Supplementary File 1

Methods

Patient Recruitment, demographics and arthroscopy

PsA patients with active arthritis were recruited from the Rheumatology Department, St. Vincent's University Hospital. All subjects gave fully informed written consent as approved by the institutional ethics committee and all research was performed in accordance with the Declaration of Helsinki. Eleven patients with PsA were recruited, 7 female and 4 male with median age (range) 49 years (35-60). These patients had a disease activity score (DAS28) of 3.8$\pm$0.39, macroscopic synovitis of 74$\pm$5.3 and macroscopic vascularity of 75$\pm$5.3. Thirty six % sixty were naïve to treatment, 63.6% on synthetic disease modifying anti-rheumatic drug methotrexate and 0.9% on methotrexate+biologic. No significant difference in DAS28 was observed between patients on no treatment compared to DMARD or biologic. Synovial tissue biopsies were obtained at arthroscopy under local anaesthetic using a Wolf 2.7 mm telescope (Storz, Tuttlingen, Germany) as previously described.[1] Biopsies were also obtained from OA patients as a disease comparator. Biopsies were either OCT embedded (TissueTek, Zoeterwoude, The Netherlands) for immunohistochemical analysis, protein lysates, established as ex-vivo PsA whole tissue synovial explant cultures or primary PsA synovial fibroblasts.

Tissue Morphology and Cell viability of PsA synovial biopsies following culture.

To examine cell viability and morphology of PsA synovial tissue explants following culture ex vivo, explants were stained with live viability marker calcein. Following PsA explant culture in 96 well plates, media was removed and explants washed with PBS and incubated for a further 15 mins in calcein (1:1000 in PBS). Explants were then removed from culture, and whole mounted on a glass slide and fluorescent viability marker assessed using a fluorescent microscopy. Further biopsies were snap frozen in OCT embedding media and

stored at 80°C for H&E staining. 7µm OCT sections were cut with a cryostat, placed on glass slides coated with 2% 3aminopropyltriethoxysilane (Sigma-Aldrich Ireland Ltd, Dublin, Ireland) and dried overnight at room temperature. Tissue sections were allowed to reach room temperature, fixed in acetone for 10 mins and air-dried. Sections were incubated with haematoxylin for 2 mins, washed, dehydrated through alcohol and xylene and mounted. Section morphology was examined by light microscopy.

Culture of PsA Synovial fibroblasts.

Primary Psoriatic Arthritis synovial fibroblasts (PsAFLS) were isolated from PsA biopsies by digestion with 1 mg/ml collagenase type 1 (Worthington biochemical, Freehold, NJ, USA) in RPMI 1640 (Life Technologies, BRL, Paisley, UK) for 4 hours at 37°C in humidified air with 5% CO2. Dissociated cells were plated and cultured in RPMI 1640 (Life Technologies, BRL) supplemented with 10% FCS (Life Technologies BRL), penicillin (100 units/ml; Biosciences, Dublin, Ireland), streptomycin (100 units/ml; Biosciences), fungizone (0.25 µg/ml; Biosciences) and HEPES (20 mM; Life Technologies BRL). PsAFLS were grown to confluence and used between passages 38. Cells were cultured in the presence of tofacitinib (1µM) or DMSO vehicle control. Protein lysates were prepared and supernatants collected for cytokine analysis.

Protein preparation

PsA synovial tissue biopsies were powdered using a mikrodismembrator U (B. Braun Biotech International, Melsungen, Germany) as previously described;[12] PsAFLS were trypsinized and collected prior to cell lysis. Icecold RIPA (Radio-Immunoprecipitation Assay) buffer (SigmaAldrich) containing 10µg/ml phosphatase inhibitor cocktail and 10 µg/ml protease inhibitor cocktail (Sigma Aldrich) was used to extract protein from powdered biopsies and PsAFLS pellets. Measurement of protein concentration was performed using a BCA assay

(Pierce Chemical Co, Rockford, IL, USA).

Transwell Invasion assay

Matrigel Invasion Chambers (Becton Dickinson, UK) were used to assess PsAFLS cell invasion in the presence tofacitinib (0.5μM-1μM) or DMSO (1μM) vehicle control for 24 hours. Cells were seeded at a density of $4x10^4$ per well in the invasion chamber on 8 μm membranes pre-coated with matrigel. EGM containing tofacitinib or DMSO control was placed in the lower well of the chamber. Cells were allowed to invade for 24 hours in EBM medium containing 1% FCS. Non-invading cells were removed from the upper surface by gentle scrubbing. Invaded cells adherent to the lower surface were fixed in 1% gluteraldehyde (VWR, Dublin, Ireland) and stained using 1% crystal violet solution (Prolab, Merseyside, UK). The quantification of invading cells was assessed by counting five random high powered fields (Magnification 40x).

Matrigel network formation

Matrigel (50μl) (Becton Dickenson) basement membrane matrix was plated in 96well culture plates and allowed to polymerize at 37^0C in 5% CO2 humidified for 30 minutes.[15] PsAFLS were removed from culture, trypsinized, and resuspended at 2×10^4 cells/ml in RPMI 1640. Cells were cultured with tofacitinib (1μM) or vehicle control for 16 hours. Network formation was examined using phase contrast microscopy and photographed (Mag 20x).

Cell migration

PsAFLS were seeded into 48well plates and serum starved upon confluence in FCS free RPMI 1640 for overnight. A single scratch wound was induced across the middle of each well with a sterile pipette tip. Cells were subsequently cultured in the presence or absence of tofacitinib (1 μM) or vehicle control for 16 hours. Cell migration across the wound margins was assessed using phase contrast microscopy and photographed**.**

Cytokine Quantification Cytokines IL-6, IL-8, IP-10, MCP-1, IL-17, IL-10, MMP3 and TIMP3 were quantified by ELISA(R&D Systems, Abingdon, UK) or MSD multiplex (Mesoscale, Rockville, Maryland , USA) according to manufacturer's instructions. Absorbance was measured in a microtiter plate spectrophometer (Dynatech MR4000, Alexandria, VA) or using MSD Sector Imager 2400.

Gelatin Zymography

The activities of MMP2/9 secreted in cultured supernatants were measured in supernatants by in-gel zymography. Briefly, 1015 µl of supernatants were added into 7.5% polyacrylamide gels consisted of 1 mg/ml gelatin, following incubation with substrate buffer (50 mM Tris, 5 mM CaCl2, pH 7.5), gels were stained with Coomassie brilliant blue R 250 and destained with distilled water.

Statistical Analysis

SPSS15 system for Windows was used for statistical analysis. Nonparametric Wilcoxon Singed Rank test for analysis of PsAFLS and PsA synovial tissue was performed. $p<0.05$ were considered statistically significant.