# Manufacture of biomaterials by a novel printing process

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Tricalcium phosphate (TCP) scaffolds with controlled internal porosity were fabricated with a suspension of TCP in diacrylate cross-linking monomers using a mold prepared by ink-jet printing. Scaffolds were removed by selective dissolution of the mold. They were heat treated for removal of the acrylic binder followed by sintering. Despite a considerable linear shrinkage, scaffold porosity was retained after sintering. Composite scaffolds were fabricated from TCP in poly(ethylene glycol) diacrylate using an identical gel casting route.


## 1. Introduction

Recent advances in cell isolation and culture procedures, combined with growing understanding and use of molecular biology and biochemistry techniques, have resulted in the establishment of a new field of biological/biomedical research: cellular and tissue engineering. A key requirement in this field is the development of scaffold structures on which cells adhere. There has been much work in developing an understanding as how cells interact with, both natural and artificial, supporting structures [1, 2]. In parallel with these advances in the life sciences, new methods of manufacture have been developed in the field of rapid prototyping that allow the fabrication of complex shapes with features in the 10–100 μm range directly from computer aided design (CAD) files [3–5]. These are also termed freeform fabrication or layered manufacturing to identify the moldless manufacturing method and their sequential deposition of slices to build up an object. These developments offer exciting possibilities for tissue engineering applications [6].

The scaffold is a key component of tissue engineering. Several requirements have been identified as crucial for the production of tissue engineering scaffolds. These are that the scaffold should: (1) have appropriate surface chemistry to favor cellular attachment, differentiation, and proliferation; (2) be made from a biodegradable or bioresorbable material, that degrades at an appropriate rate with no undesirable by-products, so that tissue will eventually replace the scaffold; (3) possess adequate mechanical properties to match the intended site for implantation and handling; (4) be easily fabricated into a variety of shapes and sizes; and (5) possess interconnecting porosity so as to favor tissue integration and vascularization [7, 8].

Porous hydroxyapatite (HA) has a long history of development and is now regularly used in clinical practice. Conventional methods of forming porous HA use ion exchange with coralline structures or the use of sacrificial organic or inorganic particles to generate porosity. These provide only a very crude level of control over the internal architecture, size, and distribution of the porosity within a HA implant. An ability to control the porosity of HA implants is important because internal porosity is known to control the extent of bone regeneration [9–11] and also to influence the mechanical properties of the material [12–14].

Chu and Halloran have developed the rapid prototyping technology of stereolithography to produce HA based porous implants. A suspension of HA, with 40% solids loading by volume, was prepared in a bifunctional acrylate, which was the cured using a thermally initiated free-radical mechanism [15]. A sacrificial mold was fabricated using stereolithography, into which the slurry was poured and thermally cured using the gel-casting process [16]. The resulting polymer/ceramic composite was processed to remove the polymer and sintered to form a porous HA structure. These HA scaffolds have been developed to the extent that extensive *in vivo* studies have now been carried out in animal models [17].

HA is one of a family of calcium phosphate phases that have chemical and structural similarity to the biomineralized apatite phase found in skeletal tissue. Another phase, tricalcium phosphate (TCP), has also attracted interest as a biomaterial for skeletal prosthesis. HA is bioactive and through the attachment of osteoblasts can integrate successfully with biological hard tissue. However, this is a surface reaction and any HA implant, whether solid or porous, will remain intact within an organism after implantation. TCP behaves in a different way and can be resorbed by dissolution and biointeraction with animal tissue after implantation. TCP can thus be used, in principle, as a temporary scaffold for tissue engineered skeletal prostheses. For such a TCP structure, controlled internal architecture is more important than with HA implants because the structure has to act as a scaffold for the support of cells throughout its interior.

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Indeed, the internal porosity may be required to provide for vascularization during tissue regeneration.

In this study we report on the manufacture of porous TCP and polymer/TCP composite porous structures using fabrication methods different to, yet inspired by, those used by Chu and Halloran. These porous structures are made using a lost mold technique but the technique used to fabricate the sacrificial molds is ink-jet printing. The ceramic suspensions are prepared using a gel-casting technique. We have chosen to study polymer/ceramic composites in addition to porous ceramic structures as these provide a method of controlling cell adhesion by the choice of polymer surfaces with appropriate surface chemistry. Here we will describe the use of a polymer based on a highly cross-linked polyethylene glycol (PEG). This has been chosen because it is a hydrophilic polymer and this is believed to promote cell adhesion.

## 2. Materials and methods

### 2.1. Materials

A gel-casting system based on isobornyl acrylate (IBA, Sigma-Aldrich, Gillingham, Dorset, UK) and propoxylated neopentoglycol diacrylate (PNPGDA, Sigma-Aldrich, Gillingham, Dorset, UK) was used with ammonium persulfate (BDH, Poole, UK) as a thermal initiator and quaternary amine N,N,N′,N′-tetramethylethylene diamine (TEMED, Sigma-Aldrich, Gillingham, Dorset, UK) as a catalyst. This is identical to the originally used by Chu and Halloran for gel casting HA. In this study TCP (Plasma Biotal, Buxton, UK) was used as the ceramic phase. This had a mean particle size of 0.33 μm and a specific surface area of 4.899 $m^2/g$ as measured by Mastersizer (Malvern Instruments, Malvern, UK) and BET (Coulter SA3100, Coulter Corporation, Miami, Florida, USA), respectively. Dispex A40 (Allied Colloids, Bradford, UK) was used as a dispersant.

Poly(ethylene glycol) diacrylate oligomer (PEGDA, Sigma-Aldrich, Gillingham, Dorset, UK) and poly(ethylene glycol) dimethacrylate oligomer (PEGDMA, Sigma-Aldrich, Gillingham, Dorset, UK) were used without further purification for composite scaffold studies. Quaternary ammonium acetate (Variquat CC-55, Goldschmidt Chemical Corporation, Dublin, OH, USA) was used as a dispersant and benzoyl peroxide (BPO, Sigma-Aldrich, Gillingham, Dorset, UK) was used as a thermal initiator for conventional free-radical polymerization.

### 2.2. Mold preparation

Molds were fabricated using a commercial ink-jet machine developed for rapid prototyping applications, Modelmaker II (MMII: Solidscape, Merrimack, NH, USA). This prints two materials: a build material (Protobuild: Solidscape), which defines the shape to be manufactured, and a sacrificial support material (Protosupport: Solidscape), which supports thin vertical structures and provides a base to develop overhanging features. Both materials have low melting points close to 100 °C and are printed at elevated temperature onto a substrate at room temperature. On impact the materials solidify to leave a layer approximately 70 μm in thickness. Three-dimensional objects are constructed using a layer building strategy. The MMII accepts the design to be built in a standard STL format file and converts this into a series of slices with a defined thickness. The MMII then prints each layer before lowering the printer table and overprinting a subsequent layer. In order to prevent cumulative errors developing in the *z*-direction from the deposited surface roughness, each layer is milled flat by a rotary cutter after deposition. The Protosupport material is removed after printing by dissolution in a solvent (Bioact: Solidscape) to leave the desired 3D structure.

### 2.3. Ceramic scaffold preparation

IBA and PNPGDA were mixed in the ratio of 1 : 1 by weight. This blend was then mixed with distilled water to prepare a 30 vol % monomer solution. TCP powder and Dispex A40 (5 wt % based on TCP) were then dispersed in the monomer solution using ball milling in a polyethylene container with $ZrO_2$ milling media for 20 h to make a suspension with 35% by volume solids. After milling, the catalyst and initiator were added to the suspension and mixed vigorously. The suspension plus catalyst and initiator were then immediately cast into the porous negative mold. This mold then was put in a vacuum chamber (water pump) for de-airing for 30 min. The suspension was then gelled by holding at 60 °C for 30 min. After a solid ceramic/polymer compact had formed, the negative mold was removed by dissolving in acetone at ambient temperature, leaving only the porous specimen. This specimen was then heat treated at 550 °C for 1 h, to burn out the polymer binder. The ceramic body was then heated to 1350 °C at a heating rate of 4 °C/min and held for 2 h to sinter.

### 2.4. Composite scaffold preparation

Scaffolds were fabricated by gel casting into molds as detailed in the previous section. The suspension was prepared by ball milling as previously described but with PEGDA oligomer (m.w. 258) and Variquat as the dispersant. Viscosities of the suspensions with varying amounts of TCP were studied using Brookfield digital rheometer, model DV-III + (Brookfield Engineering Laboratories, Middleboro, MA, USA). This uses a concentric cylinder geometry and was fitted with a small sample chamber.

For thermal polymerization, 0.5 wt % BPO (based on oligomer) was added to the suspension and the polymerization was carried out at 80 °C. Scaffolds were prepared using a lost mold technique as described earlier.

## 3. Results and discussion

### 3.1. Ceramic scaffolds

In order to fabricate reliable scaffold structures by the gel casting method, ceramic slurries must possess a sufficiently low viscosity for penetration to occur throughout a complex mold with minimum section size of the order of 100 μm. From Chu and Halloran's work [16, 17] we chose an arbitrary maximum viscosity of

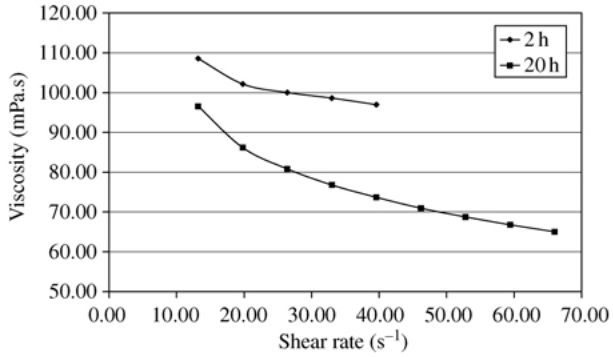


*Figure 1* Viscosity of the 35% volume TCP in IBA/PNPGDA suspension as a function of shear strain rate.

100 mPas as that allowable for our TCP suspensions in IBA-PNPDGA. Fig. 1 shows the viscosity of the 35% volume suspension as a function of shear strain rate. The slurries show thinning as the strain rate increases and this can be interpreted as the slurries developing a structure, possibly with alignment, at higher strain rates. The slurries show an interesting aging behavior, with viscosity significantly reduced 20 h after the addition of the dispersant. The reason for this is the subject of further study.

Fig. 2 shows an image of the thermoplastic mold and a sintered ceramic scaffold. It is evident that there has been considerable shrinkage after polymer removal and sintering. Fig. 3(a) shows a detail of the mold structure viewed by optical microscopy, and Fig. 3(b) the sintered TCP ceramic produced from it. The average pore size defined by the mold is 460 μm. After sintering the pore size in the ceramic is approximately 360 μm. This shows a linear shrinkage of approximately 22%. Given that our initial volume fraction of ceramic in suspension is 35%, a linear shrinkage of 30% would be predicted. Thus the sintered TCP in the structure is expected to contain a significant porosity.

## 3.2. Composite scaffolds

Polymer/ceramic composites have many potential attractions as tissue scaffold materials. In order to be degradable within the body polyester or other hydrolysable bond units are needed within the polymer chain

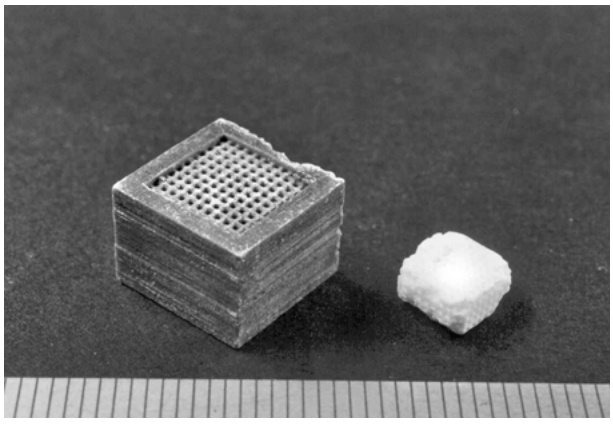

*Figure 2* Mold fabricated by ink jet printing shown alongside a sintered TCP scaffold produced from the mold; scale division 1 mm.

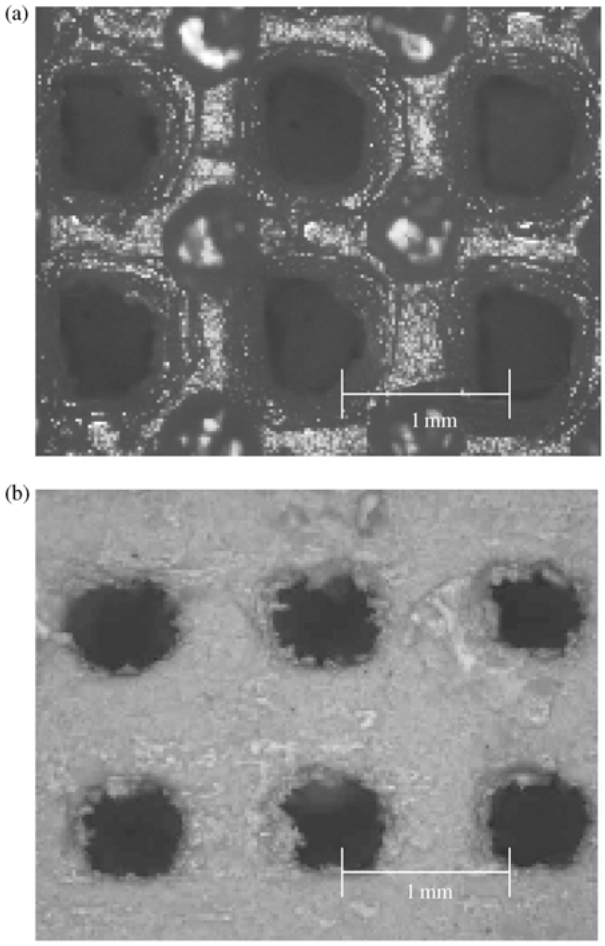


*Figure 3* Detail of interior of scaffold structures, (a) printed mold, and (b) sintered TCP ceramic.

or network. Many polyester biomaterials are well characterized and have regulatory approval for surgical applications. These are often synthesized from cyclic oligomers (e.g. ε-caprolactone) and these show low viscosity (Table I). However, many polyesters have hydrophobic surfaces and show poor cell adhesion characteristics. Model hydrophilic polymers based on PEGDA and PEGDMA oligomers were considered and their viscosities are given in Table I. Because of the relatively high viscosity of the oligomers, it is important that an efficient dispersant is selected to minimize TCP suspension viscosity. Two commercial dispersants, Emphos CS1361 (aromatic phosphate ester, Witco S.A., Paris, France) and Variquat CC-55 were screened with a dilute, 5% by volume, suspension of TCP in PEGDA (m.w. 258) and their viscosities measured. These are given in Table II and it can be clearly seen that Variquat generates the lowest viscosity. Fig. 4 shows the

TABLE I Viscosity of a range of monomers considered for polymer/ceramic composites

| Monomer/oligomer | Molar weight | Viscosity (mPa · s) | Shear rate ($s^{-1}$) |
|---|---|---|---|
| ε–Caprolactone | 114 | 6 | 200 |
| PEGDA258 | 258 | 13 | 200 |
| PEGDA575 | 575 | 70 | 50 |
| PEGDMA330 | 330 | 13 | 200 |
| PEGDMA550 | 550 | 40 | 100 |

TABLE II Viscosity of 5% TCP suspensions using 5% by weight dispersant, measured at a shear rate of $180\,s^{-1}$

| Dispersant | Manufacturer | Viscosity (mPa · s) |
|---|---|---|
| Variquat CC-55 | Goldschmidt, Dublin, OH, USA | 16 |
| Emphos CS1361 | Witco S.A., Paris, France | 23 |
| Variquat + Emphos | 1 : 1 by weight | 21 |

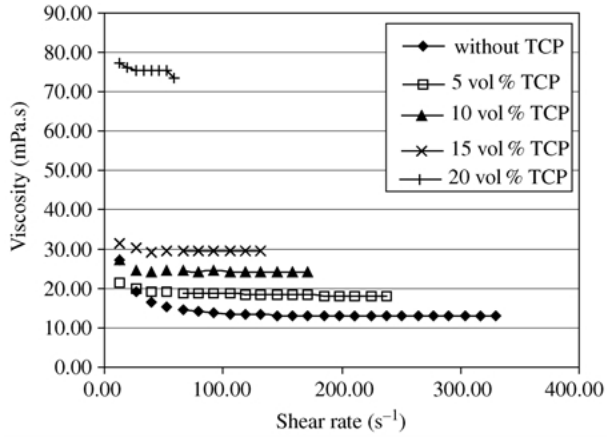


*Figure 4* Viscosity of TCP in PEGDA 258 suspensions with different fractions of solid, made using Variquat CC-55.

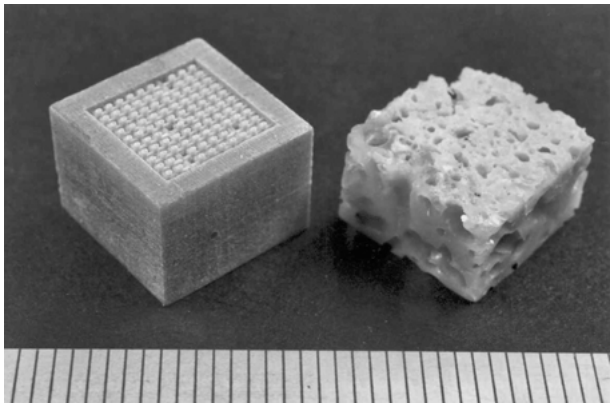

*Figure 5* Printed mold and thermally crosslinked composite scaffold; scale division 1 mm.

viscosity of TCP/PEGDA 258 suspensions with different fractions of solid, made using Variquat. The suspension viscosity shows a rapid increase with a solids fraction of 20% and this was the highest solid fraction judged to be practical for use with the printed molds.

Fig. 5 shows a printed mold adjacent to a thermally crosslinked scaffold produced from a suspension of 20% by volume TCP in PEGDA258 using Variquat as the dispersant. There has been considerable distortion and discoloration of the scaffold. This can be attributed to a thermal runaway reaction that occurred after gelation was initiated. Gelation occurs by a free-radical mechanism which evolves heat. As the gelation is initiated the local increase in temperature rapidly generates more free radicals, these cause a rapid increase in crosslinking rate and a rapid evolution of heat. This heating raised the temperature of the gelling oligomer to greater than the melting temperature of the mold, which caused the distortion and incorporation of the mold material within the cross-linked composite.

## 4. Conclusions

We have demonstrated that a lost mold process can be successfully used to prepare scaffolds from TCP with a controled internal porosity. The molds were produced by an ink-jet printing process and could, in principle, be used to produce molds with an engineered internal structure with a feature resolution $< 100\,\mu m$. Despite a linear shrinkage of about 22%, the sintered ceramic shows a regular internal porous structure as defined by the mold. A similar route was used to produce a TCP/PEGDA composite which was believed to show a greater potential for cell adhesion. In this case mold distortion occurred because of excessive heat evolution from the gelling composite suspension. Despite this, we believe that the lost mold process shows considerable potential for tissue scaffold applications.

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