Peer reviewed article

Bone tissue engineering using foetal cell therapy

Dominique P. Pioletti[a], *Marc-Olivier Montjovent*[a], *Pierre-Yves Zambelli*[b], *Lee Applegate*[c]

[a] Laboratoire de Biomécanique en Orthopédie EPFL-HOSR, Institut de Biomécanique Translationnelle, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland
[b] Hôpital Orthopédique de la Suisse Romande, CHUV, Lausanne, Switzerland
[c] Unité de Thérapie Cellulaire Orthopédique, UNIL, Lausanne, Switzerland

Summary

Different cell sources for bone tissue engineering are reviewed. In particular, adult cell source strategies have been based on the implantation of unfractionated fresh bone marrow; purified, culture expanded mesenchymal stem cells, differentiated osteoblasts, or cells that have been modified genetically to express rhBMP. Several limiting factors are mentioned for these strategies such as low number of available cells or possible immunological reaction of the host. Foetal bone cells are presented as an alternative solution and a review of actual treatments using these cells is presented. Finally, foetal cells used specifically for bone tissue engineering are characterised and potentially interesting therapeutic options are proposed.

Key words: cell therapy; foetal; cell bank

Introduction

Bone regeneration is based on the hypothesis that healthy progenitor cells, either recruited or delivered to an injured site, can ultimately regenerate lost or damaged tissue. Three-dimensional bone grafts may enhance bone regeneration by creating and maintaining a space that facilitates progenitor cell migration, proliferation and differentiation [1]. Several techniques have been developed to provide the surgeon with needed material for bone grafts. Pieces of bone are collected either from the patients undergoing surgery (autograft, which is considered as the gold standard and remains the most used therapy for bone repair) or obtained from a tissue bank (allograft). However, these techniques have some drawbacks such as traumatic procedures, morbidity or increased operating times for autografts and limited supply, risks of contamination or high costs for allografts. These limitations have led to new research aiming to provide a bone graft engineered in the laboratory and available when needed by the surgeon. The ultimate goal of this new treatment strategy is the regeneration rather than just the repair of skeletal tissue [2]. From a broad point of view, this approach can be defined as "bone tissue engineering".

As for any application in tissue engineering, the cell origin and type are essential aspects in bone tissue engineering. Technically, the cells used should be relatively simple with respect to their collection, culture, expansion and storage. Therapeutically, the cells used should have high bone formation potential, no immunological-induced reactions and no pro-inflammatory properties. In the following, we will i) review the existing options available for adult cell sources and their limitations for bone tissue engineering applications, ii) evaluate the potential of using foetal cells for general tissue engineering applications, and iii) present results obtained in our group with foetal bone cells.

This study was supported by grants from the Swiss National Science Foundation (PNR 46), the Fondation Lémanique pour la Recherche sur le Tissu Osseux, and by the Lausanne Center for Bone Tissue Engineering.

Adult cell sources for bone tissue engineering applications

Cell sources for bone tissue engineering applications can be categorised with respect to their state of differentiation. With this idea, four different cell-based tissue engineering approaches have been described for the regeneration of bone. These strategies are based on the implantation of (1) unfractionated fresh bone marrow, (2) purified, culture expanded mesenchymal stem cells. (3) dif-

ferentiated osteoblasts, or (4) cells that have been modified genetically to express rhBMP [3]. Generally, the less differentiated cells will be more easily expanded in vitro due to their high proliferation rate, while the differentiated cells will be more effective in vivo due to their higher production of mineralised extracellular matrix.

For each type of cells used, advantages and disadvantages can be found. The cells from unfractionated fresh bone marrow are relatively easy to collect, but it will not be possible to use these cells in allotransplantation as bone marrow contains T lymphocytes that encounter and respond to host antigens in virtually all tissues in the body, leading to multi-system graft-versus-host syndrome [4]. Mesenchymal stem cells, isolated from bone marrow aspirate, adult peripheral blood, neonatal cord blood or liver for example, could present advantages from an immunological point of view [5]. However, as one of every 100,000 nucleated cells derived from bone marrow is a stem cell, a procedure of isolation is required in order to decrease the volume of material injected [6]. Compared to unfractionated bone marrow, mesenchymal stem cells have been shown to generate greater bone formation in preclinical studies [7, 8]. However, gradual loss of both their proliferative and differentiation potential has been observed during in vitro expansion [9]. To overcome this difficulty, telomerase therapy (hTERT) has been recently used and effectively extended stem cell life-span while maintaining or even enhancing their osteogenic potential [10, 11]. Alternative sources of autologous precursor cells were investigated in fat by liposuction [12] or in skeletal muscle by biopsy [13]. It has to be mentioned that pluripotential cells from mesodermal tissues other than bone marrow did not show bone healing in animal models [14].

The use of predifferentiated osteoblasts has been shown to enhance the rate and extent of bone regeneration [3, 15]. Their expansion in vitro may be however difficult and is highly depending on the donor [16]. Finally, cells genetically modified to express bone formation cytokines could be used to take advantage of genetic therapy, combining gene therapy and tissue engineering methodologies to enhance tissue regeneration [17]. The transfection of the cells can be done either in two steps with an in vitro transfection followed by injection of the modified cells (procedure called ex vivo gene therapy) or in one step by transfecting directly the cells in the body (procedure called in vivo gene therapy). With ex vivo gene therapy approach, cells overexpressing BMP have been developed and used in animal studies eg [18]. Concerns on possible host immune reaction need to be clarified.

A list of requirements for ideal cell sources for bone tissue engineering applications is summarised in table 1. No adult cells seem to fulfil all the requirements leaving room to propose an alternative approach based on foetal cells.

Table 1

List of requirements for an ideal cell source for bone tissue engineering applications. A high facility for the technical or therapeutical requirement is denoted by +++, on a scale where NA means non-adequate. The immunological reaction refers to the use of cells in an allotransplantation. The quotation for bone marrow cells, MSC, pre-osteoblasts and foetal bone cells is based on the authors experiences with these cells, while the quotation for genetically modified cells comes from the literature [30, 31].

	Technical requirements				Therapeutical requirements		
	Collection	Culture	Expansion	Storage	Bone formation	No-immunological reaction	No pro-inflammatory reaction
Bone Marrow cells	++	NA	NA	NA	+	NA	++
Mesenchymal stem cells	+	++	++	++	++	++	++
Pre-osteoblasts	+	++	+	+	++	NA	++
Genetically modified cells[1]	++	+[2]	++	+	+++	+[3]	++
Foetal bone cells	++	+++	+++	+++	+++	+++	+++[4]

[1] Ex vivo gene therapy
[2] Effectiveness stability of the transfection may be difficult to obtain. Thus, the duration of the transfect expression could be affected.
[3] Depending on the vectors used even for autologous transplantation [30]
[4] Foetal cells not only induce no pro-inflammatory reaction, but were able to decrease inflammation at the wounded site [29].

Foetal cell sources for tissue engineering applications

Foetal associated tissues such as placenta, amniotic liquid or umbilical cord are described to be potential sources of cells for tissue engineering [19–22]. In contrast to embryonic tissue derived up to the end of the 8th week, foetal tissue begins at the 9th week and is considered as an organ donation.

The idea of using foetal tissues or cells for cell therapy is mainly coming from the field of neurology. Indeed, several clinical studies have already been performed to treat neurodegenerative disorders. Neuronal affections such as Huntington's [23] or Parkinson's disease [24] have been treated by transplantation of fresh foetal neuroblasts. Unfortunately, these cells are difficult to expand in culture and have to be transplanted freshly therefore needing large quantities of fresh tissue [25]. Still within the neurology but following accidents, cell therapy for stroke has also tried to benefit from foetal cells, with a clinical trial treating patients with foetal cells obtained from the porcine primordial striatum [26]. In this situation, safety of the

treatment was verified, but none of the patients showed improvement. Treatment of spinal cord injury also tested the use of intraspinal foetal central nervous system grafts. Three clinical studies have been performed where the results mainly focused on security of the procedure. However, encouraging results were obtained. In particular translational correspondence was observed between preclinical locomotor performance in a cat before and after intraspinal foetal transplant surgery compared with a human subject before and after human foetal cell grafting [27]. Transplantation of foetal central nervous system tissue is considered actually as a gold standard in neurobiology.

Beside neurology, human foetal liver cells have been used already more than 10 years for transplantation to treat severe immunodeficiencies, haematological disorders and inborn errors of metabolism when there was no perfectly matched donor for marrow transplantation [28].

Recently, human foetal skin cells derived from one cell bank (1–4 cm^2 tissue results in over 10.5 million foetal skin constructs) were used in clinical trials and new advances in tissue therapy are possible with cellular constructs obtained from ex vivo cultures [29]. Following this approach, engineered regeneration of human skeletal adult tissues could be also developed using human foetal bone cells. Surprisingly, tissue engineering using foetal cells has barely begun to be investigated. To evaluate their potential integration in a bone engineering strategy and following the experience gained in neurobiology, a biological characterisation of these cells is necessary, especially by evaluating the potential of foetal cells to produce mineralised bone extracellular matrix.

Lately, a study was performed to specifically study the characteristics of human primary foetal bone cells for a better comprehension of their biology in vitro and to evaluate their potential use for tissue engineering in comparison to adult bone cells and mesenchymal stem cells [16]. A summary of this study is presented below.

In vitro evaluation of human foetal cells for bone tissue engineering applications

Human primary foetal bone cells were compared to adult bone cells and mesenchymal stem cells for their ability to proliferate and to differentiate into osteoblasts in vitro (for details see the original article [16]). Cell proliferation, gene expression of bone markers, alkaline phosphatase (ALP) activity and mineralisation were analysed during a time-course study. Human primary foetal bone cells were compared to osteoblasts and mesenchymal stem cells obtained from adult tissues for their ability to proliferate and to differentiate into osteoblasts in vitro.

The doubling time of foetal bone cells was comparable to mesenchymal stem cells but significantly shorter than for adult osteoblasts. Gene expression of cbfa-1, ALP, α1 chain of type I collagen and osteocalcin were upregulated in foetal bone cells after 12 days of treatment with osteogenic factors, showing higher inductions than for adult osteoblasts and mesenchymal stem cells. The increase of ALP enzymatic activity was stronger for foetal than for adult osteoblasts reaching a maximum at day 10, but lower than for mesenchymal stem cells. Importantly, the mineralisation process of bone foetal cells started earlier than adult osteoblasts and mesenchymal stem cells. The human primary foetal bone cells have the advantages of high proliferation rate as for mesenchymal stem cells and effective production of mineralised extracellular bone matrix as for adult osteoblasts. As seen in table 1, human primary foetal bone cells represent an interesting and promising potential for therapeutic use in the bone tissue engineering field as these cells can be easily stocked "frozen for use" when necessary.

Correspondence:
Dominique P. Pioletti, PhD
EPFL/STI/IBME/LBO
Station 15
CH-1015 Lausanne
E-Mail: dominique.pioletti@epfl.ch

References

1 Shea LD, Wang D, Franceschi RT, Mooney DJ. Engineered bone development from a pre-osteoblast cell line on three-dimensional scaffolds. Tissue Eng 2000;6:605–17.
2 Caplan AI, Goldberg VM. Principles of tissue engineered regeneration of skeletal tissues. Clin Orthop 1999:S12–16.
3 Bruder SP, Fox BS. Tissue engineering of bone. Cell based strategies. Clin Orthop 1999:S68–83.
4 Weissman IL. Translating stem and progenitor cell biology to the clinic: Barriers and opportunities. Science 2000;287: 1442–6.
5 Javazon EH, Beggs KJ, Flake AW. Mesenchymal stem cells: Paradoxes of passaging. Exp Hematol 2004;32:414–25.
6 Connolly J, Guse R, Lippiello L, Dehne R. Development of an osteogenic bone-marrow preparation. J Bone Joint Surg Am 1989;71:684–91.
7 Inoue K, Ohgushi H, Yoshikawa T, Okumura M, Sempuku T, Tamai S, et al. The effect of aging on bone formation in porous hydroxyapatite: Biochemical and histological analysis. J Bone Miner Res 1997;12:989–94.

8 Kahn A, Gibbons R, Perkins S, Gazit D. Age-related bone loss: A hypothesis and initial assessment in mice. Clin Orthop Relat Res 1995:69–75.

9 Mauney JR, Volloch V, Kaplan DL. Role of adult mesenchymal stem cells in bone tissue engineering applications: Current status and future prospects. Tissue Eng 2005;11:787–802.

10 Shi S, Gronthos S, Chen S, Reddi A, Counter CM, Robey PG, et al. Bone formation by human postnatal bone marrow stromal stem cells is enhanced by telomerase expression. Nat Biotechnol 2002;20:587–91.

11 Simonsen JL, Rosada C, Serakinci N, Justesen J, Stenderup K, Rattan SI, et al. Telomerase expression extends the proliferative life-span and maintains the osteogenic potential of human bone marrow stromal cells. Nat Biotechnol 2002;20:592–6.

12 Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, et al. Multilineage cells from human adipose tissue: Implications for cell-based therapies. Tissue Eng 2001;7:211–28.

13 Bosch P, Musgrave DS, Lee JY, Cummins J, Shuler T, Ghivizzani TC, et al. Osteoprogenitor cells within skeletal muscle. J Orthop Res 2000;18:933–44.

14 Whang PG, Lieberman JR. Clinical issues in the development of cellular systems for use as bone graft substitutes. In: Laurencin CT, ed. Bone graft substitutes. ASTM International 2003:142–63.

15 Kadiyala S, Young RG, Thiede MA, Bruder SP. Culture expanded canine mesenchymal stem cells possess osteochondrogenic potential in vivo and in vitro. Cell Transplant 1997;6: 125–34.

16 Montjovent MO, Burri N, Mark S, Federici E, Scaletta C, Zambelli PY, et al. Fetal bone cells for tissue engineering. Bone 2004;35:1323–33.

17 Lauffenburger DA, Schaffer DV. The matrix delivers. Nat Med 1999;5:733–4.

18 Gazit D, Turgeman G, Kelley P, Wang E, Jalenak M, Zilberman Y, et al. Engineered pluripotent mesenchymal cells integrate and differentiate in regenerating bone: A novel cell-mediated gene therapy. J Gene Med 1999;1:121–33.

19 Kaviani A, Guleserian K, Perry TE, Jennings RW, Ziegler MM, Fauza DO. Fetal tissue engineering from amniotic fluid. J Am Coll Surg 2003;196:592–7.

20 Kadner A, Hoerstrup SP, Tracy J, Breymann C, Maurus CF, Melnitchouk S, et al. Human umbilical cord cells: A new cell source for cardiovascular tissue engineering. Ann Thorac Surg 2002;74:S1422–8.

21 Kaviani A, Perry TE, Barnes CM, Oh JT, Ziegler MM, Fishman SJ, et al. The placenta as a cell source in fetal tissue engineering. J Pediatr Surg 2002;37:995–9; discussion 995–9.

22 Mitka M. Amniotic cells show promise for fetal tissue engineering. JAMA 2001;286:2083.

23 Rosser AE, Dunnett SB. Neural transplantation in patients with huntington's disease. CNS Drugs 2003;17:853–67.

24 Clarkson ED. Fetal tissue transplantation for patients with parkinson's disease: A database of published clinical results. Drugs Aging 2001;18:773–85.

25 Freeman TB. From transplants to gene therapy for parkinson's disease. Exp Neurol 1997;144:47–50.

26 Savitz SI, Dinsmore JH, Wechsler LR, Rosenbaum DM, Caplan LR. Cell therapy for stroke. NeuroRx 2004;1:406–14.

27 Reier PJ. Cellular transplantation strategies for spinal cord injury and translational neurobiology. NeuroRx 2004;1:424–51.

28 Touraine JL, Roncarolo MG, Bacchetta R, Raudrant D, Rebaud A, Laplace S, et al. Fetal liver transplantation: Biology and clinical results. Bone Marrow Transplant 1993;11(Suppl 1):119–22.

29 Hohlfeld J, de Buys Roessingh A, Hirt-Burri N, Chaubert P, Gerber S, Scaletta C, et al. Tissue engineered fetal skin constructs for paediatric burns. Lancet 2005;366:840–2.

30 Park J, Ries J, Gelse K, Kloss F, von der Mark K, Wiltfang J, et al. Bone regeneration in critical size defects by cell-mediated bmp-2 gene transfer: A comparison of adenoviral vectors and liposomes. Gene Ther 2003;10:1089–98.

31 Verma IM, Somia N. Gene therapy: Promises, problems and prospects. Nature 1997;389:239–42.

Swiss
Medical Weekly

Official journal of
the Swiss Society of Infectious disease
the Swiss Society of Internal Medicine
the Swiss Respiratory Society

The many reasons why you should choose SMW to publish your research

What Swiss Medical Weekly has to offer:

- SMW's impact factor has been steadily rising, to the current 1.537
- Open access to the publication via the Internet, therefore wide audience and impact
- Rapid listing in Medline
- LinkOut-button from PubMed with link to the full text website http://www.smw.ch (direct link from each SMW record in PubMed)

- No-nonsense submission – you submit a single copy of your manuscript by e-mail attachment
- Peer review based on a broad spectrum of international academic referees
- Assistance of our professional statistician for every article with statistical analyses

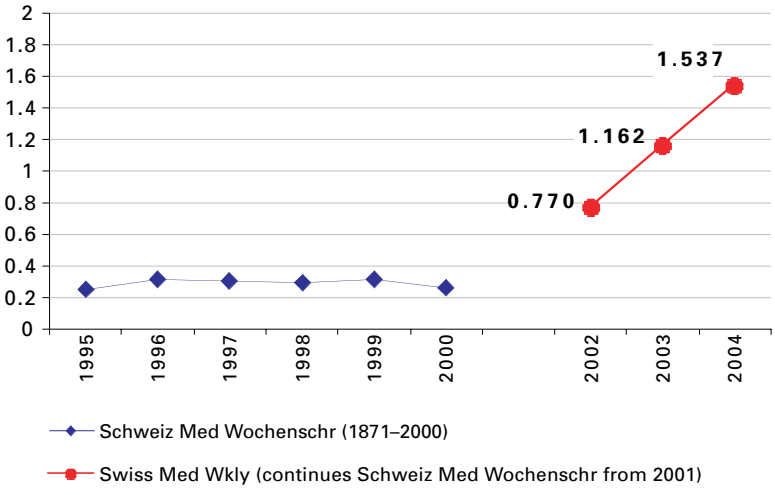
- Fast peer review, by e-mail exchange with the referees
- Prompt decisions based on weekly conferences of the Editorial Board
- Prompt notification on the status of your manuscript by e-mail
- Professional English copy editing
- No page charges and attractive colour offprints at no extra cost

We evaluate manuscripts of broad clinical interest from all specialities, including experimental medicine and clinical investigation.

We look forward to receiving your paper!

Guidelines for authors:
http://www.smw.ch/set_authors.html

Impact factor Swiss Medical Weekly



EMH FMH Schwabe
Editores Medicorum Helveticorum

All manuscripts should be sent in electronic form, to:

EMH Swiss Medical Publishers Ltd.
SMW Editorial Secretariat
Farnsburgerstrasse 8
CH-4132 Muttenz

Manuscripts: submission@smw.ch
Letters to the editor: letters@smw.ch
Editorial Board: red@smw.ch
Internet: http://www.smw.ch