# Investigation of the Presence of Telocyte-like Cells in Human Patellar Fat Pad Tissue

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Stromal cells remain the focus of research in tissue biology from past to present. It is known that the stroma hosts mainly fibroblasts, pericytes, neurons, endothelial cells and immune cells. These cell types also have various distinctive features due to their different morphologies and functional properties. In addition, other different cell types have also been described in the stroma, including the interstitial cells of Cajal and telocytes. Telocytes (TC), which are characterized with small cell bodies and long telopods, have been identified in the connective tissue of many organs and are strategically positioned between target cells, near nerve endings and capillaries. Telocytes coordinate tissue homeostasis by integrating information from multiple sources and their extracellular vesicles provide bidirectional communication between them and the other stromal cells. They are able to regulate stem cell proliferation and differentiation through their secretome and play crucial roles in embryogenesis, angiogenesis, and various diseases, including cancer. Human telocytes have so far beenidentified in the heart, lung, brain, eye, thyroid, skeletal muscles, skin, gastrointestinal tract and accessory glands, urinary system, and male and female reproductive systems. But there is no information about the relationship between the telocyte cells and the Infrapatellar Fat Pad (IPFP). The approach to serious joint diseases such as osteoarthritis (OA), which is highly prevalent in our society and causes significant morbidity in the population and treatment is currently limited to microfracture treatments, autologous chondrocyte transplantations and prosthetic surgery. The incidence of symptomatic OA in the obese and elderly population and the high costs of joint replacement surgeries are increasing every year. Therefore, in addition to current treatments, the search for tissue engineering and stem cell-based research continues to meet the needs for alternative treatment methods. In recent years, tissue engineering studies have emerged as promising methods in orthopedic treatment approaches. Difficulties that may be experienced in obtaining mesenchymal stem cells (MSCs) obtained from various sources may also lead to undesirable pathological conditions such as heterogeneity in the obtained stem cell populations and teratoma formation in new cartilage cell differentiation. For this reason, researchers are now focusing on different cellular sources. Infrapatellar fat pad (IPFP), also known as Hoffa; fat pad due to its proximity to articular cartilage, in recent years has been the focus of researchers working on the relationship between cartilage regeneration and the surrounding stem cells. Studies have shown that IPFP has a good stem cell reserve and is very suitable for studies based on cartilage retrieval. On the other hand, there are also studies showing that the use of IPFP-derived stem cells in repairing joint cartilage damage may be limited due to their heterogenity. In recent years, the presence of a unique cell group called telocytes, which is in close relationships with the stem cell niche, has been described in the stroma of many organs. These cells can stimulate stem cells with the microstructures they secrete called exosomes and induce tissue regeneration by creating cellular differentiation. The existence of infrapatellar fat pad cells with surface markers same as telocytes have been reported in only one article in the literature. TC markers including CD34, vimentin, PDGRF-α and PDGRF-β, c-kit, and α-SMA are frequently used in telocyte studies and are regarded trustworthy. Double immunofluorescence is the most accurate and precise method for identifying these cells.

Aims:
This research aims to advance our understanding of the heterogenity of IPFP cells and detect the presence of TCs by employing techniques such as immunohistochemistry and immunofluorescence. Through the investigation of their morphology and identification of telocytes in the IPFP stroma, this study endeavors to provide novel insights into IPFP architecture and its implications for regenerative medicine.

Methods:
In this study, IPFP tissues were obtained from 5-10 patients preparing for knee replacement surgery at Sadi Konuk Training and Research Hospital, Orthopedics and Traumatology Clinic. After obtainiing the specimen one small piece of it underwent tissue preparation procedures for light microscopy and immunohistochemical staining was performed using CD34 which is the most common marker for telocyte identification. The rest of the specimen was used for cell culture from which these cells were isolated using tissue digestion agent such as collagenase II and cell strainers of different diameters in order to separate them from other stromal cells with the cell adhesion method and subculturing the media in which the telocytes were present during 96 hours. The cells were photographed at different incubation times such as at 24h, 48, and 96h and were examined under the inverted microscope. Finally the cells resembling to telocytes with their long projections were analyzed by double immunofluorescence staining used CD34/c- kit, CD34/vimentin, CD34/PDGFR- α and β, and CD34/ α-SMA markers for telocyte identification. This comprehensive approach aimed to illuminate telocyte presence and distribution in the IPFP stroma, offering future insights into IPFP physiology and pathophysiology.

Results:
In this study, the existence of telocytes within the IPFP was identified by their different shape and immunophenotypes. We identified telocytes by immunohistochemical analysis, labeling with CD34, the most extensively utilized telocyte marker. We used immunofluorescence analysis with double labeling of CD34/c-kit, CD34/vimentin, and CD34/PDGFR α and β, CD34/α-SMA in order to distinguish these cells from fibroblasts and pericytes in cell culture.

We found out that TCs were strongly positive for CD34, PDGFR α and β, and vimentin and weakly positive for α-SMA. On the other hand fibroblasts and were found to be CD34 negative, while strongly positive for vimentin and PDGFR α and β. Notably, our findings delineate telocyte cells from fibroblasts and pericytes, underscoring their unique cellular identity within the IPFP microenvironment. TCs were recognized for their long and thin prolongations and small cell bodies primarily situated around blood vessels and dispersed throughout the IPFP stroma.

Conclusion:
Our study adds greatly to our understanding of IPFP telocyte biology by shedding light on their presence, distribution, and morphological properties. As a result, these cells have the potential to be a unique progenitor cell source for cartilage regeneration, and they need full attention from researchers in the tissue engineering field.

**Keywords:**

Infrapatellar Fat Pad, Telocytes