http://dx.doi.org/10.1590/1678-4162-10362

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***Arq. Bras. Med. Vet. Zootec.*, v.71, n.3, p.917-928, 2019**

Bone marrow mononuclear cells *versus* mesenchymal stem cells from adipose tissue on bone healing in an Old World primate: can this be extrapolated to humans?

[*Células mononucleares de medula óssea versus células-tronco mesenquimais de tecido adiposo em cicatrização de lesão óssea em primata: pode ser extrapolado para humanos?*]

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ABSTRACT

In veterinary medicine, the cell therapy is still unexplored and there are many unanswered questions that researchers tend to extrapolate to humans in an attempt to treat certain injuries. Investigating this subject in nonhuman primates turns out to be an unparalleled opportunity to better understand the dynamics of stem cells against some diseases. Thus, we aimed to compare the efficiency of bone marrow mononuclear cells (BMMCs) and mesenchymal stem cells (MSCs) from adipose tissue of *Chlorocebus aethiops* in induced bone injury. Ten animals were used, male adults subjected, to bone injury the iliac crests. The MSCs were isolated by and cultured. In an autologous manner, the BMMCs were infused in the right iliac crest, and MSCs from adipose tissue in the left iliac crest. After 4.8 months, the right iliac crests fully reconstructed, while left iliac crest continued to have obvious bone defects for up to 5.8 months after cell infusion. The best option for treatment of injuries with bone tissue loss in old world primates is to use autologous MSCs from adipose tissue, suggesting we can extrapolate the results to humans, since there is phylogenetic proximity between species.

Keywords: cell therapy, *Chlorocebus aethiops*, bone injury, mesenchymal stem cells from adipose tissue

RESUMO

Na medicina veterinária, a terapia celular ainda é inexplorada e há muitas perguntas não respondidas, o que leva os pesquisadores a uma tendência a estender a terapia para os seres humanos, na tentativa de tratar certas lesões. Investigar esse assunto em primatas não humanos revela-se uma oportunidade sem precedentes para compreender melhor a dinâmica das células-tronco contra algumas doenças. Assim, objetivou-se comparar a eficiência das células mononucleares de medula óssea (BMMCs) e das células-tronco mesenquimais (MSCs) do tecido adiposo de Chlorocebus aetiops na lesão óssea induzida. Foram utilizados 10 animais, adultos do sexo masculino, submetidos à lesão óssea nas cristas ilíacas. As MSCs foram isoladas e cultivadas; de forma autóloga, as BMMCs foram infundidas na crista ilíaca direita e as MSCs de tecido adiposo na crista ilíaca esquerda. Após 4,8 meses, a crista ilíaca direita foi totalmente reconstruída, enquanto a crista ilíaca esquerda continuou apresentando defeito ósseo evidente por até 5,8 meses após a infusão. A melhor opção para o tratamento de lesões com perda de tecido ósseo em primatas do Velho Mundo é a utilização de MSCs autólogas de tecido adiposo, sugerindo que se podem estender os resultados para seres humanos, uma vez que há proximidade filogenética entre as espécies.

Palavras-chave: terapia celular, Chlorocebus aethiops, lesão óssea, células-tronco mesenquimais de tecido adiposo

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Recebido em 24 de outubro de 2017
Aceito em 26 de março de 2018
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INTRODUCTION

Several stem cell sources have been used in studies involving cell therapy; however, bone marrow was the first to be used in therapeutic applications, due to its high daily production of cells in the body (Körbling *et al*., 2003). Among the various cell types that comprise bone marrow, there are hematopoietic stem cells, and mesenchymal stem cells (Abdelhay *et al.,* 2009; Wislet-Gendebien *et al.,* 2012).

For a stem cell to be considered of mesenchymal origin, it must meet three minimum prerequisites determined by the International Society for Cellular Therapy, namely the following: adherence to the plastic surface of the tissue culture flask, which must be kept under suitable conditions to have the ability to differentiate into osseous, cartilaginous and adipose tissues (Dominici *et al.,* 2006); and positively express the surface markers CD105, CD73 and CD90 (Monteiro *et al.,* 2009), and negatively express CD45, CD34, CD14, CD11b, CD79, or CD19 and HLA-DR (Dominici *et al.,* 2006).

Bone marrow can be collected through a puncture with a biopsy needle and previously heparinized syringe, in different locations, such as the iliac crest (Branco *et al*., 2012), the most common harvesting location; humeral tuberosity, femoral trochanteric fossa (Olsson *et al*., 2009) and sternum (Alves *et al*., 2009). In addition, subcutaneous adipose tissue can be collected from the gluteal and abdominal region through liposuction or a biopsy, which is a minimally invasive procedure compared to harvesting bone marrow (Vieira *et al*., 2010).

Several studies involving cell therapy in osteoinduction processes have been conducted (Cuenca-López *et al.,*2014; Dave *et al.,* 2014; Santiago-Torres *et al.,* 2015), but, experimental models have been restricted to rats, mice, sheep, dogs, and human cell culture; nonhuman primates have rarely been used in bone healing studies and have not focused on stem cells (Ripamonti *et al.,* 2008; Emerton *et al.,* 2011).

Due to their phylogenetic similarity to human species, nonhuman primates (especially *Chlorocebus aethiops*) are among the most appropriate animal models for clinical trials involving stem cells (Wolf *et al.,* 2004a*;* Wolf *et al.,* 2004b), which makes them the main experimental model for common diseases in humans (Sasaki *et al.,* 2011, Gonçalves *et al.,* 2014). However, there is a shortage in the literature regarding the isolation, cultivation and application of MSCs in regenerative medicine for *C. aethiops.*

Given the above and the apparent regenerative capacity of these cells, this study evaluated which cell group (mononuclear bone marrow cells or mesenchymal stem cell of adipose tissue) is most effective at regenerating bone in *Chlorocebus aethiops* that had an injured iliac crest, with the goal of recommending the cell group with the shortest healing time of induced bone loss.

MATERIALS AND METHODS

The procedures were performed according to the requirements of animal welfare and were approved by the Ethics Committee on the use of animals at the Instituto Evandro Chagas in Ananindeua, Pará, Brazil (protocol number 018/2009 and 0010/2011).

Ten *Chlorocebus aethiops* were used, which were all adult males, with an average weight of 4 kg. The animals belong to the National Primate Center (CENP-IEC), in Ananindeua, Pará, Brazil, and were equally divided into two groups (treated animals and control animals). The entire breeding stock of *Chlorocebus aethiops* is submitted annually to a health check up, and to date no viruses that affect these animals have been identified in the colony that could compromise the validity of this research.

After a 12-hour water and solid food fast, the animals were sedated with an intramuscular injection of tiletamine hydrochloride and zolazepam association (8mg/kg) and maintained on inhalation anesthesia with 5% isoflurane.

The harvest of bone marrow was conducted from the (shaved) iliac crest, with the aid of a disposable hypodermic needle (40x12) coupled to a 10mL syringe flushed with heparin sodium (0.2mL of heparin sodium 5000I.U./mL). An average of 13mL of bone marrow was collected from each animal in the treated animals group and transported to the laboratory.

From each animal in the treated group, after shaving and antisepsis of the skin, about 1cm^2 of adipose tissue from the left flank region was extracted from a 3cm incision. The material was packaged in sterile conical tubes containing appropriate culture medium and transported to the laboratory, to perform the isolation, cultivation and expansion of the mesenchymal stem cells.

The cell separation was established by density gradient, by using Ficoll-Paque (Branco *et al.,* 2012), and a 2µl aliquot was subjected to a viability test using trypan blue. The BMMCs were washed with 0.9% saline solution and suspended in 0.5mL of the same solution. They were then introduced into the bone injury.

The adipose tissue was washed in PBS and then triturated until a homogeneous mass formed. Enzymatic digestion was performed with collagenase type I, diluted in PBS (1mg/mL) for 2 hours at 37°C. After incubation, the collagenase was neutralized by adding an equal volume of culture medium (Dulbecco's modified Eagle's medium - DMEM) supplemented with 10% foetal bovine serum, 3.7g / l $NaHCO_3$ and 50µg/mL gentamicin.

The treated tissue was centrifuged for 5 minutes at 200g, the supernatant containing mature adipocytes was discarded and the sediment containing the stromal vascular fraction (SVF) was centrifuged again under the same conditions and suspended in DMEM. A 2µl aliquot SVF was used for the cell viability assay, by staining with trypan blue. The cells were seeded and incubated and the Non-adherent cells were removed from the culture during the first medium change (24 hours after isolation).

The morphological characteristics of the MSCs were evaluated using an inverted microscope (Nikon Eclipse 50i) with 100x magnification during passages 0-4 (P0 - P4) to verify the development of a fibroblastic shape, which is a characteristic observed in MSCs.

The growth characteristics of the MSCs derived from the adipose tissue were evaluated (day 2 to 12) in accordance with the literature (Gade *et al.*, 2013). Cells at P4 were incubated at a concentration of 10^{4}cells/well and the culture medium was changed every three days. Cell counts were performed in a Neubauer chamber every 48 hours.

Cellular senescence was measured using a β-galactosidase staining kit (β-Gal) (Cell Signaling). The MSCs at P4 were incubated in a 24-well plate with 1x10^4 cells/well density. Upon reaching 80% confluence, the cells were fixed for 15 minutes at room temperature (formaldehyde 1x /cell signaling) and washed with PBS. A β-Gal staining solution was added, and the plate was incubated overnight at 37˚C without CO_2. β -Gal evaluation was then performed with a fluorescence microscope (Nikon Eclipse TE 300) using 100x magnification.

Nuclei were stained with 10mg/ml Hoechst 33342 diluted in PBS for 15 minutes at room temperature. The senescent cells were counted from ten different microscopic fields (fluorescence microscope/Nikon) and the percentage was obtained by overlapping images (senescent cells and stained nuclei).

The cells were characterized by MSC-specific surface markers (CD105 (endoglin), CD90 (Thy-1 and CD73) and also by the absence of hematopoietic lineage markers (CD34, CD45, and CD79a), according to the manufacturer's instructions.

For adipogenic differentiation, cells at passage 4 were incubated in a 24-well plate containing DMEM at a density of 1x10^{4}cells/cm^2. After three days, this expansion medium was replaced by the adipogenic differentiation kit (STEMPRO, Gibco) according to the manufacturer's instructions. Cells were cultured for 21 days and the medium was changed every 3 days. For the negative control, the same number of cells was incubated with culture medium for the same amount of time. To confirm differentiation into adipocytes, the cells were washed twice with PBS, fixed in 4% paraformaldehyde for 1 hour at room temperature, washed again in PBS and stained with 1.25% Oil Red O for 5 minutes to visualize the intracellular accumulation of lipid-rich vacuoles.

For osteogenic differentiation, cells at P4 were incubated in 24-well plates containing DMEM at a density of 5x10^{3}cells/cm^2. After three days, this expansion medium was replaced by the

osteogenic differentiation kit (STEMPRO, Gibco) according to the manufacturer's instructions. Cells were cultured for 21 days and the medium was changed every 3 days. For the negative control, the same number of cells was incubated with culture medium for the same amount of time. To confirm differentiation into osteocytes, cells were washed twice in PBS, fixed in 4% paraformaldehyde for 20 minutes at room temperature, washed again in PBS and stained with 2% Alizarin Red S for 5 minutes to visualize calcium deposits.

For chondrogenic differentiation, cells at P4 were plated at a density of 0.25×10^7 cells/mL as a micro mass for 2 hours. Afterward, a chondrogenic differentiation kit (STEMPRO, Gibco) was added according to the manufacturer's instructions. Cells were cultured for 21 days and the medium was changed every 3 days. For the negative control, the same number of cells in a micro mass was incubated with culture medium for the same amount of time. To confirm differentiation into chondrocytes, the protocol used was the same as described for osteogenic cells. Cells were then stained with 1% Alcian Blue for 5 minutes to visualize proteoglycans.

The same protocol described above for anesthetic bone marrow and adipose tissue was adopted. The iliac crest region was injured bilaterally in the treated animals group and in the right iliac crest of the control animals. The iliac crest region was injured bilaterally in the treated animals group and in the right iliac crest of the control animals.

While the specimen was in the lateral decubitus position, it was shaved, the iliac crests region, an incision in the skin was made, and the adjacent muscle was dissected to expose the iliac crest and part of the wing of the ileum. With the aid of surgical cutters (pliers to cut bone), the bone was injured, which was approximately 2cm, wedge-shaped, juxtaposed between the sacral tuberosity and the coxal tuberosity, and included removing a portion of the wing of the ileum.

For the treated group animals, after the bone injury was established, a hemostatic lyophilized collagen sponge was placed in the tissue injury and, with a 1ml syringe, 0.5ml of bone marrow mononuclear cells suspended in 0.9% saline (right iliac crest) and 0.5ml of mesenchymal stem cells from adipose tissue suspended in 0.9% saline (left iliac crest) were injected into the sponges. The muscle and skin were then closed with catgut sutures 3.0 and polyvicril 3.0, respectively. As with the bone marrow harvest procedure and adipose tissue, all animals were given analgesics by intramuscular injection (2mg/kg tramadol hydrochloride) over a three-day period.

The animals were radiographed immediately after establishing the surgical bone lesions and were again subjected to the same evaluation at the following time intervals: 7, 20, 40, 70, 104, 144 and 174 days. The timelines increased from 20 to 30 and 40 days, as we watched the osseous callus formation and complete reconstitution of the tissue.

RESULTS

Bone marrow mononuclear cells from *Chlorocebus aethiops* had, on average, 97% cell viability, whereas the viability of the mesenchymal stem cells from adipose tissue reached 90% at P0.

Given that the mononuclear fraction of bone marrow was immediately infused into the lesion site, the following describes only tests performed with mesenchymal stem cells from adipose tissue. Several days after plating, cells presented fibroblast-like colony shapes that were proliferating rapidly. The MSCs from adipose tissue maintained this fibroblastic morphology during the entire in vitro culture process, becoming uniform with increasing passages (Figure 1A).

Cell proliferation of MSCs derived from *Chlorocebus aethiops* adipose tissue was analyzed by producing a growth curve with cell number at 48h intervals for 12 days of culture (in triplicate). The lag phase was approximately 12 - 24 hours. There was exponential growth (days 2 to 8) (log phase) (6 days), and a significant increase in the number of cells. After the period of high cell proliferation, the MSCs reached the third stage (plateau) (days 8 to 10), when the growth rate stabilized. At days 10 - 12, there was a decline in proliferation (Figure 1B).

A cellular senescence assay identified senescent cells in the culture at P4 by detecting cells with cytoplasm stained blue due to the β-Gal reaction (Figure 1C). After staining of nuclei with Hoechst (Figure 1D), ten different microscopic fields were evaluated, totaling 770 cells. Of these, 96 (12.5%) demonstrated β-Gal activity (Figure 1E).

Cells (P4) were positive for CD73, CD105 and CD90 (Figure 2A-2F) and negative for CD34, CD45 and CD79 (Figure 3A-3F).

Differentiation potential of the MSCs into mesodermal lineages was tested in vitro. The onset of morphological changes (accumulation of intracellular lipids) was observed in cells treated with adipogenic induction medium in the first week of treatment, whereas the cells treated with osteogenic and chondrogenic induction medium presented changes such as cell clustering and accumulation of calcium deposits, respectively, after 10 days. These changes intensified with the progression of the cultures, and after 21 days, cell differentiation could be seen throughout the plate.

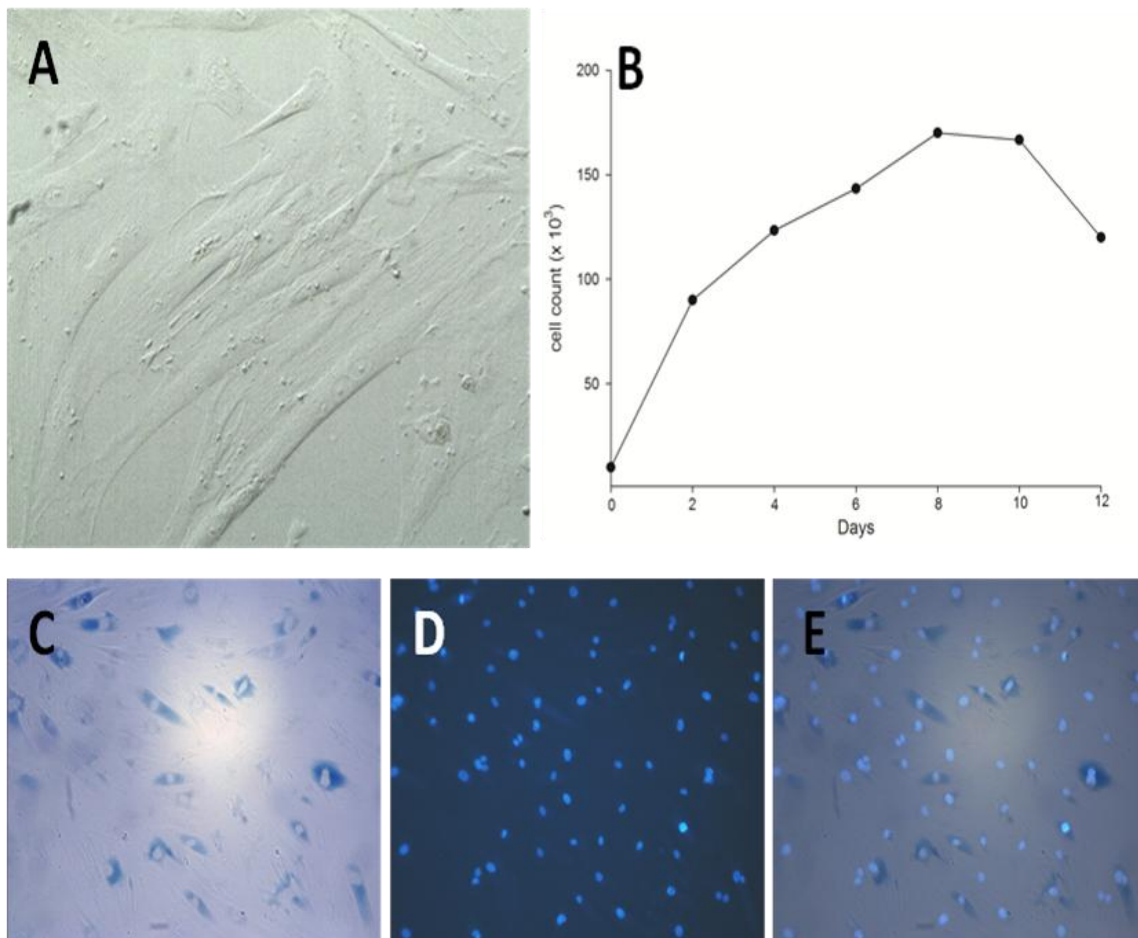

Figure 1. Photomicrograph of the MSCs derived from *Chlorocebus aethiops* adipose tissue. A: Adherent cells with fibroblastic morphology (P3) (100x magnification). B: Growth curve of the MSCs derived from *C. aethiops* adipose tissue. Cells were counted every 48 hours for 12 days. All phases of the curve were observed: lag phase (12 – 24 hours), log phase (6 days), plateau phase (days 8 - 10) and the decline in the cell growth rate (days 10 - 12). C – E: Photomicrograph of cultured MSCs derived from *C. aethiops* adipose tissue at passage 4 (P4) submitted to the cellular senescence assay. C: Cells positive for β-Gal, D: Nuclei from MSCs stained with 10µg/mL Hoechst 33342, E: Overlay of images C and D (100x magnification).

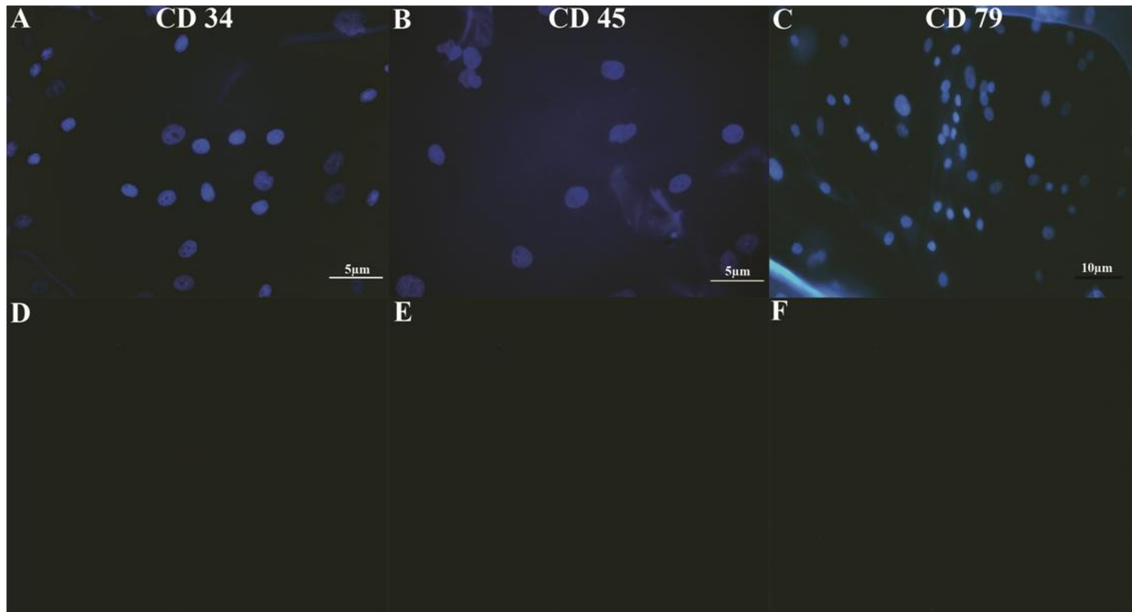

Figure 2. Immunophenotypic characterization of the MSCs derived from *C. aethiops* adipose tissue at passage 4. The nuclei of all cells were stained with 10µg/mL Hoechst 33342. (A, B, C): Immunophenotypic staining positively of MSCs with CD73, CD105 and CD90, respectively. (D, E, F): Negative control, respectively. Scale bar = 5µm.

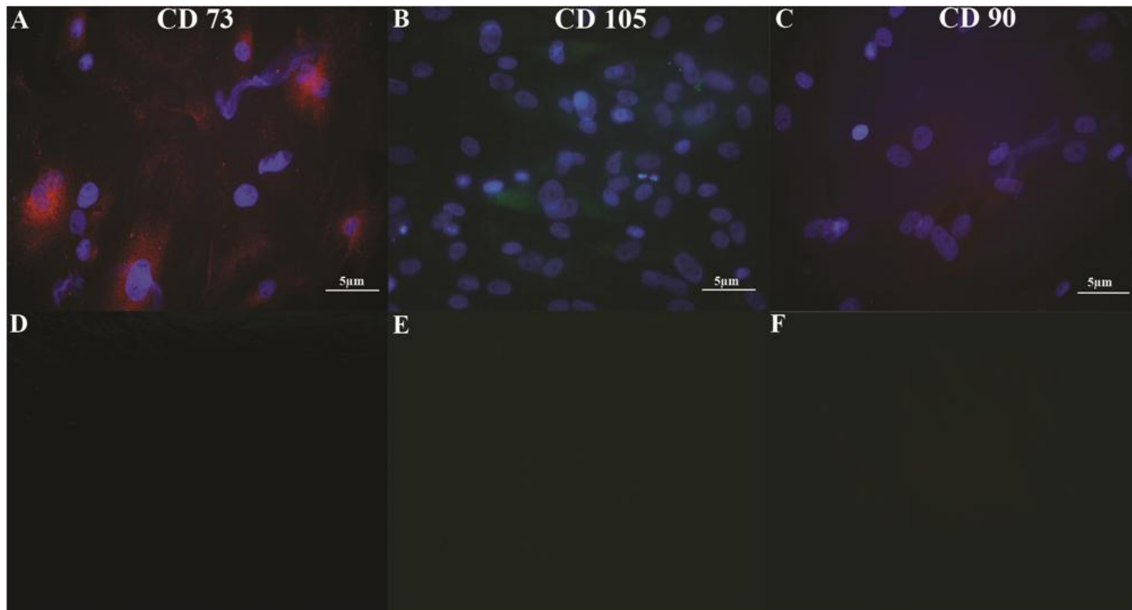

Figure 3. Immunophenotypic characterization of the MSCs derived from *C. aethiops* adipose tissue at passage 4. The nuclei of all cells were stained with 10µg/mL Hoechst 33342. (A, B, C): Immunophenotypic staining of MSCs with CD34, CD45 and CD79, respectively and negatively. (D, E, F): Negative control, respectively. Scale bar = 5µm.

After 21 days of cell differentiation induction, adipogenic cells presented intracellular lipid vacuoles, which stained red with Oil Red O (Figure 4A and 4D). Chondrogenic cells stained with Alcian Blue presented a matrix in blue, demonstrating the production of proteoglycans (Figure 4B and 4E). Osteogenic differentiation presented mineralization of the extracellular

matrix, which was demonstrated by calcium deposits stained with Alizarin Red S (Figure 4C and 4F).

A 2cm injury to the iliac crest was standardized in both groups of animals evaluated, affecting virtually all of the iliac crest and essentially leaving the coxal tuberosity and sacral tuberosity (Figure 5A-C).

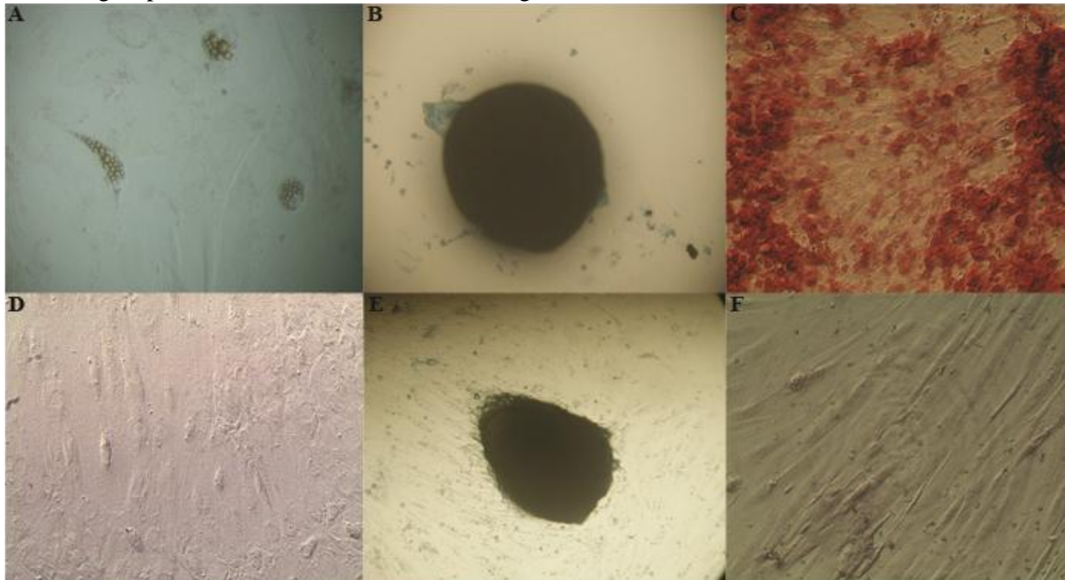

Figure 4. Photomicrograph of in vitro cell differentiation of MSCs derived from *C. aethiops* adipose tissue 21 days after induction. (A and D): Lipid vacuoles in adipogenic cells stained with Oil Red O and control group, respectively. (B and E): Extracellular matrix with proteoglycans in chondrogenic cells stained with Alcian Blue and control group, respectively. (C and F): Calcium deposits in osteogenic cells stained with Alizarin Red S and control group, respectively (100x magnification).

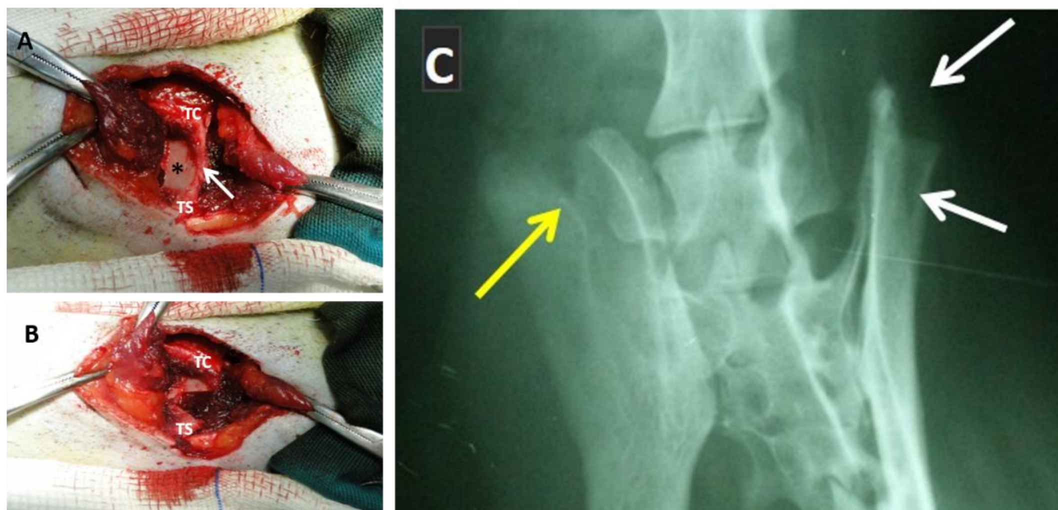

Figure 5. A and B: Photomacrograph of the establishment of surgical lesions in the iliac crest of *C. aethiops*. A: exposition of iliac crest (arrow) between the coxal tuberosity (TC) and the sacral tuberosity (TS) and part of the wing of ileum (*). B: observe the bone defect. C: postsurgical radiograph of the pelvis, highlighting the injury of the right iliac crest (yellow arrow) and left iliac crest (white arrows).

At intervals of 7, 20, 30 and 40 days, the animals were radiographed in order to check which of the cell types was capable of producing an osseous callus first and faster. After 7 and 20 days, radiographic images revealed no changes in the bone tissue of the iliac crests in all of the animals of both groups (Figure 6A and B). Forty days after surgical induction of bone loss, the left iliac crests, which were treated with mesenchymal stem cells from adipose tissue, began to show signs of tissue reconstruction, indicating an advancement in bone callus formation, while the right iliac crests, which received bone marrow mononuclear cells, still had the same post-surgical lesion (Figure 7A).

New radiographs were made after 104 days of cell therapy and the left iliac crests, treated with mesenchymal stem cells from adipose tissue, were fully repaired, while the right iliac crests, treated with bone marrow mononuclear cells, had obvious bone defects (Figure 7A-B). This result remained unchanged when new radiographic evaluations were carried out at 144 and 174 days of treatment (Figure 8A-B).

Animals of the control animals group, during the 174 days taken to evaluate the treated animals group, presented no bone repair in the iliac crest (Figure 8B).

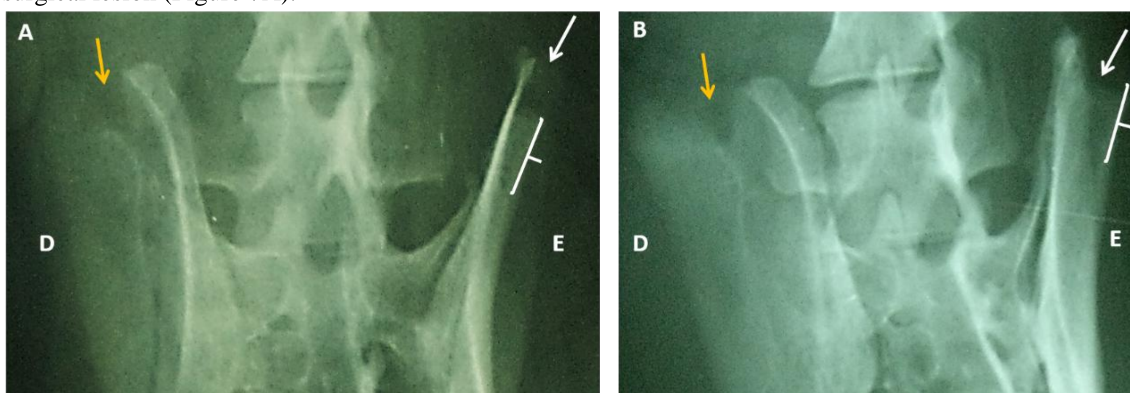

Figure 6. Coxal radiography of *C. aethiops* indicating the right iliac crest treated with BMMCs (D) and left treated with ADMCs (E). A: 7 days. B: 20 days. In A and B**,** highlighting the injury of the right iliac crest (yellow arrow) and left iliac crest (white arrow and key), changes in bone defects cannot be seen, regardless of the cell type used.

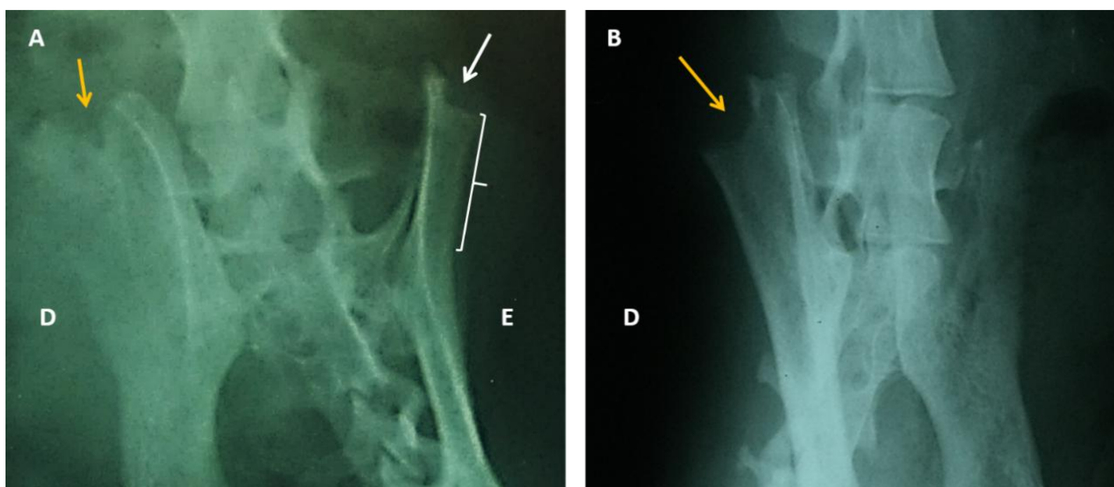

Figure 7. A and B: Coxal radiography of *C. aethiops* indicating the right iliac crest treated with BMMCs (D) and left treated with ADMCs (E). A: 40 days after cell infusion. B: 104 days after cell infusion. A e B: Changes in bone defects cannot be seen in Right iliac crest (yellow arrow), while in left iliac crest bone It is partially recovered in A (white arrow and key), and in B It is fully recovered (white arrow and key).

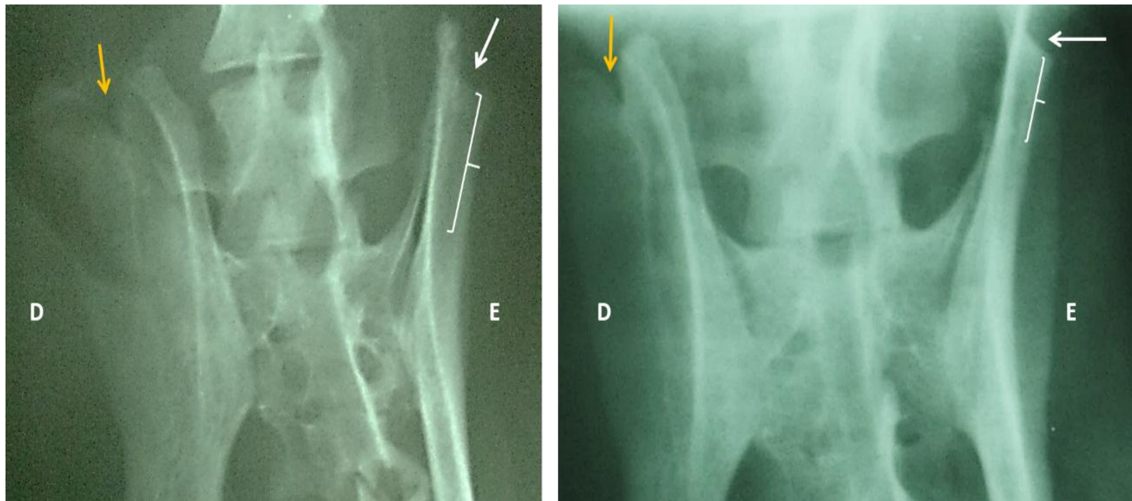

Figure 8. A: Coxal radiography of *C. aethiops* indicating the right iliac crest treated with BMMCs (D) and left treated with ADMCs (E),144 days after cell infusion. B: Animal control, 174 days after surgical lesions in the iliac crest. In **A** the right iliac crest still has the bone defect (yellow arrow), and the left iliac crest bone has no defect and is fully recovered (white arrow and key).

DISCUSSION

Several studies have been conducted using BMMCs and adipose tissue MSCs for therapeutic purposes, however, the gap that exists in this area is still large when considering the use of nonhuman primates as experimental models. As the closest relatives of man, some species of nonhuman primates are more interesting than others as a model, which is especially true for Old World primates, such as *C. aethiops* due to its phylogenetic compatibility with man (Thomson and Marshall, 1997). This supports the statement of that said animal models allow the assessment of natural, induced or behavioral biological phenomena that can be compared to human phenomena in question (Ferreira and Ferreira, 2003).

Initiating via cellular viability, our findings corroborate with Zuk *et al*. (2001) in which bone marrow mononuclear cells and mesenchymal stem cells from adipose tissue of *C. aethiops*, had 97% and 90% cell viability, respectively. The mesenchymal stem cells used herein were obtained from adipose tissue SVF, comprising a heterogeneous cell population; thus, 24 hours after plating, cells were adhered to the plastic surface of the culture flasks and presented an initial heterogeneous and flattened morphology. During subsequent passages, the cell monolayer became more uniform and fibroblastic and proliferated more rapidly. These morphological characteristics are attributed to MSCs and were also identified in the pioneering work of Zuk *et al.* (2001) in humans and other species, such as the Rhesus monkey (Izadpanah *et al.,* 2005) and rats (Zhang and Chan, 2010), and, to date, are considered the most promising cell types for therapeutic usage due to their capacity to segregate regenerative bioactive molecules (Marx *et al.,* 2015).

MSCs derived from *C. aethiops* presented a growth curve characterized by an initial lag phase that was followed by an exponential log phase and ended with a plateau phase that is known as the stationary phase. From days 10 to 12, there was a pronounced decline in the curve, which can be explained by factors intrinsic to the cells. The growth curve of the MSCs obtained from *C. aethiops* was similar to those reported in other studies using different sources of MSCs, such as a goat umbilical cord, buffalo amniotic fluid (Wagner *et al.,* 2008) and buffalo (Gade *et al.,* 2013), equine (Ranera *et al.,* 2012) and ovine bone marrow (Fernades *et al.,* 2014).

Although the MSCs have the ability to proliferate in culture over many passages, after successive cell divisions the SCs can become senescent. Moreover, the microenvironment of in vitro culture is very different from an in vivo environment, causing changes in properties typically observed in vivo due to artificial (Noer *et al.,* 2007). The percentage of senescent cells in

the cultures (P4) was 12.5%, with no chromosomal alterations. Zuk *et al.* (2001) tested late-passage (P7 - P15) human ADSCs and observed a 5% increase in the percentage of senescent cells from P7 to P10 and an increase up to 15% at P15. Wagner *et al.* (2008) also observed an increase in senescence in human BMSCs at late passages (P6 - P16).

The MSCs are phenotypically heterogeneous (Heggebö *et al.,* 2014), therefore, the International Society for Cellular Therapy (ISCT) has established minimum markers for MSC characterization, considering CD73, CD105 and CD90 as positive markers and CD34, CD45 and CD79 (specific for hematopoietic cells) as negative markers. In this study, cells were positive for CD73, CD105 and CD90 and negative for CD34, CD45 and CD79, which is in accordance with the minimum criteria required by the ISCT.

The potential of MSCs to differentiate into the three mesodermal lineages is extremely important and has also been used as morphological and functional criteria for MSC characterization (Gade *et al.,* 2013). Thus, MSCs from *C. aethiops* adipose tissue were defined as multipotent cells because they differentiated into adipocytes, chondrocytes and osteocytes. In this context, the results obtained for differentiation into bone tissue explain the recovery of the bone defect caused in the iliac crest of *C. aethiops*. Therefore, according Heggebö *et al.* (2014), osteosynthesis occurs by differentiation of mesenchymal stem cells into osteoblasts, with the deposit of a mineralized extracellular matrix, calcium, phosphorus and other minerals, and because the concentration of MSCs in adipose tissue is greater than in the bone marrow, this tends to be the reason for the shorter time for bone consolidation in the left iliac crests that were treated with adipose tissue MSCs. These required 3.4 months (102 days) for the total repair of bone defects, while the right iliac crests, treated with BMMCs continued to have obvious bone defects for up to 5.8 months (174 days).

Cell therapy in veterinary medicine is still something that needs to be explored much further because, to date, mesenchymal stem cells are well characterized only in horses and dogs, and there is little information about felines. However, the immunophenotype, proliferation and differentiation potential, and expression of pluripotency genes are similar to the characteristics described for human cells (Marx *et al.,* 2015). Thus, findings in nonhuman primates not only contribute information related to cell therapy in animals, but may represent a step forward in science toward the treatment of certain human diseases.

CONCLUSION

Despite the easy of collecting and separating BMMCs, the result of recovery of bone tissue in *C. aethiops* is not satisfactory when compared with the application of adipose tissue MSCs. Furthermore, these cells meet the requisites recommended by the International Society for Cellular Therapy, and were capable of promoting complete recovery of the lesion in a little over 4 months. In this context, and because of the gene compatibility of this species of nonhuman primate when compared to humans, we can suggest that in cases of severe bone injuries and loss of this tissue, the patient can be subjected to autologous therapy with mesenchymal stem cells from adipose tissue.

ACKNOWLEDGEMENTS

The members of the Laboratory of Tissue Culture and Cytogenetics Laboratory of the Environment Sector Evandro Chagas Institute, the medical clinic sector team of the National Center of Primates and members of the In Vitro Fertilization Laboratory at the Federal University of Para.

REFERENCES

ABDELHAY, E.S.; BRAGA, F.H.; BINATO, R.; BOUZAS, L.F.S. Células-Tronco de origem hematopoiéticas: expansão e perspectivas de uso terapêutico. *Rev. Bras. Hematol. Hemoter*., v.31, p.2-8, 2009.

ALVES, A.L.G.; VIEIRA, M.E.M.; BARREIRA, A.P.B. *et al*. Protocolo de isolamento de células mononucleares de medula óssea de equinos. *Vet. Zootec*., v.16, p.650-655, 2009.

BRANCO, E.; CABRAL, R.; GOMES, B.D.; KFOURY, J.R.JR. *et al*. Bone marrow cells of swine: collection and separation. *Microsc. Res. Technol*., v.75, p.917-920, 2012.

CUENCA-LÓPEZ, M.D.; ANDRADES, J.A.; GÓMEZ, S. *et al*. Evaluation of posterolateral lumbar fusion in sheep using mineral scaffolds seeded with cultured bone marrowcells. *Int. J. Mol. Sci*., v.5, p.23359-23376, 2014.

DAVE, P.C.; DINGAL, P.; DISCHER, D.E. Combining insoluble and soluble factors to steer stem cell fate. *Nat. Mater*., v.13, p.532-537, 2014.

DOMINICI, M.; LE BLANC, K.; MUELLER, I. *et al*. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy, v.8, p.315-317, 2006.

EMERTON, K.B.; DRAPEAU, S.J.; PRASAD, H. *et al*. Regeneration of periodontal tissues in non-human primates with rhGDF-5 and beta-tricalcium phosphate. *J. Dent. Res.*, v.90, p.1416-1421, 2011.

FERNANDES, M.B.; GUIMARÃES, J.A.; CASADO, P.L. et al. The effect of bone allografts combined with bone marrow stromal cells on the healing of segmental bone defects in a sheep model. *BMC Vet. Res*., v.5, p.10-36, 2014.

FERREIRA, L.M.; FERREIRA, L.R.K. Experimental model: historic and conceptual revision. *Acta Cir. Bras*., v.18, p.1-3, 2003.

GADE, N.E.; PRATHEESH, M.D.; NATH, A. *et al*. Molecular and Cellular Characterization of Buffalo Bone Marrow-Derived Mesenchymal Stem Cells. *Reprod. Domest. Anim*., v.48, p.358-67, 2013.

GONÇALVES, N.N.; AMBRÓSIO, C.E.; PIEDRAHITA, J.A. Stem cells and regenerative medicine in domestic and companion animals: a multispecies perspective.

HEGGEBÖ, J.; HAASTERS, F.; POLZER, H. *et al*. Aged human mesenchymal stem cells: the duration of bone morphogenetic protein-2 stimulation determines induction or inhibition of osteogenic differentiation. *Orthop. Rev*., v.6, p.5242, 2014.

IZADPANAH, R.; JOSWIG, T.; TSIEN, F. Characterization of multipotent mesenchymal stem cells from the bone marrow of rhesus macaques. *Stem Cells Dev*., v.14, p.440-451, 2005.

KÖRBLING, M.; ESTROV, Z.; CHAMPLIN, R. Adult stem cells and tissue repair. *Bone Marrow Transplant*., v.32, p.23-24, 2003.

MARX, C.; SILVEIRA, M.D.; BEYER, N.N. Adipose-derived stem cells in veterinary medicine: characterization and therapeutic applications. *Stem Cell Dev*., v.24, p.803-813, 2015.

MONTEIRO, BS.; ARGOLO, N.M.; DEL CARLO, R.J. Células-tronco mesenquimais. *Ciênc. Rural*, v.18, 2009.

NOER, A.; BOQUEST, AC.; COLLAS, P. Dynamics of adipogenic promoter DNA methylation during clonal culture of human adipose stem cells to senescence. *BMC Cell Biol.*, v.8, p.18, 2007.

OLSSON, D.C.; PIPPI, N.L.; MARTINS, D.B. *et al*. Colheita de medula óssea em cães: modelo para obtenção da fração total de células Mononucleares. *Ciênc. Rural*, v.39, p.141-147, 2009.

RANERA, B.; REMACHA, A.R.; ÁLVAREZ-ARGUEDAS, S. *et al*. Effect of hypoxia on equine mesenchymal stem cells derived from bone marrow and adipose tissue. *BMC Vet. Res*., v.8, p.142, 2012.

Reprod. Domest. Anim., v.4, p.2-10, 2014.

RIPAMONTI, U.; RAMOSHEBI, L.N.; TEARE, J. *et al*. The induction of endochondral bone formation by transforming growth factor-beta(3): experimental studies in the non-human primate Papio ursinus. *J. Cell Mol. Med*., v.12, p.1029-1048, 2008.

SANTIAGO-TORRES, J.E.; LOVASZ, R.; BERTONE, A.L. Fetal vs adult mesenchymal stem cells achieve greater gene expression, but less osteoinduction. *World J. Stem Cells*, v.7, p.223-234, 2015.

SASAKI, M.; HONMOU, O.; RADTKE, C. *et al.* Development of a middle cerebral artery occlusion model in the nonhuman primate and a safety study of I.V. infusion of human mesenchymal stem cells. *Plos One*, v.6, p.e26577, 2011.

THOMSON, J.A., MARSHALL, V.S. Primate embryonic stem cells. *Curr. Top. Dev. Biol*., v.38, p.133-165, 1997.

VIEIRA, N.M.; BRANDALISE, V.; ZUCCONI, E. *et al.* Isolation, characterization, and differentiation potential of canine adipose-derived stem cells. *Cell Transplant*., v.19, p.279-289, 2010.

WAGNER, W.; HORN, P.; CASTOLDI, M.; et al. Replicative senescence of mesenchymal stem cells: a continuous and organized process. *Plos One,* v.3, p.e2213, 2008.

WISLET-GENDEBIEN, S.; LAUDET, E.; NEIRINCKX, V.; ROGISTER, B. Adult bone marrow: which stem cells for cellular therapy protocols in neurodegenerative disorders? *J. Biomed. Biotechnol*., v.2012, 2012.

WOLF, D.P.; KUO, H.C.; PAU, K.Y.; LESTER, L. Progress with nonhuman primate embryonic stem cells. *Biol. Reprod*., v.71, p.1766-1771, 2004a.

WOLF, D.P.; THORMAHLEN, S.; RAMSEY, C. *et al*. Use of assisted reproductive technologies in the propagation of rhesus macaque offspring. *Biol. Reprod*., v.71, p.486-493, 2004b.

ZHANG, L.; CHAN, C. Isolation and enrichment of rat mesenchymal stem cells (MSCs) and separation of single-colony derived MSCs. *J. Vis. Exp*., v.37, p.e1852, 2010.

ZUK, P.A.; ZHU, M.; MIZUNO, H. *et al*. Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng*., v.7, p.211-28, 2001.