

# BUŇKY, KTERÉ NESTÁRNOU, MÁME NA DOSAH

## Cells, that do not age, are reachable

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### Treatment of chondral lesions:

**Microfractures therapy: Patients over 40 years of age, not satisfactory benefit**

Application of cells on 3D scaffold increase the treatment efficacy in these patients

### 2 options of cells from Bone Marrow (BM)

- Mononuclear cells (MNCs)
- Cultured mesenchymal stem cells (MSCs)

### The aim of preclinical study

- Assess **two different sources** of the bone marrow.
- **Proximal tibia vs. the iliac crest** (the commonly used bone marrow source) in terms of quantity and quality of the cells.
- **HYPOTHESIS:**  
**Comparable features of material would enable us to perform the bone marrow collection from the tibia perioperatively during the arthroscopic surgery.**

### Material and Methods :

**Bone marrow** was collected using **aspiration biopsy** needles from the iliac crest and the proximal metaphysis of the tibia from ten **volunteers older than 40 years of age** during **total knee replacement surgery**.

- 10 patients : 4xW , 6xM , Avrg. 51Y
- Aspirate 13-15ml of BM from each source (tibia vs. Iliac crest)
- Cytometric data analysis: FlowJo (Tree Star) a FSC Express (De Novo Software)
- Flow cytometry: BD FACSCanto II
- Population doubling time:  $Pd_t = \frac{t}{\log N - \log N_0} \cdot 0.301$
- Microscopic pictures: EVOS FL Cell Imaging System (Thermo Fisher Scientific)
- Data analysis: MS Excel and SigmaPlot, (Systat Software), t-test



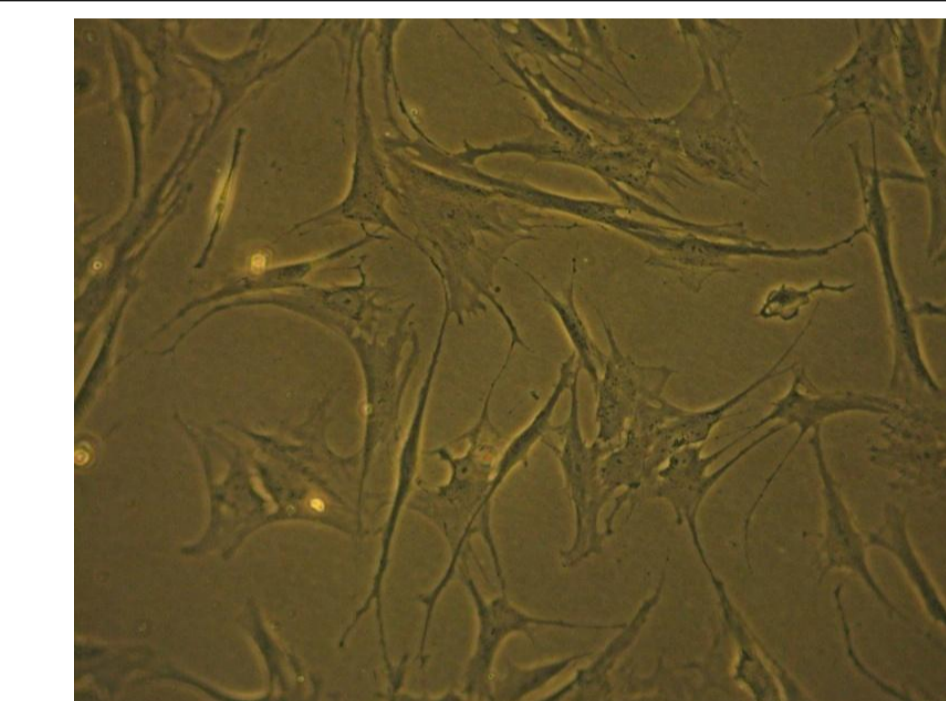
Pic. 1: The chondral defect of medial femoral condyle. Treated with microfracture technique. This type of surgery allowed us to release the cells from the subchondral bone marrow.



Pic.2: Consumption of bone marrow aspirate form iliac spine (Ortopedic's surgery, Masaryk hospital Ústí n.L.)

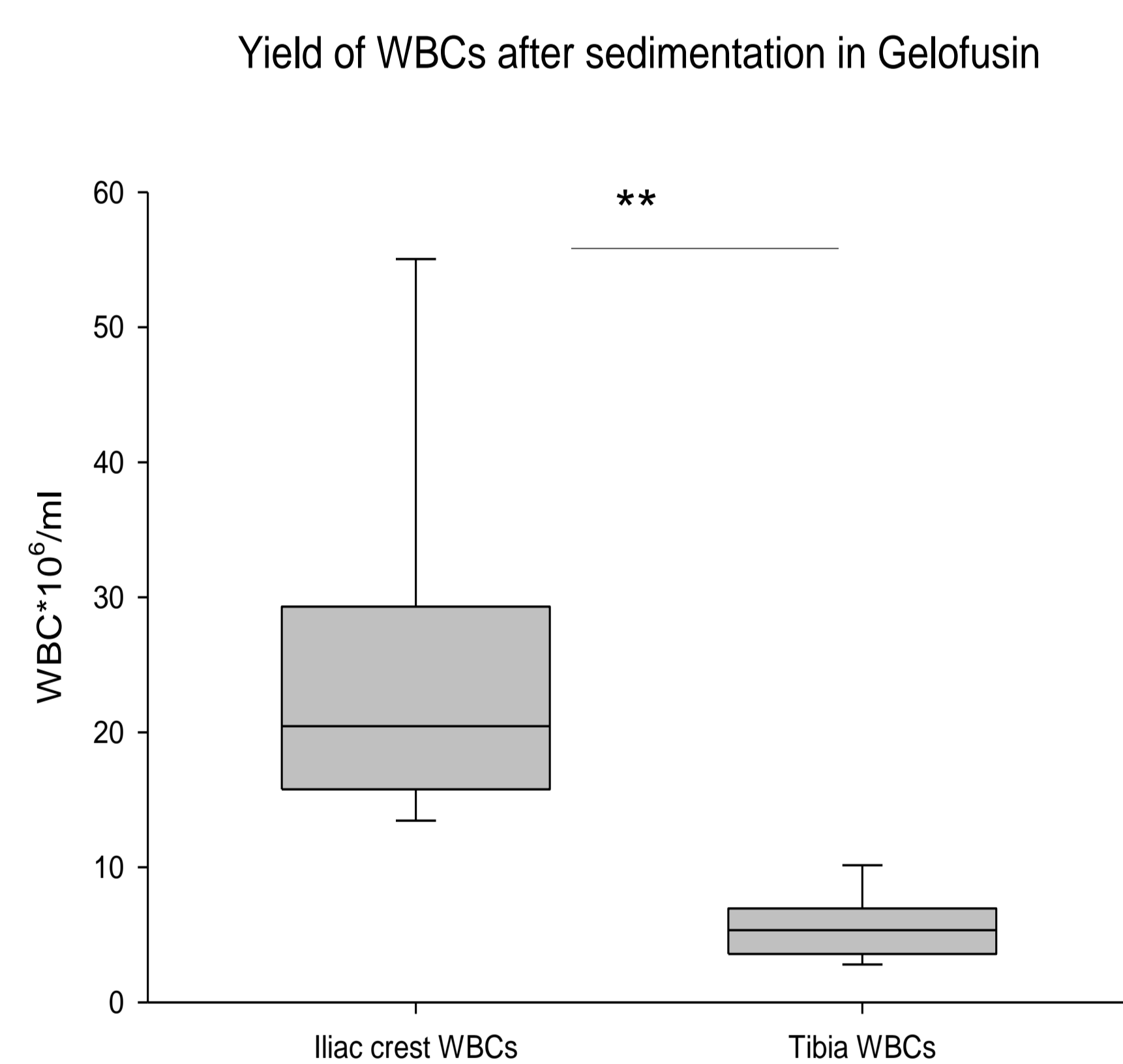


Pic.3: Microscopic picture: Mononuclear cells fraction

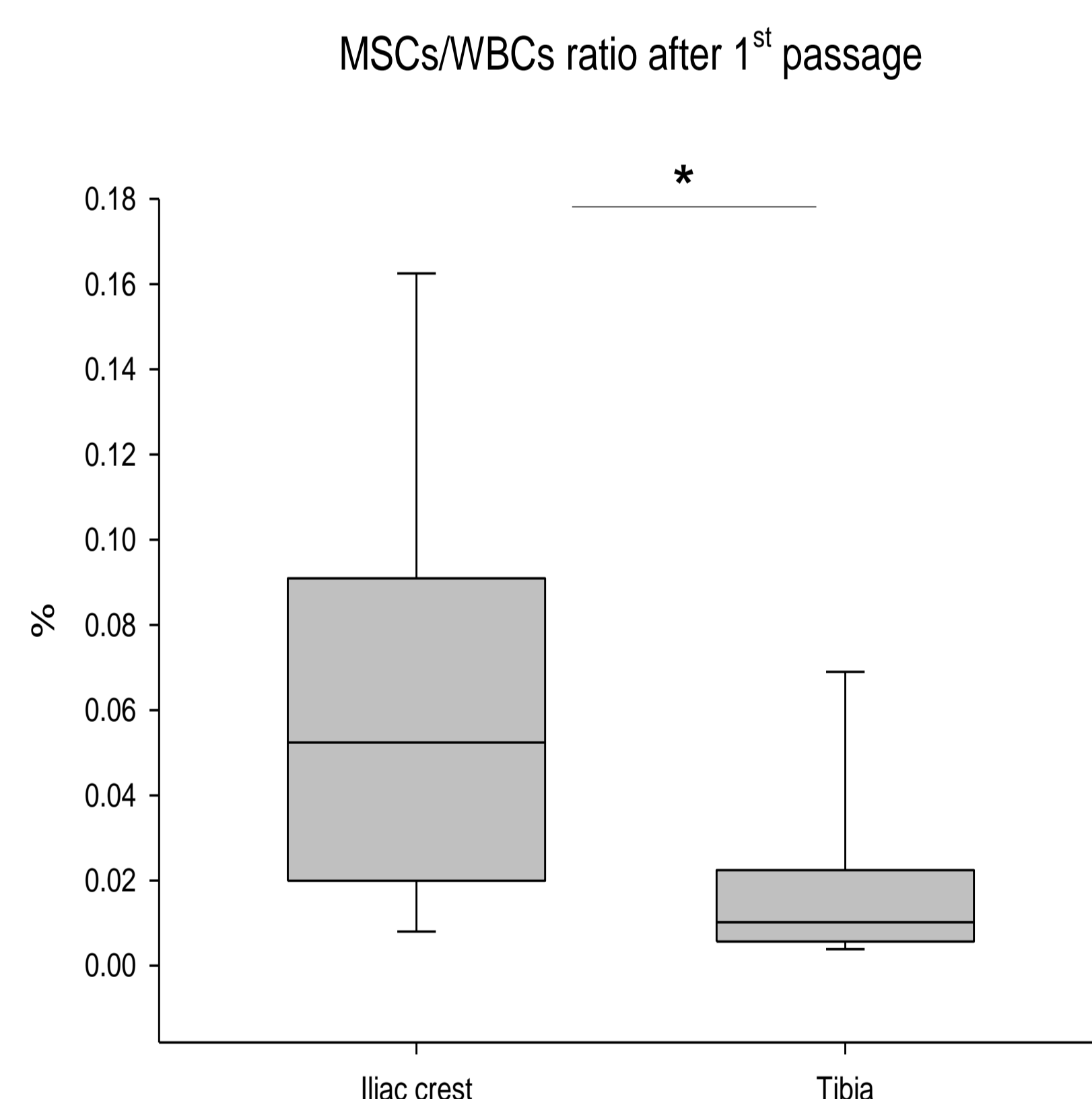


Pic.4: Microscopic picture: Mesenchymal stem cells

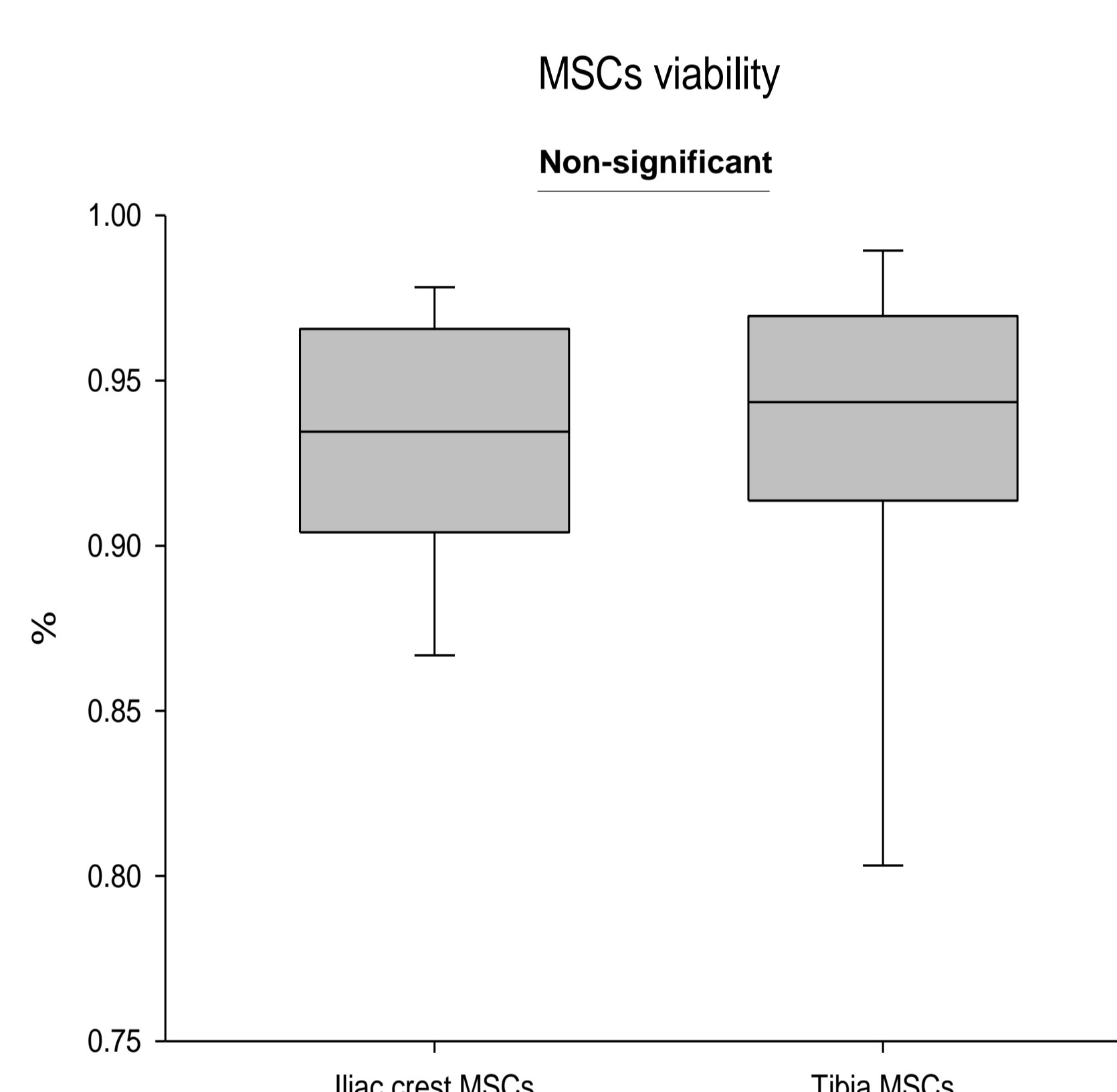
### Results



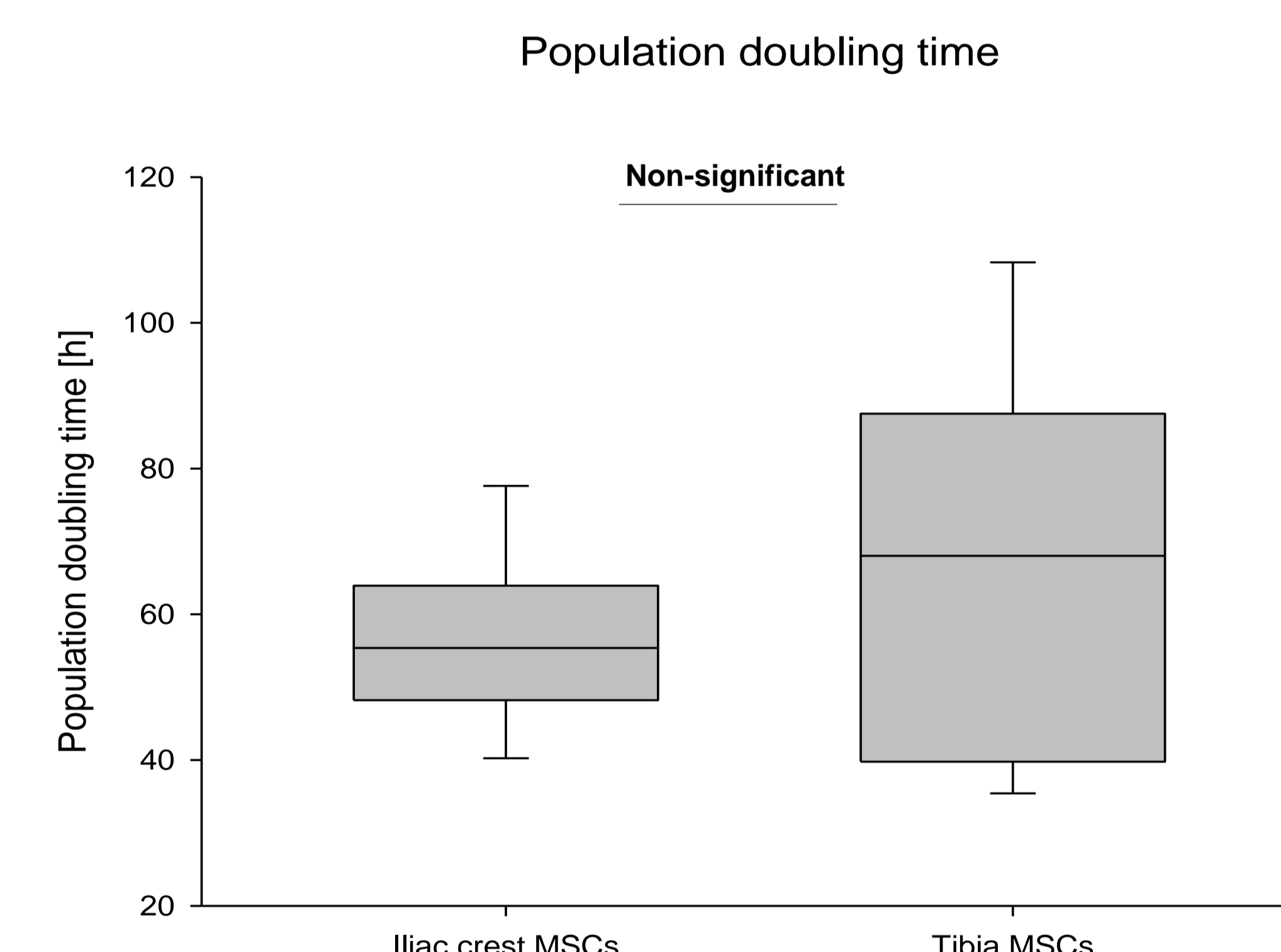
The **MNCs** concentration/yield was **significantly higher** in the samples from the **iliac crests**.



**Similar results** were obtained with the **cultured MSCs** after the first passage when the **MSCs/MNCs** ratio was compared.



Nevertheless, the **qualitative analysis** that included MSC immuno-phenotyping, viability and population doubling time **showed no difference between the two tested bone marrow sources**.



p-value:  
< 0.0001 \*\*\*\*  
0.0001 to 0.001 \*\*\*  
0.001 to 0.01 \*\*  
0.01 to 0.05 \*  
≥ 0.05 non-significant

### Conclusions:

• Bone marrow located in pelvic bone and metaphysis of long bones (distal femur, proximal tibia) contains mononuclear cells that possess features of MSCs.

1. The **iliac crest** represents a **quantitative superior** bone marrow source as for the MNCs and MSCs yield in **patients over 40 years**. However there was **no qualitative difference** between the isolated and cultures cells.
2. The population doubling time analysis showed that the **tibia** is a **good alternative source of the MSCs** which can be obtained at **therapeutically relevant number** for the treatment of chondral lesions of the knee.

• Moreover, in contrast to several reports, the **quality of these cells does not appear to decrease with the patients` age**.



Pic.5: Laboratory coworker separate the cells from bone marrow (Bioinova s.r.o.)



Pic.6.: Preparing of MSCs in laboratory (Bioinova s.r.o)

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