Introduction: Human fibroblast-like synoviocytes play a central role in the pathogenesis of joint destruction primarily by the secretion of a wide range of pro-inflammatory mediators including cytokines, growth factors and lipid mediators of inflammation. Pro-inflammatory agents produced by fibroblast-like synoviocytes are detrimental to articular cartilage in different joint diseases such as osteoarthritis and rheumatoid arthritis (1).

Pulsed electromagnetic fields (PEMFs) appear as a potential therapeutic approach to control inflammation and manage joint diseases as suggested by the results of several studies. In cartilage explants cultured in vitro, PEMFs increase proteoglycan synthesis preventing the catabolic effect of the pro-inflammatory cytokine IL-1 and in acting synergizing with insulin-like growth factor I (2). In vivo, PEMFs preserve the morphology of articular cartilage and retard the development of osteoarthritic lesions in guinea pig and in clinics they have been successfully used for the treatment of osteoarthritis (3). In addition, the analysis of various studies reporting the use of PEMF for arthritis care has conclusively shown that PEMF not only alleviates the pain in the arthritis condition but it also affords chondroprotection, exerts antiinflammatory action, helps in bone remodeling and this could be developed as a viable alternative for arthritis therapy (4,5).

Adenosine is an endogenous modulator, interacting with four G-protein coupled receptors named as A₁, A₂A, A₂B and A₃, which acts as a potent inhibitor of inflammatory processes in several tissues (6). Recently, mRNA, the protein levels, the functional role of adenosine receptors and their pharmacological modulation in human fibroblast-like synoviocytes isolated from patients with osteoarthritis have been investigated (7). It has been demonstrated that PEMFs evoke an upregulation of A₂A and A₁ adenosine receptors in bovine chondrocytes and fibroblast-like synoviocytes (8). In addition, the presence of PEMFs and adenosine agonists inhibited PGE₂ release in TNF-α or LPS-treated fibroblast-like synoviocytes (1). The aim of this study was to investigate if PEMF exposure could modulate the adenosine receptors of synoviocytes as these cells are heavily involved in joint inflammation. With this background we investigated the presence of A₁, A₂A, A₂B and A₃ adenosine receptors in primary culture of human fibroblast-like synoviocytes in the absence and in the presence of PEMFs. Adenosine receptors were evaluated from a functional point of view to complete their pharmacological characterization. The effect of adenosine agonists and antagonists was investigated on cyclic AMP production in the absence and in the presence of PEMFs.

Materials and Methods: Fibroblast-like synoviocytes were obtained by human synovial tissues and cultured in monolayer in complete medium (Dulbecco’s modified Eagle’s Ham’s F12 (1:1) medium (DMEM/F12) supplemented with 10% FBS. Total cytoplasmic RNA was extracted by the acid guanidinium thiocyanate phenol method. Quantitative real-time RT-PCR assay of A₁, A₂A, A₂B and A₃ mRNAs was carried out using gene-specific fluorescently labelled TaqMan MGB probe (minor groove binder) in a ABI Prism 7700 Sequence Detection System (7).

In western blotting assays human fibroblast-like synoviocytes were lysed in Triton buffer and aliquots of total protein sample were analyzed using antibodies specific for human A₁, A₂A, A₂B and A₃ adenosine receptors. Western blotting assays were also normalized against the housekeeping protein β-actin (7).

Saturation and competition binding experiments on human fibroblast-like synoviocytes membranes were performed by using [³H]-1,3-dipropyl-8-cyclopentyl-xanthine ([³H]-DPCPX), [³H]-2-(7-azano-2-(2-furyl) [1,2,4]-triazolo [2,3-a] [1,3] triazin-5-ylamino) ethylyphenol ([³H]-ZM 241385), [³H]-N-Benzol-[3,1]dioxol-5-yl-2-(5-(2-dixo-1,3-dipropyl-2,3,6,7-tetrahydro-1H-purin-8-yl)-1-methyl-1H-pyrazol-3-yl-oxyl)-acetamide ([³H]-MRE 2029F20) and [³H]-5N-(4-methoxyphenylcarbamoyl) amino-8-propyl-2-(2-furyl) pyrazol [4,3-e,1,2,4-triazolo [1,5-c]pyrimidine ([³H]-MRE 3008F20) as radioligands to study the presence of A₁, A₂A, A₂B and A₃ARs, respectively (9). Non specific binding was determined in the presence of DPCPX, ZM 241385, MRE 2029F20 or MRE 3008F20 at the 1 μM concentration, respectively. At the end of incubation time, bound and free radioactivity were separated by filtering, in a Brandel cell harvester, the assay mixture through Whatman GF/B glass-filter fibers. The filter bound radioactivity was counted in a liquid Scintillation Counter Tri Carb Perkin Elmer 2810TR. To perform cyclic AMP assays well-known adenosine agonists were used including the non-selective adenosine agonist 5’-Nethylcarboxamidoadenosine (NECA), the A₁ agonist N(6)-cyclohexyladenosine (CHA), the A₂B agonist 2-[p-(2-carboxethyl)-phenylamino]-5’-N-ethyl-carboxamido adenosine (CGS 21680) and the A₃ agonist N6-(3-isobenzyl)2-chloroadenosine-5’-N-methyluronamide (CI-IB-MECA). All treatments were performed both in the absence and in the presence of PEMFs (75 Hz, 1.5 mT) (Ikea, Carpi, Italy).

Results: A₁, A₂A, A₂B and A₃ARs mRNA and protein are expressed in human fibroblast-like synoviocytes. In particular high levels of A₂A and A₁ mRNA were found in human fibroblast-like synoviocytes. PEMFs exposure mediated a statistical significant increase of A₂A and A₁ adenosine receptor mRNA. This increase was also confirmed by western blotting assays showing an high A₂B and A₃ protein levels after PEMFs exposure. Saturation binding experiments reveal the increase of A₂A and A₁ receptor density (Bmax values) in PEMF-treated human fibroblast-like synoviocytes. PEMFs treatment did not modify the density of A₂B and A₃ adenosine receptors. Competition binding experiments performed in human fibroblast-like synoviocytes suggested that the affinity values of the adenosine agonists and/or antagonists were not affected by the presence of PEMFs. Cyclic AMP experiments demonstrated that PEMFs treatment was able to increase the potency of the A₁ and A₃ adenosine agonists, CGS 21680 and of the A₂B adenosine agonist, CI-IB-MECA in comparison with untreated cells.

Discussion: In this study we show that PEMFs can modulate the expression and the presence of the anti-inflammatory adenosine receptors as A₁ and A₂ subtypes. These findings suggest that the activation of adenosine receptors following PEMF exposure may significantly contribute to control joint inflammation and open new perspectives to develop local anti-inflammatory treatments finally resulting in a chondroprotective effect for the joint cartilage, especially in early phase of osteoarthritis.