Treatment of periprostetic bone-loss with Mesenchymal Stem Cells (MSC)

Frequency, proliferative potential, expansion of MSC in patients undergoing THA in different age groups.

M. Podestà, M.D.*, F. Frassoni, M.D.*, N. Sessarego, M.D.*, S. Castello, M.D.*
G. Grappiolo, M.D.°, G. Burastero, M.D.°, G. Moraca, M.D.°, L. Spotorno, M.D.°

*Cell-Therapy Unit - S. Martino Hospital - Genoa, Italy
°Hip & Knee Surgery - S. Corona Hospital - Pietra L. (SV), Italy

Introduction

MSC are found in bone marrow and belong to a clonogenic subpopulation which is capable of adhesion. They can be cultivated in vitro in an undifferentiated state; when stimulated, they are capable of expansion and give rise to different cellular types such as osteocytes, adipocytes, chondrocytes, muscle cells, astrocytes and hepatocytes. (Fig. 1)

Identification and isolation of MSC has made it possible to develop experimental techniques in tissue engineering which can also be applied in the orthopaedic revision surgery. When MSC are cultivated in vitro and expanded they must, at any rate, be combined with an appropriate carrier such as hydroxyapatite, polyglycolic and polylactic acid which provide a tridimensional skeleton where they can adhere and then differentiate.

The purpose of our research was to study the ability to form mesenchymal colonies (CFU-F) in patients undergoing hip arthroplasty. This is meant to be a preliminary approach for the preparation of an animal model of tissue engineering and, for clinical approach.

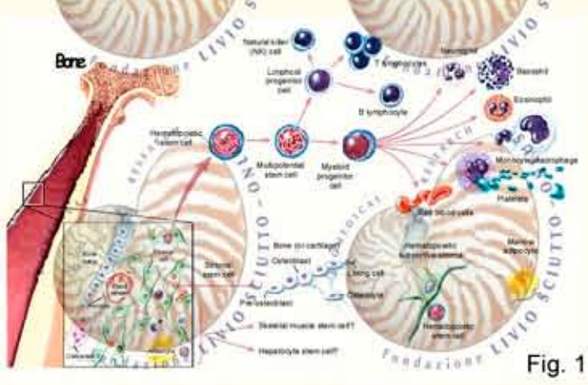

Fig. 1

Aim of the study were to establish whether...

- the **patient age** can influence the number of MSC
- different site may influence **proliferative potential** of MSC
- the explant anatomical site may influence the **frequency of cells**
- different site may influence **expansion capacity** of MSC
- the number of MSC in older patients is enough for clinical approach in revision surgery

Material & methods

Materials & Methods

CFU-F (Figg. 2-3)
The work of Fridenstein et al. provided definitive evidence that bone marrow contains a population of clonogenic fibroblast precursors termed CFU-F (Fibroblast - Colony Forming Unit). This term has been gradually abandoned and replaced by MSC (Mesenchymal Stem Cells).
At present the CFU-F assay is used to confirm the role of HSC in stromal cell formation in vitro and is used extensively to evaluate the microenvironmental progenitors.

MNCs from femour (11 patients), pelvis (11 patients) and nucleated cells from bone filings (11 patients) were plated at a density of 10 5 cells / 35cm 2 flasks in MesenCult medium (StemCell Technologies Inc., Vancouver, BC-Canada) and incubated at 37°C with 5% humidified CO2 atmosphere (Fig. 2).
After 24 h nonadherent cells were discarded, fresh medium was added and half of the culture medium was replaced with fresh medium twice a week for 14 days.
Then, removed the medium, the adherent cells were washed in PBS 1X (Euroclone) and stained in May-Grunwald / Giemsa for the count (Fig. 4).

MSC- expansion
To assess expansion ability of hMSCs we cultured MNCs from femour (11 patients), from pelvis (11 patients) and nucleated cells from bone filings (11 patients).
Cells were plated at density of 10 6 cells / ml in 75 cm 2 flasks in MesenCult medium (StemCell Technologies Inc., Vancouver, BC-Canada) and incubated at 37°C with 5% humidified CO2 atmosphere.
After 24 h nonadherent cells were discarded, fresh medium was added and half medium replaced twice a week.
When cultures reached more than 90% confluence, adherent cells were detached with 0.05% trypsin (Euroclone), washed twice with complete medium, counted with a nuclear stain (0.1% methyl violet, in 0.1M citric acid), and replated at a concentration of 5 x 10 5 cell per flask.

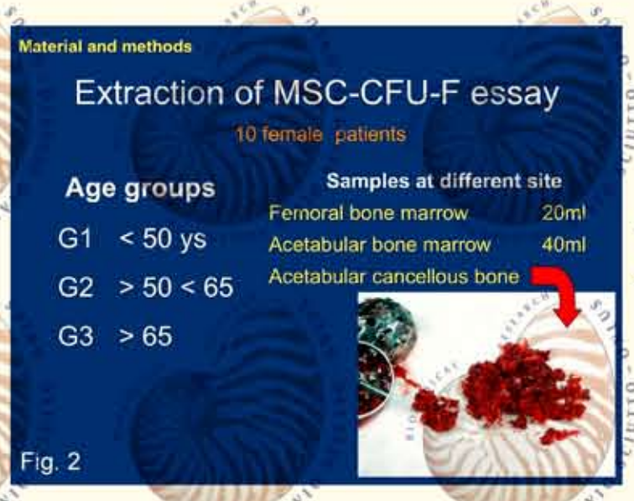

Fig. 2

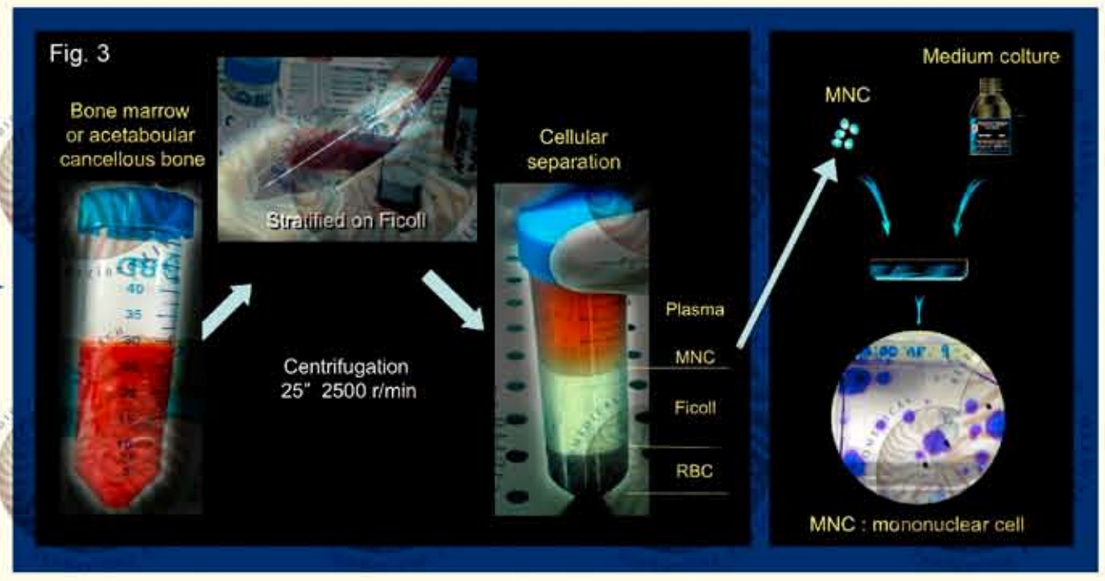

Fig. 4

Age related MSC (tab. 1)

		MSC/10^6 MNC BM pelvis	BM femur	Acetabular bone
Group 1 < 50 y° (n. 3)	median	63	57	90
	range	7 - 80	33 - 82	90 - 90
Group 2 50 - 65 y° (n. 4)	median	56	34	90
	range	14 - 80	19 - 78	39 - 92
Group 3 > 65 y° (n. 3)	median	42	38	70
	range	9 - 95	7 - 88	48 - 90

Patients were divided into three groups and bone marrow samples were tested for their colony-forming capacity.
CFU-F frequency was similar within the three groups and not different to normal donors (median=46, range: 15-85/10^6MNC, p= 0.6).
However, growing patients age, overall results showed a reduction trend in non-hemopoietic progenitors.

Proliferative potential vs. sampling area (tab. 2)

	MSC/colony BM pelvis	BM femur	Acetabular bone
median	2000	1600	4,300
range	1100/2700	700/9500	1760/23,400

In six patients we compared the **proliferative potential of single CFU-F** considering the sampling area. Colonies grown from bone fragments showed an higher proliferative potential compared to those grown from femur or pelvis: median 4300 cells/colony (range 1760-23400) vs 1600 cells/colony (700-9500) vs 2000cells/ colony (range 1100-2700) respectively.

...we find that:

- MSC reduction in older patient is not statistically significant (Tab. 1)
- Cancellous bone has higher proliferative potential of MSC (Tab. 2)
- Cancellous bone has higher frequency of MSC (Tab. 3)
- Cancellous bone has higher capacity to generate progeny (Tab. 4)

Frequency and vitality of MSC in older patients allows their use in revision surgery

Frequency vs. sampling area (tab. 3)

	MSC/10^6 MNC BM pelvis	p	Bone	p	BM femur
median	45	0,008	77	0,001	33
range	7 - 80		39 - 92		7 - 82

Marrow samples were harvested from pelvis and femur, bone fragments from acetabular cancellous bone.
The frequency of MSC was assessed considering the different sampling areas.
Bone fragments grew higher frequency of MSC in comparison with pelvis ($p<0.01$) and femur ($p<0.08$).

Amplification vs. sampling Area (tab. 4)

	Cells recovery	F^st exp	S^nd exp	T^rd exp
Days culture	+15	+25	+35	+45
BM femur	5%	1,5	2,8	4,6
	3.3 - 23	1 - 7	1,7 - 8,9	4,1 - 11,3
BM pelvis	8%	2,6	3,6	4,9
	3 - 20	1,3 - 3,8	1,9 - 4,9	2,9 - 6,3
Acetabular bone	20%	4,3	6,5	9,5
	10 - 55	1,1 - 12	3,7 - 13,8	6,2 - 16,7

MSC grown from the different sampling areas were expanded in culture to assess whether the lodgement may affect their capacity to generate a large progeny.
Samples from six patients aging between 55 and 76 years were tested.
Cell recovery after primary culture was higher using bone (median 13% of the total MNC plated; range 1.1-24) compared to pelvis (median 6.3%; range 5-11, $p<0.03$) and/or femur (median 5%; range 1.3-10, $p< 0.02$).
Cultures were carried on until the third generation.
The expansion rate was not different in the first generation, but resulted significantly higher using bone (1.8 fold) with respect to pelvis (0.7 fold) or femur (0.9 fold) in the secondary generation ($p<0.05$ for both) as well as in the third generation (2.5 fold vs 1.4 vs 1; $p<0.05$ for both). (Table 4).

As consequence, using median values drawn from our experiments, 10^6 MNC from pelvis will recover a median of 6.4x10^4 MSC after fourteen days until to reach 1.45x10^5 cells in the third generation. 10^6 MNC from femur will recover 5x10^4 MSC during the first generation and will reach 1.03x10^5 at the end of the culture. Finally, 10^6 MNC will recover 1.3x10^5 in the first generation reaching 2.05x10^6 MSC in the third generation.