A growth factor-based strategy recently has been shown to induce the intrinsic repair of partial-thickness articular cartilage lesions, thereby obviating the need for transplanting cells or tissue. It was the purpose of the current study to ascertain whether this principle could be applied to full-thickness articular cartilage defects created in adult rabbits and mature miniature pigs. The transforming growth factor-β1 contained within the authors’ chondrogenic matrix-complex proved to be potent in its osteogenic capacity when liberated into the bone compartment of such lesions, inducing the rapid upgrowth of osseous tissue and vascular buds into the cartilage compartment. This is an unwanted response that must be prevented. A cell and blood vessel-excluding membrane (Millipore® in rabbits and Goretex® in miniature pigs) was inserted at the interface between cartilage and bone compartments, both of which were filled at the appropriate juncture with the chondrogenic matrix-complex. These structural barriers were effective in obstructing the upgrowth of blood vessels into the cartilage compartment and in preventing the osteogenic tissue differentiation attributable to the presence of a vasculature.

It is a well-known circumstance that lesions confined to the substance of articular cartilage fail to heal spontaneously in adult organisms, whereas defects that penetrate the subchondral tissue spaces (wherein lie the bone marrow and vasculature) undergo spontaneous repair if they span a breadth of no more than a few millimeters. This spontaneously formed repair articular cartilage is neither biochemically, structurally, nor biomechanically on a par with native tissue; nor does it persist, but begins to degenerate a few months after having been laid down. Although many basic problems associated with healing of these small full-thickness defects, which do undergo spontaneous repair, remain to be solved, current efforts are focused more on the development of biologically-based resurfacing strategies for the healing of very large lesions (defects with diameters of 1 cm or more) which do not repair spontaneously. Currently, ideals regarding hyaline qualities, physical properties, and durability of the repair tissue that is laid down, have not been realized.

Using a small partial-thickness articular cartilage defect model as a starting point, the authors recently defined the biologic princi-
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ples and basic conditions required for intrinsic repair, thereby obviating the need for cell or tissue transplantation. The system consists of a space-filling, biodegradable matrix containing a free chemotactic and mitogenic substance (TGF-β1 at a low concentration) to attract cells from the synovial membrane and subsynovial spaces, and a liposome-encapsulated chondrogenic differentiation agent (TGF-β1 at a high concentration) to induce the timely transformation of primitive repair tissue into cartilage.

The current authors evaluated the value of this system when applied to small full-thickness defects. Specifically, the authors wished to identify the biologic stumbling blocks encountered when applying a growth factor-based repair induction system to lesions that penetrate the subchondral bone tissue, bone marrow, and vascular spaces, and to attempt to overcome these obstacles.

Application of the growth factor-based chondrogenic matrix to small full-thickness defects led to the induction of unwanted osteogenic activity within the cartilage compartment. The insertion of a porous, blood vessel- and cell-excluding (structural) membrane at the interface between cartilage and bone (similar to the guided tissue regeneration principle) helped to prevent osseous upgrowth into the former, therefore permitting the tissue compartment specific repair of full-thickness articular cartilage defects.

MATERIALS AND METHODS

Surgical Procedure

Six adult rabbits and eight mature Goettingen miniature pigs (2 to 4 years of age) were used for this study. General anesthesia was induced by intravenous injection of Narketan® (Chassot AG, Belp, Switzerland) and maintained by administering Halothan® (Halocarbon Laboratories, River Edge, NJ) and nitrous oxide. In rabbits, both knees were exposed and two full-thickness defects (approximately 0.7 mm [depth] × 1.2 mm [breadth] × 7 to 8 mm [length]) were created through the articular cartilage layer of each femoral groove facet using a custom-built planing instrument. In the miniature pigs, two circular full-thickness defects (3 mm [depth] × 5 mm [diameter]) were drilled through the articular cartilage layer of each femoral groove facet. In both animal models, defects were blotted dry and then filled with an aqueous solution of chondroitinase ABC (1 unit/mL [Sigma-Aldrich, Buchs, Switzerland]), which was removed by swabbing after 4 to 5 minutes. Each defect was rinsed thoroughly with physiologic saline and again blotted dry.

Half of the defects created in each animal were filled to the point of bulging with fibrinogen solution (19 mg/mL of 0.15 mol Tris-buffered saline [Merck, Dusseldorf, Germany]), which was purified from bovine blood (obtained from a local abattoir) and prepared according to the procedure described by Mosher and Blout. A solution of thrombin (1 unit/mL [Immuno, Vienna, Austria]), containing free TGF-β1 (R&D Systems Inc, Minneapolis, MN) at 4 ng/mL (as a chemotactic and mitogenic factor) and liposome-encapsulated TGF-β1 (prepared according to the procedure described by Kim et al) at 600 ng/mL (as a differentiation factor) were added to the fibrinogen immediately before use. In the remaining defects, only the bone tissue compartment was filled to the point of bulging with the growth factor-containing fibrinogen solution described above. After in situ polymerization of the fibrinogen solution, a structural barrier membrane (a fine Millipore® filter with a pore diameter of 0.2 μm [Millipore, Nuernbrecht, Germany] in rabbits and a cell-excluding Goretex® membrane [Goretex, Flagstaff, AZ] in miniature pigs) was introduced at the cartilage-bone interface and macroscopically adapted to the defect borders. The fibrinogen solution then was applied on top of the membrane and the cartilage compartment was filled to the point of bulging above the articular surface.

After in situ polymerization of the fibrinogen solution, the wounds were closed in layers. The animals were permitted free joint movement during the followup (10 weeks for rabbits and 8 weeks for miniature pigs), after which they were anesthetized again and then euthanized by administering a lethal dose of potassium chloride. The current study was approved by the State of Bern’s Animal Protection Commission, and experiments were done in accordance with their regulations. Rabbits and miniature pigs were housed in the animal care units at the authors’ institution and cared for by people who were trained professionally to undertake this task.
Tissue Processing and Analysis

Hind limbs were amputated and the knees were freed of soft tissues. The distal part of each femur was removed and cut into one (rabbits) or two (miniature pigs) pieces (upper and lower halves of the femoral groove) using a diamond band saw (Exact Medical Instruments, Oklahoma City, OK). Tissue pieces then were fixed by immersion in 2.5% (volume per volume) glutaraldehyde (Merck, Dusseldorf, Germany) and 2.5% (volume per volume) formaldehyde (Merck) solution (buffered with 0.1 mol sodium cacodylate (Merck pH7.4) for 2 to 4 days at ambient temperature. They then were dehydrated in ethanol and embedded in methylmethacrylate. Tissue slices, 1.2 mm in thickness, were cut perpendicular to the defect axis using a diamond saw (Leco, Warrendale, PA), glued onto polished Plexiglas object holders (Altumax France SA, Paris La Défense, France), milled to a thickness of approximately 100 to 150 μm with a Polycut E (Reichert-Jung, Heidelberg, Germany), polished, surface-stained with McNeil’s tetrachrome, Toluidine Blue O, and basic Fuchsin, and examined in a Vanox AH-2 microscope (Olympus, Tokyo, Japan). Five to six tissue slices were produced along the length of each defect; three of these were selected randomly for the semiquantitative histologic analysis.

The tissue that was laid down within the cartilage compartment of each defect was assessed qualitatively for its resemblance to the native structure, cell morphologic features, and staining characteristics of the intercellular matrix being used as the appropriate criteria. Within the bone compartment, bone and bone marrow tissues were identified using morphologic criteria. The proportion of bone tissue formed within the cartilage compartment was assessed semiquantitatively using the following grading scheme: 0%, no bone formed; 1% to 10% of the compartment’s volume occupied by bone; 11% to 50% of the compartment’s volume occupied by bone; greater than 50% of the compartment’s volume occupied by bone.

RESULTS

In all full-thickness defects that were filled with a chondrogenic matrix but not fitted with structural barriers between the cartilage and bone compartments, upgrowth of osseous tissue from the bone into the cartilage zone was observed (Figs 1,2). The degree of encroachment varied considerably, not only from location to location within a given lesion but also between defects. In some regions, newly formed bone extended all the way up to the articular surface. At the 8- (miniature pig) or 10-week (rabbits) postoperative juncture, bone tissue was predominantly of the woven trabecular kind. Active intramembranous ossification processes were observed in many places, whereas enchondral activity was apparent in only a few locations. No specific pattern of osteogenic activity was exhibited other than its overriding tendency to be upward directed.
away from the base of the bone compartment. Zones that were not occupied by bone or bone marrow were filled with connective tissue or newly formed cartilage. The semiquantitative analysis revealed that on average more than 50% of the volume of the cartilage compartment was occupied by osseous tissue (Table 1).

In virtually all of the specimens in which no structural barrier membrane had been fitted, the bone compartment was filled completely with osseous tissue; but at a few sites, some osteogenic activity still was apparent (Fig 2D). The bone tissue encountered was predominantly of the woven trabecular kind; in some regions, remodeling into lamellar bone had occurred (Fig 2).

Light microscopic inspection of defects fitted with cell-excluding barriers at the interface between cartilage and bone compartments revealed the placement of these to have been far from optimal, even though the membranes had appeared to be well seated macroscopically at the time of surgery. Not only did the height level of structural barriers deviate considerably (Fig 2C–F) from that of the natural cartilage-bone interface, but local undulations in their contours also were observed. Although Milipore® filters (rabbits) had become infiltrated thoroughly with tissue and/or had been partially resorbed (Fig 2E–F), Goretx® membranes (miniature pigs) were patent and unchanged in structure. Despite these imperfections, osteogenic activity was confined almost exclusively to the region underlying the membranes (Fig 2C–F). In instances in which a small quantity of bone upgrowth into the presumptive cartilage compartment was encountered (Table 1), it was restricted to the peripheral zones of incongruity between membrane and defect wall.

The compartment above the structural barrier was filled to a variable degree with cartilagelike and connective tissue, analytical details were whose composition published previously.10 Below the structural barrier, the presumptive bone compartment was filled with bone and cartilage tissue undergoing ossification. Although osseous tissue was predominantly of the woven trabecular kind, some portions had been remodeled into lamellar bone.

**DISCUSSION**

The introduction of a chondrogenic (TGF-β1-containing) matrix into shallow, full-thickness articular cartilage defects resulted in the upward progression of osteogenic processes from the base of the bone compartment into the cartilage compartment, wherein osteogenesis overpowered chondrogenic activity. This situation may arise partially because connective tissue ingrowth and vascularization processes, conducted within and from the bone compartment, proceed more rapidly and at a higher level of efficiency than does the chondrogenic activity within the cartilage compartment. However, TGF-βs are known to be capable of inducing not only cartilage, but also bone6 and connective tissue6,23 formation within a conducive environment containing osteoprogenitor and other types of precursor cells.16,30 The cartilage compartment is not dependent on vascular upgrowth from the bone compartment for a supply of precursor cells, because the synovial membrane and subsynovial tissue spaces can serve equally well as a source of such cells.10–12

The upgrowth of bone tissue into the cartilage compartment is an undesired effect that must be prevented. With this in mind, a cell-excluding membrane was inserted at the interface between bone and cartilage compartments, both of which were filled (before and after placement of the structural barrier, respectively), with the chondrogenic matrix-complex. As a result, blood vessels and perivascular cells, playing important roles in osteogenesis, were physically prevented from penetrating the cartilage compartment, which consequently showed little signs of osteogenic activity. The osteogenic process initiated by enchondral activity was arrested at the stage of cartilage differentiation in the absence of a blood vascular system. The indispensability of the
Fig 2A–F. (A) Light micrograph of a full-thickness articular cartilage defect created in a miniature pig and filled with a chondrogenic matrix. No structural barrier was inserted at the presumptive border between cartilage and bone compartments. Eight weeks after surgery, repair cartilage (R) occupies the upper half of the cartilaginous defect space. This tissue has a higher numerical density of cells and is more fibrous than native cartilage (N). The lower half of the cartilaginous defect space is occupied by repair bone tissue (T), which has grown upward from and completely fills the underlying bone compartment. Repair bone (T [dark red]) is principally of the woven type, whereas native subchondral bone tissue (S [light red]) is lamellar. Bar = 100 μm. (B) Light micrograph of a full-thickness articular cartilage defect created in a miniature pig and filled with a chondrogenic matrix. No structural barrier was inserted at the presumptive border between cartilage and bone compartments. Eight weeks after surgery, repair cartilage (R) occupies no more than approximately one-fifth of the depth of the cartilaginous defect space. As in Figure 2A, this tissue has a higher numerical density of cells and is more fibrous than native cartilage (N). The bulk of the cartilaginous defect space is occupied by repair bone tissue (T), which has grown upward from and completely fills the underlying bone compartment. Repair bone (T) is of the woven type, whereas native subchondral bone tissue (S) is lamellar. Bar = 200 μm. (C) Light micrograph of a full-thickness articular cartilage defect created in a miniature pig. The bony defect space was filled with a chondrogenic matrix and a porous structural barrier membrane (Goretx®) (continues)
presence of blood vessels for successful bone tissue formation is well known from studies on fracture repair. In the current model, it is the lack of blood vessels rather than the absence of osteogenic precursor cells that is responsible for the interruption of the osteogenic process.

The concept of a tissue compartment-separating membrane can be regarded as a variation of the guided tissue regeneration principle, which was developed in the 1960s. This principle was implemented first in orthopaedics; then, after appropriate modifications, in dentistry, with a view to building up the bony maxillary or mandibular ridge around implants. In these latter cases, bone tissue formation processes had to be shielded from the more rapid and osteoinhibitory growth of epithelial cells or subepithelial connective tissue.

Using the authors’ growth factor-based repair system, it was not surprising that bone tissue upgrowth was so exuberant in the absence of a barrier membrane, because TFG-βs are known not only for their orthotopic osteogenic activity but also for their inflammatory side effects. Transforming growth factor-β1 most probably precipitated an indirect angiogenic (vasculogenic) response within the zones of mild inflammation which facilitated osteogenesis. That bone tissue formation extended all the way up to the articular cartilage interface. The cartilaginous defect space then was filled with the same chondrogenic matrix. Eight weeks after surgery, the primitive mesenchymal type of repair tissue initially laid down within the cartilaginous defect space has been transformed only partially into cartilagelike repair tissue. The bony defect space contains repair bone tissue. The Gorex® membrane (arrowheads) prevented repair bone from growing upward into the cartilaginous compartment except at the periphery on the left side. This encroachment is a consequence of imperfect membrane fitment against the defect wall. The Gorex® membrane has not been resorbed and it is not infiltrated with bone tissue. The membrane was inserted at a level lower than the presumptive cartilage (N)-bone (S)-interface, which has led to a corresponding lowering of the border between the two repair tissue compartments. Bar = 500 μm. (D) Light micrograph of a full-thickness articular cartilage defect created in a miniature pig. The bony defect space was filled with a chondrogenic matrix and a porous structural barrier membrane (Goretex®) inserted at the presumptive cartilage-bone interface. The cartilaginous defect space then was filled with the same chondrogenic matrix. Eight weeks after surgery, the cartilage compartment contains cartilagelike and traces of mesenchymal-type repair tissue. The bony defect space contains repair bone tissue (T) and cartilage tissue (L) undergoing ossification (E). Repair bone (T), which is principally of the woven type, is typically more cellular than native subchondral bone tissue (S), which is lamellar. The Gorex® membrane has effectively impeded repair bone tissue from encroaching on the cartilage compartment except in peripheral regions (see Figure 2C for an explanation of this phenomenon). The membrane has not been resorbed. As in Figure 2C, the membrane was inserted at a level lower than the presumptive cartilage (N)-bone (S) interface, which has led to a corresponding lowering of the border between the two repair tissue compartments. Bar = 500 μm. (E) Light micrograph of a full-thickness articular cartilage defect created in a rabbit. The bony defect space contains repair bone tissue (T) and cartilage tissue (L) undergoing ossification (E). Repair bone (T), which is principally of the woven type, is typically more cellular than native subchondral bone tissue (S), which is lamellar. The Millipore® membrane has effectively impeded repair bone tissue from encroaching on the cartilage compartment except in peripheral regions (see Figure 2C for an explanation of this phenomenon). The membrane has not been resorbed. As in Figure 2C, the membrane was inserted at a level lower than the presumptive cartilage (N)-bone (S) interface, which has led to a corresponding lowering of the border between the two repair tissue compartments. Bar = 500 μm. (F) Light micrograph of a full-thickness articular cartilage defect created in a rabbit and treated in exactly the same manner as the lesion represented in Figure 2E. Ten weeks after surgery, the repair result is similar to that depicted in Figure 2E. N = native cartilage; R = repair cartilage; S = native subchondral bone; T = repair bone tissue. Bar = 200 μm.
cartilage surface in the absence of a structural barrier was, however, somewhat surprising, because the joint environment, especially the synovial fluid, usually is hostile to osteogenic activity. By using such an aggressive osteogenic substance as TGF-β1, the impediments were overcome.

Whether structural barriers are of practical use in growth factor-based repair strategies for full-thickness articular cartilage defects is a difficult question to answer. As a general rule, artificial membranes such as those used in the current study should be avoided, because they are either of poor biocompatibility or incompletely degraded within the biologic environment, as the current data show. In the current study, these membranes served a useful purpose in proving a principle, but they probably are not appropriate for clinical applications, because undegraded or only partially resorbed material affords a poor substratum for the desired firm anchorage of newly formed articular cartilage tissue. Additionally, histologic findings revealed that the fitting of membranes by macroscopic inspection alone during surgical intervention seldom resulted in a precise alignment with the natural cartilage-bone interface (Fig 2C, D). Local undulations in the surface contour of membranes also were encountered frequently (Fig 2C, D). Moreover, in peripheral regions, numerous focal incongruities between the membrane and lesion walls were observed, through which osteogenic cells and vascular buds may, and did pass, although to a limited degree.

As an experimental tool, the use of these structural barriers has not been exhausted. In the future, these membranes may be of value in assessing the relative importance of bone marrow-derived chondrogenic precursor cells in determining the structure and composition of repair tissue generated within the cartilage compartment of full-thickness defects, there being indications that cells arising from this source compromise the quality and durability of newly formed cartilage. Therefore, by cutting off one source of chondroprogenitor cells, and thereby ensuring that the cartilage compartment of full-thickness defects is furnished with a more homogeneous population of cells (derived from the synovium), these structural barriers may help us to ascertain whether the quality and durability of repair cartilage tissue are improved.

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References


TABLE 1. Degree of Bone Tissue Upgrowth into Cartilage Compartment

<table>
<thead>
<tr>
<th>Barrier Membrane</th>
<th>Number of Defects</th>
<th>Number of Defects With the Indicated Proportions of Bone Tissue Within the Cartilage Compartment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 28</td>
<td>0%</td>
</tr>
<tr>
<td>Without</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>With</td>
<td>28</td>
<td>14</td>
</tr>
</tbody>
</table>

The number of defects represents the total of those created in eight miniature pigs and six rabbits.


