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A Comparison of Culture Characteristics between Human Amniotic Mesenchymal Stem Cells and Dental Stem Cells

[1]Nurul Hidayat Yusoff, [1]Saaid Ayesh Alshehadat, [1,2]Ahmad Azlina*, [1,2]Thirumulu Ponnuraj Kannan and [3]Suzina Sheikh Abdul Hamid

[1]Basic Sciences and Oral Biology Unit, School of Dental Sciences,
[2]Human Genome Centre, School of Medical Sciences,
[3]Tissue Bank, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia

Abstrak: Semenjak sedekad yang lalu, bidang biologi sel stem menjadi tarikan utama dalam kalangan penyelidik kerana potensi terapeutiknya yang luas. Sel stem adalah satu kelas sel tak terbeza yang berupaya membeza kepada sel jenis khusus. Sel stem boleh dikelaskan kepada dua jenis iaitu sel-sel stem dewasa (tisu dewasa) dan sel-sel stem embrio (embrio yang terbentuk semasa fasa blastosis dalam pembentukan embrio). Artikel ini akan membincangkan mengenai dua jenis sel stem mesenkim dewasa; sel stem gigi dan sel stem amnion dari segi keturunan pembezaan, bilangan pemindahan dan kajian model haiwan. Sel stem amnion mempunyai bilangan keturunan pembezaan yang lebih tinggi berbanding sel stem gigi. Sebaliknya, peringkat bilangan sub-kultur sel stem gigi adalah lebih banyak berbanding sel stem amnion. Untuk pertumbuhan semula tisu, sel stem amnion mengambil masa yang paling singkat bagi penjanaan semula berbanding sel stem gigi, berdasarkan kajian model haiwan.

Kata kunci: Sel Stem Gigi, Sel Stem Amnion, Keturunan Pembezaan, Peringkat Bilangan Sub-Kultur

Abstract: In the past decade, the field of stem cell biology is of major interest among researchers due to its broad therapeutic potential. Stem cells are a class of undifferentiated cells that are able to differentiate into specialised cell types. Stem cells can be classified into two main types: adult stem cells (adult tissues) and embryonic stem cells (embryos formed during the blastocyst phase of embryological development). This review will discuss two types of adult mesenchymal stem cells, dental stem cells and amniotic stem cells, with respect to their differentiation lineages, passage numbers and animal model studies. Amniotic stem cells have a greater number of differentiation lineages than dental stem cells. On the contrary, dental stem cells showed the highest number of passages compared to amniotic stem cells. For tissue regeneration based on animal studies, amniotic stem cells showed the shortest time to regenerate in comparison with dental stem cells.

Keywords: Dental Stem Cells, Amniotic Stem Cells, Differentiation Lineages, Number of Passages

INTRODUCTION

Stem cells (SCs) are one of the recent scientific findings of the 21st generation and have led to some parts of the fundamental knowledge of biological cells

*Corresponding author: azlinakb@usm.my

being rewritten. Embryonic SCs are derived from embryos, while adult, or somatic SCs, come from somatic cells. Both types are generally characterised by their plasticity. However, the pluripotentiality of embryonic SCs surpasses adult SCs. Although embryonic SCs seem to be the gold standard in terms of plasticity, ethical issues have capped it from being used widely, while adult SCs are multipotent SCs with less plasticity (Raff 2003) but generally raise no ethical issues. Adult SCs derived from bone marrow have been extensively studied (Woodbury *et al.* 2000; Terada *et al.* 2002). The cells are capable of differentiating into haematopoietic lineages (Huang & Terstappen 1992) and non-haematopoietic lineages (Le Blanc *et al.* 2003). Another type of multipotent SC is mesenchymal SCs (MSCs). However, the sources of these SCs pose some limitations because the procedure for obtaining adult SCs is usually invasive, painful and occasionally associated with morbidity (Baksh *et al.* 2007). In addition, sometimes only a handful of specialised SCs can be isolated (Pittenger *et al.* 1999; Sakaguchi *et al.* 2005). Taking all those factors into consideration, adult SCs remain the favourite SC because of ethics and the growing list of SC manipulation techniques, especially in the field of tissue engineering (Tuan *et al.* 2003; Caplan 2007). Thus, identifying alternative sources of adult SCs remains an important issue.

This review discusses two types of adult SCs: namely, dental and amniotic SCs. One of the best sources of SCs is dental SCs. Dental SCs are suggested to be remarkably resilient (Zhang *et al.* 2006) and have the capacity to differentiate into many specific cell types (Gronthos *et al.* 2000). In addition, dental SCs have a high number of passages before losing their stem cell markers (Kerkis *et al.* 2007), compared to SCs from the human amniotic membrane (HAM) (Miki *et al.* 2005). Thus, to understand these two types of SCs, the abilities of these two cell types must be understood, and these topics form the basis of the below sections. The induced pluripotent stem (iPS) cell, a synthetically derived SC that is recently becoming a popular source of SCs, is out of the scope of this review article.

CHARACTERISTICS OF MESENCHYMAL STEM CELLS

Several features of MSCs include phenotypic, morphological, cell lineage and stem cell marker characteristics. The plastic adherence nature of MSCs to tissue culture flasks constitutes its phenotypic characteristic (Horwitz *et al.* 2005). MSCs have a fibroblastic-like cell morphology (Väänänen 2005). SCs are considered mesenchymal if they can differentiate into osteogenic, chondrogenic and adipogenic lineages (Pittenger *et al.* 1999; Toda *et al.* 2007). However, recent studies showed that MSCs can also differentiate into myogenic and neurogenic lineages (Alviano *et al.* 2007; Portmann-Lanz *et al.* 2006). In 2006, the International Society for Cellular Therapy (ISCT) proposed a cell surface marker panel for the minimal identification of human MSCs (Takata *et al.* 2004). Under that recommendation, MSCs should be positive for CD73, CD90, and CD105 and lack typical hematopoietic antigens, which are CD45, CD34, CD14 (Pittenger *et al.* 1999), CD11b or CD19 or CD79α, and HLA-DR (Takata *et al.* 2004). Other

expressed cell surface markers are CD44, CD166 (Sánchez *et al.* 2011), CD29 (Kern *et al.* 2006), and CD271 (Bühring *et al.* 2007).

Dental Stem Cells

Adult MSCs come from many sources, including the tooth (Fig. 1) and HAM (Fig. 2). Dental SCs can be isolated from a few locations of the tooth. The SCs isolated from the permanent third molars of adult human dental pulp are termed dental pulp SCs (DPSCs) (Shi & Gronthos 2003), while SCs isolated from the pulp of deciduous teeth are known as SCs from human exfoliated deciduous teeth (SHED) (Miura *et al.* 2003). The SCs from the apical papilla (SCAPs) are the SCs isolated from the tooth root apex (Sonoyama *et al.* 2008), while periodontal ligament (PDL) SCs (PDLSCs) are those SCs from the PDL (Demarco *et al.* 2011).

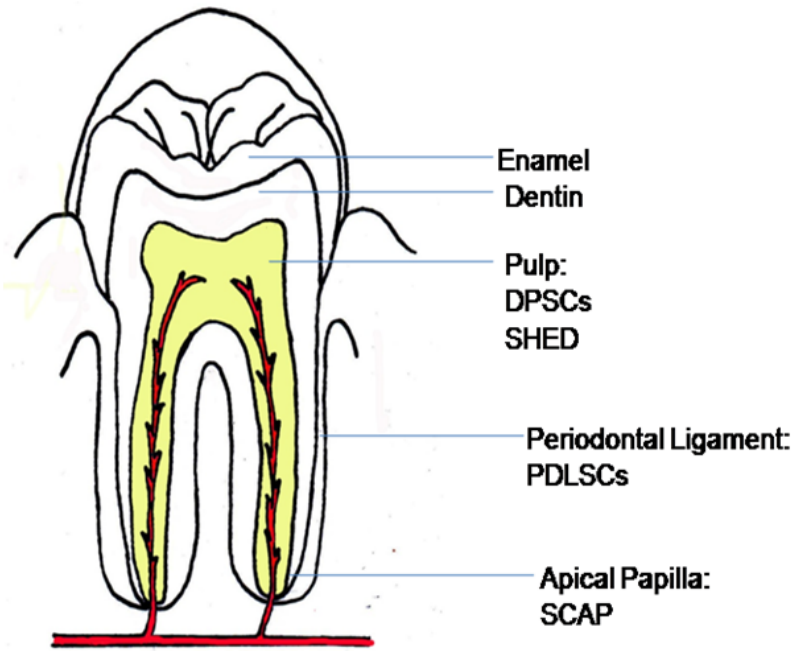

Figure 1: Diagram showing sources of MSCs from the tooth.
Note: DPSCs: dental pulp SCs; SHED: SC from human exfoliated deciduous teeth; PDLSCs: periodontal ligament SCs; SCAP: SC from apical papilla

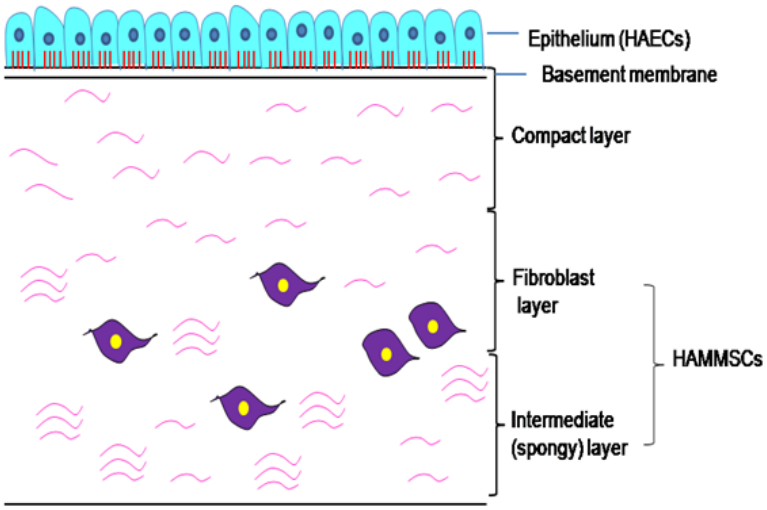

Figure 2: Diagram showing sources of MSCs from the human amniotic membrane.
Note: HAECs: human amniotic epithelial SCs; HAMMSCs: human amniotic mesenchymal SCs

Amniotic Stem Cells

Two types of SCs can be isolated from HAM: human amniotic epithelial SCs (HAESCs) (Alonso & Fuchs 2003) and human amniotic membrane mesenchymal SCs (HAMMSCs) (Alviano *et al.* 2007). HAECs are cuboidal to columnar cells that form a monolayer lining on the membrane and are in direct contact with the amniotic fluid (Caruso *et al.* 2012). HAECs, which arise from the embryonic epiblast, are amongst the first cells to differentiate from the conceptus (Parolini *et al.* 2008). The conceptus includes all structures that develop from the zygote; it comprises the embryo as well as the embryonic part of the placenta and its associated membranes: the amnion and chorion (Gitlin *et al.* 1972; Jauniaux *et al.* 2005). In contrast, HAMMSCs are dispersed in an extracellular matrix largely composed of collagen and laminin and are derived from extraembryonic mesoderm (Boury-Jamot *et al.* 2006).

CULTURE CHARACTERISTIC COMPARISONS

Differentiation Lineages

Dental and amniotic SCs differ in terms of their differentiation lineages; Zhang *et al.* (2006) found that DPSCs can differentiate into 5 lineages: osteogenic, adipogenic, chondrogenic, myogenic and neurogenic. SHED can differentiate into 6 lineages: dentinogenic (Minguell & Erices 2006), chondrogenic, myogenic (Sakaguchi *et al.* 2005), adipogenic, neurogenic and osteogenic (Miura *et al.* 2003). Some studies found that SCAPs differentiate into 3 lineages, dentinogenic, adipogenic (Caplan 2007), and neurogenic (Sonoyama *et al.* 2008), while PDLSCs differentiate into 4 lineages, osteo/cementogenic, adipogenic (Le Blanc *et al.* 2003), chondrogenic (Seo *et al.* 2004), and neurogenic (Huang *et al.* 2009). Among all the dental SCs, SHED showed the

highest differentiation capacity because they can differentiate into 6 lineages. As for amniotic SCs, HAECs can differentiate into 9 lineages: adipogenic, chondrogenic, lung (Díaz-Prado *et al.* 2011), myogenic, osteogenic, cardiomyogenic (Ilancheran *et al.* 2007), neural, hepatic, and pancreatic (Miki *et al.* 2005). On the other hand, HAMMSCs can differentiate into 8 lineages: adipogenic, chondrogenic, neurogenic, angiogenic (Alviano *et al.* 2007), osteogenic and myogenic (Portmann-Lanz *et al.* 2006), hepatic (Tamagawa *et al.* 2007), and cardiomyogenic (Zhao *et al.* 2005). Based on the above research, HAECs differentiate into more lineages than HAMMSCs, and amniotic SCs have the maximum number of differentiation lineages based on the differentiation potential of HAECs.

Number of Passages

In research, the number of passages is one of the important determinations for SC studies. Dental SCs showed a higher number of passages compared to amniotic SCs. DPSCs have been passaged for up to 25 passages (Zhang *et al.* 2006; Kerkis *et al.* 2007). Sakaguchi *et al.* (2005) stated that the maximum passage number for SHED was up to passage 5 based on their research. Minguell and Erices (2006) reported that the highest passage number for SCAPs was passage 10, and the highest passage number for PDLSCs was passage 4 (Le Blanc *et al.* 2003). Among the dental SCs, DPSCs showed the highest passage number compared to SHED, SCAPs and PDLSCs. HAECs and HAMMSCs can also be compared in terms of passage numbers. Miki *et al.* (2005) found that HAECs can be maintained up to passage 8. A study (Bilic *et al.* 2008) postulated that HAMMSC proliferation nearly stopped beyond passage 5, while another study (Parolini *et al.* 2008) reported that HAMMSCs proliferate for 2 to 6 passages before proliferation ceases. Thus, dental SCs showed the highest passage numbers.

Animal Model Studies

Dental SCs and amniotic SCs also can be compared based on studies conducted on animal models. Dental SCs, DPSCs, SHED, SCAPs and PDLSCs have been used for pulp dentin/tissue engineering and regeneration in animal studies. DPSCs and SCAPs have been used to regenerate dentin (Sonoyama *et al.* 2008; Alongi *et al.* 2010). Similar to DPSCs (Alongi *et al.* 2010), SCAPs also required 8 weeks to regenerate dentin in the presence of hydroxyapatite and tricalcium phosphate (HA/TCP) (Sonoyama *et al.* 2008). Another study employed SHED to observe the regeneration of pulp (Cordeiro *et al.* 2008). After only 2–4 weeks, SHED became pulp. Seo *et al.* (2004) used PDLSCs for periodontal repair. The PDL is similar to tendon in terms of its dense collagen fibre structure and its ability to absorb mechanical stress during normal physiological activity (Berkovitz 1990). Among dental SCs, SHED exhibited the shortest period for tissue regeneration compared to DPSCs, SCAPs and PDLSCs (Seo *et al.* 2004; Cordeiro *et al.* 2008; Sonoyama *et al.* 2008; Alongi *et al.* 2010). Some studies (Manuelpillai *et al.* 2010; Zhang *et al.* 2011) used amniotic SCs, HAMMSCs and HAECs to treat liver fibrosis using immunocompetent mice. HAECs only required 2 weeks to decrease fibrosis formation and the progression of toxic carbon

tetrachloride-induced cirrhosis; the same processes required 4 weeks for HAMMSCs. When comparing HAECs and HAMMSCs, HAECs showed the shortest time to regenerate tissues in liver fibrosis (Manuelpillai *et al.* 2010; Zhang *et al.* 2011). Both types of SCs have been successfully used in animal models but for different purposes. However, amniotic SCs showed the shortest time for tissue regeneration compared to dental SCs (Table 1).

Table 1: Comparison of culture characteristics of dental SCs and amniotic SCs.

	Dental SCs	Amniotic SCs
	6 lineages	9 lineages
Differentiation lineages	dentinogenic, chondrogenic, myogenic, adipogenic, neurogenic and osteogenic	adipogenic, chondrogenic, lung, myogenic, osteogenic, cardiomyogenic, neural, hepatic, and pancreatic
Maximum number of passages	25	9
Animal model studies	Dentin and pulp regeneration, periodontal repair	Liver fibrosis

CONCLUSION

Despite the lower passage number of amniotic SCs, they hold promise in tissue regeneration due to their greater number of differentiation lineages and shorter regeneration capacity compared with dental stem cells. The high number of differentiation lineages of amniotic SCs suggests their high multipotentiality.

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