In vitro release and expansion of mesenchymal stem cells by a hyaluronic acid scaffold used in combination with bone marrow

Marco Spoliti,
Paola Iudicone,
Rossella Leone,
Alessandro De Rosa,
Francesca Romana Rossetti,
Luca Pierelli

Department of Orthopaedics and Traumatology, San Camillo Hospital, Rome, Italy

Corresponding author:
Marco Spoliti
Department of Orthopaedics and Traumatology
San Camillo Hospital
Circonvallazione Gianicolense 81, Rome, Italy
e-mail: marcodoc@me.com

Summary

Articular cartilage injuries of the knee are difficult to treat due to the poor healing ability of cartilage and conventional treatment methods often give unsatisfactory results. Mesenchymal Stem Cells (MSCs) have generated interest as an alternative source of cells for cartilage tissue engineering due to their chondrogenic potential and their easy isolation from bone marrow. It has been reported that the use of scaffold in cartilage engineering acts as a support for cell adhesion, keeping the cells in the cartilage defects and therefore facilitating tissue formation, and that Hyaluronic acid (HA) is a molecule of particular interest for producing scaffold for tissue engineering.
In this study we evaluated the *in vitro* selection and expansion of Bone Marrow MSCs (BM-MSCs) and by residual BM+HA membrane (BM-HA-MSCs) used as scaffold. Sixty mL of BM have been aspirated by the posterior iliac crest and HA membrane (Hyalograft-C, Fidia Advanced Biopolimers) was used as scaffold. BM-MSCs were cultured with D-MEM supplemented with Desamethasone, Ascorbic Acid, -Transforming Growth Factor and Insulin. When cultured in chondrogenic selective medium MSCs from both BM and HA membrane were able to differentiate into chondrogenesis, but BM-HA-MSCs showed a higher staining intensity than BM-MSCs when they were stained with Toluidine blue. The interaction of MSCs with the HA-scaffold seems to promote by itself chondrogenesis.

Key words: bone marrow, cartilage injuries, mesenchymal stem cells, tissue engineering.

Introduction

Autologous chondrocyte implantation was the first-generation cell therapy for cartilage repair started more than 20 years ago and the treatment of articular cartilage lesions of the knee, using chondrocytes seeded into a scaffold, has been investigated in several clinical trials[1-3]. However the application of autologous chondrocytes in cartilage repair procedure is associated with several disadvantages including injury of healthy cartilage and the need of a two step procedure surgeries. Mesenchymal stem cells (MSC) have generated interest as an alternative source for cartilage tissue engineering since they have the ability to self replicate and to differentiate down multiple cell lineages, including chondrocytes[4-6]. The use of MSC in tissue repair is evolving in different approaches which include either the use of entire bone marrow (BM) or *in vitro* expanded MSC re-administered either as cell suspension or by using scaffolds or hydrogel. MSC are typically obtained by BM, but they can be isolated from several other tissues among which umbilical cord blood, placenta, adipose tissue etc.[7]
It has been reported that BM is the MSC source an appropriate in the setting of cartilage regeneration to treat focal lesions[8,9].
The use of scaffold in cartilage engineering acts as a support for cell adhesion, keeping the cells in the cartilage defects and therefore facilitating tissue formation. In recent years both natural and synthetic biomaterials have been used to create 3D scaffold capable of inducing and enhancing tissue generation through cell organization, mechanical forces and bioactive molecule delivery. A molecule of particular interest for producing scaffold for tissue engineering is hyaluronic acid (HA) since it is found natively in cartilage tissue[10,11].
In this study we evaluated the *in vitro* selection and expansion of MSC obtained respectively by samples of BM after concentration and samples of HA membrane additioned with BM and platelet enriched plasma (PRP) and used as scaffold in the treatment of patients with severe chondral lesions.

Materials and methods

Samples

Five patients (3 males and 2 females age 19-42 years) underwent knee arthroscopy. Intra-operatively 60 mL of