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Articular cartilage repair: are the intrinsic biological constraints undermining this process insuperable?

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Summary

This article reviews the experimental and clinical strategies currently in use or under development for the treatment of articular cartilage lesions. The vast majority of protocols under investigation pertain to the treatment of full-thickness defects (i.e., those which penetrate the subchondral bone and trabecular–bone spaces) rather than partial-thickness ones (i.e., those which are confined to the substance of articular cartilage tissue itself). This bias probably reflects the circumstance that partial-thickness defects do not heal spontaneously whereas full-thickness ones below a critical size do, albeit transiently. And it is, of course, a seemingly easier task to manipulate a process which is readily set in train than it is to overcome an induction-problem which Nature herself has not solved. Indeed, the reasons for this inert state of partial-thickness defects have only recently been elucidated, and these are briefly discussed. However, the main body of this review deals with the various transplantation concepts implemented for the repair of full-thickness defects. These fall into two broad categories: tissue-based (entailing the grafting of perichondrial, periosteal, cartilage or bone-cartilage material) and cell-based (utilizing chondroblasts, chondrocytes, periochondrial cells or mesenchymal stem cells). Cell-based systems are further subdivided according to whether cells are transplanted within a matrix (biodegradable, non-biodegradable or synthetic) or free in suspension. Thus far, the application of cell suspensions has always been combined with the grafting of a periosteal flap. The strengths and weaknesses of each concept are discussed.

Key words: Articular, Cartilage repair, Full-thickness, Partial-thickness, Defects.

Introduction

Trauma and disease of the synovial joint frequently involve structural damage to the articular cartilage layer. In most other tissues, such defects would be rapidly repaired without untoward consequences. However, adult articular cartilage has only a very limited capacity to heal. Indeed, an initially discrete lesion will not only fail to heal, but will almost certainly enlarge with time [1–4]. Unremitting exacerbation of this condition will ultimately lead to a debilitating state, as evidenced by the prevalence of diseases such as osteoarthritis [5]. This review will delineate the intrinsic biologic limitations undermining spontaneous repair of articular cartilage and evaluate the various therapeutic approaches thus far developed to overcome these inherent constraints.

Classification of articular cartilage defects

Articular cartilage defects are classified as being either partial- or full-thickness. The former lie entirely within the confines of cartilage tissue itself and do not penetrate beyond the calcified cartilage into subchondral bone; they thus cannot be accessed by blood-bone cells, nor by macrophages or mesenchymal stem cells located within the bone-marrow space. Hence, not unexpectedly, when a partial-thickness defect is artificially created in articular cartilage, no fibrin clot forms within its void, and there is, furthermore, little or no evidence of cell migration therein. The lesion does not heal [1–4], and its appearance several months after injury is similar to that observed at the outset; it appears to be inert [1, 3, 4, 6, 7], unlike defects of immature articular cartilage [8, 9]. Partial-thickness lesions are analogous to the clefts and fissures seen during the early stages of human osteoarthritis.

Full-thickness defects span the entire depth of articular cartilage and additionally penetrate the subchondral bone marrow; they are therefore readily accessible to blood cells, macrophages and mesenchymal cells which reside within this space [Fig. 1(A)]. When a full-thickness defect is artificially created, blood from the marrow wells up into the lesion, and a space-filling fibrin clot

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containing inflammatory cells is formed; mesenchymal cells subsequently appear, and these differentiate into chondrocytes [3, 10–12] [Fig. 1(A)–(D)]. Although full-thickness defects become filled with repair tissue that bears a superficial resemblance to hyaline cartilage [Fig. 1(B), (C)], this does not persist. Indeed, it usually undergoes degeneration within six to twelve months [10, 12] [Fig. 1(D)]. Repair of full-thickness defects is thus only transient.

Repair of full-thickness defects

SPONTANEOUS REPAIR

Articular cartilage defects which penetrate subchondral bone tissue are continuous with the marrow space, and they are therefore accessible to a number of natural stem cells located therein. Spontaneous repair consistently follows the same course, which has been characterized in detail by Shapiro *et al.* [12]. The sequence of events may be briefly summarized as follows: the void of a full-thickness defect becomes filled with a fibrin clot immediately after its creation. Two days later, this is observed to adhere tenaciously to the wound edges in the bony compartment, but not to those in the cartilaginous one. Parallel strands of fibrin are described to extend horizontally across the entire width of the defect. Mesenchymal cells begin to penetrate the fibrin clot peripherally, and, by the fifth day, completely fill it. The horizontally aligned fibrinous strands appear to furnish a guide for the ingrowth of the mesenchymal cells, since these adopt a corresponding orientation. By one week, the fibrin clot has become almost completely resorbed, and the defect is filled with mesenchymal cells [Fig. 1(A)]. Between ten and fourteen days, the mesenchymal cells differentiate into chondrocytes, which lay down a proteoglycan-rich extracellular matrix. By eight weeks, the repair tissue usually resembles cartilage [Fig. 1(B)]. By twenty-four weeks, the surface of the healed tissue is continuous with that of the surrounding native cartilage [Fig. 1(C)]. By forty-eight weeks, however, signs of degeneration are apparent [Fig. 1(D)], and this process progresses unremittingly with the advance of time. Within the bony compartment, osteogenesis proceeds in parallel with chondrogenic activity in the cartilaginous one. Subchondral bone is initially deposited along the surfaces of the defect, but progressive accretion leads to gradual filling of its void. By 48 weeks, this process is complete.

Histological inspection of the junction between repair tissue and the surrounding articular cartilage reveals frequent points of discontinuity between the two compartments. And even at sites of contiguity, there exists no true integration between repair and native articular cartilage tissue, since collagen fibrils within each compartment fail to intermingle with one another (as revealed by polarized light microscopy). This bonding problem is not confined to spontaneously-healed, full-thickness defects; it represents a major stumbling block encountered in all repair systems. The extracellular matrix of articular cartilage is rich in proteoglycans, some of which are known to have anti-adhesive properties [13–15], and herein may lie the difficulty of bonding between repair and native tissue.

Shapiro *et al.* [12] and others [1], have noted, that native articular cartilage adjacent to the defect site becomes necrotic after surgery, and with the exception of occasional chondrocyte-cluster formations, no resorption or remodelling of this tissue occurs; it remains essentially inert.

Despite the poor outcome [10, 12, 16, 17], this spontaneous process still forms the 'rationale' behind a number of orthopaedic interventions currently in use [5, 18–21].

Transplantation concepts

Endeavours to promote healing of articular cartilage lesions usually run along the lines of transplanting biological materials, the suitability of which have generally been evaluated on the basis of empirical criteria. The most commonly employed materials include osteochondral tissue, cells of osteochondral origin and tissue or cells with chondrogenic potential [19, 22, 23].

PERICHONDRIAL/PERIOSTEAL GRAFTS

The repair-promoting properties of perichondrial tissue were first recognized in 1925 [24], but thirty years passed before this observation was confirmed [25]. It was not until the 1970s that either this or periosteal tissue was utilized as an autotransplantation material for the induction of healing in cartilage defects [26–29]. Since then, this principle has been exploited in a variety of protocols for treating articular cartilage lesions [30–39]. Several investigators have reported an improved healing response if the chondrogenic potential of the perichondrial or periosteal tissue is boosted either *in vitro*, prior to transplantation [38], or postoperatively, by a passive-motion schedule [33, 35, 36, 39, 40]. Although many of the documented findings look promising, complete restoration of hyaline articular cartilage tissue

and/or long-term stability have not been achieved [34]. Furthermore, attempts to prevent the detachment of transplanted material by glueing or suturing it to the defect floor have been hampered by technical difficulties. Uncontrolled calcification of the graft may also contribute to subsequent loss. It should be borne in mind that from a biological point of view, the grafting of perichondrial or periosteal tissue cannot be considered as an exclusively uncompounded treatment approach, but rather as a combined one. The repair response triggered by such material will, of course, be superimposed upon, and influenced by, the spontaneous one. The latter involves not only the welling up of blood into the defect void and the formation of a haematoma, but also the release of signalling substances which will affect the response of cells within the transplanted material.

CARTILAGE/OSTEOCHONDRAL GRAFTS

Use of cartilage tissue itself as graft-material or as part of an osteochondral transplant, dates back to the beginning of the century [41–43], and this concept still forms the foundation of much active experimentation, both on an autograft [44–48] and allograft basis [5, 19, 45, 49, 50].

As with perichondrial/periosteal grafts, the chief problems encountered after the transplantation of cartilage/osteochondral material are its long-term stability and integration with native tissue (particularly within the cartilaginous compartment). The absence of structural bonding poses nutritional as well as functional difficulties from the outset, and these naturally promote the onset of tissue degeneration. This latter process may be exacerbated when several pieces of graft material are inserted into the defect void (such as in mosaicplasty). Tissue destined for transplantation is sometimes stored at subzero temperatures, and its subsequent thawing prior to grafting inevitably reduces its viability [51–55]. The preparation of graft material for open-joint surgery or arthroscopic procedures [47], such as mosaicplasty [46], also involves the risk of reducing chondrocyte viability. Tissue is transiently exposed to unphysiologically high temperatures during its drilling, to drying and potential metal-ion contamination from the hollow cylinders used to punch it out, and to mechanical compression during its press-fitting into the lesion void. The translocation of tissue from a low- to a high-weight-bearing region may also lead to injurious compression by the comparatively unphysiological loading at the transplantation site. When non-autologous material is employed, the situation may be further complicated by the outbreak of immunological reactions [56]. Despite the insufficiency of experimental evidence in support of this transplantation principle, the clinical grafting of cartilage/osteochondral material is nonetheless frequently carried out nowadays [5, 46, 47, 50].

CELL-BASED TRANSPLANTATION SYSTEMS

Cells used for the induction of cartilage repair are applied in one of two manners: (i) embedded within a matrix, or (ii) free in suspension.

(i) Matrix-embedded cells

This transplantation system is a popular one for which foetal chondroblasts [57, 58], chondrocytes [59–65], perichondrial cells [66, 67] and mesenchymal stem cells [68, 69] have been employed. It is generally conceived that the local conditions pertaining within the defect space will promote expansion of the cell population and the subsequent transformation of repair tissue into cartilage.

A number of interesting findings relating to this transplantation principle are worthy of mention here. An unexpected phenomenon is observed when collagen-gel embedded allogenic chondrocytes are introduced into the void of full-thickness defects [59, 70, 71]: these cells fail to hypertrophy. ‘Cartilage’ formed within the deep portion of the grafted matrix does not therefore undergo enchondral ossification. When bone-marrow-derived mesenchymal stem cells are substituted for chondrocytes in the same system [68], not only do the former transform into the latter, but these chondrocytes then become organized into a layer of cartilage-like tissue which displays all stages of differentiation—including the hypertrophic one. Enchondral ossification thus proceeds rapidly and efficiently.

Polylactic-acid-embedded perichondrial cells have been shown to elicit an overall repair frequency of 85% in the rabbit model, but the cartilage-like quality of the tissue formed was variable, and none of the specimens appeared normal after one year [66, 67]. Neoformation of subchondral bone was also inconsistent; and at best, no more than 50% of the bony compartment was filled at one year. Furthermore, only defects wherein subchondral bone formation had taken place displayed a cartilage-like tissue appearance within the respective compartment.

In all model systems which have made use of this transplantation principle, the results have been variable, and the concept cannot be considered to

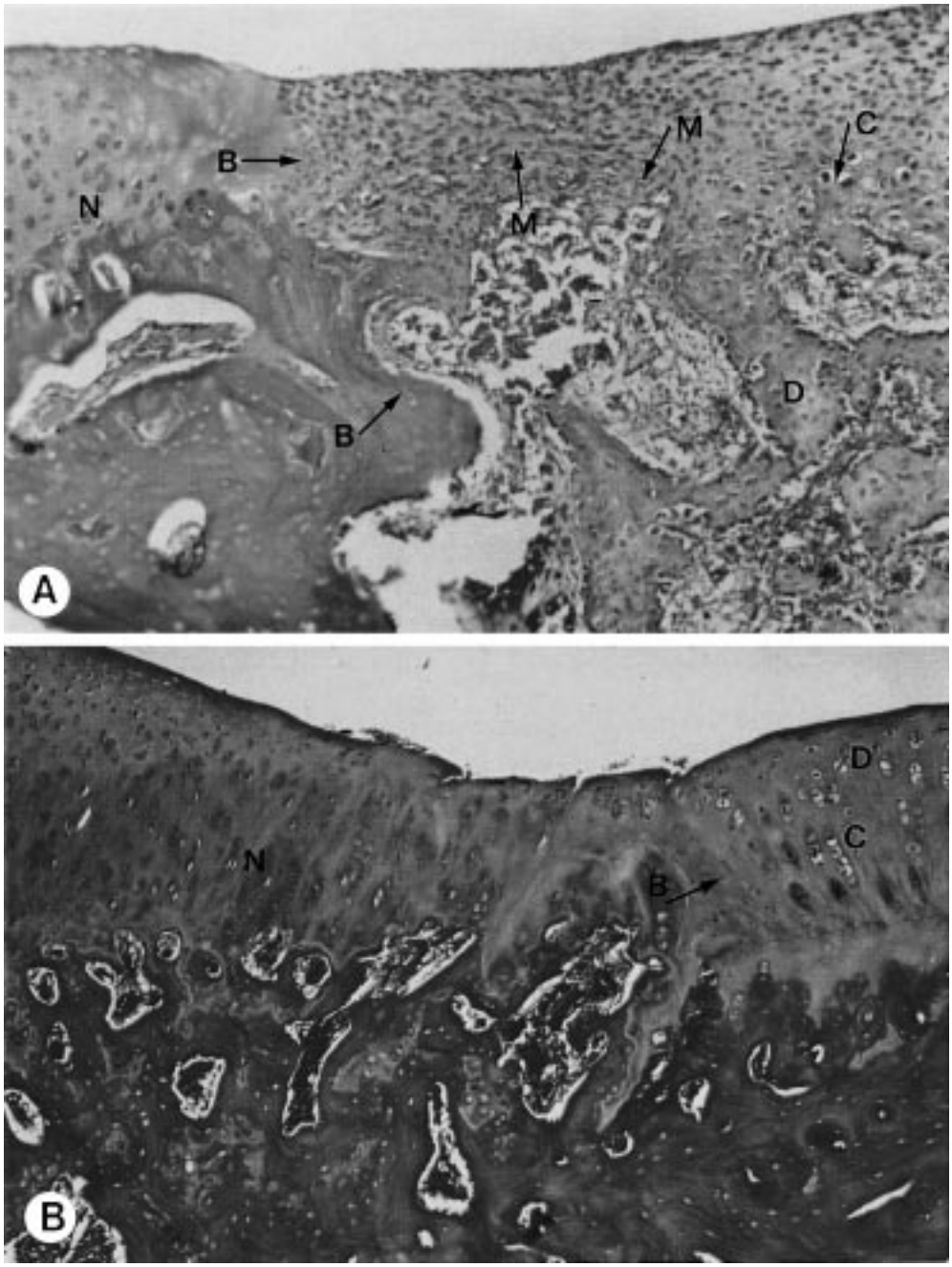

FIG. 1(a–b).

have advanced beyond the experimental stage. Several factors may contribute to the variable healing outcome. In most instances, no chondrogenic stimulating agents are applied. Differentiation of cells into chondrocytes and the formation of cartilage thus depend upon the unpredictable action of blood-associated signalling substances present within bone-marrow-derived haematomas. When matrix-embedded chondrocytes are grafted, variability in healing may also reflect the degree to which these cells have undergone dedifferentiation during culturing.

The choice of matrix used for the implantation of cells, and which serves as a scaffolding for their expansion within the defect void, is also a critical determinant of 'success'. The matrix should be composed of a material which is biocompatible, mechanically stable and amenable to rapid remodelling; it should also possess properties which ensure its adequate adhesion to the defect surfaces and facilitate the integration of repair- and native-tissue matrices. Bioincompatibility of the matrix may elicit a foreign body giant cell reaction or an immunological response,

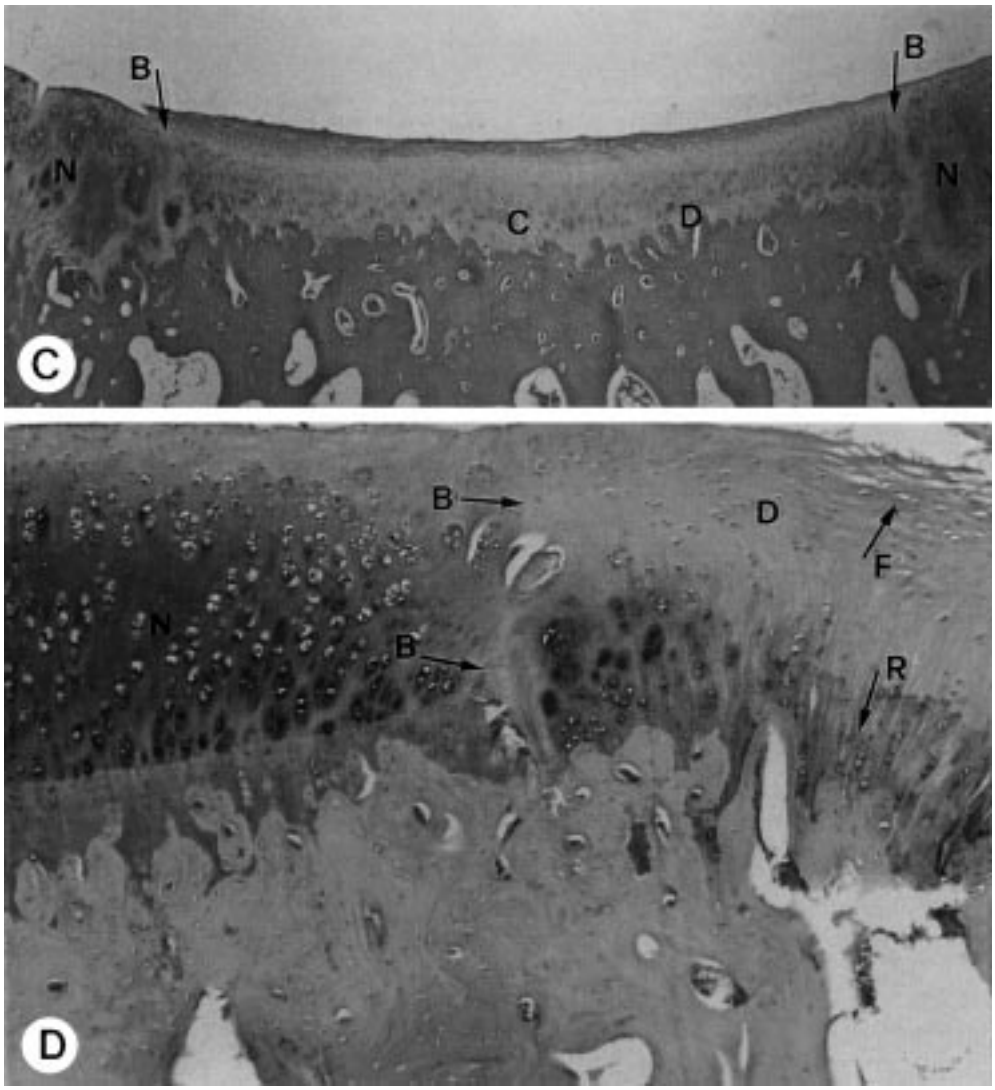

FIG. 1(c–d).

FIG. 1. Photomicrographs illustrating the appearance of repair tissue filling spontaneously-healed full-thickness defects, 10 days [A], 8 weeks [B], 24 weeks [C], and 48 weeks [D] after their creation. Each of the sections has been stained with Safranin O to demonstrate proteoglycans within the extracellular matrix. [A]: At 10 days, repair tissue still consists largely of undifferentiated mesenchymal cells (M), but there is some evidence of cartilage formation (C) on the right-hand side. B: defect border; D: defect; N: native cartilage. [B]: At 8 weeks, cartilage-like tissue (C) fills the cartilaginous compartment of the defect (D). B: defect border; N: native cartilage. [C]: At 24 weeks, cartilage-like tissue (C) within the defect (D) still appears to be healthy. B: defect border; N: native cartilage. [D]: At 48 weeks, cartilage-like tissue within the defect (D) manifests signs of degeneration, as evidenced by its decreased affinity for Safranin O (R) and by the beginning of fibrillation within the collagenous network (F). B: defect border; N: native cartilage. Reproduced with permission from Shapiro *et al.* [12].

which could delay the formation of cartilage and bone.

Although the grafting of matrix-embedded cells is a fairly straightforward undertaking in itself, this transplantation principle has the disadvantage of requiring surgical intervention on two separate occasions when autologous cell populations are employed: The first when tissue is removed for the isolation and expansion of cells in culture; the second when matrix-embedded cells are topically applied to the joint lesion.

(ii) Autologous-chondrocyte suspensions

As is the case with all transplantation systems, the cell-based principle cannot be considered in terms of the grafted cell population alone; local

biological activity thereby set in train within the defect surrounds must also be taken into account. And when other tissue or materials (even a matrix) are introduced together with the cells, then the system should, strictly speaking, be regarded as a combined one. The transplantation of autologous-chondrocyte suspensions [61, 72, 73] falls within this category, since, thus far, cells have always been applied together with a periosteal flap. One interesting aspect of this protocol deserves mention here: In this instance, the periosteal flap is placed, not in its customary position at the base of the defect (with its cambial layer facing the joint cavity), but at the top of the lesion (with its cambial layer facing the defect void), where it is sutured to the surrounding cartilage tissue; the cavity below the periosteal flap is filled with autologous, dedifferentiated (i.e., fibroblast-like) chondrocytes which had been expanded *in vitro* [72].

The periosteal flap is inserted in this reversed position in order to prevent the loss of transplanted cells from the defect void. But apart from serving the function of a lid, the reversed periosteal flap establishes an unusual and interesting microtopographic situation, in that a repair response could be activated from the cambial layer downards, towards the floor of the defect. The system appears to produce results comparable to those achieved using other set-ups [74]. However, the relative contributions made by the cambial layer of the periosteum, the transplanted dedifferentiated chondrocytes and bone-marrow-derived cells to the repair process have not been assessed. It would also have been interesting to know whether the insertion of a reversed periosteal flap alone elicites a repair response, but the authors did not undertake this experiment.

In a more recent study [75], the possible role played by transplanted autologous-cell suspensions in the repair response was evaluated in the dog using the traditional set-up. The authors came to the conclusion that the long-term outcome (one to two years) of treating defects with autologous chondrocytes did not differ from that of controls (periosteal flaps alone, no treatment). Such a deduction is, however, not well founded. In this study, postoperative animal care was not controlled, and no measures were taken to ensure that the sutured periosteal flaps were maintained in position, by partial or complete immobilization of the joints. Furthermore, the authors made no attempts to ascertain (by arthroscopy or MRI) whether the sutured flaps had indeed remained in place after one or several months. On the basis of our own experience (unpublished data), we know that such sutured flaps are inevitably lost to the joint cavity if no preventive measures are taken. If the periosteal flaps were lost (which they assuredly were), then the autologous chondrocytes were too, and it is therefore not surprising that the authors found no differences between experimental and control groups. This example points to the importance of controlling each step involved in such studies throughout the entire experimental period. Failure to do so renders interpretation of an already complex situation yet more difficult and open to misconceptions. Other simple factors, such as the thickness of the transplanted periosteal flap and how it compares to the thickness of the native hyaline articular cartilage layer, should also have been defined. In most large animal models, the periosteum is almost as thick as the layer of hyaline articular cartilage itself. Hence, in the reported animal experiments, there was unlikely to have been sufficient space available for the transplanted autologous chondrocytes within the cartilaginous compartment; only within the subchondral bone one.

SYNTHETIC MATRICES

Synthetic matrices—preferably biodegradable ones—serve as a scaffolding for the expansion of transplanted cells within the defect space. Chondrocytes or precursor cells cultured in, and subsequently implanted within, a three-dimensional system are more likely to remain in a differentiated condition (chondrocytes) or to undergo transformation (precursor cells). For this reason, as well as for optimizing the numerical density of cells and their immobilization *in situ*, the application of a matrix is advocated.

A number of natural and synthetic materials have been employed. The former group includes fibrin [58, 63, 76, 77] and (denatured) collagen-gelatine gels [78, 68]; the latter, carbohydrate-based polymers, such as polylactates [65, 79–81], polyglycolic acid [65, 79, 81, 82] and other biodegradable materials. Non-biodegradable matrices, such as carbon- [22, 83, 84], dacron- [85–87], teflon- [85, 86] and alginate-micromeshes [88], have also been tested. Although reasonable results have been obtained with some of the latter, they are nonetheless foreign materials, which will inevitably undergo changes with time and thereby influence local biomechanical properties. The conditions for obtaining optimal long-term results are thus compromised from the outset.

A number of investigators have also applied matrices in the absence of seeded cells. Carbon- [22, 83, 84], dacron- [85–87] and/or teflon-micromeshes [85, 86], as well as methacrylate

polymers [89], have been used in this capacity. In some instances, these matrices have been supplemented with growth factors, such as transforming growth factor-β [90] or growth hormone [91], in an endeavour to trigger or facilitate a repair response.

The principal advantage to be derived from filling a large full-thickness defect with a matrix is that the critical-size limit for a spontaneous healing response can be overcome by its bridging action. The utility of such a matrix depends, however, upon its becoming infiltrated with blood and blood-borne material welling up from the damaged subchondral bone and bone-marrow spaces, and the subsequent formation of a haematoma within its meshwork. A second matrix, i.e., a fibrin clot, is thereby formed within the interstices of the applied one. We are thus dealing with a composite matrix which is seeded with cells from Nature. Within this milieu, the blood and bone-marrow-derived cells form a granulation tissue, which is then resorbed and replaced by cartilage. Introduction of a matrix into the defect void thus serves as a means of supporting the spontaneous healing response over dimensions which exceed those for such a reaction in Nature. The applied matrix soaks up blood and bone-marrow-derived material, and ensures its distribution (by a kind of capillary attraction) throughout the entire defect void, which thereby becomes thoroughly populated with potential repair cells. In Nature, haematomas formed within such large defects would shrink into a small compass, such that considerable areas of the void would remain devoid of blood-borne and bone-marrow-derived cells for repair.

EVALUATION OF EXPERIMENTAL PROTOCOLS

Unfortunately, none of the numerous treatment protocols instigated for the repair of full-thickness articular cartilage defects can be considered as optimal [74]. Furthermore, the tremendous intergroup variation observed using any one particular system render it impossible to anticipate a prospective result. Hence, much more research is required before any of these methods can be adopted with a reasonable expectation of enduring success.

The cartilage-like repair tissue formed spontaneously within defects is fibrous in nature, and thus lacks the mechanical properties necessary for the fulfilment of its physiological roles, even while it persists. Many attempts have been made to improve the quality and durability of this repair tissue, but very little intrinsic progress has been made thus far. In the future, criteria used to assess the quality of repair tissue and its resemblance to hyaline articular cartilage need to be more rigorous and less subjective. Only when such objective measures are instigated can we hope to make a meaningful comparison between data gleaned from different studies [92].

Somewhat surprisingly, virtually none of the experimental approaches adopted take into account the high degree of topographical organization and compartmentalization of the tissues implicated. That is to say, although there exists a clearly delineated stratification (hyaline cartilage–calcified cartilage (non-vascularized tissues)–cell-excluding border–subchondral bone with marrow space (vascularized tissues) [93], most transplantation and other treatment procedures involve the introduction of a uniform population of cells into the defect site, with the sanguine hope that 'Nature' will undertake the necessary transformations and sort out what should go where.

A more sophisticated, rational and systematic approach is probably necessary for long-term success. One such protocol would involve the introduction of a cell-excluding barrier to separate neo-formed cartilage at the appropriate level in the defect void from the underlying bone, in imitation of the physiological situation [94].

Partial-thickness defects

Defects confined to the substance of mature articular cartilage itself [Fig. 2(A)] do not heal spontaneously [3–7, 95] [Fig. 2(A)]. Abortive repair reactions have been observed to occur within native tissue immediately below the defect surfaces, but these take the form of only very limited cell proliferation or chondrocyte-cluster formation [2, 95, 96] [Fig. 2(B)]. Only a few investigators have described the production and exudation of an extracellular matrix into the defect space [95], and even in these studies, the nature of this material was not determined. The differences in response to injury observed in partial- and full-thickness defects have fostered the belief that failure to heal in the former case reflects an inaccessibility to cells within the bone-marrow space. But recent observations indicate that partial-thickness defects do not in fact need access to bone-marrow cells in order to undergo repair, since, under appropriate conditions, mesenchymal cells can be induced to migrate from the synovial membrane and subsynovial spaces across the articular cartilage surface into the lesion void [94, 96, 97]. The difficulties thus lie elsewhere.

The anti-adhesiveness of articular cartilage matrix may pose one obstacle. Cartilage-specific molecules, such as proteoglycans—particularly

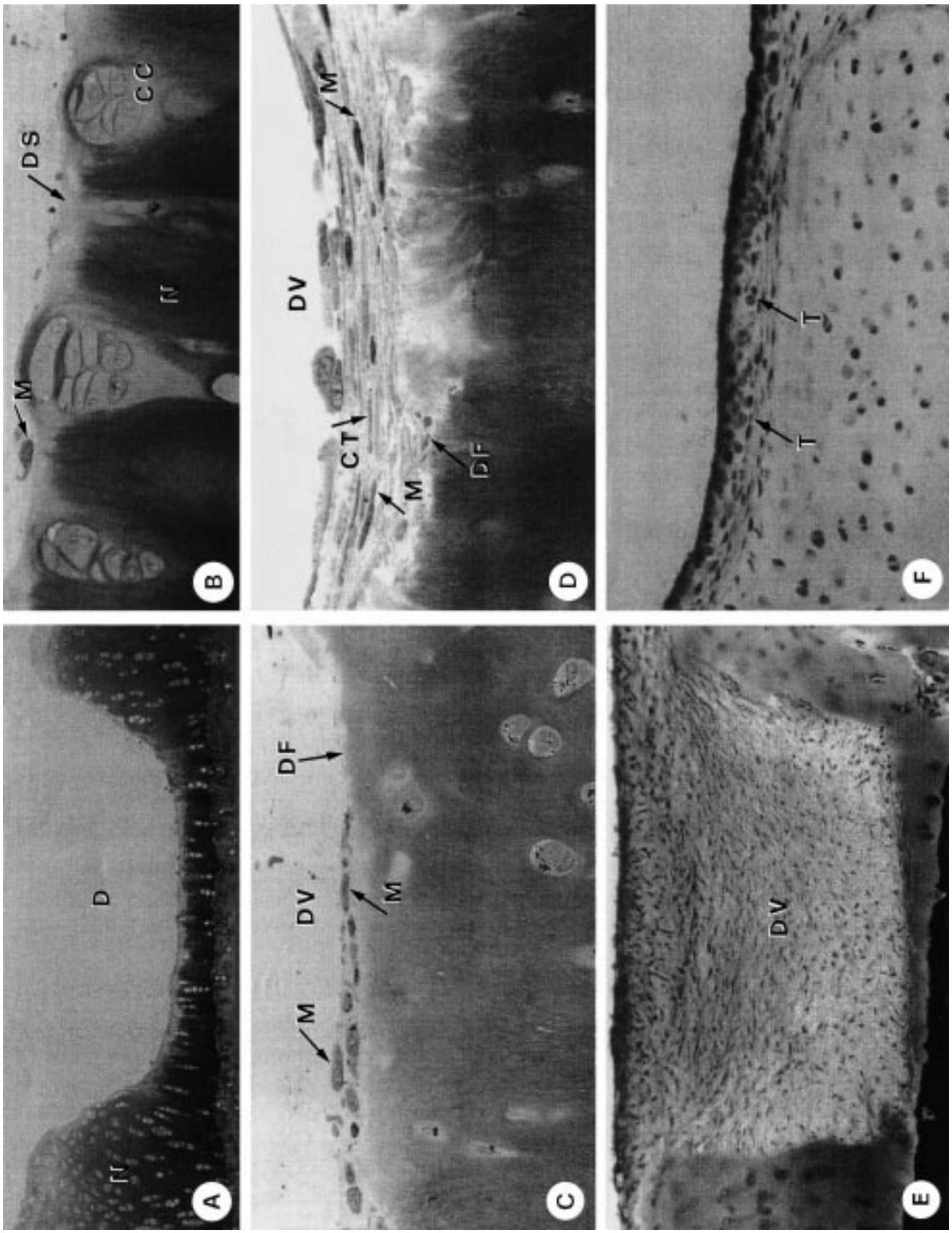

FIG. 2(a–f).

the small ones (e.g., decorin and biglycan)—are known to inhibit the adhesion of cells and blood-clot-attachment processes, both *in vitro* and *in vivo* [13, 14, 97–99]. In a recent systematic study, this and other intrinsic limiting factors were investigated, and the principles for a simple surgical treatment strategy developed [2, 94]. The anti-adhesiveness of the defect surfaces was overcome by controlled enzymatic degradation of superficial proteoglycan molecules. This step did indeed enhance the attachment of potential repair cells to the walls and floor of the lesion, but its cavity did not become populated [2, 99] [Fig. 2(C)]. The latter finding suggested that these particular repair cells have a low intrinsic mitotic activity. To test this postulate, a mitogenic growth factor was introduced into the system. This step led first to the formation of multilayers of repair cells along the defect floor and then to the laying down of a scar-like tissue within the defect space [Fig. 2(D)]. Growth factors which proved to be useful in this capacity included insulin-like growth factor I, basic fibroblast growth factor and growth hormone. When factors having a chemotactic, as well as a mitogenic, effect [e.g., transforming growth factor-β (at low activity levels, i.e., a few ng per ml) and fibroblast growth factor] were employed, cell tracks, originating from the synovial/subsynovial spaces and migrating along the articular cartilage surface into the defect void, were identified morphologically [Fig. 2(F)]. Nevertheless, complete filling of the lesion space was still not achieved. This finding implied that repair cells require spatial definition of the defect void in order to populate it entirely. To this end, a biodegradable matrix (i.e., a fibrin clot) was introduced at the time of surgery, and the entire defect space did then become sparsely populated with mesenchymal-like cells. With time, these began spontaneously to remodel the fibrin and replace it by a loose connective tissue [Fig. 2(E)]. The initial enzymatic treatment step still proved to be useful, since it not only enhanced adhesion of the fibrin-matrix to the defect surfaces [100], but also promoted integration between repair- and native tissue along the lesion borders. Numerical cell density remained low in this primitive repair tissue, even up to one year after surgery. But it could be improved by including a mitogenic and/or chemotactic factor with the fibrin matrix. Such a measure did not, however, promote tissue transformation into cartilage, a step which obviously required the timely application of a differentiation factor. This was achieved by incorporating transforming growth factor-β [at high (chondrogenic) activity levels] into liposomes, which were applied together with the biodegradable matrix at the time of surgery. These liposomes then delivered their contents at the critical juncture in time during the course of healing, i.e., at the onset of matrix remodelling [101, 102]. This final step in the treatment protocol did indeed promote the formation of a cartilage-like tissue and the healing of partial-thickness defects [101–103] (Fig. 3).

This systematic analysis has enabled us to pinpoint the inherent constraints undermining repair of partial-thickness articular cartilage defects and to establish the principles of a treatment protocol to overcome these. The advantage of this strategy over conventional ones is that it makes use of biological stimulators, thereby obviating the need for transplantation.

The very high activity of the factors employed for the repair of partial-thickness defects may not be appropriate for full-thickness ones. Bone and cartilage formation would be triggered simultaneously, and it seems unlikely that these two tissue compartments would become separated at the appropriate level within the joint. A tissue-compartment-specific treatment strategy would be

FIG. 2. A series of light micrographs illustrating the appearance of partial-thickness articular cartilage defects, 4 weeks after their creation in mature rabbits [A–D] and miniature pigs [E, F]. [A]: When left untreated, partial-thickness lesions (D) do not heal spontaneously. N: native articular cartilage. [B]: In this high-magnification view of part of the defect floor represented in [A], a few mesenchymal-like cells (M) occur sporadically along the defect surface (DS). Within the native articular cartilage (N), some chondrocyte clusters (CC) are to be seen. [C]: After controlled removal of proteoglycans from the defect surface by enzymatic digestion with chondroitinase AC, the adhesion of mesenchymal-like repair cells (M) to the floor of the defect (DF) is enhanced, but its void (DV) does not become populated. [D]: Addition of the mitogenic factor, insulin-like growth factor I, to chondroitinase AC-treated defects, leads to the laying down of multilayers of mesenchymal-like repair cells (M) along the floor of the defect (DF), but not throughout its void (DV). These cells elaborate a fibrous type of connective tissue (CT). [E]: Deposition of the space-filling biodegredable matrix, fibrin, and concomitant application of the chemotactic/mitogenic growth factor, transforming growth factor-β, leads to complete filling of the defect void (DV) with a primitive type of scar tissue. [F]: In this high-magnification view of the surface of the native articular cartilage tissue bordering the defect represented in [E], tracts of mesenchymal-like repair cells (T) are to be seen. These cells originate from the synovium and subsynovial tissue compartment, and are presumably stimulated to migrate therefrom into the defect cavity, attracted by the chemotactic factor (transforming growth factor-β). [A–D]: Semi-thin sections, stained with Toluidine Blue O. [E, F]: Thick, surface-polished saw-cuts, stained with basic Fuchsine and McNeil's Tetrachrome. Reproduced from [76] with the publisher's permission.

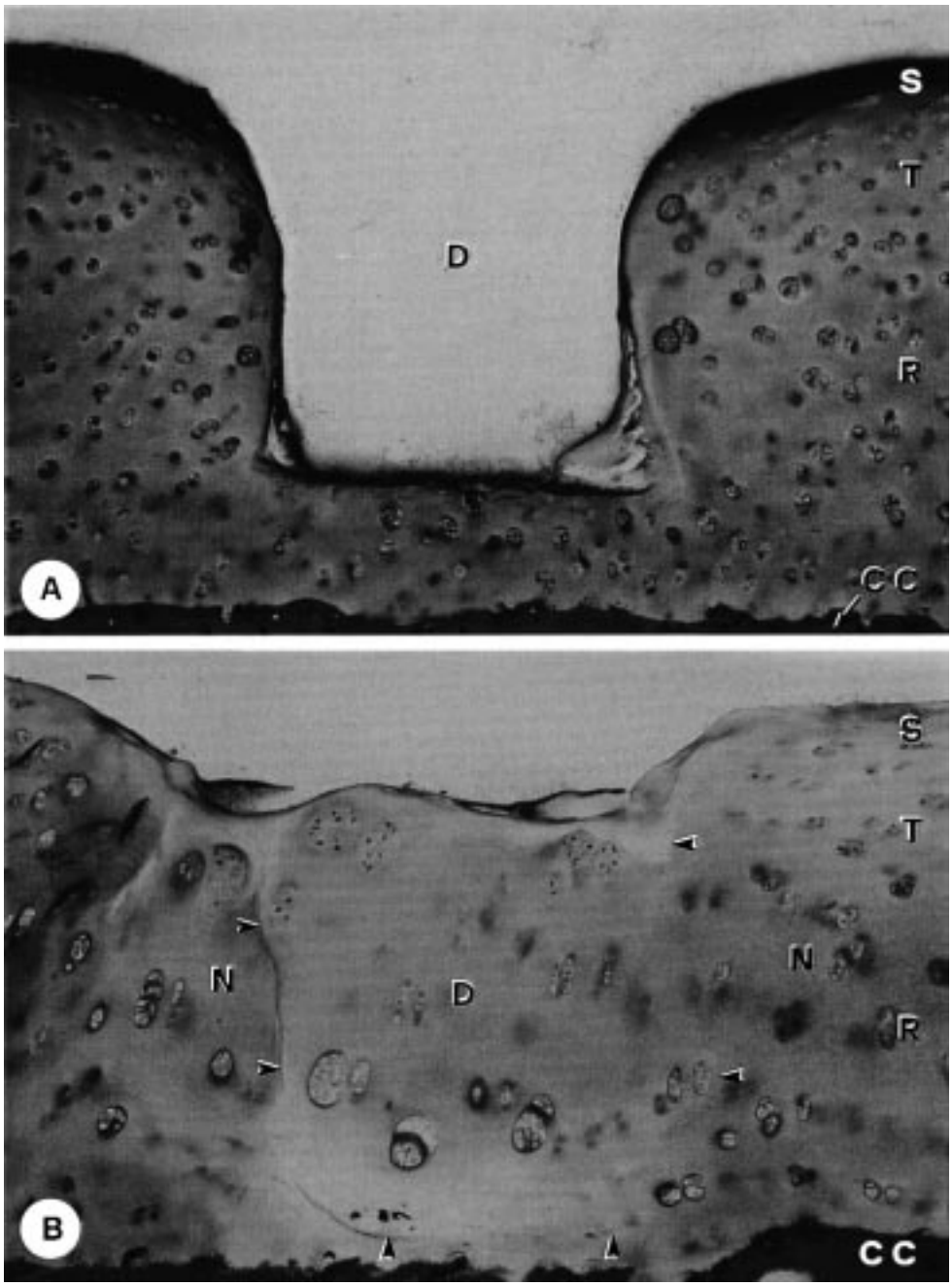

FIG. 3. Light micrographs of partial-thickness articular cartilage defects, 6 weeks after their creation in mature miniature-pig knee joints. [A]: Untreated control. No repair tissue has formed spontaneously within the defect void (D). The superficial (S), transitional (T), radial (R) and calcified (CC) zones of native articular cartilage are indicated. [B]: This defect was filled with the space-filling biodegredable matrix, fibrin, containing transforming growth factor-β at low concentration (to act in the capacity of a chemotactic/mitogenic factor), and at high concentration (to promote tissue differentiation) in a liposome-encapsulated form. The defect void (D) has become completely filled with a cartilage-like repair tissue, which is contiguous (see arrowheads) with the vicinal native articular cartilage (N). Cells within the repair tissue have a chondrocyte-like appearance. In this example, they form somewhat larger chondrones than is normal, but this was not a consistent finding. These chondrocytes are embedded within an extracellular matrix which is less intensely stained than that of native tissue, indicating that its proteoglycan content is lower than normal. The superficial (S), transitional (T), radial (R) and calcified zones of the vicinal native articular cartilage are indicated. Thick, polished saw-cuts, surface-stained with basic Fuchsine and Toluidine Blue O.

required. This could be achieved by depositing an osteogenic matrix within the bony compartment and functionally separating it from the articular cartilage one by means of a cell- and vessel-excluding barrier, which would permit cartilage tissue healing at the desired level. A barrier such

as those used in guided-tissue regeneration concepts would be an appropriate choice [94]. It is hoped that further improvement and refinement of this strategy for tissue-specific compartments will lead to the delineation of a successful treatment protocol which will promote the formation of functionally normal and structurally persistent repair tissue.

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