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Assessing the biocompatibility of a dental composite product

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Abstract

Objective: The purpose of the study was to assess the biocompatibility of a composite material considering the reaction caused at the implant site during 21 days by daily observing the subjects' behavior as well as by macroscopic examination and histological examination upon expiry of the testing period. *Materials and Methods*: We performed the tolerance test by implant of the composite material Dualcim. The implant test was made on two species of lab animals, Guinea pigs and Wistar rats in two versions: subcutaneous implant and intramuscular/perimuscular implant. *Results*: After a 21 days period, when the implant was in direct contact with the tissue, no change of the shape and consistency, color or surface of the implant occurred. Around the implants, the biocompatibility was kept under physiological limits. *Conclusions*: The product, in the structure and shape presented, could be easily placed under good conditions, both at the level of the subcutaneous tissue and at inter-muscular level. In case of both species and in all subjects, the histological exam proved a favorable development of the relationship between the implant body and the placing site.

***Keywords*:** composite material, biocompatibility, subcutaneous implants, biological test, Dualcim.

Introduction

Biocompatibility is mostly known as the capacity of a material foreign to the structure of a living organism to come into contact with the living matter (tissue or organ) and to be accepted by it within certain limits deemed as physiological [1]. The question if and what dental materials may be hazardous to patients, the environment, and dental personnel has become one of increasing public concern. Biocompatibility testing of materials that come in close contact with normal tissues is crucial for the quality of host-to-graft acceptance. Assays measuring cytotoxicity are a critical part of testing materials designed for application on human tissues.

Assessing the biocompatibility was performed according to ISO 10993–1:2003 and ISO/TC 194 norms, as well as according to the provisions of Law No. 205/ 2004 as regards lab animals welfare [2–4].

Today, in the development of biomaterials, one must consider the strength, aesthetics, or functional aspects of the material, but also its biocompatibility as well. Understanding biocompatibility requires also understanding the biological system where the materials were placed. Measuring biocompatibility is a complex process that involves *in vitro* and *in vivo* tests. These tests improved the understanding of biologic responses to a material but could not define the biocompatibility of the material with 100% certainty [5].

Biocompatibility was checked considering the reaction caused at the implant site during 21 days (three weeks) by daily observing the subjects' behavior (food ingestion, behavior, health state, toxic symptoms occurrence) as well as by macroscopic examination and histological examination upon expiry of the testing period [5, 6].

Materials and Methods

The composite materials Dualcim[RO] were prepared as a paste (Table 1), by dispersing in the organic phase the bioactive inorganic fillers. The composite materials were polymerized by light-curing and self-curing systems and the composites were exposed to a visible radiation for 60 seconds (Optilux) (Figure 1).

The chemical composition is presented in Table 1.

Table 1 – ***The chemical composition of the composite materials employed in the present study***

Dualcim	Organic phase	Inorganic phase	Initiation system
C1	Bis-GMA; TEGDMA	Glass with barium, Quartz, Colloidal silica	N,N-dimethyl-*p*-toluidine (Merck); Dimethylamino-ethyl methacrilate; Camphorquinone (Aldrich)
C2	Bis-GMA; TEGDMA	Glass with barium, Quartz, Colloidal silica	POB – Benzoyl peroxide (Merck)

Bis-GMA: 2,2-bis[4-(2-hydroxy-3-methacryloyloxypropoxy)phenyl] propane (produced in ICCRR Laboratory); TEGDMA: triethylene glycol dimethacrylate (Aldrich).

Tolerance test by implant

Local effects at the implant site requested for preclinical study were assessed within the Biobase Laboratory of "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania. According to the following norms: EUROTOX, CEE, FDA and the Order of the Ministry of Health No. 949/1991, we performed the tolerance test by implant of the composite product with low contraction upon polymerization [7–9]. The implant test was performed on two species of lab animals, Guinea pigs and rats in two versions: subcutaneous implant and intramuscular/perimuscular implant [10].

For the Guinea pig implant, we used male pigs (Peibright breed) with an average weight of 350 g. We also used male rats (Wistar breed), with average weight of 180–200 g. All specimens were healthy, their physiological status was normal, and throughout the experiment, standard maintenance and food conditions were secured [11]. The implant material was obtained from two types of paste with a dual polymerization system out of which we have modeled implants shaped as wands 0.5–0.7 cm long and 0.2–0.3 cm thick (Figure 1). The surface area was smooth. The wands thus obtained, had a compact structure, unbreakable consistency, but whose shape could be easily modified by polishing with a dental miller. The low-density material was also compact and hard. Prior to subcutaneous implant and perimuscular/ intramuscular implant, the specimens were sterilized by boiling for one hour. This procedure did not modify the implant features.

The tolerance test was performed according to the following protocol:

Composite with reduced contraction upon polymerization:

- subcutaneous inoculation (s.c.): five Guinea pigs;
- perimuscular and intramuscular inoculation (i.m.): five Guinea pigs;
- subcutaneous inoculation (s.c.): five rats;
- perimuscular and intramuscular inoculation (i.m.): five rats.

The animals were anesthetized using ether and the implant was placed under strict aseptic conditions. The subcutaneous implants were introduced by skin incision through the back right side, placing the implant by dilacerations of the subcutaneous conjunctive tissue [12–15]. After that, the incision was sutured with a single non-absorbable suture thread. In the perimuscular and intramuscular version, in case of all animals, the implant was placed deep inside the thy muscle of the back right leg, after skin incision and blunt dissociation by means of the implant of the connective inter-fascicular space between the muscular masses of the internal thy side. Here, the wound was also sutured. In case of each animal, the surgical field was prepared by mechanical toileting (hair cutting, shaving) and local area asepsis. The surgical implant procedure was performed without accidents, all animals survived, the post-op development was positive, without complications. The animal groups were fed and maintained under standard conditions being watched over a period of 21 days.

At the end of the experiment period, assessments were made regarding the local changes, which occurred at the implant site, their impact upon the general status of the animals and the relationship between the peri-implant tissue and the implant body. Fragments of the subcutaneous tissue and peri-implant muscle tissue were analyzed by classical histological technique; after that, a microscope was used for histological examination. The tissue samples were processed by means of classical histological method: fixation in 10% neutral formalin, paraffin embedding, sectioning at 5–7 μm and staining using the Goldner–Szekely thrichrome method.

Results

Presenting the results of the local and general biocompatibility of the low contraction upon polymerization dentistry material is performed according to the two testing versions: subcutaneous (s.c.) biocompatibility and intramuscular (i.m.) biocompatibility on the two species – Guinea pigs and rats.

The following questions will be provided with answers:

1. Local and general effect of the implant upon the subject.
2. Development and local site reaction to the implant throughout the testing period.
3. Local status and the reaction of the host tissue in contact with the implant: microscopic and histological examination of the implant site.
4. Conclusions upon the biocompatibility of the product under applied testing conditions.

The subcutaneous inoculation in rats

Subcutaneous inoculation in the back area was well tolerated by all subjects. After the intervention, all animals behaved normally. The health and behavioral state were fine. The implant was well tolerated with a very short convalescence period, clinically insignificant (Figure 2). After 21 days, the implant was perceivable upon touching, well anchored, without volume, no consistence or color changes. It was covered by connective subcutaneous tissue, which was moderately developed, forming a fibrous transparent wall capsule, non-painful. The implant was well integrated in the connective tissue structure without any signs of rejection. Fibro-connective proliferation is confirmed to have the normal structure of sub-dermal connective tissue. No hemorrhage, necrosis, puss creation, rejection or incompatibility signs were noticed in any of the subjects.

After removing the skin and examination of the connective tissue, we have noticed implant encapsulation by proliferating fibro-vascular connective tissue without scar characteristics. The tissue is formed as a translucent capsule, created from the subcutaneous connective tissue, enveloping the implant mass, without affecting its integrity. No necrosis (septic or aseptic) or rejection phenomena were noticed (Figure 3).

The intramuscular inoculation in case of rats

Placing the implant through the internal side from the muscle in case of the five rats was performed without any incidents. The implant, which was deeply introduced in the muscle mass, did not generate obvious movement impairment besides the 72 hours following

the procedure during which a slight sensitivity when walking was noticed. The animals healed in 4–5 days without functional traumas or vicious scars. Upon 21 days after the inoculation, the skin area was healed and we could notice a small cicatricial area marked by shaving. Removing the skin and examining the inter-muscular space revealed a fixed implant, encapsulated in a thin connective capsule, with a slight vascular reaction, materialized by neoformation vessels. No rejection reactions are noticed (peri-implant exudative inflammation (Figure 4). All animals survived normally, the inoculation area was not painful upon touch, the implant body was slightly noticeable.

At the end of the observation period (21 days), the implant was discovered between the muscle fascicles, fixed and encapsulated by proliferation of the inter-fascicular connective tissue, no rejection or incompatibility signs were noticed (Figure 5).

The subcutaneous inoculation in case of Guinea pigs

Healing of the skin wound at the implant site was without complications (Figure 6A).

The local reaction was reduced, materialized in the same non-painful and well-defined nodule. Upon 21 days after the insertion, the implant was well fixed under the skin as well as in the case of rats. No hemorrhage, necrosis or rejection reaction was noticed (Figure 6B). The subjects' general status and behavior was normal throughout the experiment. The histopathologic reaction of the Guinea pigs was similar to that of rats. It was materialized as a peri-implant capsular reaction represented by granulation (malformation) tests – composed of connective fibers placed in concentric circles. Rejection or other ill-natured process was not noticed.

The intermuscular inoculation in case of Guinea pigs

The behavior of the subjects throughout the observation period showed no abnormal changes (Figure 6B). At the end of the observation period, the body of the implant was found fixed between muscle fascicles (Figure 7) and the histopathological exam showed the same processes and phenomena as in the case of rats. No local or general rejection of the inoculated material was noticed.

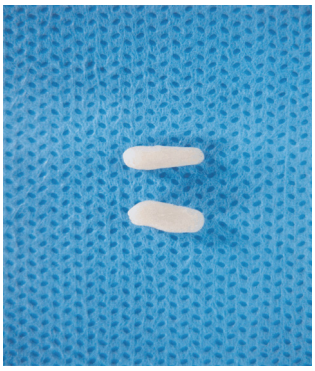

Figure 1 – *Composite material samples for implant.*

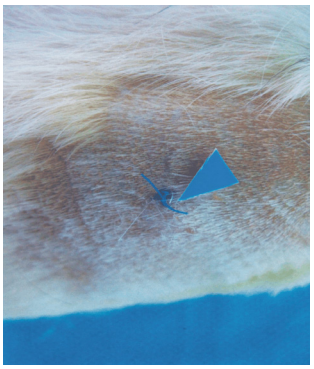

Figure 2 – *Healing without any scar tissue, skin area after 21 days from the implant (rats).*

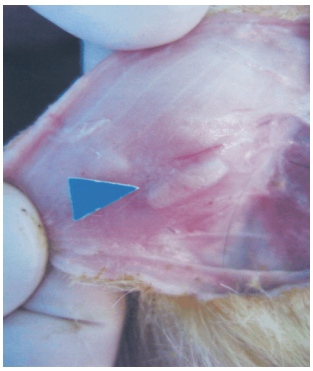

Figure 3 – *Fixation and encapsulation implanted subcutaneously in rats after 21 days post-implantation.*

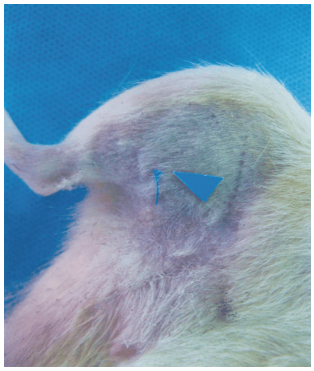

Figure 4 – *The wound healing in the inter-muscular implants in rats after 21 days.*

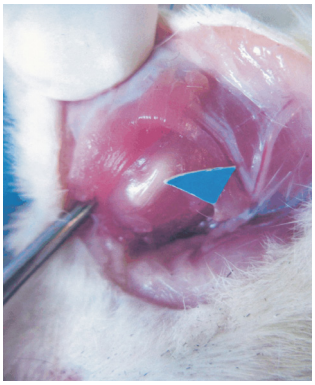

Figure 5 – *Implant body immobilized and anchored in the deep thigh in rats after 21 days post-implantation.*

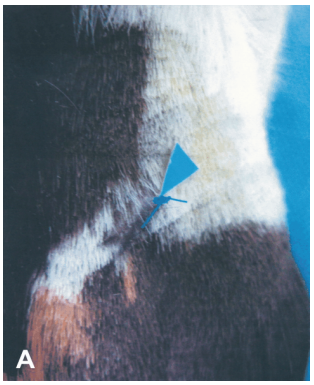

Figure 6 – *(A and B) The wound healing in the inter-muscular implants in Guinea pigs after 21 days.*

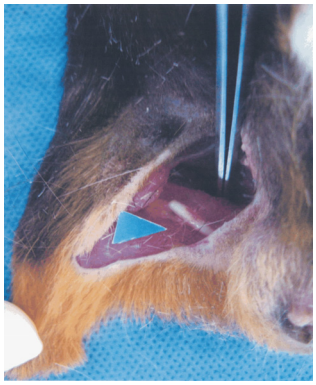

Figure 7 – *The implant body immobilized and anchored in the deep thigh in Guinea pigs after 21 days post-implantation.*

The tolerance test for biomaterial in case of Guinea pigs

Placing the implant in the intermuscular space at thy muscle level initially causes a limited alteration of the muscular fascicles in immediate contact with the implant surface. On a reduced surface, limited necrosis of muscular fibers appears. They are gradually absorbed by proliferation of connective and perimissium, around the implant body a connective capsule is formed and

consolidated. Its structure contains: neoformation capillaries, collagen fibers, young connective cells and fibroblasts, which are mobilized as one layer of macrophages/histiocytes, in close contact with the implant surface. This inclusion and fixation of the implant at the level of the intermuscular space can be noticed in Figure 8, where we also notice that the muscular tissue was affected upon contact with the implant by a slight alteration, shortly solved by connective proliferation from endo- and perimissium.

In the case of the subcutaneous implant, placing it in the connective sub-dermal tissue close to the dermis stimulated the development of a fibro-connective capsule with collagen, capillary, and fibrocyte cells. At the implant body surface, we found a monolayer of macrophages/histiocytes. The subcutaneous implant at the same species caused, similarly to the last version, the same connective proliferation with its role to isolate and encapsulate the implanted material (Figure 9).

The tolerance test for biomaterials used in rats

The implants placed in the intra-/perimuscular space of the muscle produced also morphological changes, in the same succession as in the case of the Guinea pig implants. The alteration process of the muscular tissue was limited and the reaction of the endo- and perimissium connective tissue led to a new connective-vascular capsule, fixing the implant body. This connective capsule is composed of the same morphological elements, as seen before. Introducing in the muscle the composite materials generated in this case also the same local changes, which finally led to the same capsular fibro-connective reaction separating the implant from the muscular tissue (Figure 10).

In case of subcutaneous implant, the peri-implant reaction was the formation of the same fibro-connective capsular structure as for Guinea pigs (Figure 11).

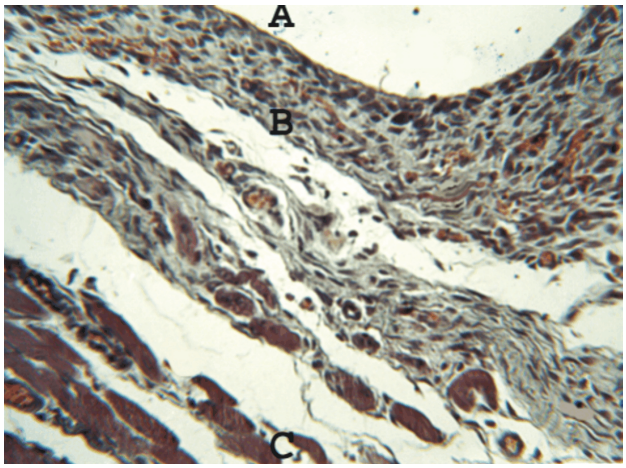

Figure 8 – ***Formation and consolidation of the connective-vascular capsule, which isolates and fixes the implant: (A) The space occupied around the implant; (B) Neoformation connective tissue constituted under a capsular shape around the implant; (C) Muscular tissue with atrophied fibers undergoing regeneration. Goldner–Szekely trichrome stain, ×150.***

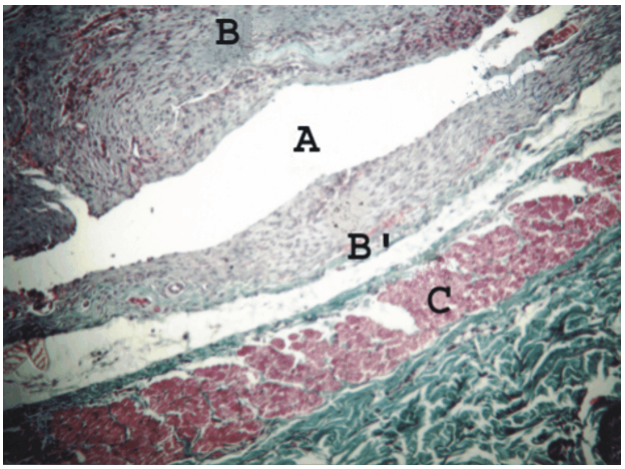

Figure 9 – ***Well-consolidated peri-implant vascular fibro-connective reaction in the subcutaneous area: (A) The space occupied around the implant; (B) Mobilization area for the macrophages/histiocytes; (B') Connective proliferation consolidated as peri-implant capsule; (C) Fibro-vascular wall of peri-implant wall. Goldner–Szekely trichrome stain, ×150.***

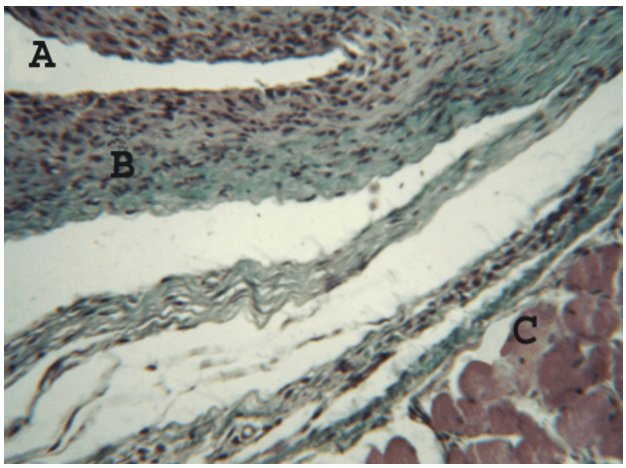

Figure 10 – ***Formation and consolidation of connective tissue around the implant body (placed in the inter-muscular space: (A) The space occupied by the implant body; (B) Vascular-connective capsule; (C) Muscular tissue undergoing regeneration. Goldner–Szekely trichrome stain, ×150.***

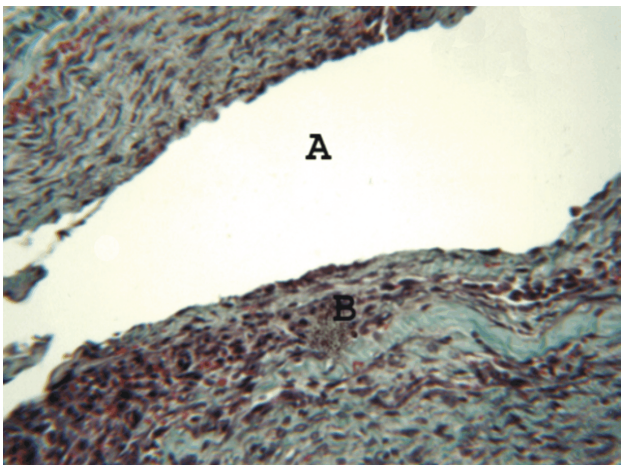

Figure 11 – ***Vascular connective reaction, peri-implant neoformation tissue: (A) The space where the implant was fixed; (B) Fibro-vascular capsule formed in the dermal contact site. Goldner–Szekely trichrome stain, ×150.***

Subcutaneous implant of the composite product with low contraction upon polymerization generated in rats the same isolation and fixing reaction of the implant by local connective reaction.

The proliferating process around the implant is well tolerated locally and the structure of the material does not stimulate phagocytosis from the monocytic–macrophagic system.

Structurally, the tissues at the implant site were slightly affected and the integration process was short.

In case of both species and in all subjects, the histological exam proved a favorable development of the relationship between the implant body and the placing site.

Discussion

The analysis and interpretation of both morphological and clinical aspects of intramuscular and subcutaneous tolerance of the two-implant versions for the two species, allows us to say that the product, in the structure and shape presented, could be easily placed under good conditions, both at the level of the subcutaneous tissue and at intermuscular level.

After a 21 days period (according to ISO 10993 for the acute experiment), when the implant was in direct contact with the tissue, no change of the shape and consistency, color or surface of the implant occurred.

Subcutaneous and intermuscular inoculation of the biomaterial in case of this two species was carried out abiding by strict asepsis and antisepsis norms and did not lead to any complications or abnormal post-op phenomena. The limited inflammation was finalized by a vascular-connective reaction of a good-natured scar type.

Upon intramuscular implant, the initial necrosis reaction of the muscular tissue manifested as fibers that were mechanically injured by the implant body was very limited, and the local connective reaction was fast and finalized by isolation and including the material.

Around the implants, the biocompatibility was kept under physiological limits. It was only materialized in a neoformation tissue, which is specific to the regeneration phenomena of local conjunctive tissue.

No changes were noticed (neither macroscopically nor microscopically – histological) at implant location which could suggest the toxicity of the implant, the fact that it contains irritating substances, neoplastic ones or which prove to be incompatible.

Locally, the presence of the product (implant) develops a moderate connective proliferation process that fades gradually, leaving behind a connective fibrous capsule, which isolates and fixes the implant body in the area where it was placed. Subcutaneous implant was much easier to tolerate, the encapsulation process being much faster and more efficient for both composites and for both species.

Histological (histopathological) aspects: according to the experimental protocol, upon the end of the observation period (21 days), the morphological and clinical exam was continued with the histological exam of the peri-implant tissues in order to finalize the tolerance test. For the composite product with low contraction upon polymerization, muscle tissue and skin samples were taken from all subjects (Guinea pigs and rats), as well as subcutaneous tissue from the peri-implant area. After 21 days, as presented in the morphological and clinical exam results, the implant bodies were removed in all cases. All implants in the experimental panel were intact, well encapsulated by the neoformation of connective-vascular tissue reaction and integrated in the anatomic structure of the subcutaneous conjunctive and muscular tissue.

Conclusions

The intra-muscular and peri-muscular implants were well tolerated by all specimens. The implant is fixed and wrapped in a thin transparent capsule conjunctiva, with light vascular reaction reflected by neoformation vessels. The composite material did not cause any changes in the general status, or any local irritant effects, throughout the testing period (21 days). Based on all the aspects presented, we may state that the dentistry product of a composite type material with dual polymerization benefits from good biocompatibility with living tissues, such as subcutaneous, conjunctive and muscular ones.

Acknowledgments

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