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DESCRIPTION

FIELD OF THE INVENTION

[0001] This invention relates to the treatment of musculoskeletal fibroproliferative disorders such as fibromatosis and, in particular, Dupuytren's disease. In particular it relates to a composition or therapeutic agent or to a combination of such compositions or therapeutic agents for the treatment, prophylaxis or prevention of progression of musculoskeletal fibroproliferative disorders, especially Dupuytren's disease, to the use of such composition/therapeutic agent or combination of compositions/therapeutic agents for the treatment, prophylaxis or prevention of progression of musculoskeletal fibroproliferative disorders, especially Dupuytren's disease and to a method of treating musculoskeletal fibroproliferative disorders, especially Dupuytren's disease.

BACKGROUND OF THE INVENTION

[0002] Dupuytren's disease, which is alternatively known as palmar fibromatosis (or in its established disease state Dupuytren's contracture), is a disease associated with the build up of extracellular matrix materials such as collagen on the connective tissue of the hand (the palmar fascia) causing it to thicken and shorten with the physical effect of causing the fingers to curl, most commonly the ring finger and little finger.

[0003] Dupuytren's disease affects approximately 5% of the white Caucasian population. The commonest manifestation is progressive flexion contracture of the digits of the hand, resulting in significantly compromised function. It affects both males and females, but the incidence is higher in males.

[0004] The causes of Dupuytren's disease are not well understood and underlying disease is not currently curable.

[0005] Treatment of Dupuytren's disease has traditionally been invasive surgical techniques. Primarily, the treatment has involved surgical excision of the offending tissue. In severe or recurrent disease, the surgical excision may be combined with excision of the overlying palmar skin and resurfacing of the cutaneous defect with full-thickness skin graft. Surgery is typically followed by prolonged rehabilitation, usually lasting 3 months and complications have been reported in up to 20% of cases. Such surgical correction is the mainstay treatment of later stage disease when secondary changes to tendons and joints have developed. A less invasive surgical intervention is needle fasciotomy in which the fibrous bands (contractures) in connective tissue are divided using the bevel of a needle.

[0006] Enzymatic cleavage of the affected tissue has been the focus of development to reduce

invasiveness associated with surgery and improve recovery time. This approach has led to trials of collagenase. A bacterial collagenase, Clostridial collagenase, has been granted FDA approval as Xiaflex™ to Pfizer and Auxilium. USRE39941, US5589171 and US6086872 describe the use of bacterial collagenase for the enzymatic cleavage of connective tissue in the treatment of Dupuytren's disease. Bacterial collagenases suffer from certain disadvantages: for example lack non-selective cleaving of various collagen materials including collagen type IV associated with blood vessels; and, in the case of Xiaflex™, possible allergic reactions and potential immunogenicity; and administration may cause haemorrhage whilst the prolonged activity of collagenase limits the dose that can be administered locally due to risk of side effects as the drug disperses.

[0007] WO 2010/102202 describes a novel temperature sensitive recombinant collagenase in which the activity is observed at significantly below body temperature, but which is comparatively inactive at body temperature. Thus Dupuytren's syndrome can be treated by administering such recombinant collagenase at lower temperatures, which it is claimed restricts the duration of activity, increases the possible local dose and reduces collagenase-related side effects.

[0008] To date collagenase therapies have appeared relatively effective in treatment of contracture of the metacarpophalangel joint, whilst the correction of proximal interphalangeal joints has been much less satisfactory. Furthermore, as with surgical interventions, recurrence can be expected, but in the case of early collagenase trials, which involve enzymatically cutting the cord, recurrence is high, especially for disease affecting the proximal interphalangeal joint.

[0009] Other non-surgical treatments that have been proposed include application of vitamin E cream applied as topical therapy, ultrasonic therapy and low-dose radiation therapy (for slowing the progression of early stage disease), such as X-rays and electron beam therapy.

[0010] Most research for treatments of Dupuytren's disease has focused on detecting pre-disposition to Dupuytren's (e.g. US-A-2004/0161761) and on the extracellular matrices produced, which has resulted in the collagenase-based treatments. There has been very little conclusive insight into potential treatments gained from studies into the biochemical pathway of Dupuytren's disease.

[0011] WO-A-2005/07419 describes compositions and methods relating to conditions associated with reduced mobility or loss of function and articulation arising from scar tissue and abnormal tissues associated with burns, ischemia, metabolic disorder, injury, surgical procedures. A large list of conditions and of compound types are included with paclitaxel formulations exemplified.

[0012] Khanna et al in 'Infliximab may be effective in the treatment of steroid-resistant eosinophilic fasciitis: report of three cases', Rheumatology, 49(6), p1184-1188, describes three cases in which patients with established symptoms of EF are treated, after steroid therapy, with infliximab with reduction in symptoms shown.

[0013] There remains a need for novel therapeutic intervention in the treatment and/or prevention of (e.g. progression of) Dupuytren's disease and other musculoskeletal fibroproliferative disorders.

[0014] The present inventors have found that administration of a TNF-α antagonist is surprisingly effective on its own or in combination with another Dupuytren's treatment in preventing the progression of early stage Dupuytren's disease and reversing later stage Dupuytren's disease as well as reducing recurrence of disease.

PROBLEM TO BE SOLVED BY THE INVENTION

[0015] There remains a need for improvements in the treatment of Dupuytren's disease and other musculoskeletal fibroproliferative disorders, particularly fibromatosis and like diseases including and preferably selected from plantar fibromatosis (or Ledderhose's disease), adhesive capsulitis (frozen shoulder) and Peyronie's disease (fibromatosis of the penis).

[0016] It is an object of this invention to provide a composition and method for the treatment or prophylaxis (e.g. prevention of progression or recurrence) of one or more of Dupuytren's disease, plantar fibromatosis, adhesive capsulitis and Peyronie's disease.

SUMMARY OF THE INVENTION

[0017] In accordance with a first aspect of the invention, there is provided a composition for use in the treatment of an early disease state musculoskeletal fibroproliferative disorder, the composition comprising a therapeutic, prophylatic or progression-inhibiting effective amount of a TNF-α antagonist, wherein the disorder is characterized by the presence of indications of disease but in the absence of significant contracture.

[0018] In a second aspect of the invention, there is provided use of a TNF-α antagonist in the manufacture of a medicament for the treatment of an early disease state musculoskeletal fibroproliferative disorder, wherein the disorder is characterized by the presence of indications of disease but in the absence of significant contracture.

ADVANTAGES OF THE INVENTION

[0019] The compositions and methods of the present invention enable progression of Dupuytren's (and other fibromatosis and like disease) to be slowed or halted. It has particular advantages in that early disease state Dupuytren's (and other fibromatosis and like disease) can be prevented from progressing to an established state disease and avoid surgical

intervention and the associated recovery time.

[0020] Compositions and methods of the present invention enable the treatment, prevention and inhibition of progression of musculoskeletal adhesions such as adhesive capsulitis and tendon adhesion (such as adhesion of the proximal interphalangeal joint in established disease state Dupuytren's disease).

BRIEF DESCRIPTION OF THE DRAWINGS

[0021]

Figure 1 shows images of nodules and cord in an intraoperative view;

Figure 2 is a chart showing a distribution of α-SMA rich cells in tissue excised from different parts of diseased Dupuytren's tissue;

Figure 3 is a photograph of a Culture Force Monitor used in *in vitro* experiments to assess contractile behaviour of cells in a three-dimensional collagen matrix;

Figure 4 shows graphs of contraction versus time for different cell cultures (in a Culture Force Monitor of Figure 3) over a 24 hour period;

Figure 5 is a chart showing the mean rate of contraction for cells of different tissue derivation from Dupuytren's patients;

Figure 6 charts mean rate of contraction for cells from different tissue from Dupuytren's patients, the amount of messenger RNA, amount and intracellular distribution of the contractile protein α-smooth muscle actin (a-SMA);

Figure 7 shows images of inflammatory cells (macrophages, CD68, mast cells, mast cell tryptase) in Dupuytren's nodule and cord;

Figure 8 shows images of sections of Dupuytren's cord samples stained for α-SMA and RAGE;

Figure 9 shows images of sections of skin samples from Dupuytren's patients stained for RAGE and showing differential distribution in non-palmar and palmar skin;

Figure 10 provides charts showing FACS analysis for cells deriving from nodular, non-palmar and palmar skin fibroblasts for expression of RAGE;

Figure 11 provides charts showing FACS analysis for cells deriving from matched sets of non-palmar and palmar skin for expression of RAGE;

Figure 12 provides a chart of contractility for palmar skin dermal fibroblasts treated with TNF-α, HMGB1 or TGF-β1;

Figure 13 is a chart showing the contractility of primary passage nodule-derived cells (from a

Dupuytren's patient) and in the presence or absence of anti-TNF-a.

Figure 14 is a chart showing the contractility of palmar dermal fibroblasts exposed to AGEs

Figure 15 is a chart showing TNF-α production from human monocytes exposed to certain DAMPs. LPS is a PAMP, shown as positive control.

Figure 16 is a chart showing TNF-α production from human monocytes exposed to certain DAMPs in the presence of certain receptor blockers.

Figure 17 is a chart showing TNF-α production from murine bone marrow cells in the presence of S100 A8 in turn in cells having TLR-4 deficiency and MyD88 deficiency.

Figure 18 is a chart showing TNF-α production from human monocytes exposed to a certain DAMP alone and in combination with LPS.

Figure 19 is a schematic of proposed mechanism of the role of trauma and Alarmins in the pathogenesis of Dupuytren's disease.

Figure 20 is a chart of fold induction in contraction of palmar fibroblasts on exposure to supernatant from monocytes stimulated with AGEs, with or without anti-TNF-a.

Figure 21 is a chart showing the contractility of palmar dermal fibroblasts and dose response to TGF-β1.

Figure 22 is a chart showing that palmar dermal fibroblasts from patients with Dupuytren's disease exposed to TNF-α become more contractile whereas non-palmar dermal fibroblasts do not.

Figure 23 is a chart showing a dose related inhibition of contractility of cells form Dupuytren's nodules exposed to TNF-α antagonist.

Figures 24 is an image showing cells from a Dupuytren's nodule in a 3-dimensional collagen gel exposed only to control IgG antibody stained with phalloidin and exhibiting alignment in axis of stress.

Figures 24b and 24c are images showing cells from a Dupuytren's nodule stained with phalloidin and α-SMA respectively, treated with a TNF-α antagonist, showing loss of alignment in axis of stress.

Figure 25 is a schematic of a proposed role of advanced glycation end products, injury and alarmins in the pathogenesis of fibroproliferative disorders, highlighting the key role of TNF-α in the final common pathway.

DETAILED DESCRIPTION OF THE INVENTION

[0022] The invention provides for an improved treatment of a musculoskeletal fibroproliferative disorder, especially Dupuytren's disease (or other fibromatosis and like disease such as plantar fibromatosis, adhesive capsulitis and Peyronie's disease), which comprises administration to a patient in need thereof, showing signs of early disease state, a therapeutic, prophylactic or progression-inhibitive amount of a TNF-α antagonist. Further, the invention provides, by administration of a TNF-α antagonist to a patient having or showing signs of developing Dupuytren's disease, prevention of disease manifestation and/or progression, optionally as an adjunctive (or concomitant) therapy to a primary surgical intervention (e.g. a fasciotomy or fasciectomy) or primary therapeutic treatment (e.g. an extracellular matrix degradation, depletion or cleaving agent, such as a matrix metalloproteinase or collagenase). Still further, the invention provides, by administration of a TNF-α antagonist to a patient, prevention of recurrence of disease as an adjunctive therapy to primary surgical intervention or therapeutic treatment of established disease.

[0023] Musculoskeletal fibroproliferative disorders are characterized by excessive or uncontrolled production of extracellular matrix in association with a musculoskeletal structure, often associated with contraction in later stage disease. As mentioned above, musculoskeletal fibroproliferative disorders include fibromatosis disorders. (The terms 'musculoskeletal fibroproliferative disorders' and 'fibromatosis disease' may be used interchangeably herein, where the context allows). The present invention is concerned with the treatment and, in particular, the inhibition of progression and recurrence (e.g. after primary treatment by surgery or therapy) of such diseases. In particular, the present invention is concerned with diseases selected from Dupuytren's disease, plantar fibromatosis, adhesive capsulitis and Peyronie's disease, especially Dupuytren's disease. The remainder of this document will discuss compositions and methods for treatment of musculoskeletal fibroproliferative disorders generally, with specific reference to Dupuytren's disease. Where the context allows, it should be understood that the disclosure may be read also with the generality or other specified diseases in place of Dupuytren's disease.

[0024] It is believed that the effectiveness of TNF-α antagonists in the treatments of the present invention is due to the dependence on TNF-α of differentiation of fibroblasts into myofibroblasts, which are understood to be the main culprits in contractile activity and induction of uncontrolled extracellular matrix generation in Dupuytren's disease (and other fibromatosis diseases). The inventors have demonstrated this TNF-α dependence and has identified antagonists of TNF-α as viable therapeutics (contrary to the teaching of Goldberg et al, J Invest Dermatol. 2007 November; 127(11): 2645-2655, which showed TNF-α suppression of myofibroblast differentiation).

[0025] The clinical consensus is currently that clinical nodules are the precursor to established Dupuytren's disease. Dupuytren's disease occurs in people with genetic predisposition and further risk factors to manifestation of Dupuytren's disease include local trauma, poor lifestyle (e.g. smoking and drinking alcohol and poor diet), liver disease and diabetes. Established disease presents as flexion contracture which may typically be presented as contracture of the metacarbophalangeal joints (MCPJ) alone, less frequently contracture of the proximal

interphalangeal joints (PIPJ) alone, and often both. A phase III clinical trial of enzymatic fasciotomy using bacterial collagenase reported (Hurst et al, N. Engl J. Med, 2009, 361, 968-979) that 77% of MCPJ contractures were effectively treated (to within 5° of full extension) compared with 40% of PIPJ contractures. An earlier stage trial (Badalamente et al, J Hand Surg Am, 2007, 32, 767-774) showed recurrence rates of 57% in patients with PIPJ contractures at 2 years follow-up.

[0026] Numerous studies have shown that the presence of myofibroblasts is concomitant with early and active disease and that such cells are implicated in proliferative extra-cellular matrix (ECM) generation or deposition and, in particular, collagen deposition. TGF-β1 leads to the development of the myofibroblast phenotype. Myofibroblasts are also believed to be responsible for contractile behavior. Myofibroblasts characteristically express α-smooth muscle actin (α-SMA), which is the actin isoform typical of vascular smooth muscle cells. α-SMA is believed to be the protein responsible for the contractility of myofibroblasts and is the most reliable marker for myofibroblasts.

[0027] As mentioned above, the present invention preferably comprises a composition and method for treating, and more preferably inhibiting or halting the progression or recurrence of, musculoskeletal fibroproliferative disorders, such as fibromatosis disease, especially Dupuytren's disease, by administering to a patient a therapeutic, prophylactic or progression-inhibiting amount of a TNF-α antagonist. Preferably, the administration is local administration (e.g. by injection into or adjacent to the affected tissue).

[0028] The invention comprises a composition and method for treating early disease state musculoskeletal fibroproliferative disorders, especially early disease state Dupuytren's disease, by administering to a patient presenting early state disease, e.g. prior to the presence of palpable cord, an effective amount of a TNF-α antagonist.

[0029] According to the first embodiment, a composition comprising a TNF-α antagonist may be administered to a patient for preventing disease progression (to established disease state) and resultant flexion contracture. Preferably, the method comprise local administration (e.g. by injection) directly into the clinical nodule(s). In a preferred embodiment, the method further comprises administering to the patient, preferably locally (and more preferably directly to the clinical nodule(s) identified), an extracellular matrix degradation, depletion or cleavage agent, which is preferably a collagen degradation, depletion or cleavage agent and may be, for example a matrix metalloproteinase (MMP) and/or a collagenase (but may be, for example, a MMP or collagenase up-regulating or inducing agent). It is believed that the matrix metalloproteinase or collagenase may disrupt collagen and extra-cellular matrix local to the clinical nodule(s) thereby enhancing access of administered TNF-α antagonist to the proliferative fibrotic foci and thus enhance efficacy of treatment. It is believed that administration of the TNF-α antagonist in this manner may be considered prophylactic or progression halting or inhibiting treatment. According to this embodiment, the primary treatment is the TNF-α antagonist to which the extracellular matrix degradation or cleavage agent is preferably adjunctive.

[0030] In a preferred embodiment which involves the combined treatment of a patient presenting early disease state musculoskeletal fibroproliferative disorders, especially Dupuytren's disease, with a TNF-α antagonist and an extracellular matrix degradation, depletion or cleavage agent (e.g. matrix metalloproteinase and/or collagenase), the TNF-α antagonist and the extracellular matrix degradation, depletion or cleavage agent (e.g. collagenase) may be administered simultaneously or sequentially, together or separately. Preferably, both TNF-α antagonist and the extracellular matrix degradation, depletion or cleavage agent (e.g. collagenase) are administered locally, for example by injection. Optionally, they may be administered simultaneously, e.g. administering a composition comprising both TNF-α antagonist and collagenase (e.g. by injectable solution) or by applying two separate compositions at the same time. Alternatively, the TNF-α antagonist and the extracellular matrix degradation, depletion or cleavage agent (e.g. collagenase) are administered separately. When administered separately, they may be administered in any order a suitable time apart. Preferably, when administered separately the extracellular matrix degradation, depletion or cleavage agent (e.g. collagenase) is administered first followed by the TNF-α antagonist, which may be administered a suitable time after the TNF-α antagonist, e.g. after no less than 5 minutes, and preferably within 48 hours, more preferably within 24 hours, still more preferably within 6 hours and most preferably within 15 minutes to 3 hours.

[0031] Preferably, the TNF-α antagonist and the extracellular matrix degradation, depletion or cleavage agent are administered simultaneously for the treatment of early disease state musculoskeletal fibroproliferative disorders. Preferably, a composition is provided for local administration (e.g. injectable solution, sustained release composition or implant) for treating early disease state musculoskeletal fibroproliferative disorders, preferably Dupuytren's disease, which composition comprises an effective amount of a TNF-α antagonist (or configured to release an effective amount of TNF-α antagonist if, for example, the composition is a sustained release composition) optionally in combination with an extracellular matrix degradation, depletion or cleavage agent (preferably a matrix metalloproteinase and/or collagenase) preferably in an adjunctive amount and a pharmaceutically acceptable carrier.

[0032] Preferably, according to this embodiment, the TNF-α antagonist is provided in an amount effective to inhibit disease progression without inducing systemic complications. Optionally, therefore, the TNF-α antagonist is provided in an amount to reduce myofibroblast activity in clinical nodule tissue by at least 10%, preferably at least 30%, more preferably at least 50%, still more preferably at least 75% and most preferably at least 90%, as indicated, for example by, an average α-SMA-positive myofibroblast cell population in clinical nodule tissue by at least 10%, preferably at least 30%, more preferably at least 50%, still more preferably at least 75% and most preferably at least 90%, which activity reduction or cell population reduction is preferably observable within 48h, more preferably 24h, from administration. Preferably, an effective amount of TNF-α antagonist is that which will result in a reduction in clinical nodule size (e.g. at least a 20%, or even at least a 50%, reduction in size, as measured by degree of protrusion or lateral or longitudinal extent) in up to two weeks post administration. Efficacy of TNF-α antagonist treatment preferably is observable by an overall reduction in the

progression of disease.

[0033] Preferably the TNF-α antagonist may be administered in an amount that is in the range 0.01 to 0.5 of the dose indicated (or would be indicated) for systemic treatment of Rheumatoid Arthritis (e.g. by reference to Marketing Authorisation or FDA approval), preferably 0.05 to 0.2 and more preferably 0.095 to 0.15 of the dose. Preferably, the TNF-α antagonist is selected from one or a combination of Infliximab, Adalimumab, Certolizumab pegol, Golimumab or Etanercept and most preferably the TNF-α antagonist is Certolizumab pegol, which is preferably administered in an amount from 1 to 100 mg, preferably 5 to 50 mg and most preferably 10 to 40 mg, e.g. as an injection into the clinical nodule(s). Where more than one injection is provided (e.g. to two distinct clinical nodules), the dose is preferably divided so the total dose provided is in the above range.

[0034] Preferably, according to this embodiment, an extracellular matrix degradation, depletion or cleavage agent, e.g. a matrix metalloproteinase and/or collagenase, is provided in a TNF-α antagonist adjunctive amount, by which it is meant an amount effective to enhance the efficacy of the TNF-α antagonist. In any case, it is preferred that the extracellular matrix degradation, depletion or cleavage agent (e.g. matrix metalloproteinase or collagenase) is provided in an amount of up to 1 mg. Preferably, the extracellular matrix degradation, depletion or cleavage agent (e.g. matrix metalloproteinase or collagenase) is administered in an amount significantly below (e.g. 0.01 to 0.5 times) the extracellular matrix degradation, depletion or cleavage agent (e.g. matrix metalloproteinase or collagenase) dose that would be required to achieve an enzymatic fasciotomy in established disease state fibromatosis. Preferably, the extracellular matrix degradation, depletion or cleavage agent (e.g. matrix metalloproteinase or collagenase) is provided in an amount of 0.01 to 0.5 mg, more preferably 0.05 to 0.2 mg.

[0035] The extracellular matrix degradation, depletion or cleavage agent, e.g. matrix metalloproteinase or collagenase, may assist the TNF-α antagonist in accessing the cell mass, as well as assisting in disaggregating of the extracellular matrix of the clinical nodule.

[0036] Any known TNF-α antagonist may be utilized in the implementation of the invention, a broad variety of which are known and disclosed in the art. The TNF-α antagonist is preferably a human TNF-α antagonist. Optionally, the TNF-α antagonist may be an antibody, such as a monoclonal antibody or fragment thereof; a chimeric monoclonal antibody (such as a human-murine chimeric monoclonal antibody); a fully human monoclonal antibody; a recombinant human monoclonal antibody; a humanized antibody fragment; a soluble TNF-α antagonist, including small molecule TNF-α blocking agents such as thalidomide or analogues thereof or PDE-IV inhibitors; a TNF receptor or a TNF receptor fusion protein, e.g. a soluble p55 or p75 TNF receptor or TNF receptor fusion protein.

[0037] Optionally, the TNF-α antagonist is a functional fragment or fusion protein comprising a functional fragment of a monoclonal antibody, e.g. of the types mentioned above, such as a Fab, $F(ab')_2$, Fv and preferably Fab. Preferably a fragment is pegylated or encapsulated (e.g. for stability and/or sustained release).

[0038] Optionally, the TNF-α antagonist is provided as a bi-functional (or bi-specific) antibody or bi-functional (or bi-specific) antibody fragment. The bi-functional TNF-α antagonist antibody or fragment thereof may be, for example, an antibody, such as a monoclonal antibody or fragment thereof, a chimeric monoclonal antibody (such as a human-murine chimeric monoclonal antibody), a fully human monoclonal antibody, a recombinant human monoclonal antibody, a humanized antibody fragment. Where the TNF-α antagonist comprises a bi-functional antibody fragment or portion, it is preferably a bi-functional $F(ab')_2$ fragment or divalent ScFv, e.g. a bi-specific tandem di-ScFv. In any case, the bi-functional (or bi-specific) antibody or fragment thereof may comprise as one variable domain (e.g. antigen binding portion) a TNF-α antagonist (e.g. a TNF-α antagonist portion of Infliximab, Adalimumab, Certolizumab, Golimumab or Etanercept) and as the other variable domain (e.g. antigen binding portion) a second variable domain other than TNF-α antagonist. Optionally, the second variable domain may comprise an antibody mobility inhibitor, which may be, for example an extracellular matrix, e.g. collagen, binder or antagonist. Thereby, a higher dose of TNF-α antagonist may be administered since the antibody or fragment thereof will be self-localising, minimizing systemic uptake and thus systemic side effects. Optionally, the second variable domain may comprise a DAMP antagonist (such as an antagonist for S100A8 and/or S100A9, e.g. as described in US-B-7553488) or an AGE inhibitor (e.g. being variable domains of DAMP antagonist antibody or AGE inhibitor antibody). Methods for the production of bi-functional antibodies, and bi-functional antibody fragments are known in the art, which methods may be applied to the present purpose.

[0039] Preferably, the TNF-α antagonist is selected from those which at administration (e.g. local administration, such as injection into clinical nodule or cord) cause administration-site irritation manifested as palpable local swelling, redness and pruritis in fewer than 40% of patients, preferably fewer than 20% and more preferably fewer than 10%.

[0040] The TNF-α antagonist may be selected, for example, from one or a combination of Infliximab, Adalimumab, Certolizumab pegol, Golimumab or Etanercept, or functional fragment thereof. Most preferably, the TNF-α antagonist is Certolizumab pegol, since it causes low injection site reaction and pain.

[0041] It is particularly advantageous according to the present invention to minimize inflammation, irritation and pain associated with administration since local irritation may limit patient acceptability and furthermore local inflammation may lead to recurrence of disease. In one embodiment, the TNF-α antagonist may be administered with or prior to an extracellular matrix (ECM) degradation or cleavage agent (e.g. collagenase) whereby the inflammatory response to ECM degradation may be minimised, thereby reducing the likelihood of treatment induced recurrence.

[0042] The extracellular matrix (ECM) degradation, depletion or cleavage agent may be any suitable agent capable of degrading, cleaving or causing or inducing degradation or cleavage of extracellular matrix, including fibronectin and collagen. For example, the ECM degradation

or cleavage agent may be an ECM degradation enzyme or an ECM degradation enzyme expression up-regulator (e.g. relaxin). Preferably the ECM degradation or cleavage agent is a matrix metalloproteinase or a collagenase, more preferably a collagenase, such as a bacterial collagenase (e.g. clostridial collagenase), human or humanised collagenase or mutant or recombinant collagenase or recombinant matrix metalloproteinase (e.g. recombinant matrix metalloproteinase I, preferably human recombinant matrix metalloproteinase I). Preferably, the collagenase is time or temperature dependent or is photodynamically activated or deactivated, to allow higher local doses to be administered without systemic or long-lasting side-effects. Optionally, it is a Cathespin-L or a mutant or recombinant thereof. Examples of suitable collagenase for use in the present invention include those described in: GB-A-2323530, US 5589171, USRE39941, US6086272 & WO-A-2010/102262 (and for established disease optionally in the amounts described therein, the disclosure of which collagenases and amounts and modes of administration are incorporated herein by reference).

[0043] By early disease state it is meant that indications of disease are present, e.g. histological markers or more particularly clinical nodules in tissue, but in the absence of, for example, palpable cord or significant contracture. By early disease state Dupuytren's disease, it is meant that indications of Dupuytren's disease are present, e.g. histological markers or more particularly clinical nodules in palmar and/or digital tissue, but in the absence of significant (e.g. at least 5°) flexion contracture (or, for example, palpable cord).

[0044] By established disease state, it is meant that clinical nodules are present, palpable cord is present and contracture is evident. By established disease state Dupuytren's disease, it is meant that clinical nodules are present on the palm and digits of the hand and flexion contracture is evident (e.g. at least 5°).

[0045] Varying histological stages of Dupuytren's disease have been categorised in the literature, most succinctly by Rombouts (J Hand Surg Am, 14, 644-652, 1989) and later authors, into three distinct stages: 1) a proliferative stage with high cellularity and the presence of mitotic figures; 2) a fibrocellular stage charactised by high cellularity but no mitotic figures and the presence of reticulin network; and 3) a fibrous stage with few cells separated by broad bundles of collagen fibres. Stage 1) disease is believed to correlate with early disease state as discussed above (i.e. presence of nodules but no contracture) and Dupuytren's stages 2) and 3) is believed to correlate with our Established Disease State (characterized by digital contracture). The present inventors have found that during early established disease state, active myofibroblasts are collected in the established nodules and cords, especially in relation to the MCP and PIP joints and these drive the progression of flexion contractures of the digit.

[0046] By clinical nodule, it is meant a palmar or digital nodule evident as a palpable subcutaneous lump.

[0047] By histological (or histopathological) nodule, it is meant a collection of cells (mainly myofibroblast cells with some inflammatory cells such as macrophages and mast cells) typically in a whorled pattern and which may range from tiny foci of cells to larger collections of cells,

but not clinically palpable.

[0048] Without being bound by theory, it is believed that the initial clinically palpable nodule(s) is the focus of proliferating fibroblasts in disease progression, but that numerous histological nodules will form at various locations in the palm and/or digits which will ultimately contribute to cord formation, contraction and flexion contracture.

[0049] Where 'nodule' is used herein it may be clinical or histological nodules (or either) as will be apparent from the context.

[0050] According to two alternative embodiments specific to Dupuytren's, a first embodiment may relate to a composition and method for treating Dupuytren's disease characterized by joint contractures of less than 20° and a second embodiment may relate to a composition and method for treating Dupuytren's disease characterized by joint contractures of at least 20°. The contracture of 20° is identified as a transition phase, since at less than 20° contracture, many patients may choose to stop progression of the disease without wishing to undergo surgery since their mobility and operative use of the hand is still largely adequate, whilst at greater than 20°, many patients will find surgery or other collagen depleting therapy (such enzymatic fasciotomy) essential to restore full function to the hand.

[0051] The inventors' investigations reveal that TNF-α as an optimal therapeutic target for early Dupuyten's disease (i.e. early disease state). In established disease state Dupuytren's disease, an ideal combination is a matrix metalloproteinase such as collagenase with a TNF-α antagonist to inhibit recurrence, which is typically associated with enzymatic fasciotomy.

[0052] As mentioned above, myofibroblasts are implicated in two ways in the development of musceloskeletal fibroproliferative disorders and, in particular, Dupuytren's disease. They are responsible for extracellular matrix production or deposition and contractile behavior. It is believed that the activity of myofibroblasts is mediated by α-SMA, which is over-expressed in active myofibroblast cells. Without being bound by theory, the present inventors have found that TNF-α is implicated in the activity of myofibroblasts in Dupuytren's disease in at least two ways - firstly, by reducing the activity of myofibroblast; and secondly by enhancing the production or attraction of myofibroblasts.

[0053] The TNF-α antagonist may be provided in a multiple administrations over an extended (or continuous) term in order to prevent or inhibit disease progression or recurrence. Where recurrence is to be avoided, intermittent treatment may be provided by, e.g. low-dose fortnightly, monthly or six-monthly administration. Alternatively, continuous treatment may be provided by low-dose releasing sustained or delayed intermittent release implant or patch. Alternatively, repeat doses may be initiated by signs of disease progression in the early disease state and may optionally comprise a combined extracellular matrix degradation or cleavage agent (e.g. a matrix metalloproteinase or a collagenase) and TNF-α antagonist treatment (e.g. consistent with the first embodiment described above).

[0054] In one embodiment of the invention, the progression of early disease state disease (e.g. Dupuytren's disease) to established disease state can be prevented, inhibited or halted by the local administration of a TNF-α antagonist.

[0055] Preferably, the TNF-α antagonist may be administered separately or simultaneously in combination with or adjunctively to a collagenase and/or matrix metalloproteinase. A collagenase, especially a photo-responsive or temperature dependent collagenase, may be administered for local effect to enhance the TNF-α antagonist disease progression inhibition effect by enhancing access to treatment sites by cleaving early stage extracellular matrix formation. A temperature dependent collagenase is one which (typically a recombinant or mutant collagenase) has collagenase activity dependent upon temperature and typically is active at below body temperature, e.g. at 25°C and below, thereby allowing extremely high doses of collagenase to act very locally (e.g. by injecting at the disease site at say 20°C without having any systemic action or other side effects associated with longevity of action). TNF-α antagonist have a further beneficial effect since it will reduce inflammation at the nodule site and thus reduce development and recruitment of further myofibroblasts.

[0056] The composition and method of the present invention may utilise any suitable means of administration, which is preferably local. In particular, the TNF-α antagonist should be administered locally, e.g. by applying directly into a surgical incision during surgery, by injection (preferably directly into the clinical nodule(s) and/or cord tissue), by release from a sustained and/or delayed release lozenge or device that may be implanted into or close to the disease site or a sustained and/or delayed release patch formulation, by topical application or any other suitable route. A composition is preferably suitably formulated and typically comprises the required dose of TNF-α antagonist along with a pharmaceutical acceptable carrier or excipient.

[0057] Formulations for parenteral administration may typically comprise a sterile aqueous preparation of the active ingredient, which is preferably isotonic with the blood of the recipient. Formulations for intra-articular administration may be in the form of a sterile aqueous preparation of the active ingredient. Formulations suitable for topical administration may include liquid and semi liquid preparations such as liniments, lotions and applications; oil-in-water and water-in-oil emulsions such as creams, ointments and pastes; and solutions and suspensions.

[0058] In a further aspect, there is provided a formulation for frequent, e.g. daily, periodic or occasional (preferably daily), topical application to the musculoskeletal fibroproliferative disorder area (e.g. the hands, and in particular palms and digits, in the case of Dupuytren's disease) for use, for example, by early disease state or post-operative patients for the inhibition of disease progression or recurrence, the formulation comprising a TNF-α antagonist suitable for topical administration (e.g. selected from such TNF-α antagonists defined above) and a suitable excipient. The formulation may be provided as a cream or lotion, a patch or a medicated glove (in which the glove is impregnated for release of the active component from the internal surface). Preferably, the formulation comprises TNF-α antagonist in a concentration for administration by topical application of a low dose, such as 0.001 to 0.05,

preferably 0.001 to 0.01, of the systemic dose of the TNF-α antagonist. Optionally, the formulation further comprises a DAMP antagonist and/or an AGE inhibitor.

[0059] Optionally, the compositions and methods of the present invention may further comprise further active ingredients that may be effective in the treatment or progression-inhibition of musculoskeletal fibroproliferative disorders such as Dupuytren's disease. For example, combination therapy or concomitant or adjunctive co-administration of a TNF-α antagonist and an agent of the vascular endothelial growth factor family, such as VEGF-A, VEGF-B, VEGF-C or VEGF-D or an agent encoding said VEGF or a functional fragment thereof (such as described in WO-A-2004/082705), which combination is preferably a development retarding combination (or composition) for use in association with surgery, or needle or enzyme fasciotomy. Additionally or alternatively such method or composition as described herein may further comprise an activator of PPARγ (such as pioglitazone) for reducing myofibroblast populations local to the disease site (and enhancing the TNF-α antagonist activity).

[0060] In the treatment of musculoskeletal fibroproliferative disorders and, preferably, Dupuytren's disease, there is as a further aspect provided a composition for use in such treatment which comprises a matrix metalloproteinase or collagenase (or matrix metalloproteinase or collagenase up-regulator) in combination with a myofibroblast activity down-regulator and/or a myofibroblast production (or differentiation) inhibitor each preferably in appropriate therapeutic amounts according to the respective embodiment as discussed above. The preferred myofibroblast activity down-regulator and/or myofibroblast production (or differentiation) inhibitor is TNF-α antagonist.

[0061] By musculoskeletal adhesions, it is meant a sub-set of musculoskeletal fibroproliferative disorders in which excess fibrotic tissue or scar tissue is formed adjacent or in association with a tendon, muscle, joint, ligament or fascia causing an adhesion. Examples of such musculoskeletal adhesions include periarticular fibrosis (e.g. about the proximal interphalangeal joint) and adhesive capsulitis. Preferably, according to this aspect, there is provided a composition and treatment for a condition selected from perarticular fibrosis (e.g. of the proximal interphalangeal joint), spinal adhesions (e.g. post-surgical) and adhesive capsulitis.

[0062] In one particular embodiment, there is a method for the prevention of recurrence of Dupuytren's disease comprising administering (e.g. post-surgery, post-needle fasciotomy or after or in association with enzyme fasciotomy) a TNF-α antagonist to the nodule(s) and/or cord and administering a TNF-α antagonist to the tissue adjacent the proximal interphalangeal joint, whereby simultaneously treatment to prevent recurrence of Dupuytren's disease (and digital contracture) and reduction in formation and persistence of fibrotic scar tissue about the joint can be achieved. It is believed that the effectiveness of, e.g. a collagenase treatment, of Dupuytren's disease (which suffers from a high rate of recurrence especially about the proximal interphalangeal joint) will be enhanced by co-therapy with a TNF-α antagonist (or other agent for the de-activation of myofibroblast and/or inhibiting the production of

myofibroblast) by administering the same to clinical nodules and/or cord tissue and to subcutaneous tissue (e.g. fibrotic scar tissue) adjacent the proximal interphalangeal joint.

[0063] For adhesive capsulitis (or frozen shoulder), preferably the treatment comprises one or both of a TNF-α antagonist and an AGE inhibitor or DAMP antagonist.

[0064] In an alternative embodiment in each of the above mentioned aspects and embodiments, a TNF-α production or activity inhibitor may be used in place of or together with a TNF-α antagonist.

[0065] The composition may be formulated for administration to and/or adjacent to the affected tissue (e.g. by injection, deposition during surgery or preferably by topical application) whereby doses in the ranges described above in relation to musculoskeletal fibroproliferative disorders (e.g. Dupuytren's disease) are achieved/provided. Topical formulations and combinations as described above are also included.

[0066] The invention will now be described and illustrated in more detail, without limitation, with reference to the following Examples.

EXAMPLES

[0067] The following studies were undertaken to understand better the progression of Dupuytren's disease. Tissue was taken from nodules and cords from Dupuytren's patients and compared with non-disease palmar tissue from the same patients. Studies were carried out in a culture force monitor developed to ensure that myofibroblast populations can be monitored in an environment more akin to that present in diseased tissue (in line with that set out in Verjee et al, J Hand Surg Am, 34, 1785-1794 and J Cell Physiol 224, 681-690). Four examples are described below - Example 1 is concerned with presence, distribution and behavior of myofibroblast cells in diseased tissue; Example 2 is concerned with the role of inflammation in Dupuytren's disease; Example 3 examines advanced glycation end products in Dupuytren's; and Example 4 examines the role of DAMPs in Dupuytren's.

Example 1

[0068] Over 100 Dupuytren's patient samples were collected to examine myofibroblast distribution. Our data on >100 Dupuytren's cords show that in the majority of patients, myofibroblasts are concentrated in nodules, located in the palm and at the level of the affected joints (see Figure 1). According to Figure 1, nodules rich in myofibroblasts are located in the vicinity of the finger joints. Figure 1 shows: A: intraoperative view of Dupuytren's cord, with location of proximal interphalangeal joint (PIPJ; 1) marked; B: Low magnification photomicrograph of histological section stained for α-smooth muscle actin. A collection of α-

SMA rich cells in a nodule is located in the vicinity of the PIPJ; C: High magnification view of nodular area, showing α-SMA positive cells (myofibroblasts).

[0069] Of over 100 cords analysed, more than 60% contained nodules. Although there was marked heterogeneity, nodules were very cellular, with approximately 2.5 thousand cells per mm^2 arranged in whorls. On average, 99% of the cells were α-SMA positive. In peri-nodular areas, there were fewer cells, approximately 800 per mm^2 and, on average, one third were α-SMA positive.

[0070] Figure 2 shows that nodules are mostly cellular and are rich in α-SMA positive cells. Examination of 24 Dupuytren's patient samples by electron microscopy showed that clinical recurrence was not related to patient age at onset, duration, or severity of disease. Histological nodules were seen as frequently in samples from both primary and recurrent disease (two-thirds of cords in each case) and there was also no significant difference in digital contracture between primary and recurrent disease. Furthermore, there was also no difference in nodular surface area between primary dermofasciectomy samples, primary fasciectomy, secondary fasciectomy or dermofaciectomy following recurrent disease (p=0.5). A similar pathogenesis in both primary and recurrent disease is likely and nodularity is unlikely to be down-regulated following previous surgery. Indeed, the increased motion following initial surgery may facilitate myofibroblast differentiation and persistence. It is possible that residual unexcised Dupuytren's tissue following fasciotomy or fasciectomy and firebreak dermofasciectomy, may serve as a trigger for recurrence. The persistence of myofibroblasts may explain the high recurrence rates seen following surgical fasciotomy or collagenase injection. Therefore, a key element of preventing recurrent disease may be to down regulate the remaining myofibroblasts.

[0071] 95% (36/38) of nodules were in the vicinity of the PIPJ and nodules were also observed over the MCPJ in the only two cases marked intra-operatively for the MCPJ and one case marked for the DIPJ. In early or active disease, tension may act intermittently on Dupuytren's tissue as active extension of the PIPJ offers resistance against the thickened, contracted palmar fascia. The increased tension sensed by cells may promote myofibroblast differentiation through recruitment of α-SMA to stress fibres and specialised attachment site formation under strict control of TGF-β1. This in turn leads to greater force generation. A densely packed cellular nodule could then theoretically exert sufficient force to promote or sustain digital contracture. The cells then remodel the surrounding matrix to a more shortened configuration. The resulting increased flexion deformity would impair function and the reduced movement at the joint would in turn lead to a reduction in tension sensed by nodular myofibroblasts. It is possible that with advanced digital contractures, reduced tension through limited active joint extension may lead to myofibroblast apoptosis, whereby myofibroblast rich nodules fail to persist. This may explain the progression from nodular to non-nodular cords and would also explain why patients with non-nodular cords tended to have more severe flexion deformities. Thus, myofibroblast aggregation in nodules in the vicinity of joints may lead to digital contracture and with subsequent matrix remodelling result in shortening of the affected fascia. Eventually fixed flexion deformity develops leading to an altered mechanical environment with loss of tension, myofibroblast apoptosis and thus may explain residual non-

nodular cords. It can be concluded that the myofibroblast phenotye depends on tension in the surrounding matrix.

[0072] The culture force monitor (CFM) utilised and culture conditions are shown in Figure 3: (A) Rectangular seeded collagen gels were cast and floated in medium, tethered between two flotation bars one of which is held stationary whilst the other is attached to a force transducer. (B) Cell-generated tensional forces in the collagen gel are detected by the force transducer, and live data are logged every 15 seconds providing a continuous output of force (dynes, $1x10^5$ *N*) generated. (C) After 24 hour contraction, gels are harvested and processed for α-SMA mRNA, protein and immunofluorescence. (D) Cells were routinely seeded in gels with a high aspect-ratio collagen lattice, although low aspect-ratio lattices (E) were also used in experiments to compare effects of less strain on cell contractility.

[0073] Surgically excised cords were bisected and half processed for cell culture, whilst the cut surface of the mirror half was processed to identify samples with α-SMA-rich nodules (condensation of cells) by immunohistochemistry. Subsequent quantification by immunofluorescence demonstrated on average 35% of cells expressed α-SMA stress fibres in histology confirmed nodular samples, as compared to 10% α-SMA stress fibres in non-nodular samples. Although this still does not constitute a homogenous population of myofibroblasts, this method of sampling α-SMA-rich cells represents a significant improvement on previous studies, which have reported on average between 9.7% and 15% α-SMA-positive cells isolated from clinical and not histologically defined nodules. 1-4% of dermal fibroblasts were found to have α-SMA positive stress fibres.

[0074] Figure 4 shows isometric contraction of collagen gels by dermal fibroblasts and Dupuytren's nodule-derived cells. Collagen gels were seeded with 1.5 million non-palmar fibroblasts (A), palmar fibroblasts (B) or Dupuytrens nodule-derived cells (C), cultured for 24h in the CFM and real-time isometric force contraction was quantified. Data shown represent triplicate experiments using cells derived from one patient. Dermal fibroblasts in fibroblast populated collagen lattices (FPCL) in our CFM reached a plateau, whereas nodule-derived cells continued to contract in a dose-dependent manner over a 24 hour test period.

[0075] Figure 5 shows that cells isolated from nodules had a much higher rate of contraction measured as the average rate of contraction between 6 and 24 hours in the CFM compared to palmar or non-palmar dermal fibroblasts. High contractility is one of the characteristics of myofibroblasts and is responsible for digital contracture in Dupuytren's disease.

[0076] In Figure 6, it is shown that the contractility of Dupuytren's nodule-derived cells is regulated by post-transcriptional changes in α-SMA: (A) [rate of contraction (dynes/hr)] Isometric force in collagen gels with nodule-derived myofibroblasts (nodule), non-palmar (NPS) and palmar dermal fibroblasts (PS) over 24 hours (±SEM). After 24 h (B) α-SMA mRNA was compared to RPLPO by quantitative RT-pcr, (C) α-SMA mRNA compared to GAPDH and (D) α-SMA protein compared with vimentin. Experiments were performed in triplicate and data are shown as the mean (±SEM) from a total of 3 different nodular and non-nodular matched

patient samples

[0077] After harvesting FPCLs following 24 hours contraction in the CFM, comparisons were made between α-SMA mRNA levels, α-SMA protein expression and α-SMA protein localisation by immunofluorescence in the matched cell types. No differences in α-SMA mRNA levels were seen between nodule-derived cells and dermal fibroblasts, although approximately 3-fold greater α-SMA protein levels were seen in nodule-derived cells compared with matched dermal fibroblasts. Furthermore, using immunofluorescence we found that in dermal fibroblasts, α-SMA was typically distributed in a 'halo' within the peri-nucleur cytoplasm, whereas in nodule-derived cells, α-SMA was frequently localised in stress fibres throughout the cell processes up to cell-matrix attachment sites, as can be seen in Figure 6E.

[0078] Cells were also cultured on glass coverslips for 24 hours, fixed and then immunofluorescently labelled using α-SMA antibodies (red), phalloidin (green) and DAPI (blue). Our immunofluoresence data demonstrate that palmar and non-palmar fibroblasts when cultured in monolayer acquired a protomyofibroblast phenotype, with the expression of de novo cytosolic α-SMA. In contrast, significantly more differentiated myofibroblasts with α-SMA incorporated to stress fibres were seen in nodule-derived cells. These differences seen between nodule-derived, non-palmar and palmar skin cells from matched samples have not been previously reported. We simultaneously examined α-SMA protein levels, protein localization and mRNA levels in cells isolated from the same patient. Our findings suggest that post-transcriptional changes in α-SMA occur in genetically matched cells to mediate the Dupuytren's myofibroblast cell phenotype.

Example 2 - Role of inflammation in Dupuytren's disease

[0079] The nodules were then examined for the presence of other cell types, specifically inflammatory cells. We found that large numbers of both macrophages and mast cells were present in nodules but not in non-nodular regions of the cords.

[0080] Figure 7 shows inflammatory cells in Dupuytren's nodule and cord. Digital cord sections were serially stained for α-SMA, CD68 positive macrophages and mast cell tryptase. The images are representative of 15 patient samples.

[0081] We systematically quantified the number of inflammatory cells observed throughout excised Dupuytren's cord tissue in 10 patient samples. For each region (nodule, cord distal to nodule and non-nodular cord), the total number of cells, the number of α-SMA positive cells and cells stained for neutrophil elastase, mast cell tryptase, CD3positive T cells, CD 4 positive T cells, CD68 positive macrophages were counted (x20 magnification) (Table 1).

Table 1. Quantification of total cell number: α-SMA positive cells, CD3 positive T cells, CD4 positive T cells, CD68 positive macrophages, and cells positive for mast cell tryptase and neutrophil elastase throughout excised Dupuytren's cord. Nodules, cord distal to nodule and

non-nodular cord were analysed (presented as mean count (±SDEV) per mm^2) Six fields of view were counted within each region.

IHC stain	Nodule		Distal to nodule		Non-nodular cord	
	mean	SDEV	mean	SDEV	mean	SDEV
Total cells	1515	181	416	104	504	163
α-SMA	1493	199	12	8	8	7
Neutrophil elastase	2	1	0	1	0	0
CD3 positive T cells	220	99	2	2	1	2
CD4 positive T cells	2	1	0	0	0	0
CD68 positive						
macrophages	282	54	1	1	1	1
Mast cell tryptase	48	11	1	1	0	1

[0082] These data show that CD68 positive macrophages, CD3 T-cells and mast cell tryptase positive cells were common within cellular nodules and sparse within cord tissue. Neutrophil elastase positive cells and CD4 positive T-cells were observed infrequently throughout Dupuytren's tissue. Dupuytren's nodules contained numerous mast cells, which are a rich source of TNF-α (Krishnaswamy et al., 2006). Whilst Dupuytren's nodular tissue is populated with highly contractile myofibroblasts, the presence of inflammatory cells suggests that inflammation may be important in pathogenesis of the disease. In non-nodular cord, almost no inflammatory cells were observed and it is also of interest that virtually no cells stained positive for neutrophil elastase in either nodular or non-nodular cord. This is in contrast to inflammation during wound healing, where neutrophils are commonly seen and they are involved with clearance of debris and bacteria and initiating myofibroblast-dependent wound contraction. However, it is important to note that whilst excised digital cord samples contain nodules, they do not necessarily reflect the processes at the earliest stages of the disease.

Example 3 - Advanced glycation end products and their receptor

[0083] We examined the distribution of RAGE in Dupuytren's tissue and both palmar and non-palmar skin. We found abundant staining for RAGE in Dupuytren's nodules, where it co-localised with the myofibroblasts (see Figure 8). Digital cord samples were longitudinally bisected and fixed in formalin. Histological sections were taken from the cut surface of cord and serial sections were stained for (A, C) α-SMA and (B, D) RAGE antibodies. Scale bars as shown. Images are representative from 15 patient samples. RAGE co-localises with α-SMA

distribution in Dupuytren's nodules.

[0084] We also found increased staining for RAGE in the superficial layers of the epidermis in palmar skin compared to non-palmar skin and FACS staining showed significantly higher RAGE expression by dermal fibroblasts from palmar skin compared to non-palmar skin. See Figure 9.

[0085] Non-palmar and palmar skin samples were fixed in formalin. Histological sections were stained for RAGE. (A, C) Non-palmar skin and (B, D) palmar skin. Scale bars are shown. Images are representative from 6 matched patient samples. Figure 9 illustrates the differential distribution of RAGE within non-palmar and palmar skin.

[0086] We also demonstrated that nodule-derived cells express higher levels of cell surface RAGE than matched dermal fibroblasts. Nodule-derived cells, palmar and non-palmar fibroblasts ($1x10^4$ cells per experiment) were stained with RAGE antibody, fluorescently labelled and mean fluorescence intensity assessed by FACS analysis. (Figure 10 **A,C**) Cell surface RAGE expression levels in nodule-derived cells as compared with matched dermal fibroblasts. **(B,D)** Cell surface RAGE expression levels in non-nodular cells as compared with matched dermal fibroblasts. Results in A and B are shown for 4 matched nodular patient and non-nodular samples (± SEM). * represents p=0.01. **(E)** RAGE fluorescent intensity trace showing nodule-derived cells, non-palmar fibroblasts, palmar fibroblasts and isotype control from 1 representative nodular matched patient sample, and **(F)** from 1 representative non-nodular matched patient sample. See Figure 10.

[0087] We demonstrated that RAGE cell surface expression is greater in palmar than non-palmar fibroblasts. Fibroblasts ($1x10^4$) from matched palmar and non-palmar skin were stained with RAGE antibody, fluorescently labelled and the mean fluorescence intensity analysed by FACS. Cell surface RAGE expression levels were consistently higher in palmar fibroblasts than non-palmar fibroblasts. Data are shown from 8 matched patient samples. See Figure 11.

[0088] In a further experiment to investigate the effect of AGE on myofibroblast formation, collagen gels were seeded with 1.5 million palmar fibroblasts and cultured for 24h in the absence (PS alone) or presence of bovine serum albumin (BSA) (15µg/ml), or AGEs-BSA (150µg/ml) for varying periods and isometric force contraction quantified in the culture force monitor. Data are shown as +/- SEM from triplicate experiments with samples from 3 different patients in Figure 14. It is apparent from Figure 14 that contractility of palmar dermal fibroblasts is not affected by exposure to AGEs.

[0089] We went on to investigate whether advanced glycation end products may also act via inflammatory cells and in other systems have been shown to lead to pro-inflammatory cytokine release (Uribarri et al., 2005). Collagen gels seeded with palmar fibroblasts were cultured for 24h with supernatants from AGE-(100µg/ml) stimulated monocytes (M) in the absence or presence of anti TNF- α (10µg/ml) and isometric force contraction quantified. Experiments were performed in duplicate. Interestingly the supernatant from human monocytes co-cultured

with AGEs stimulated palmar fibroblast contraction in a TNF-α dependent manner (Figure 20).

Example 4

[0090] We examined the effect of addition of exogenous HMGB1 to palmar fibroblasts. Collagen gels seeded with palmar fibroblasts were cultured for 24h in the absence (PS alone) or presence of TNF-α (1ng/ml), or HMGB1 (1ng/ml) or TGF-β1 (10ng/ml) and isometric force contraction quantified (utilising the culture force monitor technique, such as described in Verjee et al, Hand Surg Am, 34, 1785-1794, 2009 and Verjee et al, J Cell Physiol, 2010). Data from the experiment are shown in FIGURE 12 as +/- SD from triplicate experiments (except HMGB1 which is duplicate).

[0091] Whilst there was a trend towards increased contraction resulting from HMGB1, this was not statistically significant (Figure 12). Our data showed significant increased palmar fibroblast contractility (p=0.0001) with 10ng/ml TGF-β1 compared to untreated palmar fibroblasts.

[0092] We found that human monocytes exposed to S100A8 and to some extent S100A9 (other Alarmins) produced TNF-α in a dose dependent manner, as is illustrated by Figure 15 As can be seen from Figure 15, S100A8 is more active than S100A9 and S100A12 within the tested range. LPS, a pathogen associated molecular pattern (PAMP) is shown as positive control.

[0093] The known receptors for S100A8 are the receptor for advanced glycation end products (RAGE) and the Toll-like receptors 2 and 4. Human monocytes at $1x10^5$/ml were incubated in 10% FCS with human S100 A8 at 0.5 µg/ml with the addition of either antibody to TLR4, TLR2 or isotype controls (not shown), or soluble RAGE (sRAGE)) over 14 hours. TNF-α levels were determined by ELISA. We have found that the predominant receptor for binding S100A8 on monocytes leading to TNF-α production is TLR-4 and not RAGE or TLR-2 (Figure 16).

[0094] We have confirmed that S100A8 predominantly binds to TLR-4 and that the intracellular signalling leading to TNF- α production by monocytes is entirely dependent on adaptor protein MyD88 by comparing the effect of murine S100A8 on TNF- α production by bone marrow cells derived from TLR-4 or MyD88 deficient mice with bone marrow derived cells from wild-type C57B1/6 animals (Figure 17). In Figure 17, TNF-α produced by murine bone marrow cells of wild type, TLR4 $^{-/-}$and MyD88 $^{-/-}$ mice on exposure to murine S100A8 were measured by ELISA.

[0095] It is more difficult to show in vitro that HMGB1 also acts on monocytes to lead to pro-inflammatory cytokine release. This is because in vivo it acts in conjunction with other TLR ligands and highly purified HMGB1 alone does not lead to TNF- α production by monocytes in vitro (Figure 18). Our experiment, the results of which are shown in Figure 18, involved human monocytes at $1x10^5$/ml incubated in 10% FCS with HMGB1 or LPS alone or together at

concentrations shown over 14 hours. TNF-α levels were determined by ELISA. It was found that HMGB1 alone does not stimulate TNF-a production by monocytes but is active in combination with LPS.

[0096] Figure 19 shows a schematic of proposed mechanism of the role of trauma and alarmins in the pathogenesis of Dupuytren's disease. As can be seen, trauma (101) causes cell injury (103) and consequent release of Alarmins (105) such as S100A8, which binds to TLR-4 (107) in an inflammatory cell such as a macrophage (109) causing pro-inflammatory cytokines such as TNF-α to be produced (113) signalled via Myd88 (111). TNF-α may then bind to TNFR (115) on fibroblast precursor (117) resulting in formation of myofibroblast (119).

Example 5

[0097] Primary passage cultured cells from a Dupuytren's nodule (comprising myofibroblast cells) were treated, using the culture force monitor described above, with a monoclonal human TNF-α antibody (monoclonal IgG_1 culture # 1825, as available from R&D Systems of Canada) in an amount of 10 µg/ml. Compared with a control culture of such primary passage cells, the anti-TNF-α treated cells were found over 24 hours to contract by an amount of greater than 30% less than control (which it is believed corresponds to effective myofibroblast deactivation of greater than 30% compared with control). This is shown in Figure 13, where the gradient (or rate of contraction; in Dynes/h) over 24 hours is illustrated for each of the control cells and the TNF-α antibody treated cells.

[0098] This directly shows that myofibroblast cells cultured from a clinical from a Dupuytren's disease patient has reduced activity (e.g. reduced contractile behaviour and/or reduced abundance) when treated with a TNF-α antagonist, even over only 24 hours. Since the effect of the TNF-α antagonist on myofibroblasts in the clinical situation will be ongoing and the therapeutic regime in a patient may involve repeat applications, it is believed that this experiment shows that myofibroblast activity can be effectively managed, thereby reducing the progression of and/or inhibiting the recurrence of musculoskeletal fibroproliferative disorders and, in particular, Dupuytren's disease by local application of a TNF-α antagonist to the disease site.

[0099] We were able to confirm that addition of TGF-β1 human palmar fibroblasts from in a collagen lattice under isometric conditions enhanced contractility (see Figure 21). Collagen gels seeded with palmar fibroblasts were cultured for 24h in the absence (PS alone) or presence of TGF-β1 (10ng/ml). Data are shown as +/- SD from triplicate experiments using cells from 3 patients.

[0100] We then compared the effect of TNF-α on the rate of contraction on palmar skin and non-palmar skin from a Dupuytren's patient. Collagen gels seeded with palmar fibroblasts were cultured for 24h in the absence (PS or NPS alone) or presence of TNF-α (1ng/ml). Data are shown as +/- SD from triplicate experiments using cells from 3 patients for palmar skin and one

patient non-palmar skin (** p=0.0012, ns = not significant). We found significantly enhanced contraction in the culture force monitor (Figure 22) on addition of TNF-α to the palmar skin fibroblasts. However, there was no change, or a slight reduction, in contraction rate when TNF-α was added to non-palmar dermal fibroblasts also obtained from patients undergoing dermofasciectomy for Dupuytren's disease. It is interesting to note that Dupuytren's disease only affects the palms of the hand and rarely the soles of the feet or the tunica albuginea of the penis (Peyronie's disease).

[0101] The key next question was whether the contractility of myofibroblasts in Dupuytren's disease could be reversed by the addition of anti-TNF-α in a dose-dependent manner. Collagen gels seeded with 1.5 million Dupuytren's myofibroblasts/fibroblasts were cultured for 24h in the presence of anti TNF-α (murine anti-human R&D Systems, MAB2010) and isometric force contraction quantified. Experiments were performed in triplicate using cells from 5 consecutive unselected patients. There was no effect with isotype control antibody or with 0.1 µg/ml of anti TNF-α. Values represent mean ± SEM. The results are shown in Figure 23. Addition of anti-TNF-a at a dose range of 1-10 µg/ml to myofibroblasts from Dupuytren's cord down-regulated their contraction in the culture force monitor in a dose-dependent manner (Figure 23).

[0102] We next assessed the effect of anti- TNF-α on myofibroblast morphology. All the cells in the untreated gels or those exposed to IgG isotype control antibody were spindle shaped and aligned in the axis of maximal stress (Fig 24a). However, in the gels treated with 10 µg/ml anti-TNF-α, many of the cells showed a stellate morphology, without any alignment to the direction of stress (Fig 24b,c).

[0103] For the experiments for Figure 24, gels from the experiments shown in Fig 23 were fixed in 3% paraformaldehyde were immunofluorescently labelled using α-smooth muscle actin antibodies (red), phalloidin (green) and DAPI (nucleiblue). Figure 24a shows cells from a gel exposed to isotype control IgG antibody. Figures 24b and c show cells from a gel exposed to 10 µg/ml anti-TNF-α antibody stained with phalloidin and antibody to α-smooth muscle actin respectively. Original images photographed at x100.

[0104] Figure 25 illustrates a schematic of proposed role of advanced glycation end products, injury and alarmins in the pathogenesis of fibroproliferative disorders, highlighting the key role of TNF-α in the final common pathway. Hence TNF-α is a key therapeutic target for both early Dupuytren's disease and to prevent recurrence following treatment with collagenase.

[0105] These Examples illustrate initial findings that enhance understanding of Duputren's nodule material and contractile behaviour especially relating to the role and behaviour of active myofibroblasts. These findings implicate TNF-α in myofibroblast activity as well as DAMPs and AGE, which support the finding that TNF-α antagonists, DAMP antagonists and/or AGE inhibitors may be used to prevent or inhibit disease onset or progression from early state disease to established state disease and to prevent or inhibit disease recurrence in established disease where patients have undergone a primary corrective treatment.

REFERENCES CITED IN THE DESCRIPTION

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- **BADALAMENTE et al.**J Hand Surg Am, 2007, vol. 32, 767-774 [0025]
- **ROMBOUTS**J Hand Surg Am, 1989, vol. 14, 644-652 [0045]
- **VERJEE et al.**J Hand Surg Am, vol. 34, 1785-1794 [0067]
- J Cell Physiol, vol. 224, 681-690 [0067]
- **VERJEE et al.**Hand Surg Am20090000vol. 34, 1785-1794 [0090]
- **VERJEE et al.**J Cell Physiol, 2010, [0090]

P A T E N T K R A V

1. Sammensætning til anvendelse til behandling af et tidligt stadie af muskuloskeletal fibroproliferativ lidelse, hvor sammensætningen omfatter en terapeutisk, profylaktisk eller progressionsinhiberende effektiv mængde af en TNF-α-antagonist, hvor sygdommen er **k e n d e t e g n e t v e d** tilstedeværelsen af tegn eller symptomer på sygdom, men hvor der ikke optræder betydelig kontraktur.

2. Sammensætning ifølge krav 1 til anvendelse ifølge krav 1, hvor tegnene eller symptomerne på sygdom er tilstedeværelsen af histologiske knuder og/eller tilstedeværelsen af kliniske knuder.

3. Sammensætning ifølge krav 1 eller krav 2 til anvendelse ifølge krav 1 eller 2, hvor den muskuloskeletale fibroproliferative lidelse er fibromatosesygdom.

4. Sammensætning ifølge et hvilket som helst af kravene 1 til 3 til anvendelse ifølge et hvilket som helst af kravene 1 til 3, hvor den muskuloskeletale fibroproliferative lidelse er valgt blandt Dupuytrens kontraktur, Ledderhoses sygdom, Peyronies sygdom og muskuloskeletale adhæsioner.

5. Sammensætning ifølge krav 4, til anvendelse ifølge krav 4, hvor de muskuloskeletale adhæsioner er valgt blandt adhæsiv kapsulit og tendinose.

6. Sammensætning ifølge et hvilket som helst af de foregående krav til anvendelse ifølge et hvilket som helst af de foregående krav, hvor den muskuloskeletale fibroproliferative lidelse er Dupuytrens kontraktur.

7. Sammensætning ifølge krav 6 til anvendelse ifølge krav 6, hvor forstyrrelsen er **k e n d e t e g n e t v e d** kontraktur i led på mindre end 20°.

8. Sammensætning ifølge et hvilket som helst af de foregående krav til anvendelse ifølge et hvilket som helst af de foregående krav, som er til lokal anvendelse på områder, hvor sygdommen manifesterer sig eller giver symptomer.

9. Sammensætning ifølge et hvilket som helst af de foregående krav til anvendelse ifølge et hvilket som helst af de foregående krav, som omfatter en TNF-α-antagonist og et middel til nedbrydning, indskrænkelse eller kløvning af ekstracellulær matrix.

10. Sammensætning ifølge krav 9 til anvendelse ifølge krav 9, hvor midlet til nedbrydning, indskrænkelse eller kløvning af ekstracellulær matrix er en matrix-metalloproteinase og/eller en kollagenase.

11. Sammensætning ifølge et hvilket som helst af de foregående krav til anvendelse ifølge et hvilket som helst af de foregående krav, hvor sammensætning er formuleret til direkte injektion i sygt væv.

12. Sammensætning ifølge krav 11 til anvendelse ifølge krav 11, hvor sammensætning er til direkte injektion i knuder.

13. Sammensætning ifølge et hvilket som helst af kravene 1 til 10 til anvendelse ifølge et hvilket som helst af kravene 1 til 10, hvor sammensætningen er formuleret til topisk påføring.

14. Sammensætning ifølge et hvilket som helst af de foregående krav til anvendelse ifølge et hvilket som helst af de foregående krav, hvor TNF-α-antagonisten er valgt blandt en eller flere af Infliximab, Adalimumab, Certolizumab pegol, Golimumab eller Etanercept.

DRAWINGS

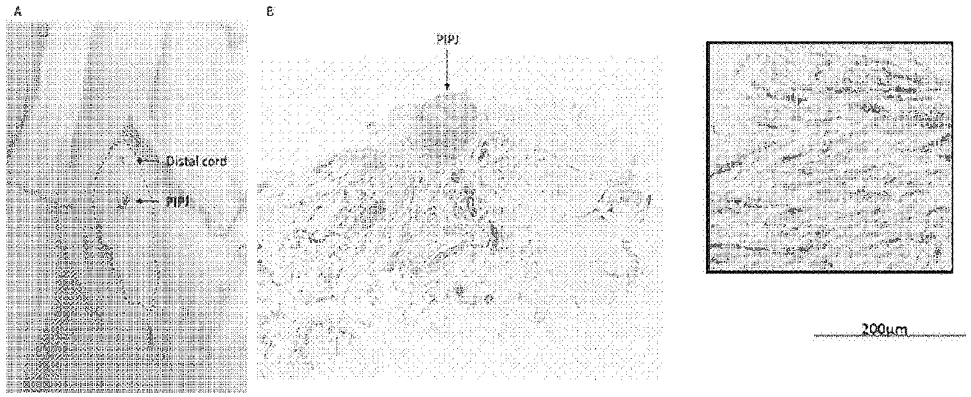


Figure 1

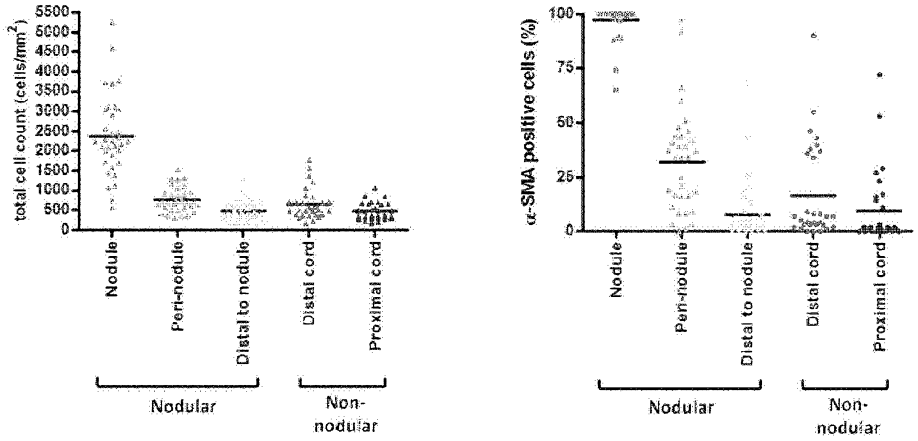
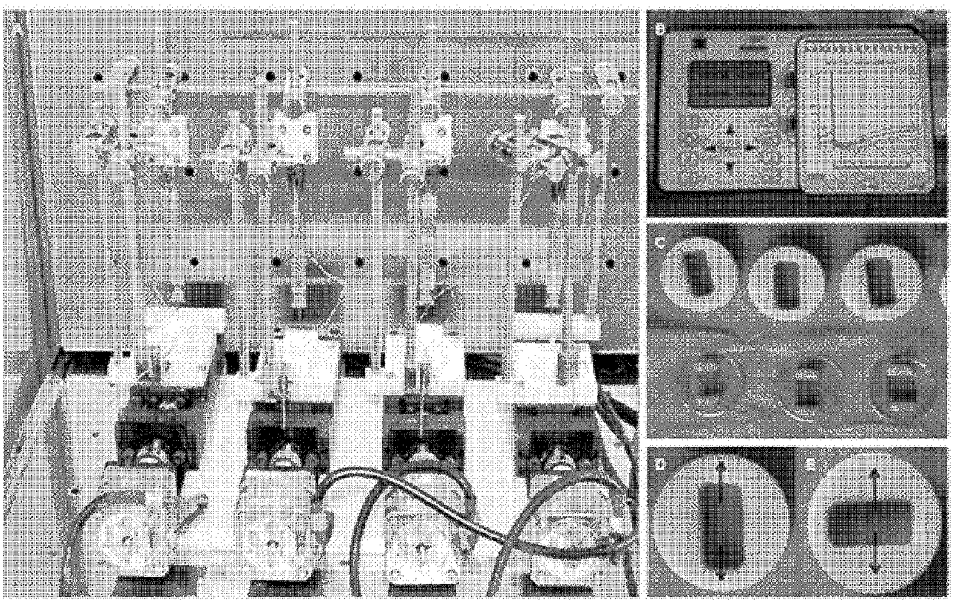


Figure 2



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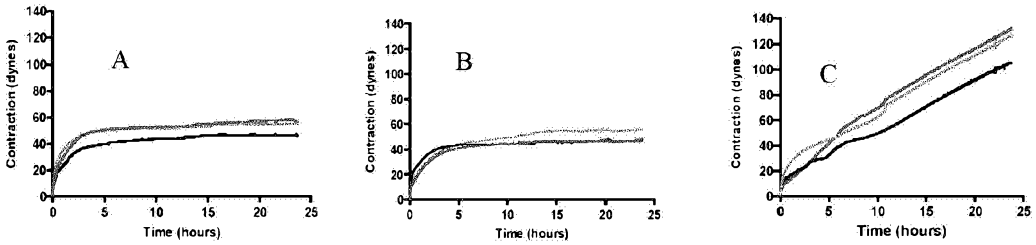

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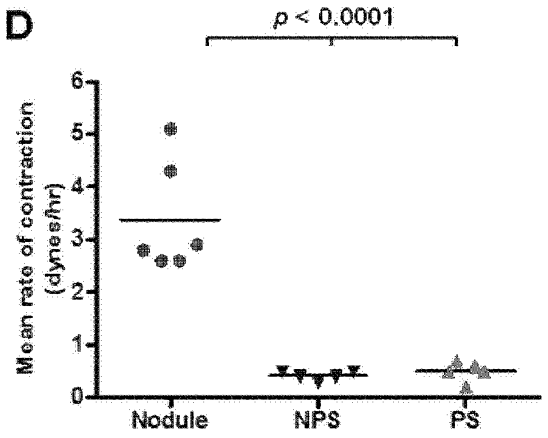

D
p < 0.0001
Mean rate of contraction (dynes/hr)
6
5
4
3
2
1
0
Nodule
NPS
PS

Figure 5

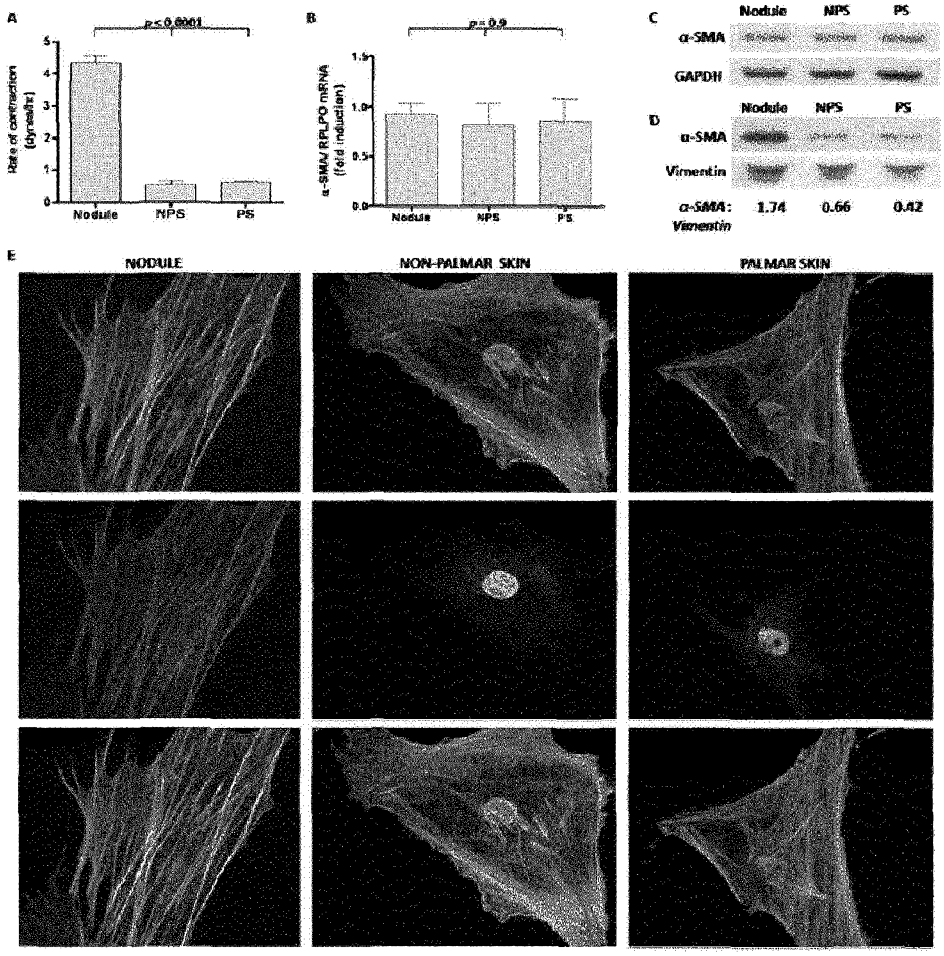

A
p<0.0001
Rate of contraction (dyne/hr)
Nodule
NPS
PS
B
p=0.9
α-SMA/ RPLPO mRNA (fold induction)
Nodule
NPS
PS
C
Nodule
NPS
PS
α-SMA
GAPDH
D
Nodule
NPS
PS
α-SMA
Vimentin
α-SMA: Vimentin
1.74
0.66
0.42
E
NODULE
NON-PALMAR SKIN
PALMAR SKIN

Figure 6

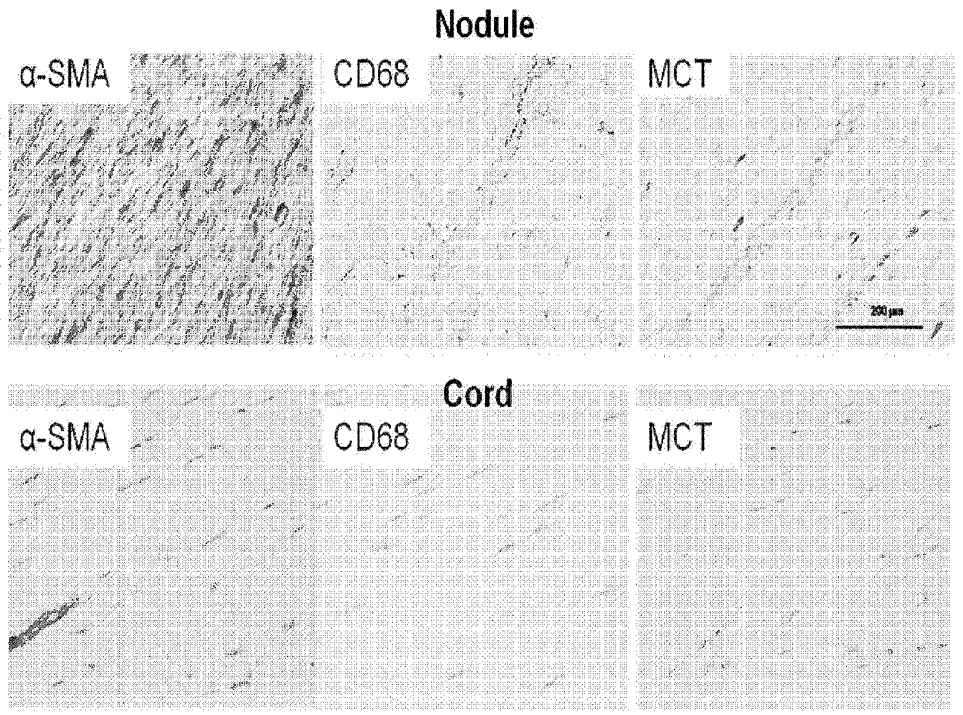


Figure 7

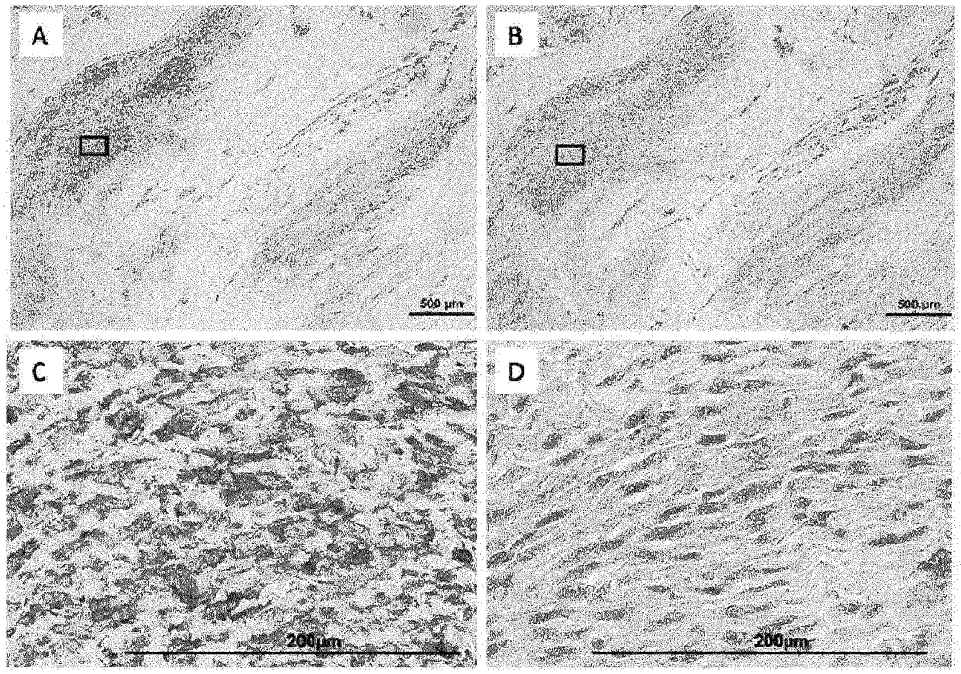


Figure 8

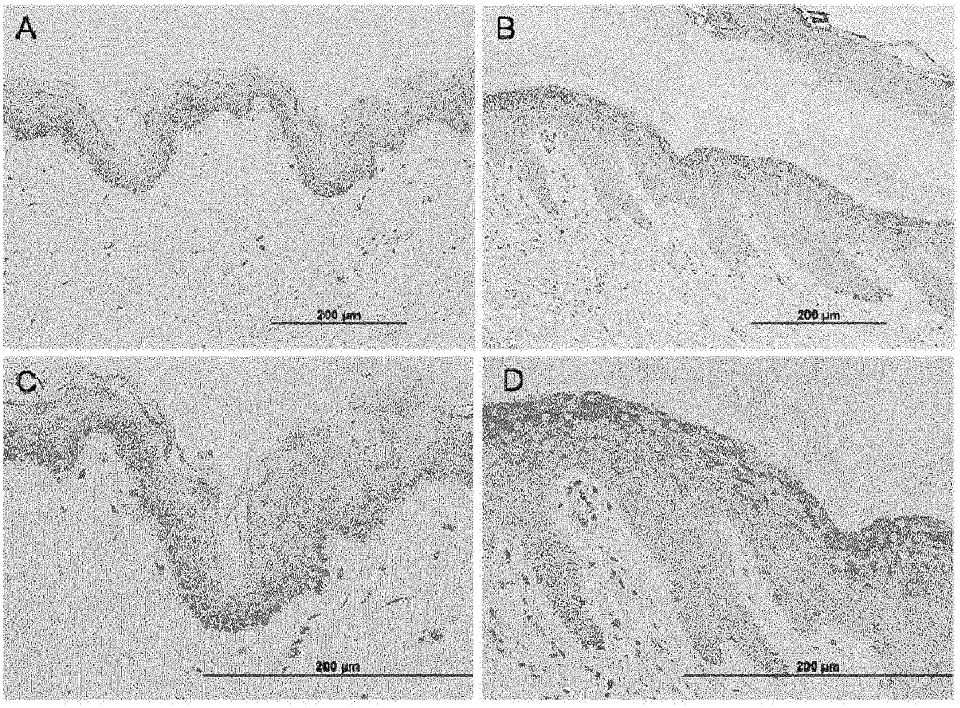


Figure 9

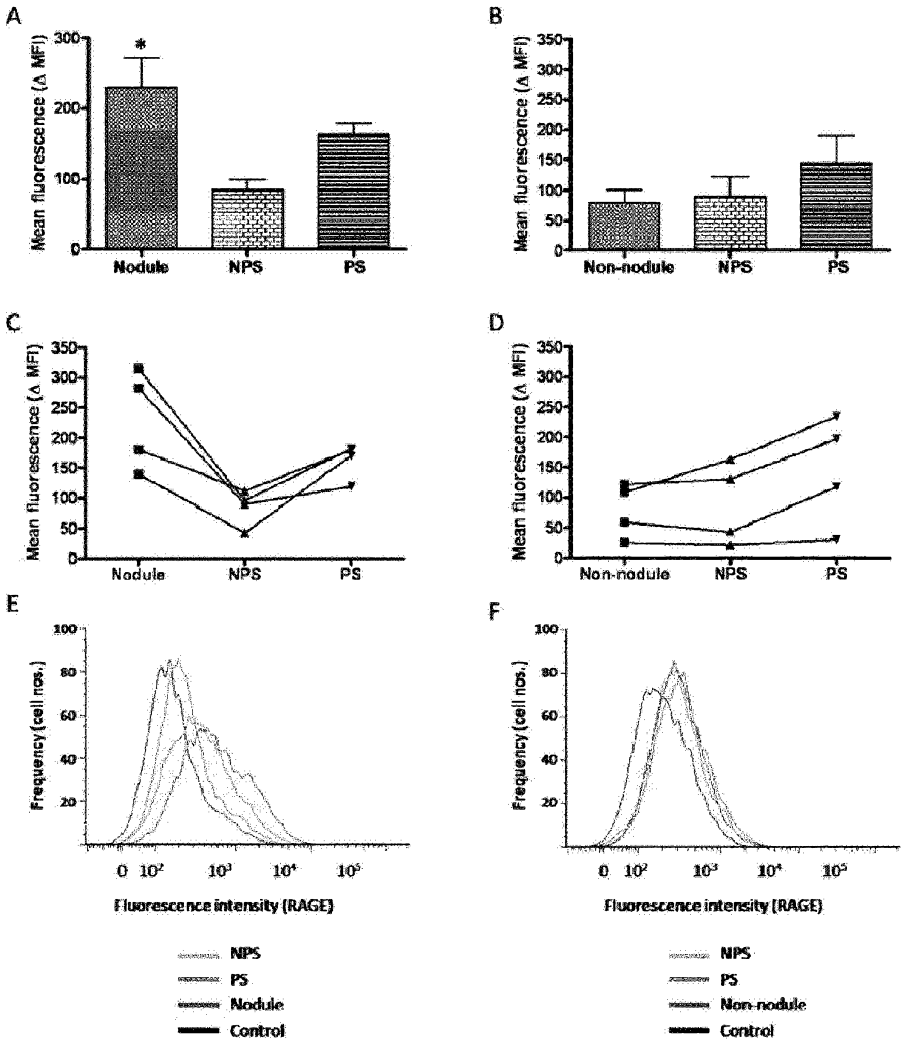
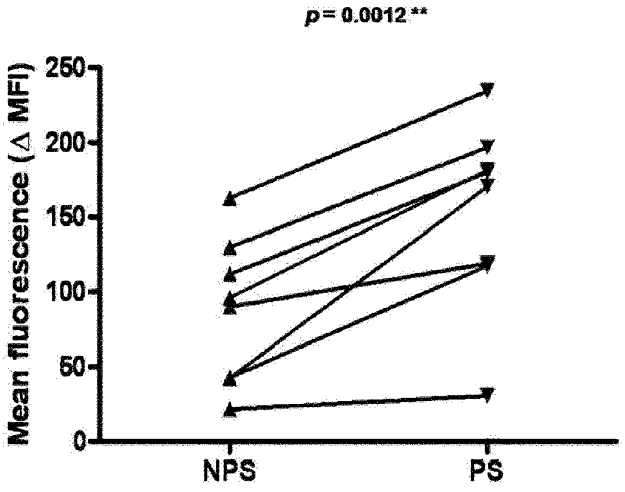

A
Mean fluorescence (Δ MFI)
300
200
100
0
*
Nodule
NPS
PS
B
Mean fluorescence (Δ MFI)
350
300
250
200
150
100
50
0
Non-nodule
NPS
PS
C
Mean fluorescence (Δ MFI)
350
300
250
200
150
100
50
0
Nodule
NPS
PS
D
Mean fluorescence (Δ MFI)
350
300
250
200
150
100
50
0
Non-nodule
NPS
PS
E
Frequency (cell nos.)
100
80
60
40
20
0
10²
10³
10⁴
10⁵
Fluorescence intensity (RAGE)
NPS
PS
Nodule
Control
F
Frequency (cell nos.)
100
80
60
40
20
0
10²
10³
10⁴
10⁵
Fluorescence intensity (RAGE)
NPS
PS
Non-nodule
Control

Figure 10



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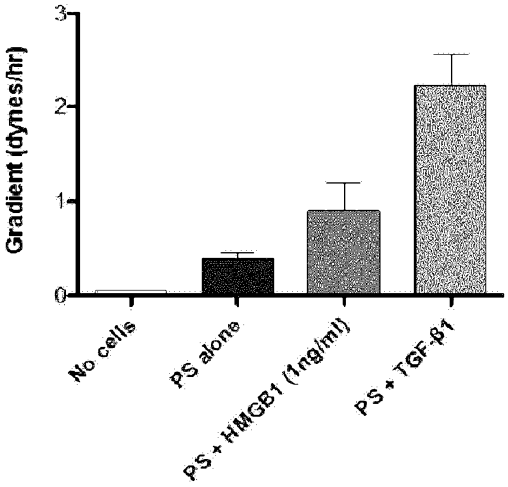

Figure 12

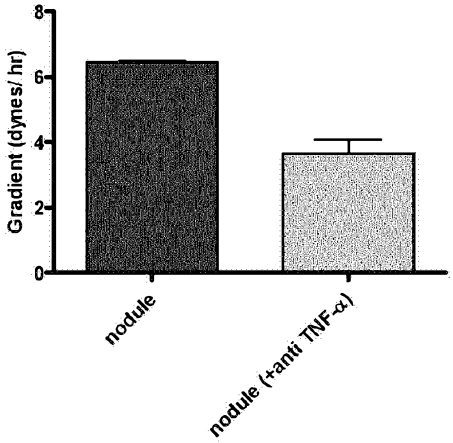

Figure 13

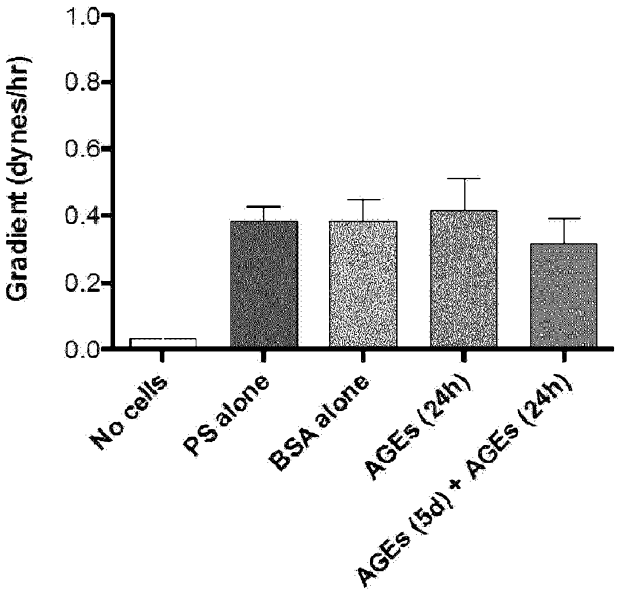

Figure 14

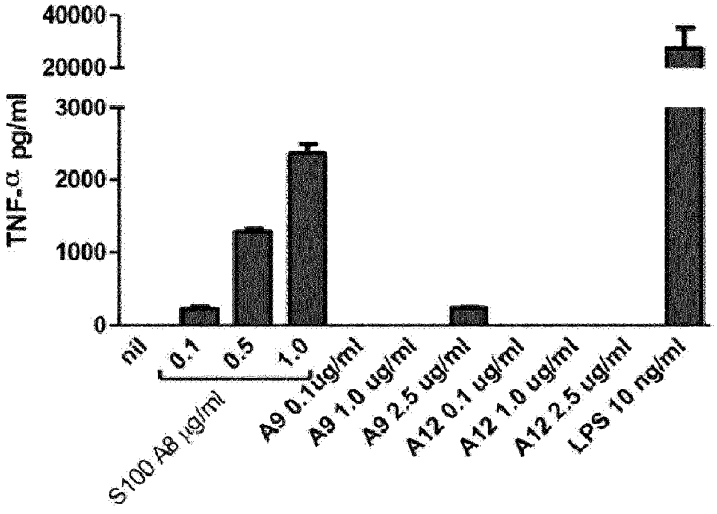

Figure 15

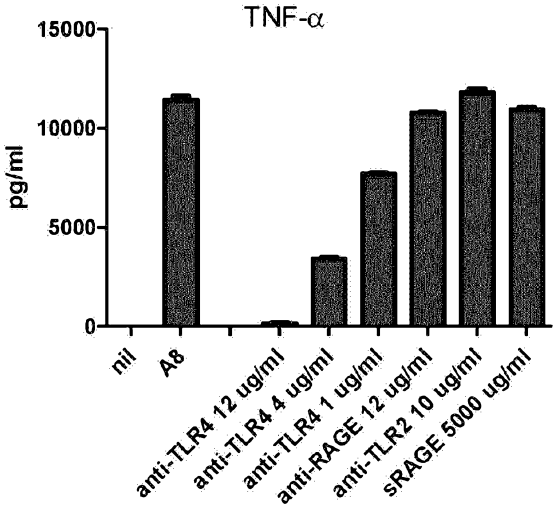

Figure 16

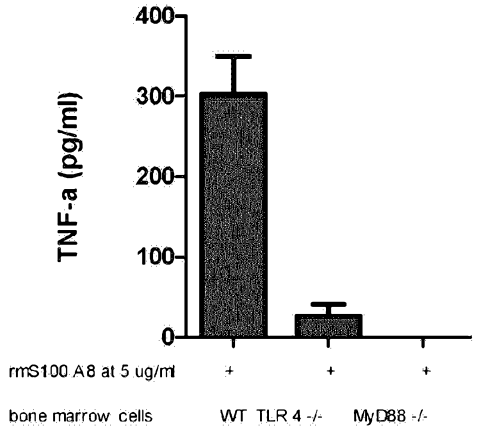

Figure 17

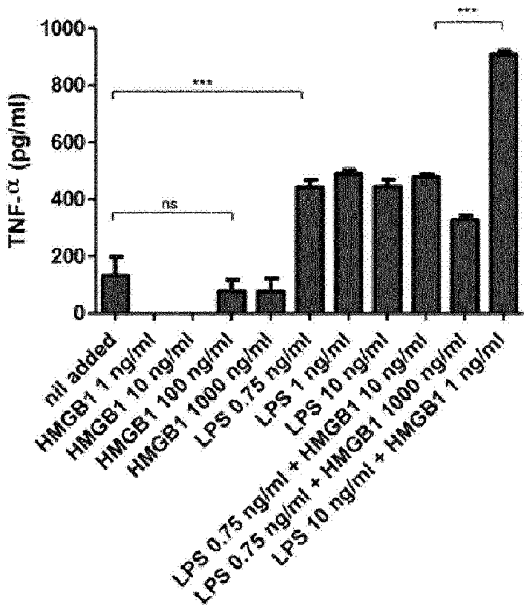

Figure 18

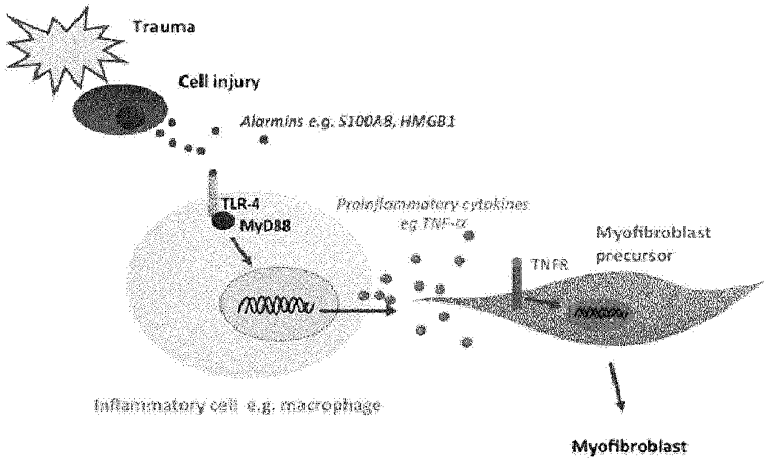

Figure 19

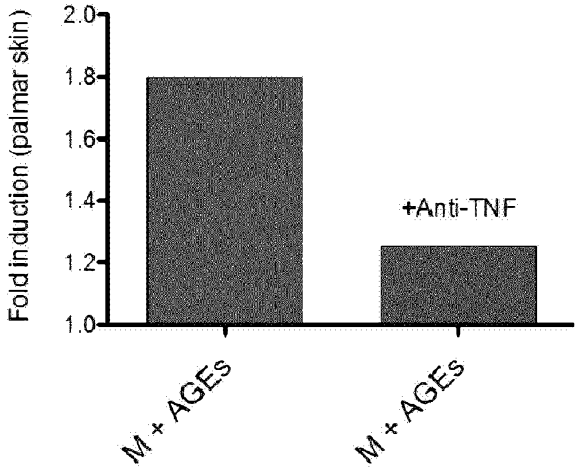

Figure 20

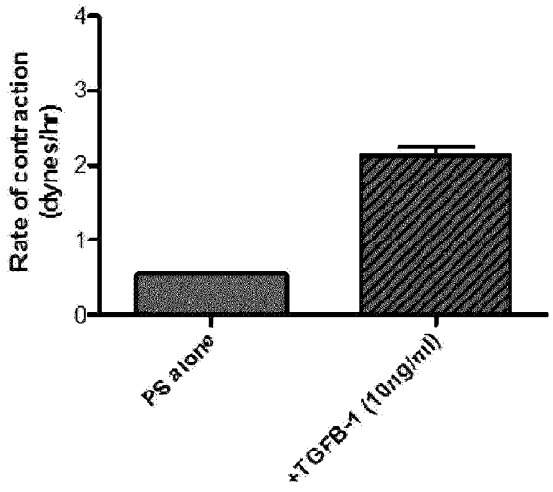

Figure 21

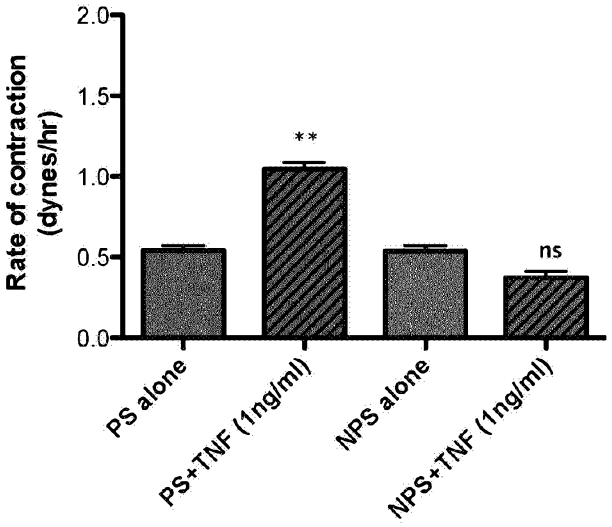

Figure 22

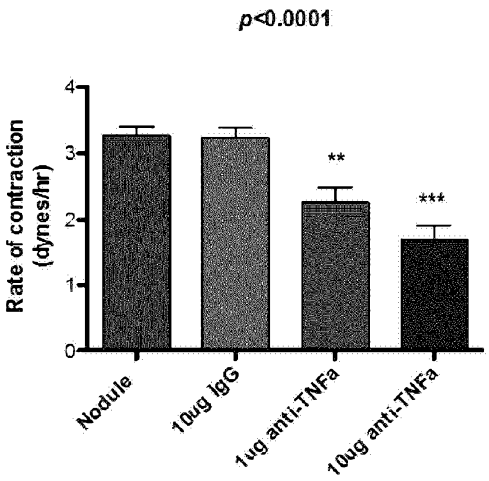
p<0.0001
Rate of contraction (dynes/hr)
4
3
2
1
0
**
***
Nodule
10ug IgG
1ug anti-TNFa
10ug anti-TNFa

Figure 23

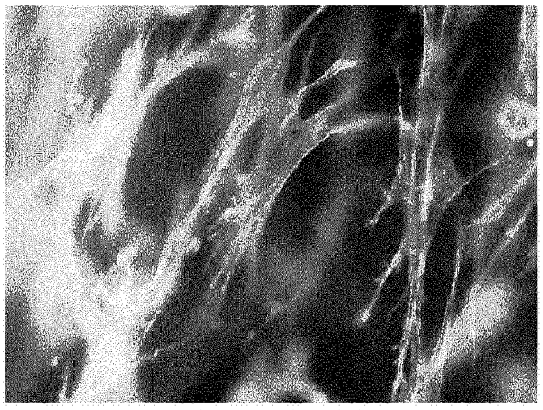

Figure 24 (a)

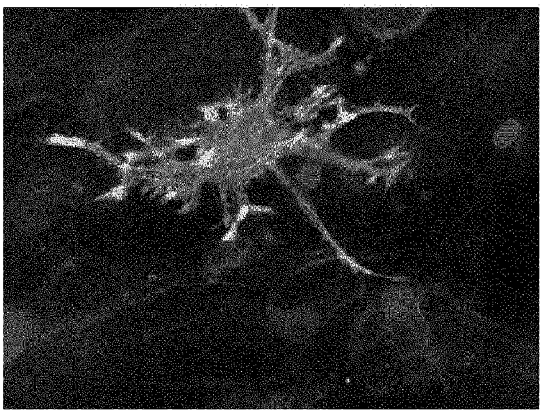

Figure 24 (b)

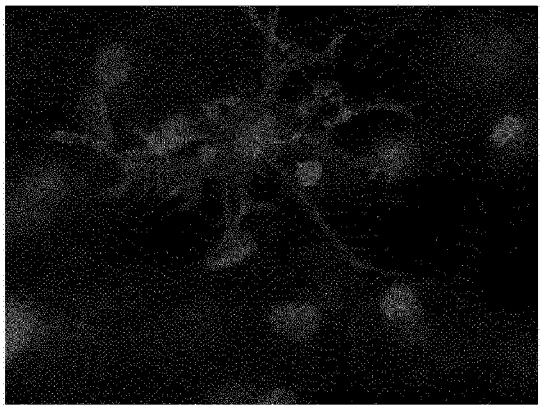

Figure 24 (c)

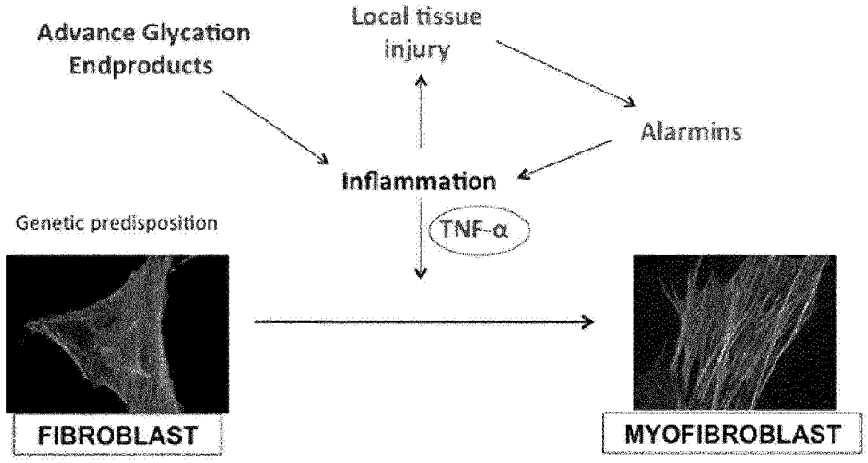
Advance Glycation Endproducts
Local tissue injury
Alarmins
Inflammation
TNF-α
Genetic predisposition
FIBROBLAST
MYOFIBROBLAST

Figure 25