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(54) Title: MATERIALS AND METHODS RELATING TO CARTILAGE REPAIR

(57) Abstract

The invention discloses use of an agent (such as a survival or growth factor) which inhibits the apoptotic response to cartilage injury and optionally a second agent (such as mitogenic competence factor, a cell cycle progression factor or an epidermal growth factor) which promotes the reparative response of cartilage in the preparation of a medicament for stimulating cartilage repair.

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Materials and Methods relating to Cartilage Repair.

Field of the invention.

The present invention relates to materials and methods involved in cartilage repair. Particularly, but not exclusively, it relates to the inhibition of apoptosis at the site of cartilage injury.

Background of the invention.

Articular cartilage has a poor reparative potential (Archer, C.W. (1994). Ann. Rheum. Dis. 53, 624-630) (Messner, K et al (1996) Acta Orthop. Scand. 67, 523-529) (Silver, F et al. Otolaryngologic Clinics of North America, 28, 847-864), but the reasons for this are not understood. The lack of tissue vascularisation and innervation are often cited as contributory factors although evidence for this is scant and details largely anecdotal. Nevertheless, poor cartilage repair poses enormous clinical problems particularly in young patients suffering from traumatic insult of major joints where prosthetic replacement is considered unsuitable. In older patients suffering from degenerative lesions of small joints such as in fingers, augmentation of an intrinsic repair response would greatly contribute to increased quality of life by facilitating every day manoeuvres through reduction in pain.

It has long been recognised that articular cartilage has a limited repair capacity (reviewed by Silver and Glasgold, 1995). Consequently, after traumatic insult or during degenerative changes in both osteo- and rheumatoid - arthritis, matrix loss is not compensated by renewed synthesis nor matrix elaboration. Furthermore, new matrix which is deposited bears little resemblance architecturally to that of normal articular cartilage.

The response of articular cartilage to injury is well documented (see review by Stockwell, 1979, of classical histological studies of cartilage repair). In essence, there are two intrinsic cellular responses; there is a band

of cell death adjacent to the cartilage lesion which is of constant width (approximately 100-150 μm). This 'necrosis' is apparent by day 1 and lasts up to two weeks depending on the system employed (Calandruccio and Gilmer, 1962; Mankin, 1962). Adjacent to this band, there is a proliferative response which is detected somewhat later than the initial cell death response and may extend through the entire cartilage depth (Mankin, 1962a; Dustmann, Puhl & Krempian, 1974; Bentley, 1974). These responses appear consistent whether the lesions are produced mechanically (Mankin, 1962), chemically and involving the disruption of the collagen network (Bentley, 1974), whether they occur in immature or mature cartilage (Dustmann, Puhl & Krempian, 1974), and whether the lesions are partial tissue depth or full thickness lesions violating the sub-chondral plate (Stockwell, 1979).

Reparative potential is always greatest during embryonic development but again, little is known about embryonic cartilage's ability for repair. Furthermore, the paucity in knowledge of joint development generally and, articular cartilage especially, must be regarded as a significant hindrance to progress, particularly since many reparative processes mimic those which have occurred during development of the tissue. In this context, it may be that during repair, the tissue reverts to a repertoire of responses which have been constrained by developmental processes such as differentiation and the establishment of the surrounding extracellular milieu. Clearly, the latter changes dramatically during repair of all tissues. In the case of cartilage, these features include renewed proliferation of chondrocytes and elevated matrix synthesis and the expression of a number of developmentally regulated glycosaminoglycan epitopes (Caterson, et al., 1990 and see Archer 1994 for a review).

Using the embryonic chick, one of the present inventors has overexpressed BMP-2, -4 and GDF5 in the developing wing using a replication competent retroviral

vector and found that overexpressed BMP-2 and -4 completely eradicated some joints apparently through massive stimulation of chondrogenesis (Duprez et al., 1996). In contrast, overexpressed GDF5 produced a larger skeleton showing normal differentiation but also possessed partially fused joints (Francis-West et al., 1996).

In most cell based studies of cartilage repair, failure of reparative and host tissue integration is widely cited to be problematical. The reasons for this failure are not known. Possibilities exist which may be related generally to the repair responses of cartilage. As stated above, the reasons for the poor reparative potential of cartilage are unknown but lack of vascularity or neural supply are often quoted, albeit, anecdotally.

Summary of the invention.

The present inventors have identified two major problems which exist in relation to cartilage repair. Firstly, there is a lack of integration of graft/repair tissue with the edge of the host defect, and secondly, the long-term survival of the repair tissue is generally poor, leading to eventual tissue breakdown.

Therefore, the present inventors have realised that there is a need for clinical strategies which seek to address either or both of these and/or other problems and improve the reparative potential of cartilage thereby leading to better intrinsic repair.

The present inventors have studied the response of embryonic chick sternal cartilage and immature/mature bovine articular cartilage to wounding *in vitro*. Interestingly, there is a remarkable similarity between the responses of embryonic and mature cartilage to wounding. In brief, the present inventors have found that the initial response involves programmed cell death, or apoptosis, at the lesion edge (extending between 80-200 μm) and is subsequently followed by elevated proliferation behind the apoptotic lesion or renewed proliferation as in the case of

mature tissue. Surprisingly, not all chondrocytes within the apoptotic lesion die and those which do not, invariably divide.

The present inventors have found that if they replace the excised tissue when making a wound, apoptosis is markedly reduced. Furthermore, injecting the wound with agarose which sets within the lesion also has an inhibitory effect on cell death suggesting that if diffusible factors are inhibited or slowed from leaving the wound edge a reduction in apoptosis can be achieved.

Therefore, at its most general, the present invention provides a method for stimulating intrinsic cartilage repair comprising the step of inhibiting the apoptotic response to injury. The apoptotic response to injury may be inhibited by the action of survival factors or by the action of a material which contacts the injury site thereby inhibiting the diffusible factors from leaving said injury site. Further, both actions may be used in combination.

As a first aspect of the present invention there is provided a medicament for stimulating intrinsic cartilage repair comprising one or more survival factors capable of inhibiting an apoptotic response to injury of said cartilage.

Use of one or more survival factors in the preparation of a medicament for stimulating intrinsic cartilage repair by inhibiting an apoptotic response to injury of said cartilage forms a second aspect of the present invention.

Preferably the medicament further comprises a pharmaceutically acceptable carrier.

In a further aspect, the present invention provides a method of treating cartilage injury in a patient, the method comprising administering a therapeutically effective amount of a survival factor to the patient.

In a further aspect, the present invention provides a pharmaceutical composition comprising a survival factor in combination with a pharmaceutical carrier.

In a further aspect, the present invention provides a

medicament for inhibiting an apoptotic response to injury of cartilage comprising a material which when in contact with the cartilage injury site prevents diffusible factors from leaving said site.

Use of such a material for inhibiting an apoptotic response to cartilage injury forms a further aspect of the present invention.

The material is preferably a pharmaceutically acceptable material, such as medical plastics or agarose. Preferably the material has the ability to change from a liquid to sold state to aid in its application to the injury site.

It will be apparent to the skilled person that the term 'injury' may be taken to include any form of change in the cartilage from the normal condition. For example, changes after traumatic insult and/or degenerative changes in both osteo- and rheumatoid - arthritis.

The term survival factor is taken to include any agents that has the ability to inhibit apoptosis, i.e. promote the survival of a cell. Preferred survival factors include growth factors that are known mitogens, for example, Insulin-like growth factor (IGF) I and II and Platelet-derived growth factor (PDGF). IGFs are particularly preferred due to their capabilities of rescuing fibroblasts (a connective tissue lineage cell) from proto-oncogene *c-myc* induced apoptosis (Harrington, 1994). Further, bone morphogenetic proteins (BMPs) may also be employed due to their importance in cartilage differentiation and morphogenesis (Storm et al., 1994; Kingsley et al., 1994; Francis-West et al., 1996).

Survival factors may also include agents used to prevent expression of genes associated with apoptosis. For example, disruption or down-regulation of proto-oncogene *c-myc* expression may inhibit the apopotic event. Such down regulation may be achieved by standard methods such as antisense mRNA, or disruption of associated promotor regions. Other proto-oncogenes known to suppress apoptosis

may also be used, for example, *Bcl-2*.

The aspects of the present invention may further comprise a promoting factor or the use of a promoting factor for optimising the reparative response of the intrinsic cartilage. These factors stimulate the rescued cells to participate in the reparative response. In other words, they may enhance the synthetic potential and proliferation of the rescued and surrounding chondrocyte cells in order to optimise the reparative response. These promoting factors may include mitogenic competence factors, e.g. basic fibroblast growth factor,(bFGF) and cell cycle progression factors such as IGFs (which co-act as synthetic stimulators) and epidermal growth factors (EGF). Each of these factors may be administered in their natural form or in a recombinant form. The promoting factors may be used in combination with BMP 2, 4, or 7 and/or GDF-5 recombinant proteins.

In a further aspect, the present invention provides a method of screening for survival factors capable of inhibiting the apoptotic response to cartilage injury, the method comprising

(a) contacting a candidate survival factor with wounded explant sections of cartilage in any assay for the biological property of apoptotic inhibition;

(b) measuring the apoptotic inhibition property; and

(c) selecting candidate survival factors which inhibit apoptosis in wounded cartilage.

As mentioned above, survival factors may be formulated in pharmaceutical compositions. These and/or promoting factors compositions may comprise, in addition, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material may depend on the route of administration. The most preferred route of administration is for the carrier to be applied

topically at the site of injury. However, other routes of administration will be known to the skilled person. For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required.

Alternatively, and more preferably, the carrier may comprise a material, such as medically acceptable plastics, e.g. biopolymers, collagen gel or agarose, which may be injected directly into the wound site in cartilage. The material, for example agarose, may be loaded with one or more survival factors such as IGF I, PDGF, a variety of BMP's. Further, anabolic growth factors may also be included in the agarose, for example, TGFβ or FGFs. All of these factors may in applied singly or in combination. The factors are preferably mixed at low temperature setting agarose in the molten state (41°C) and injected directly into the wound site in cartilage. Once cooled the agarose will set in the injury site and the factors will diffuse out into the surrounding cells.

The pharmaceutically useful compound according to the present invention that is to be given to an individual, administration is preferably in a "prophylactically effective amount" or a "therapeutically effective amount" (as the case may be, although prophylaxis may be considered therapy), this being sufficient to show benefit to the individual. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical

doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 16th edition, Osol, A. (ed), 1980.

A pharmaceutical composition may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

Preferably the survival factor and/or the promoting factors will be in form of a cream or ointment that may be applied cutaneously. The cream or ointment preferably contains a diffusing agent that allows the factors to penetrate subcutaneous and diffuse to the site of injury.

Aspects and embodiments of the present invention will now be illustrated, by way of example, with reference to the accompanying figures, Further aspects and embodiments will be apparent to those skilled in the art.

Brief description of the Figures.

Fig. 1. Seven-day bovine articular cartilage after wounding at day 0 (A,B), day 1 (C,D), day 5 (E,F), AND DAY 20 (G,H). Phase contrast images (A,C,E,G) and corresponding fluorescent TUNEL images (B,D,F,H) show that at day zero (4h) only background labelling was observed. The apoptotic lesion was most evident between days 1 and 5 but was still detectable after day 20 although by this time dead chondrocytes occupying eh lesion edge did not label. Scale bar 100 μm.

Fig. 2. Tunel analysis after 5 days post-wounding. A) Apoptosis next to lesion edge (arrowed); b) when the lesion I filled with alginate, a marked reduction in apoptosis is observed.

Fig. 3 and 4. Ultrastructural appearance of normal (3) and apoptotic chondrocytes (4) 24h after wounding. Note unclear condensation and disrupted cytoplasm compared

with the healthy chondrocyte in (3).

Detailed description.

The present inventors have employed two model systems to study the cell and molecular responses of cartilage to wounding.

First, they have used the embryonic chick sternum. This model has a number of advantages. It is a hyaline cartilage (unlike chick articular cartilage which is partly fibrous, Craig et al., 1987). The sternum comprises two distinct chondrocyte sub-populations; the anterior portion which undergoes hypertrophy and subsequently ossifies and the posterior portion which is a permanent cartilage. Thus the sternum has similarities to articular cartilage but has the advantage that the permanent cartilages and the calcifying cartilage can be separated. Additionally, the perichondrium of the sternum is easily removed in entirety making it more akin to articular cartilage. Lastly, chick sterna are easily maintained in culture, indeed, increasing their size by some 4-fold over 10 days.

Second, they have wounded full-depth explants of bovine articular cartilage from the metacarpal-phalangeal joints of 7 day and mature animals and monitored the responses over 21 days in culture. A comparison of the results is given below and reveals a consistency in the responses observed.

Embryonic chick sterna: Sterna from day 17 embryos were removed aseptically, and the adhering muscle removed with the aid of watch-makers forceps. The perichondrium was removed in entirety. A wound @ 400μm was made through the permanent cartilage of the posterior sternal keel with a seeker. The sterna were then placed in submerged organ culture and maintained in medium comprising Hams F12/DMEM (1:1), 10% foetal calf serum (FCS), 1.4mM L-glutamine, ascorbic acid (50 $\mu g ml^{-1}$) and 1% antibiotic/antimycotic. Medium was changed every 2 days and cultures were maintained for up to 14 days. At time intervals from

wounding to 14 days, specimens were harvested for a variety of analyses.

Apoptosis: Using nick end labelling (TUNEL; and confirmed using fluorescently labelled Annexin V) the inventors were able to detect fluorescently labelled nuclei within 10 mins. of wounding around the immediate lesion edge. This pattern expanded to comprise a band of apoptosis ranging from 80-100 μm after two days *in vitro*. The apoptotic nature of the chondrocyte death was confirmed at the ultrastructural level showing classic signs of programmed cell death including chromatin condensation and cell shrinkage (Fig.3 and 4). After 7 days, detection of apoptotic nuclei by TUNEL declined leaving acellular areas around the lesion. After 14 days *in vitro*, there was little evidence of repair.

Bovine articular cartilage: One cm^2 full depth cartilage explants were taken from the metacarpal-phalangeal joints of 7 day cattle. Subsequently, 500 μm full depth lesions were made with a trephine and the explants cultured for up to 21 days in media identical to that described above.

The present inventors conducted a similar TUNEL analysis as that described for embryonic cartilage. In essence, the response of articular cartilage to wounding was the same differing only in time-scale. Accordingly, the first fluorescently labelled nuclei appeared by 8 hours post-wounding. The first apoptotic chondrocytes were observed next to the lesion but subsequently spread inwards to occupy a band @ 100 μm by 24 hours and 150 μm within 5 days (Fig. 1). This pattern of TUNEL labelling did not change significantly over the next 4 days. Interestingly, incubation of wounded explants in the present of 5 μgml^{-1} cyclohexamide had no effect on apoptosis indicating that new protein synthesis (and by implication gene transcription) was not required to initiate the apoptotic response. Similarly, both light microscopical and ultrastructural examination of the lesion area again showed

cellular features consistent with apoptosis as described above.

In another set of experiments, the present inventors replaced the core of tissue back into the wound site immediately after removal with the trephine. Interestingly, the majority of the apoptotic response was abolished except in regions where there was a significant gap (100 μm.>) between the tissue interfaces. Indeed, there was a graded response such that where the two tissue interfaces were closely opposed and there was physical contact, there was little or no apoptosis. As a definite gap appeared, an apoptotic response could be observed which increased with gap size.

In Vitro Studies

Cartilage explants ($1cm^2$) may be excised from metacarpo/metatarsophalangeal joints steers which are skeletally mature. Wounds (1.7mm diam.) are made in the centre of the explant with a trephine and maintained in serum-free (Hams F12/DMEM, 1:1) submerged culture.

Early Cellular response: To investigate the immediate cellular response, explants can be harvested at 1, 2, 4, 8, 12, 16 and 24 and thereafter at 48 and 72 hr. The expression of factors can then analysed at the gene and protein level.

Gene transcription can be investigated by quantitative reverse transcription polymerase chain reaction (RT-PCR) and *in situ* hybridization of mRNA. Real time quantitative PCR can be performed on 24 samples simultaneously within 15 mins. This is a highly sensitive technique ideal for determining changes in expression of rare messages in small tissue samples. In order to investigate the distribution of the differential expression of the various components within the cartilage samples, *in situ* hybridization can be carried out using riboprobes. Probes may be made by cloning cDNAs derived from RT-PCR into Bluescript vectors and, using the two RNA polymerase promotors, the sense and

antisense probes can be generated. The riboprobes are either labelled with digoxigenin and detected by immunohistochemical techniques on thin sections or are labelled fluorescently so that thicker sections can be viewed in the confocal microscope which enables 3D reconstruction of the tissue architecture. Where bovine sequence data is not available, PCR primers can be designed covering regions of the gene that exhibit strong homology across a number of species. PCR products may be sequenced to confirm their identity.

The distribution of the expressed protein is ascertained by immunocytochemistry of protein either by indirect immunofluorescence or silver enhancement of colloidal gold conjugated secondary antibodies (Archer et al (1996) J. Anat. 189, 23-35; Morrison et al (1996) j. Anat. 189, 9-22). Both wax-embedded and cryo-processed material may be used as appropriate for the epitopes in question. Sections can be viewed by conventional light and fluorescent microscopy and scanning confocal microscopy. Specificity of the immunocytochemistry is confirmed by Western blotting. Experience with other projects utilising bovine material have found good species cross-reactivity with most antibodies.

The present applicants wished to investigate the relationship between the band of cell death which occurs at the lesion edge and the proliferation which occurs immediately behind it. One possibility is that wounding stimulates a proliferative response but, for reasons unknown, those cells at the lesion edge enter the cell cycle but apoptose. Consequently, the present inventors probe a time course of wounded explant sections for specific markers of G1 entry such as cyclin D1/D3 and cdk4 to determine if chondrocytes near the lesion edge do enter the cell cycle prior to apoptosis which may have important implications for the experiments on the rescue of these cells (i.e. if the cells are rescued, they will go on to proliferate and contribute to the repair). Other

experiments may include the probing for other cell cycle phase cyclins to determine at what phase the cells enter apoptosis.

It is also possible to probe for those proteins that are known to either promote or inhibit cell death. Consequently, this will allow the mapping of the temporo-spatial expression of known apoptosis promoters such as *fas*, *bax*, ICE (interleukin-converting enzyme) together with apoptosis suppressors *bcl-2* and *bcl-x*. The distribution of these factors may also be correlated with the expression of the early response genes *c-myc* and *c-fos*.

C-myc expression can also activate other signalling pathways involving Ras which can suppress or promote apoptosis depending on downstream effectors (Kauffmann-Zeh et al (1997) Nature, 385, 544-548). Such pathways may be important in the apoptosis/proliferative response the present inventors have seen in wounded cartilage. It is also known that oxidative products can stimulate chondrocytes (Ishizaki Y., et al (1994) J. Cell Biol., 126, 1069-1077). And that death can be prevented by the presence of anti-oxidants such as cysteine.

The present inventors have shown that a number of growth factors (both BMPs and Growth and Differentiation factors, GDFs) are up regulated in the vicinity of a wound within hours. This allows the mapping of the spatio-temporal pattern of factors known to regulate cartilage metabolism such as TGFβ, IGF I and II, and BMPs 2, 4, 5, 6 and 9. Such an analysis is useful as it can indicate an early stage modulation in phenotype. For example, BMP 6 is specific for hypertrophic chondrocytes whilst up-regulation of BMP 2 may indicate a switch to a more immature state. Furthermore, it has been shown that BMPs can induce other factors such as FGFs which have been reported to promote cartilage repair (Cuevas P., et al (1988) Biochem. Biophys. Res. Comm. 156, 611-618). Conversely, it has also been shown that some BMPs are potent promoters of apoptosis (Graham, A., et al (1994) Nature 372, 684-686). Data from

these development of articular cartilage studies also suggest that only areas that are contributing actively to growth are immunopositive for TGFβ and IGFs (Archer, C. W., et al (1994) J Anat. 184, 447-456). The data from these descriptive studies are important as they indicate on a temporal basis, possible signalling pathways which are activated as a result of wounding and indicate intervention points and/or factors to be applied.

Within a traumatised joint it is unlikely that cartilage damage will occur in isolation and any soft tissue damage will be accompanied by inflammation. Consequently, physiological levels of the cytokines IL-1 and TNFα can be added to the explant systems and the effects on the early repair responses can be analysed and compared and contrasted with non-cytokine added controls. The data can provide important information as to the possible modulatory effects these cytokines may have on early response gene expression which in turn may have significant downstream effects.

Secondary response and attempted repair: The above data can also be correlated with the downstream reparative responses on matrix synthesis (collagen, proteoglycan and glycoprotein expression including changes in integrin expression).

Effects of growth factors: The current data shows that the apoptotic lesion may result from loss of survival factors from the lesion edge. Consequently, agarose can be loaded with known survival factors such as IGF1, PDGF, a variety of BMPs together with anabolic growth factors such as TGFβ and FGFs. These factors can be applied singly and in combination and the effects on gene expression and ultimately apoptosis analysed. Factors are mixed with low temperature setting agarose in the molten state (41°C) and injected directly into the wound site in cartilage held over ice thus setting the agarose. The data indicates

factors that can be used therapeutically to inhibit apoptosis and undoubtedly enhance tissue integration during repair.

CLAIMS:

1. Use of a first agent which inhibits the apoptotic response to cartilage injury and optionally a second agent which promotes the reparative response of cartilage in the preparation of a medicament for stimulating cartilage repair.

2. Use according to claim 1 wherein the first agent is a survival factor.

3. Use according to claim 1 or claim 2 wherein the first agent is a growth factor.

4. Use according to claim 3 wherein the first agent is a mitogenic growth factor.

5. Use according to any one of claims 1 to 4 wherein the first agent is selected from insulin-like growth factor (IGF) I, IGF II and platelet-derived growth factor (PDGF).

6. Use according to claim 1 or claim 2 wherein the first agent is a bone morphogenetic protein (BMP).

7. Use according to claim 6 wherein the BMP is BMP2, 4 or 7.

8. Use according to claim 1, 2 or 3 wherein the first agent is a growth and differentiation factor (GDF).

9. Use according to claim 8 wherein the first agent is GDF-5.

10. Use according to claim 1 or 2 wherein the first agent downregulates or prevents expression of a gene associated with the promotion of apoptosis or downregulates or prevents the activity of a polypeptide encoded by a said

gene.

11. Use according to claim 10 wherein the gene is the proto-oncogene *c-myc*.

12. Use according to claim 10 wherein the survival factor is capable of rescuing fibroblasts from apoptosis induced by the proto-oncogene *c-myc*.

13. Use according to claim 1 or claim 2 wherein the first agent upregulates expression of a gene associated with the suppression of apoptosis or enhances the activity of a polypeptide encoded by a said gene.

14. Use according to claim 13 wherein the gene is the proto-oncogene *Bcl-2*.

15. Use according to any one of claims 1 to 14 wherein a said second agent is used in the preparation of said medicament.

16. Use according to claim 15 wherein said agent is a promoting factor which can stimulate cells around the site of cartilage injury to participate in the reparative response.

17. Use according to claim 16 wherein the cells are chondrocytes.

18. Use according to any one of claims 15, 16 or 17 wherein the second agent is a mitogenic competence factor, a cell cycle progression factor or an epidermal growth factor.

19. Use according to claim 18 wherein the mitogenic competence factor is basic fibroblast growth factor (bFGF).

20. Use according to claim 18 wherein the cell cycle progression factor is an IGF.

21. Use according to any one of claims 1 to 20 wherein a said agent is a non-recombinant protein.

22. Use according to any one of claims 1 to 20 wherein a said agent is a recombinant protein.

23. Use according to any one of claims 1 to 22 wherein the medicament is formulated for direct application to the injury site.

24. Use according to any one of claims 1 to 23 wherein the medicament comprises a medically acceptable plastics, biopolymer, collagen gel or agarose.

25. Use according to any one of claims 1 to 24 wherein the medicament comprises an anabolic growth factor.

26. Use according to claim 25 wherein the anabolic growth factor is TGFβ or a FGF.

27. Use according to claim 1 wherein the first agent is a material which when in contact with the cartilage injury site reduces the rate at which diffusible factors leave the injury site.

28. Use according to claim 27 wherein the material can change from a liquid state to a solid or semi-solid state.

29. Use according to claim 27 or claim 28 wherein the material comprises a medically acceptable plastics, biopolymer, collagen gel or agarose.

30. Use according to any one of claims 1 to 29 wherein the medicament is for treatment of traumatic cartilage injuries

and degenerative cartilage changes associated with osteo- and rheumatoid- arthritis.

31. A method of screening for an agent capable of inhibiting the apoptotic response to cartilage injury which comprises the steps of:

(a) contacting a candidate survival factor with wounded explant sections of cartilage in any assay to measure apoptotic inhibition;

(b) measuring any apoptotic inhibition; and

(c) selecting candidate survival factors which inhibit apoptosis in wounded cartilage.

32. A method of treating cartilage injury or degeneration in a patient which comprises administering to the patient a therapeutically effective amount of a medicament which may be made in accordance with any one of claims 1 to 30.

33. An article of manufacture which comprises a medicament which may be made in accordance with any one of claims 1 to 30 and instructions directing use of the medicament for treatment of cartilage injury or degeneration.

Fig.1.

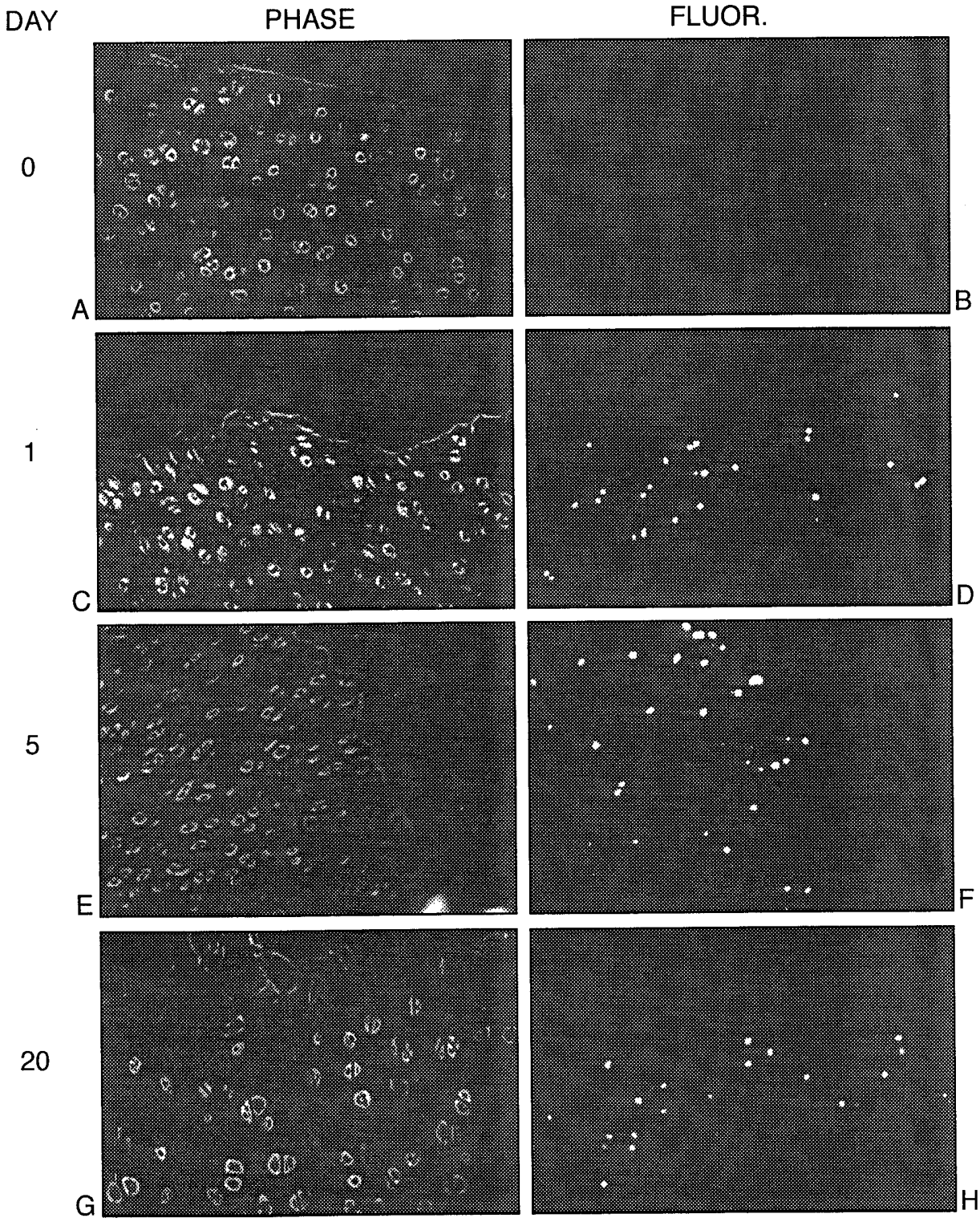


Fig.2.

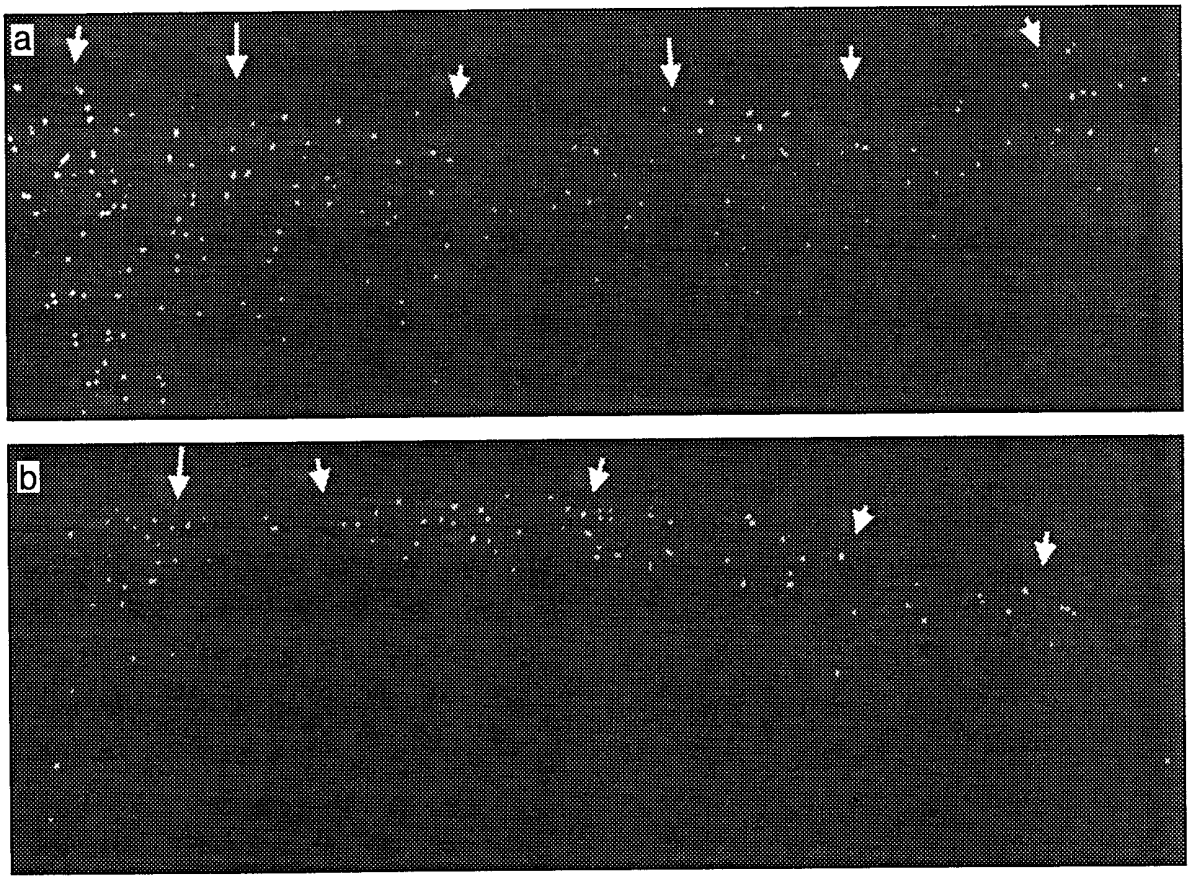
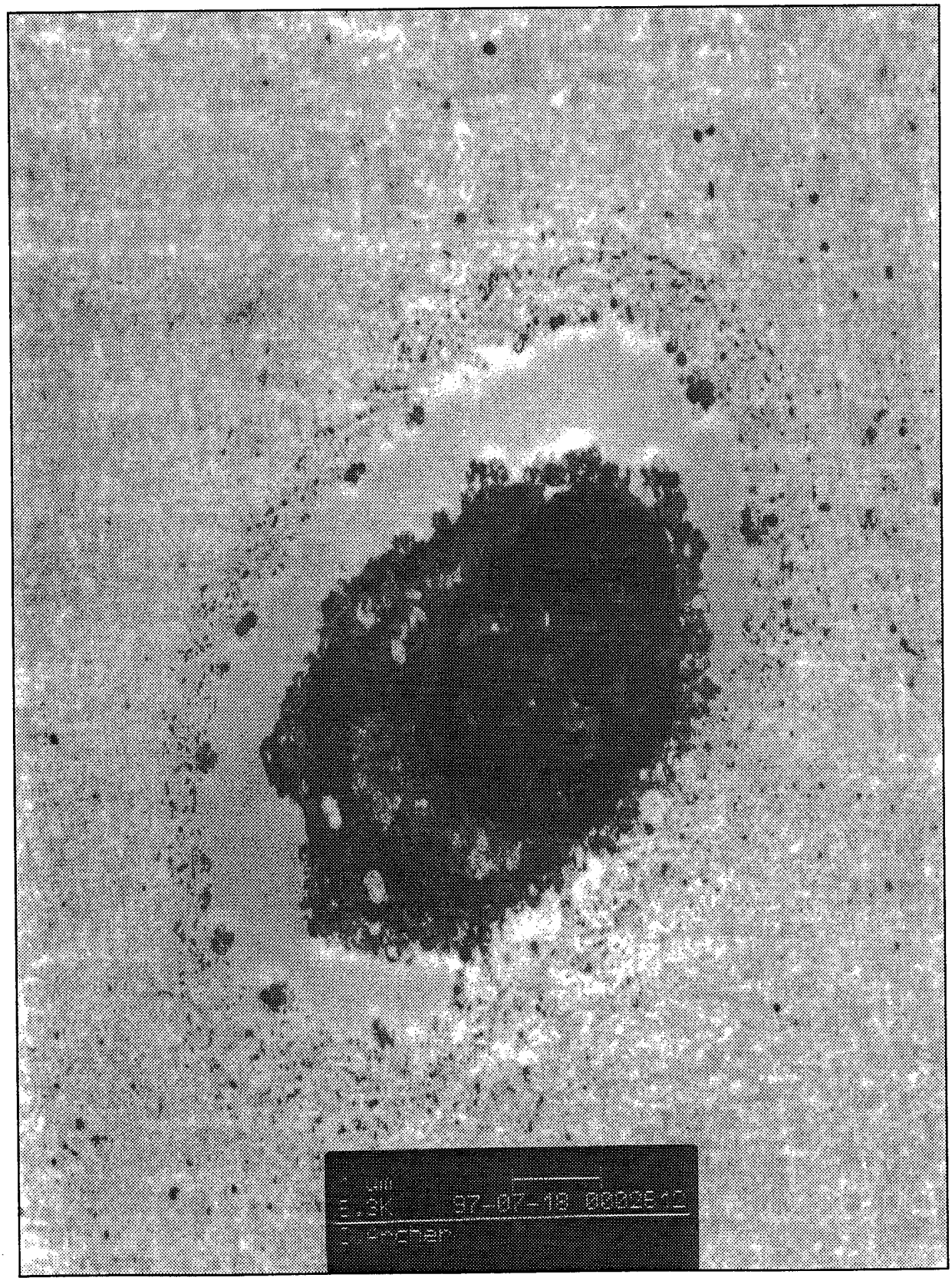


Fig.3.

Fig.4.



INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/GB 98/03089

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/18 A61K38/30

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 368 858 A (HUNZIKER E.) 29 November 1994 see the whole document ---	1-5,10, 32,33
X	HARVEY A K ET AL: "Differential modulation of degradative and repair responses of interleukin-1-treated chondrocytes by platelet-derived growth factor." BIOCHEMICAL JOURNAL, (1993 MAY 15) 292 (PT 1) 129-36, XP002092480 see the whole document ---	1,10,32, 33
A	TRIPPEL S B: "Growth factor actions on articular cartilage." JOURNAL OF RHEUMATOLOGY. SUPPLEMENT, (1995 FEB) 43 129-32, XP002092481 see the whole document ---	1-33
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 February 1999	18/02/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Moreau, J

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/GB 98/03089

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	DUPREZ D ET AL: "Overexpression of BMP -2 and BMP -4 alters the size and shape of developing skeletal elements in the chick limb." MECHANISMS OF DEVELOPMENT, (1996 JUL) 57 (2) 145-57, XP002092482 see the whole document ---	1-33
A	WO 97 35607 A (GENETICS INSTITUTE) 2 October 1997 see the whole document -----	1-33

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INTERNATIONAL SEARCH REPORT

...ernational application No.
PCT/GB 98/ 03089

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 32 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter onal Application No
PCT/GB 98/03089

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		AU 2329097 A	17-10-1997
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