

## Osteoporosis and ageing affects the migration of stem cells which is ameliorated by CXCR4.

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**INTRODUCTION:** Osteoporosis is a major health problem and is directly associated with an increased incidence of fractures and poor quality of fracture repair in the elderly (1). Bisphosphonate treatment inhibits the catabolic activity of osteoclasts and subsequent bone resorption, but does not increase bone formation. There is therefore interest in using anabolic factors such as stem cells to augment fracture repair, because these cells can differentiate into osteoblasts and increase the production of bone. Mesenchymal stem cells (MSCs) from postmenopausal women have a slower growth rate and osteogenic differentiation ability causing lower bone density and reduced fracture healing capacity compared to MSCs from premenopausal women. Additionally, cellular movement, re-localization and retention are important for many physiologic properties. Local mesenchymal stem cells (MSCs) from injured tissues and circulating MSCs aid in fracture healing. Cytokines and chemokines such as SDF1 and its receptor CXCR4 play important roles in maintaining mobilization, trafficking and homing of stem cells from bone marrow to the site of injury. However in osteoporotic patients the migration, as well as the retention of MSCs at the site of injury is poor, resulting in non-union fractures (2-4). The aim of this study was therefore to develop an osteopenic rat model, isolate stem cells from these rats and investigate the migration of cells following transfection with CXCR4. The hypothesis of this study is that the migration of MSCs from young, adult and OVX rats will have different migratory abilities but this will increase when they are genetically modified to over-express CXCR4.

**METHODS:** Ethical approval was granted and all procedures were carried out in compliance with the UK's Home Office Regulations (Animals Scientific Procedure Act 1986). Ovariectomy was performed in 6-9 month old Wistar rats and osteopenia developed over a 4 month post-op period. MSCs were harvested from the femora of young, adult and ovariectomised (OVX) rats. Cells were genetically modified to over-express CXCR4 and put in a Boyden chamber to quantify their migration towards SDF1. Cell migration in non-transfected and scrambled infected groups were also compared. Additionally, CXCR4 infected MSCs were differentiated to osteoblasts and CXCR4 expression and migration quantified. Data was analysed using a Student t-test where p values < 0.05 were considered significant.

**RESULTS:** MSCs from OVX rats migrate significantly ( $p < 0.05$ ) less towards SDF1 ( $9 \pm 5\%$ ) compared to MSCs from adult ( $15 \pm 3\%$ ) and juvenile ( $25 \pm 4\%$ ) rats. Cells transfected with CXCR4 migrated significantly more towards SDF-1 compared to non-transfected cells irrespective of whether these cells were from OVX ( $26.5 \pm 4\%$ ), young ( $47 \pm 17\%$ ) or adult ( $21 \pm 4\%$ ) rats. When the MSCs were differentiated to osteoblasts their migration towards SDF1 reduced to  $6.7 \pm 2.3\%$  and migration did not significantly increase following over-expression of CXCR4 ( $11.25 \pm 8.6\%$ ).

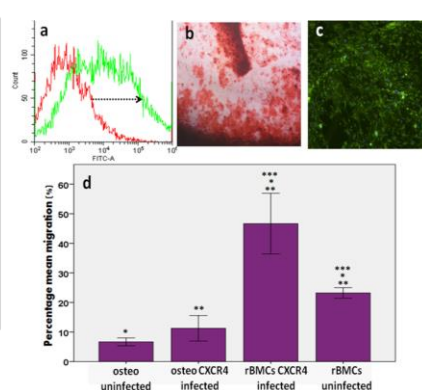
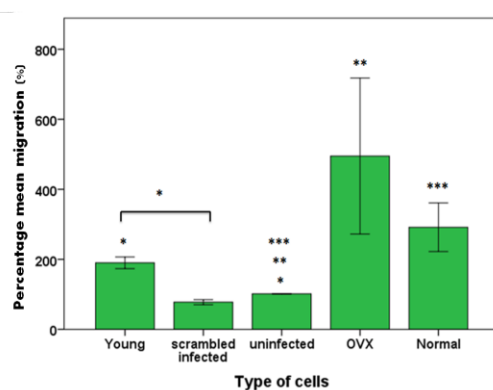
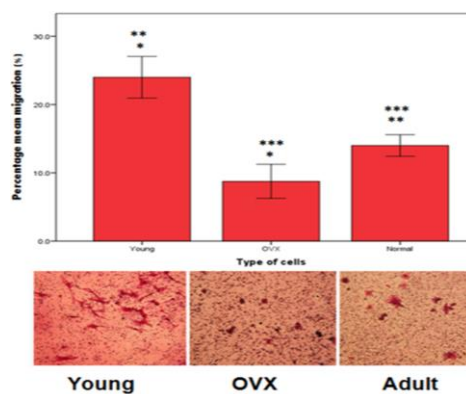


Figure 1: In vitro migration of uninfected MSCs from young, adult and OVX

Figure 2: Percentage migration of infected cells normalised against uninfected cells.

Figure 3: Migration of CXCR4 infected osteogenic cells compared to undifferentiated rBMCS

**DISCUSSION:** The impaired migration of MSCs from OVX and adult rats in comparison to MSCs from juvenile rats could be due to their diminished surface expression of CXCR4. SDF1 causes cell migration by binding with CXCR4. Our in vitro work has highlighted the pivotal role SDF1/CXCR4 axis plays in the homing of stem cells, whereby the mobility of MSCs from OVX rats towards SDF-1 was increased when their CXCR4 expression was up-regulated. Therefore increased secretion of SDF1 at the site of injury creates an environment that mediates the homing of circulating CXCR4-positive stem cells. Poor homing ability of the stem cells to bone could result in a significant reduction in bone formation which ultimately contributes to osteoporosis and delayed union in fractures (5). The impaired migration of CXCR4-osteoblasts is because the SDF1/CXCR4 axis is no longer required once the osteogenic differentiation pathway has been set into motion, as the migration of osteoblasts is no longer relevant, once the stem cells have homed to the site of injury and started differentiating to bone.

**SIGNIFICANCE:** MSCs from OVX rats have a significantly reduced migration. The maintained migration response upon up-regulation of CXCR4 illustrates the therapeutic potential of CXCR4 expressing MSCs in the treatment of osteoporotic fractures.

### REFERENCES:

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