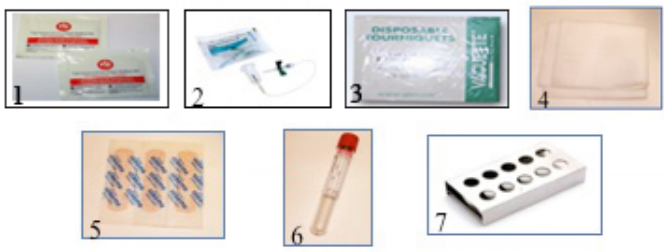
CGF Concentrated Growth Factors: Protocol and characterization

The CGF is an autologous platelet concentrate, developed by Sacco, in 2006 and obtained from blood samples through a simple and standardized separation protocol, by means of a specific centrifuge (MEDIFUGE 200, Silfradent srl, Forli, Italy), without the addition of exogenous substances. The main feature of the CGF resides in its consistency: it is an organic matrix rich in fibrin, able to "trap" platelets, leukocytes and growth factors; elements that play an important role in the regenerative processes.

CGF-(Concentrated Growth Factors)

Salient features of the CGF:
- Simple, safe and economic
- Natural - 100% autologous
- Thick Fibrin Matrix
- Leukocytes, Platelets and Growth factors
- Variable kinetics release
- Matrix for Bone Graft Material

The CGF may be a valuable aid in the field of regenerative medicine, to speed up the process of regeneration. In fact this growth factor concentrate, showed great regenerative properties and versatility (Sohn et al. 2009).
Its use has been proposed in various situations ranging from filling of extraction sockets (Tadić et al., 2014) to the filling of the cavity after cystectomy (Mirković et al., 2015), or in the sinus lift procedure (Kim et al., 2014; Del Fabbro et al., 2013; Sohn et al., 2011). Moreover, it can be used alone or with autologous bone particles or biomaterials (Gheno et al., 2014). Some authors suggest wet the surface of the implants with CGF in order to accelerate the bone-integration (Siebrecht et al., 2002).
- Scanning Electron Microscopy (SEM) studies, have shown that the CGF presents a fibrin network formed by thin and thick fibrillar elements (Rodella et al., 2014).
- Histo-morphological studies (Borsani, Bonazza et al., 2015 submitted) have allowed to see the fibrin network structure and the distribution of blood cells (leukocytes, erythrocytes and platelets) in the CGF.
- Finally, in vitro studies using different human cell lines (Borsani, Bonazza et al., 2015 submitted), have shown that the addition of the CGF to the culture medium, stimulated cell proliferation (Borsani, Bonazza et al., 2015, submitted).



CGF is obtained using polyethylene tubes (Vacuette Test Tubes), coated with silica micro particles and without the addition of exogenous substances. After collection, the blood samples are immediately centrifuged using a special centrifuge device (Medifuge, Silfradent, Italy).

CGF - MATERIALS

BLOOD COLLECTION
1. Antiseptic swab
2. Complete butterfly
3. Tourniquet
4. Gauzes
5. Patches
6. Vacuette Test Tubes (Greiner Bio-One GmbH, Kremsmunster, Austria)
7. Tube rack

CGF MEDIFUGE (Silfradent)
Characteristics

• Benchtop centrifuge dedicated to the CGF production, equipped with an appropriate rotor with alternate and controlled speed and with an acceleration always below 300 RCF.
• The medical device MEDIFUGE allows for the use of up to 8 test tubes for
the creation of CGF (fibrin);
• A microprocessor control system allows for the maintaining of a constant speed;
• The exception rotor system with self-ventilation protects the blood sample from
heat exposure;
•The rotor-holding compartment, the closing door and the test tube-holding jackets guarantee biological safety in terms of bio-containment, in the event of test tube breakage;
•The test tube-holding jackets and rotor are built from thermal, antistatic
material that is easy to clean, extract and sterilize in an autoclave at 135°;
•MEDIFUGE is equipped with a decontamination cycle with UVC reflected light;
•Cycle duration 5 minutes at 1,000 revs;
•The electronic control engine and its internal parts require no maintenance;
•Noise levels fall below the standards required and do not exceed 57 dBa.

CGF centrifugation protocol (One step protocol)
30'' acceleration
2' 2,700 rpm/ 735 g
4' 2,400 rpm/ 580 g
4' 2,700 rpm/ 735 g
3' 3,000 rpm/ 905 g
33'' deceleration and stop

At the end of the process, three blood fractions were identified: (1) the upper layer, representing the liquid phase of plasma named platelet poor plasma (PPP); (2) the lower layer, representing red blood cells (RBC) because of mainly contains erythrocytes; (3) the middle layer, representing the solid CGF consisting in three parts: the upper white part, the downer red part (about 0,5 cm from RBC) and the middle "buffy coat*" part (interface between white and red part) *(Figure 1 A,B,C).*

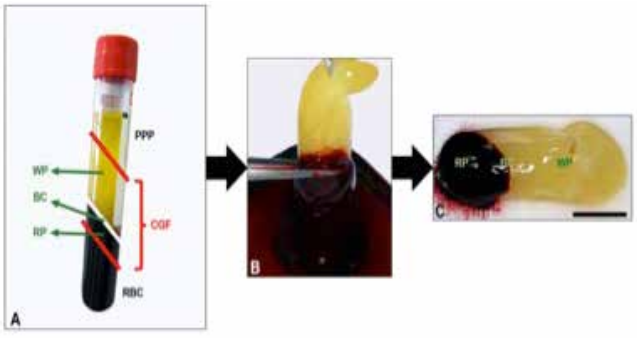


Fig.1A,B,C: phases of CGF

CGF- Morphological characterization

FIBRIN NETWORK
The use of electron microscopy (SEM), allowed to observe that the CGF fibrin network of the is constituted by thin and thick fibrillar elements *(Figure 2A).*

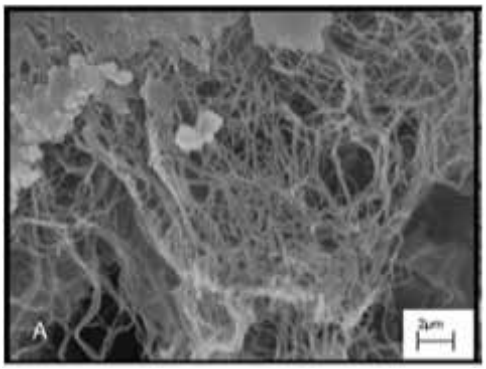


Fig.2A: SEM analysis of CGF, fibrin network

Hematoxylin-eosin staining, allowed to observe the architecture of the CGF fibrin network *(Figure 3).* The images showed that the fibrin network and architecture changed moving from the buffy coat* to the white part. In particular, near the buffy coat* the fibrin network was strictly compact *(Figure 3A)* while far from the buffy coat* became with a larger mesh *(Figure 3B).*

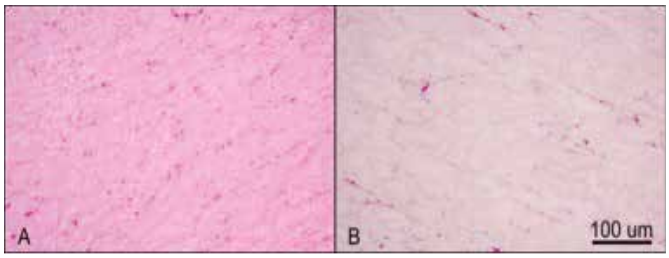


Fig.3: Architecture of the fibrin network:
A) near the buffy coat; B) far from the buffy coat;

BLOOD CELLS
The May Grunwald Giemsa histological staining *(Figure 4A)* and Hematoxylin-eosin *(Figure 4B),* allowed to localize blood cells present in the CGF. White blood cells are mainly located in the buffy coat* and dispersed in it, especially in the red part of the CGF; the red blood cells are present only in the red part of the CGF.

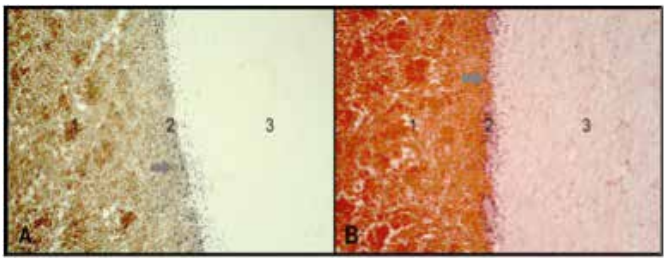


Figure 4: A) May Grunwald Giemsa; B) Hematoxylin-Eosin

Using immunohistochemical analysis, with the platelet marker CD61 *(Figure 5B)*, platelets appear principally in the buffy coat* of the CGF, although platelet aggregates have been highlighted also in the white part of the CGF.

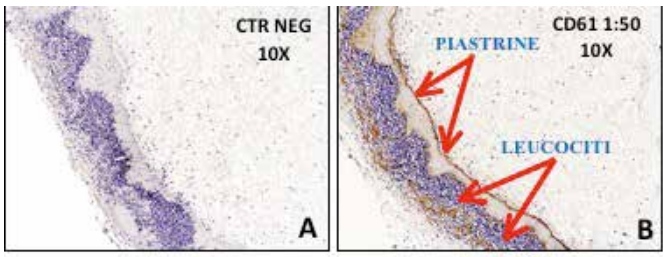


Figure 5: platelets immunohistochemical analysis using CD61: A) negative control, without CD61; B) with CD61

* interface between the white part (PPP) and the red part (RBC) of CGF.

CGF - in vitro Growth Factors Release

The in vitro kinetics release of certain CGF growth factors, showed that this is specific to each factor. In fact, some of them have a quick release (1 day) while others have a slower release (up to 6-8 days)*(Figure 6 a,b)*.

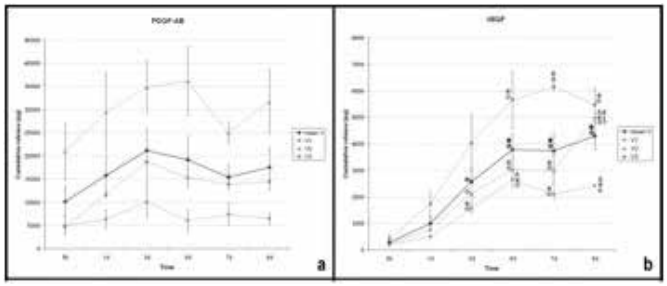


Figure 6: Kinetics release of a) PDGF-AB and b) VEGF

TNF-ß reaches its maximum accumulation at day 1 and after decreases (Figure 6 c). So it has a fast kinetic release.

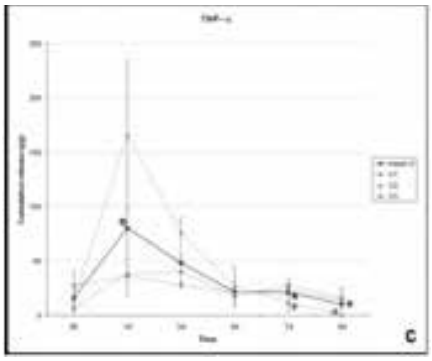


Figure 6 c: Kinetics release of TNF-ß

TGF-ß1 e BDNF have a constant accumulation *(Figure 6 d,e)*.

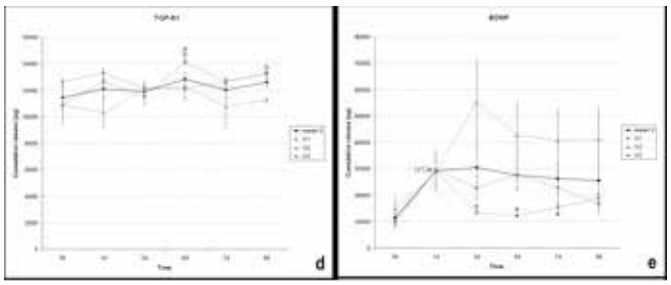


Figure 6: Kinetics release of d) TGF-ß1 and e) BDNF

BMP-2 reaches its maximum accumulation on day 8 and IGF-1 on day 6 *(Figure 6 f,g)*.

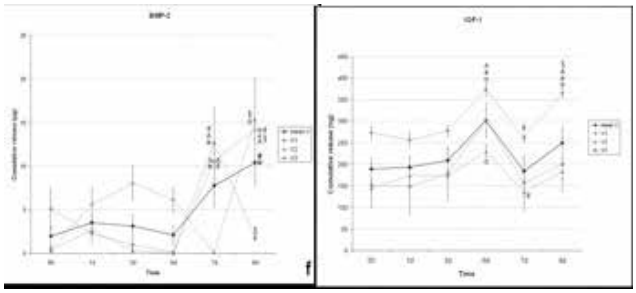


Figure 6: Kinetics release of f) BMP-2 and g) IGF-1

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