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Review

Human amniotic membrane as an alternative source of stem cells for regenerative medicine

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ABSTRACT

The human amniotic membrane (HAM) is a highly abundant and readily available tissue. This amniotic tissue has considerable advantageous characteristics to be considered as an attractive material in the field of regenerative medicine. It has low immunogenicity, anti-inflammatory properties and their cells can be isolated without the sacrifice of human embryos. Since it is discarded post-partum it may be useful for regenerative medicine and cell therapy. Amniotic membranes have already been used extensively as biologic dressings in ophthalmic, abdominal and plastic surgery. HAM contains two cell types, from different embryological origins, which display some characteristic properties of stem cells. Human amnion epithelial cells (hAECs) are derived from the embryonic ectoderm, while human amnion mesenchymal stromal cells (hAMSCs) are derived from the embryonic mesoderm. Both populations have similar immunophenotype and multipotential for in vitro differentiation into the major mesodermal lineages, however they differ in cell yield. Therefore, HAM has been proposed as a good candidate to be used in cell therapy or regenerative medicine to treat damaged or diseased tissues.

Contents

1. Mesenchymal stem cell therapy as a new clinical approach to treat osteoarthritis

Osteoarthritis (OA) is a degenerative joint disease characterized by deterioration in the integrity of hyaline cartilage and subchondral bone (Ishiguro et al., 2002). OA is the most common articular pathology and the most frequent cause of disability. Genetic, metabolic and physical factors interact in the pathogenesis of OA producing cartilage damage. The incidence of OA is directly related to age and is expected to increase along with the median age of the population (Brooks, 2002).

The prevalence of OA in the human population underscores the importance of developing an effective and functional articular cartilage replacement. Recent research efforts have focused on tissue engineering as a promising approach for cartilage

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regeneration and repair (Kuo et al., 2006). Cartilage tissue engineering is critically dependent on the selection of appropriate cells, suitable scaffolds for cell delivery and biological stimulation with chondrogenically bioactive molecules (Kuo et al., 2006).

Articular cartilage receives its nourishment through diffusion from the synovial fluid. The capacity for the self-repair of articular cartilage is very limited, mainly because it is an avascular tissue (Steinert et al., 2007; Mankin, 1982; Resinger et al., 2004; Fuentes-Boquete et al., 2008). Consequently, progenitor cells in blood and marrow cannot enter the damaged region to influence or contribute to the reparative process (Steinert et al., 2007).

There are a lack of reliable techniques and methods to stimulate growth of new tissue to treat degenerative diseases and trauma (Wong et al., 2005). Currently, there are no effective pharmaceutical treatments for OA, although some medications slow its progression (Brandt and Mazzuca, 2006; Steinert et al., 2007). There are also no surgical approaches to treat OA; however, surgery is an important tool for the repair of cartilage injuries, which if left untreated may result in secondary OA.

To date, most efforts made to repair an articular cartilage injury are intended to overcome the limitations of this tissue for healing by introducing new cells with chondrogenic capacity (Koga et al., 2008) and facilitating access to the vascular system. Of the numerous treatments available nowadays, no technique has yet been able to consistently regenerate normal hyaline cartilage. Current treatments generate a fibrocartilaginous tissue that is different from hyaline articular cartilage. To avoid the need for prosthetic replacement, different cell treatments have been developed with the aim of forming a repair tissue with structural, biochemical and functional characteristics equivalent to those of natural articular cartilage. The overall objective is not only to heal the chondral defect (repair), but to generate new tissue identical to native articular cartilage in structure, biochemical composition and functional behavior (regeneration) (Fuentes-Boquete et al., 2007).

Cell therapy is a new clinical approach for the repair of damaged tissues. Cell therapy using MSCs (Koga et al., 2008) or differentiated chondrocytes (autologous chondrocyte implantation, ACI) is one therapeutic option for the repair of focal lesions of articular cartilage, which is most successful in young people producing repair tissue of high quality (Brittberg et al., 1994; Minas and Chiu, 2000).

MSCs are multipotent non-hematopoietic progenitors located within the stroma of the bone marrow and other organs that are phenotypically characterized by the expression of several markers (e.g., CD73, CD90 and CD105) and the lack of expression of CD14 or CD11b, CD19 or CD79α, CD34, CD45 and HLA-DR surface molecules (Mrugala et al., 2009; Kastrinaki et al., 2008). Moreover, characteristics of MSCs are also the expression of surface markers like Stro-1, CD44, CD73, CD90, CD105 and CD166 (Pittenger et al., 1999). According to a recent proposal of the International Society for Cellular Therapy (Dominici et al., 2006) there are three criteria to define all types of stem cells: self-renewal, multipotency and the ability to reconstitute a tissue *in vivo*. Because there is no specific marker for MSCs, the principal criteria for identification are fibroblast-like morphology, adherence to the plastic of the tissue culture flask (Prockop, 1997), the prolonged capacity for proliferation and the potential to differentiate in vitro into cells of mesodermal lineage.

MSCs can be isolated by adherence to plastic, expanded *ex vivo* and induced, both in vitro or *in vivo*, to terminally differentiate into ectodermal (e.g., neurons) and endodermal (e.g., hepatocytes) lineages (Pasquinelli et al., 2007) and also into cell of mesodermal origin (e.g., osteocytes, chondrocytes, adipocytes, tenocytes, myotubes, astrocytes and hematopoietic-supporting stroma) (Barlow et al., 2008; Minguell et al., 2000; Caplan, 1991). MSCs derived from bone marrow show a higher potential for osteogenic differentiation (Muraglia et al., 2000), while MSCs of synovial origin show a greater tendency toward chondrogenic differentiation (Djouad et al., 2005). Under identical culture conditions for differentiation, MSCs isolated from the synovial membrane show more chondrogenic potential than those derived from bone marrow, periostium, skeletal muscle or adipose tissue (Sakaguchi et al., 2005). These results indicated that MSCs from different tissue sources can have biologic distinctions. Studies of cartilage injury repair in animal models using MSCs embedded in collagen gel (Wakitani et al., 1989) or injected into defects closed with periosteal membrane (Im et al., 2001) indicate that MSCs can differentiate *in vivo* into a number of cell types in different biologic environments.

The recent use of autologous or allogenic stem cells has been suggested as an alternative therapeutic approach for treatment of cartilage defects (Jung et al., 2009). MSCs have the capability to self-renew and are responsible for repair and repopulation of damaged tissues in the adult (Hombach-Klonisch et al., 2008; Pittenger, 2008; Tsai et al., 2007). The use of autologous MSCs represents the advantage of avoiding the problem of immunological rejection of the allotransplant and the ethical conflict of using human embryonic stem cells (hESCs). Due to the low number of MSCs that can be isolated from a tissue biopsy, proliferation in vitro is necessary to obtain adequate cell numbers for their implant into the patient. Nevertheless, the number of mitotic divisions of MSCs in culture must be limited because MSCs age during in vitro culture, causing a reduction in their proliferative and multi-differentiation potential (Banfi et al., 2000; Bonab et al., 2006; Izadpanah et al., 2006). The conservation of phenotype and differentiation capacity of MSCs is proportional to telomerization (Abdallah et al., 2005). Telomeres are normally shortened in successive cell divisions, however, in embryonic stem cells the telomere length is restored by telomerase enzyme activity. On the other hand, MSCs lack (Zimmermann et al., 2003) adequate levels of telomerase activity to achieve telomeric restoration (Izadpanah et al., 2006; Parsch et al., 2004; Yanada et al., 2006). Patient age also influences the characteristics of MSCs because their proliferative capacity is reduced by aging (Stenderup et al., 2003).

Human MSCs have been isolated from several tissues such as bone marrow (Kastrinaki et al., 2008; Yoo et al., 1998), articular cartilage (Alsalameh et al., 2004), synovial membrane (De Bari et al., 2001; Fickert et al., 2003), perichondrium (Dounchis et al., 1997), periostium (Nakahara et al., 1990), connective tissue of dermis and skeletal muscle (Young et al., 2001), adipose tissue (Zuk et al., 2001, 2002), peripheral blood (Villaron et al., 2004; Kuznetsov et al., 2001; Zvaifler et al., 2000), liver (Le Blanc et al., 2005), lung (InťAnker et al., 2003), placenta (Barlow et al., 2008, Steigman and Fauza, 2007; Fauza, 2004: Matikainen and Laine, 2005), umbilical cord (Baksh et al., 2007; McGuckin et al., 2005; Samuel et al., 2008), umbilical cord blood (Mareschi et al., 2001), amniotic fluid (You et al., 2008; Steigman and Fauza, 2007; Fauza, 2004) and amniotic membrane (Díaz-Prado et al., 2010a, 2010b; Alviano et al., 2007). Moreover, the list of tissues with the potential for tissue engineering is increasing because of recent progress in stem cell biology (Bianco and Robey, 2001).

Although bone marrow is the traditionally used tissue source of adult MSCs, it has some limitations. Among the most important limitations are accessibility and that the procedure required to obtain this kind of tissue is invasive, painful and possibility of donor site morbidity, that the number of MSCs obtained is low, and that the potential to proliferate and differentiate diminishes as the donor's age increases (Soncini et al., 2007; Baksh et al., 2007; Wei et al., 2009; Ilancheran et al., 2009). The identification of alternative sources of MSCs would be beneficial for both research and therapeutic purposes.