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The Elusive Path to Cartilage Regeneration

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Abstract

Numerous attempts have been made to develop an efficacious strategy for the repair of articular cartilage. These endeavours have been undaunted, if not spurred, by the challenge of the task and by the largely disappointing outcomes in animal models. Of the strategies that have been lately applied in a clinical setting, the autologous-chondrocyte-transplantation technique is the most notorious example. This methodology, which was prematurely launched on the clinical scene, was greeted with enthusiasm and has been widely adopted. However, a recent prospective and randomized clinical trial has revealed the approach to confer no advantage over conventional microfracturing. Why is the repair of articular cartilage such a seemingly intractable problem? The root of the evil undoubtedly lies in the tissue's poor intrinsic healing capacity. But the failure of investigators to tackle the biological stumbling blocks systematically rather than empirically is hardly a less inauspicious circumstance. Moreover, it is a common misbelief that the formation of hyaline cartilage *per se* suffices, whereas to be durable and functionally competent, the tissue must be fully mature. An appreciation of this necessity, coupled with a thorough understanding of the postnatal development of articular cartilage, would help to steer investigators clear of biological cul-de-sacs.

Introduction

Osteoarthritis is one of the most common diseases suffered by elderly persons in Western countries. This disabling joint disorder is usually multifactorial in nature and thus of unknown origin in individual cases. However, in many instances, it is precipitated by injury (e.g., by sporting or other accidents). The incidence of osteoarthritis is positively correlated not only with age but also with obesity, and due to the upward trends in both life-expectancy and youth overweight, a steady increase in the prevalence of the disease is expected. Before long, osteoarthritis will become one of the heaviest financial burdens on health-care systems in industrialized countries. The knee, the hip and the shoulder are the joints most frequently affected. During the early stages of osteoarthritis, localized lesioning of the articular cartilage layer is a characteristic occurrence [1]. Once initiated, this lesioning process is progressive, and no prophylactic measures are at hand to arrest it. Likewise, no efficacious, biologically-based treatment strategies are available to induce the healing of these structural defects. During the past few years, considerable international research efforts have been directed towards facilitating the biological repair of articular cartilage defects. The proposed strategies are diverse in nature, being based, for instance, upon tissue-engineering principles, the introduction of either novel matrices, various stem-cell types or growth-factor complexes, and gene therapy.

Current research activities

An increasing number of research teams around the world are now adopting a more biologically rational approach to the repair of articular cartilage lesions. These activities are

directed mainly towards the elaboration of novel tissue-engineering-based strategies [2]. A commonly tested model consists of chondroprogenitor cells embedded within a matrix. The chondroprogenitor cells that are used for such purposes have been derived from the periosteum [3], the perichondrium [4], articular cartilage itself [autologous chondrocytes [5]], the bone marrow [autologous/allogeneic mesenchymal stem cells [6];[7]], muscle, adipose tissue [stem cells [4]] or even foetuses [allogeneic chondroblasts]. And the search for suitable and hitherto untested types continues unabated [8]; [9]. Due to the limited availability of autologous cells, the focus is now more on allogeneic sources. However, the use of allogeneic cells raises other problematic issues, such as biocompatibility and immunogenicity [2];[9].

In most cartilage-engineering strategies, the chosen cell type is applied in conjunction with free or appropriately-encapsulated growth factors. The choice and optimization of growth-factor cocktails for cartilage repair is in itself an area of vigorous activity, which has taken a new direction of late in the field of gene therapeutics. Gene therapy has been mooted as a means of furnishing a constant local supply of the needful agents to the defect site [10].

The quest for novel scaffolding materials to serve as cell- and/ or drug-carriers is likewise frenetic. Although some of these materials are already in clinical use [11], most of them, and especially the synthetic ones, are still being tested in preclinical trials [12]. Moreover, using scaffolding materials that have a long history of use in the field of cartilage repair, such as collagen [13] fibrin [14], hyaluronan [15];[16] and polylactate [17], as well as more recent innovations, attempts are now being made either to produce them in nanofibrillar forms [18], to enhance their porosity [19] or to manufacture them with an injectable consistency [20]. The common aim of these endeavours is usually to create a resorbable, biomimetic material [21], even when using synthetic polymers, such as polydioxanone [22] or a mixture of polyethyleneoxide terephthalate and polybutylene terephthalate.

More recently, biphasic scaffolds have appeared on the scene [23]. These bilayers consist of two different materials: an upper one that is deemed to be conducive to chondrogenesis and which is destined for the cartilaginous compartment of osteochondral defects, and a lower ceramic plug which is press-fitted into the subchondral bone [23]. Apart from the obvious issues that must be addressed when testing a scaffold, such as biocompatibility, biodegradability and toxicity, its integration with native cartilage is also a troublesome problem, and one that has not yet been overcome [24].

Endeavours are also being made to obviate the need for a scaffold altogether. This so-called scaffold-free approach to cartilage engineering involves the growth and stimulation of chondroprogenitor cells under micromass conditions *in vitro*. The resulting construct, which consists of chondrocytes and a secreted extracellular matrix, is then implanted within the cartilaginous defect [25];[26]; [27]. The highly popular autologous-chondrocyte-implantation (ACL) technique (see next section) is a variation on this theme. In this instance, a suspension of expanded autologous chondrocytes is introduced directly into the defect, which is usually closed surgically with a periosteal flap. And apropos of surgery, it has recently come to light that the surgical technique itself can have a bearing on the course of cartilage repair and thus on the therapeutic outcome [12];[28];[29].

Cell-based cartilage-repair strategies in clinical use

Knutsen *et al.* [30] have recently published the results of a prospective randomized clinical trial in which patients with circumscribed lesions of the femoral condylar articular cartilage were treated either conventionally, by microfracturing (Fig. 1) [31], or according to the ACI-technique (Fig. 2) [32]. The findings of this 5-year trial revealed the latter strategy to confer no advantage over the conventional one. Microfracturing penetrates the bone marrow and

elicits a spontaneous healing response which is controlled by Nature. It is an inexpensive procedure, which has proved to be fairly successful in patients under 50 years of age. The ACI-technique took the orthopaedic world by storm when it was introduced on the clinical scene by Brittberg *et al.* in 1994 [32], generating much hope and enthusiasm. The concept was in fact the brainchild of Grande *et al.*, who tested it in a preclinical study with rabbits in 1989 [33]. But neither this study nor subsequent clinical trials formed a sufficiently rigorous basis for its establishment as a routine procedure in orthopaedics [34]. Actually, other chondrocyte-based cartilage-repair strategies were already in existence at this time, albeit that these made use of allogeneic (rather than autologous) cells, which were embedded within a matrix rather than suspended in a fluid [for review, see [34]]. But none of these techniques had met with much success in either preclinical studies or clinical trials.

The findings reported by Knutsen *et al.* confirmed the growing suspicions of sceptics that cell-based cartilage-engineering approaches do not improve the repair results that can be achieved simply by surgical stimulation of the bone marrow. The findings of this prospective randomized clinical trial underline the danger of prematurely advocating a novel therapeutic principle before it has been rigorously and systematically investigated: the hopes of surgeons and patients are raised only to be disappointed.

Neither the microfracturing nor the ACI-technique is effective in patients over 50 years of age, probably because the bone-marrow (mesenchymal) stem cells (microfracturing technique) and the transplanted autologous chondrocytes (ACI-technique) suffer an age-related loss in their potential to proliferate and differentiate [35]. Hence, especially for elderly persons, who are particularly prone to osteoarthritic lesioning of articular cartilage, there exists an undisputed need for an effective cell-based repair strategy. But we have to face the bleak fact that very little progress in this area has been made since the bone-marrow-stimulation technique – of which microfracturing is a variant – was introduced by Pridie in the 1950s [36]. During the past 5 years, about 400 publications relating to cell-based cartilage-repair strategies have appeared annually, but without much avail. However, one source of comfort is that cell-based cartilage-repair strategies, such as the ACI-technique, do not yield worse healing results than those that occur naturally. As a principle, the ACI-technique has the potential for improvement, which the knowledge and experience gained over the past 15 years since its début should render possible.

“Video meliora proboque, deteriora sequor” * (*Ovid: *Metamorphoses*)

What needs to be done to improve the clinical results of cell-based cartilage-repair strategies? A survey of publications that have appeared over the past half century brings to light one overriding circumstance, namely, that in both the preclinical and the clinical arenas, the therapeutic approaches tested have been of a largely empirical nature, viz., based on trial and error. Current cell-based cartilage-engineering strategies could thus be viewed as “alchemy” of the twenty-first century. And if this alchemistic approach is continued, then the fruitful path to cartilage regeneration will remain elusive. Ultimate success will never be achieved unless the problem of cartilage repair is tackled rationally and systematically on a step-by-step basis. But the lesson is not being learned; the research philosophy continues in the same old groove. For example, a matrix-associated variant of the ACI-technique (the so-called MACI-technique) has been recently introduced. According to this MACI-technique [37], the autologous chondrocytes are embedded within a matrix rather than suspended in a fluid, thereby obviating the need for a periosteal flap, which, in the ACI-technique, serves to trap the suspended cells within the defect. In the first place, the use of matrices as cell-carriers in cartilage-repair strategies has a long history, dating back to the 1960s [[38]; for review, see [34]], and the almost endless variations on this theme have been exhaustively pursued – with great ingenuity but to little avail. In the second place, the implantation of a

matrix, whilst dispensing with some of the disadvantages of an autologous tissue flap, introduces a new set of problems, such as the issues of biocompatibility, inflammation and degradation [for review, see [34]].

Cell selection

One of the key messages of Knutsen *et al.*'s prospective randomized clinical trial is that "younger patients did better [than older ones] in both groups". This finding suggests that a cell-based cartilage-engineering approach might confer an advantage over Nature's course if the choice of cells was an appropriate one: they should manifest a high proliferative potential and a high capacity to differentiate into chondrocytes that produce a mature and joint-specific type of repair cartilage. Since a mature type of articular cartilage can be generated only from an adult pool of chondroprogenitor cells [39], adult autologous chondrocytes that are derived from the joint that bears the defect would appear to be a logical choice for transplantation. However, an indiscriminate harvesting of chondrocytes from articular cartilage yields a heterogeneous pool of cells whose capacity for proliferation and differentiation varies according to the zone of origin. Hence, the subpopulation with the highest potential to proliferate and differentiate should be selectively isolated. At least one group of investigators has recently undertaken this task [40]. By selecting a subpopulation of chondrocytes with a high proliferative and differentiation potential, these authors have shown that the quality and the durability of the repair cartilage can be improved.

When a less committed type of precursor cell, or even stem cells, are in question, the situation becomes more complex and the chances of success are lower. Adult chondrocytes are intrinsically programmed to produce an adult type of articular cartilage [39]. Only this mature type of tissue possesses the structural anisotropy (Fig. 3) that is necessary for its functional competence and longevity. During ontogenesis, non-committed stem cells of mesodermal origin differentiate into chondrocytes that produce an immature, foetal type of articular cartilage with an isotropic structure (Fig. 3), which is neither designed nor destined to endure: it serves as a temporary scaffold for the formation of bone, the meniscus, the synovium and more immature cartilage [41];[42]. Even after the rudiments of the joint have been formed, the articular cartilage continues to act as a surface growth plate as well as a layer that facilitates articulation [39]. During the early postnatal phase of development, chondroprogenitor cells that reside within the superficial zone of the articular cartilage layer [39] still produce a rapidly-growing, immature type of tissue, which serves for the expansion of the epiphyseal bone. During this process, the immature cartilage is gradually resorbed and eliminated. Only during puberty and the post-pubertal growth phase is the pool of precursor cells within the superficial zone reprogrammed to produce a mature type of articular cartilage. In humans, the evolution of a mature type of articular cartilage spans a period of 10–18 years. During this time, the original population of stem cells undergoes successive phases of proliferation and differentiation which only at a late stage yield a mature type of articular cartilage (Fig. 4) [39]. The genetic machinery that regulates the switch for the production of a mature type of articular cartilage is unknown, and it is not reasonable to expect that a sequence of events that spans so many years in Nature can be recapitulated within a matter of weeks or months under experimental conditions.

The maturity of articular cartilage tissue is necessary for its functional competence and longevity. Consequently, repair tissue should be thoroughly characterized for its foetal- or adult-type qualities [34];[39]. If such analyses were undertaken, misleading inferences, as those made by de Bari *et al.* [43], would be avoided. Using mesenchymal stem cells of synovial origin, these investigators demonstrated the formation of repair cartilage in a nude-mouse model. The very fact that cartilage tissue was formed was deemed by the authors to be a sufficient ground for concluding that the choice of cells was an appropriate one for the

purpose of cartilage repair. However, even on the basis of structural criteria alone, the tissue formed was patently of the foetal type. The authors neither drew the readers' attention to this fact nor mentioned that such a tissue type would not endure. The degree of tissue maturity needs to be established not only on the basis of cell architecture (anisotropic vs. isotropic structural organization), but also at the molecular level for each extracellular matrix compartment. In many of the publications that have appeared during the past 5 years, the analyses have been of a very rudimentary nature, having been confined to a determination of the levels of type-II collagen, aggrecan and COMP, which are not specific markers of mature articular cartilage.

Nature affords several examples of short-cut differentiation loops in which stem cells develop directly into an adult type that produces a mature and specific tissue. During the spontaneous healing of bone fractures, stem cells are capable of differentiating into fibrochondrocytes that produce mature fibrocartilage. This fibrocartilaginous tissue serves as an anlage for endochondral bone formation. However, this fibrocartilage, like immature articular cartilage, is neither designed nor destined to persist for any great length of time. The formation of rib cartilage also involves a short-cut differentiation loop. Mesenchymal stem cells within the cambial layer of the rib perichondrium differentiate directly into chondroblasts; these mature into chondrocytes that produce an adult type of rib cartilage. Since short-cut differentiation loops do indeed occur physiologically, then it might be possible to imitate the process under experimental conditions. But it will be first necessary to elucidate the mechanisms that underlie this phenomenon in Nature.

In a recent investigation [44], a common pool of mesenchymal stem cells was induced, under different stimulation conditions *in vitro*, to differentiate into either a fibrous or a hyaline type of cartilage tissue. Although the degree of tissue maturity awaits determination, the findings are nevertheless of importance. They indicate that such an experimental set-up can be used to elucidate the regulatory pathways that drive chondroprogenitor cells along a particular tissue-specific route, namely, in either a hyaline-, a fibrous- or an elastic-cartilage direction. These regulatory pathways, a key to which should be revealed by monitoring the expression levels of specific genes, will also be impacted by microenvironmental factors, such as the biomechanical forces that act on the tissue during its formation. Such factors are of paramount importance in optimizing the differentiation process so as to yield a functionally competent type of tissue.

Prospects for the future

Tissue engineering is an applied science, which aims to restore tissue activities that have been undermined by destructive or degenerative agencies, and which either cannot be re-established by available therapeutic means, or, if they can, depend upon the transplantation of scarce auto-, allo-, or xenogeneic material. In attempting to understand the workings of a given biological system, basic scientists tend to design experiments that will reveal the nature of the processes that are at play and their underlying mechanisms. The insight thereby gained is then applied to develop tools that will selectively inhibit or enhance components of the system. Successful tissue engineering likewise depends **upon** thorough knowledge and understanding of the biological system in hand. But instead of experimentally stripping the system down to its bare elements, the tissue-engineering approach takes the basic building-blocks, or suitable substitutes, with a view to re-establishing the system both structurally and functionally. Inevitably, therefore, most tissue-engineering approaches are initially of an essentially empirical nature, the concept being tested first *in vitro* and then *in vivo*. However, many strategies that appear to be promising *in vitro* yield disappointing results when directly translated to living organisms, thereby bringing home to us the complexity of the natural system and our incomplete grasp of its intricacies. The impact of one biological

phenomenon in particular, namely, immunoreactivity and tissue rejection, cannot be fully appreciated *in vitro*. Following implantation, any engineered tissue construct will be treated by the body as a foreign object. Hence, it is not surprising that many new concepts fail from having been inadequately tested *in vivo*.

No empirical approach to tissue engineering is foolproof, and it is thus not possible to troubleshoot all difficulties. Nevertheless, by taking a broad view of the medical problem to be solved and tackling this systematically, certain stumbling blocks can be anticipated and dealt with. Hence, the nature of the specific medical problem to be solved must be first identified and the therapeutic needs established. This awareness should drive our project planning and experimental approach in a highly focused way towards establishing a successful therapeutic tool. Although such a philosophy is seemingly self-evident, a survey of the relevant literature reveals all too clearly that it is frequently disrespected. Consequently, the research activities of many investigators are misdirected and wasted.

The aim of tissue engineering is to develop methodologies whereby structural and functional bodily defects can be healed. Tissues and organs that do not heal spontaneously are induced to repair by attempting to overcome the biological limitations that undermine this process in Nature.

At the stage of its conception, a tissue-engineering principle must be screened to ascertain the size of the patient population that will be targeted, its socio-economic impact, the feasibility and likely cost of its manufacture, and its surgical practicability. Hence, tissue engineering is an interdisciplinary enterprise.

There exists no magical formula for the engineering of a product that will be universally applicable to all bodily tissues. An engineered construct must be tailor-made on a tissue-specific basis. To yield a product that will be beneficial to the targeted patient population, a tissue-engineering principle should be experimentally developed along systematic and, whenever possible, rational lines, and in the light of a thorough understanding of the biological system in hand.

We are still a long way from achieving our goal of cartilage regeneration. However, Nature affords the clues to this end if we but seek them out and have the fortitude to pursue them rationally and systematically. Only by following such a course can we hope for ultimate success. There are no short-cuts to the Holy Grail!

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Microfracture Technique

Fig. 1. Schematic representation of the microfracturing technique. The floor of a shallow, full-thickness articular cartilage defect, viz., one that does not violate the subchondral bone plate, is perforated in several places using either a sharp instrument or a small-bore drill. By this action, the bone-marrow space is opened. Blood bearing mesenchymal stem cells percolates into the defect wherein it forms a haematoma.

Fig. 2. Schematic representation of the ACI-technique. A fragment of articular cartilage is removed from the periphery of the joint that bears the lesion. This fragment of tissue is digested to release the chondrocytes, which are expanded *in vitro* for 11–21 days. A periosteal flap is then removed from the medial tibia and sutured over the lesion. A suspension of the expanded chondrocytes is injected beneath the flap into the lesion. Reproduced with the publisher's permission from Brittberg *et al.* [32].

Fig. 3.

Light micrographs of vertical sections through the tibial condylar articular cartilage of rabbits aged 1 month (A), 2 months (B), 3 months (C) and 8 months (D). One month after birth (A), the articular cartilage is of an immature, foetal type, which is characterized by an isotropic organization of the chondrocytes. This immature type of tissue persists during the pre-pubertal growth spurt [up to 2 months (B)]. By the time the rabbit has attained sexual maturity [3 months (C)], the articular cartilage manifests the anisotropic structure that is so characteristic of the adult animal (D). During the course of postnatal development, the mature type of tissue does not evolve from the immature one by a process of gradual remodelling. The immature tissue is almost completely resorbed; only the superficial zone of chondroprogenitor cells is spared, and these cells give rise directly to mature chondrocytes that produce an adult type of articular cartilage. Reproduced with the publisher's permission from Hunziker *et al.* [39].

Pre-pubertal Phase

Pubertal Phase

Immature
(isotropic Cartilage)

Mature
(anisotropic Cartilage)

Fig. 4.

Schematic representation of the sequence of events that lead to the formation of the cartilage anlage that develops into two synovial joints and three bones (A), and that are involved in its early postnatal development (B, C).

A: Mesenchymal stem cells condense and differentiate into chondroblasts, which form an anlage of cartilage. In the interzone (boxed area), a transverse split develops, which is destined to become the synovial cavity. Chondroblasts within the interzone give rise to chondrocytes – which form a layer of immature articular cartilage – to fibrochondrocytes – which form the meniscus – and to synovial cells – which elaborate the synovium.

B: During early postnatal development, the immature articular cartilage acts as a superficial growth plate for the radial expansion of the epiphyseal bone. Chondroprogenitor cells within the superficial zone proliferate exuberantly and differentiate into immature chondrocytes that produce an immature (isotropic) type of cartilage. During puberty, chondroprogenitor cells within the superficial zone continue to proliferate, but they are reprogrammed to differentiate into mature chondrocytes that produce an adult (anisotropic) type of cartilage.

C: During puberty, the expanding core of epiphyseal bone (E) divides the cartilage tissue into a superficial articular layer and a growth plate proper. The latter then serves for the elongation and expansion of the metaphysis (M) and the diaphysis (not shown).