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Synthetic biomaterials based on hydroxyapatite and tricalcium phosphate: analysis of current clinical trials

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Abstract

Introduction To date, a wide variety of synthetic materials, including metals, polymers and ceramics, have been proposed and used as a substitute for bone grafts in the field of traumatology/orthopedics, neurosurgery and oral and maxillofacial surgery (OMFS). However, the most studied materials are calcium phosphate ceramics (CPC), in particular hydroxyapatite and tricalcium phosphate, as well as their mixtures, called byphasic calcium phosphates. This interest stems from the fact that the main component of bone is the apatite mineral calcium phosphate. Hydroxyapatite and tricalcium phosphate are among the most commonly used and effective synthetic substitutes for bone grafts. They have not only osteoconductive properties, but also osteoinductive. These properties, combined with cell-mediated resorption, ensure complete regeneration of bone defects. This study will analyze existing clinical trials, registered on the clinicaltirals.gov website, on the use of hydroxyapatite and tricalcium phosphate in the field of traumatology and orthopedics, neurosurgery and OMFS.

Aim To identify the potential for clinical use, as well as possible side effects, of CPC as a replacement for bone grafts.

Materials and methods The search strategy was to use material from the clinicaltrials.gov website, which focused on key terms such as "hydroxyapatite", "tricalcium phosphate", "hydroxyapatite and tricalcium phosphate", "traumatology and orthopedics", "maxillofacial surgery", "dentistry", "neurosurgery", "bone", and "diseases of the musculoskeletal system", and the criteria for inclusion and exclusion of the data from clinical trials were divided into two stages.

Results and discussion As of November 2022, there were approximately 85 clinical trials with hydroxyapatite application, approximately 49 clinical trials with tricalcium phosphate, and approximately 16 clinical trials with the hydroxyapatite/tricalcium phosphate combination. Most of the studies were Phase 1-2, Phase 2, or Phase 4. Most focused on tibial trauma therapy, osteoporosis/osteopenia, alveolar bone resorption, and spinal surgery. It was found that full results were published only in 3, 7 and 2 clinical trials on the use of hydroxyapatite, tricalcium phosphate and their combination, respectfully. All clinical trials had similar preparation methods and all of those clinical trials produced positive results without serious side effects.

Conclusion There is a wide potential for clinical use of CPC as synthetic bone graft substitutes without reports of serious side effects. Many preclinical and clinical studies are currently underway on the use of hydroxyapatite and tricalcium phosphate, and their future results will further explore their clinical potential.

Keywords: hydroxyapatite, tricalcium phosphate, diseases of the musculoskeletal system, therapy, analysis, clinical trials

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INTRODUCTION

Bone tissue has a remarkable ability to regenerate and repair through physiological remodeling or in response to injury. In a number of situations, bone tissue repair does not occur spontaneously due to certain (unfavorable) local causes (vascular damage, infection, etc.), bone defect of a critical size, systemic causes, or a combination thereof [1, 2]. A number of surgical interventions to stimulate bone regeneration involve the use of biological support including a bone graft or a substitute, natural or synthetic [3-6]. Autogenous bone (autograft) remains to be the "gold standard" of bone grafting. Only an autologous graft provides the most desirable properties of a biomaterial, including osteoconduction, osteoinduction and osteogenesis [7-9]. However, additional surgical interventions are required to obtain autologous bone grafts, which can lead to complications, and, importantly, the volume of autologous bone tissue is always limited. The use of allografts may solve a number of problems associated with the use of autografts, but it also raises some concerns, such as the risk of infection transmission, immunological reactions of the recipient, loss of biological and mechanical properties due to their processing, increased cost and lack of availability [10, 11]. High biological safety is a desirable characteristic of synthetic bone grafts. Approximately 60 % of current synthetic bone substitutes available are ceramic biomaterials [12]. One of the most common is calcium phosphate ceramics (CPC), characterized by a chemical composition close to the mineral phase of calcified tissue, namely calcium hydroxyapatite [13]. In addition, the composition of the raw product can be controlled by adjusting the calcium to phosphate (Ca/P) ratio [13]. Hydroxyapatite and tricalcium phosphate are the most widely used CPCs, mainly in the combination in so-called biphasic calcium phosphate ceramics [14, 15]. Hydroxyapatite and tricalcium phosphate are among the most common bioactive materials currently used in neurosurgery, orthopedic and dental surgery [16–18]. This class of materials can be synthesized according to several protocols such as solid state reactions, sol-gel, precipitation methods, emulsion methods, hydrothermal reactions, mechanochemical methods, hydrolysis of other calcium phosphates and chemical vapor deposition [19, 20].

Numerous studies have demonstrated the ability of hydroxyapatite to stimulate new bone formation in vivo. Therefore, this material is generally considered osteoinductive [21]. New bone formation without the addition of bone morphogenetic proteins or osteogenic cells at extraskeletal sites has also been observed with the use of tricalcium phosphate [22]. However, it should be emphasized that at present some of the mechanisms underlying osteoinduction are not fully understood. This property is indeed related in a non-trivial way to the chemical composition and crystallinity of the material, its stoichiometry, dissolution/precipitation behavior, surface chemistry and charge, as well as microporosity and roughness. Both hydroxyapatite and tricalcium phosphate have been widely used as bone fillers in the form of powders, cements, solids, porous solids, discs and granules in many clinical trials since the early 1980s [23]. Hydroxyapatite has also been used to create bioactive coatings on metal implants to enhance the biomimetic response of such implants [24]. To date, the only commercially acceptable method for applying such coatings is plasma spraying.

Tricalcium phosphate has three recognized polymorphs: 1) β-tricalcium phosphate, which is stable below 1125 °C; 2) α-tricalcium phosphate, which is stable between 1125 °C and 1400 °C, and 3) α'-tricalcium phosphate, which can be stable at temperatures above 1430 °C [14, 15]. α-Tricalcium phosphate and β-tricalcium phosphate have different dissolution rates in the body and can be used together in appropriate combinations [25]. α-Tricalcium phosphate and α′-tricalcium phosphate have a lower theoretical density than hydroxyapatite, but β-tricalcium phosphate is more preferred for biomedical applications due to its mechanical characteristics, chemical stability and resorption rate [26].

Another important area of research into CaP-based biomaterials has focused on biphasic calcium phosphates (BCP). By definition, BCP consists of two distinct CaP phases: most often the more stable hydroxyapatite phase and the more soluble β-tricalcium phosphate phase in varying proportions [27]. This combination has significant advantages over other types of CaP bioceramics, allowing better

control of biological activity and biodegradation, which guarantees the stability of the biomaterial and promotes osseointegration of newly formed bone tissue into the implant. The combination of hydroxyapatite/tricalcium phosphate has osteoconductive properties and the possibility of acquiring osteoinductive properties [28, 29].

As of November 2022, the site *clinicaltrials.gov* lists approximately 85 registered clinical trials using hydroxyapatite alone or in combination with other biomaterials in traumatology and orthopedics, maxillofacial surgery (MFS) and neurosurgery. There have been 49 clinical trials reported on the use of tricalcium phosphate in the same areas of medicine. However, of these clinical trials, only five trials published full results using hydroxyapatite and seven using tricalcium phosphate, respectively. In addition, there are 16 clinical trials using a combination of hydroxyapatite/tricalcium phosphate, with a predominance of one or another synthetic biomaterial, two clinical trials with results were among them.

In this study, we analyzed various applications of synthetic biomaterials (hydroxyapatite and tricalcium phosphate composites) in traumatology and orthopedics, maxillofacial surgery and neurosurgery; we paid special attention to the corresponding methods of synthesis and processing of hydroxyapatite and tricalcium phosphate composites, as well as their storage conditions.

Purpose: to identify the potential for clinical use of calcium phosphate ceramics as a substitute of bone grafts, as well as possible side effects of their use.

MATERIALS AND METHODS

Search strategy

We conducted a comprehensive search for clinical trials demonstrating the use of hydroxyapatite and tricalcium phosphate composites, alone or in combination, as effective synthetic bone substitutes for various musculoskeletal conditions. Databases including *clinicaltirals.gov* were used to obtain all relevant clinical trials. Search words were: hydroxyapatite, tricalcium phosphate, hydroxyapatite and tricalcium phosphate, traumatology and orthopaedics, maxillofacial surgery, dentistry, neurosurgery, bone and diseases of the musculoskeletal system. To select studies, we used the following inclusion and exclusion criteria, which were divided into two stages.

Stage 1:

1) clinical trials on humans with the use of hydroxyapatite and tricalcium phosphate
2) clinical trials in phases 1 to 4:
   a) location;
   b) area of application;
   c) phase;
   d) study status.

Stage 2:

1) clinical trials which results were published;
2) phases of clinical trials with selected study criteria for statistical analysis:
   a) field of application;
   b) treatment;
   c) intervention model'
   d) source;
   e) selection and processing methods;
   f) results.

Selection of clinical trials

After applying the inclusion and exclusion criteria in the first stage, a total of 67 clinical trials with hydroxyapatite, 29 clinical trials with tricalcium phosphate and 12 clinical trials with the hydroxyapatite/tricalcium phosphate combination were excluded, respectively, as there were studies that were either in the early phase 1, or the phase was not specified or had an inapplicable phase (note: excluding those with results). We analyzed the remaining clinical trials (18, 20, and 4, respectively) by area of application, phase, status, and location. Only 12 clinical trials using hydroxyapatite and tricalcium phosphate alone or in combination (3, 7 and 2, respectively) were selected for the second stage because they met the stated criteria of this study (Fig. 1).

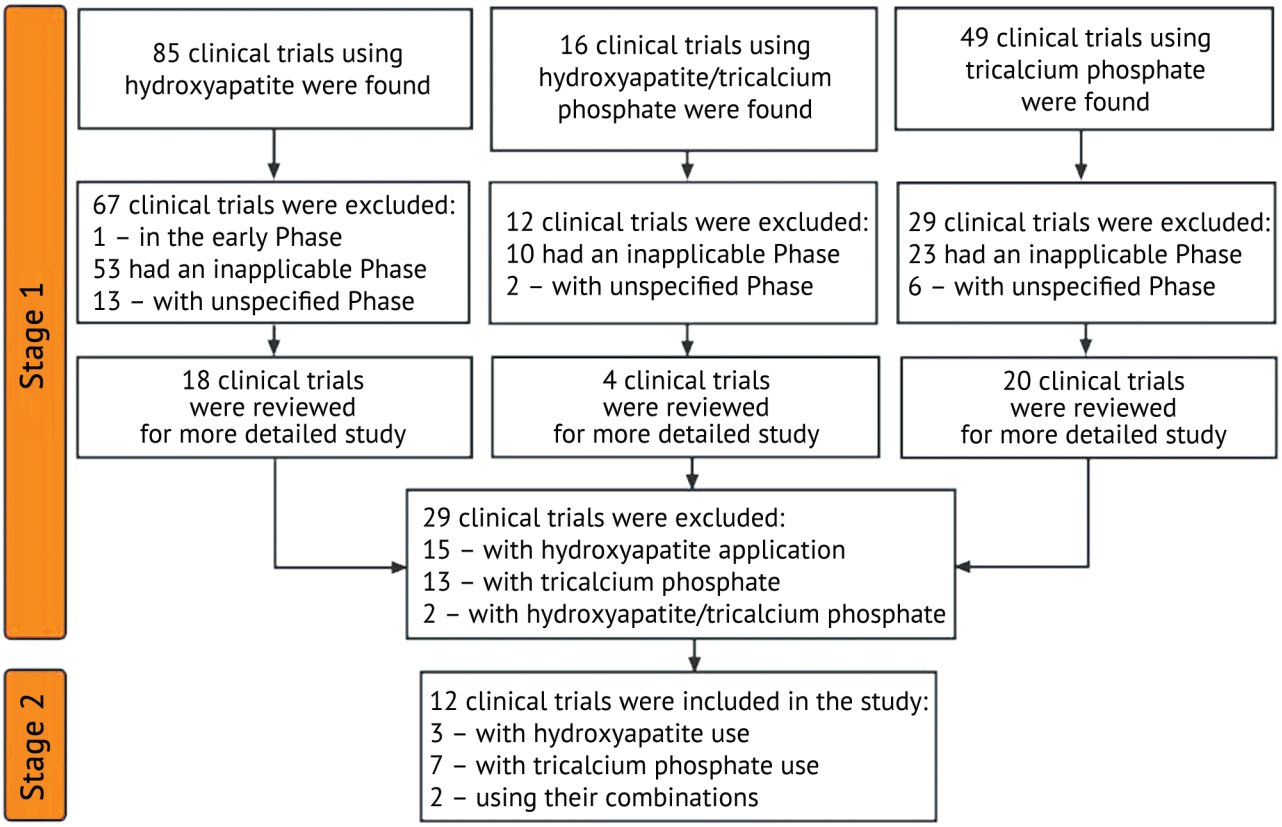

Fig. 1 Study design

Commercial products

About twenty-three major commercial bone graft products (scaffolds) have been identified from clinicaltirals.gov data: OsteoGen™, NanoBone®, Kyphos FS™, KYPHON ActivOs®, Ossix™, Bio-Oss®, CERAMENT® G, Siloss®, i-FactorTM, ReproBone®, AllogenixTM, Straumann BoneCeramic®, CustomBone, OsteoGen® Plug, CERVIOS chronOS™, AttraX® Putty, chronOS®, Easy-graft® CLASSIC, NovoMax™, Guidor®, Guidor easy-graft® CRYSTAL, PD VitalOs cement®, Neofuse®, which may differ in composition from each other, i.e. contain hydroxyapatite or tricalcium phosphate, or these synthetic biomaterials were used additionally. Moreover, four growth factor-enhanced bone grafts and two peptide-enhanced xenohybrid bone grafts were identified, respectively.

Statistical analysis

The statistical methods applied were t-test, ANOVA, chi-square analysis or Mann–Whitney test. A probability p value < 0.05 (*), < 0.01 (**), or < 0.001 (***) was considered statistically significant. Statistical analysis was performed using IBM SPSS 22.0 software and graphs were generated using Graphpad Prism 7.0.

RESULTS

Statistics from registered clinical trials on the use of hydroxyapatite and tricalcium phosphate

The use of synthetic biomaterials is a relatively new area that has great potential for solving many serious problems in neurosurgery, traumatology, orthopedics and maxillofacial surgery (Fig. 2). Additional research may be required to fully exploit this potential, but it is now confident that significant progress has been made in studying the effectiveness of the use of hydroxyapatite and tricalcium phosphate in these areas of medicine (Fig. 3).

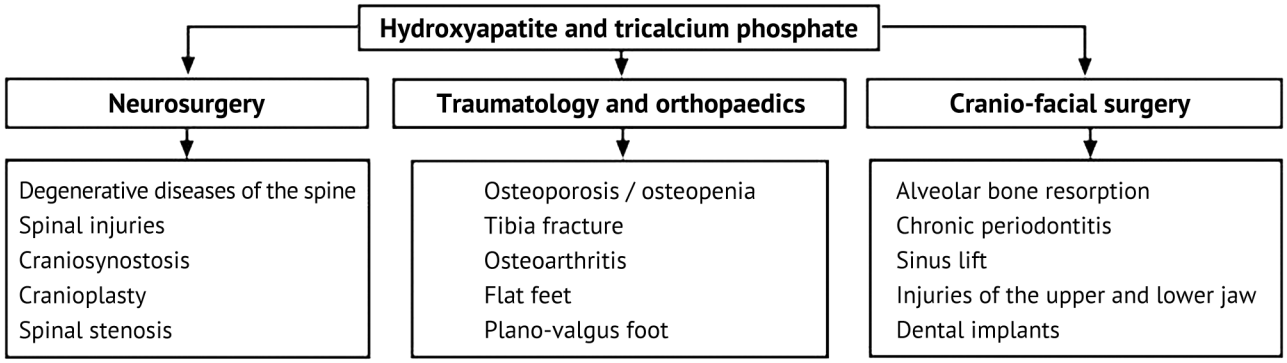

Fig. 2 Most common pathologies that can be treated with hydroxyapatite and tricalcium phosphate as bone substitutes

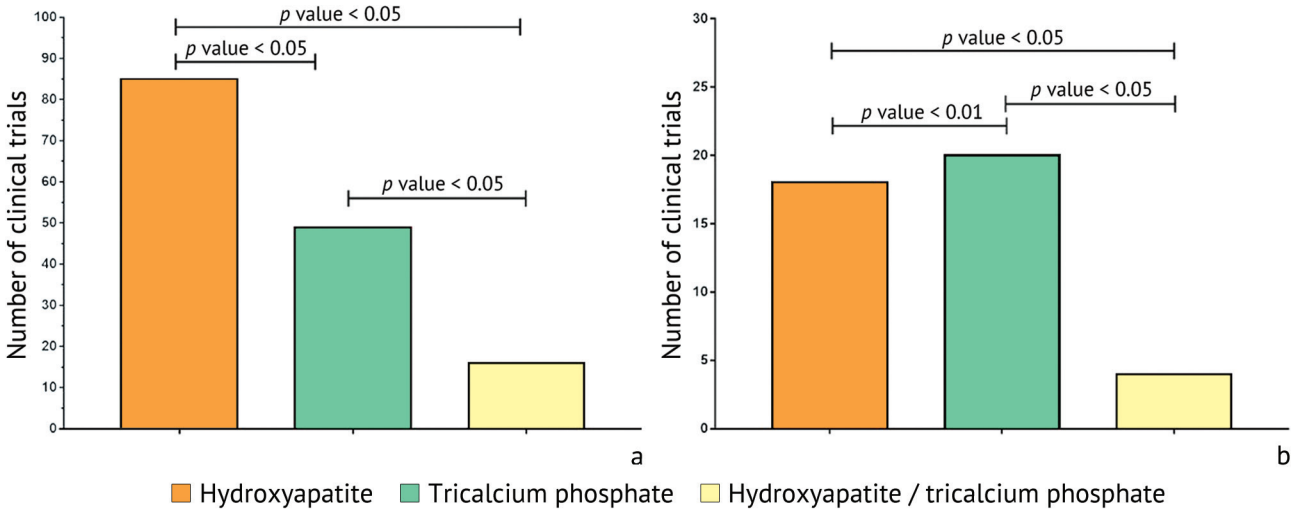

Fig. 3 Comparative analysis of clinical trials on the use of hydroxyapatite, tricalcium phosphate and their combination: *a* hydroxyapatite vs tricalcium phosphate vs hydroxyapatite/tricalcium phosphate in the first phase of this study; 85, 49 and 16 clinical trials (*p value* < 0.05 and *p value* < 0.01); *b* hydroxyapatite vs tricalcium phosphate vs hydroxyapatite/tricalcium phosphate after applying inclusion and exclusion criteria for the first stage of this study; 18, 20 and 4 clinical trials (*p value* < 0.05 and *p value* < 0.01)

At the time of the study (November 2022), 85 clinical trials using hydroxyapatite and 49 using tricalcium phosphate were registered worldwide. Also, 16 clinical trials were conducted with the combination of hydroxyapatite/tricalcium phosphate to study their clinical use in diseases of the musculoskeletal system. The number of registered clinical trials has increased significantly since their first official use that was reported on November 15, 2005, and registration on *clinicaltrials.gov* on September 11, 2006 (Figure 4).

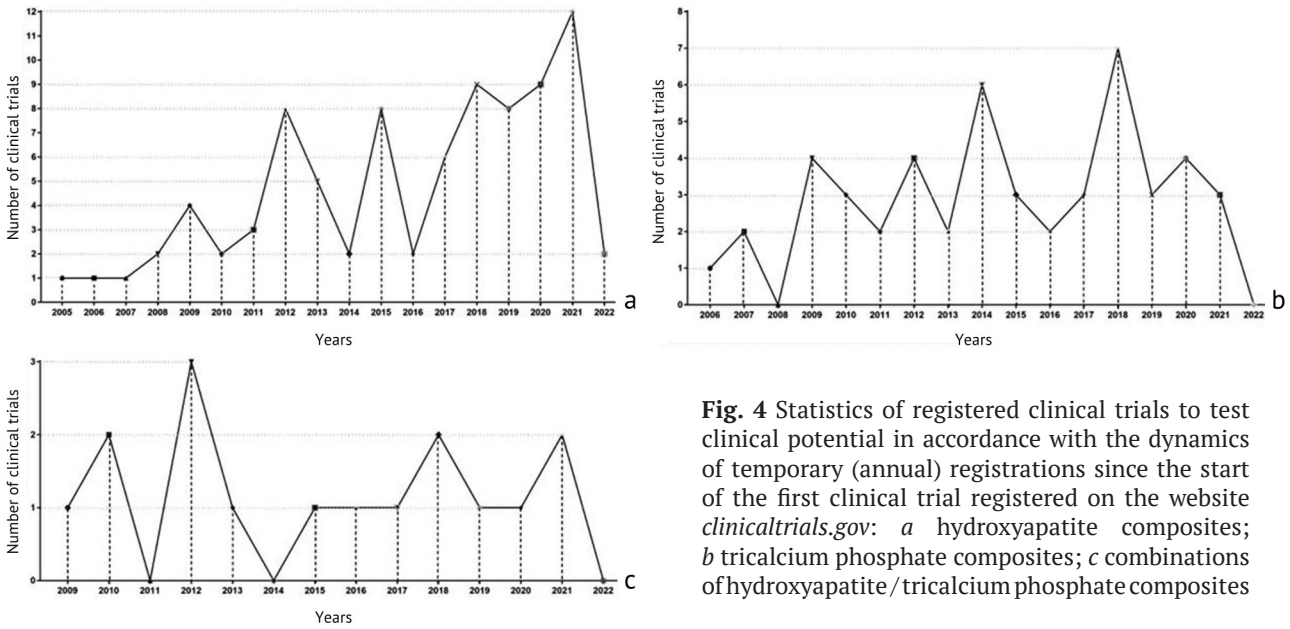

Fig. 4 Statistics of registered clinical trials to test clinical potential in accordance with the dynamics of temporary (annual) registrations since the start of the first clinical trial registered on the website *clinicaltrials.gov*: *a* hydroxyapatite composites; *b* tricalcium phosphate composites; *c* combinations of hydroxyapatite / tricalcium phosphate composites

Features of clinical trials

Based on the inclusion and exclusion criteria, 42 clinical trials that used hydroxyapatite, tricalcium phosphate and their combination were analyzed (18, 20 and 4, respectively). The reasons for exclusion in the first phase of the clinical trial study (Fig. 5) were as follows: 53 (79.1 %), 23 (79.1 %) and 10 (83.3 %) clinical trials had an inapplicable phase; 13 (19.4 %), 6 (20.9 %) and 2 (16.7 %) clinical trials did not specify the phase. There was one clinical trial that used hydroxyapatite in the early phase.

Clinical trials with hydroxyapatite are presented in 6 phases (Fig. 6 a), with tricalcium phosphate in 5 phases (Fig. 6 b), with a combination of hydroxyapatite / tricalcium phosphate – in 2 phases (Fig. 6 c).

However, of this small number of clinical trials, only 12 published full results (3, 7 and 2, respectively).

Reported clinical trial statuses were: have not started enrolling patients; are enrolling patients; active but not yet enrolling patients; discontinued; completed; withdrawn, and unknown status (Fig. 7).

Regarding the status of the 42 clinical trials analyzed, clinical trials with hydroxyapatite were mainly completed (50 % of 18 clinical trials); those using tricalcium phosphate were mostly completed (40 % of 20) and have an unknown status (40 % of 20); those using the hydroxyapatite/tricalcium phosphate combination were mainly completed (75 % of 4 clinical trials) (Fig. 8).

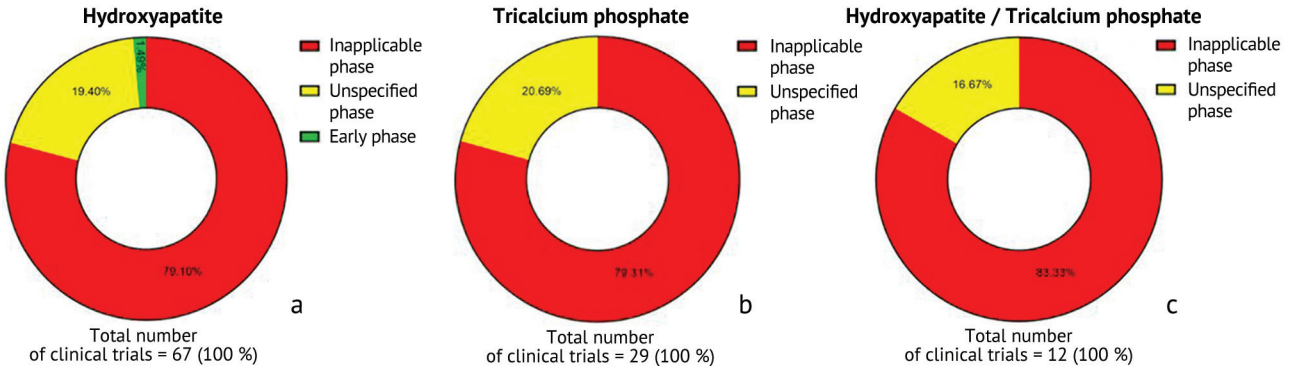

Fig. 5 Reasons for exclusion of clinical trials at the first stage of the study on the use of: *a* hydroxyapatite, *b* tricalcium phosphate; *c* combinations of hydroxyapatite /tricalcium phosphate

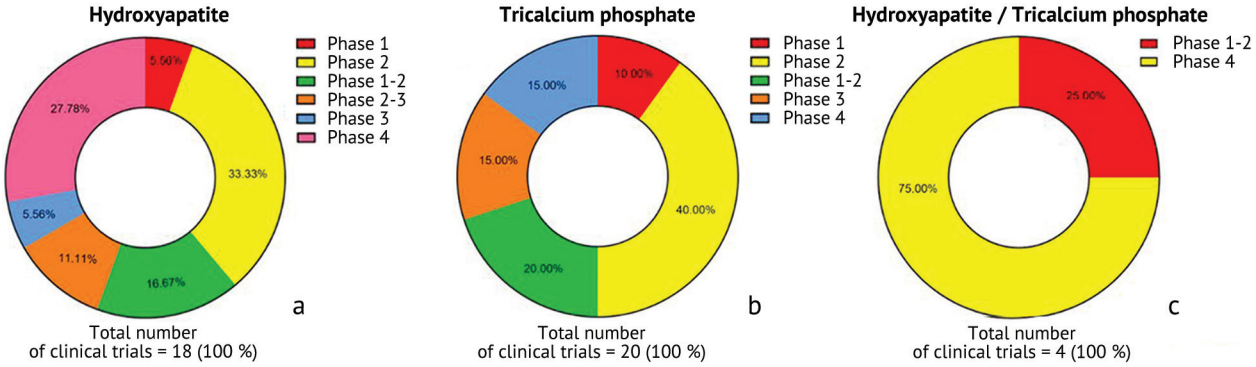

Fig. 6 Distribution by phase of registered clinical trials on application of: *a* hydroxyapatite, *b* tricalcium phosphate; *c* combinations of hydroxyapatite /tricalcium phosphate

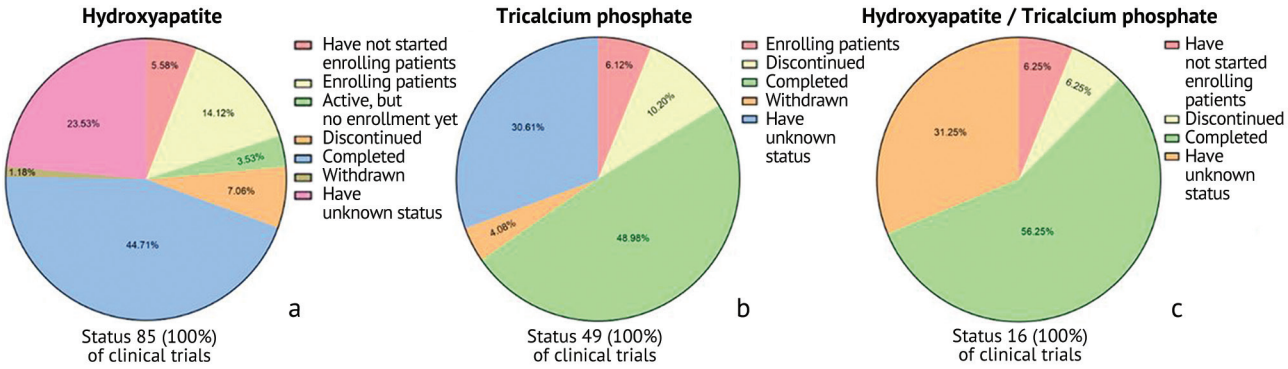

Fig. 7 Distribution based on progress of clinical trials registered in the clinicaltrials.gov database on application of: *a* hydroxyapatite, *b* tricalcium phosphate; *c* combinations of hydroxyapatite /tricalcium phosphate

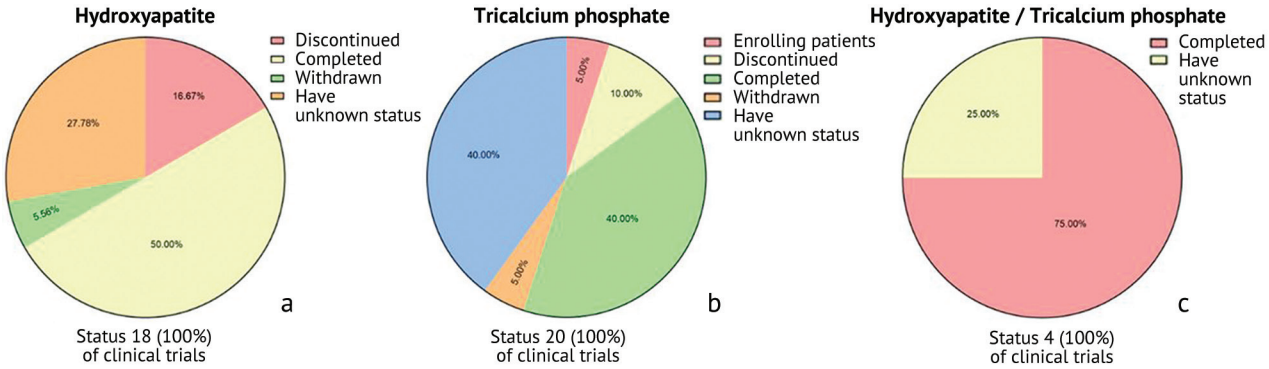

Fig. 8 Distribution depending on the progress of the analyzed clinical trials on application of: *a* hydroxyapatite, *b* tricalcium phosphate; *c* combination of hydroxyapatite / tricalcium phosphate

Geographical distribution

The analyzed 42 clinical trials with hydroxyapatite and tricalcium phosphate have been currently conducted in 19 countries. The USA, Switzerland, Iran and India lead in the number of clinical trials with hydroxyapatite; Spain, USA and Russia lead in the number of trials with tricalcium phosphate. The distribution of the number of clinical trials with the hydroxyapatite/tricalcium phosphate combination is uniform (Table 1).

Table 1

Distribution of clinical trials by regions

Country	Number of trials									
	Total	Hydroxyapatite			Tricalcium phospate			Combination of hydroxyapatite/tricalcium phosphate		
		abs.	%	Phases	abs.	%	Phases	abs.	%	Phases
USA	5	2	11.0	4	3	15.0	1. 3 и 1-2			
Spain	4	1	5.6	1-2	3	15.0	1 и 2			
Austria	3	1	5.6	1-2	1	5.0	1-2	1	25.0	1-2
Egypt	3	1	5.6	2	1	5.0	2	1	25.0	4
Brazil	2	1	5.6	2-3				1	25.0	4
Germany	2	1	5.6	3	1	5.0	2			
India	2	2	11.0	2						
Iran	2	2	11.0	2						
Russia	2				2	10.0	2 и 1-2			
Switzerland	3	2	11.0	2 и 4	1	5.0	4			
South Korea	2	1	5.6	4				1	25.0	4
Belgium	1				1	5.0	4			
Hungary	1				1	5.0	2			
Indonesia	1	1	5.6	1						
Mexico	1				1	5.0	3			
Singapore	1				1	5.0	2			
France	1	1	5.6	1-2						
Sweden	1	1	5.6	2-3						
USA+ Austria + Canada + England	2	1	5.6	4	1	5.0	3			
Unknown	3				3	15.0	2. 1-2. 4			
Total	42	18	100		20	100		4	100	

Conclusions from the techniques/protocols used for clinical application of hydroxyapatite and tricalcium phosphate

The results revealed that hydroxyapatite and tricalcium phosphate are used in a variety of different compositional and structural forms, including stoichiometric, calcium-deficient and ion-substituted, dense and porous, nanocrystalline and microcrystalline, colloidal, granular and monolithic. Each of the forms has specific properties and causes unique biological reactions. Nanostructured and nanocrystalline CaP are traditional materials used in bone tissue bioengineering. Among a dozen different CaP phases, synthetic hydroxyapatite and tricalcium phosphate have been the most commonly used as bone substitutes due to their high similarity to natural bone. Therefore, compared to other CaP materials, the number of clinical studies on the use of hydroxyapatite and tricalcium phosphate is the greatest.

There are protocols for incorporating hydroxyapatite into silk polymers. This type of composite material exhibited a higher cell adhesion and osteogenic differentiation capacity compared to its components used separately. In addition, to facilitate the bond between the scaffold and the host tissue, hydroxyapatite was used not only as a bulk component of the scaffold, but also as a surface coating. This form of hydroxyapatite can inhibit the release of certain compounds or elements limited by the bulk structure of the material. A thin layer of hydroxyapatite coating has shown to be effective in inhibiting the release of inorganic polyphosphate from the surface of osteochondral constructs

composed of calcium polyphosphate, yielding a new form of biphasic constructs. Ion-doped hydroxyapatite particles were also frequently encountered in the protocols. Some clinical trials have involved embedding bone morphogenetic proteins or other growth factors, such as recombinant basic human fibroblast growth factor-β (rhFGF-β), onto hydroxyapatite and tricalcium phosphate scaffolds to induce cell differentiation or other specific effects. CaP cements are a special form of hydroxyapatite or tricalcium phosphate materials because their main ingredients are amorphous CaP or mono-, di-, tri- or tetra-CaP, which can set and harden into hydroxyapatite or tricalcium phosphate only after they are placed in bone cavity.

There is currently no consensus on the ideal phase ratio of BCP for clinical use. Various ratios of hydroxyapatite and tricalcium phosphate in combination have been evaluated in the literature to determine the optimal ratio for bone regeneration, but only hydroxyapatite/tricalcium phosphate ratios of 65/35, 60/40, and 50/50 have been used and have been successfully tested in human clinical trials. Interestingly, two hydroxyapatite/tricalcium phosphate ratios (30/70 and 20/80) also exhibited some osteoinductive properties.

All of the clinical trials were in phase 1-2, phase 2 or phase 4. The clinical trials showed positive results without serious side effects (Table 2).

Table 2

Distribution of the most effective clinical trials (with results) registered on clinicaltrial.gov on the use of hydroxyapatite, tricalcium phosphate and their combination in traumatology and orthopedics, neurosurgery and maxillofacial surgery

Indications	Number of persons in the trial	Intervention	NCT number	Phase	Short description
Hydroxyapatite					
Spinal canal stenosis and spondylolithsesis	69	Transforaminal lumbar interbody fusion	NCT02485574	–	Evaluation of a bone bridge in patients undergoing transforaminal interbody lumbar spine fusion. The option of using autologous bone with hydroxyapatite was not inferior to the option of using only autologous bone.
Hip joint arthropathy	167	BoneMaster Coated Acetabular Sheath and Plasma Coated Acetabular Sheath	NCT00859976	–	Randomized controlled trial of patients requiring total hip replacement. A method of fixing the hip joint to the bone is by applying a coating to the implant to stimulate bone growth in the femur. It was investigated whether Bonemaster (a thin electrochemically applied coating of hydroxyapatite) stimulates bone growth on the cup, compared with plasma spraying
Cleft lip and palate	5	Use of maxillary alveolar graft	NCT01932164	–	Alveolar bone defect reconstruction in patients with cleft lip and palate using mesenchymal stem cells from primary tooth pulp associated with collagen and hydroxyapatite biomaterial (Geistlich Bio-Oss®)
Tricalcium phosphate					
Preservation of the alveolar process. Dental implants	9	Easy-graft CLASSIC (β-tricalcium phosphate)	NCT03215667	–	Clinical and histological evaluation of in situ alloplastic compaction formed by β-tricalcium phosphate and polylactide membrane bone graft in preserving the alveolar bone after extraction of non-molar teeth with unretained extraction sockets
Alveolar bone resorption and periodontal disease	88	β-tricalcium phosphate alone	NCT01728844	–	GUIDOR® growth factor-enhanced bone graft substitute (recombinant basic human fibroblast growth factor-β (rhFGF-β)) in periodontal surgery demonstrated greater gingival and bone regeneration compared to bone graft substitute alone
Periodontal diseases	8	Easy-graft CLASSIC (β-tricalcium phosphate)	NCT02221557	–	Case series to compare the effectiveness of two different treatment approaches: a new alloplastic bone graft material while preserving the alveolar process and existing methods of using allograft

Continuation of Table 2

Distribution of the most effective clinical trials (with results) registered on clinicaltrial.gov on the use of hydroxyapatite, tricalcium phosphate and their combination in traumatology and orthopedics, neurosurgery and maxillofacial surgery

Indications	Number of persons in the trial	Intervention	NCT number	Phase	Short description
Degenerative disease of the lumbar spine	104	Instrumented posterolateral fusion with interbody support	NCT00943384	–	chronOS Strip is a synthetic bone void/defect filler made from β-tricalcium phosphate granules and absorbable polymer [poly(lactide-co-ε-caprolactone)]. chronOS Strip in combination with autologous bone and/or bone marrow or autograft is intended for posterolateral spinal fusion. A prospective multicenter case series to evaluate the rates of posterolateral fusion in a prospective series of patients with degenerative disc disease. chronOS Strip in combination with bone marrow aspirate and local bone was applied to the posterolateral gutters
Dental implants	26	Sinus lift and dental implantation. Aastrom (BRCs)	NCT00980278	Phase 1-2	Determine the ability of your own bone marrow tissue to promote bone regeneration (growth) in the jaw area where the implant was installed. Process Name: Aastrom Bone Repair Cell (BRC) Therapy
Peri-implantitis	5	Easy-graft CLASSIC (β-tricalcium phospate)	NCT03213210	–	Single-arm study to evaluate the effectiveness of a treatment approach using a novel β-tricalcium phosphate and polylactide membrane moldable bone graft for peri-implantitis
Preservation of the alveolar process. Dental implants	45	Easy-graft CLASSIC (β-tricalcium phosphate). Lyophilized FDBA Bone Allograft with Collagen Plug	NCT02702609	–	A randomized controlled trial to compare the effectiveness of two different treatment approaches using a novel β -tricalcium phosphate molded bone graft for alveolar process preservation in an atraumatic extraction socket versus a collagen plug allograft
Hydroxyapatit / tricalcium phosphate					
Periodontal disease	41	Enamel matrix derivative, hydroxyapatite / β-tricalcium phosphate, flap approach operation	NCT02474498	Phase 4	Clinical evaluation of the treatment of class II mandibular furcation defects using enamel matrix derivative (EMD) and/or hydroxyapatite/tricalcium phosphate bone substitute
Intervertebral disc degeneration, intervertebral disc displacement and ossification of the posterior longitudinal ligament	85	CERVIOS chronOS™ and Bonion™	NCT01615328	Phase 4	Cervios ChronOs™ is a polyetheretherketone (PEEK) cage with β-tricalcium phosphate. Bonion™ is a PEEK frame filled with hydroxyapatite/demineralized bone matrix (DBM). However, comparative studies have not been performed between PEEK cage with β-tricalcium phosphate and PEEK cage with hydroxyapatite/DBM. The rate of bone fusion between these cervical spine cages was assessed using postoperative computed tomography (CT)

Note: type of study – interventional

DISCUSSION

Research into the use of CPC-based synthetic biomaterials has made significant progress over the past four decades since they were first used in clinical settings. This study focused on the analysis of the most widely used synthetic ceramic-based biomaterials in clinical practice, namely hydroxyapatite and tricalcium phosphate. The main areas of their clinical application are traumatology and orthopaedics, neurosurgery and maxillofacial surgery. Among the most common pathologies where they are used are injuries/fractures of long bones, osteoprosis/osteopenia, degenerative diseases of the spine and alveolar bone resorption; but there are also a significant number of clinical trials on osteoarthritis, craniopasticity and periodontal defects. The clinical trials break new ground on novel synthetic biomaterial transplantation techniques that offer potential benefits for the future of global public health as they target the field of regenerative medicine and tissue engineering of musculoskeletal diseases.

The growing number of clinical trials with application of hydroxyapatite and tricalcium phosphate represents a transition from preclinical to clinical medicine. Current clinical trials registered on *clinicaltrials.gov* site are primarily in phase 1-2, phase 2 or phase 4 and demonstrate safety and effectiveness. Given the promising results of clinical trials with the use of composite biomaterials based on hydroxyapatite, tricalcium phosphate, or a combination of both, it can be stated that they have great potential to be applied as bone graft substitutes.

Hydroxyapatite is a chemical analogue of biogenic apatite, the main component of all hard tissues of the mammalian body, with the exception of calcium carbonate otoconia of the inner ear. It attracted attention in the early days of biomaterials science due to its expected biocompatibility based on its high level of chemical and crystallographic similarity to the inorganic component of bones and teeth [30]. Bioactivity and biocompatibility of hydroxyapatite give it an advantage over bioinert ceramics such as titanium dioxide, alumina and silica and helps it interact more closely with the cells and tissues of the biological environment [31]. Moreover, many relatively simple synthesis protocols have been established for hydroxyapatite, allowing researchers to produce particles of precisely defined sizes and shapes [32]. Functionalization and surface modification of hydroxyapatite can be accomplished through equally simple non-covalent interactions, taking advantage of the strong physical sorption capacity of the polyvalent ions that make up the surface of the hydroxyapatite particles itself [33, 34]. Currently, hydroxyapatite is also widely known as a drug delivery agent (antibiotics, growth factors, nucleic acids or ligands that facilitate targeted delivery and a specially controlled rate of drug release). In addition to loading with therapeutic agents, hydroxyapatite can also be converted into a bioimaging agent using various functionalization strategies [35]. Being relatively resistant to biodegradation, but with the ability to degrade quickly in certain environments, such as near cancer cells, hydroxyapatite also has the potential to act as a smart biomaterial that responds to environmental stimuli [36]. This ability to achieve multiple functional characteristics allows it to be used for multiple drug delivery, while its location is tracked and controlled by various diagnostic methods (computed tomography), which is one of the prerequisites for theranostics applications.

The main property of tricalcium phosphate is its osteoconductivity, which is one of the main reasons for its use as a biomaterial for bone defect repair. However, only the property of osteoconductivity deprives tricalcium phosphate composite materials of a sufficient degree of bone regeneration and remodeling [37]. Today it is known that the following factors are necessary for new bone growth: bioactive materials with scaffold function; various growth factors that induce cell differentiation; stem cells with the potential to differentiate into bone tissue. Therefore, the bone-restorative effect of tricalcium phosphate can be maximized if we provide it with stem cells and/or growth factors to stimulate osteoblast differentiation. In clinical applications, degradation and mechanical properties are important physical parameters of tricalcium phosphate, which are mainly related to porosity and surface size. Continuous degradation of tricalcium phosphate provides sufficient space for cell growth, while excellent mechanical properties can maintain the space structure for long-term cell growth [38]. However, they limit each other because higher porosity leads to higher degradation and poorer mechanical properties [38]. Therefore, to improve the physical bone repair properties of tricalcium phosphate, it is necessary to combine it with other materials. Thus, the combination of hydroxyapatite and tricalcium phosphate has many advantages [27]. Tricalcium phosphate provides calcium and phosphorus ions, which are useful in differentiating osteoblast maturation.

In fact, hydroxyapatite provides a favorable microporous scaffold environment that promotes osteoblast proliferation and differentiation. Both tricalcium phosphate and hydroxyapatite can promote new bone formation [28, 29]. However, compared to the use of hydroxyapatite or tricalcium phosphate alone, their combination can produce more new bone in a shorter period of time [39]. By adjusting the hydroxyapatite/tricalcium phosphate ratio, a balance can be maintained between ceramic material absorption and new bone formation. In particular, the so-called ReproBone® is a porous resorbable ceramic bone graft substitute (composed of 60 % hydroxyapatite and 40 % β-tricalcium phosphate), similar to the mineral component of the human bone, which has already been clinically tested to create synthetic bone grafts with promising results [40, 41].

CONCLUSION

The results of clinical trials reveal that the combination of hydroxyapatite and tricalcium phosphate has many advantages. Hydroxyapatite and tricalcium phosphate used alone or in combination do not have any serious side effects.

We expect that the number of clinical studies devoted to the use of synthetic biomaterials based on hydroxyapatite and tricalcium phosphate in traumatology and orthopaedics, neurosurgery and maxillofacial surgery would help to more clearly define the potential for their therapeutic use, which can promote bone tissue regeneration and potentially improve the quality of life of patients with bone defects.

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