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## Beneficial effect of Silver fir (*Abies alba* L.) polyphenolic extract on chondrogenic differentiation of primary mesenchymal stem/ stromal cells

### Abstract

**Keywords:** osteoarthritis, mesenchymal stem cells, chondrocytes, chondrogenic differentiation, inflammation, polyphenols, Belinal®

**Background:** Osteoarthritis is the most widespread, chronic, progressive and debilitating joint disease in the elderly population. Inflammation plays a major role in osteoarthritis. Moreover, the exhaustion of the endogenous reparative potential of mesenchymal stem cells (MSCs), in particular their chondrogenic ability, has been demonstrated in patients with advanced osteoarthritis. Several natural polyphenolic extracts or polyphenols, such as Pycnogenol® (French maritime Bark extract, *Pinus maritima* L.), resveratrol and quercetin have shown to reduce symptoms of osteoarthritis due to their antioxidant, anti-inflammatory, chondroprotective and immunomodulatory properties. Another commercially available polyphenolic extract Belinal® (Silver fir branch extract, *Abies alba* L.) has in *in vitro* studies shown higher antioxidant activity as Pycnogenol®, but its antioxidant activity on osteoarthritis models has not yet been established. All of the above mentioned polyphenolic extracts or polyphenols are proven to be found in serum and synovial fluid after peroral administration.

**Objectives:** In our study we aimed to determine whether Belinal® has the ability to boost the chondrogenic potential of MSCs.

**Design and Methods:** Patients with hip osteoarthritis submitted to routine hip replacement and healthy donors *post mortem* with no evidence of musculoskeletal disorder (controls) were included in the study. Primary cells were isolated from subchondral trabecular bone harvested from femoral head in patients with osteoarthritis and medial tibia in controls. Cells were *in vitro* expanded and their MSC-like phenotype verified. First, a comprehensive screening of the effect of Belinal® and three most commonly used polyphenolic compounds (Pycnogenol®, resveratrol and quercetin) on viability and proliferation of MSCs was performed. Cell viability and proliferation were determined for four serial dilutions (1500, 750, 375 and 187 µg/ mL) of the tested compounds using flow cytometry and MTS assay. Second, selected concentration of all four compounds was tested in chondrogenesis micromass *in vitro* assay under non-inflammatory and inflammatory conditions (the addition of lipopolysaccharide).

**Results:** The highest concentration of all compounds (1500 µg/ mL) exhibited statistically significant reduction of single live cells compared to controls (one-way ANOVA with Bonferroni correction,  $p = 0.0008$ ). However, no statistical difference was found for cell proliferation between the tested serial dilutions of all four compounds (one-way ANOVA with Bonferroni correction). Based on these results the concentration 375 µg/ mL was selected for chondrogenesis. Belinal® showed significantly higher absorbance of the extracted Alcian blue (and thus chondrogenesis) in comparison with resveratrol and control in MSCs from patients with hip osteoarthritis in non-inflammatory conditions. Alcian blue absorbance (chondrogenesis) was also significantly higher in MSCs treated with Belinal® in comparison with controls when cells were pre-treated with lipopolysaccharide (inflammatory conditions).

**Conclusion:** Our results show significant effect of Belinal® on chondrogenesis of MSCs derived from patients with osteoarthritis, both in non-inflammatory as well as in inflammatory conditions. Our study thus provides the evidence on the capability of Belinal® to boost the chondrogenic potential of MSCs from patients with osteoarthritis.