The suitability of scanning electron microscopy in the evaluation of bone structure surfaces and selection of alloplastic materials for facial skeletal reconstruction

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ABSTRACT:

Introduction: Functional and aesthetic problems can arise even from minor losses in the facial skeleton. Injuries and oncological surgeries are the most frequent causes of these losses within the facial skeleton. Advances in surgical interventions have allowed for ever-increasing degrees of resections, increasing oncological radicality as well as treatment effectiveness, providing the patient with a chance for living a longer life. However, this subsequently requires the use of even more advanced reconstruction techniques in order to restore the patients’ quality of life and comfort, as well as enable their return to professional and social activities. The necessity of reconstructive surgery applies not only to patients with cancer, but also to those with impaired or failing sensory and organ function as a result of inflammatory conditions, injuries, or non-oncological surgeries. There are many available reconstruction procedures, which depend on the location of the loss, the type of tissue lost, the degree of loss and patient-dependent factors. Materials used in reconstruction surgeries may include the patients’ tissues when available, and artificial reconstruction materials otherwise.

Material and methods: The analysis involved fragments of bone tissue removed during surgery. Due to the nature of the medical procedure and the inability to replant the tissue, it was regarded as medical waste. The preparations used were observed under an optical microscope and a scanning electron microscope, and a chemical analysis was performed. The chemical composition of samples was analysed using a low vacuum detector (LVD) at an accelerating voltage of 15 kV and 10 kV and at a spot size of 4 and 3.5. The observations were performed in a secondary electron (SE) detection system.

Results: Observation of parameters under an optical microscope and of images obtained using a scanning electron microscope revealed the presence of typical compact bone tissue with varied surface shapes in each case (various degrees of unevenness and porosity). Chemical composition analysis confirmed the presence of compounds from the CaO-P2O5·H2O system. The Ca/P (calcium/phosphorus) ratio obtained from the chemical analysis varied from 1.33 to 2.1 and indicated a varied morphology of calcium phosphates forming the bone structures of the facial skeleton.

Conclusions: (1) Calcium phosphates are characterized by excellent biocompatibility because of their chemical affinity to bone and are ideal for the reconstruction of bone losses within the facial skeleton; (2) biodegradable polymers have the highest functional potential among several groups of biomaterials used in tissue engineering because of their ability to be tailored individually, in addition to their high biocompatibility.

KEYWORDS: hydroxyapatite, scanning electron microscope, secondary electron detection

INTRODUCTION

Over 500,000 cases of head and neck squamous cell carcinomas (HNSCC) are diagnosed every year around the world. The frequency of this diagnosis is increasing every year. In the case of about one third of patients, the neoplasm is diagnosed in the early stages of progression (stage I or II), and otherwise in advanced stages (stage III or IV). The rapid development of diagnostic and surgical techniques, the ability to use novel surgical tools, and interdisciplinary cooperation all allow surgeries to be performed not only in patients with the initial stages of cancer, but also in very advanced cases previously deemed to be inoperable.
The dynamic development of biomaterials in medicine began in the 20th century. The type of materials used in medicine depended on the current state of knowledge and technical capacity. As science and technology progressed, various additional metal alloys and synthetic materials were employed.

Historically, metallic implants introduced in the middle of the 20th century were associated with a high risk of implant rejection by the host and were characterised by the harmful influence of metal ions on the body. The first report on the use of artificial products for alloplastic purposes was published in 1894. A skull bone filling was attempted by Fraenkel, using a celluloid material. However, continued failures delayed further attempts for almost half a century. Another example of a stumbling block hampering the progress of using new materials in alloplastic surgery took place in 1950, when a thesis supported by research was published stating that many plastics used in experiments on animals showed carcinogenic properties.

The materials used today are biocompatible and completely safe to humans. These materials can be distinguished into different categories: biostatic, biomechanical, and aesthetic. Biostatic implants include materials that provide a scaffolding for the reconstruction or supplementation of missing tissues or organs. Biomechanical prosthetics mainly include joint prosthetics [3–5].

Current work on new materials for medical applications has brought forth new avenues for the use of polymer materials and biostable metallic materials (titanium, TiAlV alloy).

According to the Biomaterials Consensus Conference at the National Institute of Health of 1982, it is assumed that biomaterials include all substances other than a drug or a combination of synthetic or natural substances which may be used to supplement or replace tissues of an organ or a part of an organ in order to meet their respective functions [6].

Biomaterials used in reconstruction or repair procedures should show optimal physiochemical and biological properties simultaneously. These optimal physiochemical properties include material rigidity and surface, Young and Kirchhoff moduli and material density. They are combined with optimal biological properties such as environmental biostability, a lack of intense defensive reactions, a lack of intensive toxic and allergic reactions, and a lack of mutagenic and carcinogenic activity, to make up the overall characteristics of an optimal material [7, 8].
with an attachment for chemical analysis in EDS micro-areas (Energy Dispersive X-Ray Spectroscopy; EDAX). The sample was attached to the microscope table using a carbon-based conductive tape in order to perform the observation. The measurement was performed using a low vacuum detector (LVD) at an accelerating voltage of 15 kV and 10 kV and at a spot size of 4 and 3.5. The observations were performed in a secondary electron (SE) detection system.

**RESULTS**

Observation of the images obtained both under an optical microscope and using a scanning electron microscope showed the presence of typical compact bone tissue with varied surface shapes in each case (varying degrees of unevenness and porosity) (Figs. 1.–4.).

The chemical composition analysis confirmed the presence of compounds from the CaO-P₂O₅-H₂O system. The Ca/P (calcium/phosphorus) ratio obtained from the chemical analysis ranged from 1.33 to 2.1 and indicated a varied morphology of calcium phosphate formations in the bone structures of the facial skeleton (Fig. 5., Tab. I.).

By comparing the obtained results to examples of calcium phosphate representing CaO-P₂O₅-H₂O, the chemical compounds most closely resembling facial skeleton bones include Ca₁₀(PO₄)₆(OH)₂ (hydroxyapatite, Ca/P – 1.667), tricalcium phosphate Ca₃(PO₄)₂ (Ca/P – 1.5), octacalcium phosphate (Ca₈H₂(PO₄)₆5H₂O) (Ca/P – 1.33), and tetracalcium phosphate (Ca₄(PO₄)₂O) (Ca/P – 2.0) (Tab. II.).

**OBJECTIVE OF THE STUDY**

Surface evaluation and analysis of the chemical composition of facial skeleton bones in order to select the optimal biomaterial for use in reconstruction and repair surgeries.

**MATERIAL AND METHODS**

The analysis involved 30 fragments of bone tissue removed during surgery. Due to the nature of the medical procedure and the inability to replant the tissue, it was regarded as medical waste. The Bioethics Committee approved the aforementioned procedure.

The collected preparations were divided into three groups, depending on the location from which they were removed:

- Group I – bone tissue from an element of the ear ossicles, No. 10;  
- Group II – bone tissue from the bone frame of an eye socket, No. 10;  
- Group III – bone tissue from part of the nasal septum, No. 10.

Optical microscopy was used in the first stage in order to analyse the surfaces of the studied sample. The next stage involved preparing the materials for evaluation in a scanning electron microscope as well as for chemical analysis. The surface microstructure of the bone preparation was studied using a scanning electron microscope with an attachment for chemical analysis in EDS micro-areas (Energy Dispersive X-Ray Spectroscopy; EDAX). The sample was attached to the microscope table using a carbon-based conductive tape in order to perform the observation. The measurement was performed using a low vacuum detector (LVD) at an accelerating voltage of 15 kV and 10 kV and at a spot size of 4 and 3.5. The observations were performed in a secondary electron (SE) detection system.

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DISCUSSION

Reconstruction or repair surgeries hold a special significance in medicine and are an important step in any surgical procedure. The ever-increasing lifetime expectancy, the increasing numbers of oncological surgeries with increasing resection areas, and the continuously growing aesthetic, psychological, and professional problems faced by patients undergoing surgery all create the need to seek new medical solutions. In addition to materials from the patients themselves (free flaps, pedicled flaps, bone, and muscle tissue, etc.), modern medicine continues to look towards synthetic biomaterials for solutions. Modern medicine needs modern solutions to provide an answer to increasing patient requirements. Previous therapeutic methods are continuously improving thanks to the progress made not only in medicine, but also in the technology surrounding it. This work presents microscopic and chemical analyses of bone tissues from the facial skeleton and provides a starting point in the search for optimal biomaterials for the reconstruction of losses in this anatomical area. Evisceration, enucleation, and exenteration are some of the most damaging medical procedures currently performed in that area. Removal of the contents of the eye socket, often together with fragments of the bone walls, completely disrupts anatomical connections within both the eye socket and the face [9–15]. Many types of intra-eye-socket implants are used in modern ophthalmology. The first attempts to use hydroxyapatite in eye sockets were undertaken in the mid-1980s; it was previously mostly used in orthopaedics or maxillofacial surgeries. Calcium phosphates are characterised by excellent biocompatibility because of their chemical similarity to bone. They are osteoconductive, facilitating the formation of new bone tissues. Calcium phosphates may crystallise into salts, such as hydroxyapatite (HAp) or calcium triphosphate, depending on the Ca:P ratio. Hydroxyapatite (HAp) is chemically similar to the natural component of the bones and hard tissues of mammals. It occurs in nature in the form of the mineral Ca\(_3\)(PO\(_4\))\(_2\)(OH), and has the ideal Ca:P ratio of 1.66. According to our observations, the nasal septum bone is the closest to hydroxyapatite in terms of chemical structure. Use of porous eye socket implants made of hydroxyapatite or porous polyethylene provides better results during the period directly following a surgery. Porous materials enable tissue to grow in them, reducing the probability of post-transplant shifts or dislocations. After the healing stage, they also influence the application and mobility of prosthetics in the patient’s body. Despite the very similar chemical composition of hydroxyapatite to that of the bone tissue of the eye socket, there are increasing cases in which the use of other alloplastic materials or technologies is becoming necessary. In recent years, rapid prototyping technology, and 3D printing in particular, have become more popular and commonplace. This method has quickly gained popularity and currently represents an alternative in the prototype creation process in medicine, as well as in biomedical and tissue engineering. Rapid prototyping (RP), also known as solid free-form fabrication (SFF), is comprised of a group of incremental AM (additive manufacturing) technologies based on the creation of a physical, three-dimensional object layer after layer. Among the various materials in 3D printing, poly-L-lactide (PLLA) is the most frequently used and studied material in tissue engineering. PLLA is characterised by very good biocompatibility and good mechanical properties, and according to our earlier observations it is an ideal product for filling losses in the nasal septum [16–24].

Hydroxyapatite was selected on the basis of our analysis because it corresponds to bone tissues from the middle-ear area and constitutes an excellent transplant material. Reconstruction and repair procedures used in cofo surgeries are the second stage of interventions such as removing sites of inflammation in the course of treating chronic otitis media, as well as oncological procedures (reconstruction of ossicles, posterior wall or mastoid obliteration). They are often performed during the second stage of the procedure, restoring the patient’s auditory functionality, and mitigating potential limitations to athletic or professional ability. According to our observations, the recommended hydroxyapatite or its alternative (glass-ionomer cement) are ideal as materials for obliterating the mastoid part of the temporal bone, reconstructing the small bone losses in the middle-ear area, or reconstructing the posterior wall of the external auditory canal. However, the authors prefer titanium implants or implants made of thermoplastic polymers for the reconstruction of ossicles, without modifications using additional ions to prevent the possible negative influence on the inner ear function. These observations coincide with those of other authors. When planning biomaterial selection for facial skeleton reconstruction, in addition to characteristics such as chemical composition and biocompatibility with the target transplant environment, it is extremely important to keep in mind spatial structure and the adhesion surface in contact with host tissues. In our analysis, we observed compact bone tissue with varied porosity and the presence of local unevenness in all cases, both under the optical microscope and the scanning electron microscope [25–29].

It seems that biodegradable polymers have the highest functional potential among several groups of biomaterials used in cell engineering. A scaffolding made of such materials degrades in a specific manner, at a rate adapted to that of cell proliferation. This eliminates the need to remove the implant from the body at the end of therapy, increasing the probability of therapeutic success.

Currently, the inclusion of modern digital technologies allows for the design of surgical reconstruction to be individually customised in the form of a fully implanted, sterile bone tissue, intended for the
rehabilitation of lost or damaged fragments of facial skeleton bones. Individually prepared fragments are easily implanted during surgical procedures, and their surface optimally matches the bone loss compared to standard bone blocks [30, 32].

CONCLUSIONS

1. Calcium phosphates are characterised by excellent biocompatibility because of their chemical affinity to bone and are an ideal material for reconstruction of bone losses within the facial skeleton; their high biocompatibility.

REFERENCES


FINANCING

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