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IN-VIVO TRACKING OF BONE MARROW MESENCHYMAL STEM CELLS BY GOLD NANO TRACERS IN MUSCULOSKELETAL TRAUMA.

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ABSTRACT

Stem cells are multifaceted. On treating injuries, they behave as a promising facts to retain normal tissue structure and function. Once the stem cells are injected, do they reach their intended target? Will they remain at the traumatized site? How many Tracking cells actually stayed? How many diffused or died? These are all the important questions remains to be answered. The important therapeutic potential of BMSCs is their ability to migrate to site of inflammation/stress, where they balance the situation through molecular mechanism of the P38MAPK pathway. This study aims tracking of bone marrow mesenchyme cells with non-cytotoxic gold nano tracers in treatment of various therapeutic applications.

KEYWORDS: Mesenchymal Stem Cells (MSCs), Gold Nano particles (AUNPs), computed tomography (CT), Magnetic resonance imaging (MRI), Musculo skeletal trauma (MST), Transmission Electron Microscopy (TEM).

INTRODUCTION

Mesenchymal Stem Cells (MSCs) have been considered the progenitor cells for the skeletal tissues and they can differentiate along multiple lineages like osteoblasts, chondrocytes. Recently, there is evidence that Gold Nano particles (AUNPs) can facilitate stem cell therapy and bone tissue engineering through MAPK pathway. Nano technology provided valuable tools for stem cell research. Nano particle can be attached to the cell before they are transplanted into the body. They can act as contrast agent to track the cells in the body via CT

and MRI scans. Nano tracking devices allow researchers to visualise the stem cells at the site of injury.

MATERIALS

6 Adult wistar albino rats were used for this study. Cultured BMSC cells were characterised using flow cytometry. The cells were labelled with Gold Nano particle and tracked in-vivo by CT at the musculo skeletal traumatised site of wistar albino rat.

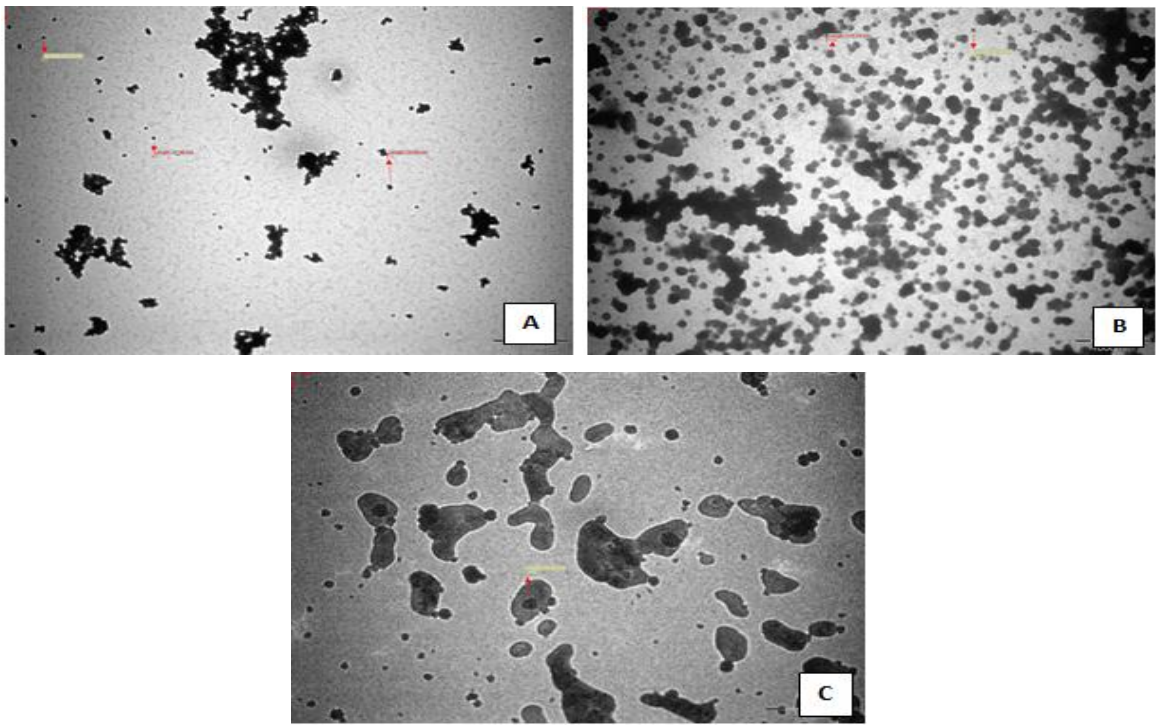
NANOPARTICLE SYNTHESIS

Gold Nano particle were synthesised via citrate reduction of Tetra chloro auric acid (HAUCL4).Under reflux, 100ml of distilled water was heated to 100cc and 1ml of 10mg/ml HAUCL4 was added while stirring, sodium citrate (11.4mg/ml) dissolved in distilled water was then added.

POLYMER COATED PEGYLATED FIBRIN GEL

Nano particles were coated with Pegylated fibrin gel and allowed to mix for 30mins. The Nano particles were then centrifuged at 3200 rpm for 30mns in order to remove the excess Pegylated gel.

NANO PARTICLE CHARACTERIZATION BY TEM

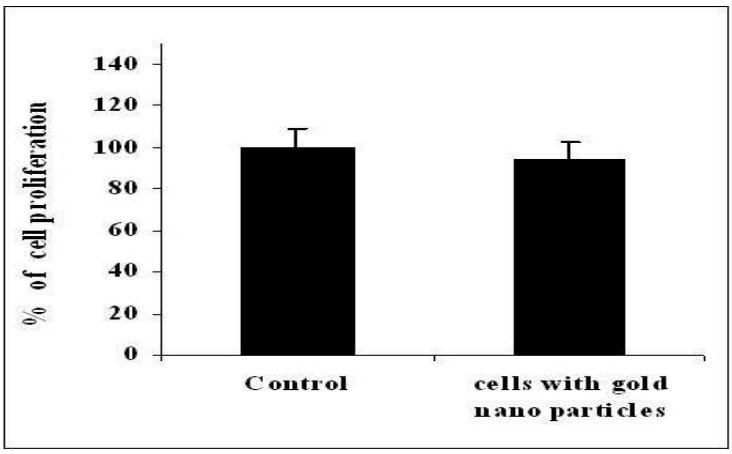


"Fig. 1", A. Red arrow shows the Gold Nano particles, B. Red arrow shoes the Stem cells with measurements in Nano scale. C. Red arrow shows Stem cell with Gold Nano particle.

NANOPARTICLE INCUBATION

Nano particle medium was prepared by centrifuging the Nanoparticle solutions (5000 rpm for 15 minutes for 20 nm–citrate-stabilized Nano particles) and suspending the pellet in phenol red free medium at a concentration of approximately 10-12 nanoparticles /ml. MSCs growth medium was aspirated from the flasks and 200ul/cm2 of Nano particle medium was added to the cell culture and allowed to incubate for 24hours. After 24hours, the nanoparticle medium was removed and the cells were washed with a phosphate buffer saline. A Leica DM12000 microscope equipped with Leica DFC290 camera was used to obtain dark field images (20 times magnification) in order to assess Nano particle uptake.

MTT ASSAY FOR CELL VIABILITY



"Fig. 2", MTT assay show proliferation of cells tagged with gold Nano particles.

After the MTT assay the gold nano particle tagged Bmsc were transplanted intraperitoneally into the traumatized (MST) rat.

ANIMALS

6 male wistar albino rats above 1year were included in the study. The animals were obtained from BRULAC, Saveetha University. The rat was maintained in a cage, containing 2 animals in each cage. Cages were labelled with body weight and group name. Animals were provided with normal food and fresh water. They were acclimatized on a 12hour day and 12hour dark schedule with (oyeyemietal 2008)

MUSCULO SKELETAL INJURY PROCEDURE

Right lower limb was used in all the experimental animals. The animal was anaesthetised under xylocaine 1.8ml of diluted stock and ketamine 1ml of diluted stock for a 250gm weighing rat. Surgical incision was made on the posterior aspect of the thigh.

The femur, sciatic nerve and muscles were identified by dissection. Then a 20gm weight dropped from 12.5mm hight on the exposed lower limb to create a musculo skeletal injury then the wound was approximated using 4-0 black silk suture. The surgical wound is dressed with betadine dressing.

INTRA PERITONIAL INJECTION OF BMSC TAGGED GOLD NANO PARTICLE INTO MUSCUTO SKELETAL TRUAMATISED ANIMAL

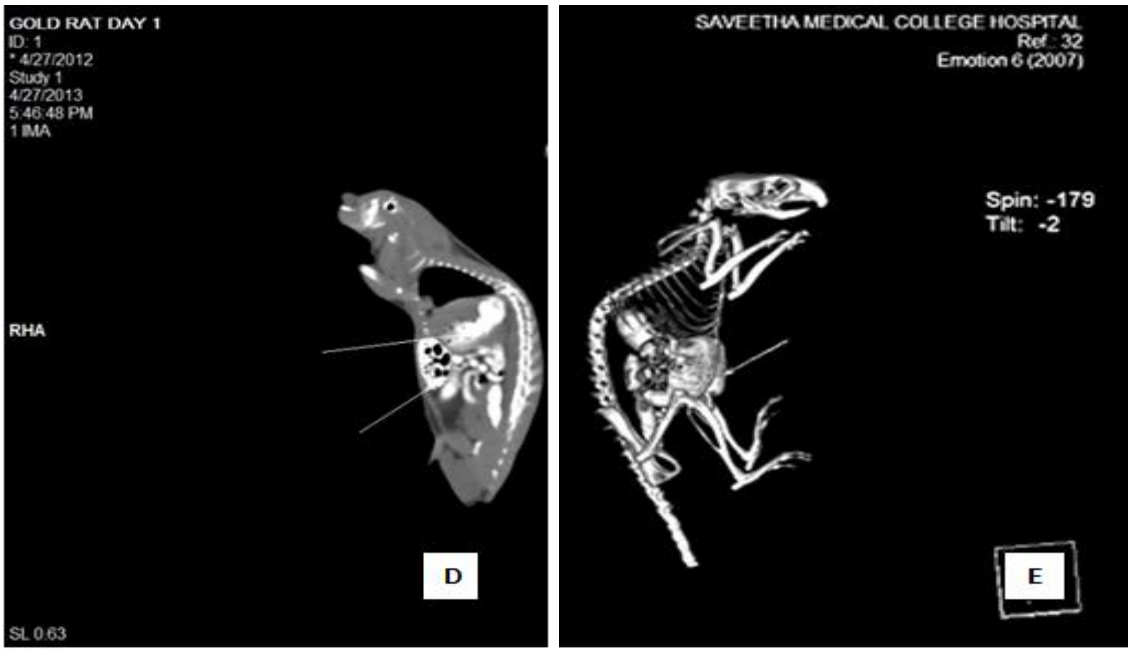
The BMSC Gold Nano particle cells were loaded in micro litre syringe at a concentration of 1 x 10^5 cells/µl of culture medium injected intraperitoneally. After 5hours of injection, 2^{nd} day and 7^{th} day the Nano practices were traced by C.T.

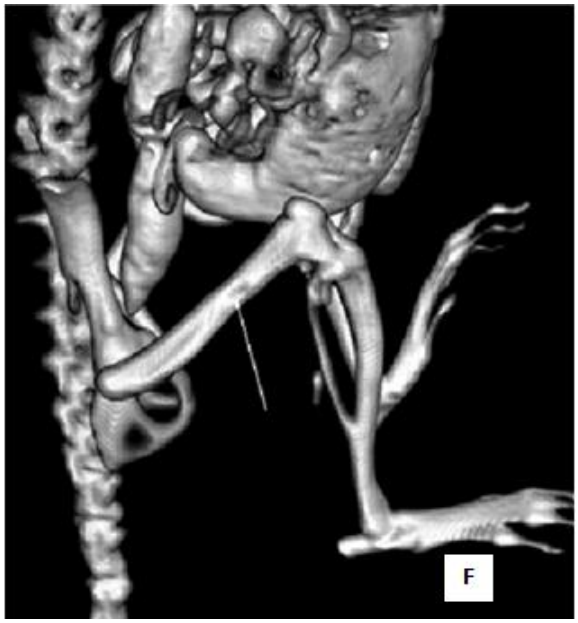
RESULTS

AUNPs loading was not cytotoxic to the cells as seen in MTT assay ("Fig. 2",).CT image was taken after 5 hours of intraperitoneal injection of BMSC loaded AUNPs.The Nano particles were seen in intestine and stomach ("Fig. 3"D,).

The CT image taken on second day slowly the Gold Nano particles migrating from stomach, intestion to the target area. No particles were seen near the trumatised area ("Fig. 3"E,).

CT taken on seventh day there is minimal amounts of particle were seen in traumatised area. Still the particles loaded in the colon, not absorbed fully by the portal circulation. It takes longer time to reach the injured area ("Fig. 3"F,).





"Fig. 3", CT image shows gold Nano particles tagged stem cells in the GI tract after, D. 5 hours of intraperitoneal injection. E. 24h ours of intraperitoneal injection. F. Arrow indicates AUNPs tagged stem cells at the traumatised area of 7thday CT.

DISCUSSION

Changquing et al 2010 stated that PEGY lated Gold Nano Particles (AUNPs) promote osteogenic differentiation of Mesenchymal Stem Cells through P38 MAPK Pathway. Saraswathy et al 2015 demonstrated the role of transplanted BMSC with scaffold in the traumatized area promote healing of muscle skeletal tissue and maintain normal physiological function by CT and ENMG respectively.

Once Stem Cells are injected do they reach their target area? Do they remain and promote healing? To find out the answers to the above questions, the Nano technology has provided valuable tool to track in-vivo by CT and MRI. AUNPs loading was not cytotoxic to the cells and did not affect the BMSC viability as seen in MTT Assay. Nano particle loading was accomplished via passive up take of the particles by the cells. No targeting agents were used to promote endocytosis.

Endocytosis of cells loaded with Gold Nano particles were seen in TEM. Transmission Electron Microscopy (TEM) used to characterize the morphology and ultra-structural change of BMSC with Gold Nano particles (ANUPs).

CT was taken immediately after 5 hours of the first day, second day, and 7th day after intra peritoneal injection. First and second day the particles were seen in intestine, stomach and liver. Seventh day Gold Nano particles has reached the traumatized area. Intra peritoneal route has taken longer time to reach the target site. Most probably the portal Circulation could have delayed the travelling of the BMSC to the target area. This method of cell tracking with CT offers a valuable tool for research and more importantly for clinical application to study the fate of BMSC.

CONCLUSION

The results of the study states that loading BMSC with Gold Nano particles does not alter cell function and long term CT imaging is feasible. Intraperitoneal route takes longer time to the target area. In addition in-vivo studies are necessary to monitor the progression of MScs growth overtime. This study may offer a whole new approach to skeletal regenerative medicine. The use of Nano particle could enable new cell culture design, new device design and would develop a new bone repair therapy.

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