(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 September 2014 (12.09.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/138372 A1

(51) International Patent Classification:
A61K 36/00 (2006.01) *A61P 19/02* (2006.01)

(21) International Application Number:
PCT/US2014/021139

(22) International Filing Date:
6 March 2014 (06.03.2014)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/773,596 6 March 2013 (06.03.2013) US

(71) Applicant: **PRIMAVERA BIOSCIENCES, INC.** [US/US]; 6253 Martin Road, Columbia, MD 21044 (US).

(72) Inventor: **TAKEDA, Yoshinori**; c/o Primavera Biosciences, Inc., 6253 Martin Road, Columbia, MD 21044 (US).

(74) Agent: **NAKAMURA, Yoshiya**; Westerman, Hattori, Daniels, & Adrian, LLP, 1250 Connecticut Avenue, Suite 700, Washington, DC 20036 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

(54) Title: COMPOSITION AND METHOD FOR TREATING OSTEOARTHRITIS

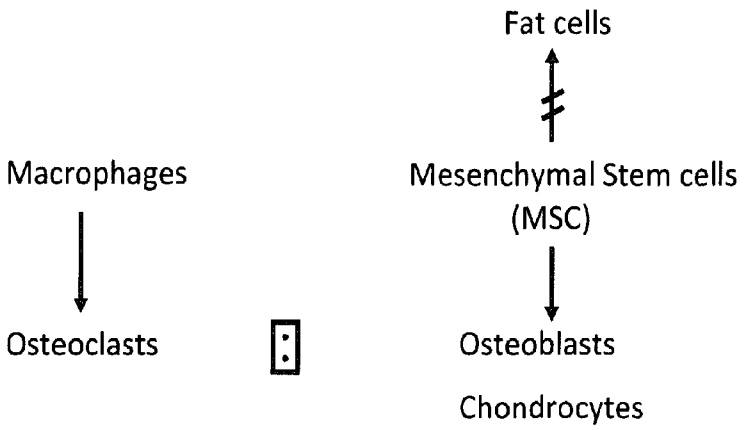

FIG.1

(57) Abstract: A composition for treating osteoarthritis includes an anti-inflammatory agent and an agent that enhances bone-formation and chondrogenesis in combination. The combined use of the anti-inflammatory agent and the agent that enhances bone-formation and chondrogenesis can synergistically ease and reverse the progression of osteoarthritis. Examples of the anti-inflammatory agent include an NF-kappaB inhibitor which is curcumin or turmeric extract. Examples of the agent that enhances bone-formation and chondrogenesis include phytonutrient that includes a PPAR-gamma inhibitor and/or phytoestrogen. The phytonutrient may be selected from the group consisting of soybean extract, kudzu extract, ginkgo extract, and celery seeds extract. The PPAR-gamma inhibitor and/or phytoestrogen may be selected from the group consisting of highly-absorbable genistein, daidzein, quercetin, kaempferol, and apigenin.

WO 2014/138372 A1

DESCRIPTION

Title of the Invention

Composition and method for treating osteoarthritis

Technical filed

[0001] The invention relates to compositions and methods for treating osteoarthritis, particularly, combined use of anti-inflammatory agent and an agent that enhances bone formation and chondrogenesisto ease and help reverse the progression of osteoarthritis.

Background art

[0002] Osteoarthritis is the most common form of arthritis, and according to the Center of Disease Control and Prevention (CDC), 25 – 27 million American adults suffer from osteoarthritis which is one of the leading causes of disability in America. 80% of older adults suffer from the disease. Osteoarthritis occurs when the protective cartilages on the ends of the bones wear down over time, and while osteoarthritis can damage any joints in the body, the disease affects most commonly joints in hands, neck, lower back, knees, and hips. Osteoarthritis gradually worsens with time, and currently no cure exists.

[0003] The current treatments for osteoarthritis focus on relieving pain, slowing the progression of the disease, and improving joint function. Medications include pain killers such as acetaminophen (Tylenol (trademark)), non-steroidal anti-inflammatory drugs (NSAIDs; e.g., aspirin, ibuprofen and naproxen), or narcotics. Physicians may recommend weight loss if patients are overweight or obese and instruct physical therapy and lifestyle changes. If osteoarthritis gets worse, there are surgical options such as lubricant injection, joint realignment, and joint replacement.

[0004] Long-term uses of Tylenol, however, at doses which are effective for pain relief (~ 4 g/day) can lead to liver damage. Use of NSAIDs is associated with adverse events of gastrointestinal bleeding, stomach upset, cramping, diarrhea, and peptic ulcer. Selective COX-2 inhibitor Vioxx (trademark) was withdrawn from the market and Celebrex (trademark) has been used under a black box warning for the elevated risk of cardiovascular disease. Oral use of corticosteroids results only in limited improvement, and the injections of corticosteroids into the joints provide a relief from pain in a short-term but do not improve osteoarthritis in a long run. A need for better management of inflammation and pain is apparent.

[0005] Since glucosamine and chondroitin are precursors in the formation of cartilage, oral consumption of these precursor molecules has been advocated as a possible method of increasing the rate of cartilage repair. However, the research has found no evidence of these

precursor molecules being incorporated into joints. Numerous clinical trials have been conducted. The GAIT reports 2006 (a summary of six months clinical trials) noted that supplements of glucosamine/condroitin reduced pain more than placebo, sometimes almost as comparable to Celebrex (trademark). However, the GAIT report 2010 (a summary of two years clinical trials) stated that there were almost no difference in effects among Celebrex (trademark), glucosamine/condroitin, and placebo and none of them improved or reversed the overall progression of osteoarthritis.

Summary of the invention

[0006] A composition according to the present invention includes an anti-inflammatory agent and an agent that enhances bone-formation andchondrogenesis in combinationat a therapeutically effective amount thereof to treat osteoarthritis.The combined use of the anti-inflammatory agent and the agent that enhances bone-formation andchondrogenesiscan synergistically ease and reverse the progression of osteoarthritis.

[0007] According to one of the embodiments of the invention, the anti-inflammatory agent is an NF-kappaB inhibitor which is curcumin or turmeric extract, and/or the NF-kappaB inhibitor includeshighly-absorbable curcumin. Highly-absorbablecurcuminis commercially availableas BCM-95 (trademark), Curcumin C3/Bioperine (trademark), and Theracurmin (trademark).

[0008] According to another embodiment, the agent that enhances bone-formation andchondrogenesis is phytonutrientthatincludes a PPAR-gamma inhibitor and/or phytoestrogen. The phytonutrient may be selected from the group consisting of soybean extract, kudzu extract, ginkgo extract, and celery seeds extract.The PPAR-gamma inhibitor and/or phytoestrogen may be selected from the group consisting of highly-absorbable genistein, daidzein, quercetin, kaempferol, and apigenin.

[0009] The composition according to another embodiment includes a combination ofcurcuminand kudzu extract, a combination of curcuminand ginkgobiloba extract, or a combination of curcumin, turmeric extract, kudzu extract and ginkgobiloba extract. According to preferableembodiments, the composition may includecurcumin, daidzein, genistein, quercetin, kaempferol and/or apigenin at a dose of 10 – 100 mg, most preferably 20 -30 mg, per day when a highly-absorbable ingredient is used; at a dose of 50 – 1000 mg, most preferably 200 – 600 mg, per day when a regular, low-absorbing ingredient is used.

[0010] The composition according to another embodiment includeshighly-absorbable curcuminin combination with at least one phytonutrient selected from the group consisting of extracts containing highly-absorvablegenistein, daidzein, quercetin, kaempferol, and apigenin.

[0011] In another aspect of the invention, a pharmaceutical composition useful in treating osteoarthritisis provided, whichincludes at a therapeutically effective amount to treat the disease a combination of synthetic compounds selected from the group consisting of curcumin, curcumin derivatives, daidzein, daidzein derivatives, quercetin, kaempferol, apigenin, and synthetic deidzein derivative equol, in the use of helping ease and reverse the progression of osteoarthritis. The composition may include each synthetic compound at a dose of 5 - 50 mg per day.

[0012] In another aspect of the invention, any of the compositionsdescribed above is used in human and veterinary uses.In another aspect of the invention, the agents listed above can be used in a process for producing a pharmaceutical composition for treating osteoarthritis.

[0013] In another aspect of the invention, an anti-inflammatory agent and an agent that enhances bone-formation andchondrogenesisare administered to a subject suffering from osteoarthritis at a therapeutically effective amount to treat the disease.The method according to one of the embodiments of the invention, the anti-inflammatory agent is an NF-kappaB inhibitor, and the agent that enhances bone-formation andchondrogenesis is a PPAR-gamma inhibitor and/or phytoestrogen.In a preferable embodiment, the anti-inflammatory agent is selected from the group consisting of curcumin, andturmeric extract, and the agent that enhances bone-formation andchondrogenesis is selected from the group consisting of soybean extract, kudzu extract, ginkgo extract, celery seeds extract, highly-absorbable genistein, daidzein, quercetin, kaempferol, and apigenin. According to preferableembodiments, the daily dose for the highly-absorbable ingredientis administered at 10 - 100 mg, preferably, 20 - 40 mg, and the daily dose for the regular low-absopbing ingredients may be administered at 50 - 1000 mg, preferably, 200 - 600 mg. Any of the combinations of agents listed above can accompany instructions in paper or online to patients or doctors to use the agents in combination for oral administration. Such instructions may include required dose or frequency of administrations which can be determined based on guidance provided in this application and a common knowledge regarding the agents.

Brief description of drawings

[0014] FIGURE 1 illustrates the roles phytonutrients play in bone formation and cartilage repair. Osteoclasts (bone-resorpting cells) are derived from monocytes and/or macrophages. Mesenchymal stem cells (MSC) are a precursor for fat cells, osteoblasts (bone-forming cells), and chondrocytes (cartilage-forming cells). Isoflavones (genistein, daidzein) and/or flavonoids (quercetin, kaempferol, apinegin), antagonizing PPAR-gamma, inhibits adipogenesis (fat formation) and promotes production of osteoblasts and chondrocytes. The same isoflavonesand flavonoids, acting as phytoestrogen, suppress activities of osteoclasts and

enhance activities of osteoblasts and chondrocytes. This leads to stronger bone formation and cartilage repair, opposing to aging that makes osteoclasts more active than osteoblasts, and adipogenesis more active than blastogenesis which leads to fat accumulation, bone loss and cartilage destruction.

[0015] FIGURE 2 illustrates improvements in walking or running capability of an osteoarthritis patient treated with a combination of curcumin and the kudzu extract (containing daidzin). X-axis: time (week); Y-axis: a speed of walking or jogging per hour. Y-axis also represents an actual distance walked or jogged since a patient walked or jogged for one hour. More explanations are given in EXAMPLE 1.

Detailed description of the invention

[0016]The present invention was inspired by the recent observations that cartilage precursor mesenchymal stem cells (MSCs) exist in synovial fluids of osteoarthritic knees. Jones et al reported in 2004 & 2008 that they found pluripotent mesenchymal stem cells (MSCs) in synovial fluids of the knees of healthy individuals as well as osteoarthritis patients; in increased numbers in the latter. The follow-up studies(for example, Sekiya et al., 2012; Patent Application US 20100178274) then reported that the MSCs in synovial fluid increased proportionally to osteoarthritis grading, even with osteoarthritis patients between age 65 and 85.These observations suggest that the body is attempting to repair degenerated joints but the joints are under unfavorable conditions; and so, if the joints are placed in a favorable environment, osteoarthritis may be reversed. In fact, there have been some indications that spontaneous joint repairs seem to have happened (Hunziker&Kapfinger 1996; Gaby 1999).

[0017] Encouraged with these observations, the inventor searched the conditions that may favor the repair of damaged cartilages. The first finding is that although “wear and tear” is said to be responsible forcartilage destructions, pro-inflammatory mediators (cytokines and metallproteinases)appear to be more responsible for cartilage destructions. Therefore, in order to reverse osteoarthritis, pro-inflammatory mediators must be down-regulated and well managed over a long period of time. The second finding is that in order to repair cartilage, bone formation must be encouraged andchondrogenesisbe activated. Bone formation and cartilage repair share many of the same growth hormones such as transforming growth factor-beta (TGF-beta) and insulin-like growth factor-1 (IGF-1). These growth hormones not only stimulate the differentiation of precursor mesenchymalstem cells (MSC) into osteoblasts (bone forming cells) and chondroblasts (cartilage forming cells) but are needed during bone formation and cartilage repair. The third finding is that the differentiation of pluripotent MSC into osteoblasts&chondroblasts is counter-balanced with its differentiation into adipocytes (fat forming cells). See FIGURE 1.The key factor that divergently

regulatesosteogenesisvsadipogenesis is nuclear transcription factor PPAR-gamma (peroxisome proliferator-activated receptor-gamma).The inhibition of PPAR-gamma promotesosteogenesis and chondrogenesiswhile the activation of PPAR-gamma promotesadipogenesis.The following ingredients among others influence the above factors and processes in favor for joint repair.

[0018] Curcumin/turmeric is an anti-inflammatory polyphenol. Oral administration of curcumin/tumeric not only eases typical osteoarthritic pain within a couple of weeks, but also reduce or stop cartilage destructions by down-regulating pro-inflammatory cytokines. NF-kappaB is chronically active in many inflammatory diseases such as osteoarthritis, rheumatoid arthritis, inflammatory bowel disease, and cancer. Curcumin inhibits transcription factor NF-kappaB (nuclear factor kappa-light-chain-enhancer of activated B cells) which regulates a variety of genes encoding pro-inflammatory cytokines (TNF-alpha, IL-1, IL-6), chemokines (IL-8, MIPI-alpha), and inducible enzymes (iNOS, COX-2). The inhibition of NF-kappaB down-regulates the production of phospholipase, lipooxygenase, cyclooxygenase 2, leukotrienes, thromboxane, prostaglandins, nitrix oxide, collagenase, elastase, hyaluronidase, monocyte chemoattractant protein-1 (MCP-1), interferon-inducible protein, TNF-alpha, and IL-12. And there is plenty of evidence that curcumin/tumeric is safe for long-term use.

[0019] Phytonutrients such as daidzein, quercetinand kaempferol are an inhibitor of transcription factor PPAR-gamma and direct the differentiation of pluripotent mesenchymal stem cells (MSCs) from adipocytes to osteoblasts & chondrocytes (Takada et al 2009; Viccica et al 2010). These phytonutrients also stimulate the production of osteoprotegrin (OPG) which down-regulates bone resorption (bone degradation) and promote bone formation&chondrogenesis.

[0020] A combination of extracts prepared from turmeric, soybean or kudzu, ginkgo bilobaor celery seeds, or a combination of bioactive compounds selected from a group of curcumin, genistein, daidzein, quercetin, kaempferol, andapigenin, not only eases pain and inflammation but synergistically promotes formation of strong bone and cartilage repair, opposing to fat accumulation, bone loss and cartilage destruction the aging process brings about.Curcumin/turmeric suppresses pain and inflammation but that is not enough for cartilage repair. It appears that cartilage repair depends on the newly-activated chondroblasts which bind damaged extracellular matrix (ECM), secret the precursor molecules and amend damaged ECM when destructive cytokines are down-regulated. Without the newly-activated chondroblasts cartilage repair may not occur even if plenty of cartilage precursor molecules such as glucosamine, chondoitin, collagen UC-II and hyaluronan are orally provided.

[0021] Osteoarthritis is the result of mechanical and biological events that destabilize the balance between the synthesis and breakdown of cartilage. This imbalance can be caused by

multiple factors: genetic, metabolic, traumatic, or wear and tear. Osteoarthritis affects all bones and tissues of the joints and manifests itself by biochemical, molecular and biomechanical changes of the cartilaginous matrix leading to softening, cracking, ulceration, a loss of articular cartilage, and sclerosis of the subchondral bone. When it becomes symptomatic, osteoarthritis causes pain and stiffness with variable degrees of local inflammation. However, the speed and severity of cartilage breakdown are highly variable; cartilage destruction is not always linear but can be interrupted with phases of restoration of the damages and the joint space. The consequences of this disease also vary. At the first step, the cartilage supporting the body weight and motion breaks down and the cartilage loses thickness. Then, if almost all cartilage breaks down and the subchondral bones are exposed, reshaping of the bone with the appearance of osteoporosis and areas of bone condensation begins. The inhomogeneous bone tissues can cause pain in surrounding tissues and also induce severe brittleness of bone tissue; then, tissue debris can form and accumulate within the joint. This causes joint inflammation and enhances further joint degeneration.

[0022] In osteoarthritis (and rheumatoid arthritis), the synovial joints (fluids) contain a variety of pro-inflammatory mediators, but at the same time they also contain proteinase inhibitors (TIMP1, TIMP2) and a variety of mitogenic growth factors (transforming growth factors (TGF), bone morphogenic protein (BMP), insulin-like growth factor-1 (IGF-1), and epidermal growth factor (EDGF)). A real cause of osteoarthritis is this imbalance between destructive factors and constructive factors: if the former dominates, cartilage destruction ensues; and if the latter dominates, cartilage repair can occur.

[0023] Pro-inflammatory mediators include cytokines, chemokines, methalloproteinases (MMP), other proteinases, tumor necrosis factor-alpha (TNF-alpha), and interleukins (IL-1, IL-6, IL-8). Primary cytokines IL-1 and TNF-alpha and secondary cytokines IL-6 and IL-8 cause inflammation in synovial membrane and cartilage. IL-8 stimulates production of prostaglandin 2 (PGE2) and PGE2 stimulates breakdown of extracellular matrix. IL-6 decreases the production of type II collagen. These cytokines increase the production of destructive enzymes and inhibit the synthesis of collagen and aggrecan.

[0024] Collagenases breakdown the collagen type II scaffolding in cartilage. Levels of collagenase-1 (MMP-1) and collagenase-3 (MMP-13) correlate with the severity of cartilage destruction. Aggrecanases degrade aggrecan, the major component of proteoglycans in cartilage. Major aggrecanases, ADAMTS-4 and ADAMTS-11, contain the disintegrin and metalloprotease with thrombospondin motif. Stromelysins and gelatinase-A (MMP-2) and gelatinase-B (MMP-9) are also involved. Stromelysin levels also correlate with the severity of osteoarthritis. Other proteolytic enzymes include elastases and hyaluronidases.

[0025] Free radical nitric oxide (NO) also has been implicated in degenerative joint disorders. NO is enzymatically synthesized from arginine by NO synthetase. There are two isoforms: constitutive and inducible NO synthetases. Inducible NO synthetase (iNOS) is responsible for NO production in the synovial fluids of osteoarthritis patients, and inhibitors of inducible NO synthetase have been reported to suppress symptoms of osteoarthritis. The activation of NF-kappaBproduces COX-2 and inducible NO synthetase. In Patent Application US 20090117072 Kealey et al disclose cocoa extracts and polyphenols enriched procyanidins in treating periodontal disease. These compounds act as antioxidants, COX- and lipooxygenase modulator, NO synthetase modular, inhibitor of ROS (reactive oxygen species), platelet aggregator modulator, apoptosis modulator, and anti-cancer agents.

[0026] Cyclooxygenase (COX, prostaglandin endoperoxidesynthetase) catalyzes the metabolism of arachidonic acid to prostaglandin H.sub.2 (PGH.sub.2), which is further metabolized to various prostaglandins (PGs), prostacyclins and thromboxane A2. PGs are ubiquitous hormones that functions as paracrine and autocrine mediators to affect a myriad of physiological changes in the immediate cellular environment. The varied physiological effects of PGs include inflammatory reactions such as rheumatoid arthritis and osteoarthritis, blood pressure control, platelet aggregation, induction of labor and aggravation of pain and fever.

[0027] COX exists in two isoforms, COX-1 and COX-2. PGs produced by COX-1 and COX-2 are identical molecules, however, COX-1 and COX-2 generate a unique pattern and various amounts of eicosanoids in different tissues; therefore, relative differences in the activation of these enzymes may result in quite dissimilar biological responses. COX-1 is expressed constitutively in most tissues whereas COX-2 is induced by pro-inflammatory stimuli including cytokines, mitogens, and bacterial lipopolysaccharide. Non-steroidal anti-inflammatory drugs (NSAIDs) are COX inhibitors used in the control of pain and inflammation of osteoarthritis.

[0028] Prostaglandins play an important role in maintenance of human gastric mucosal homeostasis. Initially, COX-1 is believed to be responsible for PG synthesis in normal gastric mucosa, but it now appears that both COX-1 and COX-2 have important physiological roles in normal gastric mucosa. For the treatment of inflammation, selective COX-2 inhibitors (which do not down-regulate PGE.sub.2 in gastric mucosa) have been explored. However, the drugs thus developed, Celebrex® and Vioxx®, showed gastric toxicity inducing spontaneous bleeding and delaying gastric ulcer healing. Vioxx® was withdrawn from the market and Celebrex® has been used under a black box warning for the elevated risk of cardiovascular disease.

[0029] Long-term uses of Tylenol, NSAIDs, and steroids are not wise to control inflammation and pain in osteoarthritis as described above. Alternatively, in Patent No.US 8,257,754 Tripp et al disclose the formulations that would selectively inhibit COX-2 and inhibit inflammation. The

compositions contain hop extracts, tryptanthrin, and rosemary extracts. In Patent No. US 8,263,069 Johnson discloses compositions comprising anthocyanin or anthocyanidin to achieve similar goals. Patent No. US 6,391,346 also discloses hop extracts to reduce inflammation and promote sleep.

[0030] In Patent Application US 20120207827 Cozean et al disclose the oral and topical use of dimethylsulfoxide (DMSO) and methylsulfornylmethane (MSM) in treating osteoarthritis. In Patent Application US 20120201781 Kamath RV discloses the use of antibody against IL-1 in treating osteoarthritis. In Patent Application US 20090280081 Vasios G discloses JAK3-specific inhibitors (hymenialdisine, debromohymenialdisine, and their derivatives) in treating osteoarthritis; JAK3-specific inhibitors down-regulate steady state mRNA levels of key cellular components involved in cartilage degradation. TNF-alpha inhibitor, Enbrel®, has been successfully used in treating rheumatoid arthritis.

[0031] In addition, a number of plants and plant-derived compounds have been explored in order to control inflammation and pain in osteoarthritis. In Patent No. US 6,514,540 Sobczak discloses therapeutic compositions based on Aloe vera. In Patent No. US 6,887,497 Gorsek WF discloses compositions including glucosamine sulfate with nettle leaf, quercetin, selenium, zinc, vitamin C, calcium, magnesium, and grape seed extract. In Patent No.US 6,949,260; Patent No.US 6,534,086; and Patent Application 20030185907 Krumhar KC discloses the compositions and methods that include boswellic acid, curcuminoids, gingerols, capsaincinoid, quercetin, genistein, daidzein, vitamin C, and linoleic acid. In Patent No. US 6,579,544 Rosenberg TD &Deffner K disclose dietary supplements containing carotenoids (lutein, lycopene, zeaxanthin), flavonoids (citrus extracts, grape seed extracts, quercetin, rutin, soy isoflavones), glucosamine &condroitin sulfate, alpha-lipoic acid, coenzyme Q10, omega-3, vitamins (A, D, K, B), and magnecium ions. In Patent No. US 6,024,960and WO02342274 Raederstorff et al disclose the compositions that include rosehip extracts, magnolia bark extract, honokiol, genistein, Licorice extracts (glycyrrhiza), tea extracts (EGCG), vitamins (E,K), polyunsaturated fatty acids, linoleinic acid and others. Anti-inflammatory compounds are isolated from numerous other plant sources including cranberry (Patent No. US 7,270,837), nutmeg seeds (Patent No.US 7,371,413), and others.In Patent No. US 6,492,429 Graus IMF discloses compositions containing glucosamine sulfate, acetylcysteine, boron, boswellic acids, curcumin, and apocynin (plant-derived inhibitor of inducible NO synthetase). In Patent No. US 8,226,987 Zoorob GK discloses herbal preparations comprised of sage, thyme and cumin. In Patent No. US 8,221,805 Myhillet al discloses compositions in alleviating inflammation and oxidative stress, comprising Bacopamonniera extract, milk thistle extract, ashwagandha powder, green tea extract, Gotukola powder, Ginkobiloba extract, Aloe vera powder, turmeric extract, and N-acetylcysteine. In Patent No. US 8,222,232 Anderson et al disclose glucosamine, N-acetylglucosamine and beta-glucan compositions. In Patent No. US 8,211,947 Selman-Housein

G discloses the compositions that are comprised of vitamin K, polyunsaturated fatty acids (blood thinner) and niacin (vasodilator and hypolipidemic agent). In Patent No.US 8,206,756 Chauhan et al disclose compositions for inflammatory disorders, comprised of an extract of flowering and fruiting heads of the plant, Sphaeranthusindicus; the compositions are intended to inhibit tumor necrosis factor (TNF-alpha), interleukin (IL-1, IL-6, IL-8), the expression of intercellular adhension molecule-1 (ICAM-1), vascular-cell adhension molecule-1 (VCAM-1), and E-Selectin. In Patent Application US 20120225053 Dushenkov et al disclose compositions containing theaflavin (black tea extract) and glucosamine; they claim that theaflavin inhibits NF-kappaB activation and COX-2 gene expression and suppresses inflammation. In Patent No. US 8,192,768 Gokaraju et al disclose anti-inflammatory and antioxidant supplement compositions comprising boswellic acid, curcumoids, glucosamine, chondroitin, garlic extract, bromelain, quercetin, gallic acid, caffeic acid, green tea, resveratrol, MSM, and serratiopeptidase. The compositions are used to inhibit COX-2 and 5-LOX enzymes and inflammation.

[0032] Boswellic acid is pentacyclictriterpene isolated from the resin of Boswelliserrata. Boswelli acid shows anti-inflammatory effects since it inhibits the synthesis of leukotriene (cytokine modulator) and the action of 5-lipoxygenase (5-LO) that is involved in prostaglandin synthesis (Ammon et al, 1993). Boswelli extracts are now seen in many supplement products made for inflammatory diseases including osteoarthritis. Boswelli extracts are also shown to have anti-cancer activities against brain tumor, leukemia, and colon cancer. A high dose is hepatotoxic while a low dose is hepatoprotective. Dosage guidelines have now been developed for safe uses (SalGenecists, Inc., Boswellia Information).

[0033] Curcumin/tumeric (Curcuma longo) is another popular component in therapeutic products made to prevent and/or treat inflammatory diseases and cancer (Aggarwal et al, 2007).Curcumin inhibits the activation of a transcription factor NF-kappaB which regulates a variety of genes encoding pro-inflammatory cytokines (TNF-alpha, IL-1, IL-6), chemokines (IL-8, MIPI-alpha), inducible enzymes (iNOS, COX-2), and other molecules (Shakibaei et al, 2007; Chainani-Wu, 2003). Thus, the inhibition of NF-kappaB down-regulates the production of phospholipase, lipooxygenase, cyclooxygenase 2, leukotrienes, thromboxane, prostaglandins, nitrix oxide (NO), collagenase, elastase, hyaluronidase, monocyte chemoattractant protein-1 (MCP-1), interferon-inducible protein, TNF-alpha, and IL-12.

[0034] NF-kappaB is a “rapid-acting” primary transcription factor involved in cellular responses to stimuli such as stress, cytokines, free radicals, bacterial and viral antigens. In unstimulated cells, NF-kappaB dimmers are sequestered in an inactive state in the cytoplasm bound to inhibitor (IkappaB). The activation of NF-kappaB occurs when IkappaB is inactivated and degraded with a kinase (IKK).

[0035] Eukaryotic cells widely use NF-kappaB as a regulator of genes that control cell proliferation and cell death (apoptosis); and NF-kappaB plays a key role in regulating the immune response to infection. NF-kappaB is activated by TNF-alpha, IL-1, bacterial lipopolysaccharides (LPS), reactive oxygen species (ROS), ionizing radiation, and others.

[0036] The activation of the NF-kappaB leads to many chronic inflammatory diseases including rheumatoid arthritis, osteoarthritis, inflammatory bowel disease, asthma, and atherosclerosis. Uncontrolled NF-kappaB production increases the risk of developing cardiovascular diseases.

[0037] In many tumor cells, NF-kappa is constitutively active; and uncontrolled NF-kappaB leads to proliferation. Blocking NF-kappaB has thus been a strategy to control cancer. Natural and synthetic NF-kappaB inhibitors have been explored for therapeutics of cancer and inflammation.

[0038] NF-kappaB is also activated via RANK (receptor activator of NF-kappaB) bound on the surface membrane of osteoclasts (boneresorption cells). Osteoprotegrin (OPG) inhibits this activation by binding RANKL bound on the surface membrane of osteoblasts (bone forming cells). OPG down-regulates activities of osteoclasts and NF-kappaB, and plays a major role on the activities of osteoblasts and bone formation. Estrogen and phytoestrogen stimulate the production of OPG, and down-regulate activities of osteoclasts and NF-kappaB: This prevents bone loss and inflammation.

[0039] TNF-alpha is a pleiotropic pro-inflammatory cytokine, produced mainly by macrophages, but by other types of cells as well. TNF-alpha initiates a cytokines cascade and increases vascular permeability, thereby recruiting macrophages and neutrophils to a site of infection or inflammation. TNF-alpha causes blood clotting. Prolonged overproduction of TNF-alpha harms cells and tissues and may result in a condition known as cachexia.

[0040] TNF-alpha demonstrates beneficial as well as pathological activities; it has both growth stimulating and growth inhibitory effects; it is self-regulated. The beneficial functions include maintaining homeostasis by regulating the body's circadian rhythm, replacing injured tissue by stimulating fibroblast growth, mounting an immune response and killing bacterial, viral, fungal, and parasitic infections, and certain tumors. On the other hand, inappropriate productions of TNF-alpha cause chronic inflammation and tissue damages. TNF-alpha is shown to play critical roles in bowel disease, rheumatoid arthritis, psoriatic arthritis, osteoarthritis, osteoporosis, coronary heart disease, vasculitis, ulcerative colitis, psoriasis, diabetes, Alzheimer's disease, and others.

[0041] The human digestive system hardly absorbs many of naturally-occurring bioactive polyphenols,isoflavones, and flavonoids: only less than 2% of orally-administered plant

compounds could be found in the blood stream.Because of the low bioavailability, many positive results obtained using these bioactive compounds in vitro and in animal systems have often suffered from being reproduced when applied to human. Low and varying bioavailability also makes accurate dosing difficult.Now, however, the bioavailability problems seem to have found a number of solutions.

[0042] DolCas Biotech, LLC (Wisconsin, USA), makes BCM-95®: seven times more absorbable curcuminoidsby adding turmeric extracts to curcumin(Patent No. US 7,736,679). Sabinsa Corporation (New Jersey, USA) makes Curcumin C3 Complex® (Patent No. US 5,861,415) and Bioperine® (Patent No. US 6,054,585). Bioperine® (blackpepper extract) enhances absorption of Curcumin C3. Qazi GN et al (Patent Application US 20080292736) report that Carumcarvi extracts, either alone or in combination with piperine, enhance the bioavailability of a wide variety of drugs. The compound isolated from the ethanol extract of Radix puerariae enhances absorption of daidzin and other isoflavones (Keung WM et al., 1996). Daidzein metabolite, equol, is highly-absorbable and when it becomes commercially available in the very near future (Patent No. US 8,399,232; Patent No. US 8,420,073; Patent Application No. 20040235758), it will suffice.

[0043] Polyphenols and flavonoids are hydrophobic compounds and hardly soluble in water. A number of efforts are done to increase water solubility of these compounds and drugs. For instance,Eidenberger T (Patent Application US 20090181901) describes that compounds with the –SH group enhance the bioavailability of carotenoids. Liu Z. (Patent Application US 20120329738) describes that a diterpene glycoside (rubusoside) increases solubility and permeability of curcumin and other compounds. Zhao G et al (2013) describe solid dispersions and self-emulsifying formulations that enhance solubility and absorption properties of isorhamnetin, quercetin and kaempferol. Luo H et al (2012) described kaempferol nanoparticles. Birbara PJ (Patent Application US 20120213842)describes manufacturing microparticulates that enhance skin penetration of a wide variety of flavonoids. Edgar KJ et al (Patent Application US 20130237609) describe cellulose derivatives for enhancing bioavailability of flavonoids including curcumin, resveratrol, ellagic acid, naringenin, and quercetin. San Ei Gen F. F. I., Inc. (Osaka, Japan) in collaboration with Theravalues Corporation (Tokyo, Japan) produces 27-30 times highly water-soluble, more absorbable Theracurmin™ by mixing gumghattiwithcurcumin and grinding them to nanoparticules(Patent Application US 20120195949/JP 2009-263638; Sasaki et al., 2011).The current invention utilizes highly absorbable curcumin and phytonutrients made by this technology.

[0044] Another way to enhance bioavailability is through chemical synthesis (Vyas et al., 2013). Ohori et al (2006) synthesized curcumin analogues and produced 30x more absorbable, potent curcumin analogues, GO-Y030 & GO-Y031. GO-Y030 & GO-Y031 appear to exhibit no harmful

effects andhave been tested for its safety and efficacy in cancer treatment (See Patent JP 5050206; Patent No. US 8,178,727; Patent Application No. US 20100152493).

[0045] Mesenchymal stem cells (MSCs) are pluripotent and differentiate into adipocytes (fat cells), osteoblasts (bone cells), chondrocytes (cartilage cells), myocytes (muscle cells) and neurons. MSCs are found in a variety of tissues but among them adipose tissue is one of the richest sources of MSCs. Compared to bone marrow, there are more than 500 times more mesenchymal stem cells in adipose tissue than bone marrow. Since MSCsgrow vigorously in vitro while maintaining their multipotency, and human MSCs avoid allorecognition, MSCs have tremendous potentials in tissue engineering (Chanda 2010).

[0046] Scientists have studied the signals and factors that determine differentiation of MSCs, bone formation, and cartilage repair. (a) The canonical Wnt/beta-catenin pathway inhibits adipogenesis and induces osteoblastogenesis, whereas activated PPAR-gamma is a prime inducer of adipogenesis and inhibits osteoblastogenesis. (b) Isoflavones (daidzin, genistin), flavonoids(quercetin, kaempferol, agigenin), and/or estrogendownregulate PPAR-gamma and direct differentiation of MSCs toward osteoblasts and chondrocytes from adipocytes. (c) Anti-diabetes drugsthiazolidinediones (TZDs such as rosiglitazone) agonize (activate) PPAR-gamma, increase marrow adipocytes, and decrease osteoblasts. Women treated with TZDs have an increased risk of bone fractures because of bone loss. (d) Differentiation of MSCs towards osteoblasts and chondrocytes are induced by morphogenic transforming growth factor-beta (TGF-beta) and Insulin-like growth factor-1 (IGF-1)(Longobandi et al 2006). Morphogenic proteins BMP-2, BMP-4, and BMP-7 (all TGF-beta family), in particular, play major roles (Miljkovic et al 2008). These growth hormones are involved in differentiation commitment as well as in actual bone and cartilage growth. (e) Hyaline cartilage formation requires the presence of Type II collagen and three-dimentionalextracellurmatrix (ECM) scaffold. In summary, active bone growth induces the production of BMPs and IGF-1, which enhances chondrogenesis and cartilage repair.

[0047] Synovial fluid is a viscous fluid found in the cavities of synovial joints. The principal role of synovial fluid is to reduce friction between cartilages of synovial joints during movement. The inner membrane of synovial joints is called the synovial membrane and secretes synovial fluid into the joint cavity. The fluid contains viscous hyaluronic acid (anionic, nonsulfated glycosaminoglycan) secreted by fibroblast-like cells in the synovial membrane and interstitial fluid filtered from the blood plasma. This fluid forms a thin layer (roughly 50 microns) at the surface of the cartilage and also seeps into microcavities and irregularities in the articular cartilage surface, filling all empty space. During movement, the synovial fluid held in the cartilage is squeezed in and out to reduce friction and absorb shock. A meniscus, made of fibrocartilage, also reduces friction and disperses gravitational stress during movement.

[0048] Synovial fluid of osteoarthritis patients contains MSCs which have chodrogenic potential (Kurth et al 2007; Fan et al 2009). Evidence suggests that the same synovial fluid also contains, at varying quantities, growth factors (BMPs, IGF-1), Type II collagen, and exposed extracellular matrix (ECM). Apparently, many reconstructive components needed for the repair of damaged cartilage are present in synovial fluid of osteoarthritis patients, along with destructive factors. Osteoarthritis develops and progresses as destructive factors become dominantand persist over constructive factors. If constructive factors become dominant over destructive factors, however, the progression of osteoarthritis would be stopped and reversed. Under favorable joint conditions minor cartilage repairs constantly occur (Hunziker&Kapfinger 1996; Gaby 1999).

[0049] The synovial fluid (joint)looks isolated, without blood supply, from the rest of the body, but it isn't. The inside of the joint is connected with outside cells and tissues through the flowing interstitial fluid; and the inside cells and tissues communicate with the outside cells and tissues via various modulators, e.g., cytokines and chemokines. For cartilage and joint repair to take place, certain levels ofpro-inflammatory modulators should be present within synovial fluid for communication and to stimulate proliferation of cells and tissues. Moderate pain thus could beconstructive rather than destructive.

[0050] Chondrogenesis during embryogenesis. The skeletal system is derived from the mesoderm germ layer. Chondrogenesis is the process by which cartilage is formed from condensed mesenchymal tissues, which differentiate into chondrocytes and begin secreting the molecules that form the extracellular matrix (ECM). Early in fetal development, the greater part of the skeleton is cartilaginous. This temporary cartilage is gradually replaced by bone (ossification), a process that ends at puberty. In contrast, the cartilage in the joints remains unossified during the whole life. Adult hyaline articular cartilage is progressively mineralized at the junction between cartilage and bone which becomes the subchondral bone.

[0051] Articular cartilage thus formed is free of blood, lymphatic vessels and nerve structures, particularly at knee joints. Within the joint, cartilage is in contact by its deepest layer with subchondral bone and its most superficial layer with synovial fluid. In the articular cartilage, the extracellular matrix (ECM) is primarily composed of water, proteoglycans (major components are aggrecans), type II collagen, and hyaluronic acid; and chondrocytes occupy roughly 5% of the tissue volume. These chondrocytes are metabolically active and consume (take up) great quantities of oxygen, glucose, glutamine and other nutrients from the synovial fluid and synthesize glucosamine, one of the major components of proteoglycans; the synovial fluid then removes carbon dioxide and metabolic wastes through the interstitial fluid. It is not known, however, whether these chondrocytes within articular cartilage are capable of proliferating and engaging in cartilage repair.

[0052] It is historically said that cartilage, once damaged, has limited repair capabilities because chondrocytes are bound in lacunae and cannot migrate to the damaged area (the surface area of articular cartilage); and because articular (hyaline) cartilage has no blood supply. Thus, several surgical interventions to repair damaged articular cartilage have taken the following approaches. One approach is to drill a hole through the subchondral bone so that bone marrow and other nutrients can freely come through to the sites of damaged cartilage surface. Another approach is to implant cultured cells into the damaged joint. Since the introduced cells hardly adhere to the pre-existing cartilage, either the surface of the damaged cartilage is pre-treated with proteinase to expose the proteoglycan matrix, or the cultured chondrocytes are pre-loaded onto a matrix and then introduced. Currently, these interventions are unsatisfactory and various technical details have yet to be worked out before these procedures can be used in actual medical practices.

[0053] Load-bearing cartilages must have visoelastic properties manifested from its extracellular matrix (ECM) composition of water (70-80%), collagen (50-75%), and glycosaminoglycan (15-30%). This composition provides cartilage with compressive, tensile, and frictional properties (Huey et al., 2012). Attempts to reconstruct a cartilage ex vivo with various growth factors (such as fibroblast growth factor-18, TGF-beta) tend to produce a weak fibrocartilage since reconstructions occur under no load or mechanical stress.Mechanical and gravitational stimulation during in vitro reconstruction increases the percentage of compression-resistant cartilage(Huey et al., 2012). A durable repair of load-bearing hyaline (articular)cartilage would thus take place in vivoapplying load or stress during repair, such as pounding by walking or jogging(see Example 1 below). Such cartilage repairs are expected to be a slow process in adults older than 45 years of age.

[0054] Osteoporosis is a bone disease that leads to an increased risk of bone fracture. Approximately 10 million people in the US are estimated to have osteoporosis, a disease that results in over 1.5 million bone fractures a year. Most bone fractures involve lumber vertebrae (the lower back), hip, thigh and wrist. Osteoporosis affects 55% of Americans after age 50. Of these about 80% are women. Osteoporosis is the most common in women after menopause because reduced estrogen levels enhance osteoclast's bone resorption activities. Estrogen (as well as testosterone) also affects calcium absorption and bone mineralization. Osteoporosis is caused by imbalance between bone resorption and bone formation. Age-related bone loss is believed to parallel to the accumulation of adipose tissues in bone marrow.

[0055] The skeletal (muscle) system is in constant renewal. Newly-born babies renew almost all of their bones in the first year, while adults renew them at a rate of about 10% a year.

[0056] The remodeling process takes place in bone multicellular units. Bone is resorbed byosteoclast cells after which new bone is deposited by osteoblast cells. Excessive bone resorption and inadequate bone formation develops fragile bone tissue which leads to osteoporosis. Bone remodeling is under hormonal control (estrogen, testosterone, thyroid hormone calcitonin, parathyroid hormone PTH); utilizes cytokines and growth hormones (TGF-beta, IGF-1) (Reddi 1997);and is affected by the calcium metabolism (skeletal bones are calcium storage sites), vitamin D, and steroids. The body must maintain proper levels of calcium within the body. Vitamin D becomes calcitriol (1,25-dihydroxyvitamin D), an active form of vitamin D, inside the body and helps calcium absorption.Calcium metabolism is also influenced by the level of phosphate, magnesium, and vitamin K (menaquinone-7).

[0057] Osteoblasts (bone forming cells) are derived from mesenchymal stem cells while osteoclasts (bone resorpting cells) are derived from monocytes/macrophages. Osteoblasts and osteoclasts cross-talk and coordinate in bone remodeling. Osteoblasts produce RANKL (osteoclast differentiation factor); RANKL binds to cell surface receptor RANK on osteoclasts and activates osteoclasts. Osteoclasts resorb tipped, cracked, or broken bones, and osteoblasts lay out new bones being helped by growth hormones BMPs and IGF-1. Osteoprotegrin (OPG) guards the skeleton from excessive bone resorption. OPG, by competitively binding to RANKL, down-regulates the activation of osteoclasts. Estrogen (or phytoestrogen) stimulates the production of OPG and down-regulates bone resorption as well as NF-kappaB. Overproduction of RANKL (and overactive osteoclasts) causes diseases and is implicated in rheumatoid arthritis and psoriatic arthritis. Denosumab (Prolia®; Amgen) is a FDA approved RANKL inhibitor used for the treatment of rheumatoid arthritis and other diseases.

[0058] PPAR-gamma (peroxisome proliferator-activated receptor-gamma) is a nuclear hormone receptor transcription factor. PPAR-gamma plays an important role in the decision of bilateral differentiation of mesenchymal stem cells to adipocytes or osteoblasts/chondrocytes.

[0059] Natural ligands of the PPAR-gamma receptors are fatty acids such as lauric acid, petroselenic acid, linolenic acid, linoleinic acid, arachidonic acid, and fatty acid metabolites such as 15-deoxy-delta 12, 14-prostaglandin J2. The synthetic ligands comprise the group of TZDs (Troglitazone, Rosiglitazone, Pioglitazone), the non-TZDs (GW1929, GW7845), and the non-steroidal anti-inflammatory drugs (flufenamic acid, fenoprofen). Anti-diabetes drugs TZDs agonize (activate) PPAR-gamma: this reduces the secretion of unestrified fatty acids and adipokines TNF-alpha and other inflammatory cytokines resistin and plasminogen-activator inhibitor-1 (PAI-1);enhances the secretion of adiponectin; and regulates the glucose transporter protein GLUT-4 in the cell membrane. The net result of these changes is to reduce insulin resistance in muscle and liver and to mitigate prothrombotic and pro-inflammatory states. Although these changes help improving the conditions of diabetes, the activation of PPAR-

gamma with TZDs increases adipocytes in bone marrow and decreases osteoblasts, resulting in bone loss. Both pre- and post-menopausal women treated with TZDs are reported to have an increased risk of bone fracture (Grey 2008).

[0060] Isoflavones are widely distributed in the plant kingdom and over 700 different isoflavones are described. However, the isoflavones which display estrogenic activity belong to a small sub-group and restricted almost exclusively to the Leguminosae family. The best known estrogenic isoflavones are daidzein (PPAR-gamma inhibitor), genistein (tyrosine kinase inhibitor), glycitin, formononetin, and biochanin A, which are found most abundantly in soybean, lentil, Puerarialobata (kudzu), alfalfa, red clover, chickpeas,peanut and other beans. Epidemiological studies show that populations eating soy protein have a lower incident of cancers, breast cancer in particular, and less menopausal symptoms. Isoflavones have been shown to prevent bone loss and improve bone mineral density (BMD) in postmenopausal women at doses of 30-90 mg/day.

[0061] In the raw plants, isoflavones principally occur as water-soluble glycosides or molonates. Glycosylated or molonatedisoflavones are hardly absorbed by the digestive system in human. Glucosidase (of gastrointestinal microbes), fermentation, or hydrolysis converts glycosides to water-insoluble aglycones which are absorbed through lumens of the lower intestines and colons. About 60% of Asians or vegetarians and about 20% of Americans or Westernsare called 'equol-producers' who carry microbes in their guts that convert daidzeinto S-equol (4, 7-isoflavandiol)(Setchell& Cole, 2006; Setchel&Clerici, 2010).Equol is efficiently absorbed by human intestines and colons;preferentially binds and activates estrogen receptor type beta (ER-beta); and acts as the most potent non-steroidal phytoestrogen.Setchell et al.(2002) proposed an equol hypothesis that equol may be responsible to many of health benefits of isoflavons: e.g., to ease menopause symptoms; to reduce heart disease risks; to reduce cancer risks; to improve bone health; and to protect against prostate problems.

[0062] The root extract of perennial leguminous vine Puerarialobata (Kudzu) contains coumarins, isoflavonoids (puerarin, daidzin, daidzein) and saponins (kudzusaponins). For thousands years Kudzu and its extracts have been used for many disorders such as fevers, gastrointestinal disorders, muscle aches, allergies, respiratory problems, skin problems, high blood pressure, migraine headache, lowering cholesterol, and treating alcoholism (Keung 1996). Reppert et al (2008) prepared radiolabeled daidzein to monitor absorption, metabolic distribution, kinetics and accumulation of daidzein and its metabolites in various organs over time.

[0063] Phytochemicals and phytonutrients, especially flavonoids(quercetin, kaempferol, myricetin, apigenin), are a group of plant secondary metabolites with a diphenylpropane

structure. They are widely distributed in the plant kingdom and are common constituents of fruits and vegetables such as: parsley, artichoke, basil, celery, grapefruit, blueberries, teas, citrus, wine, cacao, capers, apples, onions, ginkgobiloba, hypericumperforatum (St. Jogn's wart), lyciumbarbarum (goji berries), and morindacitrifolia (Indian mulberry). Epidemiological studies suggest a positive relationship between the ingestion of foods containing flavonoids and a reduced risk of developing cancer and cardiovascular diseases (Calderon-Montano et al., 2011). Flavanoids also can positively influence the bone remodeling process as well as chondrogenesis. Quercetin, kaempferol,myricetin, and apigenin are a potent anti-inflammatory agent (Kim et al., 2004; Wang et al., 2006). These flavonoids inhibit NF-kappaB, down-regulate many pro-inflammatory responses, and relieve pain just like curcumin/turmeric described above.Quercetin and kaempferol, functioning as phytoestrogen, down-regulate activities of osteoclast cells just as isoflavones (genistein, daidzein); and as a PPAR-gamma inhibitor, inhibit adipogenesis and stimulate bone formation andchondrogenesis. These flavonoids are also known for their preventive activitiesin cancer, microbial infection, Alzheimer's disease and Parkinson disease.

[0064] Watanabe et al (Patent No. US 5,650,433 & Patent JP 07-025761) showed a number of synthetic flavonoids (apigenin, luteolin, acacetin, linarin, diosmetin, baicalein, fisetin, kaempferol, quercetin, hesperetin, hesperidin, etc.) were chondroprotective when cultured chondrocytes were challenged by the proteoglycan depleting agent, PMA (phorbolmyristate acetate). Park et al (Patent Application US 20070154540) claim that compositions containing apigenin not only prevented further cartilage destructions but also promoted the proliferation of chondrocytes and was capable of restoring artificially-damaged cartilage in rabbit. The present invention extended these observations and demonstrated that kudzu extract (containing daidzein) and ginkgobiloba extract (containingquercetin and kaempferol) in combination with curcumin, inhibited PPAR-gamma, and synergistically stimulate bone-formation &chondrogenesis, and repaired cartilage in human. Although Park et al. seem to assume that the pre-existing chondrocytes in the cartilage have proliferated, it looks probable that proliferating chodrocytes might be newly derived from fibroblast-like cells on synovial membrane, synovial fluid MSCs, or bone marrow MSCs. Apigenin, quercetin and kaempferol are a potent inhibitor of cytochrome P450 2C9 (CYC2C9) and may thus interfere with the actions of numerous drugs including cyclosporine.

[0065] Hundreds of prior arts dealt with the beneficial effects of phytochemicals on osteoporosis. Forusz et al in Patent No. US 6,436,446 disclose compositions for increasing bone density, comprising isoflavone, inulin, oligosaccharide, calcium, and organic acid. Patent No. US 6,638,540 discloses plant extracts of onions, parsley, cabbage, arugula or roquette to prevent increased bone resorption and treat osteoporosis. Patent No. US 6,391,309 teaches compositions comprising isoflavones, lignana, saponins, sapogenins, catechins, phenolic acids.

Patent No. US 6,340,703 discloses soy isoflavonesformononetin and daidzein. Patent No. US 5,830,887discloses genistin, diadzein, formononetin, and biochanin A. In Patent Application No. 20090304823 Elizabeth et al disclose compositions comprising rosemary extracts that promote bone growth and maintenance. In Patent No. US 8,242,100 Pan discloses compositions comprising isoflavones or isoflavone metabolites for inducing bone growth or inhibiting bone loss. Patent No. US 8,227,013 discloses nutritional supplements comprising fermented soy (HEALAN 951™; Healan Products, Inc.), sweetener, and Curcumin C3 Complex™ with Bioperine® (Sabinsa Co.). The nutritional supplements are intended for: antioxidant support, Alzheimer's disease support, cardiovascular support, immune support and supports for wound healing, arthritis, cancer, and antiviral effects. Patent No. US 5,612,074 discloses nutrient fortified food bars containing proteins, fibers, polyunsaturated fatty acids, carbohydrates, flavones, minerals and others.

[0066] Calcium. The adult skeleton contains about 1,200 gm of calcium, 99% of which is stored in the bones and teeth; the remaining 1% of calcium (10 – 12 gm) circulates in a soluble form within the body. The role of calcium in maintaining strong bones and teeth is well known. Lesser known is the critical roles soluble calcium play in neuromuscular and cardiovascular functions, in coagulation, as an intracellular second messenger for cell surface hormone actions, and in gene transcription, cellular growth, and metabolism. Calcium and magnesium work synergistically. When the amount of magnesium in the bloodstream falls, the kidney readjusts the balance by reducing calcium, and vice versa. If a person ingests more magnesium, the more calcium is kept in person's body. The recommended daily intake of calcium is 1,000 mg for everyone over the age four; 1,300 mg for pregnant or lactating women. Dairy products (milk, cheese, yogurt) and certain plant products (tofu, kale, spinach, turnip greens) are rich sources of calcium. Vitamin D becomes calcitriol (1,25-dihydroxyvitamin D), an active form of vitamin D, inside the body which helps calcium absorption and maintains proper levels of calcium within the body.

[0067] Calcitonin. The parafollicular cells (C-cells) of the thyroid produce a small polypeptide hormone calcitonin. Calcitonin acts to reduce blood calcium, opposing the effects of parathyroid hormone (PTH), and protects against calcium loss from skeleton during the periods of calcium mobilization such as pregnancy and lactation. Calcitonin inhibits calcium absorption by the intestines and renal tubular cell's reabsorption of calcium and allowing calcium to be secreted in the urine. Calcitonin receptors are found on osteoclasts and calcitonin inhibits osteoclasts. Calcitonin also participates in phosphate metabolism. It inhibits phosphate reabsorption by the kidney tubules.

[0068] Parathyroid hormone (PTH) boosts the level of calcium in the blood; if PTH is low, bone releases calcium into the bloodstream. The hormone (and vitamin B) enhances the absorption

of calcium from the intestine and decreases the amount that is lost through urine. Low circulating vitamin D (calcitriol), which is common among the elderly, increases parathyroid hormone; this leads to bone resorption and bone loss.

[0069] Osteoporosis drugs. Strontium ranelate (Ranelic acid) stimulates OPG production as estrogen. Denosumab (Prolia®; human monoclonal antibody; Amgen) binds to RANKL as OPG. This drug is used to treat osteoporosis, bone metastasis, rheumatoid arthritis, multiple myeloma and giant cell tumor of bone. Raloxifene (Evista®; Eli Lilly) is a selective estrogen receptor modulator. Biphosphates (Fosamax®, Boniva®, Acetonel®) force osteoclasts to apoptosis.

[0070] Synthetic compounds and pharmaceutical products. In the last decade various synthetic curcumin and its analogues have been prepared and evaluated for their various pharmacological activities. Compounds GOY-030, GOY-031, and GOY-35 have now been tested for colorectal cancer in human clinical trials. Since these curcumin analogues are also shown to down-regulate NF-kappaB, they might be effective as an anti-inflammatory agent for the treatment of osteoarthritis. Synthetic daidzin, genistin, quercetin, kaempferol, apigenin and their analogues as well as daidzein metabolite equolsare also available. Clinical trials on the safety and efficacy of their combination products are thus warranted.

Examples

EXAMPLE 1

[0071] TABLE 1 explains the timing when curcumin and/or kudzu extract (daidzin) was started being taken or stopped as indicated by the numbered arrows in Figure 1.

Table 1: The Presence or Absence of Ingredients

Arrow	Curcumin	Daidzin
#1	800 mg	-
#2	800 mg	4 mg
#3	800 mg	68 mg
#4	-	68 mg
#5	800 mg	68 mg

Note: daily dosage

[0072] A 72 year old man had knee osteoarthritis and was having difficulty walking or going down stairs for over 15 years. He was given curcumin (a capsule containing 400 mg BCM-95®) twice a day (indicated by arrow #1). His knee pain was gone within a week; his movement got easier butstill could walk only at the pace of <3 miles/h. He tookadditionallyKudzu extract (a capsule containing 150 mg isoflavone; 2%daidzin) twice a day (indicated by arrow #2). Within 2-3 weeks, he started jogging 3 to 5 miles at the pace of ~4.5 miles/h. He then tookcurcumin (400 mg BCM-95®), Kudzu extract (a capsule containing 160 mg isoflavones; 21%daidzin) twice a day (indicated by arrow #3);he soon started jogging 5-6 miles at increasing speeds every week. Seven weeks later he stopped taking curcumin (indicated by arrow #4). Two weeks later knee pain came back and subsequently he could jog only at slower speeds. Four weeks later (indicated by arrow #5) he resumed taking curcumin with Kudzu extract. Soon afterward he started jogging 5-6 miles at the pace of >6 miles/h again.

Theimprovement in the walking or jogging ability reflectscumulative improvements in the conditions and functions of bones and joints(at knee, hip, ankle, in particular). Curcumin orisoflavonesalone did not produce the observed improvement; and improvement was seen only curcumin and isoflavones were taken together. Curcumin and isoflavones (daidzin, in particular) work synergistically.Isoflavone (daidzin) appears towork dose-dependently.

EXAMPLE 2

[0073] Two sisters at the age of 76 and 74 years were having difficulty walking with osteoarthritis and osteoporosis and considering knee surgery in the near future. They took curcumin (400 mg BCM-95®), Kudzu extract (a capsule containing 160 mg isoflavones; 21% daidzin), and probiotic (Jarrow-Dophilus EPS 5 billion per capsule) twice a day. Their knee pains disappeared within a week; their bones got stronger; and they were able to swiftly walk more than 5 miles a day in two months.

EXAMPLE 3

[0074] A couple of the age of 50 and 53 were having difficulty walking with osteoarthritis and osteoporosis. They took curcumin (400 mg BCM-95®), Kudzu extract (a capsule containing 160 mg isoflavones; 21% daidzin), Ginkgo biloba extract (120 mg), and probiotic (Jarrow-Dophilus EPS 5 billion per capsule) twice a day. Their pain was gone in one week and their mobility improved.

EXAMPLE 4

[0075] This test is in progress. A 65-years old man, who has been suffering from osteoarthritis for >5 years, has been receiving a combination of curcumin (400 mg BCM-95®) and flavonoids (Kudzu extract 160 mg isoflavones containing 21% daidzin& Ginkgo biloba extract 120 mg) for

three months. As his osteoarthritic conditions improve and he is able to jog at a pace of >5 miles/h, he will be deprived of flavonoids from his regimen to further confirm synergy between curcumin and flavonoids.

EXAMPLE 5

[0076] Jogging intervals have been tested to see whether shorter or longer intervals than a week (as EXAMPLE 1) are better in improving osteoarthritis.

[0077] Osteoarthritis products were made as follows. Kudzu extract (containing daidzin), or ginkgo biloba extract (containing quercetin, kaempferol) was subjected to acid hydrolysis to make them aglycons. To enhance bioavailability, isoflavone and flavonoid aglycons along with turmeric extract (curcumin) were mixed separately with gumghatti and ground using wet mill to make nanoparticles (size <1 nm) according to Patent Application US 20120195949. Product I contains turmeric extract (curcumin 30 mg) and kudzu extract (daidzein 30 mg). Product II contains turmeric extract (curcumin 30 mg) and ginkgo biloba extract (flavonoids 30 mg). Product III contains turmeric extract (30 mg curcumin), kudzu extract (daidzein 30 mg), and ginkgo biloba extract (flavonoids 30 mg). These products may be taken one to three times a day.

EXAMPLE 6

[0078] Eighty volunteers are recruited who have been at least 45 years of age and sufferfrom knee pain (with grade 2 or 3 osteoarthritis) for at least six months and on the majority of days. Group A (20 people) are given product I; Group B (20 people) Product II, Group C (20 people) Product III, and Group D (20 people) placebo, respectively, each twice a day. All are encouraged to do moderate exercise.The primary outcome over the trial period of 12 weeks measure an increase in mobility as the distance or the speed patients can travel (walk or jog). The secondoutcome measure changes in pain, stiffness, flexibility, jointfunctions, and swelling. Patient's equol-producer status is also determined.

Although many clinical trials for osteoarthritis generally measure a decrease in knee pain (the Western Ontario and McMaster University Osteoarthritis Index, WOMAC), this is not a good measure for joint repair. Pain, if not too severe, is considered rather a blessing and should not be killed since moderate pain stimulates chondrogenesis and joint repair.

EXAMPLE 7

[0079] A test is planned in which doses of synthetic curcumin, quercetin, kaempferol, and/or apigenin are tested at 5, 10, 15, 20, 30, and 50 mg for safety and efficacy in improving osteoarthritis.

EXAMPLE 8

[0080] A test is planned in which the effects of the products listed in [0077] on osteoarthritis are enhanced with systematic exercise or taking protein (amino acids). Exercise (stress) stimulates growth of muscles and bones; and maybe cartilage. Amino acids are precursors for glucosamine and chondrotin sulfate, key structural components of cartilage.

EXAMPLE 9

[0081] A 67 year-old man got a left shoulder problem. He used to bench press 160 lbs and dumbbell curl 35 lbs. After the problem he could do only 70 lbs and 20 lbs, respectively. Taking curcumin (400 mg BCM-95®), Kudzu extract (160 mg isoflavones; 21% daidzin), and Ginkgo biloba extract (120 mg flavonoids) each twice a day, he recovered over five months. He got severe flu lasting for about 10 days during recovery. He thinks flu infection aggravated inflammation and delayed the shoulder recovery probably by a month or so.

EXAMPLE 10.

[0082] A 65 year-old man got a back problem and pain ran through his legs. Taking curcumin (400 mg BCM-95®), Kudzu extract (160 mg isoflavones; 21% daidzin), and Ginkgo biloba extract (120 mg flavonoids) each twice a day, he recovered over approximately six months.

References

GAIT report 2006: Clegg et al., Glucosamine, Chondroitin Sulfate, and the Two in Combination for Painful Knee Osteoarthritis. N Engl J Med 2006 Feb 23; 358(8): 795-808.

GAIT report 2010: Sawitzke et al., Clinical efficacy and safety over two years of glucosamine, chondroitin sulfate, their combination, celecoxib or placebo taken to treat osteoarthritis of the knee. Ann Rheum Dis 2010 August; 69(8): 1459-64.

Jones et al., Enumeration and phenotypic characterization of synovial fluid multipotentialmesenchymal progenitor cells in inflammatory and degenerative arthritis. Arthritis Rheum 2004; 50: 817-27.

Jones et al., Synovial fluid mesenchymal stem cells in health and early osteoarthritis: detection and functional evaluation at the single cell level. Arthritis Rheum 2008; 58: 1731-40.

Sekiya et al., Human Mesencymal Stem Cells in Synovial Fluid Increase in the Knee with Degenerated Cartilage and Osteoarthritis. J Orthopaedic Res 2012 June; 30(6): 943-9.

Hunziker et al., Repair of partial-thickness defects in articular cartilage: cell recruitment from the synovial membrane. J bone Joint Surg Am 1996; 78: 721-33.

Gaby AR, Natural Treatments for Osteoarthritis. Altern Med Rev 1999; 4(5): 330-41.

Takada et al., Molecular switching of osteoblastgenesis versus adipogenesis: implications for targeted therapies. ExpertOpinTher Targets 2009 May; 13(5): 593-603.

Viccica et al., The role of PPAR-gamma for the osteoblastic differentiation. J. Endocrinol Invest 2010; 33(7 Suppl): 9-12.

Ammon et al., Mechanism of anti-inflammatory actions of curcumin and boswellic acids. J Ethnopharmacol 1993 March; 38(2-3): 113-9.

SalGenecists, Inc. Boswellia information.

Aggarwal et al., Medicinal and Aromatic Plants – Industrial Properties: Chapter 10, Curcumin – Biological and Medicinal Properties. Tumeric 2007; vol 45: 297-368.

Shakibaei et al., Suppression of NF-kappaB activation by curcumin leads to inhibition of expression of cyclooxygenase-2 and matrix metalloproteinase-9 in human articular chrondrocytes: Implications for the treatment of osteoarthritis. BiochemPharmacol 2007; 73 (9): 1434-45.

Chainani-Wu N. Safety and Anti-inflammatory Activity of Curcumin: A Component of Tumeric (Curcuma longa). J Alter Compl Med 2003; 9(1): 161-8.

Keung et al., Potentiation of the bioavailability of daidzin by an extract of Radix puerariae. Proc Nat AcadSci 1996 April; 93: 4284-88.

Zhao G et al., Effects of solid dispersion and self-emulsifying formulations on the solubility, dissolution, permeability and pharmacokinetics of isorhamnetin, quercetin and kaempferol in total flavones of Hippophaerhamnoides L. Drug DevInd Pharm 2013 Jul; 39(7): 1037-45.

Luo H et al., Kaempferol nanoparticles achieving strong and selective inhibition of ovarian cancer cell viability. Int J Nanomedicine 2012; 7: 3951-9.

Sasaki et al., Innovative preparation of curcumin for improved oral bioavailability. Biol Pharm Bull 2011; 34(5): 660-5.

Vyas et al., Perspective on new synthetic curcumin analogs and their potential anticancer properties. Current Pharmaceutical Design 2013; 19(11): 2047-69.

Ohori et al., Synthetic curcumin analogue GO-Y031, Synthesis and biological analysis of new curcumin analogues bearing an enhanced potential for the medical treatment of cancer. Molecular Cancer Therapy 2006; 5(10): 2563-71.

Chanda et al., Therapeutic potential of adult bone marrow-derived mesenchymal stem cells in diseases of the skeleton. J. Cell Biochem 2010 October 1; 111 (2): 249-57.

Longobandi et al., Effect of IGF-1 in the chondrogenesis of bone marrow mesenchymal stem cells in the presence or absence of TGF-beta signaling. J Bone Miner Res 2006 April; 21(4): 626-36.

Miljkovic et al., Chondrogenesis, bone morphogenic protein-4 and mesenchymal stem cells. Osteoarthritis Cartilage 2008 Oct; 16(10): 1121-30.

Kurth et al., Chondrogenic potential of human synovial mesenchymal stem cells in alginate. Osteoarthritis Cartilage 2007 Oct; 15(10): 1178-89.

Fan et al., Synovium-derived mesenchymal stem cells: a new cell source for musculoskeletal regeneration. Tissue Eng Part B Rev 2009 Mar; 15(1): 75-86.

Huey et al., Unlike bone, cartilage regeneration remains elusive. Science 2012 Nov; 338: 917-21.

Reddi AH, Bone morphogenetic proteins: an unconventional approach to isolation of first mammalian morphogens. Cytokines Growth Factor Reviews 1997; 8(1): 11-20.

Grey A, Skeletal consequences of thiazolidinedione therapy. OsteoporoInt 2008 Feb; 19(2): 129-37.

Setchell KD and Cole SJ. Methods of defining equol-producer status and its frequency among vegetarians. J. Nutr. 2006 Aug; 136(8): 2188-93.

Setchell KD and Clerici C. Equol: History, Chemistry, and Formation. J. Nutr. 2010 June 2; "Supprement: Equol, Soy, and menopause" pp1355S-62S.

Setchell et al., The clinical importance of the metabolite equol: a clue to the effectiveness of soy and its isoflavones. J. Nutr. 2002; 132: 3577-84.

Reppert et al., Isolation of radiolabeled isoflavones from kudzu (Puerarialobata) root cultures. J Agric Food Chem 2008; 56(17): 7860-5.

Calderon-Montano et al., A review on the dietary Flavonoid kaempferol. Minireview Med Chem 2011; 11(4): 298-344.

Kim HP et al., Anti-inflammatory plant flavonoids and cellular action mechanisms. J. Pharmacol. Sci. 2004; 96: 229-45.

Wang L et al., Distinctive antioxidant and anti-inflammatory effects of flavonoids. J. Agric. Food Chem. 2006; 54: 9798-804.

CLAIMS

Claim 1. A composition comprising a therapeutically effective amount of an anti-inflammatory agent and an agent that enhances bone-formation andchondrogenesis in combination to treat osteoarthritis.

Claim 2. The composition according to claim 1, wherein the anti-inflammatory agent and the agent that enhances bone-formation andchondrogenesis synergistically ease and reverse the progression of osteoarthritis.

Claim 3. The composition according to claim 1, wherein the anti-inflammatory agent is an NF-kappaB inhibitor which comprisescurcumin or turmeric extract.

Claim 4.The composition according to claim 3, wherein the NF-kappaB inhibitor comprises highly-absorbable curcuminwhich is selected from the group consisting ofBCM-95(trademark), Curcumin C3/Bioperine (trademark), andTHERACURMIN (trademark).

Claim 5. The composition according to claim 1, wherein the agent that enhances bone-formation andchondrogenesis is phytonutrientthatcomprises a PPAR-gamma inhibitor and/or phytoestrogen.

Claim 6.The composition according to claim 5, wherein the phytonutrient is selected from the group consisting of soybean extract, kudzu extract, Ginkgo biloba extract, celery seeds extract, genistein, daidzein, quercetin, kaempferol, and apigenin.

Claim 7. The composition according to claim 5 or 6, wherein the PPAR-gamma inhibitor and/or phytoestrogen is selected from the group consisting of soybean extract, kudzu extract, Ginkgo biloba extract, celery seeds extract, or highly-absorbable genistein, daidzein, quercetin, kaempferol, and apigenin.

Claim 8. The composition according to claim 1, which comprises (i) at least one of curcumin or turmeric extractand (ii) kudzu extract.

Claim 9. The composition according to claim 1, which comprises(i) highly-absorbable curcumin and (ii) kudzu extract which contains highly-absorbable daidzein.

Claim 10. The composition according to claim 1, which comprises (i) at least one of curcumin or turmeric extractand (ii) Ginkgobiloba extract.

Claim 11. The composition according to claim 1,which comprises(i) highly-absorbable curcumin and (ii) ginkgo biloba extract which contains highly-absorbable quercetin and

kaempferol.Claim 12. The composition according to claim 1, which comprises curcumin, turmeric extract, kudzu extract, and Ginkgo biloba extract.

Claim 13. The composition according to claim 1, which comprises highly-absorbable curcumin, and kudzu extract containing highly-absorbable daidzein, and Ginkgo biloba extract containing highly-absorbable quercetin and kaempferol.

Claim 14. The composition according to claim 1, which comprises highly-absorbable curcuminin combination with at least one phytonutrient selected from the group consisting of extracts containing highly-absorvablegenistein, daidzein, quercetin, kaempferol, and apigenin.

Claim 15. The pharmaceutical composition according to claim 1, which comprise a combination of synthetic compounds selected from the group consisting of curcumin, curcumin derivatives, daidzein, daidzein derivatives, quercetin, kaempferol, apigenin, and synthetic deidzein derivative equol, in the use of helping ease and reverse the progression of osteoarthritis.

Claim 16. The composition according to claims 1 to 15, whichis for human and veterinary uses.

Claim 17. A method for the treatment of osteoarthritis, comprising administering a therapeutically effective amount of an anti-inflammatory agent and an agent that enhances bone-formation andchondrogenesisto a subject suffering from osteoarthritis.

Claim 18. The method according to claim 17, wherein

the anti-inflammatory agent comprises an NF-kappaB inhibitor, and

the agent that enhances bone-formation andchondrogenesiscomprises a PPAR-gamma inhibitor and/or phytoestrogen.

Claim 19. The method according to claim 18, wherein

the anti-inflammatory agent is selected from the group consisting of curcumin, andturmeric extract, and

the agent that enhances bone-formation andchondrogenesis is selected from the group consisting of soybean extract, kudzu extract, Ginkgo biloba extract, and celery seeds extract that contain highlyabsorbablegenistein, daidzein, quercetin, kaempferol, and apigenin.

Claim 20. The method according to claim 19, whereinthe anti-inflammatory agent comprises highly-absorbable curcumin, andthe agent that enhances bone-formation andchondrogenesiscomprises kudzu extract and/or Ginkgo biloba extract.

Claim 21. The method according to claim 20, whereinthe highly-absorbable curcumin is selected from the group consisting ofBCM-95(trademark), Curcumin C3/Bioperine, andTHERACURMIN (trademark).

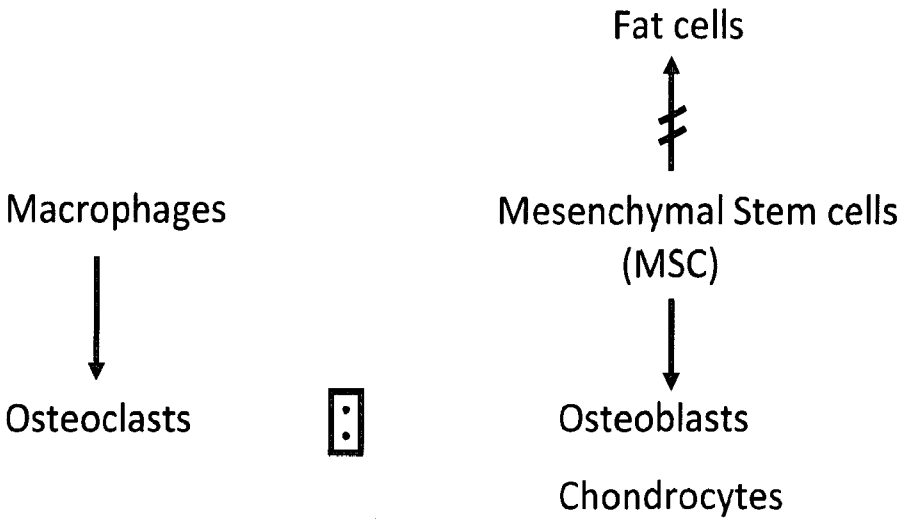
Fat cells
Macrophages
Mesenchymal Stem cells
(MSC)
Osteoclasts
Osteoblasts
Chondrocytes

FIG.1

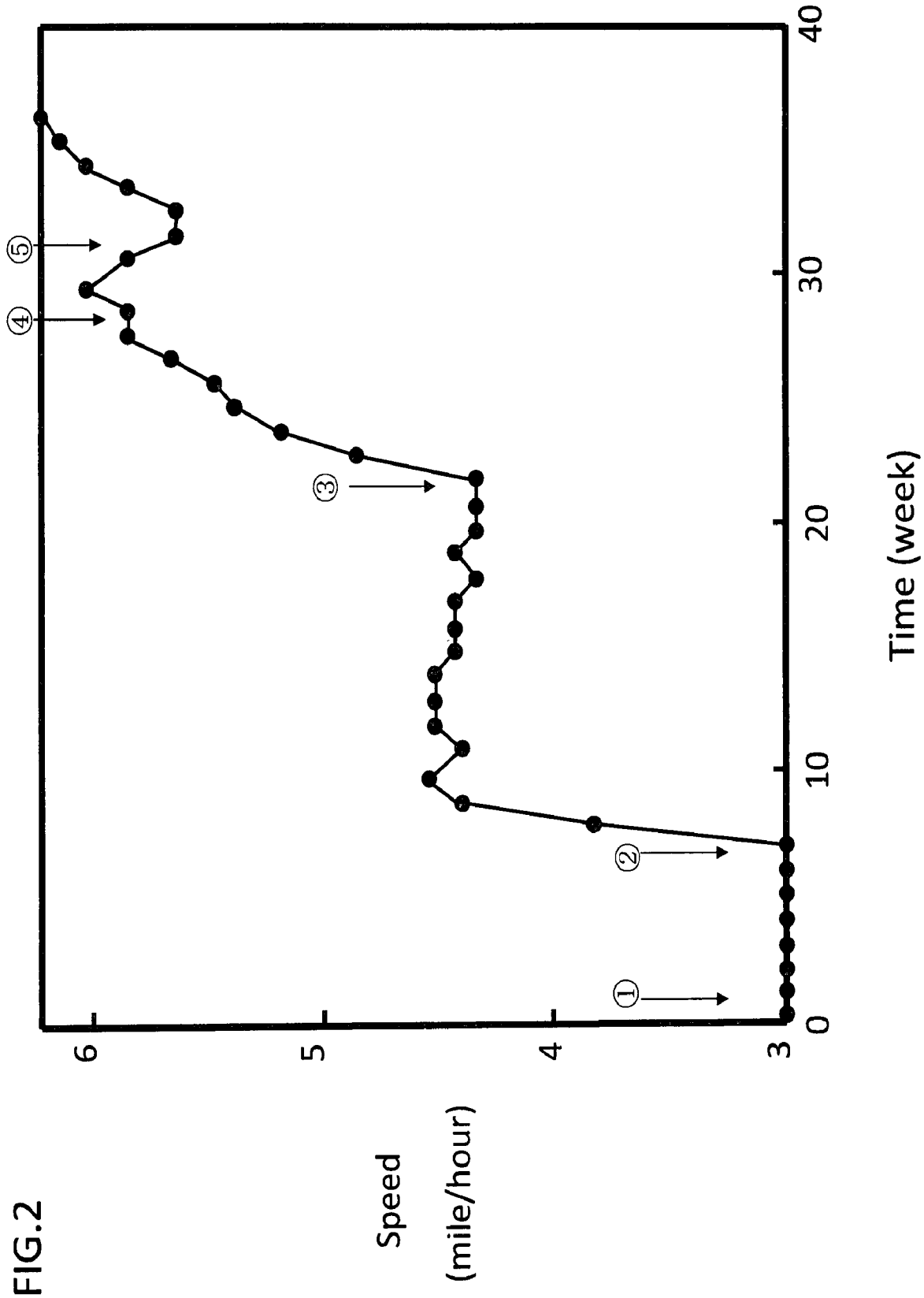

FIG.2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/21139

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 36/00, 36/9066; A61P 19/02 (2014.01)
USPC - 424/464, 756; 514/456
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(8): A61K 31/12, 36/00, 36/9066, 36/16, 9/20; A61P 19/02, 29/00, 39/06 (2014.01)
USPC: 424/725, 464, 756; 514/456

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 practicable, search terms used)
MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); Google Scholar; Google; ProQuest; Composition, osteoarthritis or osteoarthrosis, NF-κB inhibitor, antiinflammatory, curcumin, curcuminoids, turmeric extract, phytoestrogen, soybean, kudzu, ginkgo biloba, celery seeds, genistein, daidzein, quercetin, kaempferol, apigenin

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2007/0154540 A1 (PARK, CS et al.) July 5, 2007; paragraphs [0009], [0010], [0019], [0021], [0029], [0030], [0057], [0058], [0096], [0128], [0129]	1-2, 5-6, 7/5-6, 15, 17-18 ---- 3-4, 8-10, 12, 14
Y	WO 2009/097512 A1 (YI, S) August 6, 2009; paragraphs [0004], [0035], [0061], [0062], [0081]	10, 12
Y	ANTONY, B. A pilot cross-over study to evaluate human oral bioavailability of BCM-95 (biocurcumax), a novel bioenhanced preparation of curcumin. Indian Journal of Pharmaceutical Sciences. 2008. Vol. 70. pages 445–449; page 1, last paragraph; page 2, lines 22-24; page 4, lines 19-22; abstract	3-4, 8-9, 14
Y	WO 2011/135011 A1 (KARSDAL, MA et al.) November 3, 2011; page 1, lines 3-10; page 4, lines 28-29; page 5, lines 8-12; page 7, lines 7-11; claims 1-2	8-9, 12
A	US 6492429 B1 (GRAUS, IMF et al.) December 10, 2002; column 3, lines 58-67; column 4, lines 5-16	1-15, 17-21
A	US 2002/0165169 A1 (KIM, CS et al.) November 7, 2002; paragraph [0009]; abstract.	1-15, 17-21
A	HUANG, WW et al. Kaempferol induced apoptosis via endoplasmic reticulum stress and mitochondria-dependent pathway in human osteosarcoma U-2 OS cells; Molecular Nutrition & Food Research. 2010. Vol. 54. Pages 1585-1595. page 1592, left column, paragraph 2	1-15, 17-21

☐ Further documents are listed in the continuation of Box C. ☐

* 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 application or patent 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
23 May 2014 (23.05.2014)	11 JUN 2014
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US14/21139

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 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. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

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.: 16
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 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 additional fees, this Authority did not invite payment of additional fees.

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 and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.