].

The inertness of agarose has rendered it of value in studying the physiological activities of chondrocytes *in vitro*[270]. But its poor biodegradability in consequence of this property (owing to the absence of the appropriate enzymatic degradation systems in mammalian tissues) makes it an unattractive matrix candidate for cartilage repair studies *in vivo*. Rahfoth *et al*.[271] have demonstrated that when agarose is implanted alone within rabbit knee-joint osteochondral defects, it inhibits spontaneous repair processes, most probably as a result of extensive foreign body giant cell reactions to this material within the bony compartment. This inhibitory response was partially overcome by incorporating allogeneic chondrocytes into the agarose matrix. Given the dearth of experimental animal data relating to the use of agarose in articular cartilage repair and the poor prospects engendered by its poor biodegradability, it is of no surprise that this matrix has not been applied in human patients.

Alginate. Alginate is a gelatinous carbohydrate which is isolated and purified from brown algae. Polymerization depends upon the presence of calcium ions, the stiffness of the resulting meshwork being influenced by its content of guluronic acid[272]. It is a matrix that favors chondrogenesis and the maintenance of chondrocytic phenotypicity in three-dimensional culturing systems. Alginate has proved to be useful also in many basic studies relating particularly to the temporal formation of cartilage matrix, since it can readily depolymerize (in the absence of calcium ions) and as promply repolymerize. It is now available in an injectable form which, when seeded with chondrocytes, is used in the treatment of vesicoureteral reflux[273,274]. Within three-dimensional alginate cultures, dedifferentiated chondrocytes can readily redifferentiate if the appropriate nutrients are supplied[275]. Likewise, bone-marrow-derived mesenchymal stem cells can differentiate into chondrocytes when seeded within this matrix and under the appropriate nutritional and stimulatory conditions, both *in vitro* and *in vivo*[276]. Furthermore, adult human chondrocytes cultured within alginate have been shown to synthesize cartilage matrix components that bear a close resemblance to those produced in the native tissue environment[277,278]. Despite these favorable indications *in vitro*, *in-vivo* applications of alginate matrices have yielded disappointing results. When implanted alone, alginate has been shown to inhibit spontaneous repair responses[279]. Likewise, alginate-based cartilage tissue engineered *in vitro* induces severe foreign body giant cell reactions and immunological responses, as well as itself undergoing degradation, when implanted within full-thickness defects in experimental animals[276,279–281]. Hence, alginate matrices have not been employed in human patients for articular cartilage repair.

Hyaluronan. Hyaluronan is a physiological component of the articular cartilage matrix. It forms macromolecules of tremendous length and molecular weight which are perfectly biocompatible and biodegradable (for review, see Goa *et al*.[282]). In theory, hyaluronan would be an ideal matrix to support articular cartilage repair if it could be implanted in an unmodified form. But in order to achieve the physicochemical properties and structural organization requisite for this purpose, it is usually cross-linked by esterification or other means[282]. And as a result, its biocompatibility is compromised[283].

Matrices composed of hyaluronan have not been applied alone to enhance spontaneous repair responses, but they have been frequently used as carriers for chondrocytes[284,285] or bone-marrow-derived mesenchymal stem cells[286,287]. Hyaluronan matrices loaded with such cells have been shown to elicit the deposition of a cartilage-like

extracellular matrix *in vitro*; and there exists some evidence of this occurring also *in vivo*[285,288–290]. Such constructs have likewise been shown to support cell differentiation processes[291]. However, the hyaluronan degradation products yielded may lead to chondrolysis under certain conditions[292].

In an unmodified form, hyaluronan has proved to be an unsuitable matrix. Only modified (i.e., cross-linked) versions have found a use in articular cartilage repair, and these are poorly biocompatible and chondrolytic. Hence, until further chemical modifications can be elicited to produce a more functionalized derivative[293], this type of matrix is unlikely to be used in human articular cartilage repair.

Chitosan. Chitosan (polyglusam) is a copolymer of glucosamine and N-acetylglucosamine, which may be cross-linked with polyanionic chondroitin-sulfate to form a hydrogel[294,295]. Several *in-vitro* studies yield evidence of its potential value as a matrix to facilitate articular cartilage repair. It efficiently supports not only chondrogenic activities[295–297], but also the *in-vitro* expression of cartilage extracellular matrix proteins by human chondrocytes[294]. It can also serve as a carrier for growth factors[298]. Chitosan has not yet been employed as a matrix in animal or human articular cartilage repair studies, its sole clinical use thus far having been to prevent post-surgical adhesions[299]. But these are early days. And as investigators become more aware of this material, it is likely to be adopted with greater enthusiasm, owing to its excellent biodegradability.

Synthetic polymers

Carbon fibers. Meshworks composed of carbon fibers have been used for more than two decades[300] in orthopedic tissue engineering. Although chemically inert, such fibers can nevertheless carry non-biological, alien information to bodily tissues. Hence, not surprisingly, when implanted within full-thickness articular cartilage defects, carbon-fiber meshworks elicit substantial foreign body giant cell reactions and immunological responses; these adverse activities can extend even to the synovium, where they induce synovitis[300–303].

Carbon-fiber meshworks have been applied in several laboratory animals, such as rabbits[300,303–308] and guinea pigs[309], with a view to enhancing and spatially expanding the extent of the spontaneous repair reaction within full-thickness articular cartilage defects. Reported data reveal the healing response to embrace a larger volume of the lesion void than in untreated cases but with no improvement in the quality of the repair tissue formed, this being mainly of a highly-vascularized fibrous type with poor biomechanical properties.

Despite these unpromising results in experimental animals, carbon-fiber meshworks have nevertheless been applied in human patients, with unsurprisingly mixed success. Some clinicians have obtained good to excellent results (pain relief and improved joint functionality in 70–80% of cases), mainly in young individuals (20–40 years of age)[310,311], whereas others report less encouraging ones, with success rates of no more than 41%[301,302]. Regrettably, no propective, double-blinded clinical trials have been undertaken to clarify this nebulous picture. Improved results with this type of meshwork could possibly be elicited by coating the carbon fibers with hyaluronan and incorporating chondrogenic cells into the resulting matrix[312].

Dacron and teflon. Meshes composed of dacron (polyethylene terephthalates) or teflon (polytetrafluoroethylene), like those consisting of carbon fibers, have been applied in experimental animals with a view to enhancing and spatially expanding the extent of the spontaneous repair reaction within full-thickness articular cartilage defects. When tested in rabbits[313–315], such matrices have indeed elicited a considerable increase in the volume of the lesion void filled with repair tissue, but the composition of this was very variable, ranging from a highly-vascularized fibrous type of connective tissue to fibrocartilage. These materials have also been used to replace meniscal tissue in rabbits, with satisfactory results[314,316,317].

With respect to the use of these materials in human patients, one study reports on the application of polyurethane-coated teflon meshes[318]. Pain relief and improved joint functionality lay within the usual range (i.e., 70–80%), although a higher incidence of joint stiffness was encountered within this patient population.

When applied, for example, as suturing materials, artificial ligaments or membranes for abdominal surgery, dacron and teflon are known to elicit severe foreign body giant cell reactions and inflammatory responses, which would likewise be expected to occur in full-thickness osteochondral defects. This may be one of the main reasons why the testing of such matrices has not been pursued with greater vigour by scientists. Indeed, unless novel and more functionalized derivatives can be produced henceforth, these materials are unlikely to attract much attention in the future.

Various other matrices

A number of other experimental matrices are currently under development, or have been available for a while but cannot be recommended for veterinary or human use until certain serious problems have been solved. Included in the latter category is the hydrogel, polymethylmethacrylate, which was first proposed for use in articular cartilage repair 8 years ago[319,320]. Hydrogels are a class of water-swollen hydrophilic monomers which are cross-linked around a linear reinforcing polymer network. Their mechanical properties can be modulated by changing the nature of the individual monomer units, examples of which include polyurethane, acryloilmorpholine, N,N-dimethyl acrylamide, N-vinyl pyrrolidone and tetrahydrofurfuryl methacrylate. The numerous design possibilities, which relate, for example, to the control of matrix swelling and hydration, stiffness and porosity[321–324], render these hydrogels very attractive. Bovine chondrocytes have been shown to remain viable when cultured on one such hydrogel[325], and some promising results have been obtained when these materials have been implanted within rat articular cartilage defects[326]. However, the matrices thus far elaborated are but poorly biocompatible, and their very limited biodegradation yields potentially toxic products. Hence, further substantial improvements in these hydrogels are still required.

Novel matrices composed of hydroxyapatite, whose porosity and density can be varied, have been introduced and already tested in full-thickness articular cartilage defects, either in a pure form or coated with other materials[314]. Chondrocytes have been shown to bind to the surfaces of such matrices *in vitro*[327,328] and to be able to partially degrade it[329], but animal experiments undertaken with hydroxyapatite[330] have yielded conflicting results[331].

In goats, it was found to be an unsuitable matrix for supporting articular cartilage repair[331]. Indeed, when placed within the hyaline articular cartilage compartment, hydroxyapatite is clearly in an unphysiological (ectopic) situation[330].

Polyurethane has been recommended as a matrix to support meniscal repair and replacement[318,332–335]. But its biocompatibility and biodegradability are so limited as to render it of little value unless combined with other materials[336]. Indeed, its degradation products elicit very serious side-effects within the joint cavity and cause severe irritation of the synovium.

Polymers of butyric acid are currently being investigated as possible matrices for articular cartilage repair. Chemical changes therein permit modifications in, for example, fibril structure, density and porosity, which render them attractive potential candidates. However, butyric acid itself carries much information within a biological environment, such that it is poorly biocompatible. Its biodegradation is also very limited and yields products that elicit undesired side-effects. It also affects resident cell populations[337], inhibiting their growth and kinetic activities[338,339], suppressing protein secretion[340] and inducing apoptosis[341,342].

Polybutyleneterephthalate cross-linked with either polyethylene oxide or polyethylene glycol (Polyactive®) has also been suggested as a potential matrix for use in articular cartilage repair[343]. A number of basic studies pertaining to the biomaterial properties of this material have been conducted[344–347] and yielded promising data respecting its production as either a liquid, fibrillar meshwork, foam or microspheres, with various porosities. *In-vivo* testing in animal defect models has been very limited[347], but preliminary data look encouraging[348].

Matrix combinations and chemical modifications

The possibilities for chemical modification to the basic matrices described above are almost endless. Combinations of more than one matrix type[349], the formation of copolymers, and cross-linkage with various substrates to modify three-dimensional organization and geometry are also feasible. In the present article, only a few of the more promising examples will be dealt with. It must also be borne in mind that most of the materials under development have as yet been tested only *in vitro*, using cultured cells for cytotoxicity tests.

One very popular approach has been to cross-link matrices, for example, collagen-based ones[36,350,351]. The cross-linkage or functionalization of hyaluronan-based matrices has also been suggested to improve their utility in articular cartilage repair[289,293]. Likewise, very promising results have been achieved by forming copolymers between different chemical species, such as collagen and alginate[352] or polyurethane and polylactic acid[336]. The functionalization of a matrix by incorporating either agents to promote cell adhesion[353] or growth factors[354,355]—whereby it serves as a delivery system—is also a very promising approach to improving its performance in articular cartilage repair. Geometrical modifications too, elicited by the production of sponge- or foam-like matrices[252], have been shown to enhance the differentiation potential and metabolic activity of chondroprogenitor cells and mature chondrocytes, respectively.

CELLS IN SUSPENSION

The concept of using transplanted cells for articular cartilage repair is an old one, the underlying rationale being to supply the defect site with a homogeneous population of chondrocytes that would produce an optimal and enduring cartilage matrix.

In the 1980s, chondrocytes, as well as chondroblasts, were first used for transplantation purposes[356–360], but always embedded within a matrix. The idea of transplanting autologous chondrocytes suspended at low densities in culture medium was propounded as a purely empirical approach by Grande *et al*.[226] and initially tested in the clinical situation by Brittberg *et al*.[310]. There exists no experimental evidence to justify this approach, since dedifferentiated chondrocytes have never been shown to undergo redifferentiation, either in two-dimensional culturing systems[262,361] or in aqueous suspension[362], except under high-density micromass conditions[363,364]; only in three-dimensional systems, i.e., within a matrix. The use of chondrocytes in a dedifferentiated (i.e., fibroblast-like) form is necessary for their *in-vitro* expansion over two or three generations, which, in turn, is required to yield the high numbers called for to fill the large defect volumes; albeit that in native articular cartilage, the cellularity is exceedingly low (approximately 9′000 chondrocytes per mm^3 of tissue, i.e., a volume density of 1.5–2%)[365]. The cell suspensions are introduced into shallow, full-thickness defects, and their loss into the joint cavity claimed to be prevented by covering the lesion with a periosteal flap. This is sutured, cambial layer downwards, to the surrounding articular cartilage and may be additionally secured by means of fibrin glue or an alternative adhesive[310,359,360].

The application of this methodology in rabbits[366–368] results in the formation of fibrocartilage-like repair tissue. However, a close scrutiny of the experimental approach reveals a number of problematic aspects. To begin with, the periosteal flap (250 μm in thickness) occupies approximately 85% of the height of the cartilage defect void (300 μm) in rabbits[215]. Hence, the lesion is filled predominantly with this tissue from the very onset of the experiment. The true origin of the repair tissue subsequently laid down is unknown. If the periosteal flap remained in place, then one would encounter a dense, fibrous connective tissue, which is not the case. In only one study has the fate of the transplanted autologous chondrocytes been monitored[226]. In this, they were found to represent maximally 8% of the repair cell population. The origin of the overwhelming majority was not elucidated, but the subchondral bone-tissue space represents the likeliest source, as in the case of spontaneous repair responses. Indeed, the autologous chondrocyte principle has since been challenged and disproved by Breinan *et al*.[369] Using a canine model, these authors demonstrated that the transplanted dedifferentiated chondrocytes did not contribute to the repair response. Although such cells could, in theory, adhere to the floor and walls of a defect, they are unable, even then, to form cartilage tissue[262,361]. And in a recent report[370], sutured periosteal as well as other types of flap were shown to become detached within a few days of surgery unless appropriate measures were taken to immobilize the affected joint. The implication of this finding is that the periosteal flaps used to trap autologous dedifferentiated chondrocytes within the defect void are lost together with these within a matter of days following surgery and that thus neither this tissue nor the transplanted cells contribute to the repair response. This interpretation

accounts for the findings reported by Breinan *et al*.[369], which revealed no differences in the repair responses manifested by treated and untreated defects.

Another aspect that complicates the interpretation of findings relating to the autologous chondrocyte transplantation approach is that, even theoretically, it represents not a single but potentially a triple chondrogenic system, in that repair responses may originate from (1) the transplanted cells (highly unlikely), (2) the cambial layer of the periosteal flap, and (3) the subchondral bone-tissue spaces (spontaneous healing on the basis of blood-clot formation). Which of these sources is the prime contributor is still unclear. But the spontaneous response mediated by blood-clot formation is the likeliest candidate, since the periosteal flap (when not detached) will help to keep the developing hematoma *in situ* and, by itself imbibing blood flowing in from the subchondral bone-tissue spaces, will serve to increase the defect volume permeated by this fluid, thereby maximizing the extent of the repair response. That the periosteal flap contributes actively to the repair response is unlikely (see *Perichondrial/periosteal grafts*), although its position on the roof of the defect would yield a more physiologically structured type of repair cartilage than ensues when it is lodged on the floor of the lesion (see section *Perichondrial/periosteal grafts*). Hence, the autologous chondrocyte transplantation approach merely represents an alternative (though more complicated) version of surgical strategies involving no active biologics that are instigated to enhance spontaneous repair responses.

Despite the unclarity associated with this approach, it has nevertheless been introduced into clinical practice. Brittberg *et al*.[310] have applied the autologous chondrocyte implantation principle in human patients with reportedly good to excellent results (pain relief and improved joint functionality in 60–90% of cases). Similar findings have been reported by other investigators (for review, see Gillogly *et al*.)[368]. A retrospective clinical study performed by Peterson *et al*.[371] has confirmed the success rate to lie between 65% and 90%. But no prospective, double-blinded clinical trials have been undertaken to compare this methodology with alternative, well-established and less-expensive treatment protocols.

CELLS IN A MATRIX-CARRIER SYSTEM

There exist a number of chondroprogenitor cell pools within adult mammalian organisms that have the potential to differentiate into chondrocytes and thus form cartilage tissue, and that can be used for transplantation purposes. These include those within the cambial layers of the perichondrium and periosteum, adult cartilage itself (chondrocytes), the bone-marrow stroma (mesenchymal stem cells) and the synovial membrane. Fetal and embryonic precursor cells, such as fetal stem cells and chondroblasts, can also be employed. During the past few decades, many empirical approaches have been introduced to test the usefulness of these various cell pools in inducing a repair response within articular cartilage defects, and these will now be appraised. Such cells have always been transplanted within a matrix-carrier system and invariably tested in full-thickness articular cartilage defects. Hence, this type of approach has always been superimposed upon a spontaneous healing response originating from the subchondral bone-tissue spaces. It must likewise be borne in mind that blood seeping into the defect void carries not only a variety of cell types but also a whole spectrum of ill-defined signaling molecules, which most probably play an important role in triggering the chondrogenic switch. These cell–matrix transplantation strategies must therefore be considered as potentially dual chondrogenic systems, the transplanted one involving no intrinsic signaling regime wherewith to better control the chondrogenic outcome, as will be discussed under CELLS, MATRICES AND SIGNALING MOLECULES.

The transplantation approaches described below are still at experimental phases of investigation and have not yet been applied in clinical practice. The appraisal will thus be confined to descriptions of the biological principle as well as to basic scientific criticisms and suggestions for possible improvements.

Chondrocytes and chondroblasts

Embedded within a collagenous matrix, chondrocytes of both autogenic[226,372] and allogeneic origin[219,220,357,362,373–376] have been tested. Other matrices used as carriers for allogeneic chondrocytes include fibrin[234,377], carbon fibers[378] and agarose[271]. In some studies, fetal or embryonic chondrocytes or chondroblasts have been employed[223,356]. In each instance, authors reported an improvement in the healing response elicited by the treatment: the repair tissue generated was mainly fibrocartilaginous in nature and filled a greater volume of the defect void than did that laid down within untreated control lesions. The transplanted chondrocytes or chondroblasts were concluded to have a beneficial effect on the spontaneous repair response, over and above that elicited by the matrix itself. However, the long-term stability of the tissue formed has been questioned[219], which is not surprising given the incompleteness of the cartilage tissue differentiation process[373]. Moreover, immunoreactivity towards the transplanted constructs has also been described[240]. As already mentioned, such cell–matrix constructs have not been applied clinically in human patients.

Perichondrial/periosteal cells

The chondrogenic potential of certain perichondrium-derived and periosteal cells has been known for several decades[140,141,379–381]. They reside within the cambial layer (i.e., within the proliferative stratum) of the perichondrium and periosteum, respectively, and have been isolated from both autogenic and allogeneic sources for tissue engineering purposes. Following appropriate *in-vitro* testing for their chondrogenic potential[382–384], perichondrial and periosteal cells have been embedded within various matrices, including those composed of polylactic acid[257,385]. Perichondrial cells of autogenic origin have been found to perform better than those derived from allogeneic sources[258,259].

Following implantation in experimental animal defect models, such cell–matrix constructs have been shown to appreciably enhance the articular cartilage healing response. However, these perichondrial and periosteal cells do not appear to survive for long periods within the repair tissue formed, their numerical density undergoing a continual decline with time[386]. Local recruitment of cells from the bone-marrow and osseous-tissue spaces partially offset these losses, but without benefitting the long-term stability of the repair tissue.

Although the use of such precursor cell pools in adult humans would be of great advantage, owing to their high proliferative potential and capacity to differentiate into chondrocytes, irrespective of the age of the donor[383], this type of cell–matrix construct has not been applied in the clinical situation. The highly variable results achieved in experimental animals, as well as the long-term instability of the repair tissue formed and immunoincompatibility problems with allogeneic cells[386], may have contributed to this circumstance (although such difficulties have not deterred investigators in other instances). However, the clumsiness of the approach (involving two surgical interventions and complicated cell isolation procedures), as well as the absence of a simplified, commercially supported strategy, are more probably accountable.

Stromal cells of the bone marrow

That the bone-marrow stroma contains multipotential precursor cells has been known for some time[387–390], but a keen interest in them did not become manifest until the early 1990s[106,391], since when their multipotent lineage capabilities have been identified and better characterized[108,392,393]. The great value of these cells for tissue engineering purposes lies in their pluripotency; their weaknesses are that their numbers decline, and their potential to proliferate and differentiate deteriorate, as a function of age in humans[106,391,394,395].

Mesenchymal stem cells of bone-marrow stromal origin have been applied with a view to inducing articular cartilage repair in various animal models[222,396,397] and in conjunction with different matrices, such as collagen-type-I gels[398] or modified hyaluronan[288]. Although numerous *in-vitro* studies have been undertaken to follow the course of mesenchymal stem cell differentiation and to assess the influence of growth factors on the direction pursued[364,399–403], *in-vivo* experiments have been conducted in the absence of exogenous signaling substances. It has been left up to Nature to supply these, although Lennon *et al*.[397] have deemed it advantageous to incorporate fibroblasts into their system in order to enhance tissue differentiation.

Overall, the results achieved using bone-marrow stromal cells have been similar to those yielded using other cell–matrix systems, the repair tissue formed having been principally fibrocartilaginous in nature, but of variable quality and durability. One advantage gained by using such cells is that, owing to their stem-cell-like properties, they are fairly well tolerated immunologically. Hence, allogeneic cells could be made readily available on a commercial basis, which would obviate the need for bone-marrow tissue biopsies of the iliac crest, whence autologous cells would be derived in individual patients. The undertaking would not only eliminate the risk of inducing potentially long-lasting secondary pathologies at this site, but also solve sterility problems and logistic issues relating to transport.

In one instance, this type of cell–matrix construct has been applied in humans, but using bone-marrow stromal cells of autologous origin[404].

Synovial cells

Studies relating to the ontogenetic development of synovial joints have revealed articular cartilage chondrocytes and synovial cells to originate from a common precursor pool (for review, see Pacifici *et al*.)[405] and to exist in a close functional relationship not only during fetal development but also in adult life[406]. Under various pathological conditions pertaining *in-vivo*, synovial cells have been shown to possess tremendous chondrogenic potential[407–410], even within chondromatous tumors of the synovial space and chondroid osteophytes[411–415]. Furthermore, human chondroprogenitor cells of synovial origin[416,417] have been demonstrated to sustain their high proliferative potential and capacity to differentiate into chondrocytes irrespective of the individual's age.

Chondroprogenitor cells of synovial origin have, as yet, not been isolated for use in articular cartilage repair studies. However, in a series of *in-vivo* experiments conducted with adult rabbits, miniature pigs and goats[418,419], synovial cells have been indirectly shown to be recruited for the repair of small partial-thickness defects, provided that an appropriate matrix containing the necessary growth factors (of the TGF-β superfamily)[418] to induce their proliferation, migration and differentiation, is furnished.

With respect to larger partial-thickness defects, the methodology would need to be adapted to accommodate the circumstance that such lesions take longer to heal (Hunziker *et al*.: unpublished data). In the case of full-thickness defects, measures would be required to prevent osseous upgrowth into the cartilaginous compartment[171,215,420,421].

When the body of data obtained using various cell types in conjunction with different matrices is viewed overall, it becomes abundantly clear that in no instance have the findings been conclusive. In most cases, this circumstance reflects poor study design with the omission of essential controls. Empty, untreated defects do not suffice in this respect. In only a few instances has the matrix itself been tested alone, albeit that this is an absolute requirement. And particularly so since, as revealed in this review, available matrices generally have suboptimal biocompatibility and biodegradability properties, and must therefore be expected to elicit adverse reactions which need to be overcome during the course of healing. The transplanted cells may help in such a situation, but this needs to be proved experimentally. The specificity of the cellular effect must likewise be confirmed, using an alternative cell type as a negative control. But in none of the reported studies have inert control cells been tested. Furthermore, instead of resorting to the usual subjective, semi-quantitative histological analyses, investigators should adopt rigorous morphometric approaches[422–424] involving unbiased, systematic random-sampling protocols[425].

CELLS, MATRICES AND SIGNALING MOLECULES

The current trend in tissue engineering is clearly towards the elaboration of constructs that consist not only of a matrix and cells but also of signaling molecules to specifically guide the course of differentiation in the desired direction. One of the reasons for this is the emerging realization that durable, high-quality, hyaline-like articular cartilage repair tissue can be formed on a reproducible basis only if the healing response is streamlined: firstly by implanting a more homogeneous population of cells; and secondly, by inhibiting the 'contaminating' spontaneous repair reaction, which involves the uncontrolled activities of numerous cell types and signaling substances originating from the subchondral bone-tissue spaces.

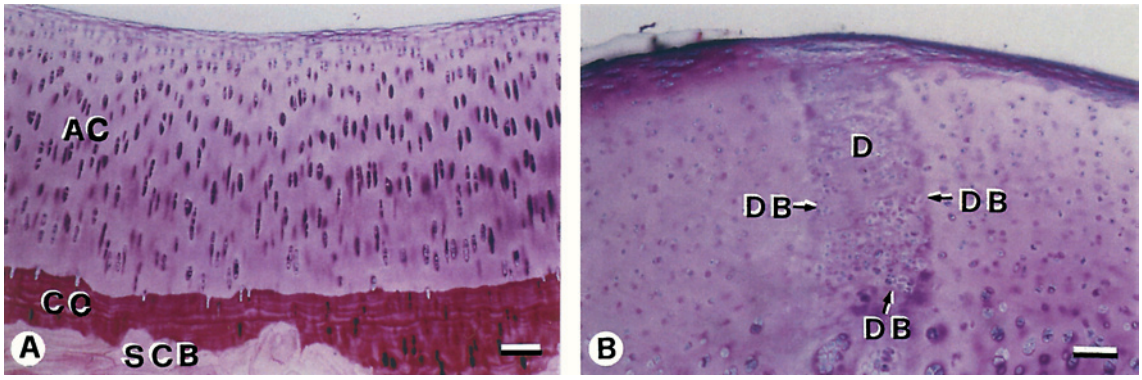

Fig. 1. Light micrographs of knee-joint articular cartilage in mature Goettingen miniature pigs. (a) Normal articular cartilage tissue (AC) together with the underlying layers of calcified cartilage (CC) and subchondral bone (SCB). (b) Partial-thickness defect (D), 2 months after treatment with chondroitinase AC and application of a fibrin matrix containing both free (4 ng/ml) and liposome-encapsulated (600 ng/ml) TGF-β1. It is filled with repair tissue which has a cartilage-like appearance. Cells have undergone transformation into chondrocytes, but are more isotropically distributed and present at a higher numerical density than those within the surrounding native tissue. A gradient in cell size and shape is nonetheless apparent. Chondrocytes in the superficial region are small and spindle-like, whereas those in deeper zones are larger and have an oblate spheroid form. The cells have laid down a matrix which stains somewhat less homogeneously than that of native tissue, indicating that it is not truly hyaline, but rather fibrous, in nature. At the defect borders (DB), repair and native tissue are well integrated. A,B: 120-μm-thick polished saw-cuts, surface-stained with McNeil's tetrachrome/Toluidine Blue O/basic Fuchsine. Bars=100 μm (modified after[438]).

One of two possible tissue engineering approaches can be adopted. According to the first, repair tissue is engineered completely *in-vitro*, the fully-differentiated construct being then implanted within the defect void. Its advantage is that cell metabolism and differentiation are subject to better control *in-vitro* than *in-vivo*, provided that the appropriate bioreactor systems, growth factors and delivery systems are available. Its drawbacks are that problems associated with repair tissue integration and mechanical fixation cannot be readily anticipated and solved. Moreover, the appropriate mechanical loading conditions are difficult to simulate *in-vitro*; hence, the composition of the extracellular matrix may not be ideally suited to the specific needs of the prospective repair site. Furthermore, biocompatibility and immunological problems are frequently associated with this approach. And given the natural curvatures of the joint surfaces, the press-fit implantation of such engineered implants poses another great challenge which remains to be solved.

The second approach, which is more frequently adopted, aims to engineer only the basic building block, namely, a matrix scaffold containing a homogeneous population of cells and signaling molecules entrapped within an appropriate delivery system, to ensure that the desired differentiation process takes place *in-vivo* and to assure the long-term maintenance of chondrocytic activity within the repair tissue formed. According to this approach, the differentiation and remodeling of repair tissue occurs *in-vivo* under physiological conditions of mechanical loading. Repair tissue formed *in situ* is more likely to adhere to, and integrate with, native articular cartilage than is that produced *in-vitro*, and it will also adapt naturally to the contours of the synovial joint. One of the disadvantages of this approach is that cell activity is more difficult to control on a long-term basis. Moreover, appropriate measures must be taken to prevent 'contamination' from cells and signaling substances involved in the spontaneous healing response, since the tissue thereby formed would compromise the quality and mechanical competence of the final repair composite.

Thus far, engineered constructs produced according to either of these approaches have been tested only *in-vitro* and using experimental animals. Illustrative examples of studies involving each approach will now be furnished, preference being given to those that embrace the current trend in articular cartilage repair to use a matrix scaffold, a homogeneous population of cells and appropriate signaling molecules.

Before embarking on a particular tissue engineering approach, both the investigator and the clinician must decide which type of defect they ultimately wish to treat, since the biology of the surrounding microenvironment is of paramount importance in determining the long-term success or failure of the implanted material. It must therefore be clear from the outset whether constructs are destined for partial- or full-thickness defects. In the former case, cell 'contamination' from the blood vasculature as well as from the bone-marrow and osseous-tissue spaces can be excluded, whereas in the latter, such influences are ever-present and must be brought under control.

The engineering of fully-mature cartilage constructs *in-vitro* has been described by a number of authors[426–429]. But, owing to the aforementioned problems of immunological rejection, mechanical fixation, tissue integration and inadequate physical properties, very little success has been achieved in experimental animals[427,430]. And whilst scientists are still struggling with these basic difficulties[429], very little progress is likely in the near future. Hence, the clinical application of such constructs in human patients seems to be a long way off.

The usefulness of different signaling substances, i.e., growth factors, in inducing chondrogenesis has been evaluated mainly *in-vitro* and to only a limited degree *in vivo*. Recent reviews covering this field have been written by Reddi *et al.*[29,431,432], Glowacki[433] and van den Berg[434]. *In-vitro* experiments have involved various combinations of matrix, cell type and growth factor, such as collagen matrices containing bone-marrow stromal cells and TGF-β1[435,436], agarose matrices containing chondrocytes and FGF-2[437], fibrin matrices containing chondrocytes and IGF-1[236], and polylactate matrices containing perichondrial cells and TGF-β1[233,384]. Successful testing *in-vivo* has been achieved using horse[237] and mature miniature pig models (Fig. 1)[438]. These latter studies serve

to show how different the *in-vivo* situation may be from the *in-vitro* one. Under *in-vitro* conditions, various types of growth factor have been shown to support chondrogenic activities[434], whereas *in vivo*, principally only those of the TGF-β superfamily are effective.

Gene therapy and gene transfer technologies also offer potent new possibilities for managing articular cartilage repair responses, the subject having been recently reviewed by Evans[13,439,440] as well as by other authors[441,442]. *In vitro*, genes carrying chondrogenic information have been successfully transfected into chondrogenic precursor (bone-marrow stromal) cells[443], chondrocytes[444,445], perichondrial cells[444] and periosteal ones[446]. *In vivo*, a successful attempt to induce a repair response using transfected mesenchymal cells or chondrocytes has been reported by Gelse *et al*.[447] This latter study demonstrated that the transfected cells could furnish sufficient quantities of BMP-2 to induce cartilage tissue formation whilst avoiding inadvertent vector spreading or immunological reactions.

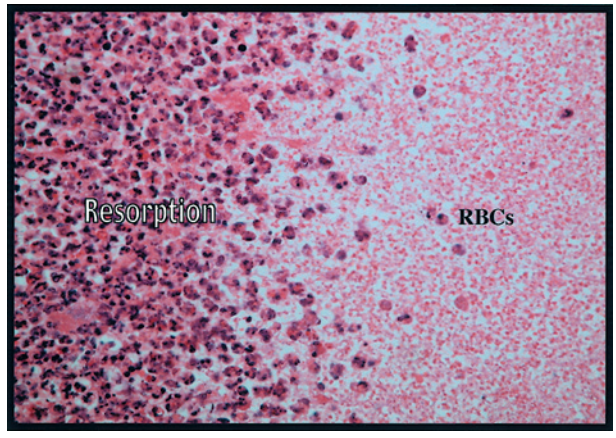

Fig. 2. Light micrograph of autologous red blood cells (RBCs) 10 days after deposition within the subcutaneous tissue of an adult rat. A very active resorption front is apparent on the left-hand side of the picture, neutrophils and macrophages being present in abundance. This finding indicates that the autologous erythrocytes have elicited a very considerable inflammatory response. 5-μm-thick, paraffin-embedded section stained with hemotoxylin-eosin (Hunziker, unpublished data).

COMMENTARY AND PERSPECTIVES

An overview of the tissue engineering literature relating to articular cartilage repair reveals the approaches thus far pursued to have been principally of an empirical nature and still very much at an experimental stage of development. Results achieved to date are far from satisfactory with respect to repair tissue quality and durability. Success in the future depends upon our now following a more rational approach to the problem, based on biological principles at the molecular, cell and physiological levels. Tissue engineering cannot be viewed simply as a recapitulation of embryological differentiation processes, as is frequently suggested[11,29,432], since the cellular microenvironment and tissue contextualities encountered in adult organisms are fundamentally different from those pertaining in embryological ones. Apart from the signaling substances, which are most probably the same, every other component is different, including the existence of an active immune system in fully-developed organisms.

Our aspiration to engineer an ideal construct *in-vitro*, consisting of a matrix, cells and signaling substances, which would undergo terminal differentiation *in situ* to yield a perfectly well incorporated and durable type of hyaline-like articular cartilage repair tissue may be overly optimistic, especially when we consider the mammalian body's own limited tissue engineering capacity, as exemplified in scar formation. If a lesion is created within the subcutaneous tissue of a rat and then filled with 'perfectly biocompatible' cells, such as autologous erythrocytes (Fig. 2), or with a 'perfectly biocompatible' matrix, such as autologous fibrin, these elements are neither directly incorporated nor directly transformed into scar tissue. Rather, they are initially met by a sterile inflammatory response which is followed by their resorption and replacement with vascularized scar tissue (Hunziker *et al*.: unpublished data). The implication of these findings is that biocompatibility is not a generalized bodily phenomenon but a tissue (compartment)-specific one. With this knowledge comes the realization that tissue engineering must be broached with a fresh outlook. In the future, it will be necessary to aim at manufacturing a construct that will be accepted by, and become integrated with, tissue neighboring its destined site without triggering the immunological responses that would result in its resorption. Following resorption, it would inevitably be replaced by a scar-like or fiber-rich tissue bearing little resemblance to the hyaline-like articular cartilage type to which we aspire. Viewed in another light, if the engineered constructs are to be merely resorbed and exchanged following their implantation, then the great pains to which investigators are put to consummate a combination of constituents and conditions conducive to cartilage tissue formation *in-vivo* will be in vain.

Various repair-associated issues

CHONDROCYTE LOSS

Surgical undertakings such as debridement, shaving, laser abrasion and tissue-edge verticalization (performed to improve the fitting of an implant), embrace, in most instances, 'healthy' articular cartilage surrounding the lesion site. The removal of such tissue is associated with cell apoptosis and/or necrosis along the cut surfaces[27,53,54,56]. Given that the cellularity of articular cartilage tissue is in any case very low and that the surviving vicinal chondrocyte population does not compensate metabolically for the loss of its neighbors[55], these effects—which are iatrogenic—can be expected to create a biologically unfavorable environment for an implanted construct.

LOSS OF FLAPS

In a number of investigations involving animal models, as well as in clinical orthopedic practice, periosteal flaps are sutured to the hyaline articular cartilage borders of defects containing cell suspensions whose loss they are deemed to prevent[226,310,366,369]. In a recent study, however, such flaps have been shown to detach following surgery unless measures are taken to immobilize the affected joint, the delamination rate being almost 100%[370]. It is thus essential to monitor the fate of these flaps during the post-operative healing phase using high-resolution, *in-vivo* imaging technologies.

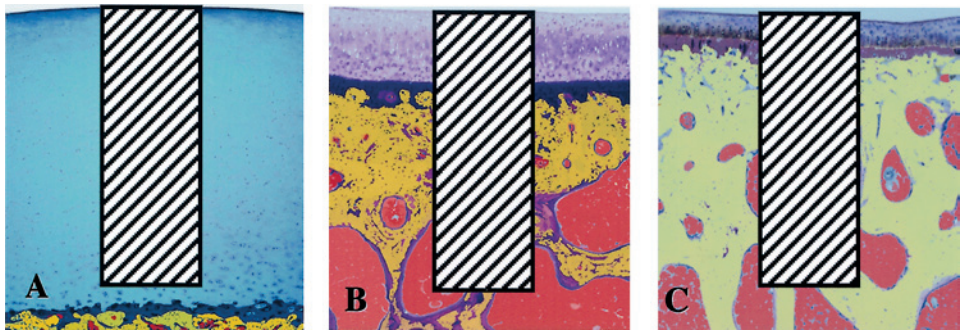

Fig. 3. Schematic representations of like-sized defects superimposed upon light micrographs of (a) human, (b) goat and (c) rabbit articular cartilage tissue (together with varying portions of the underlying subchondral bone), represented at the same magnification. Because the height of the articular cartilage layer in humans is several times greater than that in goats, and many times more so than that in rabbits, the biological environment surrounding each defect differs. In the human, the defect is a partial-thickness one, and, as such, it is surrounded exclusively by cartilage tissue. In the goat and rabbit, lesions of the same dimensions are full-thickness ones; approximately 85% and 95% of their volumes, respectively, being surrounded by osseous tissue and bone-marrow spaces. In these two latter cases, bleeding from the bone-marrow spaces will furnish the defects with an abundant supply of cells and signaling substances to which the human partial-thickness one will not be accessible (modified after[215]).

TISSUE INTEGRATION

The poor integration of repair tissue with native articular cartilage is a problem that has been recognized by several authors[36,189,438], and is one that must be resolved if we are to achieve enduring healing results and biomechanical competence. Various measures have been employed to promote this process by establishing good contact between implants and native articular cartilage, including the use of collagen cross-linkers[36], biological glues (e.g., tissue transglutaminase[84]) and other adhesives[359,448]. Another possible approach that has been shown to have a beneficial effect is the brief enzymatic degradation of proteoglycans along defect surfaces[41], these molecules being known to impede both cell and matrix adhesion[360,449–451].

MECHANICAL FACTORS

The role of mechanical factors in determining repair tissue quality has been recognized for some time[35,452,453], the outcome having been shown in a number of animal experiments to be improved by intermittent active or continuous active/passive motion[148,149,454–456]. Not surprisingly therefore, physical parameters have been identified as being of the utmost importance in tissue engineering[268,457], albeit that the mechanisms whereby such factors are transmitted to cells remain largely unknown, although under current investigation[458]. Hence, in human clinical practice, post-operative care and physical therapy will most probably play not inconsiderable roles in determining the healing outcome.

THE SCALE OF TISSUE REPAIR

Investigators pay too little heed to the dimensional aspects of articular cartilage repair. What volume needs to be filled with repair tissue? Can the biologics of the engineered construct cope with this? Such simple questions—though of utmost importance—are rarely addressed in reported tissue engineering studies. Even just a casual glance at the respective histologies of knee-joint articular cartilage in humans and experimental animals commonly used for the testing of repair concepts (Fig. 3) reveals very obvious differences, not only in structural organization and cellularity but also in height and volume[215]. In the human tibial plateau or medial femoral condyle, a shallow full-thickness defect would span a height of approximately 2–4 mm, frequently cover an area of about 3–4 cm^2 and thus embrace a tissue volume of 0.9–1.0 cm^3 (i.e., ml), which represents a vast void to be filled with engineered tissue. Indeed, routine tissue culturing methodologies cannot cope with this scale of production, which requires the use of special technologies, such as bioreactor systems[256,459–461]. Albeit so, it is still absolutely essential that investigators give due consideration to defect design in their chosen animal model, so as to be able to simulate as closely as possible the human situation aimed at (see next section).

DEFECT DESIGN IN ANIMAL MODELS

A survey of the literature pertaining to tissue engineering aspects of articular cartilage repair reveals a depressing consistency in the degree to which investigators disregard defect dimensions relative to the make-up of the tissue surrounds and fail to consider their pertinence to the human condition they ultimately wish to treat[215]. The first, most fundamental, question to be asked is: do I wish to treat a partial-thickness defect, which has no access to the blood vascular spaces, or a full-thickness one, in which case the influence of blood-borne cell populations and signaling substances must be brought under control?

The creation of partial-thickness defects in most commonly used experimental animals poses great technical difficulties owing to the small scale of the dimensions involved, and these are not easily surmounted on a reproducible basis. It is probably for this reason that most investigators have chosen to work with full-thickness defect models. But by so doing, they have, on the one hand, won a head's start for their treatment principle, in that any repair response elicited will be promoted or boosted by the spontaneously-generated one. On the other hand, they have thereby rendered an interpretation of their findings more difficult, particularly if they have failed to set up systematically the control experiments required for the drawing of unequivocal conclusions. The volumes of such full-thickness defects are, of course, more akin to those of partial-thickness ones in human patients, but then

appropriate measures must be taken to render the microenvironment alike too. One means of achieving this is to create a so-called 'virtual' partial-thickness defect, which would involve treating the floor and walls of a full-thickness one in such a manner as to render it impermeable to blood-borne cells and signaling substances emanating from the subchondral bone-tissue spaces[421].

Not only defect design but also study design needs to be better considered in the future, so as to yield more clear-cut evidence on which to base clinical decisions regarding the application of a new methodology in human patients.

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