

# FISIOGRAFT A NEW GRAFT MATERIAL

**Biocompatibility and implantation in bone**  
**Review of the pre-clinical results in animals at 1 and at 2 months**  
**Review of the clinical results in man at 4 months and beyond**

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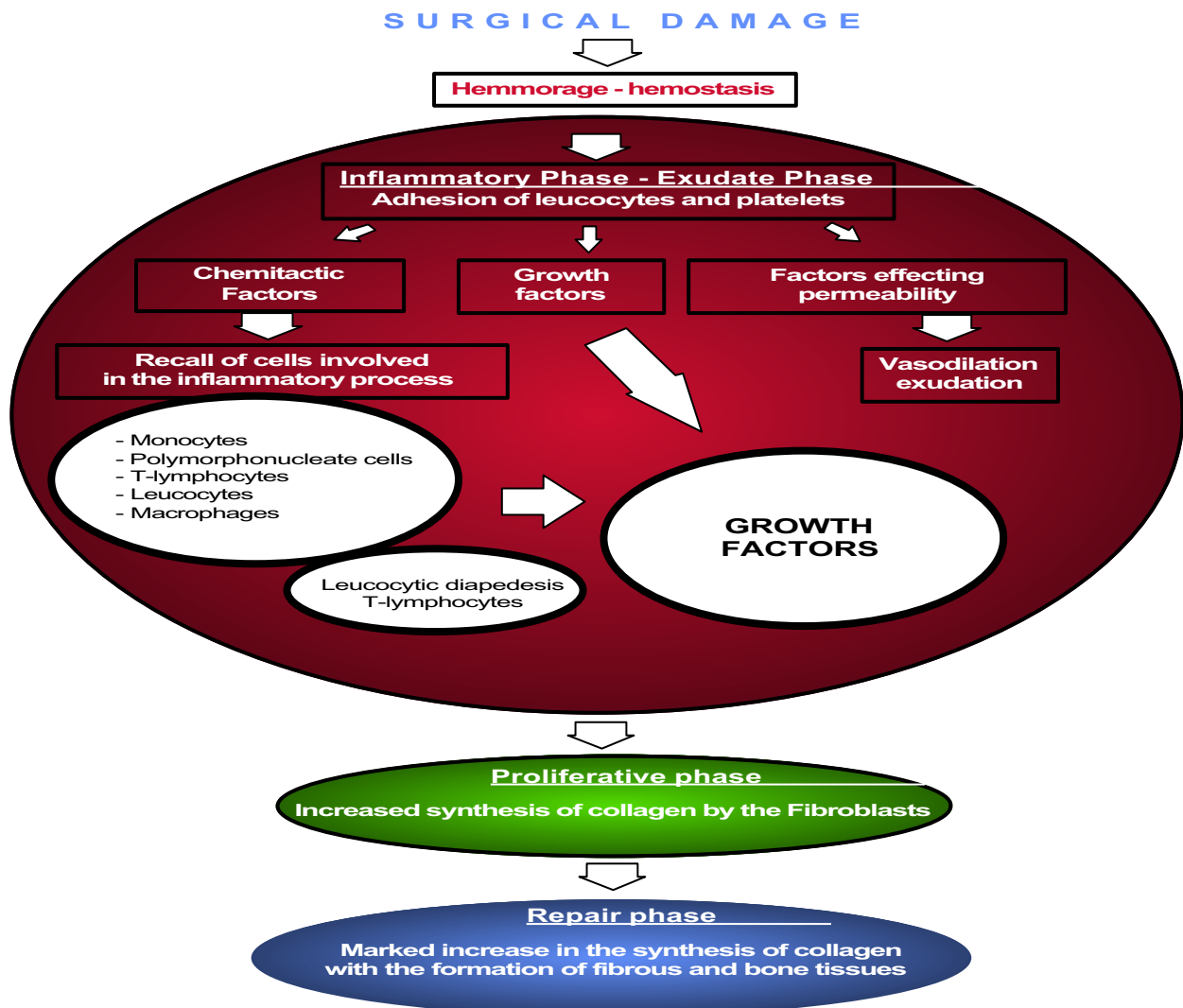
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## FISIOGRAFT: BIOCOMPATIBILITY

All medical devices that come into direct contact with a living organism must be composed of biologically compatible materials, that have the chemical physical characteristic required for the function they perform, they must not cause either systematic or local toxicity, they must not be carcinogenic or genotoxic. In addition they must not affect the blood clotting mechanisms when the material comes into contact with the blood, they must not induce an immune response by the organism or be prone to infection (Pizzoferrato et al. 1992).

The use of biologically active materials in implants has necessitated modifying the concept of biocompatibility.

Actually for biocompatibility one means the **capacity of a material to function in a specific application provoking an appropriate response** by the host, assuming of course that the material itself does not provoke a systemic toxicity, ascertained by traditional toxicological tests (Williams 1987). The appropriate response by the host in the clinical application of the biomaterial (in particular those biodegradable) and the result of the interaction between the artificial material and the biological systems, is that they are completely absorbed when their function is no longer necessary or has been completed.



### ***The repair process in the absence and in the presence of implanted materials.***

In order to better understand the correlation between biological reactions and biocompatibility, it is useful to carefully describe **the repair process resulting from surgical damage in the absence or in the presence of implanted materials.**

**In the absence of implanted materials,** a surgical intervention will always produce a trauma that is repaired by the organism by a process consisting of three phases, that in time will overlap one another:

the inflammatory or exudate phase, the proliferative phase and the repair phase (Tolone 1987).

The first phase of the wound repair starts with homeostasis which is triggered by the interaction between the walls of the vessels, the platelets and the clotting factors. The coagulum which forms as a result of this interaction, becomes colonized within a short period of time (2-4 hours) by inflammatory cells

and within 24-36 hours by fibroblasts; the three dimensional net of fibrin, which constitutes the framework of the coagulum, acts as a guide for the cellular colonization.

During the inflammatory or exudate phase, the cellular necrosis resulting from the surgical insult and activation of the homeostatic process which is initiated by the hemorrhage, brings about the production and liberation of numerous factors that guide the migration of inflammatory cells into the area that has been damaged creating the conditions for the subsequent healing that will take place.

The lysosomal enzymes, liberated from both dead and living cells whose membrane has been subjected to the action of lysing factors, stimulate the formation of active fractions of the cascade of the complement. The fractions  $C_{3a}$  and  $C_{5a}$ , the mechanical insult and other factors, induce the degranulation of the mast-cells which are distributed throughout the connective tissues. Histamine, derived from the degranulation of the mast-cells and chinine, produced by the action of factor  $XII_a$ , provoke vasodilatation and enlargement of the intercellular junctions in the endothelial.

The vasodilation in turn provokes a slowing of the blood flow that permits the leukocytes to migrate towards the margins of the vessel, adhere to the wall and pass through it. The adhesion of the leukocytes to the endothelial cells is regulated by various types of adhesion molecules which are found on the membranes of both the leukocytes and the endothelial cells, this action is regulated by cytochine which is produced by the fibroblasts situated at the site of the surgical trauma.

Enlargement of the intercellular junctions permits the leukocytes and the exudate to pass through.

Chemotactic factors cause the leukocytes present in the extravascular spaces to migrate in the direction of the inflammatory stimulus. Even the platelets, which aggregate during the formation of the clot, contribute to the development of the vascular-hematic phenomena of the angiophlogosis by means of extrusion of granules by numerous substances, vasopermeable (serotonin) and chemotactic (leucotriens synthesized by the activation of the lipossigenasis pathway) and growth factors.

The inflammatory phase is completed with the alteration of the tissue matrix provoked by the liberation of the lysosomal enzymes. The polymorphoneucleated and the macrophage cells, derived from the transformation of the monocytes,

### Healing in the presence of implanted material

In the presence of an implanted medical device the tissue reaction that brings about the repair of the surgical damage undergoes variations in intensity and duration. If the implant is biocompatible modifications of the repair process are limited and in the final phase the presence of fibrosis is minimum,

exert a phagocytic function serving to remove cellular debris and possibly, eliminating the cause of the inflammation. In addition the macrophages, have the ability to secrete substances that function like growth factors, initiating the second or prolific phase of the healing process of the wound.

The growth factors, produced by the macro-phages, exert on the fibroblasts, a chemotactic attraction, stimulate their reproduction and induce them to synthesize collagen; they stimulate the T-lymphocytes to produce chemotactic factors and factors capable of increasing the synthesis of collagen by the active fibroblasts (the difference in the roles played by the macrophages and lymphocytes resides in the fact that the first are essential for initiating and maintaining the healing process, while the lymphocytes are not required in the beginning but are necessary for regulating the continuation of the phenomena).

The platelets assist the macrophages, implicated not only for the formation of the coagulum, but also for the secretion of substances that favor tissue repair. The platelet factors act both in an indirect way, attracting macrophages and fibroblasts and in a direct way, stimulating the replication of the fibroblasts and the synthesis of collagen.

Even the endothelial and cheratinocytes are capable of synthesizing growth factors that are important above all for the repair phase of specific tissues.

The third phase of the healing process - the repair phase - is characterized by the marked synthesis of collagen with the formation of fibrous tissue more or less abundant and more or less compact, with the purpose of substituting the tissue that was damaged during the surgical procedure.



which would normally form following a typical repair of a surgical wound.

Surrounding the implanted material there is an accumulation of extracellular fluid that contains proteins (fibrinogen, albumin,  $C_3$ , immuno-globulin) and inflammatory cells.

The proteins are absorbed at the surface of the implant and undergo a variation of their conformation to such an extent that they condition the functional response of the peri-implant cells (adhesion, phagocytosis, secretion of inflammatory mediators) (Pizzoferrato et al. 1986).

In turn the inflammatory cells can modify the structure and physical-chemical properties of the surface of the implant, sometimes causing a foreign body giant cell reaction, activating the macrophages and the production of cytokines which stimulates the production of collagen and bone tissue (Pizzoferrato et al. 1993).

Any device that remains for a certain period of time in a living organism will eventually become surrounded by a layer of fibrous connective tissue, that develops with different thickness and different structural organization depending on its location, the mechanical stimuli and the chemical characteristics of the interface (Pizzoferrato et al. 1991).

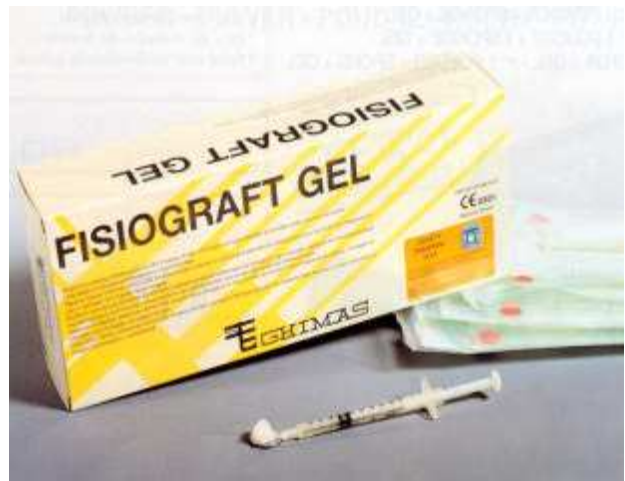
Regarding the biodegradable devices, as their absorption progresses and while performing their function for which they had been implanted, the physiologic inflammatory response will decrease up to the point where it will completely disappear.

If this is the physiologic process that leads to the healing of a wound in the presence of an implanted material, it is opportune to point out certain aspects:

- the object of the biological repair process is that of integration, where there exists compatibility between the tissues and the biomaterial,
- the biological compatibility permits for the integration of the biomaterial, on the part of the tissues or else for their absorption when the function of the biomaterial is no longer necessary,
- during the integration between the bio-material and the tissues, the organism activates physiologic mechanisms for a biologic response, that brings about the activation of specialized cells capable of synthesizing growth factors.
- the presence of such cells, sometimes defined as inflammatory cells (polymorphonucleates, macrophages, lymphocytes, platelets), indicates that at the site of the implant the organism is actively participating in the physiological healing process (even the momentary presence of a giant cell reaction associated with the demolition of substrates, has its importance in the physiological repair process).
- in the presence of materials that are not compatible, the equilibrium between the reparative response of the organism is shifted dramatically towards a rejection of the implant, in such a way that the persistence of the mitigating factor, due to a lack of integration of

the implant within the host, will result in a condition of chronic inflammation,

- the persistence of any inflammatory condition brought about by the lack of removal of the irritating stimulus by the mechanisms that are activated during the angiophlogosis is more than likely to create a condition of chronic inflammation (a classic example is that of an arthritic pathology in which the chronic deterioration of articulation cartilage brings about an attempt to remodel the articulation system, with a result that is insufficient and harmful),
- **biologically active and biologically degradable materials have the ability to stimulate the physiologic healing process, intensifying the repair capabilities of the organism, and due to the fact that the material is progressively absorbed, the onset of the phenomenon of a chronic phlogistic reaction is avoided.**



## **Investigation of biocompatibility**

The biomaterials utilized for the construction of medical devices, as has already been mentioned, must not provoke generalized toxic effects to organs or systems within the living organism. The devices for clinical use, in particular those that will be implanted, must have safety characteristics for the protection of the patient. They must not alter the components of the tissues or biological and physiological liquids with which they come into contact. The study of the biocompatibility of the materials that make up the device is aimed at guaranteeing the maximum benefit with a minimum risk to the patient.

The biocompatibility is evaluated on the basis of the type of material and the clinical application for which it is intended, keeping in mind international guidelines as they are established. Amongst the international organizations the most important of these is the International Organization for Standardization (I.O.S.) composed of the Regulatory Agencies of the most advanced industrialized nations and subdivided into Technical Committees, amongst which is the I.S.O./TC-194 which deals with establishing the guidelines for biocompatibility.

The tests reporting the pre-clinical results regarding the biocompatibility of FISIograFT and which are briefly described in the following paragraphs all follow the existing national and international guidelines and are reported in the guide entitled: "Biological Testing of Medical Devices - Part 1: Guidance on selection of tests" (ISO 10993-1).

In the various tests FISIograFT type gel, type powder and type sponge were used. In tests that required the use of eluates, the trials were performed using FISIograFT type sponge, since the two forms, sponge and powder, even though they appear different have the same chemical composition.



## **TOXICOLOGICAL TESTS**

The excellent biocompatibility of FISIograFT, was documented above all by the classic mutagenesis

tests (testing for genotoxicity and cytotoxicity) and the test for cutaneous sensitivity (Gatti et al. in press).

### **Test for genotoxicity**

**The object of the test for genotoxicity was to evaluate the potential genotoxic risk of a material when it comes into contact with yeast cultures.**

The test was performed following the procedures established by the European Community EN 30993-1 and EN 30993-3, that make reference to the OECD Guidelines for testing chemicals n° 481 (EN 30993-1, EN 30993-3, OECD). During the normal growth of yeast cultures they will undergo the phenomenon of mutation: therefore the evaluation of an increase in the number of mutations induced by any substance can be used as an index for mutagenic activity that for example, can manifest in an organism in cells that have an elevated mitotic activity, cells such as hematic cells, mucous cells, etc. (Albertini 1989, Ames et al. 1975, Zimmermann et al. 1975).

The results have demonstrated that FISIograFT in no way modified the frequency of mutations with respect to the control. A diminution of the surviving cells, especially in the case of FISIograFT type powder, is due to the intrinsic toxicity of the lactic acid, released by the co-polymer, that in this closed test was not buffered. This phenomena which occurred due to the type of methodology utilized does not occur when the device is implanted in living tissues where there is a constant blood flow that is able to buffer the lactic acid and to eliminate it by means of specific metabolic cycles.

### **Conclusion**

**The test for mutagenesis proves that FISIograFT type sponge, type powder and type gel are neither toxic or mutagenic.**

### **Test for cytotoxicity**

**The test for toxicity utilizes connective cells particularly sensitive to unfavorable survival conditions; therefore their level of vitality becomes an important sign in determining their tolerability to the material tested. This test also evaluates the potential cytotoxic risk of a material once it has been placed into contact with living cells.**

The test was performed following the guidelines established by the European Community EN 30993-1 and EN 30993-5, and based upon scientific literature (Araki et al. 1994, Assad et al. 1994, Chong et al. 1994, EN 30993-1, EN 30993-5, Harmand 1995, Wataha et al. 1994a-1994b-1995, Zentner et al. 1994).

The trials for direct toxicity did not reveal any toxic effect in the case of FISIOGRAFT type sponge. FISIOGRAFT type gel did however demonstrate a certain degree of direct toxicity which can be attributed to the rapid release of the excipient Polyethyleneglycol.

The results of the trials for indirect contact did reveal a certain degree of cellular sufferance, more than likely due to the methodology that favors a rapid release of the excipient (dextran in the case of FISIOGRAFT type sponge and the polyethyleneglycol in the case of FISIOGRAFT type gel) that alters the osmotic equilibrium of the culture media.

From the moment that the trials for direct contact gave a positive result, the material can be considered to exert an acceptable level of toxicity for a biodegradable material.

For the correct interpretation of the test, it must be emphasized that in a living organism the ambient condition in which contact is made between the material and the cells is an open type (and not closed, like the tests in the laboratory); therefore in a living organism, due to the presence of buffering and metabolic systems, the toxic action of the substances, dextran and the polyethyleneglycol which are capable of altering the osmotic equilibrium of the culture media is minimized.

## Conclusions

**The test for cytotoxicity proves that FISIOGRAFT type sponge, type powder and type gel are not to be considered cytotoxic.**

### *Test for cutaneous sensitivity*

**The object of the test for cutaneous sensitivity in guinea pigs is to evaluate the potential allergic risk of a material that is placed into contact with the skin.** It is known that an organism can have either an immediate or delayed reaction to an antigen that it comes into contact with.

The test was performed conforming to the guidelines of the European Community EN 30993-1 and EN 30993-10 and based upon scientific literature (EN 30993-1, EN 30993-10).

## Conclusions

**The test for sensibility have demonstrated that FISIOGRAFT type sponge, type powder and type gel do not have any potential allergic risks, since the response obtained in the test was identical to that of the control group.**



## TEST FOR THE BIOLOGICAL COMPATIBILITY OF FISIOGRAFT

Having already documented the absence of any toxic effects of FISIOGRAFT, more complex and selective studies were performed in order to ascertain the level of biologic compatibility of the material. During the execution of the various tests involving subcutaneous and bone implants, in addition to the evidence demonstrating an excellent level of biologic compatibility of FISIOGRAFT, a finding emerged clearly showing its action which permits it to be used as a filler in bone defects, actions confirmed by the successive clinical studies performed on humans.

### *Testing of subcutaneous implants of FISIOGRAFT*

**The testing of subcutaneous implants evaluates the response mechanisms of an organism to an implant that possibly would not be tolerated. The response to grafted material in soft tissue is without a doubt the most exasperated and therefore the easiest to see. The testing of subcutaneous implants in the rabbit verifies, within a short period of time, the biocompatibility of FISIOGRAFT type sponge, type powder and type gel when placed into contact with soft tissues.** The tests were performed in conformation with the guidelines EN 30993-6 and UNI 9582/3 (EN 30993-6, UNI 9582/3).

## Conclusions

**The testing of subcutaneous implants has demonstrated that FISIOGRAFT type sponge induces a momentary inflammatory response, that remained localized around the implanted material and that can be attributed to its rapid metabolism. Instead with FISIOGRAFT type gel, there was no evidence of any inflammatory reaction.**

These results are in line with what has been reported in the literature and shows that the initial inflammatory reaction can be partially responsible for attracting cells that promote bone neogenesis.

### *Testing implants of fisiograft in rabbit bone*

The testing of implants in rabbit bone has demonstrated, in a short period of time, the biocompatibility of FISIOGRAFT type sponge, type powder and type gel when placed into contact with hard tissues, in such a way as to study the actual integration between a biomaterial and bone tissue, the final destination of the implant and to verify the osteogenic action a function much sought after result and essential for a biomaterial that is to be defined as osteoconductive. The tests were performed in conformation with the guidelines EN 30993-6 and UNI 9582/3 (EN 30993-6, UNI 9582/3).

### Results of the test

- **Implant in bone with FISIOGRAFT type sponge after 30 days**

The microradiological analyses of the sections showed that even after only 30 days the implanted material FISIOGRAFT type sponge had stimulated the appearance of islands of newly formed bone, that had grown starting from the osteotomic line trying to fill the defect.

These islands were not found in the defects that were left empty in the control subjects (Fig. 1).

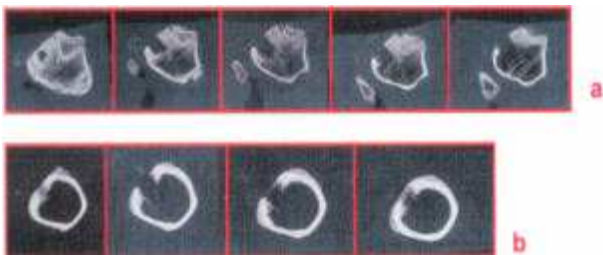


Fig. 1 - Microradiographic analysis of the sections containing FISIOGRAFT type SPONGE: a - control (without); b - 30 days after implantation

The analysis of the scanning electron microscope (SEM) conducted on the lucidate and carbonate sections showed evidence of a certain degree of remargination of the bone even only 30 days after it was implanted (Fig. 2)

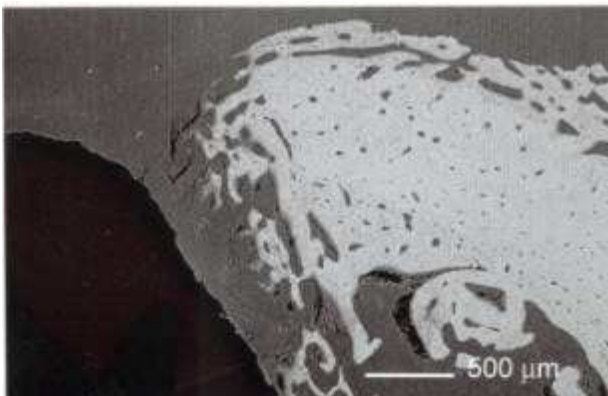


Fig. 2 - SEM analysis of FISIOGRAFT type SPONGE 30 days after implantation; it is possible to note some bone trabeculae that are growing towards the central zone of the implant

- **Implant in bone with FISIOGRAFT type sponge after 60 days**

The microradiological analyses of the sections made 60 days after being implanted show that a major portion of the defect has been filled in and substituted with newly formed bone; the cortical bone, in fact, had been almost completely reconstructed (Fig. 3).

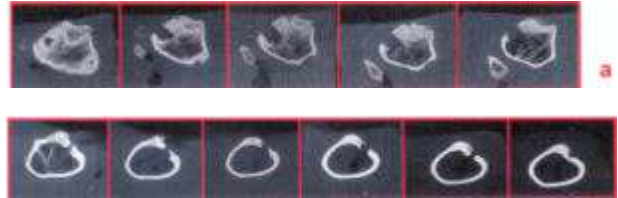


Fig. 3 - Microradiographic analysis of the sections containing FISIOGRAFT type SPONGE: a - control (without); c - 60 days after implantation.

In the SEM analysis of the section of the diaphysis of the rabbit made 60 days after being implanted, there is evidence of new bone growth starting from the osteotomic line and proceeding towards the center of the defect, attempting to close it (Fig. 4 and 5).

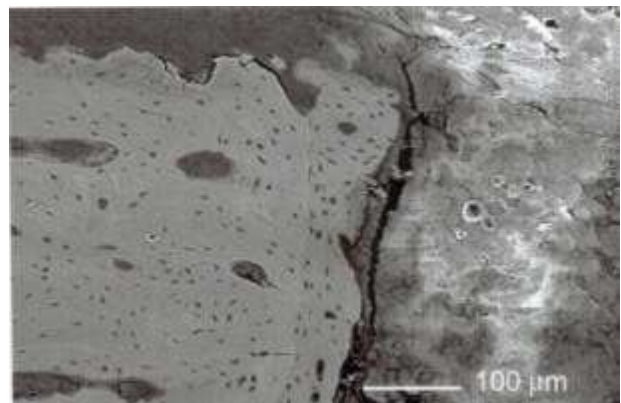


Fig. 4 - SEM photograph taken at the site 60 days after FISIOGRAFT type SPONGE had been implanted; a front of newly formed bone is visible starting from the osteotomic line

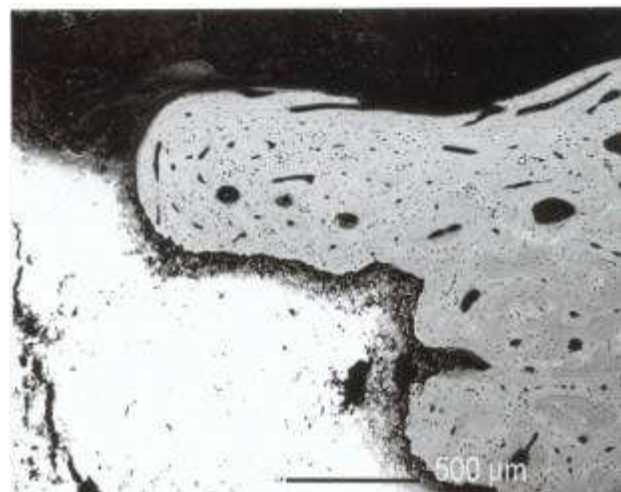


Fig. 5 - SEM microphotograph taken at the site 60 days after FISIOGRAFT type SPONGE had been implanted; a

large mass of newly formed bone has grown on the material attempting to fill in the defect

The newly formed bone had cortical structure and had an optimum level of mineralization and having the same gray level as the existing bone.

- **Implant in bone with FISIOGRAFT type gel after 60 days**

The microradiological analyses of the sections showed that after 60 days a certain level of reconstruction of the cortical wall of the femur had filled in the defect that had originally been created (Fig. 6).

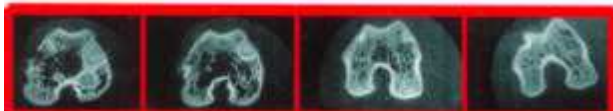
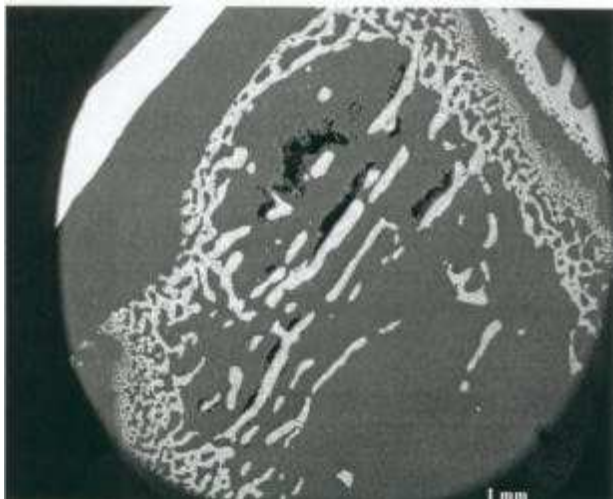


Fig. 6 - Microphotograph of the section of the femur containing FISIOGRAFT type GEL 60 days following implantation

The SEM analyses made on the sections demonstrate that 60 days after implantation it was no longer possible to recognize the implant site. The walls of the femur had been reconstructed and it was possible to note definite bone growth at the site where the material had been implanted (Fig. 7 and 8).



## IMPLANTATION OF FISIOGRAFT IN HUMAN BONE

On the basis of the excellent biocompatibility of FISIOGRAFT backed up by documentation in the tests that were previously cited for mutagenesis (test for genotoxicity and the test for cytotoxicity), the test for cutaneous sensitivity and by the test for subcutaneous and bone implantation, various clinicians have experimented with FISIOGRAFT in the fields of implantology and dental surgery, in procedures where the common denominator was the search for new formed bone deposits in pre-existing defects.

Fig. 7 - Macrophotograph of the bone tissue containing FISIOGRAFT type GEL 60 days after being implanted; the wall of the femur has been reconstructed

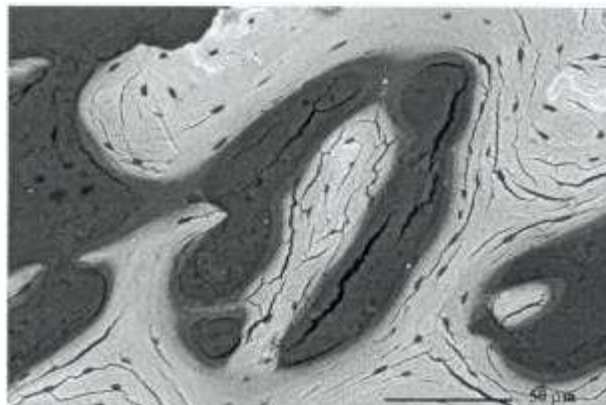


Fig. 8 - Details of a newly formed bone trabecula within the defect containing FISIOGRAFT type GEL

Thin bone trabeculae, highly vascularized, cross the cavity that had been previously filled with FISIOGRAFT type gel: the vascularization indicates the presence of a slight biological reaction, probably due to the degradation of the material.

## Conclusions

The test of implantation in bone documents that after only 30 days following implantation new bone trabeculae are formed, that have a tendency to occupy the cavity and that the material has been almost completely absorbed.

This result not only demonstrates the excellent biocompatibility of FISIOGRAFT, but also shows that it functions as a graft material which allows it to be substituted by bone tissue.

## Line of clinical research

- **FISIOGRAFT in a post-extraction reconstruction of the alveolar crest** (Minenna et al. 1998)

At the institute of Dental Science at the University of Ancona, directed by Prof. M. Procaccini, FISIOGRAFT was used as a filler for a post-extraction reconstruction of the alveolar crest by means of an auto graft of bone that was rotated and repositioned coronally. In this work a technique is

described which provides for the combined use of autologous bone and FISIOGRAFT. One can observe the excellent healing that has taken place at the site, this is an essential condition in order to obtain

an optimal positioning of the future implants. The various phases of the surgery can be observed in the following figures (Fig. 9 - 14).

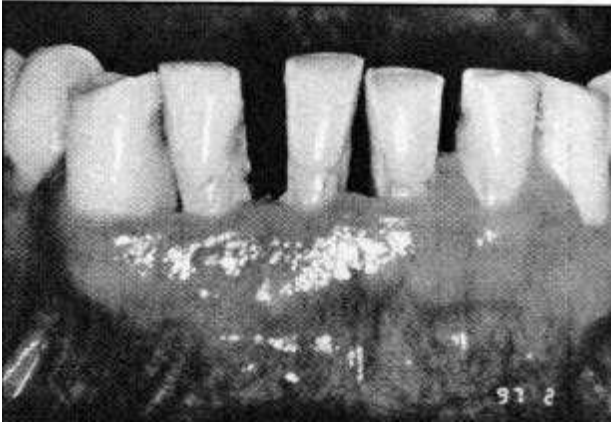


Fig. 9

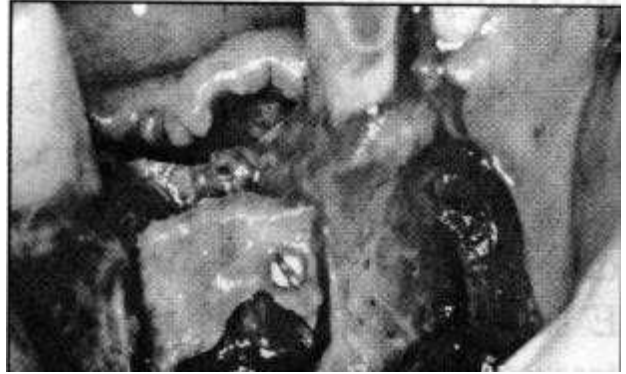


Fig. 12



Fig. 11



Fig. 14



Fig. 13

- **FISIOGRAFT used as a filling material for a periimplantar regeneration of post-extraction alveoli and atrophic crests** (Leghissa et al. 1997-1998, Leghissa & Leardi 1998)

The authors have studied the use of implants as a support for total prostheses (overdentures). In the cases in which the atrophy in the maxillary and mandibular arches was particularly pronounced the threads of the implant were exposed, these cases were treated with FISIOGRAFT type GEL in conjunction with a membrane. The results that were obtained were satisfactory and in line with those reported in other findings.

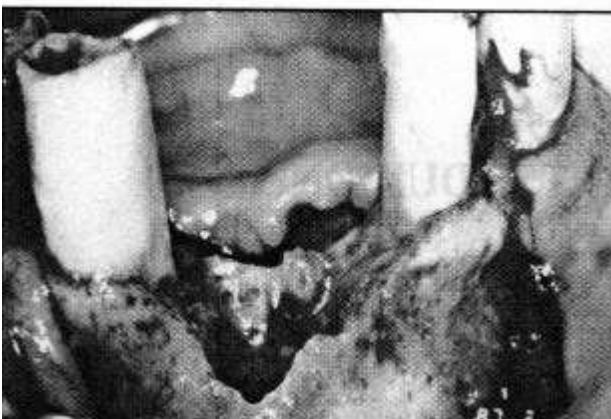
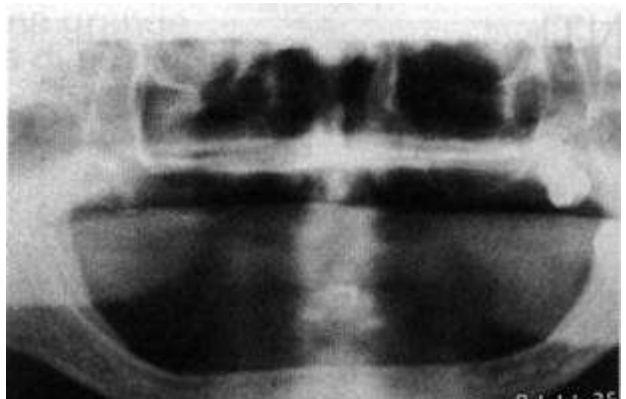
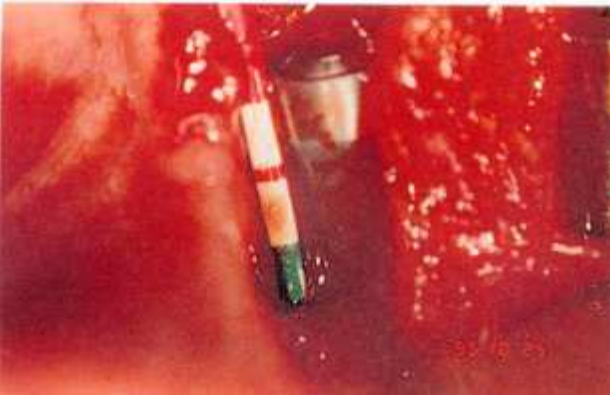


Fig. 10



□ Fig. 15: A case presenting with a particularly atrophic mandible.



□ Fig. 16: An implant with exposed threads.



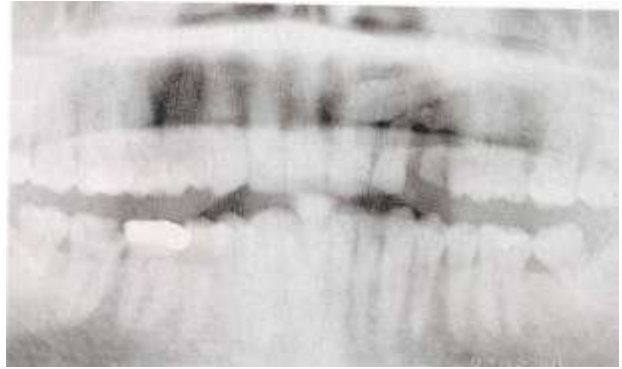
□ Fig. 17: After four months the membrane is removed and we find that the FISIOGRAFT GEL has been replaced by an abundant deposit of osteoid tissue.



□ Fig. 18: The implant in position with good regeneration of the vestibular wall.

In other clinical studied the authors experimented with the use of FISIOGRAFT type GEL in post-extraction alveoli that were candidates for implants.

In a case report a particularly ample regeneration of an impacted canine tooth is described. In this case FISIOGRAFT type GEL was combined with a titanium grill. The regeneration was excellent, as evidenced in the following figures.



□ Fig. 19: A panoramic radiograph which clearly shows the impacted upper left canine with the deciduous tooth.



□ Fig. 20: An implant with exposed threads.



□ Fig. 21: FISIOGRAFT type GEL and the titanium grill.



□ Fig. 22: Upon re-opening a good regeneration of the vestibular bone can be observed.

The bone regeneration that was obtained, evidenced by clinical observation and radiological studies, was objectively documented by means of a microscopic analysis and ultrastructural studies performed with a scanning electron microscope.

- **FISIOGRAFT in the daily clinical practice**  
(Zerbinati in press)

FISIOGRAFT was utilized in routine clinical situations both in the field of periodontology and implantology. Two personal cases will now be described.

In the first a notable vestibular periodontal compromise of 11 (Fig. 23). With the flap open we can observe a serious bone dehiscence (Fig. 24), in Fig. 25 the positioning of FISIOGRAFT type GEL, in Fig. 26 suturing. Figures 27 and 28 the healing and the excellent results at 20 days and three months.

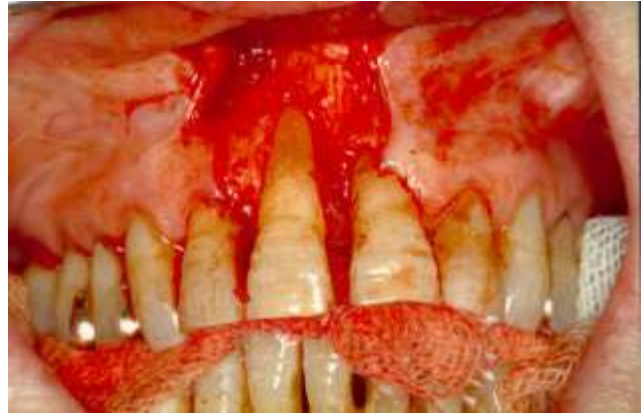


Fig. 24



Fig. 25



Fig. 26



Fig. 23





Fig. 27

Fig. 28

In the second case we observe, in Fig. 29, element 36 prior to extraction, and in Fig. 30 the filling of the

alveolus with FISIOGRAFT type SPONGE after the extraction.



Fig. 29



Fig. 30

Four months later (Fig. 31) the oral radiograph shows an excellent regeneration at the site, which permits the successful placement of an implant (Fig. 32).



Fig. 31



Fig. 32

- **FISIOGRAFT used in the major lift of the maxillary sinus** (Ghinzani 1998)

Ghinzani describes a clinical case in which FISIOGRAFT type sponge was used during the surgical procedure for a major lift of the maxillary sinus.

In this type of procedure the cavity that is to be created is so large that filling it with autologous bone taken from the chin, would have been very traumatic to the patient.

The use of FISIOGRAFT, permitted him to avoid resorting to an autologous bone graft rendering the major lifting of the floor of the maxillary sinus a procedure accessible to any dental surgeons.

The figures below describe a case example.



□ Fig. 33: Here we see the preparation of the cavity.



□ Fig. 34: The positioning of the implants.



□ Fig. 35: The filling of the cavity is obtained by wedging the FISIOGRAFT type sponge, which can also be combined with the other forms, powder and gel.



□ Fig. 36: The results after the cavity has been filled.

- **simplified and miniinvasive technique for the lifting of the floor of the maxillary sinus**  
(Bucci-Sabbatini et al. 1999a-b, Bucci-Sabbatini & Salvatorelli 1999)

The authors have proposed a new innovative procedure for lifting the floor of the maxillary sinus, taking advantage of the physical characteristics of FISIOGRAFT type GEL. This material, in fact, has a consistency and plasticity very similar to that of silicone, therefore it is soft and plastic and not traumatic. This new technique involves creating an operculum with a 3 mm bur, the fracture of the floor of the sinus, is performed manually with the use of an osteotome. The lifting of the sinus is obtained by means of the hydraulic force produced as the FISIOGRAFT type Gel is extruded from the syringe in which it is supplied. The operculum and the tip of the syringe have in fact, the same diameter.

The following figures describe this case.

- Fig. 37: The hole in the bone crest
- Fig. 38: injection of the FISIOGRAFT type GEL at the level of the operculum
- Fig. 39: Radiograph at three months - the area which has been filled is still, in part, radiotransparent due to the early stage of this control. However, we can observe a line which is more radiopaque (see arrow) which can be interpreted as the most apical portion of the filling
- Fig. 40: Implant positioned at the site with excellent primary stability,
- Fig. 41: Post-implant radiographic control.



Fig. 38

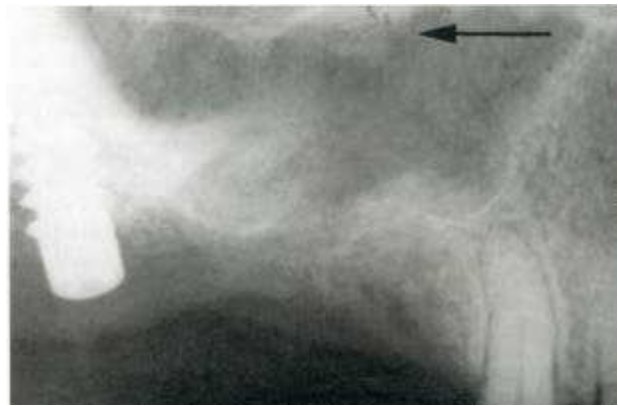


Fig. 39



Fig. 37

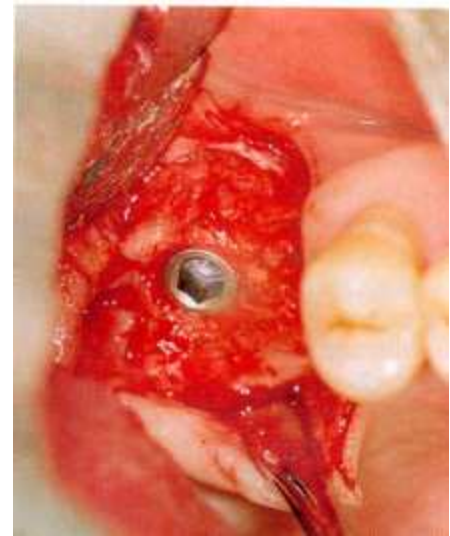


Fig. 40

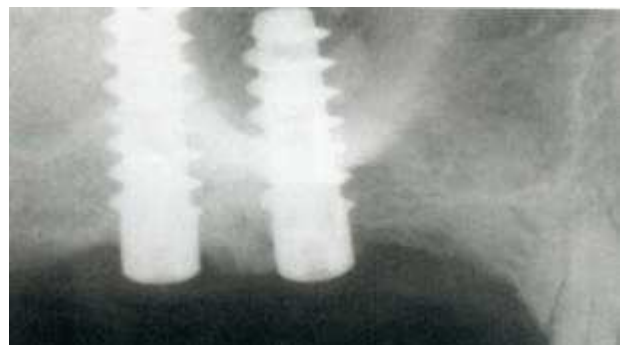


Fig. 41

Upon surgical re-entry for placement of the implants approximately four to five months after the material had been implanted a trephine bur was used to obtain biopsies of the bone in order to perform histological studies. Six to eight months following the implant the patients underwent a CT scan with bone density studies in order to verify the results of the surgery. On the basis of the clinical results that were obtained at eight months - one year, the use of FISIOGRAFT in this surgical technique for the lifting of the maxillary sinus gave positive results in over 95% of the cases, for the stability of the implants, the absence of pain either spontaneous or provoked by pressure, the absence of pathological findings and the positive radiographic results that were obtained (Bucci-Sabattini et al. 1999a-b).

- **use of FISIOGRAFT in periodontology and in extractive and implant surgery** (Stancari et al. 2000)

At the Department of Periodontics at the University of Bologna directed by Prof. Marcello Calandriello, FISIOGRAFT was tested in patients with periodontal, post-extraction and implant pathologies. The studies conducted with clinical and radiological controls which permitted a base screening of FISIOGRAFT in all three of its types, POWDER - GEL and SPONGE, identifying the best use for the various clinical conditions that were treated.

Each case was documented with photographs and monitored over a period of time with periodic controls made every three months, both clinically and by means of intraoral radiographs.

In some cases the newly formed bone deposits were evaluated objectively by means of histological and ultrastructural exams, performed on biopsies that were taken, during the course of the implant surgery, nine months after the material had been implanted.

In the case that follows:



□ Fig. 42: The complete absence of the vestibular wall of 25 that was extracted can be noted,



□ Fig. 43: Positioning of FISIOGRAFT type SPONGE at the site,



□ Fig. 44: Radiograph taken immediately after the surgery,



□ Fig. 45: Radiograph taken after eight months shows good bone regeneration,



□ Fig. 46: Bone regeneration is appreciable even when the site was re-opened.

## ***Histological analyses***

Upon microscopic examination (Fig. 47) the sections, obtained from biopsies 4 months following the placement of the material, appear to be composed of lamellar bone, in which were found numerous osteons characterized by an elevated number of concentric lamellae around Haversian canals. The presence of interstitial lamellae, residuals of preceding generations of osteons, indicates a previous healing process that has already occurred at the level of the bone tissue. The osteons appear well cellularized by osteocytes contained in the bone lamellae. It is not possible to find signs of either inflammation or traces of the implanted material, which demonstrates that during the period comprised between implantation of the material and the taking of the biopsy the material had been completely absorbed and substituted with newly formed bone tissue (Leghissa et al. 1997).

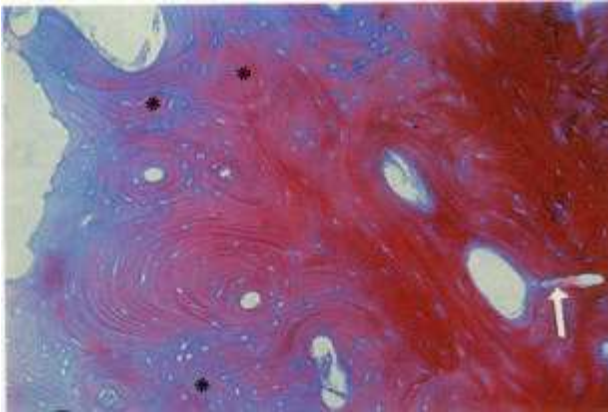


Fig. 47

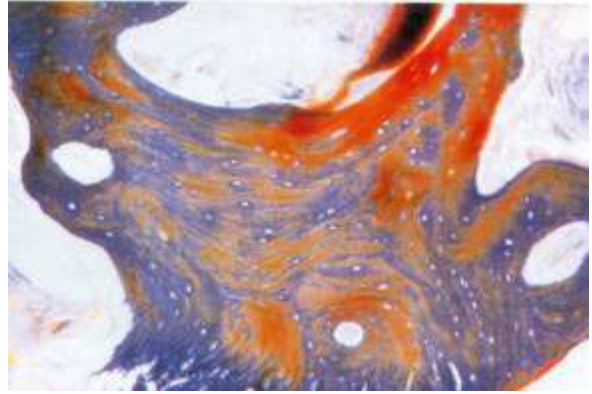


Fig. 48



Fig. 49

The histological analyses made at a later date (after 5 and 9 months) show a situation analogous to that which has just been described (Fig. 48, 49 and 50) (Bucci-Sabattini et al. 1999a, Stancari et al. 2000).

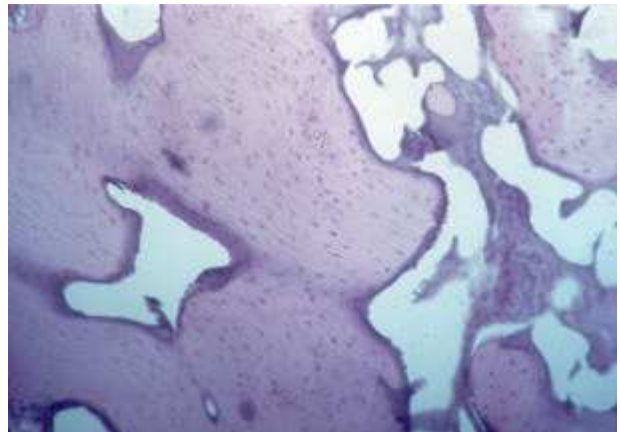


Fig. 50

### Ultrastructural analyses

The scanning electron microscope (SEM) shows Haversian canals which appear to be well lined internally with osteoprogenitor cells (Fig. 51). The fundamental substance, which is highly mineralized, demonstrated by the presence of crystals of hydroxyapatite in the company of bundles of collagen fibers (Fig. 52), appears cellularized by elements identified as osteocytes (Fig. 53). The collagen fibers, in which the bands are well visualized, present a regular disposition, but with different orientation in the contiguous bone laminae (Fig. 52). No substantial differences are seen in the ultrastructure and controls performed 4, 5 and 9 months after implantation of the material (Leghissa et al. 1997, Stancari et al. 2000).



Fig. 51

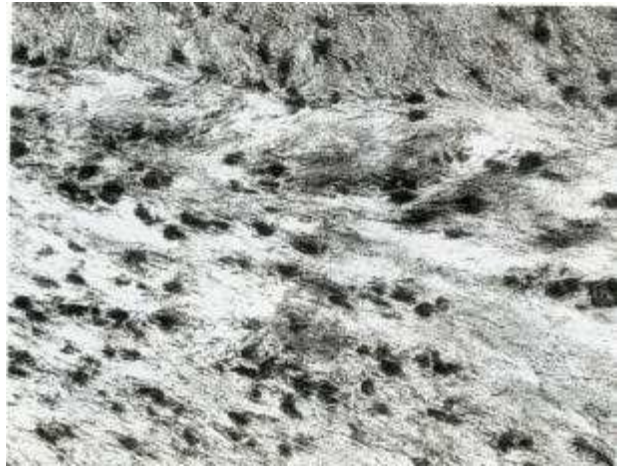


Fig. 52



Fig. 53

### Radiological analyses

The radiological analyses, taken every three months for one year monitored the progressive filling of the defect in the subject that had undergone the periodontal procedure (A) and those that had tooth extractions and implants (B). The greatest newly

formed bone deposits was found in the cases in which a membrane had been applied (Stancari et al. 2000).

RADIOLOGICAL FINDINGS	3 MONTHS		6 MONTHS		9 MONTHS		12 MONTHS	
	A	B	A	B	A	B	A	B
No filling	10	1	0	0	0	0	0	0
Filling < 50%	6	7	10	5	7	2	5	0
Filling > 50%	2	4	8	6	10	4	11	1
Total Filling	0	0	0	1	1	6	2	11

## **CT scan with bone density analysis in the lifting of the floor of the maxillary sinus**

The radiological images at six-eight months from the time of the implantation, in patients that had undergone a surgical procedure for the lifting of the floor of the maxillary sinus, by means of a mini-invasive technique document the lifting of the floor of the sinus to the planned dimensions and the stabilization of the implants in the newly formed bone, as summarized in the table below. In certain intraoral radiographs it is possible to visualize the neoformation of new cortical bone which is well mineralized in an apical position with respect to the implant (Bucci-Sabattini et al. 1999a-b).

The dimensions of the increases obtained range from a minimum of 3 mm to a maximum of 16 mm, with an average lift of 9,6 mm.

Only one implant of the 23 performed had to be removed, for the reason that six months after the procedure it was judged mobile; the success rate obtained was 95.65%.

<b>RESULTS</b>
14 surgical procedures for the lifting of the floor of the maxillary sinus in 11 patients
minimum lift obtained = 3 mm maximum lift obtained = 16 mm
<b>average lift obtained = 9.6 mm</b>
total number of implants performed = 23 number of implants removed = 1
<b>Percentage of success rate = 95.65%</b>

## **CONCLUSIONS**

The **synthesized co-polymer polylactic-polyglycolic (FISIOGRAFT)** utilized for these studies has been shown to be extremely easy to work with, on the basis of the different forms that are available, **sponge, powder and gel** which adapt to the variety of cases which require a **filler for the eventual formation of new bone, with the combined use of the three different forms**, which guarantees a more ideal placement and consequently provides an easier integration with the tissues, a more rapid absorption and a more complete colonization. Whenever an absorbable membrane is used, it becomes necessary to resort to the use of a space maintainer which will prevent the membrane from collapsing which would nullify any results: FISIOGRAFT, as has already been pointed out, is ideal to use under these conditions. Clinical and practical data indicate that the new biomaterial FISIOGRAFT is **biocompatible, non mutagenic, non allergenic and non inflammatory**, confirming the data presented in the literature (Hollinger 1983, Meikle et al. 1994, Vert et al. 1994, Winet & Hollinger 1993).

It is important to emphasize, given the synthetic origin of FISIOGRAFT, the absolute lack of any risk to cross contamination, such as BSE, hepatitis, AIDS and other similar diseases, which qualify it as a biomaterial totally absorbable making it a first choice for utilization in periodontics and implantology. The histological findings show that the co-polymer remains at the site where it was implanted until the natural healing processes have

terminated and that during this time it is penetrated by and progressively replaced by trabecular bone; in between the trabeculum one can observe spaces containing adipose cells and vascular cells, these findings also indicate the formation of bone marrow (Schakenraad & Dijkstra 1991, Winet & Hollinger 1993). Studies made on animals and in man indicate that **the product is totally absorption, in tissues that are well irrigated by the blood supply even after only four months from when the material had been implanted, and complete, in any case, within six-eight months even in more ample grafts, such as in a major lifting of the maxillary sinus** (Bucci-Sabattini & Salvatorelli 1999, Ghinzani 1998, Leghissa et al. 1997-1998, Leghissa & Leardi 1998, Lundgren et al. 1992, Minenna et al. 1998, Paivarinta et al. 1993, Saitoh et al. 1994, Zerbinati in press); the histological aspects after four-six months and nine months were identical.

Going beyond the positive results of FISIOGRAFT obtained with bone regeneration, one of the uses which appears to be very promising for the future is the possibility to chemically bind bone growth factor to the polylactic-polyglycolic co-polymer. In effect even from the first experiences (Miyamoto & Takaoka 1993) it appears that the **co-polymer acts as an excellent carrier** for these factors, representing a new frontier in the fields of periodontics and implantology. **The possible and useful use of FISIOGRAFT combined with autogenous bone** permits, in addition, to resolve or at least significantly simplify the problems derived from the

fact that often there is insufficient quantities of this material.

Actual experience permits us to be optimistic about the future of this synthetic osteoconductive

biomaterial which has demonstrated ample practical and flexible characteristics and optimal biocompatibility.

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