Tendon conditioned media and its effect on THP-1 cell morphology

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Introduction

Tendons are tissues with a fibrous nature, that consist of densely aligned collagen fibrils that connect muscle to bone. Tendon injuries account for a significant health care burden(Dunkman *et al*., 2013). Tendon injuries affect around 30 million people annually (Maffulli et al 2003). There are a variety of ways that these injuries arise from; ageing, trauma, overuse or obesity. These injuries cause pain, dysfunction and inflammation.The main draw back with both conservative and surgical approaches is that none provide complete restoration of native tendon functionality. There is a growing need for treatments with better outcomes for patients

Tenocytes share similarities with fibroblasts , they are the cells that eventually form mature tendon. Tenocytes main function is to synthesis the Extracellular matric(ECM), assembly of collagen fibers , that provide the basic units of the tendon. The collagen fibrils are crosslinked to one another and are wrapped within a tendon sheath. Each tendon or ligament differs in its specific ECM composition along with size and strength (Maeda *et al*., 2010).

The specific control of the immune response provides a goal for future research within regenerative medicine to allow for regeneration of the damaged tissue. Harnessing the ability to produce more of the regulatory macrophages has great therapeutic potential to treat a variety of inflammatory musculoskeltal conditions and eventually a bank of M1 and M2 macrophage bank that utilizes instructions from SFCM from a Tenocytes(Aldo *et al*., 2013). The present study tested the efficacy of SFCM collect from Tenocytes cultured in 21% o2, upon activation of the THP-1 cells to M1 and M2 differentiation via morphological and cell number analysis to identify the activity of SFCM on the M1 and M2 cells, based on previous research that confirms THP1 differentiate into macrophage. Previous research also states that tendon cells produce a number of cytokines such as TGFb (Lavagnino *et al*., 2015).

Immortalized cell line THP1 will be used due to their cost effectiveness , easy use and don't need the same ethical approval as human or primary cells. Also cell lines are characterized by their pure population of cells that provides consistent sample and reproducible results. The drawback however is that they are genetically different , this may change their phenotype, stimulus response and native functions. Secondly cell line sub culturing also has the potential to cause heterogeneity and genetic drift over time (Barilli *et al*., 2011).

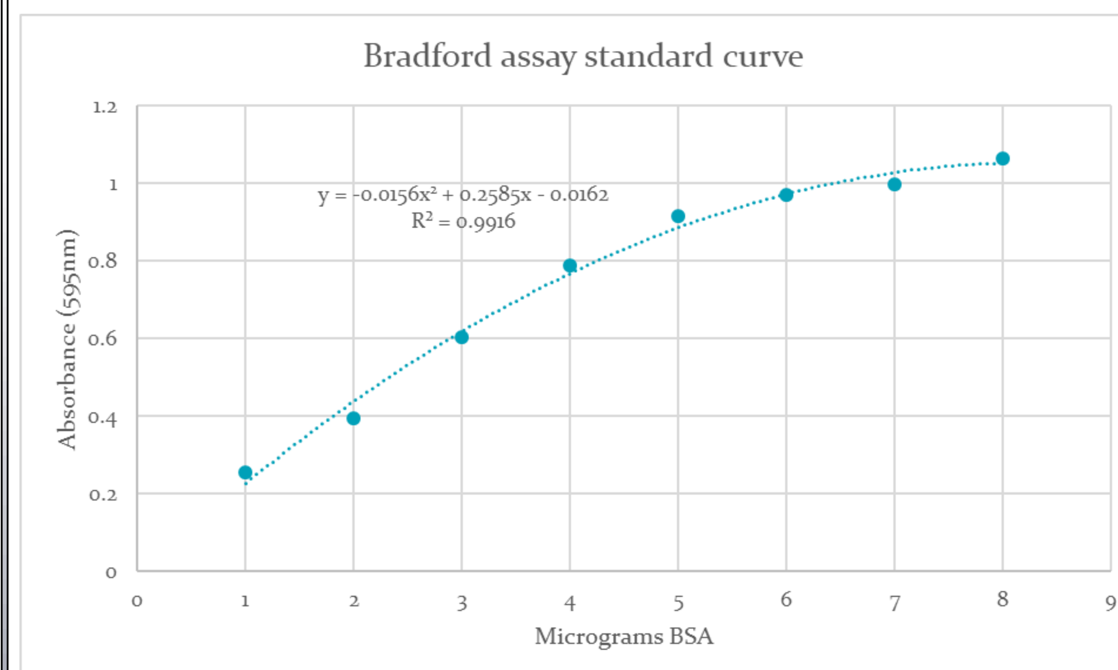
Methods

1 Preparation of serum free conditioned(SFCM) media of tenocytes

Tenocytes were grown to 70% confluency to passage 3 in T75 t tissue culture flasks in GM. To collect the conditioned media the confluent flasks were washed twice with 15ml of pbs then 16 ml of 2.5% FBS in GM was added for 24 hours , after 24 hours the media was collected in 50ml falcon tubes and filtered to remove any debris. Again cells were washed with pbs and then 16ml of SFCM was added for 24hrs and subsequently filtered through a sterile 0.2um cellulose acetate syringe filter.

2 protein quantification of conditioned media To understand how much protein is present within the conditioned media obtained from tenocytes, we utilized the Bradford assay to produce a standard curve and were able to calculate the amount of protein in the conditioned media. The standard curve was produced using Bovine serum album (BSA) for protein standards, a 96 well plate was used and standards were done in triplicate and range from 0-85ug of BSA. The plateaued area of the curve was not used to calculate the protein levels in the conditioned media(see figure 1).

Figure 1 : Bradford assay standard curve:



3 THP-1 activation via PMA cultured in conditioned media.

Media derived from tenocytes to test the immune response of THP-1 cells after their activation with PMA/PHA .The activation of the THP-1 cells was achieved by adding 50ul of PMA and 13ul of PMA at concentrations of 100mg/ml. The activation for the THP 1 cells cultured in GM, SFNCM AND SFCM.

Results

Figure 2:Protein concentration of conditioned media

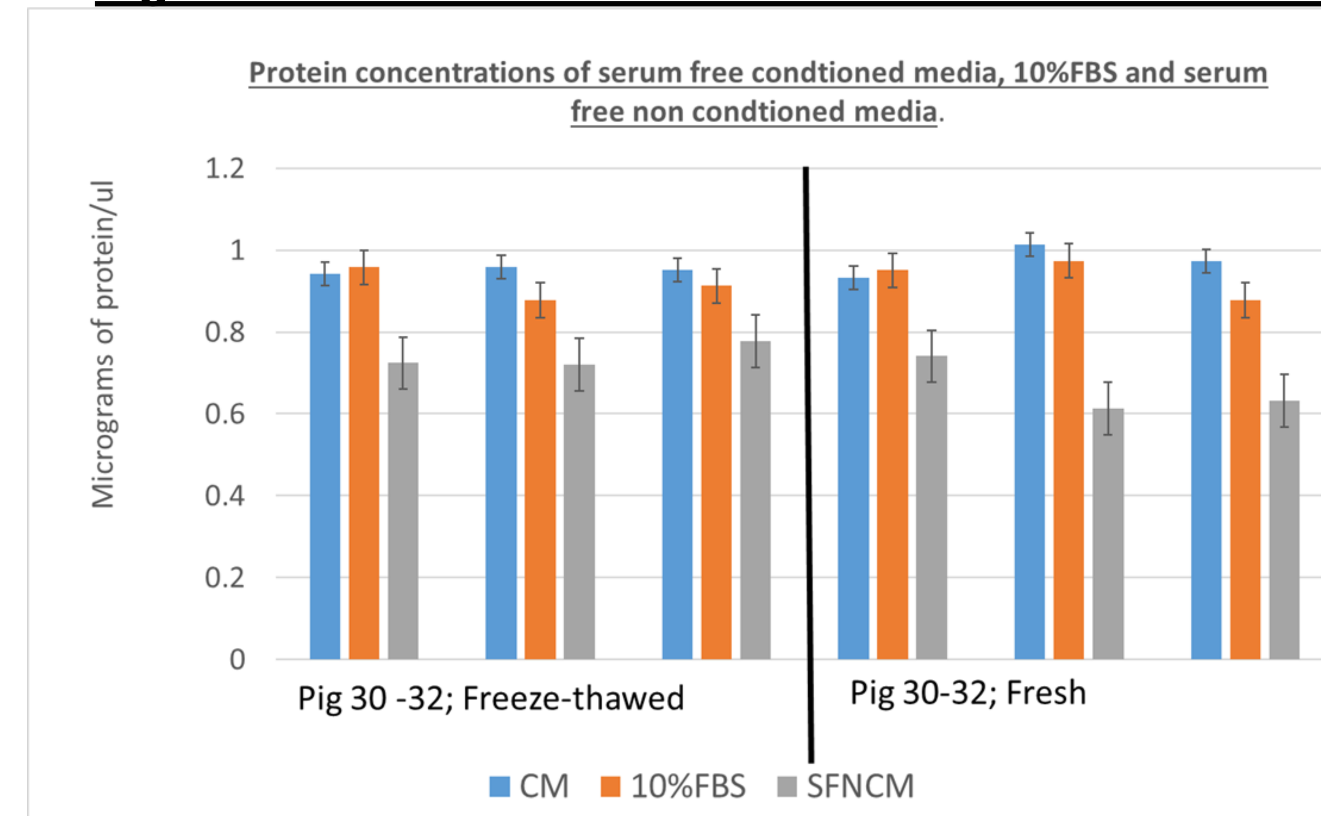
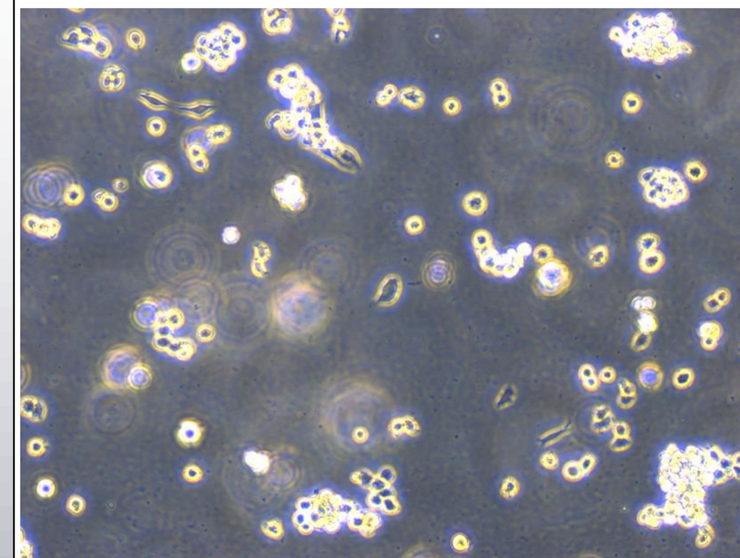
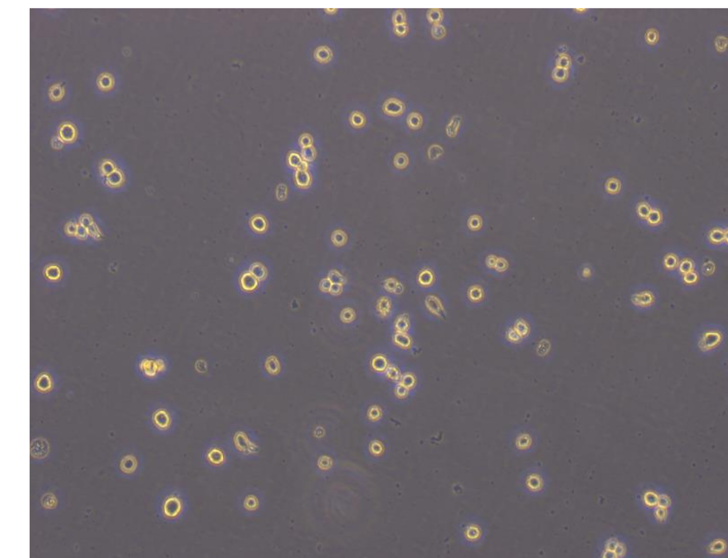


Figure 3 THP-1 cells activated with PMA/PHA in SFCM and NCM

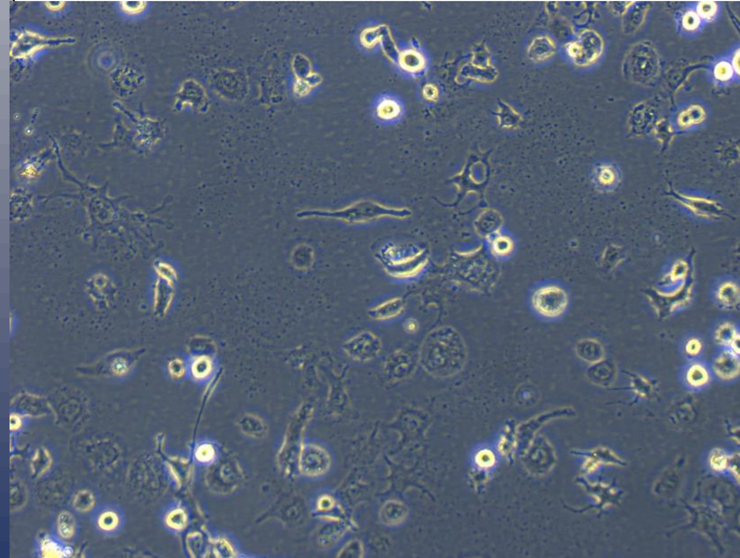
THP-1 cells grown in tendon conditioned media (21% oxygen)



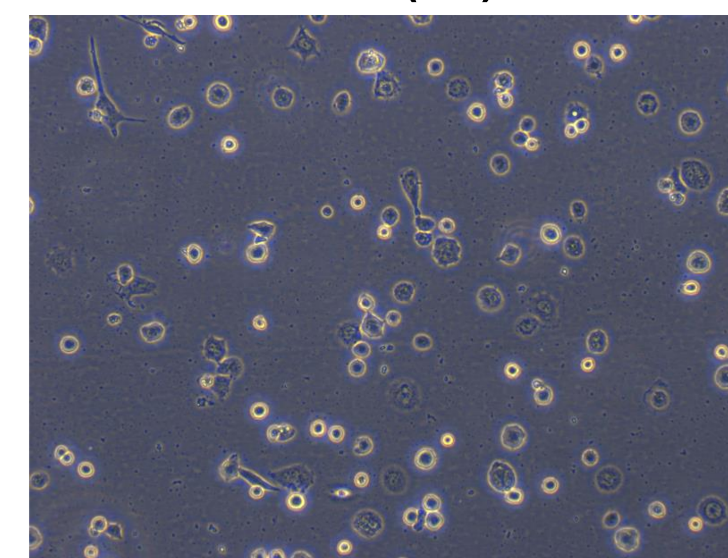
THP-1 cells grown in non-conditioned media (21%)



THP-1cells activated and grown in conditioned media (2%)



THP-1 cells activated and grown in non- conditioned media (2%)



Conclusions

In this study activated THP1 cells in serum free NCM demonstrated adherence without dramatic morphology changes (size/shape).

Macrophages are vital for the initiation of an immune response towards antigen presentation , which is a pro inflammatory action compared with regeneration which is ant inflammatory. There are few studies focusing on tenocytes ability to generate macrophages with immunoregulatory activity.

THP1 macrophages are triggered by PMA to differentiate into MO, there final differentiation utilizes the presences of cytokines for examples pro inflammatory cytokines causes differentiation into M1 and pro healing cytokines cause their differentiation into m2. In this study THP1 were activated to produced terminal differentiation in SFCM in comparison to SFNCM.

THP1 cells cultured in tendon conditioned media induced more M2 macrophage differentiation indicated by the presence of their shape when compared with the SFNCM group. To conclude this study it has confirmed that SFCM did induce terminal macrophage differentiation , this can be explained by the factors secreted by tenocytes within the SFCM that are important stimuli to trigger the conversion of monocytic THP1 cell line to the M0 phenotype(Garcia-Melchor et al., 2019). This step is directly related to the presence of either inflammatory in anti-inflammatory cytokines. further research is needed to identify the specific cytokines that direct the macrophage differentiation.

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