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(54) Title: METHODS FOR FACILITATING RECOVERY OF FUNCTIONS OF ENDOGENOUS OR IMPLANTED OR TRANSPLANTED STEM CELLS USING HIGH MOLECULAR WEIGHT HYALURONIC ACID

(57) Abstract: The glycosaminoglycan, hyaluronic acid (HA), is an essential component of tissue extracellular matrices that contribute to the architecture of stem cell niches which determine the fate of stem cells. Decreased levels of HA are found in subjects experiencing a variety of pathological conditions, as well as in subjects receiving a variety of therapeutic interventions, for example, chemotherapy or radiotherapy, to treat pathological conditions. The use of HA having a molecular weight greater than 750,000 daltons (high molecular weight HA or HMW HA) to reconstitute a tissue extracellular matrix partially or completely depleted of HA is described. More particularly, described herein is the use of exogenous forms of high molecular weight hyaluronic acid (HMW HA) as an adjuvant in the restoration of the local tissue specific stem cell microenvironment to enhance stem cell recovery or engraftment and thus tissue recovery and remodeling following stem cell transplantation or other therapies. The effect of HMW HA on hematopoietic stem cells is illustrative of the invention. Mice having severe bone marrow hypoplasia, and pancytopenia resulting from treatment with 5-fluorouracil recovered more rapidly if treated with HMW HA. Similarly, mice transplanted with hematopoietic stem cells following lethal irradiation exhibited enhanced recovery of peripheral blood cell counts when treated with HMW HA as an adjuvant therapy compared to control mice transplanted with hematopoietic stem cells without adjuvant therapy.



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METHODS FOR FACILITATING RECOVERY OF FUNCTIONS OF ENDOGENOUS OR
IMPLANTED OR TRANSPLANTED STEM CELLS USING HIGH MOLECULAR WEIGHT
HYALURONIC ACID

FIELD OF THE INVENTION

This invention relates to medical treatment protocols involving transplantation or implantation of totipotent, pluripotent and multipotent stem cells (SCs). In another aspect it relates to treatment protocols to reconstitute the extracellular matrix that is required for the tissue architecture and functions of SCs and that is damaged as a consequence of the development of or the treatment of pathological conditions.

BACKGROUND OF THE INVENTION

Tissues and organs of a mammalian organism are built by mature functional cells of different lineages. Mature cells are terminally differentiated cells that are permanently committed to a specific function(s). These mature cells have a limited life span and, therefore, have to be constantly replenished by their corresponding tissue-specific SCs. The current stage of knowledge in biomedical science is that there are three major types of SCs: totipotent (SCs that give rise to both the placenta and the embryo), pluripotent (SCs that give rise to all embryonic lineages, but not to the placenta) and multipotent (SCs that provide cells for specific organs and tissues). Over the past decade multipotent SCs specific for several tissues and organs have been isolated and characterized. For example, hematopoietic SCs provide for blood cells (erythrocytes, platelets, lymphocytes, monocytes, etc); mesenchymal SCs give rise to a connective tissue (stromal cells, osteoblasts, adipocytes, myocytes, chondrocytes, etc); and neuronal SCs build brain. Other multipotent SCs include adult stem cells, pancreatic stem cells, epithelial stem cells, and endothelial stem cells.

Recent developments arising from stem cell research has generated great interest in the already demonstrated and theoretical applications of stem cells to treat a wide variety of medical conditions. For example, in combination with cytotoxic ablative chemotherapy and irradiation

hematopoietic stem cells are already used with success to treat a variety of leukemias and lymphomas. Implanted neuronal stem cells from nasal tissue have been used to treat severed spinal cords in an effort to restore function with a measure of success in the form of at least partial restoration of function below the point of severance. It also has been proposed to use neuronal stem cell implants to treat Parkinson's disease, stroke, and Alzheimer's disease. It has been proposed to use neuronal stem cells from a variety of sources, for example cells from the subventricular zone of the forebrain and the subgranular zone of the dentate gyrus of cadavers, for other applications as well.

Mesenchymal stem cells have been implanted in damaged heart tissue resulting from infarcts and after cardiac surgery and substantial restoration of heart function has been observed. Among the proposed applications for mesenchymal stem cells can be mentioned their use to augment local repair or regeneration of bone, cartilage and tendon; to facilitate the engraftment of hematopoietic stem cells following myeloablative therapy; and to treat osteogenesis imperfecta, osteoporosis, osteoarthritis, meniscectomy, and muscular dystrophy.

Laboratory experiments involving the transplantation of pancreatic stem cells in a diabetic strain of mice have alleviated the diabetic condition of the mice. This strongly suggests that pancreatic stem cell transplantation could be an effective treatment of diabetes mellitus in humans.

It is known that the successful transplantation or implantation of stem cells to achieve therapeutic benefit is dependent on many factors and adjuvant therapies are used to improve the success of these procedures. For example, a variety of soluble factors, cytokines and interleukins are used with varying degrees of success in hematopoietic stem cell transplantation and with many attendant, undesirable side effects. Accordingly, there exists a substantial need for additional adjuvant therapies to be employed with stem cell transplantation and implantation procedures to improve the result obtained using such procedures and to reduce the incidence of undesirable side effects.

SCs constitute a very small population (less than 0.01%) of the mammalian organism. However this number of cells is sufficient to constantly produce billions of new mature cells throughout life. The major features of SCs that distinguish them from all other progenitor cells in the body are 1) the ability for self-renewal, and 2) multipotency. Self-renewal can be defined as the ability of SCs to undergo multiple divisions without also undergoing differentiation, thereby retaining the ability to maintain a pool of SCs. Multipotency is the ability of SCs to differentiate into different lineages, e.g. various cell types. Upon differentiation, SCs lose their "stemness", i.e. they became mature terminally differentiated cells with mortal fate. Once a SC has chosen a differentiation path, it is believed it can never become a SC again. The behavioral choices (self-

renewal, proliferation, or differentiation) of a SC are regulated by multiple signals provided by its microenvironmental niche in response to physiological and pathophysiological demands (Schofield R. *Biomed Pharmacother* (1983) 37:375-380). These microenvironmental structures have been described for various organs, such as bone marrow, brain, pancreas, etc. The cellular composition of such a niche is very heterogeneous and is comprised of cells of different origin (reviewed in Minguell JJ, et al. *Braz J Med Biol Research* (2000) 33:881-887; Bianco P, Robey PG. *J Clin Invest* (2000) 105:1663-1668). Over the past decade, the understanding of molecular mechanisms mediating the regulatory signals provided by the cells of the microenvironmental niches has significantly advanced (Heckney JA, et al. *PNAS* (2002) 99:13061-13066). Soluble and cell surface associated factors and extracellular matrix (ECM) molecules are produced by the cells that compose the niche and contribute to the highly complex structure of the niche (Gupta P, et al. *Blood* (1998); 92:4641-4651, and reviewed in Verfaillie C. *Blood* (1998); 92:2609-2612; Chabannon C and Torok-Storb B. *Curr Top Microbiol Immunol* (1992) 177:123-136; Klein G. *Experientia* (1995) 51:914-926). While the cellular composition of niches is tissue specific, extracellular matrix molecules (ECM) represent common features of all niches. ECM components, such as collagens, fibronectin, laminin, and hemonection, were shown to participate in the tissues' regulatory network, whereas the role of numerous other ECM molecules, including HA, is not yet completely understood.

While I do not wish to be bound by any particular theory, it is my belief that HA is a component of ECMs that is essential for tissue homeostasis. Importantly, CD44, a major receptor for HA, is expressed on the surface of SCs including but not limiting to hematopoietic, neuronal, and mesenchymal SCs. In addition, these SCs demonstrate HA binding ability (Khaldoyanidi, unpublished observations). Therefore, I believe that HA is required for structuring microenvironmental niches to optimally support the ability of SCs to self renew, proliferate and differentiate. HA, a member of the glycosaminoglycan (GAG) family, is a large negatively charged polymer containing multiple copies of the disaccharide N-acetyl-D-glucosamine (GlcNAc) and D-glucuronate (GlcA). HA is present in all organs and tissues and biological fluids of mammalian organisms. It was initially believed that by binding salt and water, HA expands and maintains extracellular space. Later studies demonstrated that, by interacting with a variety of extracellular molecules, such as aggrecan, versican, neurocan, etc., HA participates in local ECM assembly (Fraser J, et al. *J Intern Med.* (1997) 242:27-33). Identification of receptors that bind HA demonstrated that HA is implicated in the specific receptor-ligand interactions that ultimately influence cell behavior. Thus, it was revealed that HA is involved in the regulation of multiple cell functions, including cell proliferation (Brecht, M., et al. *Biochem. J.* (1986) 239:445-450; Hamann, K.J., et al. *J. Immunol.* (1995) 154:4073-4080), migration (Andreutti, D.,

et al. J. Submicrosc. Cytol. Pathol. (1999) 31:173-177), cytokine production (Noble, P.W., *et al. J. Clin. Invest.* (1993) 91:2368-2377; Hodge-Dufour, J. *et al. J. Immunol.* (1997) 159:2492-2500; Khaldoyanidi, S., *et al. Blood.* (1999) 94:940-949), and adhesion molecule expression (Oertli, B., *et al. J. Immunol.* (1998) 161:3431-3437).

While the involvement of HA in normal cell and tumor biology is generally appreciated, little is known about HA contribution to the assembly of microenvironmental niches that support SCs. Using the hematopoietic system as an example, we have previously reported that HA is not a passive structural element of the bone marrow ECM, but a necessary and specific signal-inducing molecule for hematopoiesis (Khaldoyanidi S, *et al. Blood* (1999) 94:940-949). Specifically, hyaluronidase (HA'ase) treatment of bone marrow cultures inhibits, or even prevents, lymphopoiesis and myelopoiesis, whereas addition of HA to bone marrow cultures enhances lymphopoiesis and myelopoiesis. It appears that HA regulates a decisive step before the commitment of hematopoietic SCs and is required for SC maintenance and self-renewal. With respect to other tissues and organs, HA was found in central nervous system (CNS) in perineuronal microenvironment in brain (Giarard *et al. Histochem J.* 1992;24:21-4), as well as in peripheral nervous system where it is required for myelination of growing nerve (Seckel *et al. J Neurosci Res* 1995;40:318-24). HA was also shown to be essential in the microenvironment for pancreatic Langerhans islets to support insulin release (Velten *et al. Biomaterials* 1999;20:2161-7). Since HA synthase-2 knockout mice do not survive *in utero* as embryos, it appears that HA is required for pluripotent SCs (Camenisch *et al. J Clin Invest.* 2000;106:349-60).

Although HA is essential for many cell functions, it is an unstable molecule. Total-body irradiation sharply decreases the amount of HA in tissues, including in the spleen and bone marrow (Noordegraaf, E.M., *et al. Exp. Hematol.* (1981) 9:326-331). Degradation of HA or alteration of its synthesis and accumulation can be induced by various other factors, such as UV irradiation (Koshishi, I., *et al. Biochim. Biophys. Acta.* (1999) 1428:327-333; Schmut, O., Ansari, and A.N., Faulbom, J. *Ophthalmic. Res.* (1994) 26:340-343) or administration of 5-fluorouracil (5-FU) (Young, A.V., *et al. Histol. Histopathol.* (1994) 9:515-523; Matrosova V., *et al. Stem Cells* (2004) in press), hydrocortisone or other chemicals (Yaron, M., *et al. Arthritis. Rheum.* (1977) 20:702-708). In addition to its depletion as a result of such treatments, a low amount of HA in tissues can be associated with pathological developments such as hormonal imbalance (D'avis *et al., Biochem J* 1997;324:753-60; Engelbrecht-Schnur *et al., Exp Eye Res* 1997;64:539-43), sclerosis (Bodo *et al., Cell Mol Biol.* 1995;41:1039-49), aging (Lamberg *et al., J. Invest. Dermatol.* 1986;86:659-67; Matuoka *et al., Aging* 1989;1:47-54; Schachtschabel *et al., Z Gerontol.* 1994;27:177-81), etc. Thus, I believe that disease- and treatment-induced alterations of

the amount of HA in tissues leads to an imbalance of microenvironmental homeostasis and, therefore, affects the function of tissue-specific SCs and aggravates pathological development.

In addition to providing specific receptor-mediated regulation of functions in the microenvironment of SCs, HA is essential for three-dimensional structuring of the niche by binding salt and water and by presenting growth factors. It appears that therapeutic interventions that lead to a decreased amount of HA in tissues can also alter the physicochemical structure of the niche. For example, 5-FU (a drug used in chemotherapy) induced bone marrow hypoplasia and its administration correlates with decreased levels of cell-surface associated HA (Matrosova V. et al. *Stem Cells*, in press), resulting in negative extravascular pressure outside of bone marrow sinusoids (Narayan et al., *Exp Hematol.* 1994;22:142-148).

Various pathological conditions or treatments can result in the shedding of HA receptors or down-regulation of their gene expression by cells, including stem cells, progenitor cells, mature cells and microenvironmental cells (Matrosova et al, *Stem Cells*, 2004, in press). These changes can result in decreased levels of cell surface associated HA and contribute to the development of sequelae. Therefore, it is important to develop improvements to therapies that enhance the anchoring of endogenous or exogenous HA to the cell surface of stem cells, progenitor cells, mature cells and microenvironmental cells in selected tissues and organs.

Chemotherapy is used alone or in conjunction with radiotherapy for the treatment and cure of a large variety of malignancies. The most undesirable consequences of chemotherapy are severe bone marrow aplasia and pancytopenia. The major reason for this is that chemotherapeutic drugs eliminate not only rapidly dividing cancer cells, but also the pool of cycling hematopoietic progenitor cells. Since mature blood cells have a limited life span they have to be constantly replenished by the committed, actively proliferating progenitors that in turn originate from SCs. Thus, the recovery of mature blood cells following chemotherapy requires a prolonged period of time and is generally accompanied by pancytopenia. Obviously this prolonged period of hematopoietic recovery places patients at a greatly increased risk of infection, bleeding and hypoxia and the attendant consequences, up to and including loss of life, in the hospital setting following transplantation,

Engagement of SCs in proliferation is strictly regulated, and this complex process is controlled by a number of soluble factors, including cytokines and interleukins. Soluble factors mediating SC proliferation are well characterized and are divided into two groups: positive regulators (colony stimulating factors (CSF) such as G-CSF, GM-CSF, M-CSF, erythropoietin (Epo), thrombopoietin (Tpo), interleukins (IL), stem cell factor (SCF); and flt-3 ligand (FL)) and negative regulators of SC proliferation (such as TGF- β , TNF α , LIF, MIP-1 α and interferons). It is vital to maintain the correct balance between the positive and negative regulators in order to

prevent exhaustion of stem cells and maintain the right ratio of proliferating and quiescent cells in the bone marrow, especially under conditions of physiological demand following chemotherapy, radiotherapy or chemoradiotherapy

Use of recombinant hematopoietic growth factors has promoted the development of cytokine therapy. Thus, G-CSF and GM-CSF are used to shorten the period of neutropenia in cancer patients following chemotherapy. When used in the appropriate setting, Epo ameliorates anemia following chemotherapy and decreases the need for erythrocyte transfusion in those patients. However, some cytokines, in particular G-CSF, give rise to consistent, severe thrombocytopenia in patients and mice (Momin, F., *et al. Proceedings of ASCO.* (1992) 11:294. (Abstr.); Scheduling, S., *et al. Brit. J Haematol.* (1994) 88:699-705). Thus, the "lineage competition" effect of G-CSF places patients at increased risk of bleeding, besides exhibiting high toxicity and immunogenic activity. In addition, one of the most important concerns about using growth factors, especially in combination with repeated cycles of chemotherapy, is the potential for stem cell exhaustion. The administration of growth factors not only results in an expansion of the committed progenitor compartment, but also in an increased number of quiescent multipotent SCs entering the proliferative state. Engagement of normally quiescent SCs in the cycling places them at increased risk of massive depletion upon repeated courses of proliferation-dependent chemotherapy (reviewed in Moore M, *Blood.* (1992) 80(1):3-7). Identification of the molecular mechanisms that prevent quiescent stem cells from entering the proliferative state has a significant potential for clinical applications, especially in view of using repeated cycles of proliferation-dependent chemotherapy.

Thus, it has become clear that there is a need for new approaches in improving the recovery of hematopoiesis following chemotherapy.

Another approach used in the clinic to alleviate sequelae of chemo- and radiotherapy is SC transplantation. Transplantation of hematopoietic SCs is generally used to facilitate hematopoietic recovery following high-dose chemotherapy and total-body irradiation. The efficiency of SC transplantation is reflected by the dynamics of the recovery of peripheral blood cells following transplantation. The efficacy of SC transplantation depends on the homing ability of intravenously infused SCs. As used herein, homing of hematopoietic SCs is defined as the ability of hematopoietic SCs to find the bone marrow hematopoietic niche, to lodge within it, and to produce progeny (Tavasolli M, Hardy C. (1990) *Blood* 76(6):1059-1070; Hardy C, Minguell J. (1993) *Scanning Microscopy* 7(1):333-341; Hardy C, Megason G. (1996) *Hematol Oncol* 14:17-27). Therefore, homing is divided into two major phases: extravasation followed by seeding of the bone marrow. According to this definition, a SC arrested on the bone marrow sinusoidal endothelium is not yet considered a homed cell. Similarly, the extravasated SC that has not

reached an appropriate hematopoietic niche and has not produced progeny under the conditions of physiological demand cannot be regarded as a homed cell, either. Extravasation is the first multi-step phase in SC homing and involves interaction of SCs with the bone marrow vascular endothelium under the conditions of physiological flow and includes tethering of cells (e.g., rolling), adhesion to the luminal surface of endothelial cells, and diapedesis (e.g., transmigration) across the endothelium. In the seeding phase, which completes the "homing program," the extravasated SC must be able to migrate through the bone marrow ECM either using its own enzymic activities or by inducing such activities in the surrounding cells. Finally, the homed cell must (i) find the appropriate microenvironment that produces hematopoiesis-supportive factors and (ii) respond by proliferation and self-renewal (Verfaillie, C. *Blood*. (1998) 92:2609-2612; Turner, M. *Stem Cells*. (1994) 12:22-29; Quesenberry, P., and Becker, P. *Proc. Natl. Acad. Sci. USA*. (1998) 95:15155-15157; Hardy, C., Megason, G. *Hematol. Oncol.* (1996) 14:17-27; Tavassoli, M., Hardy, C. *Blood*. (1990) 76:1059-1070).

It should be particularly noted that hematopoietic SC homing/engraftment, which involves facilitation of adhesion of hematopoietic SCs in their microenvironment, namely the bone marrow, their proliferation and self-renewal, is to be distinguished from SC mobilization, which involves the release of anchored SCs and stimulation of their migration from bone marrow into the peripheral blood system. Thus, SC homing/engraftment is the opposite of SC mobilization.

While little is known about the molecular mechanisms mediating directed SC migration, a basis for the understanding of SC homing has been created over the past decade, in which a variety of molecules are implicated, including chemokines such as SDF-1 and cell surface molecules such as P and E selectins, VCAM-1, $\alpha 4\beta 1$ and $\alpha 4\beta 7$ integrins, and CD44 (Khaldoyanidi et al., *J. Leuk. Biol.* 1996;60:579-92; Frenette et al., *Proc Natl Acad Sci USA* 1998;95:14423-14428; Williams et al., *Nature* 1991;352:438; Papayannopoulou et al., *Proc Natl Acad Sci USA* 1995;92:9647). CD44 was originally described as a homing molecule required for the binding of lymphocytes to high endothelial venules (Jalkanen et al., *Science* 1986;233:556-558). It has been shown that CD44, in addition to selectins, can mediate the rolling of activated lymphocytes on primary endothelial cells (DeGrendele et al., *J Exp Med* 1996;183:1119-1130). It has also been demonstrated that CD44 mediates the *in vitro* adhesion of lymphocytes and hematopoietic progenitors to HA and fibronectin, important components of the bone marrow ECM (Legras et al, *Blood* 1997;89(6):1905-1914; Verfaillie et al., *Blood* 1994; 84(6):1802-1811). Finally, the cytoplasmic part of CD44 specifically binds to cytoskeletal proteins such as ankyrin, and the CD44 variant isoform(s) is/are closely associated with the active form of MMP-

9, suggesting that CD44 may be involved in SC migration in extracellular space (Bourguignon et al., *J Cell Physiol* 1998;76(1):206-215).

In line with these observations, we have previously demonstrated that pretreatment of bone marrow cells with HA-binding blocking CD44-specific antibodies results in a reduction in the ability of hematopoietic SCs to repopulate the bone marrow of lethally irradiated recipients, suggesting that CD44 might interfere with hematopoietic SC homing. Furthermore, we had previously demonstrated that CD44 regulates the initial hematopoietic SC-stromal cell interaction, and therefore might be involved in hematopoietic SC seeding (Khaldoyanidi, S., *et al. J Leukoc. Biol.* (1996) 60:579-592.). Thus, I believe that CD44/HA pathway is important for regulation of SC-stromal cell and SC-endothelial cell interactions and, therefore, contributes to the regulation of SC homing/engraftment.

Total-body irradiation results in degradation of HA. Furthermore, reconstitution of lethally irradiated bone marrow with syngeneic bone marrow cells results in a secondary relapse in the GAG concentration in the bone marrow and spleen as compared to non-reconstituted mice. The absence of detectable amounts of HA in the reconstituted mice was remarkable, whereas in the non-reconstituted mice a slow recovery of HA was observed (Noordegraaf, E.M., *et al. Exp. Hematol.* (1981) 9:326-331). In addition, it was shown that irradiation affects the ratio of sulfated versus unsulfated GAGs, which can be essential for normal hematopoiesis. Therefore, a decrease of the amount of HA resulting from irradiation and the infusion of cells can interfere with homing/engraftment of transplanted SCs.

Transplanted SCs have to repopulate irradiated bone marrow and produce committed hematopoietic progenitors in order to replenish the pool of mature terminally differentiated functionally active blood cells. Thus, recovery of the mature blood cell population following transplantation of hematopoietic SCs requires a prolonged period of time and is generally accompanied by pancytopenia. To facilitate proliferation and expansion of the pool of committed progenitors in post-transplant patients, growth factors are used, in particular GM-CSF. However, in addition to stimulating proliferation of the progenitor cells, GM-CSF mobilizes hematopoietic SCs from bone marrow to peripheral blood. This effect of GM-CSF negatively affects long-term reconstitution by multipotent SCs as a result of these cells becoming sensitive to the growth factors upon mobilization. In view of these and other side effects of GM-CSF, such as bone pain, myalgia, fever and erythema, the use of GM-CSF is not desirable. Similarly to GM-CSF, low molecular weight (LMW) HA (MW<750,000 daltons) exhibits the capacity for mobilization of SCs, including mature and progenitor hematopoietic cells, from tissues to the periphery (Canadian Patent Application No. 2,199,756). Therefore, it has become apparent that LMW HA and high molecular weight (HMW) HA have different biological functions, likely as a result of

different affinities for the receptors involved. While LMW HA acts as a mobilization agent, I have found that HMW HA provides homeostatic equilibrium to the tissues. Thus, the very different functions of LMW HA compared to HMW HA makes it undesirable to use LMW HA in post-chemotherapy and post-transplant clinical settings.

It is, therefore, an object of this invention to provide alternatives or complements to the use of G-CSF, GM-CSF and Epo to stimulate post-chemo and post-transplant recovery of tissues.

It is therefore an object of this invention to provide improved treatments of chemo- and irradiation-induced sequelae.

It is an object of this invention to provide an improved method for engraftment of SCs following therapy that depletes SCs.

SUMMARY OF INVENTION

The present invention provides a method for treating pathological conditions that are associated with decreased levels of HA in tissues and organs comprising administration to the subject of an effective dose of high molecular weight HA.

High Molecular Weight Hyaluronic Acid (HMW HA)

HMW HA used in the practice of the present invention has an average molecular weight of greater than 750,000 daltons and can be obtained from any suitable source, such as purified from natural sources or produced using synthetic chemical or recombinant methodologies. The HMW HA may also be administered in the form of a pharmaceutically acceptable salt, for example, it can be administered as the sodium salt. I have found that the use of high molecular weight HA (>750,000 daltons) enhances the recovery of endogenous SCs or engraftment of transplanted or implanted SCs and, thus, tissue recovery and remodeling following stem cell transplantation and other therapies. This result is particularly surprising in that it has been observed that low molecular weight HA (HA having an average molecular weight lower than 750,000) has the opposite effect on cells to engraftment, i.e. it induces mobilization of mature cells and SCs. HA or its pharmaceutically acceptable salts having a molecular weight higher than 750,000 daltons may also be used in the invention. For example, HA having an average molecular weight of 1,000,000 daltons or greater or 2,000,000 daltons or greater, or 3,000,000 daltons or greater can be used.

The high molecular weight HA is preferably dissolved in an aqueous carrier prior to administration, such as normal saline or any other physiologically acceptable aqueous injectible diluent. Other excipients may include buffers, preservatives, and the like, so long as they are

physiologically acceptable. The concentration of the HA solution can be adjusted based on well-known pharmacological principles, but may be between 5 and 500 µg/ml.

While I do not wish to be bound by any particular theory, it is my belief that the beneficial effects that can be obtained using HMW HA result from one or more of the following:

- stimulation of the cells of microenvironmental niches to produce soluble factors supportive of SCs
- stimulation of the cells of microenvironmental niches of SCs to express the cell surface factors that support self-renewal of SCs and proliferation and differentiation of committed progenitors
- stimulation of microenvironmental niches of stem cells to produce components of tissue specific ECMs
- stimulation of microenvironmental niches of SCs to produce anti-apoptotic factors
- provision of a direct signal to SCs for their proliferation and self-renewal
- provision of a direct signal to committed progenitor cells for their proliferation
- sequestration and presentation of growth factors and cytokines
- induction of expression and production of endogenous biological activities that stimulate proliferation of committed progenitors without SC exhaustion
- provision of conditions for lodgment of SC in tissues and organs
- anchoring of HA under conditions of disease- or treatment induced decrease in the expression of HA receptors in order to maintain extracellular space and pressure
- directing the migration of cells of any origin
- induction of expression and production of endogenous biological activities with enzymatic properties that mediate SC homing and engraftment
- facilitation of the extravasation of transplanted SC
- facilitation of the seeding/engraftment of bone marrow with intravenously injected SC
- induction of expression of adhesion molecules that mediate SC-endothelial cell and SC-microenvironment cell interactions
- maintenance of the volume and pressure of extracellular sites after therapeutic interventions, including, but not limited to chemotherapy and radiotherapy.

Treatment Conditions

The invention provides a method to improve/treat the microenvironment of SCs in a wide variety of tissues and organs including, but not limited to, bone marrow, brain, pancreas, liver, and skin damaged by therapeutic interventions involving, for example, the use of drugs or ultraviolet, x-ray or other types of radiation.

The invention also provides a method to improve/treat the microenvironment of endogenous and transplanted or implanted SCs in tissues and organs such as bone marrow, brain, pancreas, heart, liver, and skin damaged by pathological development of a disease or pathological condition. Examples of such pathological developments are degenerative disorders, primary or subsequent hormonal disorders, aging, pathology of HA synthesis, heart attack and the like.

Accordingly, the practice of the present invention is broadly applicable to treatment of any subject that exhibits lower-than-normal ($>10\%$ decrease) HA levels as a result of any pathological condition or treatment thereof.

The invention also provides a method to improve/treat microenvironment of SCs in tissues and organs (bone marrow, brain, pancreas, liver, skin, etc) damaged by pathologically expressed HA receptors, such as CD44 and RHAMM (decreased cell surface expression due to shedding or specific down-regulation of gene expression).

It is particularly useful in the treatment of subjects experiencing therapy-induced bone marrow aplasia/hypoplasia, which may be brought on following chemotherapy, irradiation, hormonal therapy, for example, using prednisone, or other therapies known to lead to bone marrow suppression or ablation.

The invention also includes a method for enhancing engraftment of exogenously transplanted SCs comprising administration of a therapeutic amount of a composition comprising HMW HA, or a pharmaceutically acceptable salt thereof, in an aqueous diluent into the peripheral blood or intra-organ or intraperitoneally.

Accordingly, the present invention includes the use of high molecular weight HA to enhance recovery of functions of endogenous or engrafted SCs, including multipotent SCs, e.g., hematopoietic SCs (HSCs), mesenchymal stem cells (MSCs), neuronal stem cells (NSCs), epithelial stem cells (EpSCs), endothelial stem cells (EnSCs), hepatic stem cells (HeSCs), pancreatic stem cells (PSCs), umbilical cord blood SCs and adult stem cells (ASCs), as well as pluripotent, and totipotent SCs. More particularly, it relates to the use of HMW HA to enhance engraftment of the SCs after implantation/transplantation, including the recovery of their "stemness" properties of self-renewal and multipotency and their ability for proliferation and differentiation. In some cases, more than one kind of stem cell can be transplanted or implanted at the same time. For example, MSCs can be implanted with HSCs to support engraftment of the HSCs.

Stem cells useful in the invention for implantation or transplantation purposes can be acquired by isolation procedures well known in the art from any appropriate source, including from the bone marrow, peripheral blood, umbilical cord blood, brain, pancreatic, liver or skin cells, mucosal tissue and the like. These cells can be obtained from the tissue of living donors or

cadavers or from stem cells cultured in vitro. Useful multipotent stem cells can also be obtained by causing differentiation of totipotent or pluripotent stem cells or from the corresponding stem cell lines. Stem cells useful in the invention can also be obtained by the nuclear transfer process. This process involves removal of the nucleus of a pluripotent cell from blastocysts followed by introduction into the enucleated cell of a nucleus extracted from an adult cell of the intended recipient of the stem cell implantation or engraftment.

A population of pluripotent or multipotent stem cells can be expanded and differentiated prior to implantation or transplantation or for other purposes using culturing methods known to the art. Among such processes can be mentioned the coculturing of the stem cells with a feeder layer containing fibroblasts or stromal cells. Pluripotent stem cells can also be cultured in the presence of leukemia inhibitory factor (LIF). In one embodiment of the invention, high molecular weight hyaluronic acid can be included in the culture of pluripotent stem cells containing a feeder layer or LIF or in the culture of multipotent stem cells containing a cocktail of cytokines and growth factors.

In order to demonstrate the utility of the invention, experiments using hematopoietic SCs and the hematopoietic system have been conducted and the results are set out as examples herein. It was found that high molecular weight HA facilitates post-chemotherapy (5-FU injection) recovery of hematopoiesis in bone marrow and shortens the time of bone marrow hypoplasia. An increased number of hematopoietic SCs and progenitor cells were indicated in the marrow of mice treated with high molecular weight HA. Furthermore, it was found that an elevated number of cells in mitosis, indicative of a higher proliferating rate. The argumentation of bone marrow hematopoiesis by high molecular weight HA subsequently resulted in higher numbers of mature terminally differentiated functionally active peripheral blood cells, including lymphocytes, neutrophils, monocytes, platelets and megakaryocytes, and erythrocytes in comparison with control animals. Thus, administration of high molecular weight HA proved effectual in fighting the 5-FU-induced bone marrow hypoplasia and pancytopenia. This suggests that high molecular weight HA substantially contributes to the requisite microenvironment in the bone marrow for hematopoietic SCs, which accelerates the rebound of hematopoiesis. I believe that high molecular weight HA induces the balanced production of soluble and membrane-associated regulators of SC proliferation, differentiation and self-renewal. The correct composition of factors induced by high molecular weight HA would, therefore, help to maintain a balanced mature cell production, to avoid lineage competition and to prevent SC depletion/exhaustion.

The present invention also demonstrates that high molecular weight HA significantly improves chemotherapeutically perturbed hematopoiesis in mice and is therefore an appropriate therapy for treatment-induced bone marrow hypoplasia and aplasia. The use of high molecular

weight HA will result in a better prognosis for, and more rapid recovery of, patients who undergo chemotherapy.

Since both total-body irradiation and transplantation of bone marrow cells sharply decrease the amount of HA in bone marrow (Noordegraaf et al., Exp Hematol. 1981;9:326-331), we investigated the effect of exogenous high molecular weight HA on SC engraftment after lethal irradiation (15.25 Gy γ -rays at a dose rate of 0.85 Gy/h) followed by bone marrow transplantation. While the number of white blood cells (WBC) in control groups remained low, a complete recovery of leukocyte numbers in recipients of high molecular weight HA was observed on day 13. In addition, recovery of platelets (PLTs) and red blood cells (RBCs) in the peripheral blood of mice treated with high molecular weight HA was monitored. The enhanced recovery of peripheral blood cell counts in the HA-treated group is a result of facilitated engraftment of transplanted SCs, since analysis on bone marrow revealed an increased number of mature cells as well as their progenitors in the HA-treated group.

These findings demonstrate that high molecular weight HA helps restore and/or maintain a requisite microenvironment in the bone marrow, which facilitates homing and engraftment of SCs.

Overall, the results presented herein demonstrate the beneficial use of high molecular weight HA in clinical hematology to improve bone marrow recovery after chemotherapy and body irradiation, as well as other treatment-induced damage of tissues and organs.

Modes of Administration

The high molecular weight HA compositions of the present invention can be administered as an aqueous solution, or they may be incorporated into carrier vehicles such as liposomes or microparticles, especially those that are targeted specifically for any tissue/organ, and administered as suspensions of these carriers. Such targeted carrier vehicles are described in the literature.

To assure more tissue-specific delivery of injected high molecular weight HA, it can be conjugated with a carrier that targets a particular tissue, for example, any type of SC, stromal cells, endothelium cells or other cell type to which it is desirable to direct the high molecular weight HA. A suitable tissue specific carrier can be, for example, a fusion protein composed of an HA-binding protein and an antibody, particularly an IgG, or antibody fragment specific for the target tissue. Suitable antibody fragments include, for example, F(ab)' and F(ab)2' fragments. The use of such target specific carriers will provide improved anchoring of the injected HA when treating pathological conditions associated with the loss of HA receptors.

Pharmaceutical compositions of high molecular weight HA can be administered intraperitoneally, intravenously, or intra-organ.

The composition of high molecular weight HA can be administered any time following recognition of low levels of HA in a subject.

In some applications, for example, following chemotherapy with drugs such as 5-FU under conditions that deplete, but do not eliminate stem cells, the composition of high molecular weight HA can be administered alone. Alternatively, the composition of high molecular weight HA can be combined with a suspension of SCs or, for that matter, a suspension of any other type of cell, prior to implantation or transplantation.

In another embodiment, the composition of high molecular weight HA can be pre-incubated with the cellular suspension, for example, a SC suspension, prior to implantation or transplantation. In yet other embodiments, high molecular weight HA can be administered in conjunction with therapies involving administration of colony stimulating factors such as G-CSF, GM-CSF, M-CSF, Epo, and Tpo, interleukins, stem cell factor, flt-3 ligand, or negative regulators of SC proliferation such as TGF- β , TNF- α , LIF, MIP-1 α , and interferons, and other agents used in such therapies.

The high molecular weight HA composition can be administered once, or multiple times, so long as the subject continues to demonstrate symptoms suspected of being alleviated by high molecular weight HA therapy, or low levels of HA. If used in conjunction with transplanted or implanted cells, administration can be before, with or after treatment with a suspension of cells such as SCs.

The total amount of high molecular weight HA to be administered to a subject in each dose for a particular application that constitutes an effective therapeutic dose can be readily determined by those skilled in the art. In a preferred embodiment of the invention, the dose may be any dose between 0.1 to 100 mg/kg, and, more preferably any dose between 1 to 10 mg/kg. The term "therapeutic dose" is meant to express the amount necessary to result in an observable increase in HA levels in the subject to which the composition is administered. As such, the precise amount that represents a "therapeutic dose" can easily be determined on the basis of monitoring of the HA levels post-administration, and multiple dosing until a therapeutically effective amount has been administered.

BRIEF DESCRIPTION OF FIGURES

Figures 1-4 depict the results of experiments designed to demonstrate the effects of HMW HA on the recovery of bone marrow hematopoiesis in mice after chemotherapy.

Figures 5-6 depict the results of experiments designed to demonstrate the effects of HMW HA on engraftment of hematopoietic SC and hematopoietic tissue recovery after lethal irradiation.

DETAILED DESCRIPTION OF FIGURES

EXAMPLE 1

Figure 1 demonstrates the effects of HMW HA on recovery of peripheral blood cells after 5-FU administration. 5-FU was intraperitoneally injected in mice at 150 mg/kg. The counts of white blood cells (WBC), red blood cells (RBC), platelets (PLT), hemoglobin (HGB) and hematocrit (HCT) were monitored daily for two weeks. As expected, the treatment of mice with 5-FU induced severe bone marrow hypoplasia and pancytopenia. The numbers of WBC and PLT dropped from $8.4 \pm 1.5 \times 10^6/\text{ml}$ and $678.4 \pm 82 \times 10^6/\text{ml}$ before 5-FU administration to $2.52 \pm 0.5 \times 10^6/\text{ml}$ and $388 \pm 50 \times 10^6/\text{ml}$, respectively, 7 days later (Figure 1A, B). The total number of mononuclear cells in the bone marrow decreased from $15.3 \pm 2.2 \times 10^6/\text{femur}$ before to $5.00 \pm 0.65 \times 10^6/\text{femur}$ 7 days after 5-FU administration (Figure 2A). All parameters were recovered to normal in 14 days after 5-FU administration. To examine the effect of high molecular weight HA on 5-FU-perturbed hematopoiesis, 5-FU-treated (day 0) mice were administered 100 $\mu\text{g}/\text{mouse}$ HMW HA (from Sigma-Aldrich, average molecular weight 750,000-2,000,000 daltons as a 0.05% solution in PBS) on days 4, 6, 10, and 13. A control group of animals was treated with a 200- μl injection of PBS. The peripheral blood from HA- and PBS-treated mice was collected daily and examined for numbers of WBC, RBC, PLT, HGB, and HCT. The numbers of WBC in HA-treated mice were significantly higher starting from day 5 (2-2.5 fold) as compared to the control PBS-treated group (Figure 1A). To demonstrate a dose-dependent effect of high molecular weight HA on WBC recovery, mice were administered with various doses of HMW HA (0-1000 $\mu\text{g}/\text{mouse}$), and the peripheral blood samples were evaluated for the leukocyte number on day 7. The most effective concentration of high molecular weight HA was found to be 100 $\mu\text{g}/\text{mouse}$ (or 3mg/kg). The number of PLT in HA-treated mice was increased starting from day 5 and was elevated by a factor of 1.7 on day 8 (Figure 1B). From week 2 the parameters observed in the HA-treated group corresponded to those in normal mice prior to 5-FU treatment. Thus, administration of high molecular weight HA rescued mice from 5-FU-induced leukocytopenia and thrombocytopenia.

Because mature blood cells are a product of proliferation and differentiation of hematopoietic progenitors, we next examined the effect of high molecular weight HA on bone marrow hematopoiesis in 5-FU-treated mice. 5-FU-treated mice (day 0) were administered PBS or 100 $\mu\text{g}/\text{mouse}$ HMW HA (as a 0.05% solution in PBS) on day 4, 6, 10, and 13. Bone marrow

cells were harvested on days 7, 14, 21, and 28. On day 7 the total number of bone marrow cells in the HA-treated mice was 3.5-fold higher in comparison to the PBS-treated mice (Figure 2A). To evaluate the effect of high molecular HA on different hematopoietic lineages, a morphological analysis of the bone marrow cells was performed as followed. Smears of bone marrow cells were fixed on glass slides in methanol at -20°C for 20 min and dried at room temperature, the slides were incubated with Filipson's dye (25% Giemsa dye in 96% ethanol) for 15 min, extensively washed with distilled water (pH 7.0), dried, and covered with a cover slip. Examination of the slides under the microscope revealed that the numbers of mature myeloid and lymphoid elements in the bone marrow were increased in the mice that received high molecular weight HA treatment following 5-FU administration (Table 1).

Table 1
Effect of HA on bone marrow cell counts after 5-FU administration

Cell types:	non-treated	control			HA		
	Day 0	Day 7	Day 14	Day 21	Day 7	Day 14	Day 21
promyelocyte	0.3	0.2	1.5	0.1	0.8	1	0.3
melocyte	3	5.4	4	3	4.2	6	4.7
metamyelocyte	3.7	4	6	5.3	4.3	5	5.7
band-neutrophil	17	4.5	18	15	13	25	18.3
segmented neutrophil	27.3	2	22	39	17.6	19	49
eosinophil	1	0	0.5	0.3	0.6	0	0.3
monocyte	0.7	0.5	2	2	1	2.5	2.8
lymphocyte	21.3	11.1	6.5	13.3	33	20.3	29.7
basophilic normocyte	4.5	3.5	2.5	1	5	2	2
oxyphilic normocyte	0.3	0.4	0.5	0.3	0.5	0.3	0.5
polychromatophilic normocyte	28.2	17.2	20	19.6	17.9	19	21.2
plasma cell	0.5	1	0.5	0.7	1.5	0.4	0.5
platelet/field	7	2	2	5	25	20	10

Interestingly, the animals treated with high molecular weight HA also showed an increased number of bone marrow cells in mitosis (1.7/100) as compared to control (0.3/100). These data are suggestive of a higher number of proliferating progenitor cells in the bone marrow of HA-treated mice. To examine this assumption, bone marrow cells were cultured in methylcellulose to evaluate the number of proliferating lineage-committed progenitors. Bone marrow cells were harvested and plated at a concentration of 1×10^4 cells/ml in 24-well plates in semisolid methylcellulose containing 30% FCS, 1% BSA, 10^{-4} M 2-mercaptoethanol, 2 mM L-glutamine (StemCell Technologies, Vancouver, Canada). Conditioned medium from WEHI-3B was added (15% v/v) as a source of interleukin-3. The cultures were incubated at 37°C in a humidified atmosphere of 5% CO₂. Colonies containing more than 20 cells were counted under the inverted microscope after 7 days of culture. To culture erythroid burst-forming units (BFU-e), 10 U/ml of erythropoietin (Boehringer-Mannheim, Germany) was added. Colonies containing at least 500 cells were counted after 14 days. The number of myeloid progenitors in the mice

treated with high molecular weight HA was 2.9-fold higher, and the number of early erythroid progenitors was 21.5-fold higher as compared to control (Figure 2B). The number of megakaryocytes in the bone marrow of HA-treated mice showed a 3.7-fold increase. We next investigated whether high molecular weight HA stimulation benefited the pool of committed progenitors at the cost of damaging more primitive progenitors that were measured by using a long-term culture-initiating cells (LTC-IC) assay. The number of LTC-IC in the bone marrow of mice treated with high molecular weight HA was evaluated using a limited dilution assay. We monitored a trend in the increase in number of LTC-IC in the bone marrow of 5FU/HA vs. 5FU/PBS mice. Although the difference was not statistically significant ($p>0.1$), the number of LTC-IC was elevated from 14.8 ± 9.6 /femur in control to 30.6 ± 13.3 /femur in HA-treated animals (Figure 2C). These findings suggest that the increase in the number of mature cells and committed progenitors in the bone marrow of HA-treated mice does not result in the exhaustion of the pool of more primitive stem cells as measure by LTC-IC. Thus, HA promoted bone marrow hematopoietic activity, which had been impaired by 5-FU.

Since the mobilization effect of LMW HA (<750,000 Da) on hematopoietic cells in healthy individuals has been demonstrated (L. Pilarski, Canadian patent application 2,199,756), we further tested the effect of HMW HA on cell mobilization in 5-FU treated mice. Mice were administered 150mg/kg 5FU followed by HMW HA (from Sigma-Aldrich, average mol. wt. 750,000 – 2,000,000 daltons, as a 0.05% solution in PBS) or PBS infusion (3 mice per group) on day 4. Twelve and 24 hours after the HA infused mice were sacrificed, peripheral blood (PB) or bone marrow (BM) cells were harvested and analyzed on a flow cytometer. Each sample was measured for 10^4 total events (100%). Size (as X-Mean), granularity (as Y-Mean) and the percentage of the total cells for each cell population was evaluated and expressed as mean $\pm$ SD. FACS analysis revealed that neither after 12 hours (data not shown) nor 24 hours (Figure 3) was the composition of cell populations in the peripheral blood or bone marrow changed. It can be conclude, therefore, that HMW HA does not induce mobilization of cells from the bone marrow to the peripheral blood in 5FU treated mice. We anticipate several possible reasons for this observation: 1. Chemotherapy might affect the expression of HA receptors, resulting in an impaired mobilization response to HA treatment; 2. Chemotherapy induces hypoplasia of bone marrow and therefore eliminates cellular resources for detectable mobilization; 3. HMW HA and LMW HA can have different biological functions by targeting different HA receptors/isoforms.

Table 1. Analysis of the peripheral blood (PB) and bone marrow (BM) cell composition in 5FU-administered mice 24 hours after HA administration

Population	PBS	HA
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	X-Mean	Y-Mean	% Total	X-Mean	Y-Mean	% Total
R1	289.8±2.9	120.7±0.85	56.4±5.8	291.4±0.9	117.7±1.7	60.14±8.1
R2	485.9±10.3	270.7±9.6	17.8±4.2	471.4±10	262±6.9	13.6±3.8
R3	543.9±13.7	738±0.9	2.8±0.9	535.3±12.8	746.8±10.2	2.3±1.2
R4	293±2.5	572.4±15.8	9.7±1.2	291.2±4.6	587.7±2.2	11.23±0.96
Population BM	PBS			HA		
	X-Mean	Y-Mean	% Total	X-Mean	Y-Mean	% Total
R1	305.3±0.4	38.5±0.3	85.5±0.8	301.3±0.6	40.8±0.1	87.2±0.64
R2	463.3±2.3	27.7±0.3	1.2±0.23	470.2±2.3	27.7±0.6	1.5±0.12
R3	642.6±3	74.9±0.7	4.1±0.47	653.2±4	78.1±1.88	3.4±0.22
R4	313.6±1.7	185±1.4	2±0.29	310.8±3.5	191.7±4	1.43±0.16

We have previously demonstrated that high molecular weight HA does not promote proliferation of hematopoietic progenitors directly (Khalidoyanidi, S., *et al. Blood.* (1999) 94:940-949). Subsequent studies demonstrated that high molecular weight HA up-regulates the production of hematopoiesis-supporting cytokines IL-1 and IL-6 by the cells of the bone marrow hematopoietic microenvironment. However, the experiment with IL-1 and IL-6 neutralizing antibodies suggested that in addition to IL-1 and IL-6, other hematopoiesis-supportive soluble factors are produced by the HA stimulated hematopoietic microenvironment. To identify other molecules that mediate HA effects, we performed gene expression profiling using Affymetrix chip technology. Mice were administered 150mg/kg 5FU at day 1, followed by the infusion of 100µg HMW HA (Sigma-Aldrich, average mol. wt. 750,000-2,000,000 daltons, as a 0.05% solution in PBS) at day 4. Twenty hours later the animals were sacrificed, the bone marrow harvested and total RNA isolated using a Qiagen RNA isolation kit. Probe preparation and chip hybridization was performed according to the manufacturer's recommendations (Affymetrix, Alameda). Differentially expressed genes were analyzed with the Affymetrix Data Mining Tool software. In this log transformed graph the hybridization signals for over 10,000 genes are plotted. Hybridization signals obtained from control samples (5FU/PBS) (X-axis) are compared to samples from mice treated with high molecular weight HA (5FU/HA) (Y-axis). Genes that were statistically significantly detected in the samples are represented by black spots at higher hybridization intensities (top right of the data trend). Non-detected genes are shown by gray spots. Spots that deviate from the main trend in the plot are differentially expressed between the two samples. We identified a total of 179 genes that were called as highly significantly differentially expressed in the bone marrow of HA-treated mice (5FU/HA) vs. control mice (5FU/PBS). A replicate from a separately treated mouse gave almost completely concordant data (Figure 4). The differentially expressed genes could be grouped as follows: (1) Transcription regulation, hormone receptors and DNA replication factors; (2) Signal transduction cascade regulators; (3) Apoptosis regulation; (4) Migration mediating enzymes; (5) Cell surface

associated molecules; (6) Soluble factors. Overall, our results suggest that HA is a biologically active component of microenvironment and is involved in regulating the expression of genes and their products which mediate stem cell behavior.

EXAMPLE 2

Total-body irradiation sharply decreases the amount of GAGs, including HA, in the spleen and bone marrow. Furthermore, transplantation of bone marrow cells results in a second relapse of HA concentration in hematopoietic tissue. Thus, we investigated the effect of HMW HA on the peripheral blood and bone marrow cell recovery after total body irradiation followed by bone marrow transplantation. Recipient mice were lethally (15.25 Gy at a dose rate of 0.85 Gy/h) irradiated to eliminate endogenous bone marrow hematopoiesis. HSPC were obtained from donor mice, pretreated with 5-FU (150 mg/kg body weight) to eliminate the proliferating committed progenitor cell pool, and transplanted into the recipient mice (10^4 cells/mouse) 24 hours after irradiation. The recipient mice were administered 200 μ l/mouse PBS (control group) or 100 μ g/mouse of high molecular weight HA (Sigma-Aldrich, average mol. wt. 750,000-2,000,000 daltons, as a 0.05 % solution in PBS) on day 4, 6, 10, and 13 after transplantation. The number of peripheral WBC was measured daily. A significant increase in WBC number in the peripheral blood of mice treated with high molecular weight HA was detected on day 11 ($p < 0.01$) as compared to control. While the number of WBC in control groups remained low, a complete recovery of leukocyte numbers in HA-treated recipients was observed on day 13 (Figure 5A). In addition, facilitated recovery of PLT and RBC in the peripheral blood of HA-treated mice was monitored. The numbers of PLT and RBC were elevated 3-fold and 2-fold, respectively in the HA-treated group. PLT and RBC levels are shown as a percent increase, where counts from control PBS administered group are taken as 100% (Figure 5B and 5C). The increased number of RBC correlated with greater values of HCT and HGB in mice administered high molecular weight HA in comparison with the control mice.

To demonstrate that the enhanced recovery of peripheral blood cell counts in mice treated with high molecular weight HA is a result of facilitated engraftment of transplanted HSPC, we further evaluated hematopoietic activity in the bone marrow. Seven days after the transplantation of HSPC, the bone marrow was harvested and examined. A morphological analysis of the bone marrow revealed that the total number of blast cells in the group treated with high molecular weight HA was 10 times higher (Figure 6A) than in the PBS-treated control group. In addition, a 3-fold increase in the number of erythroblasts (Figure 6A) and an 18.8-fold increase in megakaryocyte count was observed in the bone marrow of the HA-treated mice in comparison to control (Figure 6B). The elevated number of megakaryocytes correlated with higher numbers of

platelets in the bone marrow of the HA-treated mice: the platelet count in these mice was $20 \pm 1.5/\text{field}$, while in the control group it remained $4 \pm 0.2/\text{field}$.

Thus, we have demonstrated that HA provides more favorable conditions for engraftment of SC and subsequently tissue recovery/remodeling.

We claim:

1. A method of treating a pathological condition in a subject that is associated with decreased levels of hyaluronic acid in tissue or organs comprising administration to the subject of an effective dose of hyaluronic acid, or a pharmaceutically acceptable salt thereof, having a molecular weight greater than 750,000 daltons.
2. A method according to claim 1 wherein the dose is from 0.1 to 100 mg/kg.
3. A method according to claim 2 wherein the dose is from 1 to 10 mg/kg.
4. A method according to claims 1, 2, or 3 wherein the dose is administered intraperitoneally, intravenously or intraorgan.
5. A method according to claim 1, 2 or 3 wherein the hyaluronic acid is incorporated into a carrier vehicle.
6. A method according to claim 5 wherein the carrier vehicle is a liposome or microparticle.
7. A method according to claim 1, 2 or 3 wherein the hyaluronic acid is conjugated with a tissue specific carrier.
8. A method according to claim 7 wherein the tissue specific carrier comprises a fusion protein of HA binding protein and a F(ab)2 or F(ab) fragment.
9. A method for treating a subject to improve the engraftment of implanted or transplanted stem cells comprising the administration to the subject of an effective dose of hyaluronic acid or a pharmaceutically acceptable salt thereof having a molecular weight greater than 750,000 daltons.
10. A method according to claim 9 wherein the stem cells are selected from totipotent, pluripotent and multipotent stem cells.
11. A method according to claim 10 wherein the stem cells are multipotent stem cells.
12. A method according to claim 11 wherein the multipotent stem cells are obtained by causing the differentiation of totipotent or pluripotent stem cells.
13. A method according to claim 12 wherein the pluripotent stem cells are manipulated by the nuclear transfer process.
14. A method according to claim 11 wherein the multipotent stem cells are selected from hematopoietic, neuronal, mesenchymal, epithelial, endothelial, pancreatic hepatic, and adult stem cells.
15. A method according to claim 14 wherein the stem cells are from bone marrow, peripheral blood, umbilical cord blood, brain, pancreas, liver, mucosal tissue, or skin.
16. A method according to claim 14 wherein the stem cells are primary stem cells isolated from the tissue of a living donor of a cadaver or stem cells cultured in *in vitro* conditions.
17. A method according to claim 9 wherein the subject is implanted or transplanted with stem cells to treat a pathological condition.

18. A method according to claim 17 wherein the hyaluronic acid is administered before, with or after the implantation or transplantation of the stem cells.

19. A method according to claim 18 wherein the subject receives therapy prior to transplantation of the stem cells.

20. A method according to claim 19 wherein the therapy is cytotoxic therapy..

21. A method according to claim 19 wherein the therapy is chemotherapy, radiotherapy or hormonal therapy.

22. A method according to claim 19 wherein the therapy is ablative therapy.

23. A method according to claim 18 wherein the stem cells are hematopoietic stem cells.

24. A method according to claim 23 wherein the therapy is cytotoxic therapy.

25. A method according to claim 24 wherein the therapy is chemotherapy, radio therapy or hormonal therapy.

26. A method according to claim 25 wherein the therapy is ablative therapy.

27. A method according to claims 9, 10, 11, 14, 17, 18, or 19, wherein the dose is from 0.1 to 100 mg/kg.

28. A method according to claim 27 wherein the dose is from 1 to 10 mg/kg.

29. A method according to claim 28 wherein the dose is administered intravenously, intraperitoneally or intraorgan.

30.. A method according to claims 23 or 26 wherein mesenchymal stem cells are coadministered with the hematopoietic stem cells.

31.. A method according to claim 1 wherein the administration of hyaluronic acid is to facilitate hematopoiesis following therapy.

32. A method according to claim 31 wherein the dose is from 0.1 to 100 mg/kg.

33.. A method according to claim 32 wherein the dose is from 1 to 10 mg/kg.

34. A method according to claim 33 wherein the dose is administered intravenously, intraperitoneally or intraorgan.

35. A method according to claim 1 wherein the subject to whom hyaluronic acid is administered exhibits a condition selected from the group consisting of pancytopenia, neutropenia, thrombocytopenia, anemia, lymphocytopenia or any combination or subcombination thereof.

36. A method according to claim 35 wherein the condition is the result of therapy or disease.

37. A method according to claim 36 wherein the therapy is cytotoxic therapy.

38. A method according to claim 36 wherein the therapy is chemotherapy, radiotherapy or hormonal therapy.

39. A method according to claim 17 wherein the stem cells are pancreatic stem cells and the pathological condition is diabetes.

40. A method according to claim 39 wherein the dose is from 0.1 to 100 mg/kg.

41. A method according to claim 40 wherein the dose is from 1 to 100 mg/kg.

42. A method according to claim 39, 40 or 41 wherein the dose is administered intravenously, or intraorgan.

43. A method according to claim 17 wherein the stem cells are mesenchymal stem cells.

44. A method according to claim 43 wherein the pathological condition is heart damage.

45. A method according to claim 44 wherein the heart damage is the result of an infarct or surgery.

46. A method according to claim 43 wherein the mesenchymal stem cells are administered directly to tissue selected from bone/bone marrow, cartilage, muscle, tendon or brain tissue, or intravenously; alone or in combination with other SCs.

47. A method according to claims 43, 44, 45, or 46 wherein the dose is from 0.1 to 100 mg/kg.

48. A method according to claim 47 wherein the dose is from 1 to 10 mg/kg.

49. In a method of treating a subject with a drug or radiation that results in bone marrow dysfunction, the improvement comprising treating the subject having the bone marrow dysfunction with an effective amount of hyaluronic acid, or a pharmaceutically acceptable salt thereof, having a molecular weight greater than 750,000 daltons.

50. A method for improving the recovery of the number of stem cells and their functions in a subject having a depleted population of stem cells as the result of a pathological condition or of a therapy which depletes the stem cell population or proper function of stem cells comprising administering to the patient an effective amount of hyaluronic acid, or a pharmaceutically acceptable salt thereof, having a molecular weight greater than 750,000 daltons.

51. A method according to claim 50 wherein the stem cells are multipotent stem cells.

52. A method according to claim 51 wherein the multipotent stem cells are a member of the group consisting of hematopoietic, neuronal, mesenchymal, epithelial, endothelial, liver, hepatic, pancreatic and adult stem cells.

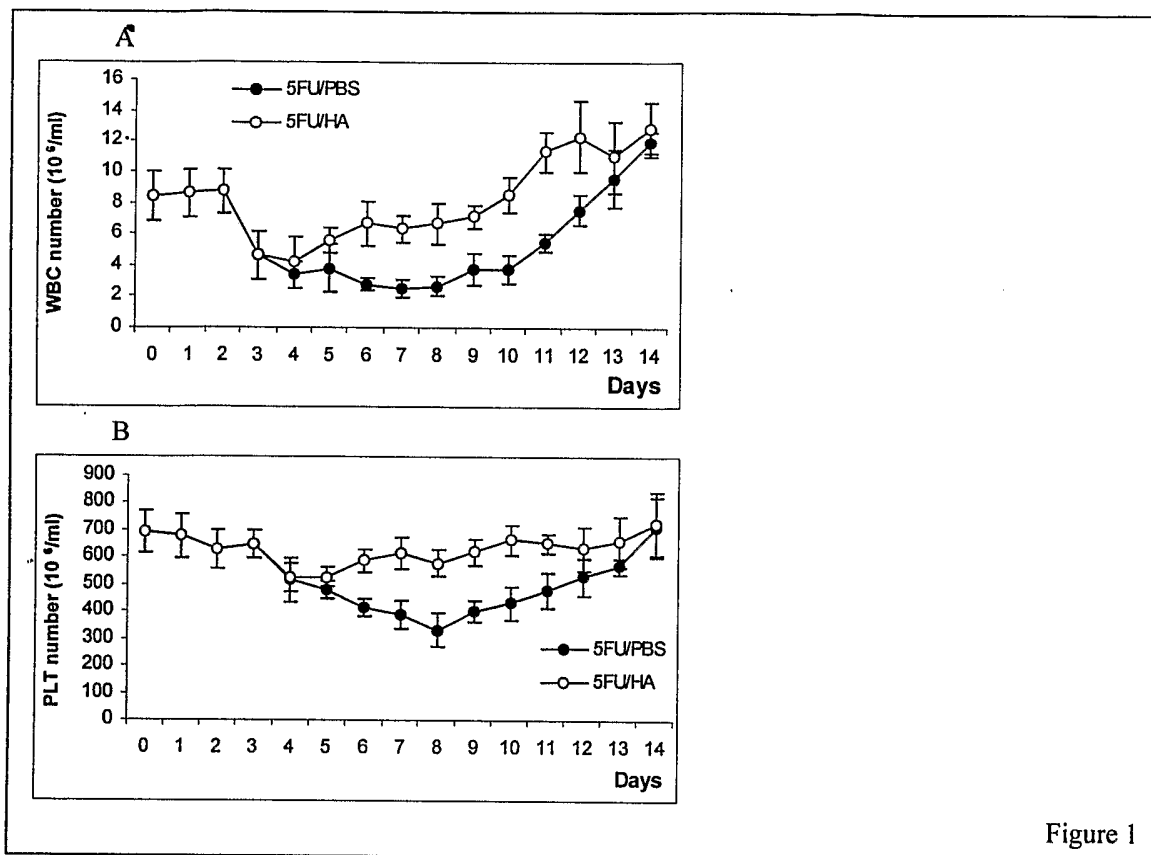
53. A method according to claim 51 or 52 wherein the multipotent stem cells are obtained by causing the differentiation of totipotent or pluripotent stem cells.

54. A method according to claim 50 wherein the pluripotent stem cells are manipulated by a nuclear transfer process.

55. A method according to claim 50 wherein the stem cells are depleted by therapy.
56. A method according to claim 55 wherein the population of stem cells are hematopoietic stem cells.
57. A method according to claim 55 or 56 wherein the therapy is cytotoxic therapy.
58. A method according to claim 57 wherein the therapy is chemotherapy, radiotherapy or hormonal therapy.
59. A method according to claim 50, 51 or 58 wherein the dose is from 0.1 to 100 mg/kg.
- 60.. A method according to claim 59 wherein the dose is from 1 to 10 mg/kg.
61. A method according to claim 1, 9, 11, 51, or 52 wherein the hyaluronic acid is administered with an effective amount of an agent selected from positive or negative regulators of stem and committed progenitor cell proliferation.
62. A method according to claim 1, 9, 11, 51, or 52 wherein the hyaluronic acid is administered with an effective amount of a chemokine
63. A method according to claim 62 wherein the chemokine is SDF-1.
- 64.. A method according to claim 1, 9, 11, 51 or 52 wherein the hyaluronic acid is used in a form conjugated with tissue specific carrier, which is a fusion protein consisting of HA binding protein fused to an F(ab)₂ or F(ab) fragment directed against a tissue specific cell surface antigen.
65. A method according to claim 11 wherein the stem cells are neuronal stem cells.
66. A method according to claim 64 wherein the neuronal stem cells are implanted or transplanted to treat a disease of central and peripheral nervous system.
67. A method according to claim 11 wherein the stem cells are hepatic stem cells to treat a disease of the liver.
68. A method according to claim 1, 17 and 64 wherein the pathological condition is the result of aging.
69. In a method for the culturing of pluripotent or multipotent stem cells, the improvement comprising including hyaluronic acid having a molecular weight of greater than 750,000 daltons in the culture medium.
70. A method according to claim 69 wherein the culture conditions employ a feeder layer of fibroblasts or stromal cells.
71. A method according to claim 69 wherein the stem cells are pluripotent stem cells and the culture medium contains LIF.

72. A method according to claim 16 wherein the stem cells are cultured in vitro in a medium employing a feeder layer of fibroblasts or stromal cells and the medium contains hyaluronic acid having a molecular weight of greater than 750,000 daltons.

73. A method according to claim 16 wherein the stem cells are pluripotent stem cells cultured in vitro in a culture medium that contains LIF and hyaluronic acid having a molecular weight of greater than 750,000 daltons.



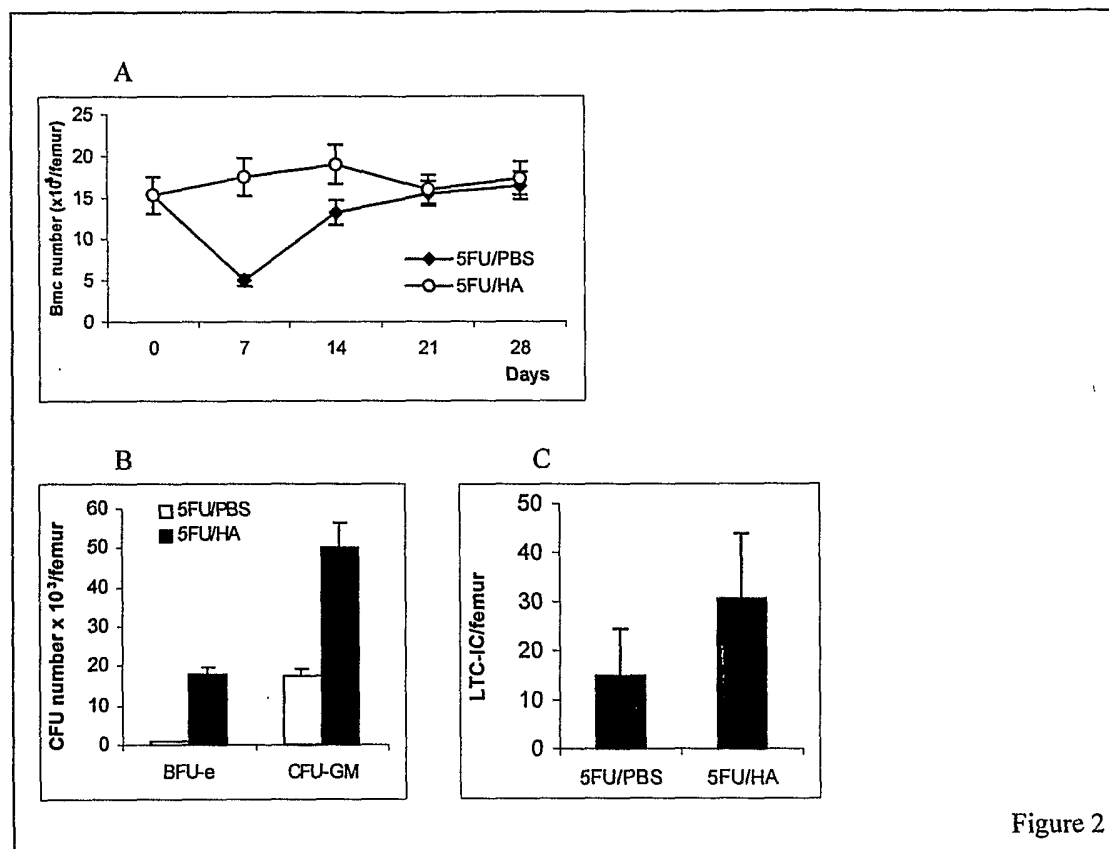


Figure 2

