Bone Grafting with a New Implantable Medical Device. Design and the Biocompatibility

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Abstract

In dentistry, bone is one of the most commonly repaired and reshaped, high load organ of the body. Currently, functional treatment of fractures and bone loss associated with trauma, cancer or revision of post-extraction maxillo-facial resorption, remains a significant challenge in the field of reconstructive dentistry and orthopaedics. This is why the development of new medical devices as biomaterials, supporting complete bone reconstruction, reshaping and providing anatomical osseointegration, should be one of the key objectives to design next-generation bone-substitute implantable medical devices. Composite poly-DL-lactide-co-glycolide (DLPLG)-betaTCP combines the advantages of different materials, offer high flexibility and versatility in biomedical and tissue engineering applications. Results showed that composites is resorbable and biocompatible. This composites have neither cytotoxic nor negative effects on osteoblast in culture and cell proliferation is significantly improved in the presence of this Ca based biomaterials, indicating an early stage of attachment to the composite substrate. Morphological and functional in vitro tests evidenced that the three particulate composite formulations have excellent cytocompatibility and provide a good, promising substrate for osteoblast proliferation, attachment and differentiation. Therefore, proposed composites, with all three particulates, make them suitable as implantable scaffolds for new bone cells ingrowth and thus for bone engineering. Results of the biocompatibility testing of this medical device, significantly contribute to the benefit and safety of the patients.

Keywords : Biocompatibility; Biodegradable; Bone Graft; Dental Implant Scaffold; Design; Implantable Medical Device; Orthopaedics.

Introduction

In the past two decades, considerable progress has been made in the design and development of new biocompatible implantable medical devices as alloplastic biomaterials with biomechanical and physiological features more similar to those of autologous bone [28, 3 and 8]. At the same time, the search for new technologies and biocompatible biomaterials with the scope to improve bone regeneration has evolved at a fast pace [15, 16 and 6]. These breakthroughs are expected to lead to better orthopaedic bone reconstructions, cosmetic and functional results in patients treated for craniofacial tumours, malformations and traumas, giving them a better quality of life. Clinicians' ability to create fully functional tissue equivalents will have a dramatic impact on the future of implantable orthopaedic medical devices and improving craniofacial surgery [1]. The approach that is taken in implantable medical devices for tissue engineering depends on multiple factors, including: size of the defect, the cell supply from adjacent areas, cell migration speed, surrounding vasculature and an availability of a new biomaterials. With a scientific foundation on biomaterials firmly established, we now need a vigorous infusion of biology-based engineering analysis to move the tissue engineering field from an era of phenomenological observation to one of commercially viable products [33, 21 and 24]. Patient-oriented orthopaedics ad maxillo-facial tissue engineering research on bone regeneration with reconstructive composites' biomaterials is an attractive field for Industry [8, 25 and 26].

Of particular interest to orthopaedics and maxillo-facial applications are biodegradable polymer scaffolds for filling irregularly shaped defects with minimal surgical procedures. In the future, the availability of shapable, biodegradable and biocompatible bioactive polymer formulations might be of great interest in order to avoid the inconvenient surgical insertion of large implants. Recently, the development of hybrid polymer systems (composites, copolymers, complexes, hydrogels, blends, etc.), based on natural and synthetic polymers, and their wide range of applications in biomaterial science has received tremendous attention [30].

Most of the polymers previously studied include polyesters,

such as poly (lactic acid), poly (glycolic acid), and their copolymers [20, 28 and 3] which undergo degradation to biocompatible products through hydrolysis of their ester linkage. Poly (glycolic acid) (PGA) was first marketed in 1970 as a biodegradable suture, while poly (lactic acid) (PLA) has been introduced as a drug delivery material since 1971. By varying monomer ratios in polymer processing, as well as the processing conditions, the resulting polymer exhibits drug release capabilities that last for months or even years [8]. The main advantages of these polymers consist in their mechanical properties, which may be engineered for a wide array of applications [2, 9]. These biomaterials require that also their reagents are biocompatible. PLA-based biomaterials due to their bioresorbability are used for bone plates or temporary internal fixation of broken or damaged bone [25, 26, 24 and 23]. The resorption rate is controllable through the degree of polymerization or copolymerization with aliphatic polyesters. The modulus of elasticity of PLA or PGA, however, is relatively low (2-7 GPa) compared with that of natural cortical bone (3-30 GPa) [10]. However, manufacturing composites that consist of PLA copolymer and osteoconductive ceramics, such as hydroxyapatite (HA) or beta-tricalcium phosphate (betaTCP) this situation can be favourable changed [25, 7 and 11].

Some bioresorbable polymer mixtures with HA have been proposed for use in bone repair, because they support bioactive osteogenesis [13]. Bioresorbability is a very important property that is expected from bone equivalent implants. Bone reconstruction should follow the resorption of the polymer; in particular, bone should nucleate calcification around each grain of ceramics, especially if the latter is bioactive. TCP is a biomaterial that was extensively studied in the past [33]. Its particles, which may be obtained in various sizes, have a high porosity and a diameter ranging between 5 and 15 /um. This material was clinically shown to biodegrade without adverse reactions [32, 33]. Composites of polymers and osteoconductive TCP ceramics are ideal bioresorbable materials, as they limit the invasion of fibrous tissue into bone defects, but facilitate bone ingrowth [32, 5]. The bone substitutes designed on the basis of the above criteria provide secure and more complete bone proliferation allowing fast and excellent vascularization [19].

The target of the study covered in this paper was to design and develop a new multi-functional, shapable composite product as a new bone equivalent to be used successfully in three-dimensional bone regeneration thanks to their complete biocompatibility, bioactivity, form adjustment, easy surgical manipulation, and biodegradability. The research activity included technological processes for creating a very specific biocompatible, resorbable composite formulation consisting of polymer poly-DL-lactide-co-glycolide (DLPLG) and ceramic beta-tricalcium phosphate (TCP). Biological and morphological analysis were done in vitro confirm safety, biocompatibility and suitability of these materials as biochemical substrates, scaffold, for new bone deposition.

Materials and Methods

Biomaterials

Biocompatible microporous TCP was prepared according to the protocol suggested by [8] modified procedure. Spherical granules were dried and calcined at 1,100°C for 12h. The heating and cooling rate was 10 C/min. The solid TCP was allowed to cool to room temperature. Evaluation of chemical composition and purity of TCP was carried out by Energy Dispersive spectroscopy (EDS) and Fourier transform-infrared spectroscopy (FTIR). Energy dispersive x-ray spectroscopy (EDS) is a chemical microanalysis technique performed in conjunction with a scanning electron microscope (SEM). The technique utilizes x-rays that are emitted from the sample during the bombardment by the electron beam to characterize the elemental composition of the analysed volume. The EDS x-ray detector measures the number of emitted x-rays versus their energy. The energy of the x-ray is characteristic of the element from which the x-ray was emitted such as TCP. A spectrum of the energy versus relative counts of the detected x-rays is obtained and evaluated for qualitative and quantitative determinations of the elements present in the sampled volume.

FTIR spectra were recorded using the KBr pellet method by using a Perkin-Elmer 782 spectrometer. Three particle sizes of TCP (25-100 /um, 150-300 /um and >300 /um) were collected and used to prepare three main composite formulations using the solvent evaporation technique:

- Formulation I - poly-DL-lactide-co-glycolide (DLPLG) 50:50, MW 40 000-75 000 (P2191, Sigma-Aldrich), reinforced with osteoconductive ceramic TCP, particle of the size 25-100 /um.
- Formulation II - DLPLG 50:50, MW 40 000-75 000 (P2191, Sigma-Aldrich), reinforced with TCP, particle of the size 150-300 /um.
- Formulation III - DLPLG 50:50, MW 40 000-75 000 (P2191, Sigma-Aldrich), reinforced with TCP, particle of the size >300 /um

Each formulation was prepared in the desired shape. After DLPLG dissolution in acetone (5 ml of acetone on 0.5 g of copolymer), the solution was stirred with a blade mixer at 100 rpm for 5 hours. TCP granules of a given size were added to the DLPLG solution, until reaching the ration by weight an 80/20 acetone/ DLPLG. The material was mixed at 50 rpm/h. Then, it underwent vacuum vaporization. The final DLPLG/TCP ratio was 90/10-30/70 by weight. By adopting the same technological process and changing a few parameters (e.g. temperature and pressing time), the same material may be produced with variable compressive strength and porosity values. The mixture was cast in the desired shaped using chilled Teflon moulds. For this study, discs of 5 mm were prepared. After complete solvent evaporation, the samples were air dried for 48 h, freeze-dried for 24h, lyophilized and sterilized. All samples were sterilized with ethylene oxide.

Scanning Electron Microscopy

Representative samples of cells attached to the composite were processed for SEM. Samples were rinsed 3 times with

PBS and fixed for 60 minutes with 2.5 % glutaraldehyde and 2% paraformaldehyde in 0.1% cacodylate buffer (pH 7.4), and fixed afterwards with osmium tetroxide, critical point-dried and sputter-coated with gold-palladium. Morphological analysis and element analysis (KEVEX) were performed by SEM (Etec Autoscan, Etec, Haywood, CA).

Cell Cultures

The osteosarcoma cell line, MG-63, was obtained from the European Collection of Animal Cell Cultures (Porton Down, Salisbury, UK) originated from ATCC (USA www.atcc.org) and maintained in DMEM supplemented with 10% FCS. No antibiotics were added. All cells were cultured in a humidified 5% CO2, 37°C incubator. Cells were, also, sub cultured (0.05% trypsin/0.02% EDTA wt/vol) and seeded in 75 cm2 flasks. The cells were released at the trypsin/EDTA confluence, counted and used for experiments. Six samples of each material (Form I, Form II and Form III) were placed in 24-well culture plates, and the cell suspension (1 x 104 cell/ml in 100/ul) was directly applied onto each sample. The same amount of cells was also plated onto the remaining 6 empty wells for control. Cells were allowed to attach for 2 hours; then 900/ul of culture medium (DMEM, ascorbic acid 50mg/ml and beta-glycero-phosphate 10-8M) were added. The culture was kept under the same conditions as those described above for 48 hours. No contamination was found during the experiments. At the end of the experiment, osteoblasts were characterized according to well-established parameters of osteoblast phenotype: Alkaline Phosphatase activity (ALP, Sigma, UK Kinetic method kit), Lactate Dehydrogenase (LDH, Sigma UK kit), Calcium (Ca, Sigma, UK kit), Phosphorus (P, Sigma UK kit), all tests as indicators of osteoblast activity. The MTT test (Sigma, UK) was performed to assess cell proliferation in four random samples of each group: 80 /ul of MTT solution (5mg/ml in phosphate buffer) and 720/ul of medium were added to each well and the plates were incubated at 37°C for another 4h. After discarding the supernatants, the dark blue crystals of Formazan were dissolved by adding DMSO (800/ul), and quantified spectrophotometrically at 550nm. Results were analysed and described as optical density (OD).

Degree of swelling of the composites

Samples for swelling tests were cut from hot press sheets with a size of 1x1x0.2 cm3. The swelling test was carried out in distilled water at room temperature (23 ± 1.5°C). The degree of swelling of the composites was calculated according to the following equation:

$$Sw = (Wt - Wo) / Wo$$

Where Sw is the swelling degree in a given time interval, Wt the weight of the tested specimens after immersion in water at time t , and Wo the weight of the tested specimens at the beginning of the test. DLPLG copolymer without ceramic was used for control.

Hydrolytic degradation

For degradation, the specimens under study were placed into small flasks filled with 20 ml of 0.13 M isoosmolar phosphate buffer containing 0.02% NaN_3 to prevent bacteria development. The flasks were allowed to stand in a thermostatic oven for predetermined periods of time. For degradation at 37° C, the pH value 3.7 was selected. Two specimens were withdrawn from the aging media at each degradation time, and washed with distilled water. Each data point represents the mean of two measurements. DLPLG polymer without ceramic was used for control.

Result

Surface and chemical analysis of the Beta-TCP

The size and quality of the betaTCP particles were as follows: 25-100 /um to 150-300 /um (100% pure, 70% interconnected microporosity) and >300 /um (100% pure, 70% interconnected macroporosity). Constructed composites shape and SEM microporosity shown in Fig. 1. The FTIR profiles and SEM microporosity for TCP are displayed in Fig.1A. Stoichiometric EDS analysis of the beta TCP is shown in Fig.1B. The Specific Surface Area (SSA) of the TCP particles, measured by the multiple point BET nitrogen adsorption method, yielded 1.07 m2/g.

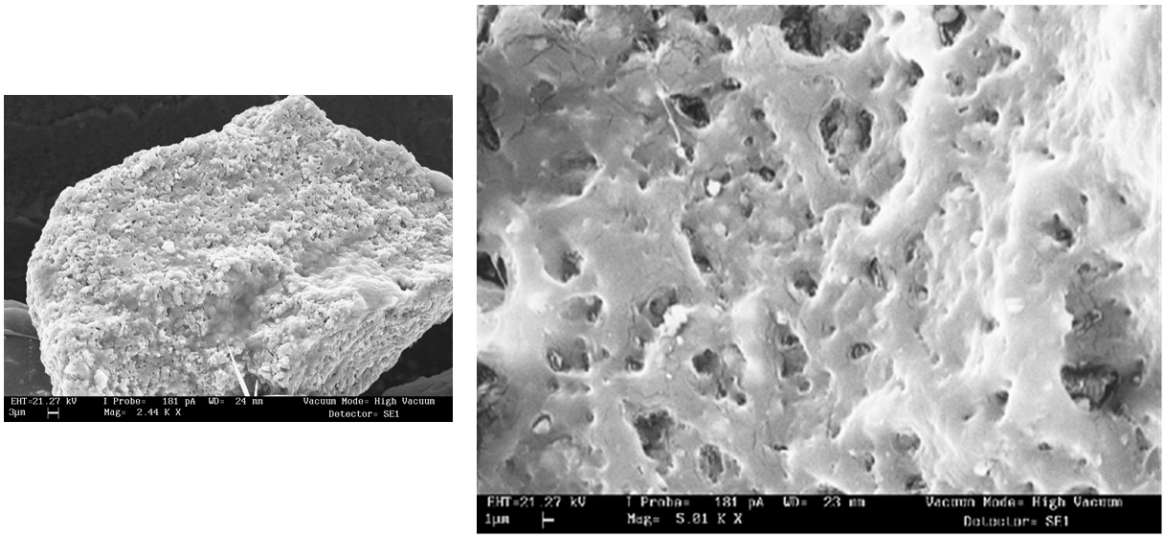

Figure 1: Form and SEM microporosity of the new composites βTCP/DLPLG.

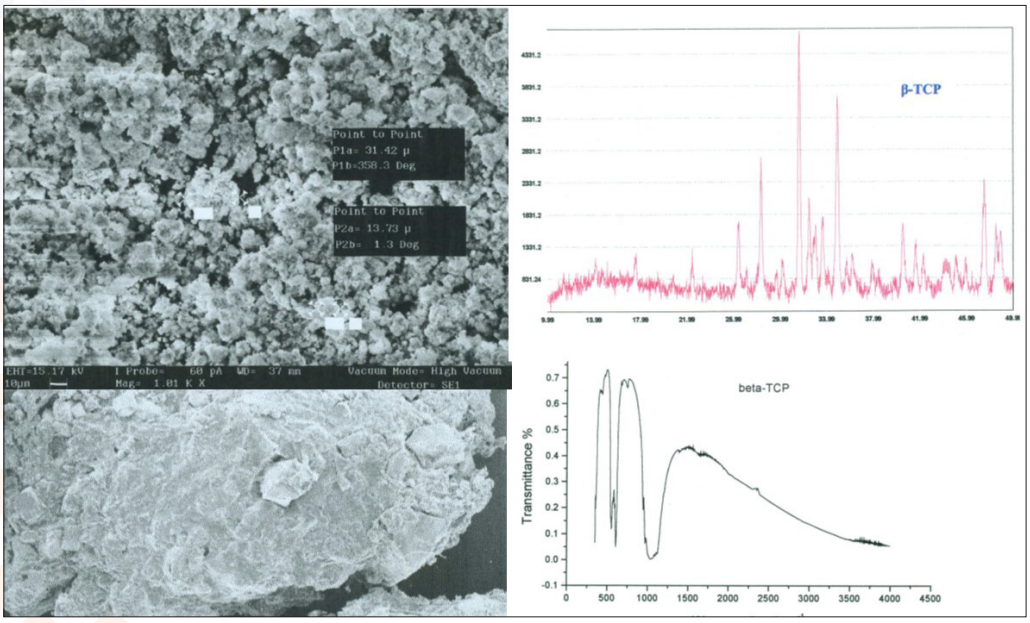

Figure 1A : Fourier transform infrared spectroscopy (FTIR) for beta tricalcium phosphate (βTCP) used for optimisation of new composites TCP/DLPLG;

Physical characteristics of copolymers in our formulations

Degree of swelling:

The stability of the composite structure in water and water incorporation were studied by using standard swelling experiments as a measure of mutual interactions between the components. The degree of swelling of the composites DLPLG with three different particle sizes of TCP was studied and the results were compared. The results are shown in Fig. 3A. All the tested structures were saturated with solvent during 1 hour. Within time swelling degree increased up to the value 0.6%. The composite retained a compact structure and disintegration was not observed, even after several days. The first signs of degradation were recorded only after 10 days.

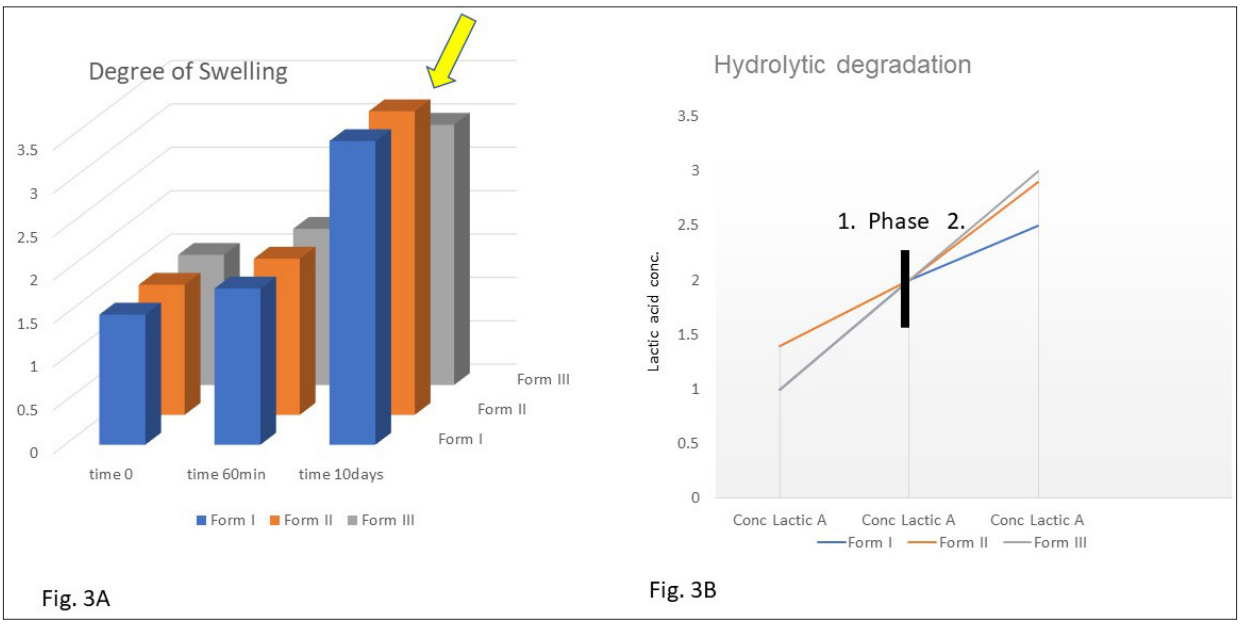

Fig. 3A

Fig. 3B

Figure 3A : The degree of swelling of the copolymers in Forms I, II and III in vitro. Note that the degree of swelling nearly reached equilibrium after 24 h immersion in water. Control was polymer of DLPLG without any ceramic. Arrow shows significant degradation after 10 days.

Figure 3B : Hydrolytic degradation of copolymers Form I, II and III in vitro. Generally two phase degradation observed. Control was polymer of DLPLG without any ceramic.

Hydrolytic degradation in vitro

Hydrolytic degradation generally occurred in two steps, Fig, 3B. In the first one, the structure became saturated and showed a very slowly increasing concentration of free lactic acid, attributable to the breakage of its physical bonds, which is particularly marked in the poly (lactic acid) part of copolymer. In the second step, the degree of degradation was slightly lower, with no significant differences between the groups (formulations) and with respect to the controls. The results are shown in Fig.3B.

Cell culture and biochemical and cytotoxic testing

Biocompatibility results showed that during the incubation of MG63 osteoblast-like cells on the three formulations of composites, no signs of cytotoxicity appeared (Table). In fact, the LDH values measured in sample cultures of the materials matched those of the control cultures. The ALP values showed that the presence of bone substitute biomaterials did not negatively interfere with osteoblast activity. Calcium (Ca) and Phosphorus (P) detected in the supernatant were significantly lower than in the controls. The MTT test revealed that cell proliferation significantly improved in the presence of biomaterials with respect to the control values.

MG63 culture	LDH (U/L)	ALP (U/L)	Ca (mg/dL)	P (mg/dL)	MTT (OD550nm)
DMEM-control	3.35 ± 0.07	19.55 ± 0.70	7.32 ± 0.26[a]	3.17 ± 0.17[a]	0.329 ± 0.053[a]
Form I	3.40 ± 0.14	19.32 ± 1.10	3.00 ± 0.42	1.12 ± 0.15	0.502 ± 0.032
Form II	3.30 ± 0.01	18.32 ± 0.65	5.10 ± 0.53b,c	1.77 ± 0.15b	0.473 ± 0.030
Form III	3.35 ± 0.07	19.87 ± 1.73	2.97 ± 0.26	1.35 ± 0.29	0.538 ± 0.040
ANOVA F	0.44, ns	1.25, ns	113.51, p<0.0005	85.89, p<0.0005	21.27, p<0.0005

Table: Biochemical values of MG63 osteoblast-like cells after 48 hours of culturing with three formulations of bone substitute composites (Mean ± SD, n=6 triplicates for each group).

Scheffé post hoc multiple comparison test:
a - DMEM-control vs others materials, p< 0.001;
b - Form II vs Form I, p< 0.0005;
c - Form II vs Form III, p< 0.005.

Scanning Electron Microscopy

After culturing on the Formulation I substrate (Form I), osteoblast-like cells displayed extremely long and thin cell processes (Fig. 4A and B). Numerous cells proved to have close relations with the substrate in terms of cell processes and cell bodies. Additionally, these cells gave rise to many filamentous processes towards an extra-cellular matrix substrate. On the cell surface, there was a great number of very fine cytoplasmic extensions which tended to attach to the substrate. The long lamellar and granular structures in the extra-cellular spaces were closely related to osteoblast-like cells and to the substrate. The small granular structures (probably particles of ceramics) in the extra-cellular spaces demonstrated to have close relations with the cells. Higher magnification (Figs.5 C and D) revealed miniature granular structures attached to the cell surface, where also lamellar structures were found.

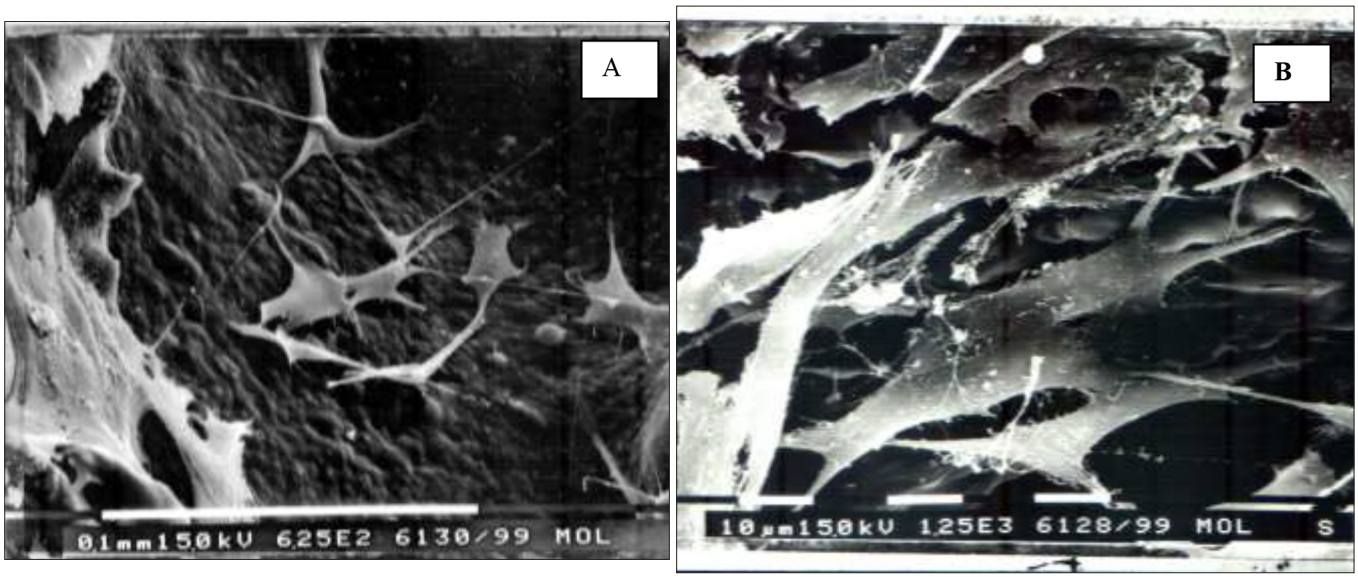

Figure 4A and B: Scanning electron micrograph (SEM) appearance of MG63 osteoblast-like cells cultured on βTCP/DLPLG composite. After cultivation on the **Formulation I substrate (Form 1)** osteoblast-like cells show extremely long and thin cell processes. The numerous cells make close relations with substrate by cell processes and by cell bodies. Bar: A and B - 0.1 mm;

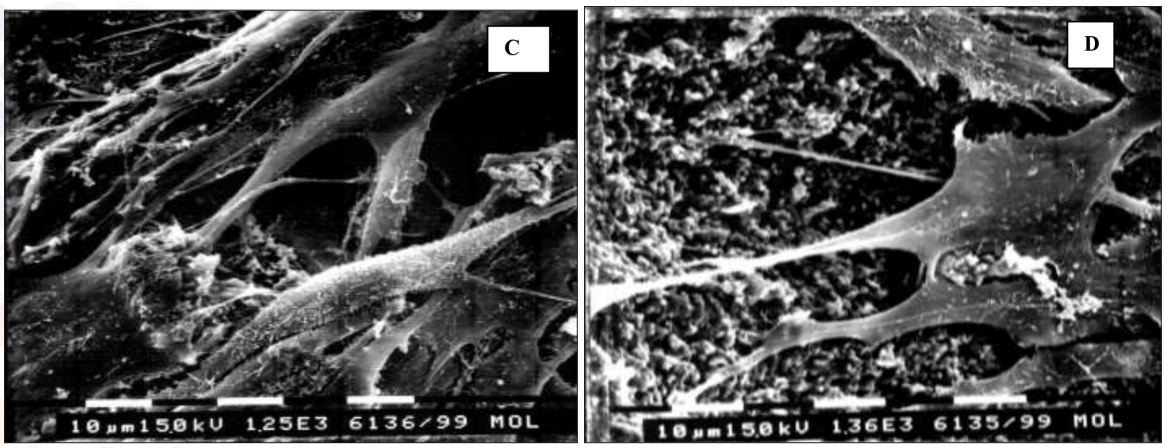

Figure 5.C and D: Scanning electron micrograph (SEM) of MG63 osteoblast-like cells cultured on βTCP/DLPLG composite. Cell morphology of osteoblast-like cells cultivated on **Formulation II (Form II)** show great number of short thick and some very long and very thin (Fig.4B and C) cytoplasmic continuations on cell surface. After cultivation osteoblast-like cells didn't show the same colour. Bar: 10 µm.

Morphologically, the osteoblast-like cells cultured on Formulation II (Form II) were elongated cells with a large number of cell processes (Fig. 4A, B and 5C, D). During culturing on Formulation II, we observed that these cells made numerous contacts with one another via processes and cell bodies. Additionally, these cells were firmly and widely attached to the substrate structures. On the cell surface, a high number of short thick (Fig. 4A) and some very long and very thin (Figs.4B and 5.C) cytoplasmic extensions were detected. After culturing, the osteoblast-like cells did not show the same colour but a white to grey colour range. "White" osteoblast-like cells had a lower number of cell processes (Fig. 4B). In osteoblast-like cells with grey bodies and white processes (Fig.5C) and totally grey osteoblast-like cells, we identified a much higher number of processes (Fig. 4A). Higher magnification of the osteoblast-like cells revealed a fine lamellar and granular structure on the cell surface (Fig.6F). On these cells, attached particles of substrate were observed (Fig. 6E and F). The osteoblast-like cells proliferated on the Formulation II substrate, and showed to have close relations with it (Fig.5D). The osteoblast-like cells appeared to have high affinity with the composite substrate and propensity to attach to it. In the extra-cellular spaces, we observed short and thin fibrillar granular structures, probably representing extra-cellular matrix (ECM) components and binding to the osteoblast-like cells.

The osteoblast-like cells cultured on Formulation III substrate (Form III) showed a wide variety of forms and appearance. On this substrate, osteoblasts were abundant, highly proliferative, closely connected and firmly attached to the substrate (Fig. 6E and F). A sharp increase in the number of cells (Fig.6F) on a small surface was indicative of a high degree of confluence. These cells were centrally widened (Fig. 6E) and had long cell processes on both poles (Fig. 6F), although there were osteoblast-like cells with numerous processes. A high number of long and short processes were firmly attached to the Form III composite substrate (Fig. 6E and F). Osteoblasts were located close to each other (Fig. 6F). In extra-cellular spaces, rough, globular structures of the substrate were attached to or in close relation with the osteoblast-like cells. Interestingly, in this experimental group, we found cells, which lost their peaked structure and acquired a rounded contour (Fig. 6E). The cell bodies accommodated numerous short processes, which were attached to the substrate.

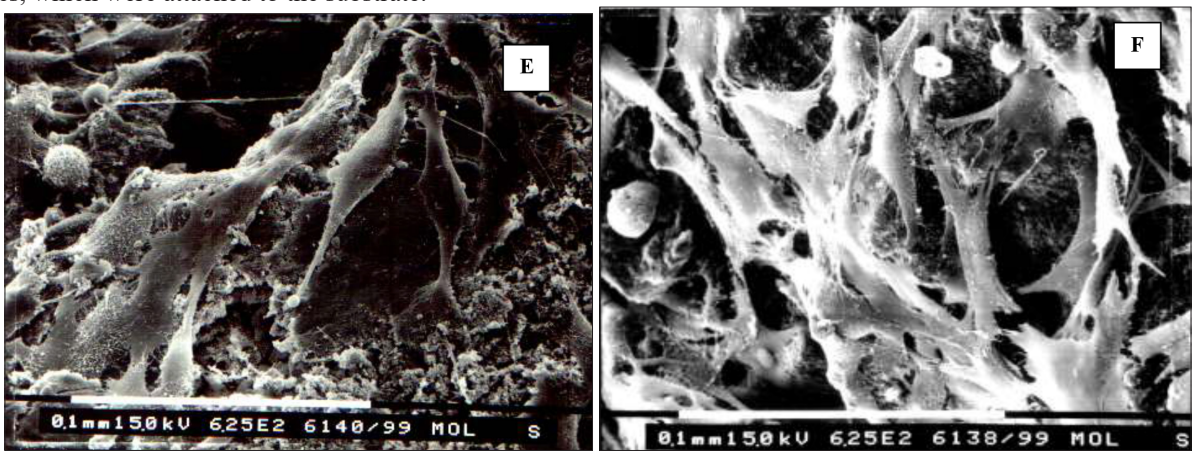

Figure 6 E and F: Scanning electron micrograph (SEM) of MG63 osteoblast-like cells cultured on βTCP/DLPLG composite. The osteoblast-like cells cultivated on Formulation III substrate (Form III) show a great variety of forms and cellular appearance. On this substrate osteoblasts are abundant, highly proliferated, extremely connected and firmly attached to the substrate. Significantly increased number of cells, on the small surface, indicate high degree of confluence. Bar: 0.1 mm.

Discussion

An ideal reconstructive biomaterial should combine: i) dynamic mechanical performance matching the one of the tissue to be replaced (regenerated); ii) adequate degradation as a function of the tissue healing kinetics; and iii) biocompatible performance. Polymer materials are not commonly used as oral or maxillo-facial implants. Amongst the different classes of biodegradable polymers, thermoplastic aliphatic poly(esters), such as poly(lactide) (PLA) and poly(glycolide) (PGA), have aroused great interest thanks to their properties (e.g. good biocompatibility, biodegradability, and mechanical strength) [8, 29]. Our experimental results demonstrate that all three formulations of betaTCP/DLPLG composites that we prepared and tested are biocompatible in vitro. These formulations have an outstanding propensity to be colonized with osteoblasts, thereby promoting their osteogenic activity. All three formulations (with some differences depending on the TCP grain size) succeeded in improving the adhesion of cultured cells in the boundary layers and thus in raising the strength of the cellular and chemical bonds between the implant and bone [25, 26, 18]. We chose TCP/DLPLG because the DLPLG with TCP ceramics can offer some distinct advantages over other similar biomaterials. With our formulations, the mechanical properties and degradation time of polymers can be easily adjusted by adding calcium phosphate material TCP, and also their bioresorbability increases significantly. The most common types of calcium phosphate ceramics used so far in composites are hydroxyapatite (HA = Ca10 (PO4)6(OH)2) and beta tricalciumphosphate (TCP = Ca3(PO4)2) [13, 14, 23]. They have different characteristics in vivo, although both forms have Ca/P ratios within the range known to promote bone ingrowth. In general, HAp is more osteogenic, while TCP degrades much faster. TCP is commonly used as a filling biomaterial in reconstructive surgery [5, 27, 13, and 14]. TCP is degradable at a rate that depends on its crystalline forms and surface area [31]. We used high SSA of 1.07 m2/g which easily facilitate bone ingrowth. However, the features of the TCP-bone interaction on the microscopic level and the effect of TCP on bone regeneration in vivo are poorly understood. Hench and Polak [6] speculated that an apatite layer on the surface plays an active role, preferentially by absorbing extracellular matrix proteins (EMP) that serve as growth factors. Moreover, the release of soluble calcium phosphate may positively influence osteoblast behaviour. The results of our in vitro study provide strong evidence of good osteocompatibility of the proposed composite formulations. It is very obvious with the biochemical values of osteoblast metabolism in our tests. Alkaline phosphatase activity (ALP) is a parameter of bone cell differentiation showed no significant differences vs. control probably because of a very short period (48 h) of culturing, but ALP activity seems to be increased when measured in OBs cultured on the Form III composite substrate. This is in agreement with literature reports on the impact of matrix geometry on osteogenesis [12] and also with our results concerning other biochemical parameters and SEM observations. The MTT test is a very important indicator of OBs proliferation. This test indicates that, in all three cases, strong proliferation takes place. Changed levels of Ca and P concentrations in a liquid microenvironment further corroborate these findings. Biochemical results demonstrate that our TCP/DLPLG biodegradable composites, when cultured in vitro on all three formulations, enhance the performance of OBs (in terms of cell proliferation, synthesis of alkaline phosphatase, and concentration of Ca and P in the extracellular matrix). Furthermore, all three composite substrates undergo significant swelling and hydrolysis in the presence of water (Figs. 2A and B). The design and development of time-controllable biodegradable device for therapeutic applications require a thorough understanding of in vivo biochemical processes, because the final polymer products resulting from degradation should be spontaneously catalysed to H2O and CO2. Biodegradation of a reconstructive devices followed by new bone ingrowth is important for successful tissue reconstruction [17]. The mechanism of degradation of aliphatic polyesters is generally considered as a hydrolytic mechanism, and some studies also hypothesized an enzyme-catalysed degradation. So far, both in vitro and in vivo studies have identified numerous factors influencing the hydrolytic degradation behaviour of polyesters [4]. Biodegradable materials used for craniofacial devices for bone reconstruction must be biodegradable and biocompatible. Moreover, as the process of degradation takes place in vivo, not only the intact material but also its degradation products should be biodegradable and biocompatible. While the specific requirements and characteristics of a biodegradable biomaterial depend on the specific application, there is a general set of criteria for "a good biodegradable polymer biomaterial" and we believe that our composites fulfil these criteria. Firstly, during the period when the material is in the body, there should be no sustained inflammatory reaction or foreign body response that necessitates removal. Secondly, the material should be completely resorbed with no histological evidence of residuals. Thirdly, upon complete resorption of the material, there should be little or no physiological histological evidence of the former implant, that is, the body should "forget " that the implant was ever there.

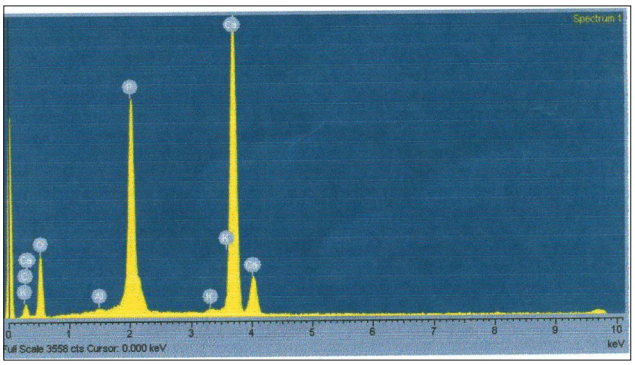

Figure 2: Energy Dispersive spectroscopy (EDS) for tri-calcium phosphate (βTCP) used for optimisation a new composites βTCP/DLPLG;

Cellular attachment to the implant surface is an important step in the process of tissue-implant interaction and thus long-term successful implantation. We noticed that, in the presence of our three different formulations (particularly with the formulation III, granule size 300/um), MG-63 osteoblast-like cells react very positively. Biochemical and SEM results confirmed

that qualitative differences in the grain size of our three formulations (probably due to different surface geometry, as reported by [12] induce different OBs morphologies after the same incubation period (Fig. 4 and 5). These differences may not be quantitative, but they are definitively significant for qualitative cellular response and final cellular function, i.e., osteogenesis. There are insufficient data at this time to indicate whether the future use of resorbable or bioactive polymer coatings on metallic dental implants already commercialized offer advantages over sprayed HA coatings. In general, polymer surfaces should be designed to act as shock (stress) absorber after dental implantation. Additionally, some polymeric materials appear to have the potential to act as carriers for growth factors and thus may be used in conjunction with dental implants in some forms.

Conclusion

Thus research led to the development of new functional biomaterials that may translate into new surgical biomaterials for non-loaded small and big craniofacial bone reconstructions [27] and anti-stress limited time coating on the metallic implants. Examples of conditions requiring new bone tissue include missing bone in cleft palates, bony nasal pyramid defects following removal of fistulous tracts and defects following removal of sinus and mandibular cysts, etc. The composite formulations proposed in this study have significant clinical advantages and, depending on future developments in tissue engineering, they may be multi-functional, i.e. used both as a valuable biomaterial and as an in situ drug delivery device (growth factors, antibiotics, vaccine etc.). Furthermore, the proposed formulations have a very important potential as scaffolds for cell transfer, thus offering also the possibility of rapid tissue regeneration. To date, results have not been satisfactory, since the final cosmetic and functional results in patients could not be anticipated and guaranteed. Thus, our future goal is to promote combined clinical, pharmaceutical and medical device research clinical trial efforts, so as to obtain bone equivalent products for successful craniofacial reconstruction. Preclinical, biocompatibility trial would be very important to validate safety of patients. This reduces risk and safety of patients or healthy volunteers in the first human trial depends on data from experimentation in animals and the specific design of the trial. Because of that, recommendations on the sort, duration and extend of preclinical safety study, have been developed by the ICH EU Commission for Proprietary Medicinal Products for human use. This is collection of designed study to characterized local and systemic tolerance, general toxicity and genotoxicity of the implantable medical device product. This article add value to the recommendations for preclinical biocompatibility testing which are of obvious benefit to the safety of the patient.

Conflicts of Interests

The authors declare that they have no conflicts of interest in relation to this article.

References

1. Alsberg E, Hill EE, Mooney DL (2001). Craniofacial Tissue Engineering. *Crit Rev Oral Biol Med* 12:64-75.
2. Beckett LE, Lewis JT, Tonge TK, Korley LTJ. (2020). Enhancement of the Mechanical Properties of Hydrogels with Continuous Fibrous Reinforcement. ACS Biomater Sci Eng. 12;6(10):5453-5473. doi: 10.1021/acsbiomaterials.0c00911. *Epub* 2020 Sep 25.
3. Davin JE, Attawis MA, Laurencin CT (1996). Three-dimensional degradable porous polymer-ceramic matrices for use in bone repair. *J Biomater Sci Polym Ed* 7: 661-669.
4. Dunn AS, Campbell PG, Marra KG (2001). The influence of polymer blend composition on the degradation of polymer/hydroxyapatite biomaterials. *J Mater Sci: Mater Med* 12: 673-677.
5. Foitzik C, Stamm M (1997). Einsatz von phasenreinem -Trikalziumphosphat zur Auffullung von ossfaren Defekten Biologische Materialvorteile und klinische Erfahrungen. *Quintessenz* 48:1365–1377.
6. Hench LL and Polak JM (2002). Third-Generation Biomedical Materials. *Science* 295: 1014-1017.
7. Higashi S, Yamamuro T, Nakamura T, Ikada Y, Jamshidi K (1986). Polymer-hydroxyapatite composites for biodegradable bone fillers. *Biomaterials* 7:183-187.
8. Jain RA (2000). The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices. *Biomaterials* 21:2475-2490.
9. Jones DS (1999). Dynamic mechanical analysis of polymeric systems of pharmaceutical and biomedical significance. *Int J Pharm 179*(2): 167-178.
10. Kasuga T, Ota Y, Nogami M, Abe Y (2001). Preparation and mechanical properties of polylactic acid composites containing hydroxyapatite fibers. *Biomaterials* 22: 19-23.
11. Kikuchi M, Suetsugu Y, Tanaka J, Akao M (1997). Preparation and mechanical properties of calcium phosphate/copoly-L-lactide composites. *J Mater Sci* 8:361-364.
12. Kuboki Y, Saito T, Murata M, Takita H, Mizuno M, Inoue M, Nagai N, Pool R (1995). Two distinctive BMP-carriers induce zonal chondrogenesis and membranous ossification, respectively; geometrical factors of matrices for cell-differentiation. *Connect Tissue Res* 32: 219-226.
13. Kurashina K, Kurita H, Kotani A, Kobayashi S, Kyoshima K, Hirano M (1998). Experimental cranioplasty and skeletal augmentation using an alpha-tricalcium phosphate/dicalcium phosphate dibasic/tetra calcium phosphate monoxide cement: a preliminary short-term experiment in rabbits. *Biomaterials* 19:701–706.
14. Kurashina K, Kurita H, Hirano M, Kotani A, Klein CP, de Groot K (1997). In vivo study of calcium phosphate cements: implantation of an alpha-tricalcium phosphate/dicalcium phosphate dibasic/tetra calcium phosphate monoxide cement paste. *Biomaterials* 18:539–543.
15. Letic-Gavrilovic A, Gavrilovic I (2020). Cytokine's and Growth Factors from Bone and Dentine Extracellular Matrix as Regulators of Bone Engineering. *EC Dental Science 19*(4): 17-19.

16. Letic-Gavrilovic A, Scandurra R, Abe K (2000). Genetic potential of interfacial guided osteogenesis in implant devices. *Dent Mater J 19*(2): 99-132.
17. Merolli A, Gabbi C, Cacchioli A, Ragionieri L, Caruso L, Giannotta L, Tranquilli Leali P (2001). Bone response to polymers based on poly-lactic acid and having different degradation times. *J Mater Sci:Mater Med* 12:775-778.
18. Neo M, Herbst H, Voigt CF, Gross UM (1998). Temporal and spatial patterns of osteoblast activation following implantation of beta-TCP particles into bone. *J Biomed Mater Res* 39:71–6.
19. Oftadeh R, Perez-Viloria M, Villa-Camacho JC, Vaziri A, Nazarian A. (2015). Biomechanics and mechanobiology of trabecular bone: a review. *J Biomech Eng. 137*(1):0108021-01080215. doi: 10.1115/1.402917.
20. Oikonomidis S, Ashqar G, Kaulhausen T, Herren C, Siewe J, Sobottke R. (2018). Clinical experiences with a PEEK-based dynamic instrumentation device in lumbar spinal surgery: 2 years and no more. *J Orthop Surg Res*. 9; 13(1):196. doi: 10.1186/s13018-018-0905-z
21. Persidis A (1999). Tissue engineering. *Nature Biotechnol* 17:508-510.
22. Pioletti DP, Takei H, Lin T, van Landuyt P, Ma QJ, Kwon SY, Sung KLP (2000). The effects of calcium phosphate cement particles on osteoblast functions. *Biomaterials* 21:1103–1114.
23. Rejda BV, Peelen JG, de Groot K (1977). Tri-calcium phosphate as a bone substitute. *J Bioeng* 1: 93–97.
24. Schincaglia GP and Nowzari H (2001). Surgical treatment planning for the single-unit implant in aesthetic areas. *Periodontol* 2000 27:162-182.
25. Shikinami Y, Okuno M (1999). Bioresorbable devices made of forged composites of hydroxyapatite (HA) particles and poly-L-lactide (PLLA): Part I. Basic characteristics. *Biomaterials* 20:859-877.
26. Shikinami Y, Okuno M (2001). Bioresorbable devices made of forged composites of hydroxyapatite (HA) particles and poly-L-lactide (PLLA): Part II. Practical properties of mini screws and miniplates. *Biomaterials* 22: 3197–3211.
27. Tada H, Hatoko M, Tanaka A, Kuwahara M, Mashiba K, Yurugi S, Iioka H, Niitsuma K (2002). Pre-shaped hydroxyapatite tricalcium-phosphate implant using three-dimensional computed tomography in the reconstruction of bone deformities of craniomaxillofacial region. *J Cranio Surg 13*(2); 287-292.
28. Thomson RC, Yaszemski MJ, Powers JM, Mikos AG (1995). Fabrication of biodegradable polymer scaffolds to engineer trabecular bone. *J Biomater Sci Polym Ed* 7: 23-38.
29. Van de Velde K and Kiekens P (2002). Biopolymers: overview of several properties and consequences on their applications. *Polymer Testing* 21: 433-442.
30. Wang M, Chen J, Ni J, Weng J, Yue CY (2001). Manufacture and evaluation of bioactive and biodegradable materials and scaffolds for tissue engineering. *J Mater Sci: Mater Med* 12: 855-860.
31. Wiltfang J, Merten HA, Schleger KA, Schultze-Mosgau S, Kloss FR, Rupprecht S, Kessler P (2002). Degradation characteristics of alpha and beta tri-calcium-phosphate (TCP) in minipigs. *J Biomed Mater Res* 63: 115-121.
32. Xie Z, Yan D, Zhou Q, Wu Z, Weng S, Boodhun V, Bai B, Shen Z, Tang J, Chen L, Wang B, Yang L. (2019). The fast degradation of beta-TCP ceramics facilitates healing of bone defects by the combination of BMP-2 and Teriparatide. *Biomed Pharmacother*.112:108578. doi: 10.1016/j.biopha.2019.01.039. Epub
33. Zerbo IR, Bronckers ALJJ, de Lange GL, van Beek GJ, Burger EH (2001). Histology of human alveolar bone regeneration with a porous tricalcium phosphate. A report of two cases. *Clin Oral Impl Res* 12: 379-384.