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Cell Therapy in Chronic Obstructive Pulmonary Disease: State of the Art and Perspectives

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1. Introduction

The pulmonary diseases of obstructive character have high prevalence in the human population and has been subject of several clinical and experimental studies in order to seek a wider understanding of their pathogeny, physiopathology and, especially, the establishment of more rational ways for their treatment. Accordingly, this great effort has led to an extraordinary widening in the concepts of obstructive diseases in the last years, involving the integration of mechanical factors, inflammatory agents, autonomic regulation of airways and environmental aspects.

The COPD may be understood as a pathologic condition in which a non-reversible and limited gas exchange occurs. There are two clinical entities that constitute the COPD: chronic bronchitis and emphysema. Within the COPD spectrum, the main characteristic of pulmonary emphysema is air flow blockage and progressive dyspnea, arising out of the impairment of alveolar walls and increase of air spaces distal to terminal bronchiole, without significant pulmonary fibrosis (Barnes et al., 2003; GOLD, 2009; Oliveira et al., 2000).

The oxidative damage to which lungs are submitted to, as well as the inflammation occurring as a response to irritant agents, such as those coming from air pollution and cigarette smoke, contributes to the induction of the pulmonary degeneration (Lee et al., 2011). Therefore, it may be concluded that chief characteristic of COPD is the acceleration of functional and morphologic loss, with limitation of gas exchanges, resulting in progressive dyspnea, disability and premature death.

Therefore, the development and progression of the pathology are resulting from the interaction of genetic and environmental factors (Ribeiro-Paes et al., 2009). About 1-3% of cases of emphysema are generated by enzyme α1-antitrypsin deficiency, that characterizes a genetic abnormality as an inheritance of autossomal recessive pattern. The other risk factors include: age, infections, as well as social and economic factors (Mannino & Buist, 2007).

Smoking, however, has been established as the major cause related with COPD, resulting for active or passive exposure to the cigarette smoke, and corresponds to 15-20% of cases of

pulmonary emphysema (Ribeiro-Paes et al., 2009). The cigarette smoke in their gas and particle stages has a significant quantity of oxidant substances. A high number of particles and oxidant agents are contained in cigarette smoke. Oxidant agents are capable of reducing the effect of the anti-protease system through the oxidation of the active site of those enzymes and leading to a direct injury to the extracellular matrix (Barnes 2000; Barnes et al., 2003; Bast et al., 1991; Rufino & Lapa e Silva, 2006).

The Global Initiative for Chronic Obstructive Lung Disease (GOLD, 2009) has pointed out COPD as a serious public health issue. The pathology is considered the fifth largest cause of death worldwide, and has 210 million patients, with 80 million already in the moderate and/or serious stage of the disease. Estimates put it at the third ranking of cause of death in 2020 (GOLD, 2009; WHO, 2008). Moreover, faced with the ageing of world population, the economic burden of COPD should represent a significant parcel of the future global investments in health (Mannino & Buist, 2007).

Several clinical strategies, associated with the pulmonary rehabilitation techniques have contributed to the extension and improvement of the quality of life of emphysema patients. Notwithstanding the significant advances resulting from the introduction of new therapeutic approaches and rehabilitation, there has not been any efficient form of treatment up to now, other than the one in the palliative scope. The surgery treatment entails highly complex procedures and, in the specific case of lung transplant, a shortage of donors. By taking these aspects into account, experimental models have been proposed, in order to advance the knowledge about the physiopathological processes and new therapeutic approaches to the pulmonary emphysema (Gross et al., 1965; Hele, 2002; Mahadeva & Shapiro, 2002; Martorana et al., 1989; Nikula et al., 2000; Ribeiro-Paes et al., 2009).

2. Experimental models in COPD

Experimental models represent an important tool, since they enable the broadening of knowledge about COPD physiopathology, besides allowing the application of new therapeutic approaches.

The methodology of papain intratracheal instillation, proposed by Gross and coworkers in 1965, represented an original model for pulmonary emphysema induction. Starting from the proposition of this pioneer methodology, a series of studies were conducted, which led to the development of the models of induced pulmonary emphysema by the instillation of other proteases. (Pushpakom *et al.*, 1970; Fusco *et al.*, 2002).

The use of proteolytic enzymes, chiefly of porcine pancreatic elastase (PPE) for the generation of DPOC in an animal model is a widely employed methodology for the conduction of experimental studies, since it is mainly a simple and fast method and produces physiopathological effects similar to the human disease (March, 2000; Shapiro, 2000). The experimental models of pulmonary emphysema induced by proteases instillation have not reproduced precisely the mechanisms of alveolar destruction ensuing the inhaling of smoke and other toxic particles, and, therefore, they do not mimic exactly the sequence of pathological events that occur in the disease in humans (Cendron, 1007).

The use of animal models of cigarette-smoke-induced emphysema means seeking an accuracy in experimental models to match the human, chiefly with respect to the

physiopathological mechanisms involved in the formation of the emphysema. Up to the beginning of the 80's decade, the studies involving induced pulmonary emphysema in animals by exposure to cigarette smoke were scarce and their reliability questioned (March, 2000). In 1981, Huber and coworkers proposed a study based on the model of induced emphysema through exposure to cigarette smoke. Some achieved results in the study, with respect to morphometric and physiological aspects provided the basis for the ensuing research. According to the report from the First Siena International Conference on Animal Models of COPD held at the University of Siena in 2001, the induced lesions with the use of this model are similar to those observed in emphysematous humans, highlighting the importance of the stimulus through cigarette smoke in COPD experimental models. (Hele, 2001).

At our laboratory, a new apparatus (Figure 1) for induced emphysema through exposure to cigarette smoke is under test. The present device has a series of innovations when compared to the already existing inhaling models, such as the fact that the animals are contained inside acrylic containers making up the device, while in other cages the animals stay freed. Another important aspect worth highlighting relates to the smoke, which is pumped inside the box. In the device created by our team, the smoke pumped into the box interior comes from puffing on the cigarette; therefore, the situation of an active smoking human is mimicked. This apparatus is expected to lead to a model which mimics, as close as possible, the human pathology and, accordingly, which can be applied to research projects oriented to the analysis of physiopathological processes and to the development of new therapies in chronic degenerative pulmonary diseases.

Fig. 1. Apparatus created by the team of the Laboratory of Genetics and Cell Therapy – GenTe Cel to induce emphysema by cigarette smoke.

Notwithstanding the challenges involved in some parameters related to the cigarette-smoke-induced emphysema models, mainly with respect to the age of the animals, exposure time and reproducibility difficulty due to the required resources and time, this is a promising approach to turning animal models closer to the human, chiefly in relation to the physiopathological processes featured in the human pulmonary emphysema (March, 2000).

Currently, the creation and use of genetic models is a very important tool for DPOC study, since the strains mimic a series of aspects related to the human disease, mainly with respect to the α_1-antitrypsin deficiency (March, et al., 2000). At present, several mice strains are known to have natural or laboratory-induced mutations (gene targeting), which generate abnormal conditions in the animal development and are completed with the spontaneous arise of DPOC (March, 2000; Shapiro, 2000). Other methodological approaches to emphysema induction entail animal models with genetic modifications. Martorana *et al*. (1995), showed the installation and development of pulmonary emphysema in Tight skin transgenic mice, which show mutation in the fibrillin-1 gene, a protein related to the elastic fibers assembly making up the pulmonary tissue (Kietly, 1998).

The Table 1 shows some advantagens and disadvantages of the main animal models of induction of COPD.

Experimental model of COPD	**Advantages**	**Disadvantages**	**References**
Protease-induced Emphysema	- Simple method, easy to apply, quick results, high reproducibility - Morphological features similar to human disease	- Lack of inflammatory constituents - Physiological process is different from human	March *et al*. (2000)
Genetic models	- Reproduction of human pathology, mainly in relation to deficiency of α_1-antitripsin - Demonstrate the role of proteases in the development of the disease	- The aspects of pathology are reproduced just individually - Need for further studies with respect to inflammatory mechanisms	Shapiro (2000) Fujita and Nakanishi (2007)
Smoke cigarette	- Reproduction of aspects related to the inflammatory processes of COPD - Changes in the airways similar to the human disease	- Long time to onset of symptoms - Time of exposure highly variable	Zheng *et al*. (2009)

Table 1. Comparison of the main experimental models of COPD.

Taking into account these aspects, is evident the great importance of experimental models of COPD, even though none of them entirely mimic all the features making up the human

disease. The use of these models affords the broadening of knowledge, especially related to the physiopathology. The achieved results in animal models may be the grounds for the development of new therapeutic alternatives with the ensuing impact on the survival and improvement of the quality of life of COPD patients.

3. Stem cells and cell therapy: The rationale for use in the lung

The employment of cells for treating diseases is an ancient therapeutic practice, which dates back to the transfusion of whole blood or platelet concentrate in different acute or chronic clinical conditions. The first hematopoietic stem cells (HSC) transplantations were made according to the works of Till and Mculloch in 1961, on the response of mice with the bone marrow transplanted after lesion by ionizing radiation. Since then, new ranges and possibilities of use of other tissues according to the experimental model adopted by the authors have arisen.

The potential of differentiation of stem cells (SC), i.e., the wide range of options of commitments available for the cell (Smith, 2006), has aroused a growing and great interest, bearing in view the employment in the therapy of several types of degenerative diseases and in tissue bioengineering (Atala, 2008). According to The National Institutes of Health (NIH), SC can be defined as cells able to divide for indefinite time *in vitro* and to give origin to specialized cells. Melton and Cowan (2004) proposed a working definition of SC: "a clonal self-renewable entity which is multipotent and can generate several types of differentiated cells." Notwithstanding the concept variation, SC have two basic characteristic aspects: self-renewal, in order to maintain the pool of undifferentiated cells for tissue replacement, remodeling, and repair, as well as the differentiation into at least one mature cell type. These inherent properties for SC are afforded through particular asymmetric divisions, where undifferentiated cells are originated, or, alternatively, differentiation into specialized cells (Figure 2).

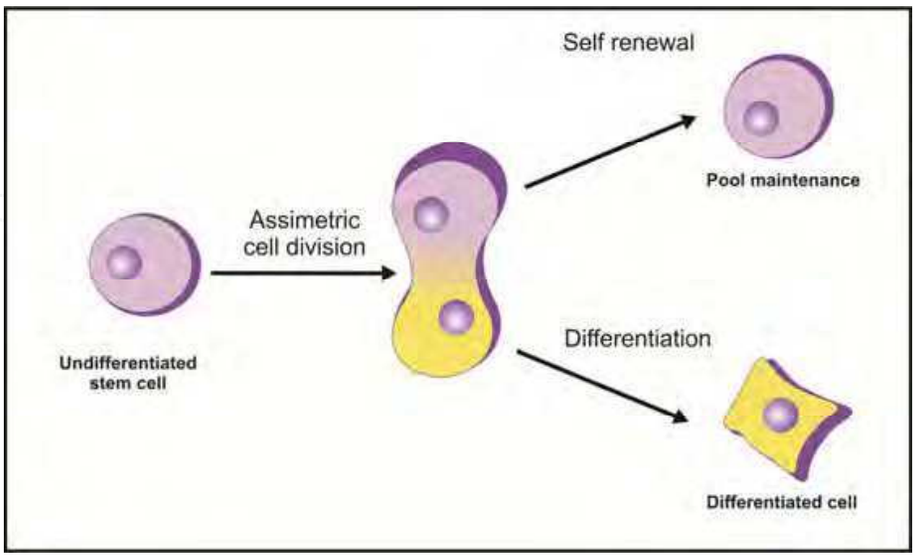


Fig. 2. Assimetric SC division. An undifferentiated SC under microenvironment stimulation start assimetric divisions producing two distinct daughter cells. One cell, undifferentiated, maintain the SC pool. In contrast, the differentiated cell acquires a new mature and specialized phenotype.

Considering their origin, SC are classified in three general types: embryonic stem cells (ESC), germinative stem cells (GSC), and adult or tissue-specific stem cells (ASC). The ESC are derived from the inner cell mass of the blastocyst, capable to generate any differentiated cellular type of the three primary germ layers (ectoderm, mesoderm and endoderm), as well as the GSC originated from the gonadal crest (Geijsen et al., 2004). On the other hand, ASC are undifferentiated cells, found in differentiated cell types in a tissue where they can renew themselves for long periods of time, and can differentiate to yield specialized cell types of the host tissue. By and large, ESC maintains the undifferentiated stage for a long period of time without losing their differentiation potential (Draper et al., 2004). Moreover, the ASC have a limited number of generations, and at each division there is loss of response to differentiation signals (Jiang et al., 2002).

The knowledge that undifferentiated cells exist in the bone marrow has been verified since the 40's decade; by the way, the blood progenitors are the first well characterized SC. Both in humans and in animal models, the literature reports consistent data with evidence for the existence of stained SC from the bone marrow in lungs after bone marrow transplant (Bittmann et al ., 2001; Kotton et al., 2001; Krause et al., 2001; Lama et al., 2007; Ribeiro_Paes et al., 2009; Schrepfer et al., 2007; Suratt et al., 2003; Yamada et al., 2004). At different experimental situations, these and others classical works have shown evidence for the migration of SC to the lung and have provided the theoretical reference which gives grounds for the idea of employing cell therapy in the regeneration of pulmonary tissue.

The experimental evidence of migration of SC from the bone marrow to the lungs was pioneering described in the work of Pereira et al. in 1995. Authors cultured murine cells expressing a collagen human gene and injected the expanded mesenchymal precursor cells into irradiated mice. The presence of transplanted cells in recipient animals for a period of up to 5 months was showed by PCR *in* situ assay. There was incorporation into the pulmonary tissue, where the cells disseminated through the mesenchymal parenchyma and could continue the replication process *in* vivo. Therefore, bone marrow cells can migrate and populate the pulmonary tissue and act as precursors of local cells.

Experimental animal models and clinical trials in regenerative tissue therapy by intravenous (IV) SC or BMMC infusion indicate a "pulmonary first-pass effect" as proposed by Fischer et al. 2009. The lungs act as a barrier, where administered cells are preferentially attracted and retained. Cell size and adhesion receptors of the stem and progenitors cells IV infused can determine this effect through pulmonary microvastulature (Fischer et al., 2009). Five minutes after labeled MSC IV infusion was verified, in animal model, a significant greater bioluminicensce signal in the lungs, in relation to several other organs, such as heart, spleen, liver and kidney. Therefore, the mean size of injected cells larger than the caliber of lung capillaries provides an efficient and fast cell trapping in lungs (Schrepfer et al., 2007).

Interesting works found on literature indicate the initial migration and chimerism in lungs after cell transplantation. Krause et al. (2001) transplanted male mice cells into females with bone marrow depleted by ionizing radiation and tracked the presence of Y chromosome in gastrointestinal tract, liver, lung and skin. It was verified co-staining of pneumocytes type II and Y chromosome in bronchi and alveoli showed by FISH assay (Y chromosome and surfactant B mRNA staining) and immunohistochemistry (anti-cytokeratin antibodies for the detection of epithelial cells). However, authors proposed that the significant damage to

the lungs, arising out of the radiation, provided high levels of incorporation in the alveolar tissue.

In the same year, Kotton *et al*. (2001) IV infused Lac-Z stained cells of transgenic mice into recipient wild animals, which underwent pulmonary lesion by intratracheal instillation of bleomycin. There was typical staining of lac-Z expression (Incubation in medium containing X-gal), with statistically significant increase in the animals sustaining lesion with bleomycin. The grafted cells showed evidence for morphologic and molecular phenotype of pneumocytes type I. So, cultured or fresh aspirates of bone marrow cells can express pulmonary markers. Thereby, these cells could represent a potential therapy in extensive alveolar degeneration.

An elegant experimental model of suppression of bone marrow and later lesion with bleomycin was elaborated by Rojas *et al*., (2005). The authors obtained full survival index and protective effect in mice which underwent MSC transplantation. The immunohistochemistry analysis of the pulmonary tissue of the animals with suppression of bone marrow disclosed, when compared to group without suppression, that the transplanted cells (GFP^+) were present in the organ and in a large number, even 14 days after the administration of bleomycin.

As in the animal models, cell migration and chimerism were also observed in human patients who received, for different reasons, bone marrow allogeneic transplant, as in the models of animal studies. Suratt et al. (2003), in a pioneer work, showed pulmonary chimerism upon the incorporation of cells with Y chromosome in women receiving HSC allogeneic transplant from male donors. Another study, 7 patients who underwent pulmonary transplant between (donor and recipient) individuals of opposite sexes showed, by means of different assays of histochemistry staining and molecular analysis (RT-PCR), the presence of mesenchymal stem cells (MSC) in lungs of recipients with cytogenetic expression of the sex of donor. In a period of up to 11 and a half years after the transplant was verified donor cells in the recipient patients (Lama et al., 2007).

Nevertheless, the SC migration to the lungs can be overestimated and, therefore, they are allegedly present at a much lower rate with a questionable clinical meaning. So, the results obtained and reported have been evaluated more carefully by some authors, who challenge the accuracy of the employed detection techniques. For example, after transplanting MSC GFP^+ in mice which had previously received an LPS intraperitoneal injection, Xu et al. (2008) did not find, in the immunohistochemistry analysis of the pulmonary tissue conducted 14 days after the transplant, circumstantial evidence for a significant presence of cells with positive sign of GFP. However, although the authors did not find evidence for an actual integration of MSC to the pulmonary tissue and the presence of cells with the pulmonary phenotype, there was demonstration that the SC transplant afforded a decrease in the lungs inflammation and edema induced by the LPS. There are, accordingly these results, the indication that the action mechanism of cells would be mediated by paracrine factors that stimulate tissue regeneration rather than cell engraftment into lungs (Huh et al., 2011).

More recently, Katsha and collaborators (2011) reported a significant improvement resulting from the use of MSC from the murine bone marrow for the repair and regeneration of the pulmonary parenchyma, in an elastase-induced experimental model of emphysema. The

authors suggest in the same study the importance of paracrine factors derived from MSC as the regenerative mechanism operating in the pulmonary parenchyma.

Notwithstanding the diversity of used methodologies, in human patients and animal models, has been proposed that ASC from several tissue sources can migrate and populate injured areas in the lung. It is propounded that the regenerative property of SC involves cellular migration to the site of tissue damage and probable promotion of functional and structural organ repair. This mobilization process (homing) is related to liberation of chemotactic mediators by injured organ (Chen et al., 2011).

4. Use of stem cells in chronic obstructive pulmonary disease: Experimental basis

In lungs, affected by chronic inflammation, there is intense production of molecules that signal and can recruit SC (endogenous and transplanted) capable of tissue reconstruction (Rojas *et al*., 2005). In this context, the rationale for cell therapy in COPD comprehends the ability of SC homing toward injured pulmonary tissue, allowing repair of the lung parenchyma and probable clinical efficacy.

Two groups of Japanese researchers reported in 2004 the first consistent results of pulmonary regeneration in an experimental mouse model (C57BL/6 strain) of lesion and later infusion of SC from bone marrow. The mice were submitted to lipopolysaccharide (LPS) intranasal treatment after irradiation. An experimental group received bone marrow-derived progenitor cells transplant from transgenic mice donors expressing GFP. There was protection of the lungs against the lesion of the emphysematous type in the animals transplanted with BMMC. It was also detection of stained cells (endothelial and epithelial) only in the recipient animals in which the induced pulmonary lesion (Yamada et al, 2004).

In a model of elastase-induced pulmonary emphysema, Ishizawa et al. (2004) reported that the treatment with retinoic acid or granulocyte colony-stimulating factor (G-CSF) led to the alveolar regeneration and the treatment, concurrently with both factors, resulted in an additive effect. There was BMC mobilization to injured alveoli by retinoic acid and G-CSF besides regeneration process.

Several authors around the world reported experimental and interesting results with cell therapy in animal models of COPD. Some of these works are shortly described in the Table 2.

At our laboratory, several research projects have been directed for the study of morphologic and functional pulmonary recovery after the treatment with ASC in mice with experimentally-induced COPD. Our model basically consists of the induction of emphysema by intranasal instillation of papain or elastase and later treatment with BMMC or MSC pool originated from the bone marrow (Figure 3).

Female mice of the C57BL/6 act as recipients. Transgenic male mice (with C57BL/6 background), which express the green fluorescent protein (GFP) are used as donors of BMMC and MSC for the purpose of cellular tracking and validation of the post transplant chimerism.

The achieved results both in quality and in quantity have shown the regeneration of the pulmonary tissue in animals with emphysema and treated with BMMC pool or MSC (Figure 4).

Animal	COPD induction	Stem cell type / source	Therapeutic effects	Probable action mechanism	Reference
Rabbit	Elastase	BMMC	Improves pulmonary function, decreases airspace enlargement	-	Yuhgetsu et al., 2006
Rat	Papain Co-60	MSC / bone marrow	Improves alveolar parameters (mean alveoli area and linear interval)	Inhibition of the apoptosis of alveolar cell wall	Liu et al., 2008
Rat	Cigarette smoke for 6 months	BMC MSC Conditioned medium of MSC/bone marrow	Attenuates cigarette induced emphysema, restores the increased Lm, increase pulmonary microvastulature,	Paracrine effects	Huh et al., 2011
Sheep	Elastase	MSC/ lung	Increases tissue mass, lung perfusion, cellularity and ECM content.	Paracrine effects	Ingenito et al., 2011
Mice	Cigarette smoke for 6 months	Human or murine MSC / cell-free contidioned medium adipose tissue	Decreases inflammation and airspace enlargement, prevents cigarette-induced weight loss, restores cigarette-induced BM dysfunction	Paracrine effects	Schweitzer et al., 2011
Mice	Elastase	MSC / bone marrow	Ameliorates alveolar structure, restores increased Lm and destructive index	Paracrine factors	Katsha et al., 2011

Table 2. Experimental animal models of cell therapy for COPD.

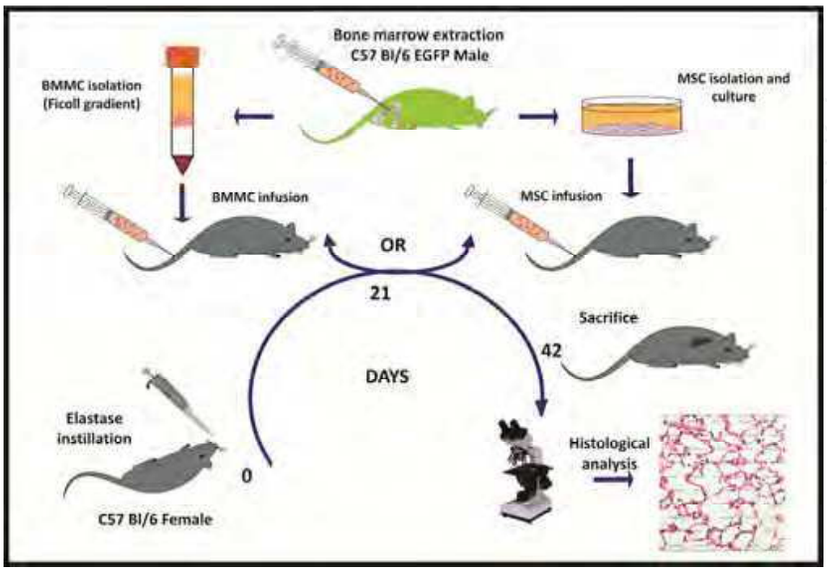


Fig. 3. Experimental design of protease-induced emphysema and ASC treatment.

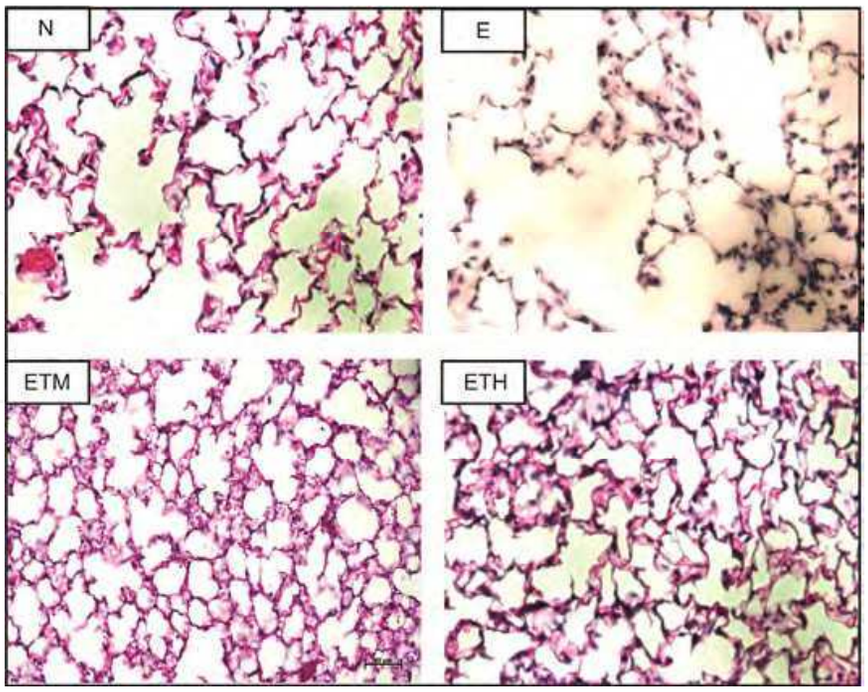


Fig. 4. Pulmonary tissue from female mice C57Bl/6 in representative histological cuts. Hematoxylin and eosin staining. Groups: N - no treatment, E - instillation of elastase only, ETM - instillation of elastase and MSC transplant and ETH - instillation of elastase and infusion of BMMC. Original magnification 200 x.

The regeneration of the pulmonary tissue, expressed in a quantitative way as the measurement of the mean linear intercept (Lm), had a significant statistical difference between animals treated with ASC and controls.

In accordance with the data showed in Figure 5, there is a statistically significant difference between E group, treated with elastase only, and N group, with no treatment, which shows evidence for the efficacy of elastase via intranasal administration in the induction of pulmonary emphysema. Between groups treated only with elastase (E) and treated with elastase and growth medium (EME) there is no statistically significant difference, which suggests the inability of the infusion vehicle in the regeneration of the pulmonary parenchyma. Furthermore, the experimental groups, treated with HSC or MSC have not shown statistically significant difference in comparison with N group, with no treatment. It is worth noting that groups treated with HSC and MSC have not turned out significant difference, which shows the therapeutic equivalence between the two stem strains originated from the bone marrow.

The comparison between the achieved values of Lm equivalent to the groups undergoing the elastase instillation (E) and treated with DMEM (EME), as well as the groups with experimentally-induced emphysema and treated with HSC or MSC has shown statistically significant difference, according to Figure 5 ($p>0.05$).

Accordingly, it is possible that MSC and BMMC hold a potential role to deliver the required cellular strain diversity during the tissue regeneration process, possibly by paracrine mechanisms (Katsha et al, 2011) and to check, in a significant and effective way, the repair of the lesion in the pulmonary tissue.

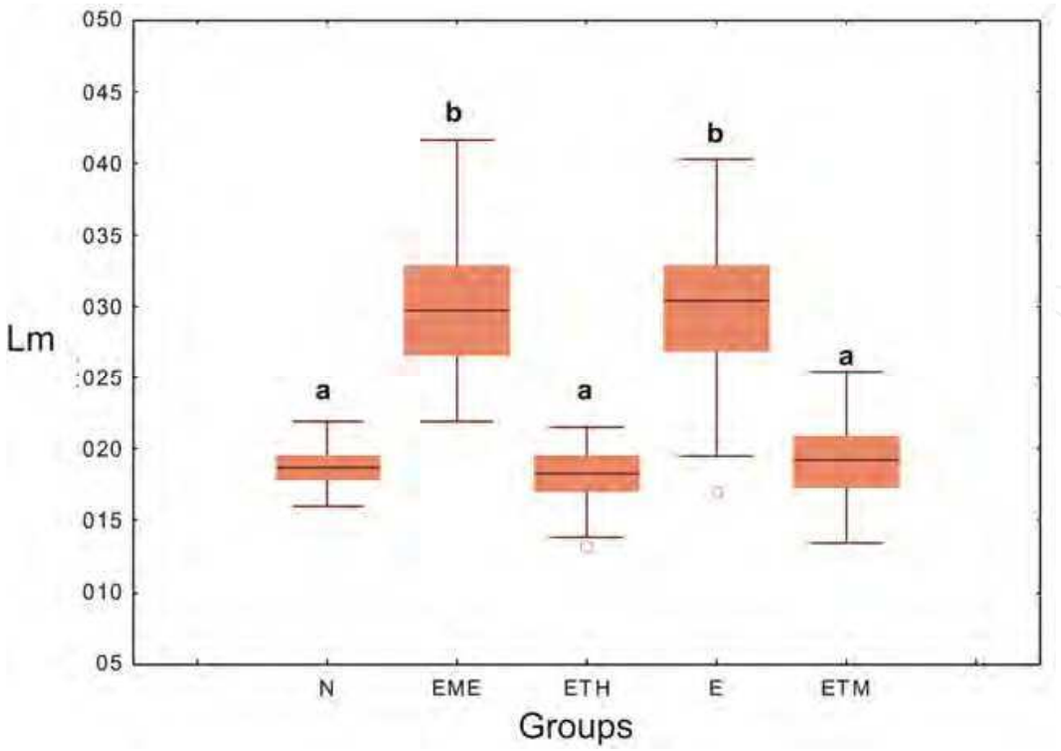


Fig. 5. Mean linear intercept (Lm) of the animals in the control and treated groups.
N Group - no treatment, EME - instillation of elastase and infusion of DMEM growth medium, ETH - instillation of elastase and infusion of BMMC, E - instillation of elastase only, ETM - instillation of elastase and MSC transplant. Medians followed by the same letter indicate no significant difference (p>0,05).

As it can apprehended from the literature, there is a consistent set of results generated by several laboratories, including those achieved by our research group, which supplied the experimental basis and afforded the cell therapy application by our group in COPD patients.

5. Clinical application: Cell therapy as a new therapeutic approach for COPD

Due to the high prevalence and significant economic and social impact caused by COPD, there are, as already presented, several researches in cell therapy, described in animal models, which sustain the use of ASC in human patients with COPD.

The results arising out of the basic research in animal models of COPD cell therapy, at our laboratory, have shown regeneration of the pulmonary parenchyma both in the qualitative and in the quantitative forms, as demonstrated by the histological analyses and by the measurement of the Lm. These results were the grounds for the preparation of a research project submitted to the National Committee of Ethics in Research (CONEP-Brazil) in April 2008. The clinical protocol was approved in April 2009 (registration nº 14764, CONEP 233/2009) and, on May 11th, 2009, the first patient, with CPOD in advanced stage, was submitted to BMMC pool infusion (Ribeiro-Paes et al., 2011).

This first work corresponds to a phase 1 clinical screening for the evaluation of safety concerning SC infusion in COPD patients and it was registered with Clinical Trials – NIH – USA (NTC01110252). The experimental outlining consists, basically, of the autologous

transplant of Bone Marrow Mononuclear Cells (BMMC) pool in patients with COPD in advanced stage, higher than 3 according to the Modified Medical Research Council (MRC) Dyspnea Scale Score (Curley, 1997; Mahler & Wells, 1988). The study design is shown in Figure 6.

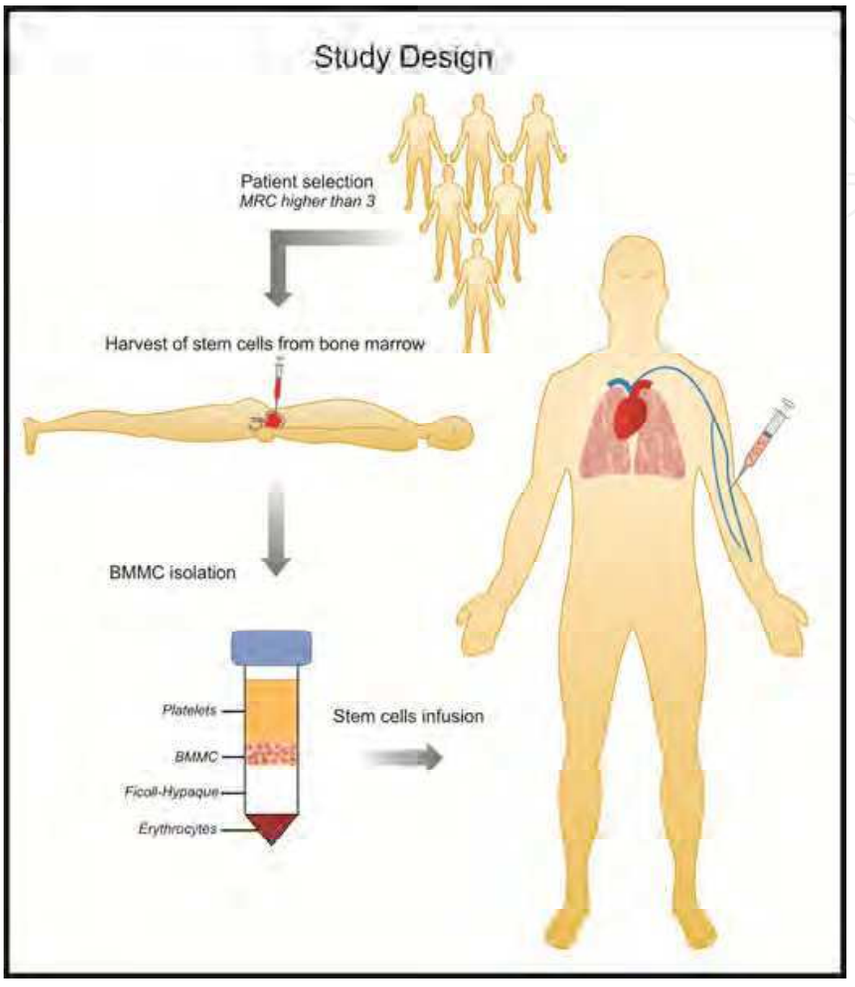


Fig. 6. Clinical protocol adopted for cell therapy in patients with advanced pulmonary emphysema (Ribeiro-Paes et al., 2011).

In the pre-procedure period, the selected patients were submitted to a full pulmonary and cardiac evaluation. Routine laboratory tests were also performed and the Dyspnea Scale Score test, modified according to the British MRC, was also conducted.The selection criteria is presented below.

Inclusion criteria: 1) age between 40 and 76 years; 2) severe obstructive pulmonary disease; 3) ineffective clinical treatment; 4) limited life expectancy; 4) limitation in daily physical activities; 5) possibility of pulmonary rehabilitation physiotherapy; 6) acceptable nutritional condition; 7) acceptable cardiac function; 8) no tobacco use for at least six months; 9) satisfactory psychosocial and emotional profile and family support and 10) Dyspnea Scale Score greater than 3.

Exclusion criteria: 1) active pulmonary or extra-pulmonary infection; 2) serious coronaropathy and/or ventricular dysfunction; 3) significant renal illness and/or hepatitis;

4) detected immunosuppressive illnesses, including HIV; 5) hepatitis B or C; 6) smoking habit; 7) carrier of known neoplasies; 8) pregnancy; 9) noncompliance with established medical protocol; 10) psychosocial problems, including drug or alcohol abuse; 11) lack of family support. After the selection, the participants received written and verbal information explaining the study and written consent was obtained from all participants before the beginning of the procedure.

After a thorough clinical evaluation, bone marrow of the voluntary patients was collected, processed and the BMMC pool achieved after isolation in Ficoll density gradient. The infusion of the achieved mononuclear fraction was made by peripheral IV (brachial medial) way and the clinical evolution of patients after the transplant has been monitored until the present date by the conduction of pulmonary function tests.

The use of BMMC pool for cell therapy in COPD patients has shown to be quite safe. No intercurrent disease occurred that could put the research's voluntary subjects in clinically serious situations or long lasting discomfort.

All the voluntary subjects of the research had some kind of clinical improvement. The spirometry tests showed a very slight improvement, as shown in Figure 7. The VEF 1 showed an improvement in all patients after thirty days.

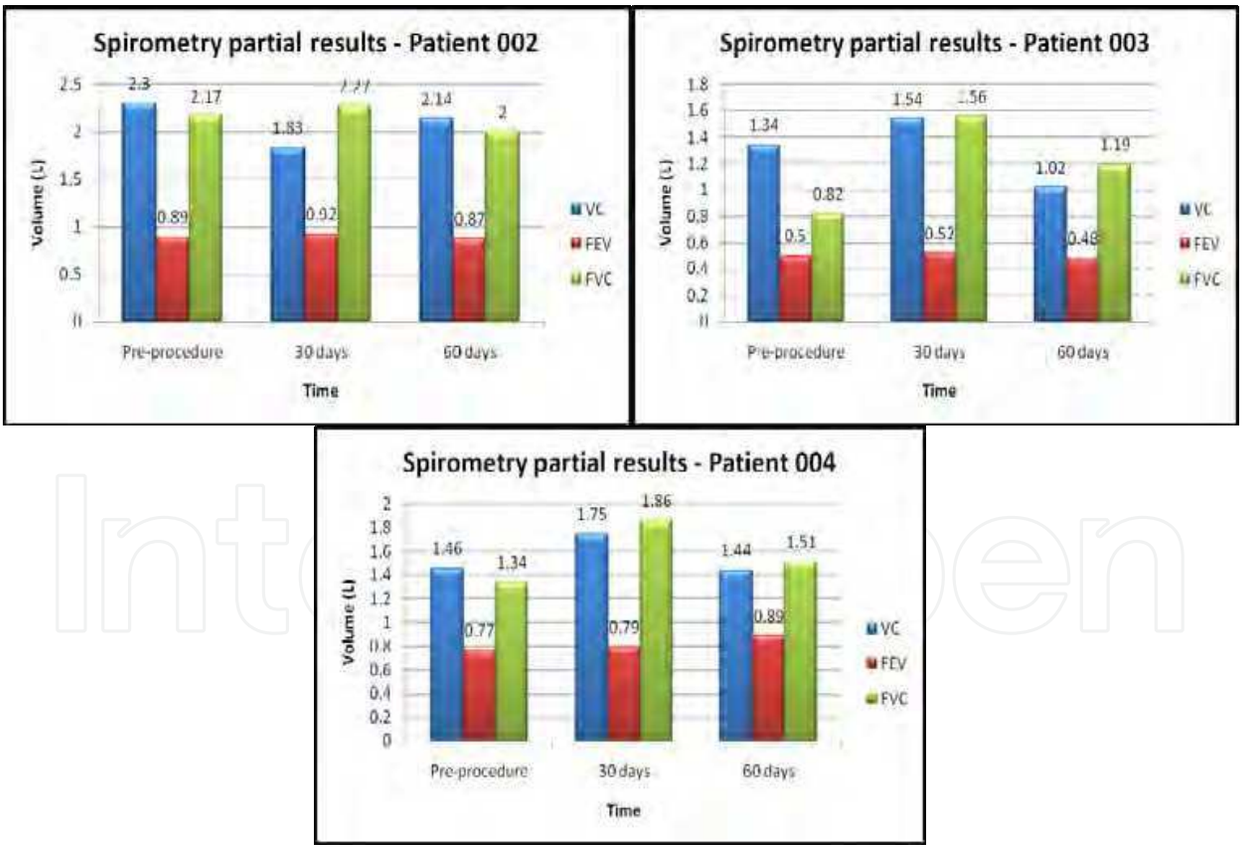


Fig. 7. Spirometry absolute values from 3 research patients included in clinical protocol and submitted to autologous BMMC transplantation.

Likewise, the increase in the CVF and CV parameters occurred in all patients after 30 days had lapsed from the procedure (Figure 7). However, after this period, there was a decrease

in CVF; in spite of this fact, an important aspect is that the functional parameters remained always higher than the ones found before the procedure.

An interesting information turned out in the long term results, approximately 2 years of clinical monitoring. The spirometry parameters along the post transplantation period, by and large, maintain a certain regularity and similarity to those found before the procedure. One of the research subject disclosed a significant increase in the forced vital capacity, after 1 year and 3 months of treatment (Figure 8); The analysis of this parameter suggests a proximity to normality and reduction of the severity of the disease.

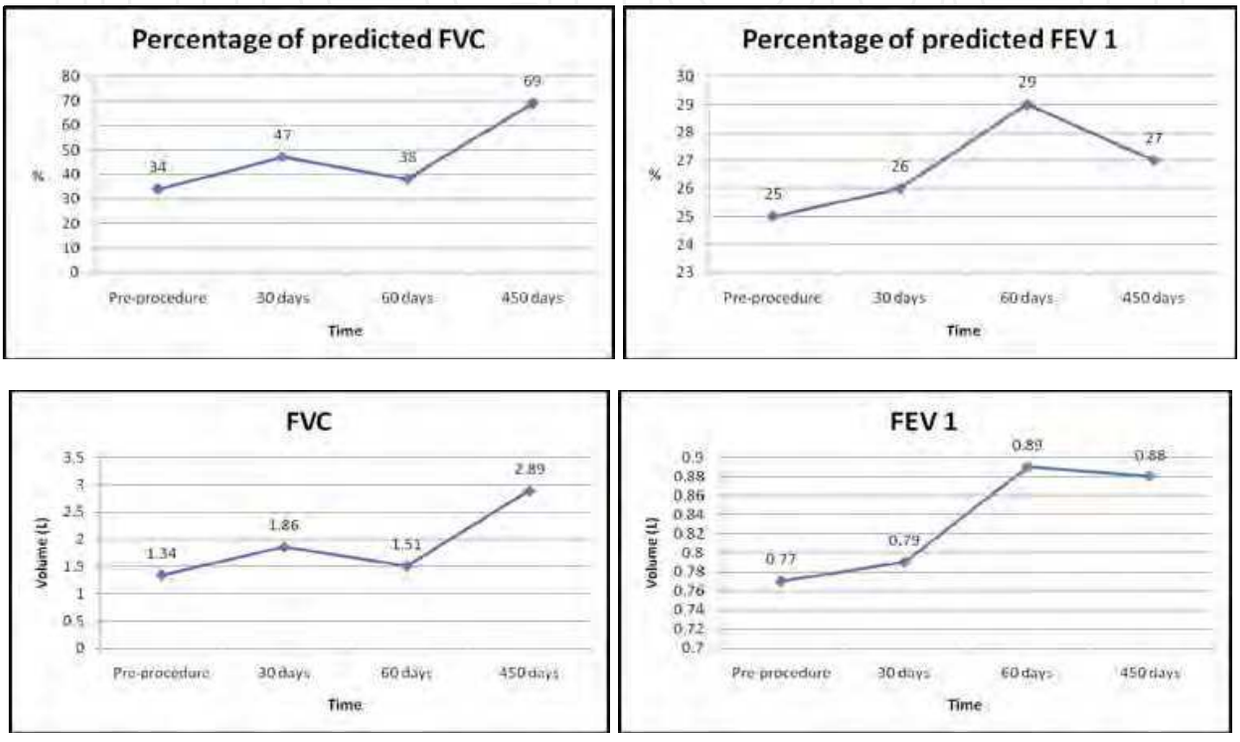


Fig. 8. Percentage of predicted and absolute values from a patient spirometry until 1 year and 3 months after BMMC autologous transplant.

The results from this clinical protocol show the procedure should be conducted at an earlier stage, that is, at a less advanced stage of the pathology. As mentioned, the laboratorial analysis, confirmed by clinical response, has reported a significant improvement in all patients, chiefly in the first 30 days after the procedure was carried out. After this period, laboratory tests displayed a tendency to decrease; however they did not drop to the base values obtained before the BMMC therapy treatment. These results advance the possibility that cell therapy may be applied in repeated doses from time to time for the purpose of stimulating pulmonary regeneration.

Another protocol under registration with Clinical Trials (NTC00683722) corresponds to a multicenter, double-blind, placebo controlled phase II study for patients with moderate to severe COPD. The clinical protocol, sponsored by Osiris Therapeutics Inc. (Columbia, MD), concerns the employment of *ex vivo* cultured adult human SC (PROCHYMAL) in the treatment of pulmonary emphysema. The purpose comprehends the evaluation of safety and efficacy of MSC multiple infusion.

As proposed by Osiris Therapeutics "Preclinical and clinical data suggest that Prochymal's unique mechanism of action may provide a first-in-class treatment option with the ability to reverse the underlying disease". However, there is no publication to date reporting the results arising out of the screening made in 62 patients. By virtue of the lack of results from the use of PROCHYMAL cell therapy in COPD, it is not possible to check and uphold the effect of regression of chronic inflammation in lungs as a response to the MSC treatment. Therefore, no critical evaluation may be made about the results of the protocol proposed by Osiris Therapeutics.

More recently, a phase 1 clinical study sponsored by Leiden University Medical Center (Leiden, Netherlands) was registered with Clinical Trials.gov (NCT01306513). The clinical protocol consists of the autologous transplant of bone-marrow-derived MSC in patients with COPD (MRC 3) before the surgery to reduce pulmonary volume. The purpose of the work, still in progress, is the evaluation of the cell therapy safety, as well as the feasibility of cultivating MSC.

The results achieved by our group, as well as the registration of clinical protocols concerning cellular therapy by other research centers, have led to the opening of new strategies of therapeutic investigation. Thus, it is possible to establish ew perspectives in regard to the formulation of cell therapy experimental designs which will be surely incorporated into future research projects for the purpose of optimizing the clinical effect and the quality of life of COPD patients.

6. Perspectives and challenges

COPD represents a serious public health problem, which, according to the latest projections of the World Health Organization, should gradually change for the worse in the coming years, with a great impact on the economy, on a global scale.

The incorporation of new drugs having more effectiveness and longer effect unquestionably has contributed to the improvement in quality of life of the patients; however, up to now, no significant change in the natural history of the disease has been achieved. In this context, cell therapy turns out as a potentially promising treatment option, which, perhaps, may represent a change of paradigm in therapeutics and in the natural course of the disease.

The results achieved at our laboratory and by several other coworkers, at different research centers, have shown a morphological recovery of the pulmonary parenchyma in animals with experimentally-induced emphysema by the employment of proteases and/or cigarette smoke. From said results, a pioneer treatment with BMMC pool was administered for patients with emphysema in advanced stage. It is a project in an initial phase and the sample of treated patients is still small, which limit the analyses from the statistical point of view. At our research center, a new project will soon start. It will comprehend a larger sample (about 40 patients) and the employment of a new methodology, which the use of MSC.

Notwithstanding the statistical limitations, the pioneer publication of the results by our research group (Ribeiro-Paes *et al*., 2011), has afforded the preparation of some logical inferences and methodological suggestions which will be incorporated into future projects. The use of MSC obtained from adipose tissue has disclosed a highly promising future perspective. Furthermore, the feasibility of establishing a protocol with repeated SC infusions should also be taken into account, just like in chronic treatments with drugs.

There are, finally, a series of questions and possibilities that arise from this pioneering studies and results obtained in our laboratory. The sample of treated patients is still small. There is, indeed, in these beginnings of research, far more doubts than certainties. Also, extreme caution should be exercised so as not to arouse false expectations and unrealistic hopes in COPD patients. Only the first trials have been carried out. We do not know exactly how this story is going to unfold. However, the first steps of a long and challenging journey have been taken, but surely it looks potentially promising, in therapeutic terms. For our group, it means a very stimulating journey of research and work.

7. References

Atala, A. (2000). Advances in tissue and organ replacement. *Current Stem Cell Research & Therapy,* Vol.3, No.1, (January 2000), pp. 21-31, ISSN 1574-888X

Barnes, P. (2000). Chronic obstructive pulmonary disease. *The New England Journal of Medicine*, Vol.343, No.4, (July 2000), pp. 269-280, ISSN 1533-4406

Barnes, P.; Shapiro, S. & Pauwels, R. (2003). Chronic obstructive pulmonary disease: molecular and celular mechanisms. *The European Respiratory Journal*, Vol.22, No.4, (October 2003), pp. 672-688, ISSN 0903-1936

Bast, A.; Haenen, G. & Doelman, C. (1991). Oxidants and antioxidants: state of the art. *The American Journal of Medicine*, Vol.91, No.3C, (September 1991), pp. 2S-3S, ISSN: 0002- 9343

Bittmann, I.; Dose, T.; Baretton, G.; Muller, C.; Schwaiblmar Lohrs, I.; Kur, F. & Lohrs, U. (2001). Cellular chimerism of the lung after transplantation. An interphase cytogenetic study. *American Journal of Clinical Pathology*, Vol.115, No.4, (April 2001), pp. 525-533, ISSN: 0002-9173

Cendon, S.; Battlehner, C.; Lorenzi-Filho, G.; Dohlnikoff, M.; Pereira, P.; Conceição, G.; Beppu, O. & Saldiva, P. (1997). Pulmonary emphysema induced by passive smoking: an experimental study in rats. *Brazilian Journal of Medical and Biological Research*, Vol.30, No.10, (October 1997), pp. 1241-1247, ISSN 1678-4510

Chen, F-M.; Wu, L-A.; Zhang, M.; Zhang, R. & Sun, H. (2011). Homing of endogenous stem/progenitors cells for *in situ* tissue regeneration: Promisses, strategies and translational perspectives. *Biomaterials*, Vol.32, No.12, (April 2011), pp. 3189-3209, ISSN: 0142-9612

Curley, F.(1997). Dyspnea, In: Diagnosis and Treatment of Symptoms of the Respiratory Tract, Irwin, R.; Curley, F.; Grossman, R, (Ed), pp.56-115, Future Publishing, ISBN 978-0879936570, Armonk, NY

Draper, J.; Smith, K.; Gokhale, P.; Moore, H.; Maltby, E.; Johnson, J.; Meisner, L.;Zwaka, P.; Thomsom, J. &Andrews, P. (2004). Recurrent gain of chromosomes 17q and 12 in cultured human embryonic stem cells. *Nature Biotechnology*, Vol.22, No.1, (January 2004), pp.53-54, ISSN : 1087-0156

Fischer, U.; Harting, M.; Jimenez, F.; Monzon-Posadas, W.; Xue, H.; Savitz, S.; Laine, G. & Cox, C. (2009). Pulmonary passage is a major obstacle for intravenous stem cell delivery: the pulmonary first-pass effect. *Stem Cells and Development*, Vol.18, No.5, (June 2009), pp. 683-692, ISSN: 1557-8534

Fujita, M. & Nakanishi, Y. (2007). The pathogenesis of COPD: Lessons learned from in vivo animal models. *Medical Science Monitor*, Vol.13, No.2, (February 2007), pp. 19-24, ISSN: 1234-1010

Fusco, L.; Pêgo-Fernandes, P.; Xavier, A.; Pazetti, R; Rivero, D.; Capelozzi, V. & Jatene, F. (2002). Modelo experimental de enfisema pulmonar em ratos induzido por papaína. *Jornal de Pneumologia*, Vol.28, No.1, (January 2002), pp.1-7, ISSN 1806-3713

Geijsen, N.; Horoschak, M.; Kim, K.; Gribnau, J.;Eggan, K. & Daley, G. (2004). Derivation of embryonic germ cells and male gametes from embryonic stem cells. *Nature*, Vol.427, No.6970, (January 2004), pp. 148-154, ISSN: 1476-4687

GOLD – Global Initiative for Chronic Obstructive Lung Disease. (2009). Global Strategy for the Dagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease, Executive Sumary

Gross, P.; Pfitzer, E.; Tolker, M.; Babyak, M. & Kaschak, M. (1965). Experimental emphysema: its production with papain in normal and silicotic rats. *Archives of Environmental Health*, Vol.11, (July 1965), pp. 50-58, ISSN 0003-9896

Hele, D. (2002). First Siena International Conference on animal models of chronic obstructive pulmonary disease, Certosa di Pontignano.Universidade of Siena, Italy, September 30-October 2, 2001. Respiratory Research, Vol.3, No.12, (November 2001) ISSN: 1465- 9921

Huh, J.; Kim, S-Y.; Lee, J.; Lee, J.; Ta, Q.; Kim, M.; Oh, Y.; Lee, Y. & Lee, S. (2011). Bone marrow cells repair cigarette smoke-induced emphysema in rats. *American Journal of Physiology. Lung Cellular and Molecular Physiology*, (Epub ahead of print). ISSN: 1522-1504

Ingenito, E.; Tsai, L.; Murthy, S.; Tyagi, S.; Mazan, M. & Hoffman, A. (2011). Autologous lung-derived msenchymal stem cell transplantation in experimental emphysema. *Cell Transplantation*, (Epub ahead of print). ISSN: 0963-6897

Ishizawa, K.: Kubo, H.; Yamada, M.; Kobayashi, S.; Numasaki, M.; Ueda, S.; Suzuki, T. & Sasaki, H. (2004). Bone marrow-derived cells contribute to lung regeneration after elastase-induced pulmonary emphysema. *FEBS Letters*, Vol.556, No.1-3, (January 2004), pp. 249-252, ISSN: 0014-5793

Jiang, Y.; Jahagirdar, B.; Reinhardt, R.; Schwartz, R.; Keene, C.; Ortiz-Gonzalez, X.; Reyes, M.; Lenvik, T.; Lund, T.; Blackstad, M.; Du, J.; Aldrich, S.; Lisberg, A.; Low, W.; Largaespada, D. & Verfaille, C. (2002). Pluripotency of mesenchimal stem cells derived from adult marrow. *Nature,* Vol.418, No.6893, (July 2002), pp. 41-49, ISSN: 1476-4687

Katsha, A.; Ohkouchi, S.; Xin, H.; Kanehira, M.; Sun, R.; Nukiwa, T. & Saijo, Y. (2011). Paracrine Factors of Multipotent Stromal Cells Ameliorate Lung Injury in an Elastase-induced Emphysema Model.*The American Society of Gene & Cell Therapy*, Vol.19, No.1, (September 2010), pp. 196–203, ISSN:1525-0016

Kielty, C.; Raghunath, M.; Siracusa, L.; Sherratt, M.; Peters, R.; Shuttleworth, C. & Jimenez, S. (1998). The tight skin mouse: Demonstration of mutant .brillin-1 production and assembly into abnormal microfibrils. *The Journal of Cell Biology,* Vol.140, No.5, (March 1998), pp. 1159–1166, ISSN: 1540-8140

Kotton, D.; Ma, B.; Cardoso, W.; Sanderson, E.; Summer, R.; Williams, M. & Fine, A. (2001). Bone marrow-derived cells as progenitors of lung alveolar epithelium. *Development*, Vol.128, No.24, (December 2001), pp. 5181-5188, ISSN: 1098-2795

Krause, D.; Theise, N.; Collector, M.; Henegariu, O.; Hwang, S.; Gardner, R.; Neutzel, S. & Sharkis, J. (2001). Multi-organ, multi-linage enfragment by a single bone marrow-derived stem cell. *Cell*, Vol.105, No.3, (May 2001), pp. 369-377, ISSN 1097-2765

Lama, V.; Smith, L.; Badri, L.; Flint, A.; Andrei, A-C.; Murray, S.; Wang, Z.; Liao, H.; Toews, G.; Krebsbach, P.; Potere-Golde, M.; Pinsky, D.; Martinez, F. & Thannickal, V. (2007). Evidence for tissue-resident mesenchymal stem cells in human adult lung from studies of transplanted allografts. *The Journal of Clinical Investigation*, Vol. 117, No.4, (Abril 2007), pp. 989-996, ISSN: 0021-9738

Lee, J., Sandford, A., Man, P.,.(2011). Is the aging process accelerated in chronic obstructive pulmonary disease? .*Current Opinion in Pulmonary Medicine*, Vol.17, pp. 90-97, ISSN: 1531-6971

Liu, H., Zhen, G., Zhang, Z., Zhang, H., Cao, Y., Wang, T., Gu, N. & Xu, Y. (2008). Effects of bone marrow mesenchymal stem cells transplantation on the apoptosis of alveolar wall cells in papain and Co60-induced pulmonary emphysema rats. *Chinese Journal of Applied Physiology*, Vol.24, No.2, (May 2008), pp.210-214, ISSN: 1000-6834

Mahadeva, R. & Shapiro S. (2002). Chronic obstrutive pulmonary disease: Experimental animal models of pulmonary emphysema. *Thorax*, Vol.57, pp.908-914, ISSN: 1468-3296

Mahler D. & Wells C. (1988). Evaluation of clinical methods for rating dyspnea. *Chest*, Vol.93, No.3, pp.580–586, ISSN: 0012-3692

Mannino D. & Buist A. (2007). Global burden of COPD: risk factors, prevalence and future trends. *Lancet*, Vol.370, pp.765-773, ISSN: 0140-6736

March T., Green F., Hahn F. & Nikula K. (2000). Animal models of emphysema and their relevance to studies of particle-induced disease. *Inhalation Toxicology*, Vol.12(Supplement 4), pp.155–187, ISSN: 1091-7691

Martorana, P., Even, P., Gardi, C. & Lungarella, G. (1989). A 16-Month Study of Development of genetic emphysema in Tight-skin mice. *The American Review of Respiratory Disease*, Vol.139, No.1, (January 1989), pp. 226-232, ISSN: 0003-0805

Martorana, P., Cassella, A. & Lungarella, G. (1995). Animal model of human disease. Genetic deficiency in α-1 proteinase inhibitor (α-1 PI) associated with emphysema. *Journal of Comarative Pathology,* Vol.27, No.4, pp. 3–6, ISSN: 0021-9975

Melton, D. & Cowan, C. (2004). "Stemness": Definitions, criteria, and standards, In: Handbo of Stem Cells Adult and Fetal Stem Cells, Lanza, R. (Ed), pp.xxv-xxxi, Elsevier/Academic Press, ISBN 0-12-436644, New York

NIH - Stem Cells: Scientific Progress and Future Research Directions. Department of Health and Human Services. June 2001. </info/scireport/2001report>.

Nikula K., March, T., Seagrave, J., Finch, G., Barr, E., Ménache, M., Hahn, F. & Hobbs, C. (2000). A mouse model of cigarette smoke-induced emphysema. *Chest*, Vol.117, No.5, (April 2006), pp. 246S-247S, ISSN:0012-3692

Oliveira, J., Jardim, J. & Rufino, R. (2000) I Consenso Brasileiro de Doença Pulmonar Obstrutiva Crônica (DPOC) *Jornal de Pneumologia*, Vol.26 No.1, (April 2000), pp. S1-S3, ISSN 1806-3713

Pereira, R., Halford, K., O'Hara, M., Leeper, D., Sokolov, B., Pollard, M., Bagasra, O. & Prockop D. (1995). Cultured adherent cells from marrow can serve as long-lasting precursor cells for bone, cartilage, and lung in irradiated mice. *Proceedings of The National Academy of Sciences of United States of America*, Vol.92, No.11, (May 1995), pp. 4857-61, ISSN 1091-6490

Pushpakom, R., Hogg, J., Woolcock, A., Angus, A., Macklem, P. & Thurlbeek, W. (1970). Experimental papain-induced emphysema in dogs. *The American Review of Respiratory Disease*, Vol.102, No.5, (Novembro 1970), pp. 778-779, ISSN: 0003-0805

Ribeiro-Paes, J., Bilaqui, A., Greco, O., Ruiz, M., Alves-de-Moraes, L., Faria, C. & Stessuk, T.(2009). Terapia celular em doenças pulmonares: Existem perspectivas? *Revista Brasileira de Hematologia e Hemoterapia*, Vol.31, (August 2009), pp. 140-148, ISSN: 1516-8484

Ribeiro-Paes, J., Bilaqui, A., Greco, O., Ruiz, Marcelino, M., Stessuk, T., Faria, C. & Lago, M. (2011). Unicentric study of cell therapy in chronic obstructive pulmonary disease/pulmonary Emphysema. *International Journal of COPD*, Vol.6, (January 2011), pp. 63-71, ISSN: 1178-2005

Rojas, M., Xu, J., Woods, C., Mora, A., Spears, W., Roman, J. & Brigham, K. (2005). Bone marrow-derived mesenchymal stem cells in repair of the injured lung. *American Journal of Respiratory Cell and Mollecular Biology*, Vol.33, No.2, (August 2005), pp. 145-52, ISSN:1044-1549

Rufino, R. & Lapa e Silva, J. (2006). Bases celulares e bioquímicas da doença pulmonar obstrutiva crônica. *Jornal Brasileiro de Pneumologia*, Vol.32, No.3, (May 2006), pp. 241-248, ISSN 1806-3713

Schrepfer, S., Deuse, T., Reichenspurner, H., Fischbein, M., Robbins, R. & Pelletier, M. (2007). Stem Cell Transplantation: The Lung Barrier. *Transplantation Proceedings*, Vol.39, No.2, (March 2007), pp. 573-576, ISSN: 0041-1345

Shapiro, S. (2000). Animal Models for COPD. *Chest*, Vol.117, pp. 223–227, ISSN: 0012-3692

Schweitzer, K., Johnstone, B., Garrison, J., Rush, N., Cooper, S., Traktuev, D., Feng, D., Adamowicz, J., Van Demark, M., Fisher, A., Kamocki, K., Brown, M., Presson, R., Broxmeyer, H., March, K. & Petrache, I. (2011). Adipose stem cell treatment in mice attenuates lung and systemic injury induced by cigarette smoking. *American Journal of Respiratory and Critical Care Medicine*, Vol.183, No.2, (January 2011), pp. 215-225, ISSN: 0003-0805

Smith, A. (2006). Glossary A glossary for stem-cell biology. *Nature*, Vol. 441, (June 2006), pp.1060, ISSN: 1476-4687

Suratt, B., Cool, C., Serls, A., Chen, L., Varella-Garcia, M., Shapall, E., Crown K. & Worthen G. (2003). Human pulmonary chimerism after hematopoetic stem cell transplantation. *American Journal of Respiratory and Critical Care Medicine*, Vol.168, No.3, (August 2003) pp. 318-322, ISSN: 0003-0805

Till, J. & Mccullough, E. (1961). A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiation Research*, Vol.14, (February 1961), pp. 213-222, INSS : 0033-7587

Xu, J., Qu, J., Cao, L., Sai, Y., Chen, C., He, L. & Yu, L. (2008). Mesenchymal stem cell-based angiopoietin-1 gene therapy for acute lung injury induced by lipopolysaccharide in mice. *Journal of Pathology*, Vol. 214, No.4, (March 2008), pp. 472-81, ISSN: 1096-9896

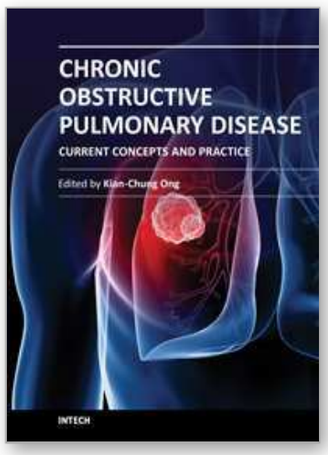
WHO - WORLD HEALTH ORGANIZATION. (2008). Global alliance against chronic respiratory diseases (GARD). General Meeting Report, ISBN 978 92 4 1597 13 5, Istanbul, Turkey, May, 2008

Yamada, M., Kubo, H., Kobayashi, S., Ishizawa, K., Numasaki, M., Ueda, S., Suzuki, T. & Sasaki, H. (2004). Bone marrow-derived progenitors cells are important for lung

repair after lipopolisaccharide-induced lung injury. *The Journal of Immunology*, Vol.172, No.2, (January 2004), pp. 1266-1272, ISSN: 1550-6606

Yuhgetsu, H., Ohno, Y., Funaguchi, N., Asai, T., Sawada, M., Takemura, G., Minatoguchi, S., Fujiwara, H., & Fujiwara, T. (2006). Beneficial effects of autologous bone marrow mononuclear cell transplantation against elastase-induced emphysema in rabbits. *Experimental Lung Research*, Vol.32, No.9, (October 2006), pp.413-426, ISSN: 1521-0499

Zheng, H., Liu, Y., Huang, T., Fang, Z., Li, G. & He, S. (2009). Development and characterization of a rat model of chronic obstructive pulmonary disease (COPD) induced by sidestream cigarette smoke. Shantou: 2009. *Toxicology Letters,* Vol. 189, (June 2009), pp.225–234, ISSN: 0378-4274



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A decade or so ago, many clinicians were described as having an unnecessarily 'nihilistic' view of COPD. This has certainly changed over the years... This open access book on COPD provides a platform for scientists and clinicians from around the world to present their knowledge of the disease and up-to-date scientific findings, and avails the reader to a multitude of topics: from recent discoveries in the basic sciences to state-of-the-art interventions on COPD. Management of patients with COPD challenges the whole gamut of Respiratory Medicine - necessarily pushing frontiers in pulmonary function (and exercise) testing, radiologic imaging, pharmaceuticals, chest physiotherapy, intensive care with respiratory therapy, bronchology and thoracic surgery. In addition, multi-disciplinary inputs from other specialty fields such as cardiology, neuro-psychiatry, geriatric medicine and palliative care are often necessary for the comprehensive management of COPD. The recent progress and a multi-disciplinary approach in dealing with COPD certainly bode well for the future. Nonetheless, the final goal and ultimate outcome is in improving the health status and survival of patients with COPD.

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