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On the synthesis and characterization of β-tricalcium phosphate scaffolds coated with collagen or poly (D, L-lactic acid) for alveolar bone augmentation

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ABSTRACT

Objectives: After tooth loss, dimensional alterations on the alveolar bone ridge can occur that can negatively affect the placement of dental implants. The purpose of this study was to evaluate the synthesis, and mechanical properties of β-tricalcium phosphate (β-TCP) scaffolds coated with bioabsorbable polymers, namely, collagen and poly (D, L-lactic acid) (PDLLA). **Materials and Methods:** β-TCP powder was obtained by reactive milling and then characterized by X-ray diffraction and scanning electron microscopy/energy dispersive X-ray spectroscopy (SEM/EDS). β-TCP scaffolds were obtained by replica method, in which polyurethane foams are immersed in β-TCP suspension and thereafter submitted to a thermal treatment to remove the polyurethane and sinter the ceramic. Type-I collagen or PDLLA were used to coat the β-TCP scaffolds by dip-coating method. Scaffolds were separated in four groups depending on the coating material: noncoated (Group A), double immersion in collagen (Group B), double immersion in PDLLA (Group C), and ten immersions in PDLLA (Group D). Samples were characterized by compressive tests and SEM/EDS. Data were statistically analyzed through two-way ANOVA ($p = 0.05$). **Results:** Chemical and microscopic analyses revealed proper morphology and chemical composition of powder particles and scaffolds with or without polymeric coatings. Scaffolds coated with PDLLA showed higher compressive strength (0.11 ± 0.054 MPa) than those of collagen (0.022 ± 0.012 MPa) or noncoated groups (0.024 ± 0.012 MPa). **Conclusions:** The coating method of β-TCP with PDLLA revealed a potential strategy to increase the mechanical strength of porous ceramic materials while collagen can enhance cell migration.

Key words: Bioceramics, bone tissue engineering, dental implants, scaffolds

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INTRODUCTION

After tooth loss, dimensional alterations take place on alveolar bone ridge, resulting in complications for oral rehabilitation supported by dental implants.[1] When the insertion of narrow diameter implants is not possible, bone augmentation procedures previous to implant placement is a viable option.[2] Autogenous bone grafts are classically used due to their potential of bone formation, bio-absorption, and angiogenic characteristics.[3] However, the need of a donor site for bone harvesting, increased surgical morbidity, and high rates of bone remodeling lead to the development of alternative materials for atrophic alveolar ridge treatment.[4]

Synthetic bio-absorbable scaffolds have emerged as an interesting possibility, decreasing the surgical morbidity, and therefore showing low rates of bone remodeling.[5,6] The porous three-dimensional (3D) structure of synthetic scaffolds stimulates growth, migration, and differentiation of human cells for bone reparation.[7] Those scaffolds should follow certain requirements, such as: possessing interconnected pores of proper size (100–800 μm in diameter) to allow the integration and vascularization of bone tissue; high wettability of the surface inducing cell adsorption and proliferation; controlled bio-integration and bio-absorption; adequate compressive strength ranging from 2 up to 10 MPa (a compressive strength comparable to cancellous bone); and industrial viability to be synthesized on specific shapes and sizes.[8]

Calcium phosphate (Ca/P)-based bone substitutes such as hydroxyapatite (HA), and β-tricalcium phosphate (β-TCP) have been safely used in dentistry.[5,6,9,10] Those ceramic scaffolds might have a proper pore size, which mainly depends on the manufacture technique. Several methods have been used to generate porosity in scaffolds, namely, replica foaming, direct gas foaming, freeze casting, model subtraction, and more recently, 3D printing.[9,11,12] Those graft materials have been shown to resorb fully, being replaced by bone over a period from 6 up to 12 months.[13,14] Even though ceramic scaffolds possess excellent biological behavior, their mechanical strength is low, especially because of the brittle porous structure. After sintering, a HA/β-TCP scaffold acquires compressive strength mean values of about 0.05 MPa.[15,16]

A large variety of polymer-based synthetic and natural biomaterials have been studied in the last years revealing good results.[8,9,15,17] The most common examples of polymers are cellulose, collagen, polyvinyl alcohol, poly(ε-caprolactone), poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly(hydroxyl butyrate), poly(propylene fumarate), poly(lactic acid-co-glycolic acid) (PLGA), and poly(D, L-lactic acid) (PDLLA). They can be used as graft materials themselves or in association with metallic or ceramic structures.[8-10,17,18] Considering the degradation of those polymers in nontoxic components, PLA, PGA, and their copolymers have been extensively used in the field of tissue engineering.[8] In addition, those materials can be mixed with fragile structures to improve mechanical properties.[15-17] In addition, antibiotics and growth factors can be embedded into polymeric structure to be released in bone tissue, providing a drug delivery capacity to the scaffold.[9,17]

Nowadays, the field of bone tissue engineering has investigated structures and materials that mimic human tissues properties. Bone is a complex tissue described as a natural composite of 70% HA and 30% collagen, possessing a natural functionally gradated porous structure.[17] Thus, synthetic scaffolds for bone formation should have a similarly porous composite structure with interpenetrating ceramic and polymer phases.[15] Considering application, scaffolds are subjected to mechanical loads since their handling before bone placement up to osseointegration.[17] Thus, the scaffold structure must withstand loading applied by the surgeon during placement into the defect site.[19] In addition, load-bearing capacity is important to maintain bone volume and avoid tissue remodeling after placement of the scaffold. This fact might be highlighted when applying graft materials for periodontal and peri-implant regenerative therapies, once these materials can be under several mechanical stresses originated from occlusal forces.[18]

In fact, bone tissue engineering has experienced many challenges in the pursuit of a functionally scaffold for alveolar bone defects. The mechanical properties of the scaffolds during bone reparation are a clinical concern that can be overcome using different materials. The purpose of this study was to synthesize and evaluate the compressive strength of β-TCP scaffolds coated with bioabsorbable polymers, namely, PDLLA and collagen, for alveolar and peri-implant reparation. In addition, the influence of PDLLA layers on the compressive strength of the scaffolds was assessed in this study. It was hypothesized that bio-absorbable polymers such as PDLLA can increase the compressive strength of β-TCP scaffolds.

MATERIALS AND METHODS

Synthesis and characterization of β-tricalcium phosphate powders

The β-TCP powder was synthesized by reactive milling using stoichiometric proportions as seen in Equation 1:

$$2NH_4H_2PO_4 + 3CaCO_3 \rightarrow Ca_3(PO_4)_2 + NH_3 + 3CO_2 + 3H_2O \quad (1)$$

The dosage of the compounds was performed by gravimetric analysis (Bel, MARK210, Monza, Italy). Each synthesis route consisted of 30 g $CaCO_3$, 22.986 g $NH_4H_2PO_4$, and 500 ml ethanol. Ethanol was selected as solvent due to its inertness to the compounds and easy removal after the synthesis. The mixture was milled in agate milling device, containing alumina milling balls of different sizes, following company's recommendations (U. S. Stoneware, Youngstown, Ohio, USA). After the process, the mixture was compacted into a stainless steel mold covered with filter paper up to full evaporation of ethanol. The dry powder was then roughly milled in an agate mortar (Jung, LF0612, Blumenau, SC, Brazil) and calcined.

X-ray diffractometry was carried out to identify crystalline phases using a Philips X'Pert equipment (PANalytical, Almelo, Netherlands). The radiation source was Cu Kα radiation (λ = 0.15141 nm), with 40 kV and 30 mA. The scan was performed continuously in 2-theta setting from 0°C to 100°C with step size of 0.02°C, and a counting time of 2 s per step. The lattice parameters of the resulting powders were measured by energy-dispersive X-ray diffraction (XDR) (Philips X'pert, Almelo, Netherlands). Particle morphology and presence of agglomerations were analyzed by scanning electron microscopy (SEM) coupled to energy dispersive X-ray spectroscopy (SEM-EDS, JEOL USA JSM-6510 LV, Tokyo, Japan).

Scaffold manufacturing

The scaffolds were manufactured by replica method using 45 pores per inch (ppi) polyurethane foam pattern with 1 cm^3 dimension and a β-TCP slurry. The replica method consists on dipping polymeric foam into ceramic slurry resulting in a porous structure coated with the ceramic material. Balanced rheological properties are required to ensure the coating since the fluid must flow when shear stress is applied, to remove the excess of fluid within the foam. After drying, the assembly was submitted to a thermal treatment to remove the polymeric foam and sinter the ceramic powder. The result of the process is a ceramic replica from the polymeric foam pattern.

In this study, the ceramic slurry was composed of 57% wt H_2O, 43% wt β-TCP, and the following additives: 2% wt carboxymethyl cellulose (1922 EMFAL, Jardim Piemonte, Brazil), 3% wt Poly (vinyl alcohol) (P1763 Sigma Aldrich, St. Louis, USA), and 3% wt citric acid (A1026.01. AH, Synth, Diadema, Brazil). Those components were mixed in the ball milling device (U. S. Stoneware, Youngstown, Ohio, USA), at 300 rpm for 1 h. The rheological behavior of all slurry compositions was evaluated directly after the milling process in a rotational coaxial cylinder (SV2P) using a viscometer (Viscotester VT550, Haake, Germany) at shear rate of 1/s from 0 up to 600 Pa and then from 600 down to 0 Pa at room temperature (25°C).

After immersion of the polymeric foam in the β-TCP slurry, samples were air dried for 24 h prior to thermal treatment. The initial heating rate at 1°C/min was performed to degrade slowly the polymeric foam maintaining the scaffold structure and to avoid the residues of incomplete firing.[20]

β-tricalcium phosphate scaffolds coating with bio-absorbable polymers

Ceramic scaffold samples were divided into four Groups (A, B, C, and D). Samples from Group B were coated with 0.5% Type I porcine collagen (Collagen Research Institute, Kiel, Germany) dissolved in distilled water. Samples from Group C and D were coated with 0.5% PDLLA dissolved in chloroform (Proquímios, Bangu, Brazil). The polymer veneers were deposited on ceramic scaffolds by dip coating method into a solution of 2% of each polymer. After immersion, samples were dried at room temperature before each repetition of the dip coating process. Samples from the Groups B and C were immersed twice while samples from Group D were immersed ten times. Samples of Group A (control group) were not coated.

Total porosity of the scaffolds was measured using gravimetry, according to Equation 2:

$$\text{Porosity } (\%) = (1-\rho_{app}/\rho_{th}) \times 100 \quad (2)$$

Where ρ_{th} is the theoretical density of the material, obtained by combining the molecular weight with unit cell volume, and ρ_{app} is the apparent density, obtained by the weight divided by the geometrical measurement of the volume.

Compressive strength tests

The compressive strength of the scaffolds was obtained by compressive tests at a rate of 1 mm/min, using a load cell with 100 N capacity (DL3000, EMIC, Brazil). The tests were performed at 23°C using a universal testing machine (Instron 8874, MA, USA). Specimens (n = 5 from each group) were positioned on the testing machine base and subjected to axial compressive loading up to fracture. The compressive force curves were recorded using the Trapezium (Shimadzu Corporation, Tokyo, Japan) software and the compressive strength values were calculated using the equation F/w^2, whereas F is the maximum load at fracture and w the cross-section area of the specimen.[21]

Scanning electron microscopy

Scaffolds were dehydrated through a series of graded ethanol (50, 70, 80, 90, and 100%) baths and then embedded in histological methacrylate resin. After, they were cross-sectioned in 100 μm thick slices using a precise cutting machine (EXAKT 300/310 CP, Norderstedt, Germany). Samples on each group were sputter-coated with Ag-Pd and then inspected using SEM (JEOL JSM-6390 LV, USA) coupled to an energy-dispersive X-ray spectroscope EDX (JEOL JSM-6390 LV, USA). Analyses were performed at secondary and back-scattered electron mode at 15 kV. The software Adobe Photoshop (Adobe Systems Software, Ireland) was used to analyze black and white images, with the black representing the pores and the white the bulk material. The porosity percentage was quantified by using ImageJ software (National Institutes of Health, USA).

Statistical analyses

Data were statistically analyzed through two-way ANOVA at a significance level of 95% (P = 0.05) using the Statistical Package for Social Sciences 17.0 software for Windows (IBM SPSS, Chicago, IL, USA). In addition, Tukey's analysis was applied to compare groups.

RESULTS

Chemical, rheological, and microscopic characterization of powders

XDR and SEM images on β-TCP powders as well as rheology of ceramic slurry for scaffolds are shown in Figure 1. As seen in Figure 1a, XRD analyses of the powder confirmed the presence of β-TCP in accordance with the database.[22] SEM analysis of the powder showed nanoscale particles disposed in agglomerates [Figure 1b]. EDS analysis revealed the

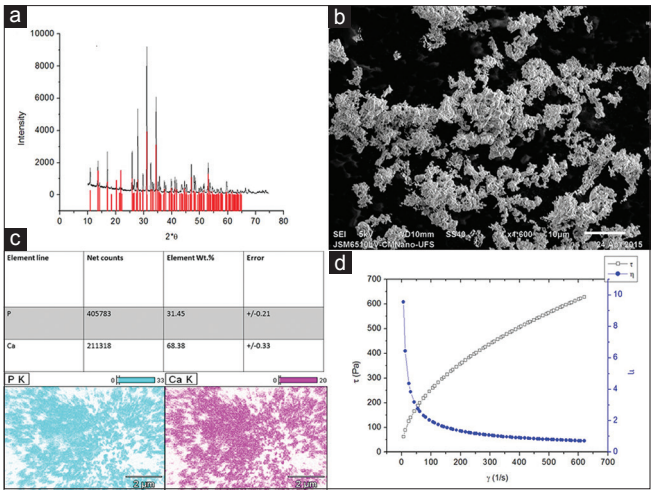

Figure 1: (a) X-ray diffraction patterns of β-tricalcium phosphate. Cu-Kα radiation (1.54056 Å), scanning rate of 1.5° min $^{-1}$, voltage of 40 kV and current of 30 mA. (b) SEM images micrograph of the powder particles obtained by secondary electron mode at 15 kV. (c) EDX map of the β-tricalcium phosphate powder. (d) Rheological behavior of the β-tricalcium phosphate slurry

presence of Ca and P at 1.68 ratio [Figure 1c]. The slurry showed a pseudoplastic behavior, decreasing its viscosity when shear stress was increased [Figure 1d]. Such behavior is desirable on the replica method since the slurry which block the pores can be released during compression of the sample while it can adhere to the struts when the stress is ceased.

Compressive strength

The mean values of compressive strength recorded on the β-TCP scaffolds coated with 10 layers of PDLLA [Figure 2] were significantly higher than those recorded on the other groups (P < 0.05). The highest mean values of compressive strength recorded on scaffolds coated with 10 layers of PDLLA were at 0.11 ± 0.054 MPa. The scaffolds coated with double layer of PDLLA revealed compressive strength mean values of about 0.025 ±0.015 MPa while scaffolds coated with double layer of collagen showed compressive strength mean values at 0.022 ± 0.012 MPa. The noncoated β-TCP scaffolds showed compressive strength mean values at 0.024 ± 0.012 MPa.

Morphological analyses of the scaffolds

SEM images of the scaffolds obtained by backscattered electrons are shown in Figures 3 and 4. The interconnected pore network of the polyurethane template was well replicated with very little blocking of the pores as seen in Figure 3. The sintered ceramic foams maintained the previous geometry and pore shape of the template foam. The spherical macro pore size of previous 45 ppi (~1000 μm) resulted in structures with mean pore size of about 738 μm [Figure 3a].

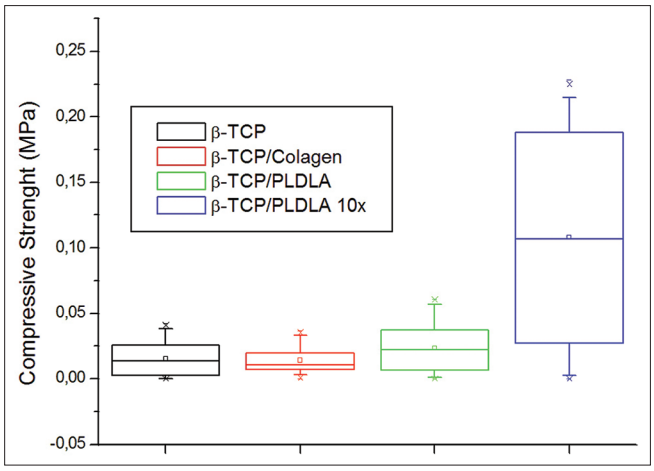

Figure 2: Mean values of compressive strength recorded on β-tricalcium phosphate scaffolds coated with poly (D, L-lactic acid) or collagen

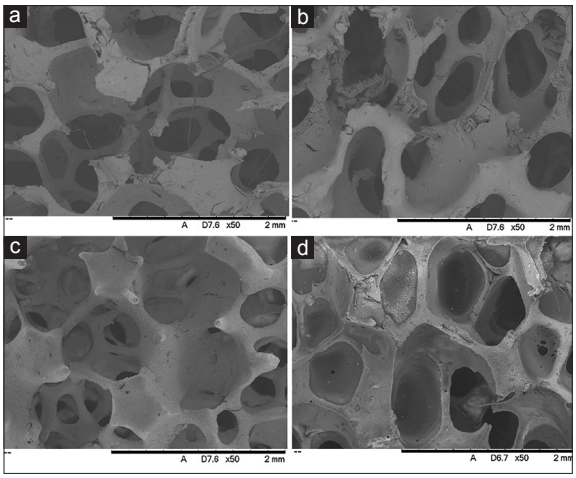

Figure 3: Scanning electron microscope images of the scaffolds. (a) Noncoated β-tricalcium phosphate scaffold. β-tricalcium phosphate scaffold coated with double layer of (b) collagen or (c) poly (D, L-lactic acid). (d) β-tricalcium phosphate scaffold coated with 10 layers of poly (D, L-lactic acid)

However, the scaffolds coated with 10 layers of PDLLA revealed a pore size ranging from 200 up to 400 μm [Figures 3d and 4]. All the porous structures formed a highly interconnected pore network [Figures 3 and 4]. The mean porosity β-TCP scaffolds free of coatings was around 86% while scaffolds coated with 10 layers of PDLLA had a mean porosity of about 44.6%.

DISCUSSION

This study aimed to evaluate the synthesis route as well as the morphology and mechanical strength of β-TCP scaffolds, coated with two different polymers: collagen and PDLLA. The results of the present study reject the null hypothesis. Results showed that β-TCP scaffolds synthesized by the replica method provide proper size and morphology of the interconnected pore network. In addition, the coating of the β-TCP scaffolds with PDLLA (in the Group D) significantly increased the mechanical strength of the porous structures ($p < 0.05$).

In this study, the chemical composition used to synthesize the scaffolds was based on *in vitro* and *in vivo* results concerning biocompatibility and bone reparation.[5,7,8,10,11,13,18] Several ceramic materials composed of Ca/P have been used in bone reparation with predictable results.[5,6,19,23-27] Apatite-like ceramics such as β-TCP are commercially available due to its excellent osteoconductivity and biodegradability. The pursuit of a biodegradable synthetic bone substitute has led clinicians and the scientific community to look on the ceramic scaffolds as a feasible possibility.[5,6,19,23,25,27]

On the β-TCP scaffolds synthesized in this study, the porosity around 86% and pore size at about 738 μm [Figure 3] are proper for angiogenesis, cellular migration, and bone growth, as found in the previous studies.[4-7,9,11,13,19,23,24] Scaffolds coated with 10 layers of PDLLA showed porosity around 44.6% and pore size at about 200–400 μm and therefore revealed an interconnected pore network [Figures 3d and 4]. Depending on manufacture method, scaffolds can display a porosity ranging from 50 up to 80% and a pore size ranging from 150 up to 800 μm.[9-11,13,19,23,24] A network of interconnected micro- and macro-sized pores is critically important to induce bone growth into the structure. The ability of retain blood clot throughout the scaffold provides a pathway where osteogenic cells can migrate (osteoconduction). In fact, scaffolds might have a 3D architecture that mimics natural human trabecular bone.[5-13,15-19,23,24] The trabecular bone left after the degradation of the structure allows functional loading and a dental implant placement.[23]

Although increased porosity and pore size facilitate bone ingrowth, porosity negatively affects the mechanical properties of scaffolds[11] as found in the present study [Figure 2]. A proper mechanical behavior is important since the manipulation during the surgical procedure until loading after implantation in the bone defect.[10,11,17] Thus, scaffolds for bone augmentation procedures must resist to compression from soft tissues as well as occlusal stress from mastication.[17,18,23] In this study, PDLLA was used as coating material for the ceramic structure increasing the compressive strength of the scaffold [Figure 2]. Samples with PDLLA coating (especially Group D) revealed significantly higher compressive strength

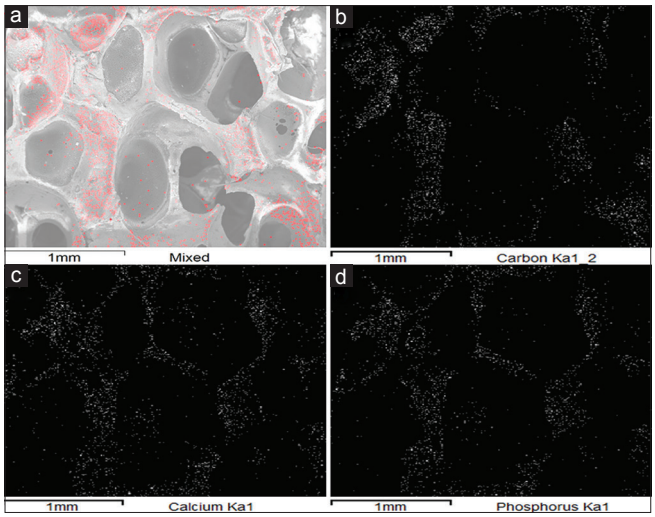

Figure 4: β-tricalcium phosphate scaffold coated with 10 layers of poly (D, L-lactic acid). (a) EDX mapping (red area) indicating (a) the poly (D, L-lactic acid), (b) carbon and (c) calcium and (d) phosphorus elements

when compared to scaffolds free of PDLLA ($p < 0.05$). The significant increase in compressive strength recorded for scaffolds coated with a thick layer of PDLLA (Group D) should be highlighted in our study. That indicates the influence of the polymeric thickness on the compressive strength of the scaffold.

Scaffolds composed of polymer and ceramic phases combine desirable properties of both materials to achieve a synergistic effect on their resultant properties.[5,7,9,13,15-18,23] Indeed, β-TCP-based scaffolds coated with bioabsorbable polymers are a novel strategy to achieve good compressive strength, maintaining high biocompatibility, biodegradability, and osteoconductibility. The interpenetrating structure formed is probably responsible for the strengthening of the scaffold. The infiltration of the polymer in micropores and microcracks forms layered structures that reduce the propagation of cracks on loading.[25] Another mechanism that can provide toughness to scaffolds is the crack bridging, once the polymer can stretch avoiding crack opening.[16,26] Miao *et al*.(2008) stated that the compressive strength of HA/β-TCP scaffold coated with PLGA was higher (0.6 MPa) than that recorded on scaffolds free of polymer (0.05 MPa).[15] Kang *et al*.(2011) also pointed to a significant improvement on mechanical performance of porous β-TCP scaffolds coated with PLGA.[16]

Recently, natural polymers such as collagen have been studied to coat ceramic scaffolds enhancing biological effects for bone augmentation. Alcaide *et al*. (2010) showed that coating HA/agarose scaffolds with Type-I collagen stimulated osteoblasts adhesion and therefore decreased the programmed cell death.[27] In the present study, ceramic scaffolds coated with collagen (Group B) were evaluated concerning mechanical strength. Even though the collagen has not shown any influence on the compressive strength of the scaffolds, it could accelerate the bone reparation considering biointegration, the cell migration, and angiogenesis.[14]

CONCLUSIONS

Within the limitations of this study, it can be concluded that β-TCP scaffolds synthesized by the replica method showed proper morphology and size of interconnected pores to be used as bone substitute. In addition, coating β-TCP scaffolds with biodegradable polymers such as PDLLA is an advantageous method to increase mechanical strength of porous ceramic materials. Despite the fact that collagen coating did not reveal mechanical benefits on the scaffold structure, collagen can enhance cell migration, vascular organization, and consequent bone growth. Further studies should address the synergistic effect of combining synthetic and natural bioabsorbable polymers on cell migration and *in vivo* bone augmentation.

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Conflicts of interest

There are no conflicts of interest.

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