REVISTA BRASILEIRA
DE HEMATOLOGIA
E HEMOTERAPIA

Review / Revisão

Regenerative medicine: A review

Medicina regenerativa: Uma revisão

Adolfo Porcellini

*Regenerative medicine is a technique to replace or repair defective or diseased tissue or organs by in vitro design with in vivo usage. It can be considered a relatively new branch of medicine born in 1997 when Whithman DH et al. proposed to integrate platelet enriched plasma (PRP) in fibrin glue. In 1998 Marx et al. demonstrated that PRP was able to induce bone regeneration of the jaw. In the same period it was discovered that a fraction of stem cells of bone marrow origin was able to repair several mesenchymal tissues or organs. Rev. Bras. Hematol. Hemoter. 2009;**31**(Supl. 2):63-66.*

Key words: *Regenerative medicine; hematopoietic stem cells; mesenchimal cells.*

Introduction

Regenerative medicine is a technique to replace or repair defective or diseased tissue or organs by *in vitro* design with *in vivo* usage. It can be considered a relatively new branch of medicine born in 1997 when Whithman DH *et al.*[1] proposed to integrate platelet Rich plasma (PRP) in fibrin glue. In 1998 Marx *et al*. demonstrated that PRP was able to induce bone regeneration of the jaw. In the same period it was discovered that a fraction of stem cells of bone marrow origin was able to repair several mesenchymal tissues or organs.

Therefore, regenerative medicine is based on the employment of either stem cells with multipotent differentiating potential and/or biological products (PRP, or its gel formulation Platelet Gel, PG) that have the ability to induce the migration of stem cells to the damaged tissue, to stimulate their proliferation and to eventually obtain tissue repair. In some cases, particularly in the regeneration of bone, it is necessary to add some biomaterials to the PRP and/or stem cells, that besides, having a support function operate as a guide for stem cells to obtain spatial repair.

Tissue repair is an extremely complex biological process in which several factors interplay: age, site and depth of the lesion, co-morbidity (i.e. diabetes, concomitant infections).

Such a complex process is facilitated by so-called growth factors (GF), molecules of crucial importance that interplay and exchange biochemical information. GF are produced by the cells involved in the regenerative process and when they reach a proper concentration they trigger the reparation process. Cells sensitive to the GF migrate into the site of the lesion and give rise to support tissue, extracellular matrix, while endothelial precursor cells give rise to newly formed vessels (neo-angiogenesis).

Several clinical applications employing cell infusions, PG or both, sometimes in combination with biomaterials, are today possible and are currently applied in situations in which no other therapy is available. The combination of these clinical applications is now called Regenerative Medicine.

The clinical applications in which the best results have been obtained are: vascular surgery, maxillo-facial surgery, orthopedic surgery and esthetic medicine.

Modern techniques employed in Regenerative Medicine involve using various fractions of autologous stem cells, biomaterials and PRP/PG, alone or in combinations.

Platelet gel

Platelet gel (PG) stimulates cells involved in tissue repair to migrate to the lesion area, stimulates cell proliferation and stimulates the production of collagen and connective tissue. PG is able to induce the migration of mesenchymal stem cells in the area of the damaged tissue to stimulate their proliferation.

Servizio di Ematologia, Medicina II, Centra Trapianto Midollo Osseo, Cremona, Italy.

Correspondence: *Adolfo Porcellini*
Centra Trapianto Midollo Osseo, Servizio di Ematologia, Medicina II
Cremona 26100, Italy
E-mail:aporcellini@gmail.com

Basic Biology

Since 1990, several components in blood were recognized as being part of the natural healing process and if they are added to wounded tissues or surgical sites as a concentrate they have the potential to accelerate healing. These specific components, also called growth factors are released by the alpha granules of the platelets; they include: PDGF (platelet derived growth factor), *TGF-ß* (transforming growth factor-ß) that regulates proliferation and differentiation of several cell types, *VEGF* (vascular endothelial growth factor), *IGF I/II* (insulin growth factor I/II), *FGF b* (fibroblast growth factor b), and *EGF* (epidermal growth factor). It is now well understood that platelets play a fundamental role in wound healing. In fact the release of growth factors does not occur by platelet disruption or fragmentation, rather by platelet alpha granules actively extruding the growth factors involved with initiating wound healing. In response to platelet aggregation or platelet to connective tissue contact, as occurs in injury or surgery, the cell membrane of the platelet is "activated" to release these alpha granules. Alpha granules release growth factors via active extrusion through the cell membrane.

Platelet Derived Growth Factor (PDGF)

PDGF initiates nearly all wound healing. The main functions of PDGF are to stimulate cell replication (mitogenesis) of healing capable stem cells. It also stimulates cell replication of endothelial cells. This will cause budding of new capillaries into the wound (angiogenesis), a fundamental part of all wound healing. In addition, PDGF seems to promote the migration of perivascular healing-capable cells into a wound and to modulate the effects of other growth factors.

Transforming Growth Factor-beta (TGFß)

The so-called "super family" of TGFßs numbers about forty-seven, and includes all of the well-known bone specific morphogen growth factors of the 13 known bone morphogenetic proteins (BMPs). The types of TGFß found in platelets are TGFß1 and TGFß2, which are the most generic connective tissue growth factors involved with matrix formation (i.e. cartilage and bone matrix as well as vascular basal lamina matrix.). Cells which are activated by TGFß1 or TGFß2 include fibroblasts, endothelial cells, osteoprogenitor cells, chondroprogenitor cells, and mesenchymal stem cells.

Fibronectin and Vitronectin

Both of these are proteins called cell adhesion molecules. As part of cellular proliferation and migration, particularly in bone and cartilage healing, cells move to new positions to lay down their products such as bone or cartilage. In relation to bone, this is termed osteo-conduction.

Fibrin

Fibrin contributes to cell mobility in the wound. The role of fibrin, which is a cross linked protein derived from the fibrinogen in plasma, is not only to serve as a scaffold or surface for cell migration, but to entrap platelets.

Through these recognized components in blood, natural wound healing is initiated, directed, and controlled. As a young science, blood component concentrates have not been completely studied. In fact, only a small percentage of the knowledge base is known today. Clinically, adding enriched platelets and plasma to various clinical systems has proved to accelerate bone graft healing and maturation of the graft, accelerate skin graft healing and its maturation, and to enhance hemostasis in bone and soft tissue defects. PRP/PG under optimal clinical conditions accelerates healing of both hard and soft tissues, because of its content of GF. The clinical applications of PRP/PG are several and continue to expand.

PRP/PG has been extensively used in maxillo-facial and oral surgery with predictable clinical outcomes. Available data indicate that PRP in combination with bone chips or biomaterials, enhances the early wound-healing cascade by the interactions of activated PDGF with the extra cellular matrix giving a potent anabolic effect.[2,3,4]

Another important clinical application of this biological preparation is the treatment of chronic ulcers of the limbs and particularly of diabetic foot ulcerations. These are multifactorial syndromes that are known for their slow healing rate and resistance to treatment. PG is very effective and safe in accelerating the healing of diabetic foot ulcers and vascular ulcers of the lower limbs.[5]

PRP/PG has also many unique and biological mechanisms of action in the practice of orthopedic surgery,[6,7] the main indications being lesions of tendons and osteo-necrosis. Current treatments for chronic tendon injuries are temporary in nature and do not provide long term relief. PRP therapy is a treatment option for non-healing tendon injuries such as tennis elbow, Achilles tendonitis and knee tendonitis.

Mesenchymal stem cells

Definition

Mesenchymal stem cells, or MSCs, are multipotent stem cells that can differentiate into a variety of cell types. Cell types that MSCs have been shown to differentiate into in vitro or in vivo include osteoblasts, chondrocytes, myocytes, adipocytes, and, as recently described, beta-pancreatic islets cells.

While the terms Mesenchymal Stem Cell and Marrow Stromal Cell have been used interchangeably, neither term is sufficiently descriptive as discussed below:

- Mesenchyme is embryonic connective tissue that is derived from the mesoderm and that differentiates into

hematopoietic and connective tissue, whereas MSCs do not differentiate into hematopoietic cells.

• Stromal cells are connective tissue cells that form the supportive structure in which the functional cells of the tissue reside. While this is an accurate description for one function of MSCs, the term fails to convey the relatively recently-discovered roles of MSCs in tissue repair.

• Because the cells, called MSCs by many laboratories today, can encompass multipotent cells derived from other non-marrow tissues, such as adult muscle or adipose tissue, yet do not have the capacity to reconstitute an entire organ, this term has been proposed.

Multipotent Stromal Cell/Mesenchymal Stem Cells (MSC) are non-hemopoietic multi-potent stem-like cells that are capable of differentiating into both mesenchymal and non-mesenchymal lineages. In fact, in addition to bone, cartilage, fat and myoblasts, it has been demonstrated, *in vitro* and *in vivo*, that MSC are capable of differentiating into neurons and astrocytes.[8-10] This discovery has addressed one of the most intriguing questions in both biology and medicine, that is, how do complex organisms repair injured tissue.[11] At the moment it is difficult to resolve all of the apparent discrepancies in observations of the presence of reparative cells in bone marrow, but several generalizations can be made. Mesenchymal cells of several tissues have a considerable degree of plasticity and are able to cross the boundary of their tissue specificity (in other words they are able to trans-differentiate into cells of other lineages). Recent studies suggest that the tissue reparation process is led by cells with stem cell characteristics residing in several tissues but they are fed from precursor cells residing in the bone marrow.[11]

The results of these studies would indicate the interesting possibility that MSC repair the tissues through three mechanisms: the creation of a milieu with the property of stimulating the proliferation of endogenous cells, trans-differentiation and perhaps cell fusion.[11,12]

From the bench to the ward only a few years have elapsed[13] and various clinical applications have been published where, up to now, nothing or little could be done. These clinical applications relate to the treatment of chronic refractory angina,[14] which was the first ever attempt of cell therapy, followed by a number of clinical attempts in a small number of patients[15] with several subsets of progenitor cells, MSC or unselected bone marrow for therapeutic angiogenesis in patients with limb ischaemia[16,17] and skin and bone diseases.

More recent is the discovery that hemopoietic MSCs have a marked capacity to modulate the immune system[18] and several studies are ongoing to assess in large studies this capacity to control post-allogeneic transplant GvHD refractory to the standard immunosuppressive therapies.

MSC are of interest because they are isolated from a small aspirate of bone marrow and can be easily expanded *in vitro.* As such, these cells are currently being tested for their potential use in cell and gene therapy for a number of human diseases. Nevertheless, there are still some open questions about origin, multi-potentiality and anatomical localization of MSC. Not only the medical community but also the media are currently experiencing a wave of enthusiasm for clinical trials in which adult stem/progenitor cells are used to repair tissue. This is reasonable in view of several reports in which promising results were obtained in animal models for a variety of diseases and the encouraging initial reports from a number of clinical trials. It is also driven by the prospect that stem cell therapy may offer new hope for patients with end-stage diseases for which there are no therapies. In the wave of enthusiasm, however, several essential precautions are not being fully addressed. Therefore, there is a great danger that potentially important new therapies will be prematurely discarded because of poorly designed clinical trials with small numbers of patients.[19] We should remember that currently, the largest number of clinical trials is for patients with heart disease. Here, a confusing variety of cells and strategies for different syndromes have been tested (different subsets of cells or unfractionated bone marrow, different site of infusion etc). To date only a limited number of adverse reactions have been described. However, the number of patients enrolled in well controlled trials is still limited.

In conclusion, the use of MSCs or related cells presents problems in that cultures of the cells are heterogeneous. As a result, there is a considerable variability in the properties of different preparations of MSCs used in clinical trials. Unfortunately, adequate markers to identify most of the stem/progenitor cells being used are not yet available. Accordingly, there is a great need to standardize protocols to prepare cells and to develop more definitive markers.[19]

Resumo

*Medicina regenerativa é uma técnica de substituir ou reparar defeitos ou tecidos ou órgãos doentes por outros desenhados in vitro para uso in vivo. Estas técnicas podem ser consideradas relativamente novas já que nasceu em bancada em 1997 quando Whithman DH et al propuseram a utilização da cola de fibrina obtida em plasma rico em plaquetas (PRP) para uso terapêutico. Em 1998, Marx et al demonstraram que a PRP foi capaz de induzir regeneração óssea da mandíbula. No mesmo período, foi descoberto que frações de células-tronco de origem na medula óssea foram capazes de reparar tecidos e órgãos a partir de células mesenquimais. O autor faz aqui uma breve revisão do assunto altamente contemporâneo. Rev. Bras. Hematol. Hemoter. 2009;**31**(Supl. 2):63-66.*

Palavras-chave*: Medicina regenerativa;células-tronco hemato-poéticas; células mesenquimais.*

References

1. Whitman DH, Berry RL, Green DM. Platelet gel: an autologous alternative to fibrin glue with applications in oral and maxillofacial surgery. J Oral Maxillofac Surg. 1997;55(11):1294-9.
2. Marx RE. Platelet-rich plasma: evidence to support its use. J Oral Maxillofac Surg. 2004;62(4):489-96.
3. Okuda K, Kawase T, Momose M, Murata M, Saito Y, Suzuki H, *et al*. Platelet-rich plasma contains high levels of platelet-derived growth factor and transforming growth factor-beta and modulates the proliferation of periodontally related cells in vitro. J Periodontol. 2003;74(6):849-57.
4. Graziani F, Cei S, Ducci F, Giuca MR, Donos N, Gabriele M. In vitro effects of different concentration of PRP on primary bone and gingival cell lines. Preliminary results. Minerva Stomatol. 2005;54(1-2):15-22.
5. Saldalamacchia G et al. Platelet gel for the therapy of the ulcers of the diabetic foot. GIDM 2004;24:103-105
6. Roukis TS, Zgonis T, Tiernan B. Autologous platelet-rich plasma for wound and osseous healing: a review of the literature and commercially available products. Adv Ther. 2006;23(2):218-37.
7. Schnabel LV, Mohammed HO, Miller BJ, McDermott WG, Jacobson MS, Santangelo KS, *et al.* Platelet rich plasma (PRP) enhances anabolic gene expression patterns in flexor digitorum superficialis tendons. J Orthop Res. 2007;25(2):230-40.
8. Prockop DJ. Marrow stromal cells as stem cells for non-hematopoietic tissues. Science. 1997;276(5309):71-4.
9. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, *et al*. Multilineage potential of adult human mesenchymal stem cells. Science. 1999;284(5411):143-7.
10. Deans RJ, Moseley AB. Mesenchymal stem cells: biology and potential clinical uses. Exp Hematol. 2000;28(8):875-84.
11. Prockop DJ, Gregory CA, Spees JL. One strategy for cell and gene therapy: harnessing the power of adult stem cells to repair tissues. Proc Natl Acad Sci U S A. 2003;100 Suppl 1:11917-23.
12. Minguell JJ, Erices A, Conget P. Mesenchymal stem cells. Exp Biol Med (Maywood). 2001;226(6):507-20.
13. Koç ON, Lazarus HM. Mesenchymal stem cells: heading into the clinic. Bone Marrow Transplant. 2001;27(3):235-9.
14. Briguori C, Reimers B, Sarais C, Napodano M, Pascotto P, Azzarello G, *et al*. Direct intramyocardial percutaneous delivery of autologous bone marrow in patients with refractory myocardial angina. Am Heart J. 2006;151(3):674-80.
15. Tse HF, Kwong YL, Chan JK, Lo G, Ho CL, Lau CP. Angiogenesis in ischaemic myocardium by intramyocardial autologous bone marrow mononuclear cell implantation. Lancet. 2003;361(9351):47-9.
16. Tateishi-Yuyama E, Matsubara H, Murohara T, Ikeda U, Shintani S, Masaki H, *et al*. Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial. Lancet. 2002;360(9331):427-35.
17. Akar AR, Durdu S, Arikbuka M et al. Implantation of autologous bone marrow mononuclear cells for Bürger's disease with retractable limb ischaemia. Bone Marrow Transplantation 2005; 35 supp 2 47.
18. LeBlanc K, Rasmusson O. Treatment of severe acute GvHD with third party haploidentical mesenchymal stem cells. Lancet (2004); 363:128-32.
19. Prockop DJ, Olson SD. Clinical trials with adult stem/progenitor cells for tissue repair: let's not overlook some essential precautions. Blood. 2007;109(8):3147-51.

O tema apresentado foi proposto pela Organização do 8º Simpósio da Associação Ítalo-Brasileira de Hematologia, realizado durante o XIII Congresso de Transplante de Medula Óssea.
Publicado após concordância do editor.

Conflito de interesse: sem conflito de interesse

Recebido: 07/07/2009
Aceito: 12/07/2009