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Original article

Proliferation and differentiation of stem cells in contact with eluate from fibrin-rich plasma membrane

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ABSTRACT

Objective: To evaluate the ability of the eluate from fibrin-rich plasma (FRP) membrane to induce proliferation and differentiation of isolated human adipose-derived stem cells (ASCs) into chondrocytes.

Method: FRP membranes were obtained by centrifugation of peripheral blood from two healthy donors, cut, and maintained in culture plate wells for 48 h to prepare the fibrin eluate. The SCATh were isolated from adipose tissue by collagenase digestion solution, and expanded *in vitro*. Cells were expanded and treated with DMEM-F12 culture, a commercial media for chondrogenic differentiation, and eluate from FRP membrane for three days, and labeled with BrdU for quantitative assessment of cell proliferation using the High-Content Operetta® imaging system. For the chondrogenic differentiation assay, the SCATh were grown in micromass for 21 days and stained with toluidine blue and aggrecan for qualitative evaluation by light microscopy. The statistical analysis was performed using ANOVA and Tukey's test.

Results: There was a greater proliferation of cells treated with the eluate from FRP membrane compared to the other two treatments, where the ANOVA test showed significance ($p < 0.001$). The differentiation into chondrocytes was visualized by the presence of mucopolysaccharide in the matrix of the cells marked in blue toluidine and aggrecan.

Conclusions: Treatment with eluate from FRP membrane stimulated cell proliferation and induced differentiation of the stem cells into chondrocytes, suggesting a potential application of FRP membranes in hyaline cartilage regeneration therapies.

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Proliferação e diferenciação de células-tronco em contato com eluato de membrana de fibrina

Palavras-chave:
Plasma rico em plaquetas
Membranas
Cartilagem
Regeneração

RESUMO

Objetivo: Avaliar a capacidade do eluato proveniente da membrana de plasma rico em fibrina (PRF) de induzir proliferação e diferenciação das células-tronco humanas isoladas de tecido adiposo (CTDAh) em condrócitos.

Método: As membranas de PRF foram obtidas por centrifugação de sangue periférico de dois indivíduos saudáveis, cortadas, colocadas em poços de placa de cultivo por 48 h para obtenção do eluato de fibrina. As CTDAh foram isoladas do tecido adiposo por digestão com solução de colagenase e expandidas *in vitro*. As células foram expandidas e tratadas com meio de cultivo DMEM-F12, meio comercial para diferenciação condrocítica, e eluato de fibrina durante três dias e marcadas com BrdU para avaliação quantitativa da proliferação celular com o uso do sistema de imagens High-Content Operetta®. Para o ensaio de diferenciação condrogênica, as CTDAh foram cultivadas em micromassa por 21 dias e coradas com azul de toluidina e agrecana para avaliação qualitativa em microscópio óptico. As avaliações estatísticas foram feitas por meio dos testes Anova e Tukey.

Resultados: Houve uma maior proliferação das células tratadas com o eluato de fibrina comparativamente com os outros dois tratamentos, nos quais o teste Anova apontou significância ($p < 0{,}001$). A diferenciação em condrócitos foi visualizada pela presença de mucopolissacarídeos na matriz das células tratadas com meio de diferenciação ou eluato e marcação positiva para agrecana.

Conclusões: O tratamento com o eluato da membrana de fibrina estimulou a proliferação celular e induziu a diferenciação das células-tronco em condrócitos, o que sugere uma potencial aplicação da membrana de PRF nas terapias de regeneração de cartilagem hialina.

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Introduction

Among the degenerative diseases that affect the elderly, degradation of articular cartilage tissue, a process known as osteoarthritis or osteoarthrosis (OA) is one of the most common.[1]

The functionality of the cartilage depends on the integrity of its extracellular matrix (ECM) and on the arrangement of its molecular components. The susceptibility of articular cartilage to progress to OA is due to its limited autoregeneration capacity, caused by the low mitotic activity of chondrocytes and their avascular nature.[2,3]

In large weight-bearing joints that are subjected to friction, cartilage defects do not regenerate spontaneously and require therapeutic intervention. Conventional treatment for the repair of cartilage defects, such as non-surgical approaches (*e.g.*, glucosamine, steroids, and hyaluronic acid injections) or surgical treatment (*e.g.*, debridement) only relieve pain and do not restore the joint surface.[4] Therefore, traditional techniques are palliative. Washing and chondroplasty promote symptomatic relief of pain without hyaline tissue formation. These techniques remove the superficial layer of the cartilage, including the collagen fibers, which are responsible for the mechanical resistance and create a cartilage tissue with inferior functionality. The subchondral debridement or microfracture technique has been considered as a stimulant for the production of hyaline-like tissue, whose properties and durability are comparable to that of normal cartilage. However, in many cases a formation of fibrocartilaginous tissue that degenerates over time has been observed. Autologous osteochondral transplantation and mosaicplasty (cartilage autotransplantation) may restore cartilage tissue, but its applications are restricted to small defects; there are also some concerns regarding morbidity of the donor site.[5] The treatment of OA and local articular cartilage defects remains challenging. There are currently no surgical or non-surgical treatments that repair or restore the damaged surface.

Accordingly, current treatment methods do not provide a satisfactory long-term outcome; this fact has stimulated studies into innovative approaches in tissue engineering with the use of biomaterials as frameworks for new tissue formation.[6,7] Some of the biomaterials are natural and autologous, such as a membrane of fibrin-rich plasma (FRP). The fibrin membrane can be readily isolated from plasma from the patient's peripheral blood through centrifugation, when a dense FRP membrane is formed and can be readily used after exudation. The FRP exudate contains significant amounts of growth factors in addition to the glycoprotein matrix, particularly fibronectin and vitronectin, two key proteins that allow extracellular and cell-matrix contact.[8]

Fibrin membrane, therefore, is an autologous natural biomaterial that is rich in glycoproteins and growth factors, easy to prepare, and inexpensive. Due to its autologous nature, there is no risk of infection or onset of autoimmune processes. Therefore, this study is aimed at evaluating the ability of FRP membrane to induce proliferation and

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