

## Why is important the use of the right anticoagulant in PRP/CGF?

### CGF: why is it different?

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Platelet-rich plasma (PRP) is a concentrated preparation of platelets now called “first-generation platelet concentrate”. PRP is a rich source of growth factors and promotes significant changes in monocyte, mediated pro-inflammatory action through cytokine/chemokine release. Leucotrine A4 (LXA4) was increased in PRP, suggesting that PRP may suppress cytokine release, limit inflammation, and, thereby, promote tissue regeneration.<sup>1</sup> Platelet activation allows access to autologous growth factors which by definition are neither toxic nor immunogenic and are able to accelerate the normal processes of bone regeneration. In general, a large body of PRP studies demonstrated that PRP stimulates the proliferation and differentiation of fibroblasts, osteoblasts, chondrocytes, and mesenchymal stem cells.<sup>2,3</sup> PRP can thus be considered a useful instrument for increasing the quality of regenerated bone tissue,<sup>4</sup> wound healing,<sup>5</sup> healing of isoft tissue defects<sup>6</sup> for non-healing chronic tendon injuries including lateral epicondylitis, plantar fasciitis and cartilage degeneration.<sup>2</sup>

New technologies (as Silfradent<sup>®</sup> cell concentrator) make possible the extraction of CGF (Concentrated Growth Factor). CGF is an autologous rich leukocyte and platelet-rich fibrin (L-PRF) biomaterial called “second-generation platelet concentrate”. CGF contains autologous osteoinductive platelet growth factors and an osteoconductive fibrin matrix.<sup>7</sup> Is also present in CGF: TGF-b1, VEGF and CD34 positive cells.<sup>8</sup> The application of CGF leads to an excellent healing of critical-size bone defects *in vivo*,<sup>7</sup> hair loss<sup>9</sup> and promissory in periphery and myocardial ischemia.<sup>10</sup>

The Silfradent<sup>®</sup> concentrator uses a speed gradient that facilitate the recovery of different fractions.<sup>8</sup> Variable clinical results using PRP probably respond to the use of different preparation protocols. For aesthetical application (e.g. hair loss or facial regeneration) PRP or PRP fraction in CGF should be liquid to facilitate the injection, and the clot formation should be avoided using anti-coagulant agents. The anticoagulants play an important role in the activation of the platelets.

Anticoagulants either heparin (calcium salt, 18/30 U.I./ml blood) normally used for therapeutic purposes or ACD (Citric acid, Na citrate, Glucose) were used during the isolation of PRP platelet

fraction in CGF. It has been demonstrated that the release of growth factor from platelets take place more efficiently in presence of heparin.<sup>11; 12</sup>

In addition, it has been demonstrated that during the activation in presence of heparin, is significantly higher the amount of platelet-derived growth factor (PDGF), transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1) and interleukin-8 (IL-8) are released in a dose-dependent manner after ozonation of heparinized platelet-rich plasma samples, in contrast with the procedure that use ACD.<sup>12</sup>

Moreover, numerous extracellular proteins, growth factors, chemokines, cytokines, enzymes, lipoproteins, involved in a variety of biological processes, interact with heparin and/or heparin sulfate at the cell surface and in the extracellular matrix. The interaction of some important growth factors derived from platelets, as the Fibroblast growth factor (FGF) with its receptor: Fibroblast growth factor receptor (FGFR), is more efficient in presence of heparin.<sup>13</sup> The heparin-binding protein site near to the FGFR regulates the angiogenesis, then the presence of heparin induce an enhancement of the mitogenic activity of FGF.<sup>14</sup>

For clinical use, when heparin is re-injected, the use Na or Ca heparin is appropriate, on the contrary, lithium heparin salts are only useful when blood samples will be used for analytical purpose (not for re-injections). For the listed reasons, when CGF PRP fraction /CD34+ produced with the technologies Silfradent<sup>®</sup> is use for direct injections (aesthetic or hair loss) is appropriated the use of heparinized (Na salt, green cap test tube). None heparinized (White cap test tube) can be used, but with the previous addition (injection) of 18/30 U.I./ml blood of heparin.

Using the white cap test tubes, you can remote the PPP fraction, with an albumin denaturation process, you get an autologous gel.

The albumin denaturation by frequency heating leads to the construction of a web structure, scaffold (APAG Activated Plasma Albumin Gel).

Apply APAG and inject the concentrated growth factors or CD34+.

Cells imbricate into the gel, growing in the application point.

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