The Effect of Sodium Doping on Calcium Polyphosphate

by

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Abstract

Calcium polyphosphate (CPP) is a suitable substrate in a novel tissue-engineering strategy. The strategy aims to culture articular cartilage in vitro onto porous CPP and then implant the biphasic construct into the joint to replace damaged cartilage. CPP substrates should degrade faster to enhance repair.

This project examined the structural and degradation effects of doping CPP with sodium phosphate, sodium hydroxide, and sodium carbonate. Doping concentration was narrowed to 0.01 Na₂O/CaO for comparable phase composition to pure CPP. All doped groups sintered and crystallized at lower temperatures than pure CPP. Hydroxide-doped CPP did not form adequate sinter necks. At similar open porosity, Phosphate-doped CPP had similar diametral strength than pure CPP, while Carbonate-doped CPP had greater diametral strength. Degradation in vitro showed that Phosphate-doped CPP had greater strength loss, while Carbonate-doped CPP had similar strength loss, compared to pure CPP. Both doped groups degraded more slowly than pure CPP.
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1. Introduction

1.1. Osteoarthritis

1.1.1. Disease and Prevalence

Osteoarthritis (OA), alternatively known as degenerative joint disease, describes a set of degenerative symptoms of the articular cartilage and the underlying subchondral bone[1, 2]. These include the development of focal fissures and irregularities on the smooth, continuous cartilage surface, leading to further erosion and propagation of damage to the subchondral bone[3]. Risk factors for OA include age, developmental defects, trauma and inflammation[4]. As the cartilage tissue is avascular and lacks nerve endings, focal defects generally remain undetected until damage has developed into the subchondral bone and the individual experiences pain and loss of movement in the joint. Early signs of abnormalities may be detected in more athletic populations during arthroscopic examination of other injuries to the joint[5]. It should be noted by Smith et al. that these early defects are often not sufficiently developed to warrant intervention[5].

Although the cartilage tissue has its own repair mechanism, in which chondrocytes rebuild the collagen matrix in the cartilage to a limited degree, this process is much slower than the rate of degeneration. As a result, osteochondral defects accumulate throughout an individual's lifetime, and the individual experiences increasing discomfort and difficulty with movement as the disease advances. A variety of modalities are available[1, 6], and these were appraised by a panel of experts appointed by the Osteoarthritis Research Society International in 2005 [7, 8].
This panel of surgeons and researchers assessed existing guidelines and evidence-based modalities for OA, and condensed these modalities to twenty-five recommendations, categorized as general recommendations, followed by non-pharmacological, pharmacological, and surgical modalities of treatment [7, 8]. The panel agreed that the "optimal treatment of OA requires a combination of non-pharmacological and pharmacological modalities". Non-pharmacological modalities of treatment include patient education, regular telephone contact with health professionals, physical therapy evaluation and instruction, appropriate forms of exercise, weight loss, walking aids and appropriate braces and footwear, thermal modalities, transcutaneous electrical nerve stimulation and acupuncture. Pharmacological modalities recommended include acetaminophen and non-steroidal anti-inflammatory drugs (NSAIDs) as oral analgesics for treatment of mild to moderate pain, with topical NSAIDs and capsaicin as alternatives or substitutions; intra-articular (IA) injections with corticosteroids or hyaluronate for moderate to severe pain; treatment using glucosamine and/or chondroitin sulphate for symptomatic or potentially structure-modifying benefit; and as a last pharmacological treatment resort, weak opioids and narcotic analgesics for treatment of refractory pain. Surgical modalities listed by the OARSI include partial or total joint replacement surgery, unicompartmental knee replacement, osteotomy and joint preserving surgical procedures for young adults, joint lavage, arthroscopic debridement, and as a final surgical treatment beyond joint replacement and revision surgeries, joint fusion was suggested [8]. Each of the listed recommendations was assigned a strength-of-recommendation (SOR) score based on the panel's expertise.

The recommendations listed by OASRI showed clearly, with the exception of surgical interventions, that most other modalities are aimed at OA pain relief. These palliative options are also temporarily effective, as discomfort inevitably increases as the arthritic joint continue to
deteriorate. Further, pharmacological modalities such as NSAIDs are not recommended for long-term use due to increased risk of adverse gastrointestinal events. Samuels et al. and Goldring et al both pointed out that research towards identifying OA disease modifying pharmaceuticals are underway, with the aim of arresting cartilage degeneration or providing aid in cartilage structure preservation [1, 4]. However, this has not become a viable clinical modality.

1.1.2. Current surgical approaches for OA

Surgical recommendations listed by the OASRI have varying degrees of joint preservation. Options such as joint lavage and debridement for knee or hip OA did not attain high SOR scores, and the literature showed that these techniques did not provide statistically significant improvement by known pain scoring matrices [8-10]. In fact, the placebo effect appears to contribute to pain relief as assessed by these matrices [8-11].

Microfracture is also an available option, and has been shown to have positive clinical outcomes in a case-study of patients less than 45 years old, with chondral defects of the knee, over the course of 7 to 17 years of follow-up [12]. Steadman et al. first debrided damaged cartilage from lesions to neatly expose the subchondral bone, and then made holes of approximately 3 to 4 mm deep, spaced also 3 to 4 mm apart, using an awl to produce perpendicular microfracture around the subchondal bone perimeter [12]. While variations for the technique exist [13], the principle of these marrow stimulation techniques take advantage of undifferentiated mesenchymal cells from the bone marrow to repair the subchondral bone and the articular cartilage surface [5, 14]. Compared to the Pridie drilling method, which is also based on the same principle, this has the advantage of avoiding excessive thermal damage to the microfracture sites. However, both methods fall short of the ideal cartilage reparation, as the regenerated fibrocartilage is not suitable for proper joint support, compared to the highly
organized hyaline cartilage. Davies and Jones (2004) also noted that microfracture is typically suitable for smaller defects, of less than 2 cm² [15].

Of the surgical treatment modalities, joint replacement won overwhelming support from the OSARI panel as an "effective and cost-effective intervention with significant symptoms, and/or functional limitations associated with a reduced health-related quality of life."[8] The surgery involves removing the components of the defective joint, and replacing it with implants made of non-biological materials. In hip arthroplasty, an acetabular cup fits into the hip socket, and the femoral head and neck are exercised and replaced with a femoral stem. An estimated 200 000 total hip replacements and 35 000 total knee replacements are performed in the United States, and post-operative assessment predicts that 80-90% of patients will have improvement in joint function, and near complete pain relief [16].

Designs of replacement implants vary in terms of materials (stainless steel, cobalt chrome, titanium alloy, alumina, zirconia) [17, 18], size, extent of femoral head replacement (with the aim of preserving more bone stock), the angle between the implant femoral neck and stem, and tissue-implant contact methods (cement, smooth contact surface, porous coated surfaces, roughened/abraded surfaces) [19, 20]. Although joint function is rapidly restored and pain relief is largely overcome after surgery, total joint replacement has specific shortcomings. Osteolysis occurs in the repaired joint, and was associated with the presence of polyethylene wear debris [21], particularly in metal-on-polymer hip (acetabular cups) or knee (tibial plate inserts) implants [22]. Metallic debris and cement particulates are also identified. In addition, the bone surrounding the implant experiences less mechanical load as a result of the stress-shielding effect from the implant material, and so it does not rebuild sufficiently to fulfill its function [23]. These factors lead to aseptic loosening of the implant, and revision surgery is required after ten to fifteen years. Combined with other factors such as joint instability [24], increased high-activity
levels, and longer average life expectancy, there is increasing demand from both a health and an economic standpoint to improve current designs for joint replacement [25], or to identify strategies that can delay the surgery.

1.1.3. **Cell-based interventions approaches for OA**

Several cell-based surgical strategies are available to repair and restore osteochondral defects, thus returning the tissue, and thus the articulating joint, to its load-bearing function. These strategies vary in terms of level of clinical development, tissue manipulation, and use of synthetic biomaterials. Autologous chondrocyte implantation (ACI) involves several steps. Chondrocytes are isolated and cultured *in vitro* following extraction from the patient's own healthy cartilage tissue. When sufficient cell volume is reached, the chondrocytes are injected to fill the defect cartilage site, which has been cleared of damaged tissue. The defect is enclosed with a periosteum flap. Postsurgical rehabilitation and walking aids are used to provide controlled mechanical stimuli without overloading the recovering joint while cartilage tissue develops [6, 15, 26]. Minas *et al.* suggests that the tissue reaches maturation and stabilization between one to three years after transplant [27]. Peterson *et al.* showed that the procedure is applicable to patients of wider age groups compared to total joint replacements, and can be used for other forms of articular cartilage disorders (i.e., lesions) [28]. Mithöfer *et al.* showed that that sixty percent of adolescent athletes who underwent ACI due to full-thickness articular cartilage lesions in the knee returned to high impact sports at a level equal or higher than their pre-injury levels [29].

Although chondrocytes were injected into the enclosed periosteum-enclosed "pouch", reported recovery showed that a variety of tissue types, including hyaline cartilage, fill the defect. Preoperative and postoperative radiographic examination identified osteophyte growth in fifteen of twenty-seven patients who underwent ACL due to osteochondritis dissecans of the
knee [28]. In another study, the authors identified fibrocartilage by histological examination of biopsies from four out of twelve patients at an average of 54 months post transplantation. The authors did not specify the graft locations for this part of the study, although the study included 61 patients with lesions of various types from various locations on the knee [30]. Despite the positive clinical scores for the treatment, these varying results suggest that the cartilage is not entirely repaired to its "healthy" state. Furthermore, Horas et al. found that recovery from ACI is slower at all of the follow-up time points (6-, 12-, 24-month) compared to another cell-based surgical modality, mosaicplasty (also known as osteochondral autologous transplantation, OATS) [31].

Osteochondral autologous transplantation (OATS) or mosaicplasty, is another cell-based alternative [32, 33]. The procedure involves filling osteochondral defects with "plugs" of bone and developed cartilage, which were harvest from a healthy site in the patient's own articulating joints. The press-fitted plugs are sized to maximize coverage of the defects, but since the defect size for this form of intervention is typically large (1 to 4 cm²) compared to the size of plugs (varying diameters, from 3 to 6mm [33], up to 10mm [34], the resulting transplantation often resembles a mosaic of plugs. In a trial that included mosaicplasty of various parts of the knee, the researchers found that good-to-excellent clinical scores (pain, ease of mobility, activity levels) were achieved in at least 80% of the 831 procedures documented in a ten-year span [32]. In addition, arthroscopic observations showed that the restructured defect formed a good gliding surface, and histology showed that the transplanted hyaline cartilage survived [32]. Other studies showed similar positive results [35].

Typical harvest sites for mosaicplasty grafts for the knee include the minimal load-bearing periphery of the patellofemoral joint, or of the medial femoral condyle. Individual "plugs" were extracted with specialized tools spaced evenly to preserve local joint stability. Donor sites are
either left "unfilled" after the surgery [32, 33], or are filled and covered with periosteum, with either bone grafted from the iliac crest, or bone substituted material [35, 36]. In the ten-year study conducted by Hangody *et al.*, three percent of the patients experienced donor-site disturbances, and fibrocartilage was observed at the "unfilled" donor sites during post-operative arthroscopy [32]. Feczko *et al.* assessed donor site repair using rods made of hydroxyapatite, carbon fibre, polyglyconate-B, compressed collagen, or two types of polycaprolactones, in a dog model [36]. They found that with the exception of compressed collagen rods, repairs using the other bone substitutes resulted in poor articular gliding surfaces, and/or scar tissue formation that was weaker compared to even fibrocartilage when probed. Repairs made using compressed collagen rods results in fibrocartilage formation of similar macroscopic appearance compared to the unfilled, control donor sites at thirty weeks post surgery.

Although the osteochondral defects are effectively filled with hyaline cartilage anchored onto bone, thus recreating much of the biological conditions of the pre-defect joint, there are notable concerns with the procedure. Foremost is the possibility that the donor cartilage tissue is diseased, since it may not be possible to detect the extent of the pathology in the joint. In fact, OATS as practiced by Hangody and collaborators listed osteoarthritis and rheumatoid arthritis as absolute contraindication for the procedure [33]. Secondly, there is a limited supply of "healthy tissue", as there is a limited "minimal" load-bearing tissue source. Furthermore, harvesting can influence subsequent stress distributions at donor sites [37], leading to donor site morbidity. Allografts harvested from cadaver joints may address these concerns, and reports have shown that they are used in large defects or where a large amount of bone stock is desired [38], but in this case additional concerns over disease transmission, tissue viability (dependent upon the tissue stock storage [39]), and graft immunogenicity dominate in the success of the modality. Finally, although the restructured defect surface appears smooth and has similar stiffness to that
of hyaline cartilage, the individual plugs as well as the periphery of the defect are surrounded by fibrocartilage [15, 33]. Histologically, gaps were noted in some cases between adjacent hyaline cartilage, suggesting compromised cartilage integration [31]. These repair heterogeneities remain weak spots in the joint.

1.1.4. Tissue Engineering Approaches

The problems of tissue shortage and donor site morbidity could be avoided by tissue engineering, "the application of scientific principles to the design, construction, modification, growth and maintenance of living tissues" [40]. Cartilage tissues cultured in vitro presents an unlimited supply for repair, and the tissue composition and structure can be manipulated using proper culture conditions. This can also help to avoid the growth of fibrocartilage in the repair tissue, another shortcoming in both ACI and OATS. Defects affecting the cartilage and bone can be repaired using the same principle behind OATS, with tailored plugs consisting of tissue-engineered cartilage grown on an appropriate substrate. These plugs create a biphasic construct, and have been a strategy of much research interest [41-54]. Designs of the biphasic constructs varied depending upon the maturity of the developing cellular component (from mesenchymal stem cells seeding [45, 55] to cartilaginous tissue [51, 52]), the substrate material [56] (P(L)LA, P(DL)LA [53], Collagen and HA [46, 57], PGA and Collagraft [42], PLGA-β-tricalcium phosphate [58], tricalcium phosphate [44, 45]), and tissue-substrate anchorage mode (in vivo merged [55], sutured [42], integrated [46, 52, 59], fibrin glue gel with cell seeding [60]).
1.2. **Substrates for Tissue-Engineered Cartilage**

These strategies illustrate a set of requirements for the substrate component of biphasic implants. They include biocompatibility, bioactivity (towards both cartilage and bone), sufficient mechanical strength to sustain the loading conditions at the defect site, and the capability to be fabricated with an open-pore structure to facilitate subchondral bone in-growth. Furthermore, it should be bioresorbable at a rate that is in concert with the rate of bone in-growth [50, 54].

Polymers, both naturally derived and synthetic, have the advantage of versatility in fabrication and design for specificity, and have been tailored to fulfill some of these criteria [48]. Chen et al. used a PLGA sponge as the backbone structure upon which collagen were deposited to fill up parts of the void, and hydroxyapatite particles were deposited onto the organic surfaces. The collagen further partitioned the voids within the PLGA foam, but neither it nor the hydroxyapatite particles formed a continuous secondary phase [59, 61]. As a class of materials polymers have lower strength compared to that of bone mineral, which makes these substrates less desirable for osteochondral defects. Metals have been widely used as dental and orthopaedic implants. While there is a wide range of designs to tailor the substrate porosity and to enhance osteointegration at the bone-implant interface [20, 62, 63], metals are not suitable as osteochondral biodegradable substrates because of low degradation rates, and limited bioresorbability of wear particles [64].

1.2.1. **Ceramic substrates for tissue-engineered cartilage**

Ceramics such as alumina and zirconia, considered bioinert, have been incorporated into orthopaedic implant designs, owing to their improved wear resistance in load-bearing sites compared to older metal-on-polymer models [18, 56]. Ceramic for osteochondral substrates are typically calcium phosphates, since they are chemically similar to bone mineral, and are bioactive without additional surface modifications [65, 66]. Many calcium phosphates have been
shown to be osteoconductive; that they promote bone growth into the porous substrate [67, 68]. In addition, some calcium phosphates were suggested to have an osteoinductive effect, the ability of promoting bone mineralization when implanted in sites where bone does not develop (e.g., muscle sites) [69, 70]. Of particular interest amongst these ceramics is hydroxyapatite (HA) and \( \beta \)-tricalcium phosphate (\( \beta \)-TCP). Since the former is very close to the composition of bone, and the other has a faster degradation rate, composites of HA/\( \beta \)-TCP, termed BCP (biphasic calcium phosphate), has received much research attention as a bone substitute material [71-74]. Other calcium phosphates have been explored for a variety of different orthopaedic and dental applications, such as Bioglass™ as dental filler, or combined with polymers as osteochondral substrates[75], HA derived from coral, which serve as natural templates to fulfill the trabecular structure requirement, such as thermally converting coral into HA [76].

Scaffold designs consisting of composites of bioactive ceramics and polymers were also investigated to compensate for the stiff ceramics. The structure of these composites vary from a continuous polymer matrix filled with bioactive ceramic particles to two interpenetrating phases, formed by infiltrating sintered ceramic compacts with polymers [77-80]. Kim et al. used polymer-HA particle-antibiotic coating on a calcined porous HA scaffold to produce a drug carrier with greater mechanical strength than the bare HA scaffold [79]. Ghosh et al. used compression moulding to join two layers of different stiffness: a starch-P(L)LA layer (elastic modulus = 23±5 MPa) and an HA particulate/P(L)LA matrix composite layer (elastic modulus = 110 ± 25 MPa). A compacted layer of P(L)LA polymer formed between the two layers during fabrication, and NaCl particles were used to generate an open pore structure [81]. Andriano et al. evaluated in vivo biocompatibility of composites of polymer and phosphate fibres [82]. These composites were synthesized by solvent mixing (methylene chloride) of either crystalline calcium sodium metaphosphate fibres or amorphous sodium calcium aluminum polyphosphate
fibres into either poly(ε-caprolactone/L-lactide) or poly(ortho ester), followed by drying, heating and injection moulding into the desired specimen shapes. The implants were inserted in bone (rabbit lateral femoral cortex) and in muscle (rabbit back) sites, and assessed histologically after sacrifice at 4, 13 and 26 weeks [82]. The authors noted that not all combinations of polymer/fibre ratios were assessed, but as emphasized by Rezwan et al., composite scaffold design strategies should generally have detailed in vitro and in vivo characterization and degradation analyses to determine their effectiveness as scaffolds for osteochondral implants [80].

In this regard, bioactive ceramics hold an advantage since their biocompatibility in vivo is well established, particularly as load-bearing bone substitutes [72, 76]. The literature described in vivo studies of tissue-engineered cartilage-substrate strategies, using either porous interconnected hydroxyapatite [83], or porous calcium polyphosphate (CPP) [52, 84], as substrates in rabbit and sheep models, respectively. Both substrates were shown to be osteoconductive in another rabbit model [67, 85]. A recent study found that under specific culture conditions, a calcified cartilage layer would form between the tissue-engineered cartilage and CPP. This layer is generally observed as part of the anatomy of the native articular cartilage-subchondral bone structure [51], and was hypothesized to stabilize the tissue-subchondral bone interface during transfer of mechanical loads.

This strategy of tissue-engineered cartilage grown on CPP substrates was proposed by Drs, Grynpas, Kandel, and Pilliar at the University of Toronto [86]. The CPP porous substrate was formed by gravity sintering and crystallization of CPP glass particles [87] packed into a platinum mould [88]. Particle size and processing conditions were carefully controlled, as they were found to affect the degradation of the crystalline substrate in vivo [67, 89]. Literature showed that although as much as 50% of the CPP substrate surface formed intimate contact with bone tissue within three months of implant in vivo [52], the in vitro and in vivo degradation of the CPP
substrate was slower than desired for the substrate to fully bioresorb, based on the requirements for osteochondral substrate design [67, 88]. Thus there is a need to tailor the degradation rate of the substrate.
1.3. Calcium polyphosphate (CPP)

Calcium polyphosphate (CPP) has been investigated in the last sixty years for many applications, which include fertilizer [90], water softener [91], detergent [92, 93], and as a vessel for nuclear waste containment ([94], pp. 267-287). More recently, it is being considered for various biomedical applications, including a vehicle for controlled drug release [95, 96], as bone substitute [65], and as described above, as osteochondral substrates for tissue engineering.

1.3.1. Amorphous CPP

Calcium polyphosphate is an inorganic polymer with a monomer structure as shown in Figure 1.3.1, linked via bridging oxygen atoms in the form of –P-O-P– bonds between tetrahedral phosphates[97]. Cations, including hydrogen and calcium, bind to other unbound oxygen atoms, and may be located at various positions relative to the polyphosphate structure (Figure 1.3.2). These positions include the end of chains, or along the chain, where non-bridging oxygen reside. Therefore, calcium atoms may bind to oxygen atoms from two different chains, effectively connecting the two molecules. Hoppe suggests that there is additional orientation between all non-bridging oxygen atoms and the cations [98]. Depending on the proportion of cations and anions in the matrix, expressed as the ratio of cation oxide to phosphorus pentoxide, (M₂O/P₂O₅), the polyphosphate may be linear (1 ≤ M₂O/P₂O₅ < 2), or it may form a cross-linked network in (M₂O/P₂O₅ < 1), termed “ultraphosphate” [99]. The coordination between non-bridging oxygen and the cation accordingly varies, but it is also dependent on the cation (network modifier) properties, of which Hoppe illustrated the effects of valence and covalent character between the modifier and the coordinating oxygen [98]. The CPP glass synthesized for the present study carefully keeps CaO/P₂O₅ at unity, and is quenched from a relatively short melting dwell[94], thus likely arresting the formation of a stable structure [67].
**Figure 1.3.1.** Calcium polyphosphate monomer.
Non-bridging oxygen atoms (red) bind to the available calcium atom (blue). The conformation of the non-bridging oxygen atoms varies.

**Figure 1.3.2.** Schematic of calcium polyphosphate glass.
Non-bridging oxygen atoms (red) bind to available calcium atoms (blue) and hydrogen (not-shown). Cations can be found at the end of chains or along the chain. Conformation of the non-bridging oxygen atoms is randomly positioned to illustrate the metastable material used in this study.
There are two main synthesis methods for amorphous CPP. Aqueous reactions between phosphoric acid and calcium hydroxide, at the desired $\text{M}_2\text{O}/\text{P}_2\text{O}_5$, followed by heating, with [100], or without[101], controlled cooling, and ending with purification by wash and filtration generally produced crystalline CPP. Amorphous calcium polyphosphate, on the other hand, could be produced under more specific $\text{M}_2\text{O}/\text{P}_2\text{O}_5$ ratios ($1 \leq \text{M}_2\text{O}/\text{P}_2\text{O}_5 \leq 2$, [102]) and heating conditions. Another common method is by melt quenching. A melt is typically made from calcium phosphate monobasic monohydrate, $\text{Ca(H}_2\text{PO}_4\cdot\text{H}_2\text{O}$, resulting in condensation and polymerization of the phosphate groups into polyphosphates [65, 96, 103-107]. In studies that manipulate $\text{M}_2\text{O}/\text{P}_2\text{O}_5$, or that aim to include more than one type of cation, salt mixtures would be used as the starting material for thermo-synthesis [108-113]. This melt of polyphosphates is then quenched in water, resulting in an amorphous frit. Further processing produces glass of the desired particle size, which may be used as-made[114], compacted and isostatically pressed[115], compacted and sintered [116], or gravity-sintered [67, 87, 88].

Although there are some differences in the hypotheses of the reaction pathway for polyphosphate formation [94, 117], the underlying agreement is that calcium polyphosphate consist of a phosphate backbone, connected via shared oxygen atoms as $-\text{P-O-P-}$ bonds. Particularly during the melt, at temperatures typically at $1000^\circ\text{C}$ or above [113], the hydrolysis reaction:

$$-\text{[P-O-P-]}_n\text{-O-[P-O-P-]}_m^- + \text{H}_2\text{O}(g) \rightarrow -\text{[P-O-P-]}_n\text{-OH + HO-[P-O-P-]}_m^- [1]$$

competes with the condensation reaction:

$$-\text{[P-O-P-]}_n\text{-OH + HO-[P-O-P-]}_m^- \rightarrow -\text{[P-O-P-]}_n\text{-O-[P-O-P-]}_m^- + \text{H}_2\text{O}(g) [2]$$

The resulting polyphosphate glass chain length was thought to depend upon the time spent at this step [94].
The synthesis route taken also has an impact on the resulting product, as LeGero pointed out, since phosphate synthesis is sensitive to chemical impurities [118]; and for polyphosphates, a small excess of phosphorus could “contaminate” the resulting glass and affect its stability [119]. No literature to date has compared the properties of amorphous CPP synthesized from different routes.

1.3.2. *Crystalline CPP*

The structure of crystalline CPP has been under examination in a number of studies. McIntosh and Jablonski described the transformations of monocalcium phosphate hydrate into different calcium metaphosphate phases by varying the temperature of heat treatment and the treatment atmosphere (air versus steam), and provided XRD data for these phases [120]. β-CPP formed in either atmosphere by heating at 700°C. Heat treatment in air would produce β-CPP at temperatures as low as 500°C, whereas heat treatment in steam at 270°C, followed by heating in air to 340-360°C resulted in γ-CPP, which converted to β-CPP at 700°C. Qiu *et al.* heated amorphous CPP in air for one hour at 1000°C and 800°C and found that the three strongest peaks in the XRD patterns of these samples matched those of γ-CPP and β-CPP, respectively, presented by McIntosh and Jablonski [117, 120], (d-spacings of 3.49, 2.76, 4.76 Å for γ-CPP and 3.74, 3.52, 4.58 Å for β–CPP). Qiu *et al.* [117] then reported that the material they identified as γ-CPP degraded fully by the end of four weeks, as measured by weight loss in tris-buffered solution (pH=7.4), whereas the material identified as β-CPP experienced only a 12% weight loss within the same period. Considering that the synthesis temperature for γ-CPP (1000°C) reported by Qiu *et al.* [117] was above the melting point of CPP as determined by DTA (984°C), and the degradation profiles between γ-CPP and amorphous CPP were similar [117], it is likely that the increased degradation rate for these specimens was due to the presence of an amorphous phase. The story of γ-CPP is even more confusing when Guo *et al.* [107] reportedly converted mixtures
of γ-CPP/β-CPP phases into a pure β-CPP phase by increasing the sintering temperature of CPP from 500 to 900°C, based on calculations done on the XRD patterns of the samples, with standard patterns of two calcium metaphosphate phases as reference [107].

The structure of calcium polyphosphate was presented by Rothammel et al. [121], who named the chemical calcium metaphosphate in the original publication, and Schneider et al. [122], who named the chemical β-calcium polyphosphate. Simulated diffraction patterns of these two structures were identical [123]. These two reports, and another publication on the crystal structure of calcium polyphosphate are summarized in Table 1.3.1. Experimental and calculated powder diffraction patterns of β-CPP and γ-CPP are summarized in Table 1.3.2.

Table 1.3.1 showed that the PDF cards 79-0700 and 77-1953 are identical as they are calculated patterns from the identical structure as presented by Rothammel et al. [121] and Schneider et al. [122], respectively. The PDF #11-0039 includes unit cell parameters that are very close to that reported by Corbridge [124], and also matches the other two PDF cards for CPP. These powder diffraction files, and the structural information as presented in Table 1.3.1, describe β-CPP. A comparison between these patterns with the diffraction pattern for β-CPP as reported by McIntosh and Jablowski [120] (PDF #17-0500) showed that while the position of the major peaks are identical, the intensity of these peaks varied, and weaker peaks are missing in PDF #17-0500 (between 15 and 18 °2θ). Since the McIntosh and Jablowski presented d-spacings and relative intensities for peaks with intensities as low as 5% [120], a possible reason that accounts for the missing peaks could be that the β-CPP structure had preferred orientation.

Two experimental PDF cards exist for γ-CPP, but PDF #17-0498 has been replaced by the more details PDF 50-0584, which included structural information, as shown in Table 1.3.1.
Table 1.3.1.  Reported crystal structures of calcium polyphosphate.
Structural information extracted from source, while collection codes are supplied if the structure was included in the ICSD.

<table>
<thead>
<tr>
<th>ICSD</th>
<th>Source</th>
<th>Name</th>
<th>Chemical formula</th>
<th>Z</th>
<th>Space group</th>
<th>unit cell dimensions</th>
<th>unit cell volume</th>
<th>Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>065653</td>
<td>Rothammel et al. [120]</td>
<td>Calcium metaphosphate</td>
<td>Ca(PO₃)₂</td>
<td>8</td>
<td>Monoclinic, P2₁/a</td>
<td>16.960 x 7.7144 x 6.9963 (β=90.394°)</td>
<td>915.35 Å³</td>
<td>2.874 g/cm³</td>
</tr>
<tr>
<td>060117</td>
<td>Schneider et al. [121]</td>
<td>β-calcium polyphosphate</td>
<td>Ca₂(PO₃)₂</td>
<td>4</td>
<td>Monoclinic, P2₁/c = C₂h</td>
<td>6.999 x 7.717 x 16.944 (β=90.44°)</td>
<td>915.0 Å³</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Corbridge [123]</td>
<td>Calcium metaphosphate</td>
<td>[Ca(PO₃)₂]₆</td>
<td>8</td>
<td>Monoclinic, P2₁/a</td>
<td>16.95 x 7.66 x 7.04 (β=90°)</td>
<td>N/A</td>
<td>2.87 g/cm³</td>
</tr>
<tr>
<td></td>
<td>Worzala, et al.</td>
<td>Calcium phosphate</td>
<td>γ-Ca(PO₃)₂</td>
<td>8</td>
<td>Cc</td>
<td>9.5649 x 9.50140 x 10.36940 (β=93.47°)</td>
<td>940.64 Å³</td>
<td>2.797 g/cm³</td>
</tr>
</tbody>
</table>

Table 1.3.2.  Reported powder diffraction files of calcium polyphosphate.

<table>
<thead>
<tr>
<th>PDF</th>
<th>Type</th>
<th>Name</th>
<th>Structure</th>
<th>Literature appearances</th>
</tr>
</thead>
<tbody>
<tr>
<td>79-0700</td>
<td>Calculated</td>
<td>Calcium phosphate, Ca(PO₃)₂</td>
<td>Rothammel et al. [120]</td>
<td>Guo et al. [106]</td>
</tr>
<tr>
<td>77-1953</td>
<td>Calculated</td>
<td>Calcium phosphate, Ca(PO₃)₂</td>
<td>Schneider et al. [121]</td>
<td>Guo et al. [106], Ding et al. [124], Ding et al. [125], Chen et al. [126]</td>
</tr>
<tr>
<td>11-0039</td>
<td>Experimental, de Wolff et al. (ICDD)</td>
<td>Calcium phosphate, β-(Ca₅P₆O₁₈)</td>
<td>Similar to Corbridge [123] (unit cell only)</td>
<td>Omelon [89]</td>
</tr>
<tr>
<td>17-0500</td>
<td>Experimental [119]</td>
<td>Calcium phosphate, β-Ca(PO₃)₂</td>
<td>n/a</td>
<td>Omelon et al. [127]</td>
</tr>
<tr>
<td>17-0498</td>
<td>Experimental [119] (replaced by 50-0584)</td>
<td>Calcium phosphate, γ-Ca(PO₃)₂</td>
<td>n/a</td>
<td>Guo et al. [106]</td>
</tr>
<tr>
<td>50-0584</td>
<td>Experimental, Worzala et al. (ICDD)</td>
<td>Calcium phosphate, γ-Ca(PO₃)₂</td>
<td>Provided within PDF card, as presented in Table 1.3.1.</td>
<td>Chen et al. [126]</td>
</tr>
</tbody>
</table>
The x-ray diffraction pattern obtained from crystalline CPP synthesized using the method of Pilliar et al. [87, 88] matched the simulated diffraction patterns based on the structures of Rothammel et al. [121] and Schneider et al. [122], and is thus reported as β-CPP [111]. Degradation in vitro of this substrate material using tris-buffered solution (pH= 7.4) showed that the sample released 0.5% of the total available phosphorus into the solution at the end of one month.

1.3.3. Effects of CPP structure on degradation

Many different studies have reported on the effects of amorphous or crystalline CPP structure on its degradation and mechanical properties. Qiu et al. assessed the degradation rate and the mechanical strength of CPP glass made by calcining Ca(H2PO4)2•H2O at 500°C for one, five and ten hours before melting at 1100°C for one hour [117, 125]. The authors reported that the in vitro degradation rate of glass particles increased as calcining time decreased. However the authors did not fully describe the degradation behavior of crystallized samples made from these glass groups, nor did they analyze the polyphosphate glass structure. Ropp advocated increasing the length of the melting step for a chemically stable glass[94]. In an study aimed at producing a stable glass product, Ropp increased the melt hold time of CPP glass, held at 1200°C, to 144 hours. He found that increased melt hold time resulted in an increase in the glass transition temperature of CPP, and the resulting glass was nearly unaffected by boiling water degradation test. While this is not the aim for the design for a bioresorbable substrate for tissue-engineering, it nevertheless suggests that reducing the melt hold time may result in a less chemically stable product.

Wang et al. reported on the effect of phase composition of CPP on degradation and cell viability [104]. CPP was calcined at 500°C for ten hours, melted at 1200°C for 1.5 hours, quenched and milled. The CPP glass was then mixed with stearic acid as a pore-forming agent,
pressed, heated for removal of pore-forming phase, and then sintered at 550°C, 650°C and 750°C, respectively. XRD of the sintered bodies showed that at 550°C, CPP (550-CPP) remained amorphous, while at 650°C and 750°C, CPP (650-CPP; 750-CPP) crystallized and the resulting pattern matched that of β-CPP (PDF #11-0039). Degradation of the specimens in SBF showed that by the end of one month, weight loss of 550-CPP reached 80%, while that of 650-CPP and 750-CPP were approximately 8% and 5%, respectively. Accordingly, compressive strength of 550-CPP under degradation was lower than that of the other two groups. Cell culture on the three groups showed that after six days, higher cell density was observed on 650-CPP and 750-CPP. At each of the earlier time points (two and four days), higher cell volume was found at the other two substrate groups as well. The authors noted that this could be because the amorphous 550-CPP degraded more quickly than either of the crystalline groups (20% weight loss after six days in SBF, compared to less than 2% for the other two groups). In addition, Omelon[89] showed that a partially crystalline material degrades faster than highly crystalline CPP. This suggested that an amorphous structure, while favourable towards degradation, would not be suitable as a substrate for osteochondral tissue engineering.

A number of publications have reported the effects of manipulating the chemical composition of phosphate glass on various mechanical and chemical degradation properties. Knowles summarized the degradation effects of varying the composition of cation and phosphorous content in the Na$_2$O-CaO-P$_2$O$_5$, K$_2$O-CaO-P$_2$O$_5$ systems[126]. Both cation and anion contents were manipulated, and at fixed P$_2$O$_5$ (45 mol%), an increase in Na$_2$O (from 15 mol% to 37 mol%, the balance being CaO) was associated with an increase in the dissolution rate in terms of substrate mass loss and sodium ion released [126]. A similar effect was achieved by increasing the concentration of K$_2$O in the K$_2$O-CaO-P$_2$O$_5$ system.
Ahmed et al. further investigated this system at 45, 50 and 55 mol% P$_2$O$_5$, each with 30, 35, or 40 mol% CaO, and the balance being Na$_2$O, to determine the glass transformation temperatures, glass degradation rates, and phase composition upon annealing of the bulk material [113]. NaH$_2$PO$_4$, CaCO$_3$, P$_2$O$_5$ were used as precursors, and upon melting for one hour, between 1000ºC to 1050ºC, the melts were poured into a preheated (350ºC) graphite mould and slowly cooled in a furnace [113]. Differential thermal analysis of the ground glass clearly showed more than one crystallization peak, and/or more than one melting peak, for all three CaO compositions at 45 mol% P$_2$O$_5$, at 30 mol% and 40 mol% CaO when phosphate content was 50 mol%, and at 35 mol% and 40 mol% CaO when the phosphate content was 55 mol% [113]. XRD of the crystallized groups showed that all but one group possessed one or more phases of sodium calcium phosphate [113]. The authors reported that the major phase of the exception, (Na$_2$O)$_{0.05}$-(CaO)$_{0.40}$-(P$_2$O$_5$)$_{0.55}$, matched PDF #11-0039, that of β-CPP, but did not indicate whether the diffraction pattern contained unidentified peaks. Glass degradation was lowest for all three CaO concentrations at this P$_2$O$_5$ content. Expressed as glass solubility, in terms of weight loss per initial specimen area, the authors reported approximately 0.05 to 0.06 mg/cm$^2$ of material was lost at the end of nearly 200 hours of immersion in water at 37ºC. Solubility for other glass groups reached as high as 0.55 mg/cm$^2$, for (Na$_2$O)$_{0.20}$-(CaO)$_{0.30}$-(P$_2$O$_5$)$_{0.50}$. Glass groups with higher CaO concentration had lower solubility, for all three P$_2$O$_5$ compositions[113]. The same observations about degradation were made by the authors when they assessed glass fibre samples of these compositions[127]; however, they pointed out that it was impossible to produce fibres with 45 mol% P$_2$O$_5$, owing to the low viscosity of the melt. This could be due to a lack of phosphate network formers, as the (Na$_2$O + CaO)/P$_2$O$_5$ ratio in this instance is greater than one, exceeding the metaphosphate range as suggested by van Wazer [99]. Degradation rates of the crystallized material were not investigated in these studies.
Chun et al.[128] combined NaH$_2$PO$_4$•2H$_2$O with Ca(H$_2$PO$_4$)$_2$•H$_2$O to synthesize sodium-incorporated calcium metaphosphate, and assessed the degradation of the amorphous and crystalline materials in tris-buffered saline and in simulated body fluids (SBF). The ratio (CaO + Na$_2$O)/P$_2$O$_5$ was kept at one (metaphosphate region), and two levels of Na$_2$O were assessed (5 mol% Na$_2$O, and 10 mol% Na$_2$O, respectively). Although the authors did not analyze the chemical nor the phase composition of the starting materials, they found that the amorphous materials degraded faster than the crystalline materials. Comparing the crystallized groups, calcium metaphosphate (CMP) with 10 mol% Na$_2$O lost 10% of its initial weight after 21 days in tris-buffered saline, compared to no weight changes in pure CMP, despite measurable (up to 10% weight loss) at earlier time points. This “mass gain” during degradation was also observed in both groups when SBF was used as the solvent, such that pure CMP experienced no weight loss at the end of the degradation, but 10% weight loss was measured at day 3, 5% weight loss at day 7, whereas CMP with 10 mol% Na$_2$O also measured 10% weight loss at day 3, no weight loss at day 7, and 4% weight loss at day 21. Since the authors also observed precipitation on the degrading specimens, it was possible that the weight changes were underestimated values of actual material degradation. Yoon et al.[112] reported, in the crystallized form, pure CPP, and CPP with 5% Na$_2$O, experienced no more than 5% weight loss at any time points (1, 7, 14, 21 days) in SBF. Since the authors did not describe in detail their synthesis method, nor did they characterize the materials, it is difficult to put this data in context.

In another study, Chun et al.[116] assessed the degradation of mixed phase NaCa(PO$_3$)$_3$-CMP and KCa(PO$_3$)$_3$-CMP. NaH$_2$PO$_4$•2H$_2$O and KH$_2$PO$_4$ were mixed with Ca(H$_2$PO$_4$)$_2$•H$_2$O and calcined at 600°C and 650°C, respectively for twelve hours. The powders were then milled, pressed, and then sintered in air for three hours at 730°C (NaCa(PO$_3$)$_3$-CMP) and 800°C (KCa(PO$_3$)$_3$-CMP). For each monovalent salt, 5, 10, 15 and 20 mol% was incorporated, and were
measured as mol% of salt (NaCa(PO3)3 or KCa(PO3)3) in CMP. The authors found that whereas weight changes for NaCa(PO3)3-CMP fluctuated between a gain of 0.120 % (10 mol% NaCa(PO3)3-CMP) and a loss of 0.027% (20 mol% NaCa(PO3)3-CMP) for these four levels at the end of 21 days in revised-SBF, increased potassium content was associated with increased weight loss within the same period, from 0.747% (5 mol% KCa(PO3)3-CMP), to 12.16% (20 mol% KCa(PO3)3-CMP). The authors observed precipitation on NaCa(PO3)3-CMP specimens, and pore enlargement on KCa(PO3)3-CMP specimens over the course of degradation. They suggested that the difference in degradation rates between NaCa(PO3)3-CMP and KCa(PO3)3-CMP was due to preferential degradation of KCa(PO3)3, since diffraction patterns of degraded samples of the latter showed a reduction in relative intensity of peaks associated with KCa(PO3)3 over the course of degradation. The authors also reported that although the flexural strength of NaCa(PO3)3-CMP was lower than that of KCa(PO3)3-CMP before degradation (7.7 MPa vs. 16 MPa), it increased during degradation, while that of KCa(PO3)3-CMP decreased. At the end of two weeks, flexural strength of the former was 13.2 MPa, and that of the latter was 5 MPa. Unfortunately, the authors did not attempt to resolve how the monovalent cations affected the calcium polyphosphate phase in these mixed phase groups. Further, as the authors chose a different synthesis method, one without a high temperature melt, it was difficult to assess whether the mixed phases occurred via precipitation, as a result of the solubility limit of the monovalent cation into calcium polyphosphate, or from the initial phosphate mixture.

To assess the degradation effects of doping trace amounts of cations into calcium polyphosphate, Song et al.[129] incorporated small amounts of sodium carbonate, potassium carbonate, zinc carbonate, magnesium carbonate, or strontium carbonate into calcium polyphosphate. The materials were synthesized by mixing each dopant with calcium carbonate and phosphoric acid in water, dried, then calcined at 500ºC for ten hours and melted at 1200ºC
for one hour and quenched in ice. The glass frits were milled, then packed in a cylindrical mould, pressed, and sintered at 800°C for one hour. Crystallized calcium polyphosphate was found to match the β-CPP structure (PDF #17-1953) as described above, and lattice parameters for each doped group were calculated. Degradation of the specimens in SBF over 28 days showed that potassium-doped CPP degraded most (0.14%), followed by magnesium-doped CPP, sodium-doped CPP, zinc-doped CPP, pure CPP (approximately 0.025%), and finally strontium-doped CPP was most stable (approximately 0.01% weight loss over 28 days). The authors noted that these values were underestimated by the presence of variable amounts of precipitation on the specimen surface, which also affected measurements of soluble phosphate in SBF. The initial spike in degradation observed by Chun et al. [128] was noted in this study only in potassium-doped, sodium-doped and magnesium-doped CPP. This could be attributed to a difference in doping concentration. The authors were ambiguous in describing the concentration of the dopants incorporated in this study. It was further difficult to discern from the available results since XPS was used to ascertain the presence of each dopant, but the plots showed very faint signals largely masked by background noise. Since no other analyses for dopant concentration was present, it was difficult to ensure that the results presented were due to cation doping.

Underlying these studies is a keen interest in introducing impurities to effect CPP degradation. However, it is unclear whether the results reported thus far represent dopant effects on degradation of crystalline CPP, or that they are mixed-phase effects, or both. In its capacity as an osteochondral substrate, mixed-phase effects may compromise the substrate’s ability in tissue support, particularly if one phase degrades faster than the other(s). Moreover, these papers did not differentiate between cation from anion effects. Since this study focuses on sodium doping on CPP, it is therefore necessary to consider the solubility limit of sodium, in its network modifier form, Na₂O, in the CaO-P₂O₅ phase diagram, when CaO/P₂O₅ = 1.
1.3.4. \( \text{Na}_2\text{O}-\text{CaO}-\text{P}_2\text{O}_5 \text{ phase diagram} \)

Three phase diagrams of the \( \text{Na}_2\text{O}-\text{CaO}-\text{P}_2\text{O}_5 \) have been published. Morey[93] first described the binary system \( \text{NaPO}_3-\text{Ca(PO}_3)_2 \) in 1952 (Figure 1.3.3 (left)). However, since the focus of the paper was to identify calcium-sequestering ability of sodium polyphosphates in hard water, the compositions investigated leaned towards high concentrations of \( \text{NaPO}_3 \). Further details were added by van Wazer (Figure 1.3.3 (right), [130]), which provided more details on the opposite end of the phase diagram, and composition unit conversions. Griffith produced a different version of the phase diagram based on his work with phosphate glass fibres (Figure 1.3.4, [131]).

![Figure 1.3.3. Phase diagram of \( \text{Na}_2\text{O} \cdot \text{P}_2\text{O}_5 \) and \( \text{CaO} \cdot \text{P}_2\text{O}_5 \). Phase diagram by from Morey [93] (left), weight percent of \( \text{CaO} \cdot \text{P}_2\text{O}_5 \) is shown on the x-axis. Modified phase diagram by van Wazer [130], (right).](image-url)
None of the phase diagrams show a terminal solid solution of NaO•P₂O₅ in CaO•P₂O₅, which suggests that the solubility of sodium in CaO•P₂O₅ is either low, or that it is below the lowest sodium-containing composition investigated. In Morey’s phase diagram this was at 11.2 wt% NaO•P₂O₅ (approximately 0.11 Na₂O/CaO); the lower limits of the other two phase diagrams are unknown. It is interesting to note that, while van Wazer pointed out that the formula Na₄Ca(PO₃)₆ was empirical, Ahmed et al. [113] reported that XRD of their crystallized sodium calcium phosphate matched powder diffraction patterns of this name, although the material had a slightly higher M₂O/P₂O₅ ratio ( (Na₂O+CaO)/P₂O₅ =1.22). Based on their results, at M₂O/P₂O₅ = 1, one would expect to find NaCa(PO₃)₃, which only appeared in Griffith’s phase diagram (Figure 1.3.4). All these inconsistencies suggest that the information on the phase diagrams may be incomplete.
Figure 1.3.4. Phase diagram of NaPO$_3$ – Ca(PO$_3$)$_2$.
Phase diagram by Griffith [131]. Proportion of calcium polyphosphate is shown in mol % basis on the x-axis. Typographical errors are: [NaCa(PO$_3$)$_2$] should be [NaCa(PO$_3$)$_3$] between 70% and 100% [Ca(PO$_3$)$_2$]$_n$, and NaPO$_3$ should read Na$_4$Ca(PO$_3$)$_6$. 
1.4. Objectives

The overall goal of the project is to determine the effects of sodium doping on crystalline calcium polyphosphate (CPP), particularly in CPP’s role as an osteochondral tissue-engineered cartilage substrate. As stated previously, this type of substrates must possess the following qualities: biocompatibility, bioactivity (towards both cartilage and bone), sufficient mechanical strength to sustain the loading conditions at the defect site, and the capability to be fabricated with an open-pore structure to facilitate subchondral bone in-growth. Furthermore, it should be bioresorbable at a rate that is in concert with the rate of bone in-growth [50, 54]. Since CPP already possesses many of these properties [52, 67], specific objectives are to determine how sodium doping affects the mechanical properties and the degradation rate in vitro of CPP.

There are three parts to this project, listed as follows:

- To synthesize vitreous sodium-doped CPP and upon sintering, to characterize the porous sintered, crystalline substrates;

- To determine the mechanical strength loss of porous crystalline sodium-doped CPP substrates under in vitro degradation;

- To determine the in vitro chemical degradation rate of porous crystalline sodium-doped CPP substrates

This study will use three dopants, Na₂HPO₄, NaOH, and Na₂CO₃. It is important for sodium-doped CPP to have the same crystal structure as that of pure CPP, so to isolate the effects of doping. The as-made sodium-doped CPP substrates should have the same open porosity, as well as equal if not greater diametral strength, compared to pure CPP substrates. It would be desirable for sodium-doped CPP to have a faster in vitro degradation rate.
2. Methods

2.1. Pure and Sodium-doped CPP synthesis

Pure CPP glass was produced by calcining crystalline Ca(H₂PO₄)₂•H₂O (J.T. Baker, ACS grade, for composition, see Table 2.1.1 and ) at 500°C for ten hours, followed by melting at 1100°C for one hour. The melt was quenched in distilled, deionized water, and washed with anhydrous ethanol to remove excess water. The glass frit was dried in an oven (Fisherband Isotherm 600 Series) at 60°C, and then milled using an automatic grinder (Retsch RM100) equipped with agate mortar and pestle. Particle sieving using standard sieves (Fisherbrand Standard Test Sieves) and a sieve shaker (Endecotts Octagon digital CE) produced glass powders of size range 75µm to 106µm [88].

Table 2.1.1. Composition of Ca(H₂PO₄)₂•H₂O supplied by manufacturer.
Data was supplied by manufacturer (Baker Analyzed ™, ACS grade, VWR).

<table>
<thead>
<tr>
<th>Component</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay</td>
<td>99-105%</td>
</tr>
<tr>
<td>Insoluble in HCl</td>
<td>Max. 0.01%</td>
</tr>
<tr>
<td>Chloride</td>
<td>Max. 0.002%</td>
</tr>
<tr>
<td>Sulphate</td>
<td>Max. 0.01%</td>
</tr>
<tr>
<td>Ammonium</td>
<td>Max. 0.01%</td>
</tr>
<tr>
<td>Heavy metals (as Pb)</td>
<td>Max. 0.002%</td>
</tr>
<tr>
<td>Iron (Fe)</td>
<td>Max. 0.002%</td>
</tr>
<tr>
<td>Trace impurities</td>
<td>Arsenic (As ) max. 1 ppm</td>
</tr>
</tbody>
</table>

Table 2.1.2. Chemical composition of Ca(H₂PO₄)₂•H₂O
Results collected from four samples analyzed by neutron activation analysis..

<table>
<thead>
<tr>
<th>Component</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>15.85%</td>
</tr>
<tr>
<td>P</td>
<td>21.08%</td>
</tr>
<tr>
<td>Mg</td>
<td>250 ppm</td>
</tr>
<tr>
<td>Al</td>
<td>123 ppm</td>
</tr>
<tr>
<td>Sr</td>
<td>82 ppm</td>
</tr>
<tr>
<td>Na</td>
<td>44 ppm</td>
</tr>
<tr>
<td>Cl</td>
<td>36 ppm</td>
</tr>
</tbody>
</table>
Sn 32 ppm
Ti 18 ppm
Cu 6.5 ppm
Br 3.2 ppm
I 1.6 ppm
Mn 0.47 ppm
V 0.1 ppm

NaOH, Na₂HPO₄, and Na₂CO₃ were used to make sodium-doped CPP (Fisher Scientific, ACS grade for all but Na₂CO₃, which was HPLC grade, see Table 2.1.3).

Aqueous solutions of each dopant were mixed with crystalline Ca(H₂PO₄)₂•H₂O (J.T. Baker, ACS grade) to produce a slurry, and the slurry was dried at 150°C to 200°C for one hour to remove excess moisture. The viscous mixture then followed the same procedure as pure CPP synthesis as mentioned above.

Table 2.1.3. Composition of dopant chemicals.
*Data as specified by Fisher Scientific.*

<table>
<thead>
<tr>
<th>NaOH (ACS grade)</th>
<th>Na₂HPO₄ (ACS grade)</th>
<th>Na₂CO₃ (HPLC grade)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Component</strong></td>
<td><strong>Content</strong></td>
<td><strong>Component</strong></td>
</tr>
<tr>
<td>NaOH</td>
<td>≥ 97%</td>
<td>Assay</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Insoluble matter</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Loss on drying</td>
</tr>
<tr>
<td>Sodium carbonate</td>
<td>≤ 1.0%</td>
<td></td>
</tr>
<tr>
<td>Chloride</td>
<td>≤ 0.005%</td>
<td>Chloride</td>
</tr>
<tr>
<td>Nitrogen compound (as N)</td>
<td>≤ 0.001%</td>
<td></td>
</tr>
<tr>
<td>Phosphate</td>
<td>≤ 0.001%</td>
<td>Sulphate</td>
</tr>
<tr>
<td>Sulphate</td>
<td>≤ 0.003%</td>
<td></td>
</tr>
<tr>
<td>Ammonium hydroxide precipitate</td>
<td>≤ 0.002%</td>
<td>Heavy metals (as Ag)</td>
</tr>
<tr>
<td>Heavy metals (as Pb)</td>
<td>≤ 0.001%</td>
<td></td>
</tr>
<tr>
<td>Iron</td>
<td>≤ 0.001%</td>
<td>Iron</td>
</tr>
<tr>
<td>Nickel</td>
<td>≤ 0.001%</td>
<td></td>
</tr>
</tbody>
</table>
Potassium $\leq 0.02\%$
Mercury $\leq 0.1$ ppm
Potassium $\leq 0.005\%$
Silica $\leq 0.005\%$

Three size ranges of doped glass powders were produced: less than 75µm, 75µm to 106µm, and greater than 106µm. Design dopant concentrations are as follows, expressed as molar ratios Na$_2$O/CaO:

Table 2.1.4. Design composition of doped material.
*All values are expressed as molar cationic ratios of sodium oxide to calcium oxide.*

<table>
<thead>
<tr>
<th>Name</th>
<th>Na$_2$O/CaO</th>
<th>Name</th>
<th>Na$_2$O/CaO</th>
<th>Name</th>
<th>Na$_2$O/CaO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$_2$HPO$_4$</td>
<td>NaOH</td>
<td>Na$_2$CO$_3$</td>
<td>Na$_2$HPO$_4$</td>
<td>NaOH</td>
<td>Na$_2$CO$_3$</td>
</tr>
<tr>
<td>D</td>
<td>0.501</td>
<td>O</td>
<td>0.500</td>
<td>U</td>
<td>0.500</td>
</tr>
<tr>
<td>J</td>
<td>0.100</td>
<td>P</td>
<td>0.100</td>
<td>V</td>
<td>0.100</td>
</tr>
<tr>
<td>K</td>
<td>0.050</td>
<td>Q</td>
<td>0.049</td>
<td>W</td>
<td>0.050</td>
</tr>
<tr>
<td>L1</td>
<td>0.010</td>
<td>R</td>
<td>0.010</td>
<td>X</td>
<td>0.010</td>
</tr>
<tr>
<td>M</td>
<td>0.005</td>
<td>S</td>
<td>0.005</td>
<td>Y</td>
<td>0.005</td>
</tr>
<tr>
<td>N</td>
<td>0.001</td>
<td>T</td>
<td>0.001</td>
<td>Z</td>
<td>0.001</td>
</tr>
</tbody>
</table>
2.2. Chemical analysis of doped CPP glass

Dopant concentrations in CPP glass were quantified by inductively-coupled plasma atomic emission spectroscopy (ICP-AES). Approximately 0.1 g of milled powder (<75µm) was dissolved in 10mL of 1N HCl (ICP-MS grade), and heated to 60ºC for 1 hour. An aliquot (1mL) of the resulting solution was diluted to 25mL with distilled, deionized water. The solutions were analyzed using a Perkin Elmer Optima 3000DV /7300 DV, and the intensities of the Na, Ca, P spectras were quantified against calibrated standards. Four samples were taken from the glass frit, and the resulting concentration readings were averaged from three measurements. Detection wavelengths are listed below:

<table>
<thead>
<tr>
<th>Element</th>
<th>Wavelength (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>589.592</td>
</tr>
<tr>
<td>Ca</td>
<td>317.933</td>
</tr>
<tr>
<td>P</td>
<td>178.221</td>
</tr>
</tbody>
</table>

Na, Ca, P aqueous standards were made using AAS grade stock solutions (1000ppm, Spex CertiPrep, Fisher Scientific).
2.3. Powder X-ray diffraction

Powder x-ray diffraction was performed using a Rigaku Multiflex diffractometer, at 40 kV 20mA using Cu Kα. Scans were performed at 1 °2θ/min, from 10 to 60 °2θ. Data collection was performed by the Rigaku software. Milled doped samples (< 75µm) were used, and both amorphous and crystalline materials were examined. Glass doped CPP samples were analyzed to ensure that the material is amorphous, while diffraction patterns of sintered samples were used for phase identification by comparison with the ICDD database using the Jade software (v. 5.0) [132]. The search-match function was limited to compounds that definitely contain phosphorus and oxygen, and that possibly contain calcium or sodium. If residual, minor phases were presented after identification of the major phase, the pure CPP diffraction pattern was used to isolate the peaks of the minor phases. Then, the search-match function was used to identify the minor phases, with the same search parameters.
2.4. Thermal Analysis of doped CPP

Crystallization and melting temperatures of milled glass samples were identified by differential thermal analysis (Netzsch STA 409PC/PG). Pre-weighed samples (< 75µm) were heated from room temperature to 1100ºC in an inert atmosphere at 10ºC/min. Thermoelectric changes were recorded in µV/mg; energy standards were not used. Weight changes were also recorded for comparison in some groups. Data collected were used to estimate sintering temperatures for these glass samples. Glass transition temperatures, when clearly shown on thermographs, were also noted. A typical DTA curve is shown below:

![Figure 2.4.1. Schematic of differential thermal analysis of glass](Adopted from Barsoum [133]. Glass transition temperature (Tg) is described as an anomaly on the DTA baseline. Crystallization/devitrification and melting are shown as exothermic and endothermic reactions, respectively.)

A sample DTA of CPP glass that underwent 122 hours of melting at 1200ºC is presented below (Figure 2.4.2), showing clearly the glass transformations expected[94]. It was expected that glass that underwent shorter melting would show less pronounced signals.
Figure 2.4.2. Sample differential thermal analysis of pure calcium polyphosphate Ropp[94] showed that DTA of CPP glass melted for 122 hours clearly showed glass transformations, namely glass transition temperature ($T_g$), softening temperature ($T_{sp}$), devitrification/crystallization temperature ($T_d$), and melting temperature ($T_m$).
2.5. Sintering of pure CPP

Porous crystalline CPP substrates were made by gravity-sintering and crystallizing amorphous CPP powder under controlled atmosphere (air inlet adjusted to the equivalent of 35% RH at 23°C) [87, 103]. Amorphous CPP was prepared as described in Section 2.1 (above). The CPP glass particles (75-106µm) were loosely packed into Pt/Rh crucibles as described by Porter et al. [88]. Samples were sintered at temperatures below the crystallization temperature, but above the glass transition temperature, in order to promote viscous flow sintering [103, 111]. The crystallization step that followed arrested densification, resulting in a solid body with an open, interconnected porous network. Grynpas et al. quantified this porosity to be about 35% (volume basis) based on image analysis of substrate cross-section SE micrographs, and Omelon reported that specimens sintered at 585°C for one hour and crystallized at 950°C for one hour had a volume porosity of 29.9±4.2% [89]. Porous constructs of pure CPP sintered from the same size range from 75 to 106µm, as above, sintered at 585°C, and crystallized at 950°C[87, 88], were used as a control group.
2.6. Sintering of doped CPP

Initial sintering experiments were performed in a Lindberg/Blue M tube furnace. Milled samples, with a particle size range of 75µm to 106µm, were loosely packed in alumina rectangular trays lined with platinum foils. Sintering was conducted in air, with constant inlet moisture content (adjusted to the equivalent of 35% RH at 23°C). To determine sintering characteristics, samples were heated at 10°C/min, and held for one hour at temperatures near their crystallization temperatures, and then cooled to room temperature. Bulk density and porosity of the sintered constructs at each temperature were evaluated. Powder x-ray diffraction patterns of ground specimens were used to determine whether samples had crystallized.

Doped CPP rod specimens were sintered and crystallized based on the doped glass crystallization and melting temperatures, but the at a ramp rate of 10°C/min and hold time of one hour throughout. Sintering temperatures were selected near the crystallization temperature, while the temperature for the crystallization step was set at a temperature below the lowest melting temperature of the doped glass groups. These experiments were performed using a box furnace (Lindberg/Blue M LGO™) with sintering atmosphere moisture control, and samples were prepared as described above (Section 2.5).
2.7. Bulk density and open porosity measurements

Bulk density was calculated by combining the specific gravity of the specimen with its open porosity. A Sartorius balance (model BP 110S) with density apparatus (model YDK 01) was used to determine the specimen’s weights in air and ethanol. The specific gravity, \( \rho_{\text{solid}} \), was calculated from these values using the equation (Sartorius YDK 01 manual, p. 18), which included a correction factor for the apparatus (0.99983):

\[
\rho_{\text{solid}} = \frac{W(a) \cdot [\rho(fl) - 0.0012 \text{ g/cm}^3]}{0.99983 \cdot [W(a) - W(fl)]} + 0.0012 \text{ g/cm}^3
\]  
[eq. 1]

Where
\( \rho(fl) \) = density of fluid, temperature specific (in this case, ethanol, \( \rho_{\text{ethanol at } T^\circ C} \))
\( W(a) \) = weight of sample in air
\( W(fl) \) = weight of sample in fluid (in this case, W(ethanol))
The density of air is taken to be constant (0.0012 g/cm\(^3\)).
Ethanol temperatures were noted for each measurement.

As the sintered samples were porous, ethanol was expected to infiltrate into the specimen’s open pores when submerged. Thus, after measuring the submerged weight, the specimen was removed from ethanol, surface liquid was absorbed using moist Kimwipe, and the ethanol-infiltrated specimen was weighed in air. The volume of open porosity was calculated using the equation:

\[
V_{\text{open porosity}} = \frac{W(\text{ethanol}) - W(a)}{\rho_{\text{ethanol at } T^\circ C}}
\]  
[eq. 2]

Bulk density, \( \rho_{\text{bulk}} \), of these samples was calculated as the ratio of the sample’s dry weight to the total volume, made up of the solid volume and the volume occupied by open porosity. The % open porosity was calculated as the ratio of the open porosity volume to the total volume. Hence:
\[
\rho_{\text{bulk}} = \frac{w(a)}{V_{\text{open pore}} + V_{\text{solid}}} = \frac{w(a)}{V_{\text{open pore}} + \frac{w(a)}{\rho_{\text{solid}}}} \quad \text{[eq. 3]}
\]

\[
\% \text{open porosity} = \frac{V_{\text{open porosity}}}{W(a)} \times 100\% \quad \text{[eq. 4]}
\]

Four to six measurements were taken per specimen, with thirty to forty minutes drying time (at 60°C) in between measurements, depending on specimen size. Average bulk density, % open porosity and their respectively standard errors of the mean were reported for each specimen.
2.8. Pyknometry measurements of doped CPP

Density of amorphous and crystalline doped CPP powders were measured using ISO18753:2004(E), “Determination of absolute density of ceramic powders by pyknometer.”[134] Approximately one gram of fine powders (particle size < 25 µm) was placed in the glass pyknometer and the combined weight was recorded (w_{SP}). The exact sample weight (w_{S}) was back-calculated using the known weight of the empty pyknometer (w_{P}). The pyknometer was then filled with 90% ethanol, and re-weighed (w_{ESP}). The weight of ethanol was calculated from that of the sample and pyknometer (w_{ESP} - w_{SP}). The mixture was decanted and the pyknometer thoroughly dried, then re-filled completely with ethanol, and weighed (w_{EP}). The weight of ethanol in this case (w_{EP} - w_{P}) was compared against the weight of ethanol calculated previously (w_{ESP} - w_{SP}). This difference was converted to the solvent’s volume, equivalent to the volume occupied by the sample. As the sample was finely ground, all closed porosity was assumed to be exposed; the sample’s “true” density was estimated by dividing w_{S} by this volume. The density of the solvent, 90% (v/v) ethanol, at various temperatures was first measured by accurately pipetting and weighing one millilitre of the solvent into a container of known weight.
2.9. Closed porosity of doped CPP

Closed porosity of glassy and crystallized specimens were calculated by comparing the material density, as measured using the pyknometer ($\rho_{\text{solid}}$, see above), with the bulk density ($\rho_{\text{bulk}}$, see Section 2.7). The volume of closed porosity, $V_{\text{close}}$, was first calculated:

$$V_{\text{closed}} = \frac{w_S}{\rho_{\text{solid}}} - \frac{w_S}{\rho_{\text{true}}} = w_S \left( \frac{\rho_{\text{true}} - \rho_{\text{solid}}}{\rho_{\text{solid}} \rho_{\text{true}}} \right)$$  \[eq. 5\]

The closed porosity was expressed as a percentage of bulk volume, $V_{\text{total}}$. It was calculated as follows:

$$\% \text{closed porosity} = \frac{V_{\text{closed}}}{V_{\text{total}}} = \frac{w_S \left( \frac{\rho_{\text{true}} - \rho_{\text{solid}}}{\rho_{\text{true}} \rho_{\text{solid}}} \right)}{\frac{w_S}{\rho_{\text{bulk}}}} = \frac{\rho_{\text{bulk}} \left( \frac{\rho_{\text{true}} - \rho_{\text{solid}}}{\rho_{\text{true}} \rho_{\text{solid}}} \right)}{\rho_{\text{solid}} \rho_{\text{true}}}$$  \[eq. 6\]

Since total volume may vary, % closed porosity was used to describe doped CPP.
2.10. Mechanical testing of sintered/crystallized samples

Sintered and crystallized samples were mechanically tested by diametral compression, as described by Pilliar et al. [88]. Briefly, cylindrical specimens of 4 mm diameter were cut into discs 2 mm thick. A load was applied along the diameter of the specimen, resulting in fracture normal to the direction of force. The strength of the specimen was calculated as follows:

\[
Diametral \ strength = \frac{2P}{\pi Dt}
\]  

[eq. 7]

Where  
\( P = \) applied load (N)  
\( D = \) diameter, average of four measurements (mm)  
\( t = \) thickness, average of four measurements (mm)

The specimens were tested on an Instron 4465 mechanical tester (Instron Canada Inc.) using a 1000N load cell at a crosshead speed 0.2 mm/min. Only specimens that failed via brittle fracture were considered (Figure 2.10.1). Weibull statistics were used to analyze the data for each sample group (See Section 2.13).

![Sample load-displacement curves of disc samples.](image)

**Figure 2.10.1.** Sample load-displacement curves of disc samples.  
(Left- Sodium carbonate-doped at 0.1Na₂O/CaO) Parts of this specimen chipped away from the bulk as compression was applied, and the entire specimen crumbled. (Right – Sodium phosphate-doped CPP at 0.1 Na₂O/CaO) Specimen sustained compression until it ruptured along the diameter normal to the applied force. Only specimens that failed in this way were considered.
2.11. SEM of construct and fracture surface

Doped CPP specimens were gold coated and examined under a scanning electron microscope (FEI 30XL) using secondary electron mode. Micrographs of construct and fracture surfaces were used to identify porous network structures, and fracture features.

2.12. Degradation study of constructs

Sintered, crystallized disc specimens of pure and doped CPP were placed in calcium- and magnesium-free phosphate buffer saline (PBS, Mount Sinai Hospital Media Preparation Services), with pH adjusted to 7.5 using HCl. Solvent composition is listed in the table below. Discs of approximately 40mg were placed in 10mL of solution and incubated at 37°C. While in incubation, the solutions were gently agitated on a solution rocker, upon which they lie horizontally, while the rocker continuously tilted the platform to a 30° angle, and then reversed directions. Two time points were selected, five and fifteen days. It was thought that this solvent volume, being ten times greater than those used previously [89], was sufficiently high that precipitation of dissolved salts would not occur.

<table>
<thead>
<tr>
<th>Table 2.12.1. Composition of Phosphate-buffered saline pH=7.5.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solutions were made in 10L bulk quantities, decanted into 1L units and autoclaved. Final solution volumes were not found to be significantly different from pre-autoclaved volumes.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Component</th>
<th>g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>8</td>
</tr>
<tr>
<td>KCl</td>
<td>0.2</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>0.2</td>
</tr>
<tr>
<td>Na₂HPO₄</td>
<td>1.15</td>
</tr>
</tbody>
</table>
A previous study showed that the mechanical strength of pure CPP constructs are different when tested wet, compared to dry testing [89]. As a result, at the end of each time point, the degraded specimens immediately underwent diametral compression, without drying. As an additional “time zero” time point, some specimens for each group were placed in solution overnight and assessed the next morning.

Upon completion of mechanical testing, the specimens were washed in deionized, distilled water to remove surface salt precipitates due to the saline solution, then rinsed in ethanol and dried at 60°C for one hour in an oven. These specimens were then mounted and gold-coated for SEM examination (See Section 2.11). The remaining solution was diluted with deionized, distilled water in a ratio of 1:1, filtered to remove any loose particles, and the calcium content was measured by ICP-AES, using the same wavelength as in Table 2.2.1, with appropriate adjustments to the method. Since PBS was originally free of calcium, measured calcium content was attributed as the degraded species, and normalized based on the original calcium content in the specimens.
2.13. Statistical analysis

Unless noted otherwise, physical and chemical characterization data were presented as the mean, and error values were presented as the standard error of the mean, along with the number of specimens sampled. Student t-test is used to analyze the effect of one factor, and statistical significance and statistical trend is assigned where $p < 0.005$ and $p < 0.01$, respectively.

Weibull statistics were used to analyze diametral compression data of CPP discs. This described the probability of failure (or survival) as a function of the failure stress population of CPP specimens [133]. The fraction of specimens that would fail ($P$) at a given failure stress ($\sigma$) was expressed as:

\[
P = 1 - \exp\left( -\left( \frac{\sigma}{\sigma_0} \right)^m \right) \quad \text{[eq. 8]}
\]

where

- $\sigma_0 =$ characteristic strength, and
- $m =$ shape factor, or Weibull modulus.

The Weibull modulus and characteristic strength were unique to the population, and thus were determined for each doped CPP group. Diametral strength of each specimen in a group of $n$ samples were sorted and ranked from lowest to highest. A probability of failure ($P_i$) was calculated for each ranking ($i$), using the equation:

\[
P_i = \frac{i - 0.3}{n + 0.4} \quad \text{[eq. 9]}
\]
Linear regression (Microsoft Excel) of a plot of $P_i$ versus the natural logarithm of diametral strength for each group was used to solve for the Weibull modulus (slope of the regression) and characteristic strength (calculated from the y-intercept) in equation 8.

Confidence intervals (90%) of the characteristic strength were reported as error estimates. This value was expressed as a percentage of the characteristic strength, and was highly dependent on sample size [135, 136]. Since ten to twenty specimens were analyzed, the 90% C.I. was determined to be ±10% of the characteristic strength. Where necessary, student t-test (Microsoft Excel) was used to analyze characteristic strengths between two groups[137]. ANOVA was not applicable to these sets of data because the statistical mean used in the procedure is not equivalent to the characteristic strength as determined by Weibull statistics. The ANOVA procedure employs sums of squares terms, which determine the sum of squares of differences between each value and the arithmetic mean, between and within sampling groups. On the other hand, the Weibull function, based on the “weakest link hypothesis,” gives greater weight to the most serious flaw, which is not necessarily the largest flaw [133, 138, 139]. As Weibull analysis requires each group to have at least ten specimens, statistical methods were not applied to groups with insufficient samples.
3. Results

3.1. Differential thermal analysis of doped and pure CPP glass
Differential thermal analyses (DTA) were performed on all doped glass samples. Typical results of pure and doped CPP are shown (sodium phosphate-doped CPP - Figure 3.1.2, sodium carbonate-doped CPP - Figure 3.1.3, sodium hydroxide-doped CPP - Figure 3.1.4).

![Differential thermal analysis of pure CPP](image)

**Figure 3.1.1.** Differential thermal analysis of pure CPP. Peaks represent crystallization (exothermic) and melting (endothermic).
Figure 3.1.2. Sample differential thermal analysis of sodium phosphate-doped CPP. Graphs are labelled (arrows) by dopant concentration in Na$_2$O/CaO.

Figure 3.1.3. Sample differential thermal analysis of sodium carbonate-doped CPP. Graphs are labelled (arrows) using dopant concentration in Na$_2$O/CaO.
Figure 3.1.4. Sample differential thermal analysis of sodium hydroxide-doped CPP. Graphs are labelled (arrows) using dopant concentration in Na₂O/CaO. Melting peaks occurred approximately 200°C lower than that of pure CPP (red) in 0.05 Na₂O/CaO and above.

The exothermic peak indicates crystallization of the sample, while the endothermic peak indicates melting. It is important to note that the ordinate has units μV/mg, rather than mW/g, hence manipulation of the data for enthalpy of crystallization and of melting was not pursued.

Crystallization and melting temperatures decreased as dopant concentration increased regardless of dopant sources. Glass transition temperatures, indicated as an inflection on the thermograms, were not apparent (reported T_g for CPP was 550°C [140]). Groups doped with sodium carbonate and sodium phosphate below 0.05 Na₂O/CaO showed only one endothermic peak, but those above this concentration showed two peaks, a smaller peak at temperatures below 800°C, and another at approximately 950°C. This suggests that two phases formed for these groups. Although these groups did not show multiple exothermic peaks, it is possible that two or more peaks appeared as a merged, broader peak as a result of heating rate (10°C/min) despite frequent sampling (every 0.025 min). Recorded crystallization and melting temperatures are
listed in Table 3.1.1, and broad peaks are indicated by *. Thermogravimetry was conducted on some samples, but mass changes were found to be negligible; and so the analysis was not pursued in all groups.

<table>
<thead>
<tr>
<th>Source</th>
<th>Name</th>
<th>Na₂O/CaO</th>
<th>Tc</th>
<th>Tm</th>
<th>Other peaks in °C</th>
<th>Other peaks in °C</th>
<th>Thermogravimetry analysis (% weight change)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Endothermic</td>
<td>Exothermic</td>
<td></td>
</tr>
<tr>
<td>Na₂HPO₄</td>
<td></td>
<td>0</td>
<td>653</td>
<td>974</td>
<td></td>
<td></td>
<td>n/a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>652</td>
<td>972</td>
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<td>n/a</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0.5</td>
<td>545</td>
<td>748</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>0.05</td>
<td>0.05</td>
<td>598</td>
<td>747</td>
<td>894*, 981*</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>0.01</td>
<td>0.01</td>
<td>648</td>
<td>969*</td>
<td></td>
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<tr>
<td></td>
<td>0.005</td>
<td>0.005</td>
<td>644</td>
<td>975*</td>
<td></td>
<td></td>
<td>n/a</td>
</tr>
<tr>
<td></td>
<td>0.001</td>
<td>0.001</td>
<td>670</td>
<td>998</td>
<td></td>
<td></td>
<td>-0.68%</td>
</tr>
<tr>
<td>NaOH</td>
<td></td>
<td>0.5</td>
<td>512</td>
<td>undetected</td>
<td></td>
<td></td>
<td>-0.4%</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0.1</td>
<td>601</td>
<td>758</td>
<td>955*, 901*</td>
<td>714*</td>
<td>n/a</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>0.05</td>
<td>623*</td>
<td>752</td>
<td>954*</td>
<td></td>
<td>n/a</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>0.01</td>
<td>642</td>
<td>971*</td>
<td></td>
<td></td>
<td>-0.3%</td>
</tr>
<tr>
<td></td>
<td>0.005</td>
<td>0.005</td>
<td>643</td>
<td>974</td>
<td></td>
<td></td>
<td>-1.1%</td>
</tr>
<tr>
<td></td>
<td>0.001</td>
<td>0.001</td>
<td>655</td>
<td>980</td>
<td></td>
<td></td>
<td>-0.3%</td>
</tr>
<tr>
<td>Na₂CO₃</td>
<td></td>
<td>0.5</td>
<td>512</td>
<td>undetected</td>
<td></td>
<td></td>
<td>-0.3%</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0.1</td>
<td>491</td>
<td>undetected</td>
<td></td>
<td></td>
<td>-0.3%</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>0.05</td>
<td>624*</td>
<td>746</td>
<td>945*</td>
<td></td>
<td>-0.4%</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>0.01</td>
<td>647</td>
<td>976</td>
<td></td>
<td></td>
<td>-0.2%</td>
</tr>
<tr>
<td></td>
<td>0.005</td>
<td>0.005</td>
<td>643*</td>
<td>971</td>
<td></td>
<td></td>
<td>-0.8%</td>
</tr>
<tr>
<td></td>
<td>0.001</td>
<td>0.001</td>
<td>652</td>
<td>977</td>
<td></td>
<td></td>
<td>-0.3%</td>
</tr>
</tbody>
</table>

The thermal behavior of CPP samples doped at 0.5 Na₂O/CaO deviated significantly from that of pure CPP, and their melting temperatures could not be detected.
3.2. Powder X-ray diffraction of doped CPP glass

Diffraction patterns were obtained from all remaining sample groups (<75µm), and all were found to be amorphous. As an example, sodium-carbonate doped specimens are shown below (Figure 3.2.1):

![XRD pattern of sodium-doped calcium polyphosphate. Sodium carbonate-doped materials are shown for the following concentrations: 0.1 Na2O/CaO (blue), 0.05 Na2O/CaO (red), 0.01 Na2O/CaO (magenta), 0.005 Na2O/CaO (green).](image)

**Figure 3.2.1.** XRD pattern of sodium-doped calcium polyphosphate. Sodium carbonate-doped materials are shown for the following concentrations: 0.1 Na$_2$O/CaO (blue), 0.05 Na$_2$O/CaO (red), 0.01 Na$_2$O/CaO (magenta), 0.005 Na$_2$O/CaO (green).
3.3. Chemical analysis of as-made doped CPP frit

Four replicates of each doped sample were analyzed by inductively-coupled plasma atomic emission spectroscopy (ICP-AES) using the method described in Section 2.2 and concentrations of sodium, calcium and phosphorus were reported as mg/L. These values were normalized using the original sample mass, and then converted to molar ratios of Na₂O to CaO to compare with expected dopant concentrations, as listed in Section 2.1.

| Table 3.3.1. Composition of doped material. All values are expressed as ratios of mol/mol. Cations are expressed as cation oxides, and phosphorus is expressed as phosphorus pentoxide (P₂O₅). |
| Dopant Source (Sample Name) | n | Expected Na₂O/CaO | Measured Na₂O/CaO | Measured CaO/P₂O₅ | Measured (Na₂O + CaO)/P₂O₅ |
| CPP | 4 | 0 | 0.0065 ± 0.0001 | 1.0426 ± 0.0019 | 1.0493 ± 0.0039 |
| Na₂HPO₄ | 0.1 | 4 | 0.099 | 0.0987 ± 0.0001 | 0.9749 ± 0.0014 | 1.0711 ± 0.0030 |
| | 0.05 | 4 | 0.053 | 0.0397 ± 0.0003 | 1.0710 ± 0.0064 | 1.1136 ± 0.0131 |
| | 0.01 | 4 | 0.010 | 0.0185 ± 0.0000 | 1.0147 ± 0.0021 | 1.0335 ± 0.0043 |
| | 0.005 | 4 | 0.005 | 0.00651 ± 0.00005 | 1.0152 ± 0.0035 | 1.0218 ± 0.0071 |
| | 0.1 | 4 | 0.100 | 0.0991 ± 0.0003 | 1.0043 ± 0.0043 | 1.1039 ± 0.0093 |
| NaOH | 0.05 | 3 | 0.049 | 0.0479 ± 0.0002 | 1.0022 ± 0.0020 | 1.0502 ± 0.0042 |
| | 0.01 | 4 | 0.010 | 0.0108 ± 0.0001 | 1.0178 ± 0.0018 | 1.0288 ± 0.0037 |
| | 0.005 | 4 | 0.005 | 0.0053 ± 0.0001 | 0.9956 ± 0.0045 | 1.0008 ± 0.0090 |
| | 0.001 | 4 | 0.001 | 0.0023± 0.0001 | 0.9995 ± 0.0031 | 1.0018 ± 0.0062 |
| Na₂CO₃ | 0.1 | 4 | 0.100 | 0.1030 ± 0.0001 | 1.0057 ± 0.0018 | 1.1093 ± 0.0038 |
| | 0.5 | 4 | 0.050 | 0.0515 ± 0.0001 | 1.0170 ± 0.0025 | 1.0694 ± 0.0053 |
| | 0.01 | 3 | 0.010 | 0.00865 ± 0.00004 | 0.8056 ± 0.0031 | 0.8126 ± 0.0062 |
| | 0.005 | 4 | 0.005 | 0.00572 ± 0.00003 | 0.9997 ± 0.0040 | 1.0168 ± 0.0081 |
| | 0.001 | 4 | 0.001 | 0.00190 ± 0.00003 | 1.0134 ± 0.0027 | 1.0094 ± 0.0055 |

As another representation of the composition, molar values are normalized to sample mass in the table below. Expected values of each element are reported based on mass balance, taking into account the initial dopant chemical incorporated into Ca(H₂PO₄)₂•H₂O.
Table 3.3.2.  Doped material content, expressed in as mmol/(g sample).

Error values are determined by standard error of the mean and error propagation.

<table>
<thead>
<tr>
<th>Dopant Source (Name)</th>
<th>n</th>
<th>Expected mmol Na/ g sample</th>
<th>Measured mmol Na/ g sample</th>
<th>Expected mmol Ca/ g sample</th>
<th>Measured mmol Ca/ g sample</th>
<th>Expected mmol P/ g sample</th>
<th>Measured mmol P/ g sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPP</td>
<td>4</td>
<td>0.000</td>
<td>0.069 ± 0.002</td>
<td>4.970</td>
<td>5.452 ± 0.012</td>
<td>9.940</td>
<td>10.46 ± 0.03</td>
</tr>
<tr>
<td>J</td>
<td>4</td>
<td>0.917</td>
<td>1.072 ± 0.002</td>
<td>4.646</td>
<td>5.432 ± 0.010</td>
<td>9.750</td>
<td>11.14 ± 0.02</td>
</tr>
<tr>
<td>K</td>
<td>4</td>
<td>0.520</td>
<td>0.464 ± 0.004</td>
<td>4.886</td>
<td>5.852 ± 0.054</td>
<td>10.03</td>
<td>10.93 ± 0.08</td>
</tr>
<tr>
<td>L1</td>
<td>4</td>
<td>0.104</td>
<td>0.189 ± 0.001</td>
<td>5.017</td>
<td>5.138 ± 0.010</td>
<td>10.09</td>
<td>10.13 ± 0.04</td>
</tr>
<tr>
<td>M</td>
<td>4</td>
<td>0.051</td>
<td>0.075 ± 0.001</td>
<td>5.034</td>
<td>5.791 ± 0.028</td>
<td>10.09</td>
<td>11.41 ± 0.06</td>
</tr>
<tr>
<td>P</td>
<td>4</td>
<td>0.990</td>
<td>1.101 ± 0.005</td>
<td>4.935</td>
<td>5.552 ± 0.021</td>
<td>9.870</td>
<td>11.06 ± 0.08</td>
</tr>
<tr>
<td>Q</td>
<td>3</td>
<td>0.471</td>
<td>0.522 ± 0.003</td>
<td>4.842</td>
<td>5.457 ± 0.011</td>
<td>9.685</td>
<td>10.89 ± 0.04</td>
</tr>
<tr>
<td>Na₂HPO₄</td>
<td>R</td>
<td>4</td>
<td>0.101</td>
<td>0.107 ± 0.001</td>
<td>5.038</td>
<td>4.968 ± 0.010</td>
<td>10.08</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>4</td>
<td>0.052</td>
<td>0.059 ± 0.001</td>
<td>5.044</td>
<td>4.805 ± 0.012</td>
<td>10.09</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>4</td>
<td>0.010</td>
<td>0.026 ± 0.001</td>
<td>5.049</td>
<td>4.902 ± 0.013</td>
<td>10.10</td>
</tr>
<tr>
<td>NaOH</td>
<td>V</td>
<td>4</td>
<td>0.991</td>
<td>1.003 ± 0.003</td>
<td>4.935</td>
<td>4.866 ± 0.009</td>
<td>9.870</td>
</tr>
<tr>
<td></td>
<td>W</td>
<td>4</td>
<td>0.500</td>
<td>0.516 ± 0.001</td>
<td>4.992</td>
<td>5.006 ± 0.009</td>
<td>9.984</td>
</tr>
<tr>
<td></td>
<td>X1</td>
<td>4</td>
<td>0.103</td>
<td>0.071 ± 0.001</td>
<td>5.050</td>
<td>4.420 ± 0.010</td>
<td>10.10</td>
</tr>
<tr>
<td></td>
<td>Y</td>
<td>4</td>
<td>0.051</td>
<td>0.061 ± 0.001</td>
<td>5.044</td>
<td>4.924 ± 0.024</td>
<td>10.09</td>
</tr>
<tr>
<td></td>
<td>Z</td>
<td>4</td>
<td>0.010</td>
<td>0.025 ± 0.001</td>
<td>5.049</td>
<td>4.978 ± 0.018</td>
<td>10.10</td>
</tr>
</tbody>
</table>

Since trace amounts of sodium was found in pure CPP, which was comparable to sodium-doped CPP at 0.005 Na₂O/CaO, the study was further narrowed to examine doped-CPP at concentrations higher than this value. As a result, initial sintering was conducted upon CPP doped at 0.1 and 0.01 Na₂O/CaO.
3.4. Initial sintering experiments

CPP doped at 0.1 Na$_2$O/CaO and 0.01 Na$_2$O/CaO using the three dopants were sintered at near-crystallization temperatures as indicated by DTA thermograms. For simplicity, the following results refer to these groups by their dopant source and concentration, e.g., “Carbonate-doped 0.01” refers to sodium carbonate-doped CPP at 0.01 Na$_2$O/CaO. Sintering conditions are as follows:

Table 3.4.1. Heating profile of one-stage pure and sodium-doped CPP
*Sintered specimens are air-cooled before removal from furnace.*

<table>
<thead>
<tr>
<th>Samples</th>
<th>Heating rate</th>
<th>Dwell time</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPP</td>
<td>10ºC/min to 500ºC, 5ºC/min to 585ºC</td>
<td>1 hour at 585ºC</td>
</tr>
<tr>
<td>0.1 Na$_2$O/CaO (all dopant sources)</td>
<td>10ºC/min to 500ºC, 5ºC/min to 540ºC</td>
<td>1 hour at 540ºC</td>
</tr>
<tr>
<td>0.01 Na$_2$O/CaO (all dopant sources)</td>
<td>10ºC/min to 500ºC, 5ºC/min to 582ºC</td>
<td>1 hour at 582ºC</td>
</tr>
</tbody>
</table>

The purpose of this test was to identify the groups that could be sintered into intact, porous constructs. SEM images (Figure 3.4.2) of the samples indicated that Phosphate-doped 0.1 and Carbonate-doped 0.1 showed particle rounding and some sinter necks, as do Phosphate-doped 0.01. Carbonate-doped 0.01, Hydroxide-doped 0.1 and 0.01 specimens showed less particle rounding, and sinter neck formation was not obvious.
CPP doped at 0.1 Na₂O/CaO (left) or 0.01 Na₂O/CaO (right) are labelled by the dopant sources (left-most column).

XRD patterns of these samples indicated that Hydroxide-doped 0.1 and 0.01 already underwent crystallization at these temperatures (Figure 3.4.3 for CPP doped at 0.1 Na₂O/CaO, and Figure 3.4.4 for CPP doped at 0.01 Na₂O/CaO). Combined with the observation that there was little sinter neck formation in these groups, only CPP doped with sodium phosphate (phosphate-doped CPP) and CPP doped with sodium carbonate (carbonate-doped CPP) at 0.1 Na₂O/CaO appeared to be more promising candidates to produce sintered porous constructs.

<table>
<thead>
<tr>
<th>Source</th>
<th>CPP doped at 0.1 Na₂O/CaO</th>
<th>CPP doped at 0.01 Na₂O/CaO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate</td>
<td><img src="image1" alt="Phosphate doped at 0.1 Na₂O/CaO" /></td>
<td><img src="image2" alt="Phosphate doped at 0.01 Na₂O/CaO" /></td>
</tr>
<tr>
<td>Hydroxide</td>
<td><img src="image3" alt="Hydroxide doped at 0.1 Na₂O/CaO" /></td>
<td><img src="image4" alt="Hydroxide doped at 0.01 Na₂O/CaO" /></td>
</tr>
<tr>
<td>Carbonate</td>
<td><img src="image5" alt="Carbonate doped at 0.1 Na₂O/CaO" /></td>
<td><img src="image6" alt="Carbonate doped at 0.01 Na₂O/CaO" /></td>
</tr>
</tbody>
</table>

*Figure 3.4.2. Scanning electron micrographs of doped CPP.*

CPP doped at 0.1 Na₂O/CaO (left) or 0.01 Na₂O/CaO (right) are labelled by the dopant sources (left-most column).
Figure 3.4.3. Powder X-ray diffraction patterns of 0.1 Na$_2$O/CaO sintered at 540°C. Phosphate-doped CPP appeared amorphous (blue), while Hydroxide-doped CPP (red) showed crystalline patterns. Carbonate-doped CPP (magenta) appeared amorphous. Diffraction pattern of Ca(PO$_3$)$_2$ (PDF #11-0039) is shown as green marks for reference.

Figure 3.4.4. Powder X-ray diffraction patterns of 0.01 Na$_2$O/CaO, sintered at 582°C. Phosphate-doped CPP (blue) appeared amorphous, while Carbonate-doped CPP (magenta) crystallized, and Hydroxide-doped CPP (red) appeared amorphous with some crystalline content. Diffraction pattern of Ca(PO$_3$)$_2$ (PDF #11-0039) is shown as green markers for reference.
3.5. Sintering and crystallization of doped-CPP at 0.1 Na$_2$O/CaO

3.5.1. Physical Characterization of Sintered Doped-CPP

Phosphate-doped 0.1 and Carbonate-doped 0.1 were sintered at different temperatures near crystallization temperatures to determine appropriate conditions to produce an open pore structure for tissue engineering, with an open porosity of approximately 35% [52, 67, 88]. As a preliminary experiment these groups were sintered at 530ºC, 540ºC, 550ºC, 560ºC, and 570 ºC for one hour. Bulk density and open porosity were measured for these groups, and between two to three specimens were analyzed (Figure 3.5.1). As sintering temperature increased, specimens from both dopant sources show an increase in bulk density and a decrease in open porosity. This densification was reflected in SEM images of these specimens (Figure 3.5.2). XRD patterns of these specimens showed that crystallization occurs in treatments higher than 550ºC (Phosphate-doped CPP - Figure 3.5.3; Carbonate-doped CPP - Figure 3.5.4). While comparable bulk density and open porosity to those of pure CPP were achieved when these specimens were sintered at 540ºC, both Phosphate-doped 0.1 and Carbonate-doped 0.1 specimens sintered at 530ºC did not hold together during bulk density measurements, and these specimens could not be properly mounted for SEM observation. However, XRD of these specimens were performed.

![Figure 3.5.1. Bulk density of one-stage sodium-doped CPP at 0.1 Na$_2$O/CaO. Phosphate-doped CPP (purple) and Carbonate-doped CPP (blue) showed increasing bulk density and decreasing open porosity after one-stage sintering.](image-url)
<table>
<thead>
<tr>
<th>Sintering temperature</th>
<th>Phosphate-doped 0.1</th>
<th>Carbonate-doped 0.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>540ºC</td>
<td><img src="image1" alt="Image" /></td>
<td><img src="image2" alt="Image" /></td>
</tr>
<tr>
<td>550ºC</td>
<td><img src="image3" alt="Image" /></td>
<td><img src="image4" alt="Image" /></td>
</tr>
<tr>
<td>560ºC</td>
<td><img src="image5" alt="Image" /></td>
<td><img src="image6" alt="Image" /></td>
</tr>
<tr>
<td>570ºC</td>
<td><img src="image7" alt="Image" /></td>
<td><img src="image8" alt="Image" /></td>
</tr>
</tbody>
</table>

Figure 3.5.2. *SEM of Phosphate-doped 0.1 and Carbonate-doped 0.1* 
Phosphate-doped CPP (left) and Carbonate-doped CPP (right) at 0.1 Na$_2$O/CaO sintering temperatures as labelled.
Figure 3.5.3. *X-ray diffraction patterns of sintered Phosphate-doped 0.1* Phosphate-doped CPP at 0.1 Na$_2$O/CaO showed that crystallization occurred in specimens sintered at temperatures greater than 550°C. Sintering temperatures are indicated to the left of each pattern, and green markers represent PDF #11-0039 Ca(PO$_3$)$_2$. 
Figure 3.5.4. X-ray diffraction patterns of sintered Carbonate-doped 0.1. Carbonate-doped CPP doped at 0.1 Na₂O/CaO crystallized when sintered at temperatures greater than 550°C. Sintering temperatures are indicated to the left of each pattern, and green markers represent PDF #11-0039 Ca(PO₃)₂.

3.5.2. Physical Characterization of Sintered, Crystallized Doped-CPP (730°C)

To maximize crystallization, a second stage of heat treatment was applied to phosphate- and carbonate-doped CPP at 0.1 Na₂O/CaO, near their melting points. In these experiments, Phosphate-doped 0.1 and Carbonate-doped 0.1 were first sintered at 540, 550, 560, and 570 respectively for one hour, and then further heating was conducted at 10°C/min to 730°C and held for one hour. The objective was to measure the final open porosity to determine the proper combination of sinter neck formation and crystallization. Open porosity of all groups, regardless of the sintering temperature, dropped to approximately 10% from this crystallization treatment (Table 3.5.1). This was also observed from SEM images (Figure 3.5.5). Porous networks observed from sintering (Figure 3.5.2) collapsed upon crystallization treatment, and sintered
particle contours were difficult to distinguish from the bulk. Isolated pores could be observed from these fracture surfaces, and overall, this degree of densification was unexpected.

Table 3.5.1. Summary of specimens of sintered, crystallized Phosphate-doped 0.1 and Carbonate-doped 0.1.

Specimens were sintered at different temperatures and crystallized at 730°C. Sintered and crystallized CPP was used as control. Errors represent standard error of the mean.

<table>
<thead>
<tr>
<th>Group</th>
<th>CPP</th>
<th>Phosphate-doped CPP 0.1</th>
<th>Carbonate-doped CPP 0.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sintering temperature (ºC)</td>
<td>585</td>
<td>540 550 560 570</td>
<td>540 550 560 570</td>
</tr>
<tr>
<td>Number of samples</td>
<td>28</td>
<td>8  7  4  8</td>
<td>8  8  4  7</td>
</tr>
<tr>
<td>Open porosity (%)</td>
<td>36.1% ± 0.9%</td>
<td>10.8% ± 1.10%</td>
<td>15.8% ± 2.10%</td>
</tr>
<tr>
<td>Bulk density (g/cm³)</td>
<td>1.66 ± 0.02</td>
<td>2.38 ± 0.03</td>
<td>2.21 ± 0.05</td>
</tr>
</tbody>
</table>
Figure 3.5.5. SEM images of Phosphate-doped 0.1 and Carbonate-doped 0.1. Fracture surfaces of are labelled by their sintering temperature (left-most column). All specimens were crystallized at 730°C.
3.5.3. Physical Characterization of Sintered, Crystallized Doped-CPP (718°C)

Crystallization of these specimens was attempted once more at a reduced temperature (718°C), using the same heating profiles. For each dopant source, two rods specimens were sintered at each sintering/crystallization temperature. Specimens were then cut into discs approximately 2 mm thick. Bulk density and open porosity of 13 to 14 specimens for each dopant/sintering temperature was measured (Table 3.5.2).

Table 3.5.2. Summary of specimens of sintered, crystallized Phosphate-doped 0.1 and Carbonate-doped 0.1.

<table>
<thead>
<tr>
<th>Group</th>
<th>CPP</th>
<th>Phosphate-doped CPP 0.1</th>
<th>Carbonate-doped CPP 0.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sintering temperature (°C)</td>
<td>585/950°C</td>
<td>540 550 560 570 540 550 560 570</td>
<td></td>
</tr>
<tr>
<td>Number of samples</td>
<td>28</td>
<td>14 14 13 14 14 14 14 14</td>
<td></td>
</tr>
<tr>
<td>Open porosity (%)</td>
<td>36.1% ±0.9%</td>
<td>37.7% ±1.3% 34.0% ±1.5% 24.6% ±0.8% 13.3% ±0.6% 34.3% ±1.1% 13.2% ±0.6% 11.6% ±0.8% 13.3% ±1.3%</td>
<td></td>
</tr>
<tr>
<td>Bulk density (g/cm³)</td>
<td>1.66 ±0.02</td>
<td>1.67 ±0.03 1.77 ±0.04 1.96 ±0.02 2.32 ±0.02 1.73 ±0.02 2.27 ±0.02 2.35 ±0.02 2.31 ±0.03</td>
<td></td>
</tr>
</tbody>
</table>

Crystallized Carbonate-doped 0.1 sintered at 540°C, and crystallized Phosphate-doped 0.1 specimens sintered at 540°C or 550°C, all had comparable open porosity to that of pure CPP. However, open porosity decreased in all other doped specimens after crystallization. SE micrographs (Figure 3.5.6) clearly confirmed this. Crystallized Phosphate-doped 0.1 specimens have a morphology similar to as-sintered samples (Figure 3.5.2). Crystallized Carbonate-doped 0.1 specimens, however, lost the morphology of sintered particles, and sinter necks became indistinguishable. Interestingly, there were no signs of extensive melting, and some specimens appeared hollow (white arrows, Figure 3.5.6).
<table>
<thead>
<tr>
<th>Sintering temperature</th>
<th>Phosphate-doped 0.1</th>
<th>Carbonate-doped 0.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>540°C</td>
<td>![SEM image]</td>
<td>![SEM image]</td>
</tr>
<tr>
<td>550°C</td>
<td>![SEM image]</td>
<td>![SEM image]</td>
</tr>
<tr>
<td>560°C</td>
<td>![SEM image]</td>
<td>![SEM image]</td>
</tr>
<tr>
<td>570°C</td>
<td>![SEM image]</td>
<td>![SEM image]</td>
</tr>
</tbody>
</table>

Figure 3.5.6. *SEM image of Phosphate-doped 0.1 and Carbonate-doped 0.1*
All specimens were crystallized at 718°C. Hollow particles are labelled with arrows.
3.5.4. **Mechanical Properties of Sintered, Crystallized Doped-CPP**

Logarithmic survival probability plots for Phosphate-doped 0.1 and Carbonate-doped 0.1 are shown in Figure 3.5.7 and Figure 3.5.8. All groups showed more than one linear region, suggesting more than one failure mode. Linear regression was performed for these plots, from which the characteristic strength ($\sigma_c$), and Weibull modulii (m) were calculated. Error was estimated at ± 10% of the characteristic strengths of each group, as described by McLean and Hartsock [136]. R² values are listed as an indication of goodness-of-fit (Table 3.5.3). Sample sizes differed from those used for bulk density measurements (Table 3.5.2) as some samples experienced compression rather than brittle failure, and were removed from the pooled data.

**Figure 3.5.7.** Weibull plots of Phosphate-doped CPP at 0.1
Plots of sodium phosphate-doped CPP at 0.1 Na₂O/CaO are labelled with their sintering/crystallization temperatures.
Figure 3.5.8. Weibull plots of Carbonate-doped CPP 0.1
Plots of sodium carbonate-doped CPP at 0.1 Na₂O/CaO are labelled with their sintering/crystallization temperatures.

Table 3.5.3. Summary of Weibull modulus and characteristic strengths of doped-CPP at 0.1 Na₂O/CaO.

<table>
<thead>
<tr>
<th>Group</th>
<th>Phosphate-doped CPP</th>
<th>Carbonate-doped CPP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>540 °C</td>
<td>550 °C</td>
</tr>
<tr>
<td>n</td>
<td>21</td>
<td>13</td>
</tr>
<tr>
<td>% open porosity</td>
<td>34.5% ± 0.8%</td>
<td>37.9% ± 1.4%</td>
</tr>
<tr>
<td>Weibull modulus</td>
<td>3.30</td>
<td>2.24</td>
</tr>
<tr>
<td>σ_c</td>
<td>7.50</td>
<td>8.36</td>
</tr>
<tr>
<td>Δσ_c</td>
<td>0.75</td>
<td>0.84</td>
</tr>
<tr>
<td>R²</td>
<td>0.9544</td>
<td>0.8921</td>
</tr>
</tbody>
</table>

3.5.5. XRD of Sintered, Crystallized Doped-CPP

Sample XRD patterns of specimens from both dopant sources showed that doped-CPP was amorphous when sintered at 550°C, but at either crystallization temperature (718°C or 730°C), the specimens have crystallized (Figure 3.5.9 and Figure 3.5.10, for Phosphate-doped 0.1 and Carbonate-doped 0.1, respectively). Although major peaks were identified as those of crystalline β-CPP (sintered, crystallized CPP, blue patterns in Figures 1.5.9 and 1.5.10), a
number of other prominent peaks also appeared, suggesting multiple phases. It is interesting to note that these residual peaks are similar regardless of crystallization temperature used.

**Figure 3.5.9.** X-ray diffraction patterns of Phosphate-doped 0.1. Sintered (at 550ºC) and crystallized sodium phosphate-doped CPP at 0.1Na₂O/CaO showed diffraction patterns with similar major peaks compared to crystallized CPP (blue pattern), but other peaks are unaccounted for. This is observed in specimens crystallized in 718ºC (green pattern), as well as those crystallized at 730ºC (brown patterns). Red patterns are obtained from sintered specimens not exposed to a second, high temperature, crystallization heat treatment.
Figure 3.5.10. X-ray diffraction patterns of Carbonate-doped 0.1.

Diffraction patterns of sintered (at 550°C) and crystallized sodium carbonate-doped CPP at 0.1Na₂O/CaO have similar major peaks compared to crystallized CPP (blue pattern), but other peaks are unaccounted for. This is observed in specimens crystallized in 718°C (green pattern), as well as those crystallized at 730°C (brown patterns). Red patterns are obtained from sintered specimens not exposed to a second, high temperature, crystallization heat treatment.

An attempt was made to identify these other phases using Jade with the PDF-2 database. The XRD pattern of crystalline sintered CPP was subtracted from crystalline doped CPP patterns. The residual peaks underwent search and match functions to find the most likely full-pattern fits within the PDF-2 database. Phases that are associated with these other peaks are tabulated below (Table 3.5.4).

Table 3.5.4. Pattern matches with residual peaks of doped-CPP at 0.1Na₂O/CaO.

<table>
<thead>
<tr>
<th>Name</th>
<th>ICDD #</th>
<th>Doped CPP groups (# of patterns examined)</th>
<th>Phosphate-doped 0.1 patterns (5)</th>
<th>Hydroxide-doped 0.1 patterns (5)</th>
<th>Carbonate-doped 0.1 patterns (4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCa(PO₃)₃</td>
<td>23-0669</td>
<td></td>
<td>5</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Na₁.₈Ca₁.₁P₆O₁₇</td>
<td>47-0863</td>
<td></td>
<td>5</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Na₄Ca(PO₃)₆</td>
<td>25-0811</td>
<td></td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Ca₂P₂O₇</td>
<td>09-0345</td>
<td></td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Ca₂P₂O₇</td>
<td>09-0346</td>
<td></td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>33-0297</td>
<td></td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>CaP₂O₆</td>
<td>11-0039</td>
<td></td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Ca₄P₆O₁₉</td>
<td>15-0177</td>
<td></td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>
A number of standard powder diffraction patterns matched residual patterns of both doped sources. The tally above also clearly showed that if the standard and residual patterns are true matches, then the residual patterns consist of a combination of mixed phases. Interestingly, diffraction patterns of sintered, crystallized (730°C) Hydroxide-doped 0.1 also contain additional peaks than those of pure CPP (Figure 3.5.11). Even though Carbonate-doped 0.1 and Phosphate-doped 0.1 could be sintered into porous constructs with greater characteristic tensile strength compared to pure CPP of the same porosity, their lack of phase homogeneity made them undesirable substrates for tissue engineering.

**Figure 3.5.11.** X-ray diffraction patterns of Hydroxide-doped 0.1. Patterns of sodium hydroxide-doped CPP at 0.1 Na₂O/CaO, sintered (590°C – green) and sintered-then-crystallized (590°C and 730°C – brown). Peaks in addition to those of pure CPP (blue) can be observed.
3.6. Sintering and crystallization of doped-CPP at 0.01 Na$_2$O/CaO

3.6.1. Physical Characterization of Sintered, Crystallized Doped-CPP (720°C)

Two sintering temperatures for sodium phosphate-doped CPP at 0.01 Na$_2$O/CaO and sodium carbonate-doped CPP at 0.01 were selected (580°C and 590°C) since initial sintering experiments (Section 3.4) produced intact samples, with insufficient sinter necks. These rod specimens were crystallized at 720°C for one hour, and disc specimens were cut. Bulk density and open porosity were determined (Table 3.6.1).

<table>
<thead>
<tr>
<th>Sintering temperature (°C)</th>
<th>CPP</th>
<th>Phosphate-doped 0.01</th>
<th>Hydroxide-doped 0.01</th>
<th>Carbonate-doped 0.01</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sintering temperature (°C)</td>
<td>585°C/950°C</td>
<td>580°C</td>
<td>590°C</td>
<td>580°C</td>
</tr>
<tr>
<td>Number of samples</td>
<td>28</td>
<td>11</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td>Open porosity (%)</td>
<td>36.1% ± 0.9%</td>
<td>30.1% ± 1.1%</td>
<td>33.1% ± 2.5%</td>
<td>38.1% ± 0.5%</td>
</tr>
<tr>
<td>Bulk density (g/cm$^3$)</td>
<td>1.66 ± 0.02</td>
<td>1.88 ± 0.02</td>
<td>1.77 ± 0.02</td>
<td>1.61 ± 0.01</td>
</tr>
</tbody>
</table>

Sintered, crystalline pure CPP discs were used for comparison. Sample size varied in these groups as a number of the specimens crumbled during handling, particularly those sintered at 580°C. Bulk density and open porosity showed that the applied sintering temperatures had different effects on CPP doped at 0.01 Na$_2$O/CaO depending on the dopant source. Sodium phosphate-doped CPP at 0.01 Na$_2$O/CaO (Phosphate-doped 0.01) has lowest open porosity, while sodium carbonate-doped CPP at 0.01 Na$_2$O/CaO (Carbonate-doped 0.01) has the highest open porosity, when sintered at 580°C. The same ranking persists when the specimens are sintered at 590°C.
It is interesting to note that contrary to expectations, Phosphate-doped 0.01 specimens shows a slight increase in open porosity (3.0% ± 2.7%, p=0.048 < 0.05), and a decrease in bulk density (0.11± 0.03g/cm³, p=0.002 <0.01) as sintering temperature increased. Carbonate-doped 0.01 showed the largest change over a ten-degree sintering temperature difference, a decrease of 8.9% ± 1.5% and an increase of 0.28 ± 0.02g/cm³, in open porosity and bulk density, respectively. Since a large number of specimens sintered at 580ºC that could not be assessed, significance could not be reported. Hydroxide-doped 0.1 did not appear influenced by sintering temperature changes, as evidenced by statistically insignificant differences in open porosity and bulk density.

3.6.2. Mechanical Properties of Sintered, Crystallized Doped-CPP (720°C)

Diametral compression of doped CPP at 0.01 Na₂O/CaO was performed, and it was observed that Carbonate-doped 0.01 specimens sintered at 580ºC failed by compression, rather than tension, and could not be considered in the Weibull analysis. Survival probability plots for all other doped-CPP groups at 0.01 Na₂O/CaO are shown (Figure 3.6.1 to Figure 3.6.3). Since all groups appear to conform to the linear regression, visual inspection suggests that each group was influenced by only one population of flaws. While it may be an artefact of the small sample sizes, it is interesting to note that both Phosphate-doped 0.01 and Hydroxide-doped 0.01 specimens sintered at 590ºC have lower ranges of failure stresses, compared to those sintered at 580ºC. Weibull modulus (m), characteristic strength (σ_c), and coefficient of determination (R²) results are shown in Table 3.6.2.
Figure 3.6.1. Survival plot of Phosphate-doped 0.01. Groups are listed by their sintering/crystallization temperature. Sintered, crystallized CPP is shown (in blue) for comparison. Plots of each group is shown with a linear guide for a single mode of failure.

Figure 3.6.2. Survival plot of Hydroxide-doped 0.01. Groups are listed by their sintering/crystallization temperature. Sintered, crystallized CPP is shown (in blue) for comparison. Plots of each group is shown with a linear guide for a single mode of failure.
Figure 3.6.3. Survival plot of Carbonate-doped 0.01. Sintered, crystallized CPP is shown (in blue) for comparison.

Table 3.6.2. Summary of Weibull plots of doped-CPP at 0.01 Na₂O/CaO. All groups were crystallized at 720°C for one hour. Data for Carbonate-doped 0.01 sintered at 582°C was eliminated.

<table>
<thead>
<tr>
<th>Group</th>
<th>CPP</th>
<th>Phosphate-doped 0.01</th>
<th>Hydroxide-doped 0.01</th>
<th>Carbonate-doped 0.01</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>582°C</td>
<td>592°C</td>
<td>582°C</td>
</tr>
<tr>
<td>n</td>
<td>21</td>
<td>9</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>% open porosity</td>
<td>34.5%</td>
<td>± 0.8%</td>
<td>28.7%</td>
<td>± 0.8%</td>
</tr>
<tr>
<td>Weibull modulus</td>
<td>3.30</td>
<td>7.51</td>
<td>5.85</td>
<td>5.93</td>
</tr>
<tr>
<td>σ_c (MPa)</td>
<td>7.50</td>
<td>22.08</td>
<td>15.87</td>
<td>5.40</td>
</tr>
<tr>
<td>Δσ_c (MPa)</td>
<td>0.75</td>
<td>2.21</td>
<td>1.59</td>
<td>0.54</td>
</tr>
<tr>
<td>R²</td>
<td>0.9544</td>
<td>0.9537</td>
<td>0.9708</td>
<td>0.8998</td>
</tr>
</tbody>
</table>

Although linear regression showed favourable goodness-to-fit between the calculated parameters and the collected data, the Weibull distribution has a strong dependence on sample size. As a result, the parameter estimates for Phosphate-doped 0.01 and Hydroxide-doped 0.01, sintered at 580°C and crystallized at 720°C, were likely affected by higher uncertainty than others.

It is surprising that Phosphate-doped 0.01 (sintered at 590°C and crystallized at 720°C) specimens have twice the characteristic strength compared to pure CPP, when the two groups
have similar open porosity (Table 3.6.1). The same is observed from the comparison between Carbonate-doped 0.01 (sintered at 590°C and crystallized at 720°C) and the control group.

3.6.3. **SEM of Sintered, Crystallized Doped-CPP**

SEM micrographs of doped CPP of 0.01 Na₂O/CaO from all three dopant sources showed features similar to those found in 0.1 Na₂O/CaO groups. Porosity within particles was observed (black arrows), but not to the extent seen in groups of the higher doping concentration. Sinter necks observed from Carbonate-doped 0.01 specimens sintered at 580°C were narrow, with little densification, but this changed dramatically when the specimens were sintered at 590°C, in which sinter necks and densification increased visibly, and particle rounding was obvious.
Figure 3.6.4. SEM images of doped-CPP at 0.01 Na₂O/CaO sintered at 580°C. Images of sintered, crystalline CPP are shown for comparison with sintered, crystalline (720°C) doped-CPP. Discs surfaces (left) and fracture surfaces (right) are presented for material surface and fracture morphology, and for porosity observations.
<table>
<thead>
<tr>
<th>Group</th>
<th>Disc surface</th>
<th>Fracture surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPP</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>Phosphate-doped 0.01</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>Hydroxide-doped 0.01</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>Carbonate-doped 0.01</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
</tbody>
</table>

Figure 3.6.5. SEM images doped-CPP at 0.01 Na₂O/CaO sintered at 590°C. Images of sintered, crystalline CPP are shown for comparison with sintered, crystalline (720°C) doped-CPP. Discs surfaces (left) and fracture surfaces (right) are presented for material surface and fracture morphology, and for porosity observations.
3.6.4.  
**XRD of Sintered, Crystalline Doped-CPP**

Diffraction patterns of CPP doped at 0.01 Na₂O/CaO match exactly with those of pure CPP, regardless of the dopant source (Figure 3.6.6-8). The pattern of Phosphate-doped 0.01 sintered/crystallized at 590°C/720°C showed a small peak at 16° 2θ, which is attributed to the quartz sample holder. Comparison with diffraction patterns of samples sintered at 580°C (Figure 3.4.4) showed that crystallization had begun these two sintering temperatures.

![Figure 3.6.6. X-ray diffraction patterns of Phosphate-doped 0.01. Patterns from sintered (590°C, red), and sintered-and-crystalline (590°C/720°C, green) specimen compared with that of pure CPP (blue).](image)
Figure 3.6.7. X-ray diffraction patterns of Hydroxide-doped 0.01. Patterns from sintered (590°C, red), and sintered-and-crystalline (590°C/720°C, green) specimens compared with that of pure CPP (blue).

Figure 3.6.8. X-ray diffraction patterns of Carbonate-doped 0.01. Patterns from sintered (590°C, red), and sintered-and-crystalline (590°C/720°C, green) specimens compared with that of pure CPP (blue).
3.6.5. Material density of Phosphate-doped 0.01 and Carbonate-doped 0.01

Phosphate-doped 0.01 and Carbonate-doped 0.01, both sintered/crystallized at 590º/720ºC, were identified as the doped CPP groups that have comparable porosity to that of pure CPP constructs, and higher diametral characteristic strength. To examine further earlier observations of closed porosity (Figure 3.6.4-5), absolute density of these groups, and of pure CPP, were determined as described in Section 2.8. Results are tabulated below:

Table 3.6.3. Absolute density of doped-CPP at 0.01 Na₂O/CaO (glass and crystalline).
*Error shown is standard error of the mean.*

<table>
<thead>
<tr>
<th>Group</th>
<th>State</th>
<th>CPP</th>
<th>Phosphate-doped 0.01</th>
<th>Carbonate-doped 0.01</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>glass</td>
<td>crystalline</td>
<td>glass</td>
<td>crystalline</td>
</tr>
<tr>
<td>Density (g/cm³)</td>
<td>2.58 ± 0.03</td>
<td>2.90 ± 0.04</td>
<td>2.68 ± 0.02</td>
<td>3.02 ± 0.06</td>
</tr>
<tr>
<td>n</td>
<td>15</td>
<td>4</td>
<td>6</td>
<td>7</td>
</tr>
</tbody>
</table>

As expected crystallized pure and doped CPP had greater absolute density than their glassy counterparts. There were no significant differences in density among the crystallized groups.

Using these values as well as pooled specific gravity and bulk density data from the previous sections (Section 2.9), an estimate of the closed porosity was made of the sintered, crystalline constructs (Table 3.6.4).

Table 3.6.4. Closed porosity of pure CPP and doped CPP at 0.01 Na₂O/CaO.
*Error represents standard error of the mean. Number of samples refers to the number of data points collected from physical characterization of these groups.*

<table>
<thead>
<tr>
<th>Group</th>
<th>Sintering temperatures (ºC)</th>
<th>CPP</th>
<th>Phosphate-doped 0.01</th>
<th>Carbonate-doped 0.01</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>21</td>
<td>14</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>% closed porosity</td>
<td>5.94% ±0.28%</td>
<td>7.40% ±0.43%</td>
<td>4.35% ±0.19%</td>
<td></td>
</tr>
</tbody>
</table>

These closed porosity estimates, calculated on a volume-basis, confirm observations from the SEM images, which exposed some of these closed pores (Figure 3.6.5). The values also suggest that of the three groups, Phosphate-doped 0.01 had the highest closed porosity.
3.7. Degradation of doped-CPP at 0.01 Na₂O/CaO

3.7.1. Physical characterization of specimens before degradation

Phosphate-doped 0.01 and Carbonate-doped 0.01 specimens, sintered and crystallized at 590°C and 720°C, were selected for comparable open porosity (approximately 36% vol. basis) to that of pure CPP constructs used as tissue-engineered cartilage substrates. The table below summarizes the as-made specifications of the specimens used for the degradation study. Specimens are grouped by their assigned degradation time points, and are denoted “wet” to emphasize mechanical testing before drying the specimens (Section 2.12). Characterization results from the previous section have been included as reference (listed as “reference”, under “Time point”).

**Table 3.7.1. Physical characterization of pure CPP for degradation.**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>21</th>
<th>15</th>
<th>17</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk density</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>± S.E. (g/cm³)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.70 ± 0.02</td>
<td>1.66 ± 0.01</td>
<td>1.64 ± 0.01</td>
<td>1.66 ± 0.02</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| % open porosity ± S.E. | 34.50% ± 0.80% | 37.70% ± 0.50% | 38.00% ± 0.50% | 37.66% ± 0.55% |

| Time point | reference | Day 0, wet | Day 5, wet | Day 15, wet |

**Table 3.7.2. Physical characterization of Carbonate-doped 0.01 for degradation.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Carbonate-doped 0.01 590</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>39</td>
</tr>
<tr>
<td>Bulk density ± S.E. (g/cm³)</td>
<td>1.69 ± 0.01</td>
</tr>
<tr>
<td>% open porosity ± S.E.</td>
<td>36.90% ± 0.50%</td>
</tr>
</tbody>
</table>

| Time point | reference | Day 0, wet | Day 5, wet | Day 15, wet |
81

3.7.2. Changes in chemical and mechanical properties of Doped-CPP

Specimens removed from degradation were tested while wet by diametral compression and Weibull parameters are tabulated (Table 3.7.4) and graphically represented (Figure 3.7.1).

Calcium dissolved into the solution were analyzed and normalized by the total calcium content in the constructs, and are thus termed “% Ca released.”

### Table 3.7.3. Physical characterization of Phosphate-doped 0.01 for degradation.

<table>
<thead>
<tr>
<th>Group</th>
<th>Phosphate-doped 0.01</th>
<th>590</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>Bulk density ± S.E. (g/cm³)</td>
<td>1.77 ± 0.02</td>
<td>1.71 ± 0.02</td>
</tr>
<tr>
<td>% open porosity ± S.E.</td>
<td>33.20% ± 1.00%</td>
<td>36.20% ± 0.90%</td>
</tr>
</tbody>
</table>

### Table 3.7.4. Degradation and mechanical property changes of Doped CPP.

Error represents standard error of the mean.

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 0, wet</th>
<th>Day 5, wet</th>
<th>Day 15, wet</th>
<th>Day 0, wet</th>
<th>Day 5, wet</th>
<th>Day 15, wet</th>
<th>Day 0, wet</th>
<th>Day 5, wet</th>
<th>Day 15, wet</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Ca released</td>
<td>0.13% ± 0.01%</td>
<td>0.69% ± 0.09%</td>
<td>0.98% ± 0.07%</td>
<td>0.10% ± 0.003%</td>
<td>0.16% ± 0.01%</td>
<td>0.28% ± 0.01%</td>
<td>0.08% ± 0.01%</td>
<td>0.24% ± 0.03%</td>
<td>0.33% ± 0.01%</td>
</tr>
<tr>
<td>Characteristic strength, σc (MPa)</td>
<td>7.16 ± 0.8</td>
<td>4.74 ± 0.5</td>
<td>5.40 ± 0.6</td>
<td>12.7 ± 1.7</td>
<td>10.75 ± 1.2</td>
<td>5.62 ± 0.73</td>
<td>14 ± 1.6</td>
<td>12.9 ± 1.5</td>
<td>12.16 ± 1.4</td>
</tr>
<tr>
<td>Weibull modulus</td>
<td>5.27</td>
<td>5.42</td>
<td>3.96</td>
<td>5.55</td>
<td>5.93</td>
<td>1.81</td>
<td>4.89</td>
<td>5.47</td>
<td>6.88</td>
</tr>
<tr>
<td>R²</td>
<td>0.9701</td>
<td>0.9090</td>
<td>0.7779</td>
<td>0.9787</td>
<td>0.9237</td>
<td>0.7588</td>
<td>0.8995</td>
<td>0.9658</td>
<td>0.9814</td>
</tr>
</tbody>
</table>
The data showed that of the three groups, pure CPP degraded the fastest chemically, while in terms of diametral characteristic strength, Phosphate-doped 0.01 decreased the most over the course of degradation. Carbonate-doped 0.01 specimens degraded slowly both chemically and in terms of strength loss. It is interesting to note that although mechanically frail, Phosphate-doped 0.01 did not chemically degrade faster than that of Carbonate-doped 0.01.

3.7.3. **SEM images of Doped-CPP degradation**

Images for both disc and fracture surfaces are shown below, for pure CPP (Figure 3.7.2), Phosphate-doped 0.01 (Table 3.7.2), and Carbonate-doped 0.01 (Table 3.7.3). Micrographs of as-made specimens (no degradation) are included for comparison.

SEM images of partially degraded specimens revealed that construct surface changes are more prominent in both pure and doped CPP. Bristle-like features were observed on the surface of Phosphate-doped 0.01 and Carbonate-doped 0.01 degrading discs (Figure 3.7.3-4, Days 5 and 15, disc surfaces, white arrows). These were not observed on fracture surfaces of these
specimens, suggesting that they occur as a result of direct contact with the solvent during degradation. These were also generally found on the cross section of a sintered particle, which was exposed during specimen cutting. However, despite the same preparation, these fibre-like structures were not observed on pure CPP disc surfaces. Rather, CPP disc and fracture surfaces show signs of laminar material separation (Figure 3.7.2, Day 15, disc surface, red arrows). As expected, degradation up to 15 days did not influence the interior of crystalline particles, since fracture surface images appeared little different from as-made images for these three groups.
<table>
<thead>
<tr>
<th>Time point</th>
<th>Disc surface</th>
<th>Fracture surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>(as made)</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>Day 0</td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td>Day 5</td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
<tr>
<td>Day 15</td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
</tr>
</tbody>
</table>

**Figure 3.7.2.** SEM images of degraded pure CPP. Discs surfaces (left) and fracture surfaces (right) are presented for material surface and fracture morphology. Degradation length is shown on the left-most column.
Figure 3.7.3. SEM images of degraded Phosphate-doped 0.01. Discs surfaces (left) and fracture surfaces (right) are presented for material surface and fracture morphology. Degradation length is shown on the left-most column.
Figure 3.7.4. SEM images of degraded Carbonate-doped 0.01. Discs surfaces (left) and fracture surfaces (right) are presented for material surface and fracture morphology. Degradation length is shown on the left-most column.
4. Discussion

4.1. Effect of doping on crystalline phase composition of sodium-doped CPP

4.1.1. Experimental results

Doping concentrations from 0.5 to 0.001 Na₂O/CaO were considered in this study. With the aim of obtaining a single crystalline phase, thermograms of CPP doped with sodium phosphate, sodium hydroxide, and sodium carbonate (labelled Phosphate-doped, Hydroxide-doped and Carbonate-doped, respectively) were compared to that of pure CPP. Although it was expected that glass transition endotherms would be weaker in these thermograms than those reported by Ropp[94], it was surprising to find that well-defined melting endotherms of Hydroxide-doped 0.5 and Carbonated-doped 0.5 could not be identified. Two melting peaks were observed for the Phosphate-doped 0.5 thermogram, indicating that at this concentration more than one crystalline phase had formed. Therefore, the 0.5 Na₂O/CaO composition was not considered further. It is interesting to note that the thermogram of Phosphate-doped 0.5 matched the glass fibre results presented by Ahmed et al. [127], who had a similar chemical composition (50 mol% P₂O₅, 35 mol% CaO, 15 mol% Na₂O), but a different DTA heating rate (20°C/min instead of 10°C/min used here). At this concentration, Ahmed et al.[113] reported that the main peaks in the XRD patterns correspond to NaCa(PO₃)₃ (PDF# 23-0669).

The lower limit of sodium doping was established by the sodium content in the calcium phosphate precursor, Ca(H₂PO₄)₂•H₂O. The material contained the equivalent of 0.0002 Na₂O/CaO (see Section 2.1), but after processing, the sodium content of the resulting CPP glass increased to 0.0065 ± 0.0001 Na₂O/CaO, as measured by ICP-AES (Table 3.3.1). This effectively became the lower limit for the doping study, and specimens doped at 0.005 and 0.001
Na2O/CaO were discarded. This result was surprising, as it is unclear how sodium could have been introduced during synthesis. However, it would account for the observation (Table 3.3.2) that in general among the doped groups there was slightly higher sodium content than expected. Furthermore, a similar observation was noted by Illingworth, who aimed to incorporate trace amounts of sodium into CPP, with the target concentration of 0.01 wt% Na, the equivalent of 8.72x10^-5 mol Na2O/CaO. Analysis of the resulting doped CPP glass showed that the dopant concentration was 7.06x10^-4 Na2O/CaO[141].

For the rest of the study, only 0.01 Na2O/CaO, 0.05 Na2O/CaO, and 0.1 Na2O/CaO were examined. XRD patterns of crystallized sodium-doped CPP at 0.01 Na2O/CaO, regardless of the dopant, matched that of pure CPP. Although doped CPP at 0.05 Na2O/CaO were not crystallized and assessed, DTA thermograms of these specimens were very similar to those of sodium-doped at 0.1 Na2O/CaO, containing features such as asymmetrical crystallization peaks, multiple melting peaks, and shoulders on these melting peaks. Since XRD of crystallized sodium-doped CPP at 0.1 Na2O/CaO showed a number of peaks in addition to those of pure CPP, it was concluded that the thermograms of the 0.1 and 0.05 Na2O/CaO specimens indicated the presence of more than one crystalline phase. Thus, the solubility limit of sodium into CPP is between 0.05 Na2O/CaO and 0.01 Na2O/CaO.

4.1.2. \( Na_2O\cdot P_2O_5 - CaO\cdot P_2O_5 \) phase diagrams

As mentioned, there are two main phase diagrams pertaining to the system \( Na_2O\cdot P_2O_5 - CaO\cdot P_2O_5 \), that of Morey and van Wazer [93, 130], and that of Griffith[131] (Figure 1.3.3 and Figure 1.3.4). It appears that neither the Morey-van Wazer diagram nor the Griffith diagram included data of sodium concentrations below approximately 10 wt% NaPO3, thus the solubility limit of sodium in calcium polyphosphate is not indicated in these diagrams. The differences between these two diagrams are in phase labelling and Griffith's identification of NaCa(PO3)3, at
70% [Ca(PO$_3$)$_2$]$_n$, which undergoes a peritectic reaction at 770°C. As a result, two new phase fields appear. Between 70% and 100% [Ca(PO$_3$)$_2$]$_n$, the field consists of a mixture of Ca(PO$_3$)$_2$ and NaCa(PO$_3$)$_3$, while Na$_4$Ca(PO$_3$)$_6$ (erroneously labelled NaPO$_3$ in the diagram) and NaCa(PO$_3$)$_3$ are found between 30% and 70% [Ca(PO$_3$)$_2$]$_n$. Van Wazer presented this region (the equivalent of 30 to 100 mol% Ca(PO$_3$)$_2$) as one field containing a mixture of Ca(PO$_3$)$_2$ and Na$_4$Ca(PO$_3$)$_6$, with a eutectic isotherm at 725°C.

X-ray diffraction patterns of sintered, and/or crystallized sodium-doped CPP specimens at 0.1 Na$_2$O/CaO showed a number of peaks other than those that belonging to pure CPP. The attempt to identify the phase responsible for these residual peaks showed that the most frequent match of the remaining peaks was with NaCa(PO$_3$)$_3$ (PDF-2, #23-0669). Occasionally these residual peaks matched Na$_4$Ca(PO$_3$)$_6$ (#25-0811). The phase NaCa(PO$_3$)$_3$ appeared only in Griffith’s phase diagram of the Na$_2$O•P$_2$O$_5$ - CaO•P$_2$O$_5$ system, therefore it would appear that Griffith’s phase diagram is correct. This phase diagram is further supported by the DTA results. In thermographs showing more than one melting peak, the low temperature peak occurred at ~750°C, close to the peritectic temperature of ~775°C as indicated on Griffith's diagram. The melting peak occurred at ~970°C in thermographs containing only one melting peak (sodium-doped CPP at 0.01 Na$_2$O/CaO), slightly below the reported melting temperature of pure CPP.
4.2. Effects of sodium doping on CPP sintering, strength and degradation

4.2.1. Effects of doping on amorphous CPP

Incorporating sodium phosphate, sodium hydroxide, or sodium carbonate into calcium polyphosphate glass using the present synthesis method resulted in an amorphous product, as shown by XRD of the doped groups Figure 3.2.1. The groups also have different crystallization and melting temperatures, as summarized in Table 3.1.1. Crystallization temperatures decreased as sodium concentration increased, regardless of the dopant source. Melting temperatures, or in some cases, the first of several melting peaks, also decreased as sodium content increased. Similar observations in glass transition temperatures were noted by Ahmed et al. [113, 127].

The presence of sodium is believed to influence the chain length and/or crystalline structure of calcium polyphosphate. Sodium oxide is known as a network modifier in the silicate glass system [94, 133], and it is thought to serve a similar purpose in the network forming phosphate glass system. Two monovalent sodium cations would replace one calcium cation in forming two ionic bonds with non-bridging oxygen in polyphosphate chains (Figure 4.2.1). In the case of adjacent polyphosphate chains, this new arrangement would increase the mobility of the polyphosphates species, which may improve sintering (Figure 4.2.1, middle). Upon crystallization, there are a number of positions in which sodium might reside. It is possible that either one or two sodium ions would reside at a calcium site, thus creating lattice strain due to the ionic size mismatch. In the case of one-to-one atomic substitution, electronic defects would also occur. It is also possible for sodium to reside interstitially.
4.2.1. Schematic of possible sodium interaction with calcium polyphosphate. An increased proportion of sodium is used to illustrate three possible locations of sodium. As a chain modifier (dotted oval, top), longer polyphosphate chains are broken by Na$_2$O. By substituting a calcium ion that is bound to oxygen of two distinct chain (dotted oval, middle), the resulting chains are further separated from one another. It is also possible for substitution to occur upon a calcium ion that is bound to oxygen atoms of the same chain (dotted oval, bottom). Other non-bridging oxygen atoms are assumed to be bound to hydrogen ions.

4.2.2. Effects of doping on CPP sintering

Sodium-doped CPP at either 0.1 or 0.01 Na$_2$O/CaO crystallized at lower temperatures than the crystallization temperatures identified by DTA results. XRD patterns of Phosphate-doped 0.1 and Carbonate-doped 0.1 showed that these groups crystallized by 560°C, despite crystallization temperatures of 598°C and 611°C, respectively. Hydroxide-doped 0.1 crystallized when sintered at 540°C, although its crystallization peak on the DTA occurred at 601°C. Phosphate-doped 0.01, Hydroxide-doped 0.01, and Carbonate-doped 0.01 showed signs of crystallization at 590°C, although their crystallization peaks occurred at 648°C, 642°C, and 647°C, respectively. Early crystallization could be attributed to differences in heating atmosphere, since the DTA was conducted in an inert atmosphere, while sintering occurred in air with controlled humidity (inlet 35% RH at 23°C). The presence of moisture and oxygen appears to have an effect on CPP
sintering \[111\]. Heating rate and particle size also influence the observed crystallization temperatures.

CPP substrates doped with different anions had different open porosity when sintered under the same conditions. At 0.1 Na\(_2\)O/CaO, Carbonate-doped 0.1 was more sensitive to sintering temperature than Phosphate-doped 0.1. Open porosity of crystallized Carbonate-doped 0.1 decreased from 34.3\(\% \pm 1.1\%\) to 13.2\(\% \pm 0.6\%\) when sintering temperature increased from 540\(^\circ\)C to 550\(^\circ\)C, but did not change significantly beyond that temperature. On the other hand, open porosity decreased more gradually in crystallized Phosphate-doped 0.1, resulting in approximately 10\% loss for every ten degree increase in sintering temperature, from 550\(^\circ\)C to 570\(^\circ\)C. At the lower concentration, Carbonate-doped 0.01 was also more sensitive to sintering temperature than Phosphate-doped 0.01. However, as the aim was to identify the conditions to sinter and crystallize doped CPP into open porosity close to that of pure CPP, other sintering temperatures for these groups were not investigated.

SEM images reflected the low porosity of CPP doped at 0.1 Na\(_2\)O/CaO, clearly showing the collapse of porous architecture (Figure 3.5.5 and Figure 3.5.6). Micrographs of 0.01 Na\(_2\)O/CaO at either sinter temperature (580\(^\circ\)C (Figure 3.6.4), or 590\(^\circ\)C (Figure 3.6.5)) showed an open pore structure, comparable to that of pure CPP. An interesting feature common to both dopant concentrations is the appearance of internal porosity, as marked by arrows. Although sintered CPP also contains some internal voids (Figure 3.6.4-5, CPP fracture surface), as noted by Omelon and estimated to be about three percent \[89\], those found in sodium-doped CPP were larger, some of which were exposed to the surface (Hydroxide-doped 0.01, sintered at 580\(^\circ\)C, Figure 3.6.4), while others have developed into an internal, porous network (Phosphate-doped 0.1, sintered at 560\(^\circ\)C, crystallized at 718\(^\circ\)C, Figure 3.5.6). If sodium doping into polyphosphate
did allow greater mobility of phosphate chains, this could have contributed to more rapid growth of the pores within the particles.

**4.2.3. Effects of doping on CPP mechanical strength**

Diametral strengths of Phosphate-doped and Carbonate-doped CPP showed marked improvement compared to pure CPP of similar open porosity. The characteristic strengths of Phosphate-doped 0.1 and 0.01 were similar for comparable open porosity (Table 3.5.3 and Table 3.6.2). When sintered at 550°C and crystallized, Phosphate-doped 0.1 had open porosity of 34.0±1.5% and characteristic strength of 13 MPa, while Phosphate-doped 0.01 achieved 33.1±2.5% open porosity and a characteristic strength of 16 MPa, when sintered at 590°C and crystallized. Due to the small sample sizes in these two groups, which resulted in an uncertainty of approximately 10% (90% confidence intervals) in the characteristic strength, there were no statistical differences in the two strength values. It is clear though, that both groups had higher strength values than undoped CPP, whose diametral strength was 7.5 ±0.75 MPa with an open porosity of 34.5± 0.8%. Carbonate-CPP also seemed stronger than pure CPP. Carbonate-doped 0.1 and 0.01 had strengths of 9 MPa and 18 MPa, at open porosities of 34.5± 1.1% and 36.5± 0.6% respectively. The increase in strength at lower doping concentration suggests a dependence upon concentration, which was not noticeable in Phosphate-doped CPP. This also suggests that dopant source has an influence upon the sintering and mechanical strength of the sintered, crystallized substrates.

These differences are unique in that compared to pure CPP, doped CPP substrates had increased in strength without further densification. While strength increases could be explained by differences in the size of sinter necks, when combined with similarity in open porosity, the results suggest differences in the macroscopic driving forces that occur during sintering, namely the competition between densification and coarsening. Densification results in a reduction in
pores, thus producing substrates of greater bulk density. On the other hand, coarsening lead to an increase in pores [133]. Different extent of this competition may have contributed to doped and undoped CPP having similar open porosities, but different amounts of sinter necks. The presence of more mobile polyphosphate chains, having decreased viscosity, may have helped to promote coarsening, thus preventing further densification of the substrates. Having shorter chains would also produce more end-chain phosphates, which may be more susceptible to reactions with moisture in the atmosphere during sintering.

4.2.4. Effects of doping on CPP degradation

Substrate degradation was not only dramatically affected by both doping, but by dopant source as well. Both Phosphate-doped 0.01 and Carbonate-doped 0.01 degraded chemically more slowly than pure CPP in phosphate-buffered saline (pH 7.4). As shown below, % calcium released at the end of degradation was highest in pure CPP, while the two doped groups had similar values.

Table 4.2.1. Doped and pure CPP chemical and mechanical degradation.

<table>
<thead>
<tr>
<th>Group</th>
<th>CPP</th>
<th>Phosphate-doped 0.01</th>
<th>Carbonate-doped 0.01</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Ca released</td>
<td>0.98% ± 0.07%</td>
<td>0.28% ± 0.01%</td>
<td>0.22% ± 0.01%</td>
</tr>
<tr>
<td>Fractional loss in strength</td>
<td>0.25</td>
<td>0.56</td>
<td>0.13</td>
</tr>
</tbody>
</table>

This reduced chemical degradation could be explained also with decreased doped glass viscosity. In addition to greater diffusion towards sinter necks, grain growth is promoted at increased chain mobility. Compared to CPP then, crystalline doped CPP may have greater grains and less grain boundary area. Since grain boundaries have less surface energy than grain surfaces, doped CPP degradation may have been affected.
The fractional loss in strength is determined by dividing the difference between initial and final (after degradation) characteristic strengths by the initial strength. The fractional strength loss is highest for Phosphate-doped 0.01, while that of Carbonate-doped 0.01 is less than that of pure CPP. Dopant sources then, seemed to contribute to the mechanical strength differences of CPP substrates.
4.3. Dopant anion effects on CPP

4.3.1. Choice of dopants

The previous sections outlined the observed dopant anion effects for CPP doped at 0.01 Na$_2$O/CaO. In this study, sodium phosphate was chosen as it maintains the same cation oxide to diphosphorus pentoxide ratio as the calcium phosphate precursor. Sodium carbonate was introduced to the study as it was hypothesized that only sodium cations would be introduced into calcium polyphosphate. This was due to the fact that the aqueous sodium carbonate solution reacted with calcium phosphate monobasic monohydrate during slurry synthesis, and generated effervescence, which was thought to be carbon dioxide, and so sodium remained in the slurry. Sodium hydroxide was selected as the source which would introduce both sodium and hydroxyl ions to CPP.

4.3.2. Hydroxide-doping effects on CPP

One of the most evident observations about dopant source effects is that Hydroxide-doped 0.01 crystallized during the sintering step (580ºC or 590ºC), but did not form sufficient sinter necks, and open porosity of the substrates was not influenced by sintering temperatures. It possessed low diametral strength, likely due to its lack of sinter necks, as observed by SEM (Figure 3.6.4 and Figure 3.6.5). XRD patterns showed that these specimens crystallized at the end of sintering. It seems possible that hydroxide contributed to these effects, since they were not observed in doped-CPP groups using other sources.

As network modifiers, both sodium oxide and the hydroxide ions may have broken polyphosphate chains, or prevented its formation. An increase of hydroxide ions will shift the condensation/hydrolysis equilibrium towards the left (below):

$$- [P-O-P]_n-OH + HO-[P-O-P]_m \leftrightarrow -[P-O-P]_n-O-[P-O-P]_m + H_2O(g) \quad [1]$$
This may also shift the resulting chain length distribution towards shorter chains overall, which may increase polyphosphate glass mobility and thus sintering, but at the same time, shorter chains may have greater short-range order, and so it may crystallize more readily than longer chains. In the competition between densification and crystallization, it appeared that crystallization was favored, rather than densification and the formation of sinter necks.

4.3.3. Phosphate-and Carbonate-doping effects on CPP

Phosphate-doped CPP showed some improvements in as-made mechanical strength compared to mechanical strength of undoped CPP of the same % open porosity. Comparing the chemical composition of the Phosphate-doped 0.01 and pure CPP (Table 3.3.2), one finds that the two to have similar phosphate content. This suggests that the differences observed, namely: lower sintering temperature required to sinter substrates of comparable porosity as pure CPP, increased as-made diametral strength, greater strength loss during \textit{in vitro} degradation, decreased chemical degradation \textit{in vitro}, can be attributed to the effect of the sodium cation. Comparison of the micrographs showed that the laminar structure of crystalline Phosphate-doped CPP, as shown on fracture surface images, seemed more ordered than CPP (Figure 3.6.4 and Figure 3.6.5).

The behaviour of Carbonate-doped CPP is interesting. It was assumed that this dopant would only contribute sodium into calcium polyphosphate, but sintered, crystalline Carbonate-doped CPP has higher characteristic strength than Phosphate-doped CPP, for which sodium was also proposed as main influence. Carbonate-doped CPP was also found to be more sensitive to sintering temperature than Phosphate-doped CPP. These observations then, suggest that there is a hidden anion effect from this dopant source. Although it was assumed that \textit{CO}_2 bubbles generated from the reaction between sodium carbonate and acidic, slightly soluble \textit{Ca(H}_2\textit{PO}_4\textit{)}_3\textit{H}_2\textit{O} during slurry mixing subsequently escaped into the atmosphere, the exact carbonate content in the doped CPP was not determined analytically. Therefore, it is possible that
upon drying of the slurry, trace amounts of planar carbonate, or carbon dioxide, was left behind and incorporated into the glassy tetrahedral phosphate network.

This hypothesis may help to describe the marked differences in fractional strength loss after degradation between Phosphate-doped and Carbonate-doped CPP (Table 4.2.1). Assuming that Phosphate-doped CPP was only modified by sodium doping, then Carbonate-doped CPP might be affected by doping of sodium and a carbon compound. As it is unclear which carbon compound might be incorporated, a number of possibilities exist. Being tetravalent ion with smaller ionic radius than calcium, it may be possible for one carbon ion to directly replace one calcium ion, but charge balance must be satisfied either by the displacement of another calcium ion, or the reconfiguration of the polyphosphate tetrahedra. Both may translate into defects in the material upon crystallization. An additional possibility is that the carbon compound may be present as an impurity in the polyphosphate grains [142], with no chemical interaction to the polyphosphate chains structure.
5. **Summary and Conclusions**

This project investigated the doping of calcium polyphosphate (CPP) with low concentrations of sodium to avoid the formation of more than one phase in the crystalline material. This helps to maintain the favourable properties that make CPP part of the biphasic tissue-engineered cartilage/substrate strategy [52, 84, 86, 87]. In addition to CPP's bioactivity, these constructs are osteoconductive [67] and have an open, interconnected pore structure for bone integration. They also possess sufficient mechanical strength to sustain loading conditions at defect sites [52, 84]. Doped CPP constructs must also possess these favourable properties. As noted in previous work, the degradation rate of pure CPP is lower than desired [88, 89] for the observed mechanical strength loss. It would be desirable for doped CPP to have a faster chemical degradation, without increasing its mechanical strength loss.

The conclusions drawn from the results of this study are as follows:

- The solubility limit of sodium in calcium polyphosphate (M$_2$O/P$_2$O$_5$ = 1) is between 0.01 and 0.05 Na$_2$O/CaO.

- XRD of crystallized sodium-doped CPP at 0.1 Na$_2$O/CaO contains peaks that match those of pure CPP, as well as other peaks that match NaCa(PO$_3$)$_3$ (PDF #23-0669). This shows that Griffith’s phase diagram of Na$_2$O•P$_2$O$_5$ – CaO•P$_2$O$_5$ is correct.

- Doping CPP with sodium at 0.01 Na$_2$O/CaO, using any of Na$_2$HPO$_4$, NaOH, or Na$_2$CO$_3$ yields a crystalline material whose XRD patterns that match that of pure CPP.

- Sodium hydroxide-doped CPP at 0.01 Na$_2$O/CaO did not sinter.
• When compared to pure CPP of similar open porosity, Phosphate-doped CPP at 0.01 Na₂O/CaO has similar diametral strength, while Carbonate-doped CPP at 0.01 Na₂O/CaO has greater diametral strength. Hydroxide-doped CPP at 0.01 has very low strength.

• Both Phosphate-doped CPP at 0.01 Na₂O/CaO and Carbonate-doped CPP at 0.01 Na₂O/CaO released less calcium during *in vitro* degradation than pure CPP, and thus degraded more slowly. Therefore, the more rapid degradation reported in studies in which sodium was added to calcium polyphosphate should be attributed to the presence of second phases.

• Carbonate-doped CPP at 0.01 Na₂O/CaO experienced less strength loss from *in vitro* degradation than pure CPP, while Phosphate-doped CPP at 0.01 Na₂O/CaO lost more of its strength than pure CPP.

• The dopant anion was found to have a significant effect on sintering, strength and degradation of CPP substrates.
6. **Recommendations**

This study focused upon single-phased doped CPP using sodium compounds as dopants.

Recommendations based on these results and the available literature, are as follows:

- Explore CPP doping using other salts, to determine the effects of doping with cations of similar and/or different sizes and charge, compared to calcium. It would be important towards the tailoring of CPP substrates for tissue-engineered cartilage, if one can determine whether charge or size differences have a greater impact on doped-CPP. Anion selection must also be considered as they may also affect doped-CPP properties. Dopant selection should also consider compounds that may have an added biological/tissue-engineering benefit.

- Clarify the Na$_2$O•P$_2$O$_5$ – CaO•P$_2$O$_5$ phase diagram by identifying equilibrium species at low concentrations of Na$_2$O•P$_2$O$_5$, and compiling the existing diagrams.

- Determine how the $\beta$-CPP structure was affected by sodium-doping, to clarifying the dopant effect on crystalline structure.

- Explore sintering conditions to determine whether it is possible to produce an open-pore structure with internal porosity and sufficient mechanical strength to serve as substrate for osteochondral tissue-engineered cartilage. The resulting shell-like structure will increase total available pore volume for gradual bone in-growth, and the same volume will contain less material for bioresorption.
7. References


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