HSCs have the ability to differentiate into any of the blood cells and cellular blood components in the body. They have been used in medical treatment for over 25 years and currently treat over 80 blood and bone related conditions. Unlike HSCs, MSCs readily differentiate into neurons as well as bone, cartilage, muscle and fat tissue cells. MSCs aren't currently FDA approved for treatment but researchers are very excited about their potential in emerging fields of gene therapy and cellular repair [26].

Wharton's jelly matrix

Mesenchymal stromal cells (MSCs) are derived from wharton's jelly matrix. They can be isolated from perivascular, intervascular and sub-amnion zones. Wharton's jelly cells (WJCs) are not derived from umbilical blood but rather from the cushioning matrix between umbilical vessels [27]. The WJCs meet the criteria of MSCs i.e., they self-renew and can be differentiated into various cell types. A large number of WJCs can be rapidly isolated – 10000-15000 cells/cm of human UC to ten times this number of cells/cm have been reported. WJCs also have the same immune properties of other MSCs [28-30].

WJCs are similar to adult derived MSCs in that they are multipotent, CD34, CD45 and HLA class II negative and CD73, CH90, CD105 positive and can be engineered to express exogenous proteins. In addition, they proliferate faster and have a greater ex-vivo expansion compared to BM-MSCs. Another reason to use foetal cells is that there is age related exhaustion of BM-MSCs and this was elucidated by Heechen et al. in 2004 [31].

Amniotic fluid

Amniotic fluid (AF) can be sampled by amniocentesis at any point starting from week 14 until the end of pregnancy, a procedure that is already being performed in many pregnancies to identify congenital abnormalities or to determine sex. However, amniocentesis should not be performed in the first trimester because a number of fetal limb defects were shown after early amniocentesis. AF cells can be obtained from a small amount of fluid. These cells take about 20-24 h to double in number, which is faster than for UC cells [32].

Epithelial cells (AE) are readily identified as a single layer adjacent to the amniotic fluid on one side and basement membrane on the other side. While AE cells reside on the inner layer of the amniotic membrane, amniotic mesenchymal stromal cells (aMSCs) form the outer layer [33,34].

Both the cell types have been extensively investigated for their properties using a number of in-vitro and in-vivo markers. Technical issues have prevented researchers from investigating whether a single human AE or aMSC cell can differentiate into cells representative of all three germ layers after clonal expansion [35,36].

Nevertheless, it is widely accepted that multiple cell types can be derived by culturing either AE or aMSC cells under appropriate conditions. Furthermore, extracting the cells from amniotic fluid bypasses the problems associated with a technique called donor–recipient HLA matching, which involves transplanting cells [37].

16-20 ml of AF is collected. It is not known whether AF cells are purely foetal or derived partly from placenta as well. AF cells have physical characteristics of both embryonic and adult cells. Based on their morphological characteristics, they can be classified into epitheliod, amniotic fluid specific and fibroblastoid. They can be differentiated into six different lineages - endothelial, neurogenic, osteogenic, hepatic, adipogenic and myogenic [36].

Umbilical vein

MSCs have been isolated from sub-endothelium of umbilical vein. It was first described by Romanov's lab. These cells are shown to be similar in properties to MSCs from BM. They have osteogenic ability and multilineage potential.

Placental cells

Placenta derived stem cells (PDSCs) can be obtained from dissociated placental tissue based on plastic adherence, a technique widely employed in harvesting BM-MSCs. PDSCs express numerous mesenchymal surface markers including CD29 and CD44. They exist in a multi-differentiated state simultaneously expressing ectodermal, mesodermal and endodermal genes. They are highly proliferative cells [38] **(Figure 3)**.

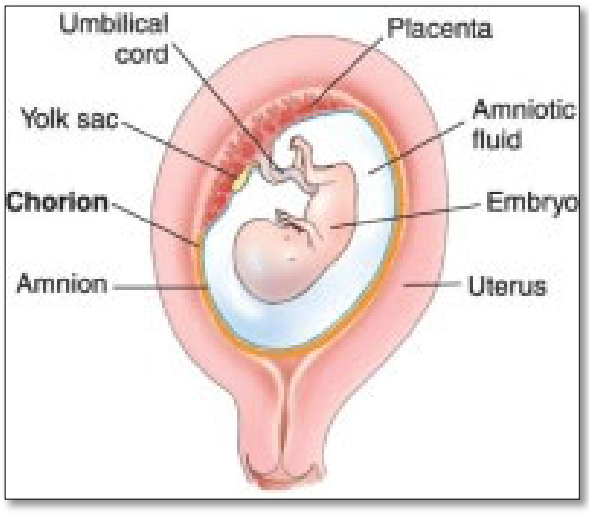

Figure 3. The human placenta.

DISCUSSION-WHOLE TOOTH REGENERATION

Tooth is a complex biological organ. Tooth loss is the most common organ failure. Tissue engineering of teeth requires the coordinated formation of correctly formed crowns, roots and periodontal ligament. The preliminary step in tooth

tissue engineering is understanding the requirements of a defined tooth crown formation. The generation of a whole tooth that can be implanted will require complete recapitulation of the odontogenic developmental program involving epithelial-mesenchymal cell interactions with the resultant structure being capable to integrate with the host vasculature. Numerous factors such as the cell lines used, the culture medium, culture time may account for the variability of results [39].

Natural structures are highly vascularised to provide teeth with proper nutrients and to remove unwanted products. The presence of a nervous system to modulate pain is also very important for longevity of tissues. Since dental implants are not innervated, it is not uncommon for them to get fractured due to excessive forces while chewing. Regenerating teeth that are vascularised and innervated would be a significant improvement over the current tooth replacement strategies [39].

There are two approaches involved in whole tooth regeneration:

- *In-vitro* cultured and expanded progenitor cell populations seeded onto scaffolds and implanted *in vivo*.
- *In-vivo* implantation of immature tooth structure grown in-vitro **(Figure 4)**.

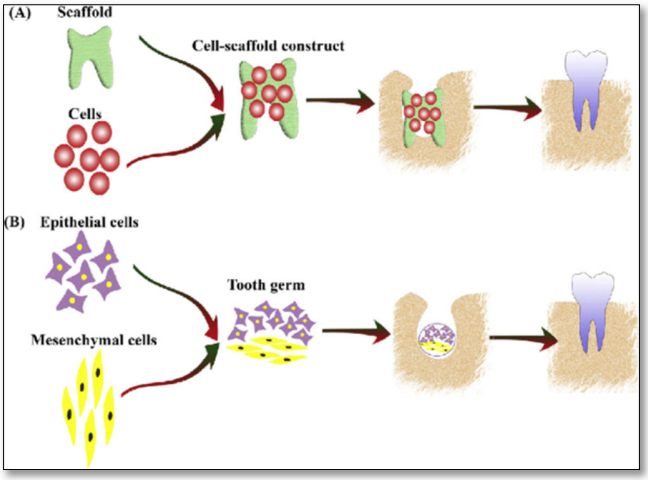

Figure 4. Two current approaches of tooth engineering.

Earlier studies showed the regeneration of tooth crowns from partially dissected tooth germs under suitable conditions. Of all the dental structures, only enamel is incapable of regenerating its original structure while the other tissues possess the capacity in varying degrees depending on multiple factors. Since the cellular component constitutes the living part of a tissue, the regenerative process is cell dependant [40].

By applying traditional tissue engineering techniques, tooth like structures can be regenerated from biodegradable polymer scaffolds seeded with stem cells. A study was performed with rat tooth germ cells grown in the omentum of mice. Although harvested implants contained anatomically correct teeth which closely resembled natural teeth, they formed in a disoriented way and did not adopt the shape and size of the scaffold. However, these results have confirmed the cell reaggregation ability of dissociated tooth germs [39].

In vitro cell culture methods are being developed and constantly improved but the task of finding stem cell populations that can replace dental epithelium and mesenchyme continues based on the findings that dental epithelium can be created from non-tooth bearing areas and non-dental mesenchyme can participate in odontogenesis on interaction with inductive dental epithelium. These experiments show that the non-dental tissues can be instructed to develop teeth [41,42].

ETHICAL CONSIDERATIONS

Human embryonic cells are valuable resources for new biotechnological developments but the controversy about the nature of human embryo and its ambivalent moral status makes it difficult to justify destroying an embryo for use as a source of raw material for tissue engineering. Because of these limitations of ESCs, pluripotent stem cells obtained from other sources besides the foetus, like the perinatal stem cells discussed in this review are gaining considerable attention. Since these tissues are discarded at the time of birth, it is a simple and safe means of harvesting stem cells. Because of their least invasiveness, amniotic fluid and placenta are considered the most appealing ones [43].

There are ethical considerations in tissue engineering research as well, like the degree of invasiveness of the methods. An *in vitro* experiment is free of risk but the implantation of cultivated cells implies the risk of contamination and incompatibility. The risks involved in the methods of tissue engineering need to be evaluated based on their effects on the body in order to engineer tissues nearer to the natural structure and function. Finally, there are unexpected risks in any new experimental therapies. The important issue is to not ignore them and the general considerations of these risks should be mentioned in the informed consent [44].

CONCLUDING REMARKS

Tissue engineering is progressing rapidly and creating a new era for therapeutic medicine. Tissue engineering was just an idea a few decades ago but today, it is a therapy for various conditions. The paradigms of tissue engineering have variable outcomes, and a true and biological tissue regeneration has not yet been achieved. From recent experiments, it has been established that teeth can be produced from stem cells of both dental and non-dental origin. The development of such teeth requires regulation of the regenerative events in order to achieve proper tooth size

and shape as well as the development of new technologies to facilitate these processes [6,7]. The practical use of perinatal stem cells for dental tissue engineering applications is at an early stage. The functional tooth eruption process in adult jaws has to be controlled. Based on the current efforts, it can be speculated that clinically relevant bioengineered functional tooth therapies for humans may be available in the near future [45].

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