SUMMARY OF PHD THESIS

Evaluation of the healing stimulation potential of composite biomaterials

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INTRODUCTION

The therapy of bone and skin defects in animals represents a real challenge in the usual work of veterinarians. Currently, there is a variety of biomaterials with various qualities, but which still do not meet all the requirements of a full-fledged product. The discovery of a multipotent biomaterial to aid healing and encourage regeneration of multiple tissue types is a topic of great interest. Even if human medicine benefits from more research in the field, it is important to implement these practices in veterinary medicine as well.

The purpose of this paper is to evaluate the effectiveness of some biomaterials on several types of tissues, considering that studies of this kind are relatively limited. Composite biomaterials are often used in dental practice, but not in bone or especially skin healing. The presence of biocompatibility and the absence of cytotoxicity are valuable elements that provide the quality of a biomaterial.

Therefore, research and integration of these bioproducts into healing strategies could lead to considerable improvements in regenerative medicine and encourage the development of feasible biomaterials for multiple tissue types.

PHD THESIS STRUCTURE

The doctoral thesis entitled "Evaluation of the healing stimulation potential of some composite biomaterials" is structured in two main parts, namely: **Part I - Current state of knowledge**, respectively **Part II** - **Personal contribution.**

CURRENT STATE OF KNOWLEDGE

Part I consists of 3 chapters and provides a synthesis of general information regarding biomaterials, skin tissue and bone tissue. In chapter 1, biomaterials are presented and notions related to the types of biomaterials, their biocompatibility, their classification, the medical applications in which they are used and their properties are provided. Chapters 2 and 3 describe notions of skin and bone anatomy, their functions, and the types of defects that can occur at their level.

PERSONAL CONTRIBUTION

Part II contains 6 chapters. Here are described the materials and methods used, the results obtained, the discussions, conclusions and recommendations, as well as the original elements of the thesis.

RESEARCH STUDIES

1. Obtaining and characterization of composite biomaterials via microscopic methods

Creating bioproducts through the controlled release of mineral fillers into the polymer matrix is both a challenge and a significant requirement. Thus, in this study, two composites were created based on a complex matrix containing Bisphenol A-glycidyl Methacrylate, Urethane Dimethacrylate, Hydroxyethyl Methacrylate and Triethylene Glycol-Dimethacrylate (BisGMA, UDMA, HEMA and TEGDMA) and a mineral filler consisting of Barium Oxide (BaO), Barium Fluoride (BaF2), Nanohydroxyapatite (nHA) and Quartz, Silica and Fluoroaluminosilicate glass.

These biomaterials were embedded in polycaprolactone (PCL) microcapsules to deliver the mineral filler. PCL contains filler particles assisted by buffalo whey as an anti-caking agent and bioactive promoter, which releases the filler gradually during matrix polymerization. During C1 polymerization, the microcapsules were completely destroyed. In contrast, the C2 biomaterial retained some parts of microcapsules well embedded in the matrix near the BaO filler particles. This was confirmed by Energy Dispersive Spectroscopy (EDS).

The reduction of mineral content in sample C2 prevents the inhibition of the polycaprolactone envelope during the polymerization of the composites. This allows the mineral particles to incorporate into a very well-mixed structure without local segregation or surface cracks.

According to SEM microscopy, the microstructure of the composite is significantly influenced by the amount of filler. The polycaprolactone core shell breaks down better and the particles are better distributed in the polymer matrix due to a higher amount of mineral filler in sample C1. In addition, the distribution of the filler is crucial.

The effective incorporation of the mineral filler in both samples C1 and C2 was evidenced by FTIR spectra, which showed the stabilization of Bis-GMA as a result of the presence of UDMA monomers. This stabilizes the carboxylic bonds in the polymer matrix, controlling the viscosity during polymerization to ensure a uniform and optimal distribution of the filler particles in the composite. The uniform appearance of the microstructural details supports this fact.

Polycaprolactone microcapsules were used to control the controlled release of the filler by photopolymerization.

C2 samples have a higher uniformity of nanostructured filler particles.

2. "In vitro" evaluation of C1 and C2 cement-type composites on mesenchymal stem cells of palatal origin

An ideal medium for testing the biocompatibility of biomaterials is mesenchymal stem cell (MSC) of palatal origin (HAN et al., 2014; KAWAI et al., 2022; XU et al., 2019). Thus, to evaluate the biocompatibility and the presence of cytotoxic effects, the composites that were previously created were cultured. According to the literature, it is believed that the biological behavior of composites could be significantly influenced by minor differences in the mineral filler ratio (LAWRENCE et al., 2023; ZHAO et al., 2023).

Cultivation of mesenchymal stem cells of palatal origin (MSC) was performed at 1, 3, 5 and 7 days. After 3, 5, and 7 days of coculture, alkaline phosphatase (ALP) levels were measured at each time interval, and cytotoxicity was assessed on composite samples. Both composites allowed strong cell proliferation according to SEM study. In 1 and 3, a mesenchymal stem cell pluripotency stage was observed, with mean ALP levels of 209.2 u/L for C1 and 193.0 u/L for C2, as well as a spindle cell morphology.

After 7 days of culture, cell differentiation was observed, which was demonstrated by SEM. Morphological changes such as flattened, stellate, and rounded shapes were associated with an increased ALP level (279.4 u/L for C1 and 284.3 u/L for C2). After seven days of co-culture, energy dispersive X-ray spectroscopy (EDX) showed increased levels of phosphorus (P) and calcium (Ca) close to the stoichiometry of hydroxyapatite. This suggests that C1 and C2 MSCs begin to exhibit osteoinductive behavior.

After three days, the colorimetric test based on the cleavage of yellow tetrazolium salt as an indicator of cell viability (MTT) showed a cell viability of 98.08% for C1 and 97.33% for C2, suggesting that the composite samples are biocompatible. Although the cell viability decreased slightly at 5 and 7 days, the results were remarkable: C1 had 89.5% and C2 had 87.3%. Therefore, both C1 and C2 are suitable for further "in vivo" testing.

The amount of bioactive filler is very important for cell viability. Better MTT assay results indicate that C1 has a higher value of MSC viability. But both C1 and C2 are viable candidates for further in vivo testing.

In addition, there was a correlation between the increased amount of bioactive filler and the level of alkaline phosphatase (ALP).

3. Evaluation of the biocompatibility of two types of composite by subcutaneous and intramuscular implantation in rats

Assessment of the biocompatibility of products that come into direct contact with normal tissues is essential for determining the degree of host-graft tolerance. Toxicity tests are essential for the evaluation of products to be used on animals or humans. To understand the phenomenon, both in vitro and in vivo experiments are needed (PATROI, 2013, EMOKE PALL, 2015). There is a possibility that certain materials may cause acute toxicity by accumulating in kidney or liver cells.

Although cements may exhibit relatively low toxicity, this decreases considerably as time passes (SCHMID-SCHWAP, 2009). This reduction in toxicity is attributed to the unpolymerized parts of the air-inhibited layer (DARMANI, 2007). The complex interactions that occur between the material and the biological system are made possible by using a living organism, as "in vitro" studies cannot replicate these interactions (GOCIU, 2013).

This study aimed to evaluate the biocompatibility of two composite cements over a period of ninety days by examining the behavior of rats and performing clinical, histological and CT scan examinations.

The materials were implanted subcutaneously and peri/intramuscularly to be tested for toxicity and biocompatibility. Days thirty and ninety were critical to the study as the implants were harvested along with adjacent tissue, kidneys and liver to identify any potential toxic deposits. Despite the fact that the biomaterial was in close contact with the tissue, its shape, color or texture remained unchanged.

There was a positive relationship between the material and the insertion site, despite a slight inflammatory response at the implantation site. The liver and kidneys showed no changes in shape or function. The results demonstrated that the biomaterials were compatible with biological tissues, as they did not cause clinical changes or specific irritations.

No changes were observed in the dimensions of both cements according to computed tomography scans.

Without negative consequences, cytotoxicity or rejection, histopathological observations confirm the biocompatibility of the biomaterials.

In our studies, the in vivo implantation model used confirmed the in vitro results, namely a feasible biocompatibility.

4. Comparative evaluation of composites C1 and C2 in stimulating the healing of skin wounds in rats

The cementitious composites in this study were developed as a result of current research in regenerative medicine. In an experimental model used in rats, the main purpose of this research is to evaluate the ability of composite cements to stimulate wound healing. Unpolymerized biomaterials were used to cover wounds after five-millimeter-thick skin defects were created.

A variety of microscopic and macroscopic protocols were used to assess defect evolution. These protocols included histopathological analysis, wound closure rate, and wound photography. While both composites demonstrated a healing-promoting effect, product C1 showed a more extensive and faster healing mechanism.

None of the treated groups had negative results, which shows that these bioproducts are effective and biocompatible. The skin of all rats healed completely by day 15. This study presents an innovative approach to skin wound healing using non-polymerized composite cements, which are traditionally used in dentistry.

All subjects in the study were healthy and did not impair the skin's ability to heal, according to paraclinical analyses.

Macroscopic evaluations showed that the C1 biomaterial facilitated healing from the third day and had superior qualities compared to the C2 biomaterial, which started wound closure on the sixth day.

Histological analysis shows that the C1 bioproduct had superior qualities: the sebaceous units were partially restored, neoangiogenesis was observed, and the C1 composite cement was completely absorbed.

After topical application of the material, histological investigations of the organs demonstrated no hepatotoxicity or nephrotoxicity. This again confirms the biocompatibility and reliability of both composite cements.

5. Comparative evaluation of C1 and C2 composites in stimulating the healing of induced bone defects in the rat

Repaired or Reconstructed bone defects represent. In this study, two composite cements were implanted into a subcritical femoral defect in rats. To compare the results, three groups were used, one control group, and two experimental groups.

The research looked at the possible osteogenic capacity and toxicological tolerance of the bioproducts through histopathology and computerized imaging analyzes performed at 14, 28, 56 and 90 days. The initial findings of this research are positive as they show little difference between the groups treated with biomaterials. The use of these bioproducts can be an effective means of treating bone abnormalities.

Compared to the C2 material, the C1 biomaterial healed the bone defects faster. Areas of bone fracture were observed in some rats. It is unclear whether the fractures were caused by the C2 biomaterial or other factors.

By the end of the ninety days of the study, the biomaterials remained the same in the bone tissue, but without creating negative consequences, rejection phenomena or cellular apoptosis or necrosis.

Both biomaterials demonstrated slight osteoinductive activity, but biomaterial C1 was more effective than bioproduct C2.

The physicochemical properties of the bioproducts match the requirements of the bone tissue, and the durability and mechanical strength are confirmed by CT scans. This could serve as the basis for a research project examining the use of dental biomaterials in the manufacture of implants for bone defects.

FINAL CONCLUSIONS AND RECOMMENDATIONS

Following the research carried out, the results form the basis of the following **conclusions**:

• The synthesized composite biomaterials have proven a good tolerance in vitro with an increased biocompatibility and the absence of cytotoxicity;

• The research conducted validated that the use of biomaterials did not change the values of the blood tests and the functioning of the kidneys and liver was not affected;

• Non-polymerized biomaterials helped skin healing and increased the biodegradability of products without producing negative consequences at the level of other tissues or organs;

• The implantation of biomaterials at the subcutaneous and peri/intramuscular level did not induce a local or systemic inflammatory response;

• The use of bioproducts in bone defects did not negatively interfere with the bone healing process;

• The addition of composites with various types of nanoparticles induce an increased therapeutic efficiency;

• The research of bioproducts in bone defects has validated that they are suitable for the manufacture of various types of prostheses, due to their high biocompatibility and low toxicity, as well as excellent mechanical properties, having a high mechanical strength.

Considering the above, the author's recommendations are: that biomaterials be used in tissue engineering and regenerative medicine. Due to the increased biocompatibility, possibilities are opened for the further development and integration of specific products in these cements (for example: microchips). However, the deepening of materials is necessary for applications in the manufacture of prostheses of various types

THE ORIGINAL ELEMENTS OF THE THESIS

The present research brings some innovative elements to the study of biomaterials:

1. Synthesis of new cement composite biomaterials that have not been produced before;
2. Obtaining materials with increased biocompatibility and low toxicity;
3. Controlled release of the mineral filler in the polymer matrix during the polymerization process which increases the uniformity of the bioproduct;
4. The use of non-polymerized cement-type biomaterials in the skin healing process;
5. Use of a microchipping syringe as a way to implant bioproducts at subcutaneous and peri/intramuscular level.