ORIGINAL ARTICLE

Preparation and properties of three dimensional printing materials made from biopolymers for medical applications

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Abstract

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A preliminary study employing a mixture of natural polymers for three dimensional printing (3DP) technology was carried out to determine the influence of mixture composition and post-processing technique on their physical and mechanical properties. Series of blended natural polymers including cassava starch, maltodextrin, cellulose fiber and gelatin with different amount were formulated. It was observed that the percentage of individual component influenced the properties and characteristics of prepared samples including part stability, dimension accuracy and flexural properties. Starch aided part stability and the fineness of the mixture. Maltodextrin and gelatin increased flexural strength whereas cellulose fiber helped in both part stability and strength. Infiltration by light-cured resin could further enhance flexural modulus and flexural strength of samples to be close to generally used acrylate resin. Preliminary *in vitro* toxicity test of infiltrated sample showed that the cells which were in contact with samples were healthy. No inhibition zone was observed.

Key words : three dimensional printing, implant, rapid prototyping, natural polymer, medical

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บทคัดย่อ

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การเตรียมและสมบัติของพอลิเมอร์ธรรมชาติที่ขึ้นรูปด้วยเครื่องพิมพ์สามมิติสำหรับการใช้งานทางการแพทย์

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บทความนี้แสดงการศึกษาเบื้องต้นในการพัฒนาส่วนผสมของพอลิเมอร์ธรรมชาติที่เหมาะสำหรับการขึ้นรูปด้วยเทคนิคการขึ้นรูปทรงแบบอิสระประเภทเครื่องพิมพ์สามมิติสำหรับการประยุกต์ใช้งานทางการแพทย์ โดยทำการศึกษาถึงผลกระทบของส่วนผสมของพอลิเมอร์ธรรมชาติต่าง ๆ ได้แก่ แป้งมันสำปะหลัง มัลโตเด็กซ์ทริน เส้นใยพืช และเจลาติน จากการศึกษาพบว่าการเปลี่ยนแปลงอัตราส่วนของส่วนผสมของวัตถุดิบดังกล่าวจะส่งผลต่อสมบัติของชิ้นงานที่ขึ้นรูปต่าง ๆ ได้แก่ ความเสถียรของรูปทรง ความถูกต้องของขนาด และสมบัติความต้านทานการดัดงอ โดยส่วนผสมของแป้งมันสำปะหลังจะช่วยในการสร้างความเสถียรของรูปทรงและความละเอียดของผิวชิ้นงาน มัลโต-เด็กซ์ทรินและเจลาตินจะช่วยเพิ่มความแข็งแรงของชิ้นงาน ส่วนเส้นใยพืชจะช่วยทั้งการเพิ่มความเสถียรของรูปทรงและความแข็งแรงของชิ้นงาน นอกจากนี้ การอัดแทรกชิ้นงานด้วยเรซินไวแสงในกลุ่มอะคริเลตพบว่าสามารถเพิ่มค่ามอดูลัสของการดัดงอและความต้านทานแรงดัดสูงสุดของชิ้นงานให้มีค่ามากขึ้นใกล้เคียงกับเรซินอะคริลิกที่ใช้งานทางการแพทย์ทั่วไป ผลการทดสอบความเป็นพิษเบื้องต้นของชิ้นงานที่ผ่านการอัดแทรกพบว่าชิ้นงานไม่แสดงความเป็นพิษแต่อย่างไร

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In reconstructive or cosmetic surgery, implants are frequently employed to replace or augment the defective or missing parts of the bones to restore the aesthetics or functionality of the organs. Allograft and autograft are commonly utilized as implants, but synthetic materials which can be produced in large quantities, including calcium phosphates, silicone, polyethylene and poly (methyl methacrylate), are also used as alternatives. However, these implants are normally available in simple forms that do not match the unique anatomical constraints of the defective sites and require manual modifications of the host bone or the implants themselves by surgeons. This process takes time and is generally not anatomically accurate especially for complex cases.

Freeform fabrication or rapid prototyping is a relatively new technology that additively builds three dimensional parts layer by layer in contrast to the traditionally substractive process. This approach allows the complex physical structures to be fabricated rapidly and accurately using graphical data in computer. Freeform fabrication has been utilized in medical applications as visualization and modeling tools to aid with pre- or per-operative planning and production of patient models or implants (Potamianos *et al*., 1988; Jee *et al*., 2000; Sanghera *et al*., 2001). Starting from the digital data of patient's organs acquired from medical imaging system such as computerized tomography (CT) or magnetic resonance imaging (MRI), the medical models can be fabricated easily and accurately in a short time by freeform fabrication technology. Implants can be designed digitally to fit the host site and to be aesthetically correct for individual patient prior to the surgery. Freeform technology is then normally employed to build positive components having the shape and size of the desired implants. This positive component is then used to create a silicone or plaster mould for further casting by biomedical materials such as bone cement or dental acrylic (Oris *et al*., 2002). Alternatively, a mould with the internal structure according to the size and shape of the implants

can also be made instead, but less frequently than the previous method. These indirect processes are multi-stepped and prone to add error in dimension to the implant model unless carefully planned. This is due to the fact that freeform fabrication techniques require the use of specific raw materials for working and these materials are usually not designed for biomedical applications. The lack of biomaterials that can be used with freeform fabrication systems limits the direct fabrication of implants. Therefore, a number of biomaterial systems that have properties and characteristics appropriate for processing by some freeform fabrication technologies have been studied recently to overcome the limitation (Cooke *et al*., 2002, Zein *et al*., 2002, Tan *et al*., 2003, Rimell *et al*., 2000).

One of the technologies that received much attention in medical application is three dimensional printing (3DP). This technology is a fast and low cost system that does not need support structure to build a model. The technique involves the spreading of a thin layer of a powdered material, followed by a selective joining of powder through printing of a binder material onto the powder bed. This operation is similar to inkjet printer, but using a powder bed instead of a paper as the medium. Subsequently, a cylinder containing the powder bed is lowered, allowing for the spread of the next powder layer. Unbound powder temporarily supports unconnected portions of the component, allowing overhang, undercut and internal volumes to be formed. These steps, printing and spreading a new layer, are repeated until the whole object is fabricated. The unbound powder is removed upon process completion by vacuum or air blowing, leaving only the finished green part. The operation also does not involve a high temperature so cells or biological materials can be incorporated, Figure 1. Until now, 3DP has been studied to directly fabricate drug delivery devices and scaffold successfully (Griffith *et al*., 1992; Giordano *et al*., 1996; Wu *et al*., 1996; Park *et al*., 1998; Kim *et al*., 1998; Lam *et al*., 2002; Koegler *et al*., 2002). However, these 3DP parts either used solvent as a binder or a polymer solution to infiltrate the part. In the case of using organic solvents as the binder, it was found that there remained 0.5 %wt (5000 ppm) chloroform on samples made by 3DP after 1 week drying (Lam *et al*., 2002), although residual chloroform extraction using liquid carbon dioxide has been investigated recently and shown to reduce the level of chloroform below 50 ppm (Koegler *et al*., 2002). On the other hand, Lam *et al*. used water as a binder with 3DP to produced starch-based polymer scaffolds to avoid the use of solvent binder. However, infiltration of the porous scaffolds with a solution of poly (L-lactide) and polycaprolactone in methylene chloride was required to increase the mechanical strength (Lam *et al*., 2002). In both ways, the residual solvent within the parts could be a possible source of toxicity. Due to its abundance and low cost, it was shown that natural polymers such as carbohydrate could be used as cost-effective biomaterials (Mendes *et al*., 2001; Gomes *et al*., 2002; Marquesa *et al*., 2002). This study was thus aimed to investigate the influence of combinations of employing natural polymers such as carbohydrate and solvent-free infiltration by low viscosity acrylate resin formulation on their physical and mechanical properties.

Materials and Methods

1. Materials

Materials employed to be mixed and processed in the 3DP machine in this study were various natural polymers including cassava starch (Thai Wah Co., Ltd), maltodextrin (Shandong Duqing Inc.), cellulose fiber (Opta Food Ingredients, Inc.) and gelatin (Geltech Co.,Ltd). These materials were supplied in the form of powders with particle size ranging 20-200 micrometres and used without further sieving. Distilled water was used as a binder for fabrication. Commercially available 3DP material type ZP 15E (Z Corporation) was also used as a benchmark for processing characteristic in 3DP machine. Infiltration material used was light cured dental sealant (Dentguard, Dentech Co., Ltd). This material is based on a combination of triethylene glycol dimethacrylate (TEGDMA), 2,2-bis[4(2-

hydroxy-3 methacryloyloxypropyloxy)-phenyl] propane (Bis-GMA) and urethane dimethacrylate (UDMA) with camphorquinone as a photoinitiator.

2. Specimen Preparation

Each type of raw material was weighed according to the proportion as shown in Table 1. They were initially stirred in a plastic bag and then thoroughly mixed by a mechanical blender. The mixture was then loaded in the 3DP machine (Z400, Z Corporation). Graphic files of rectangular bars (80 mm. x 10 mm. x 4 mm) were created in computer and the models were then arranged for printing using a layer thickness of 0.175 mm. Water was used as a binder in all formulations. After building, all the specimens were left in the machine for 2 hours before being taken out and left for drying outside for 24 hours. The specimens were then air blown to remove the unbound powders.

3. Infiltration

Post-processing of 3DP specimens was carried by infiltration method at room temperature. This was carried out by pouring a liquid infiltrant in the container. The specimen was placed on the liquid and the infiltrant allowed to enter the specimen naturally by capillary force. Then, the fully infiltrated specimen was wiped off with a tissue paper to remove excess resin on the surface of the specimens and then light cured to solidify the infiltrant portion by a halogen light for 1 hour.

4. Binder contact interaction

Prior to formulating a material system for printing, each type of material was studied to observe its individual characteristics when contacting with water that would be used as a binder. This was done by slightly pressing and leveling powder lump using a roller. A water droplet was then dropped on the powder bed using a syringe. Upon initial contact, the visual observation was made. The bed was left for 24 hours for evaporation and the wetted area was observed again.

5. Dimension measurement

Dimension of the specimen was measured by a vernier caliper (Mitutoyo) with the reading resolution of 0.01 mm. The measurement was done three times in each direction and the values were then averaged.

6. Flexural Test

Flexural tests were performed on a universal testing machine (Instron 4502) equipped with a 10 kN load cell. All the tests were carried out according to ASTM D790-03 at 23ºC and 50% RH. using three point method and a constant crosshead speed of 1.9 mm min^{-1}. The reported data are the average values from five replicates.

7. Hardness test

Hardness test was carried out using Shore D technique (Shore Instruments) according to ASTM

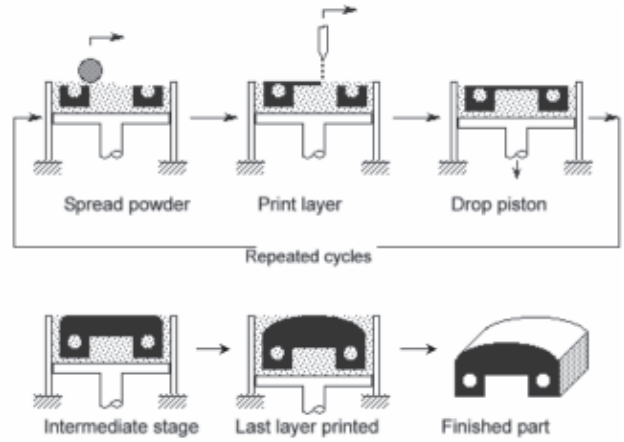

Figure 1. Three dimensional printing process.

D2240-91 at 23ºC and 50% RH. The reading was taken at 1 second after the fully applied load. The reported data are the average values from five replicates.

8. Fractography

Fracture surfaces of flexural tested samples were gold sputtered and viewed microscopically using a scanning electron microscope (JEOL, JSM-5410) at the accelerating voltage of 20 kV.

9. Raman Measurement

The cured infiltrated samples and sealant resin were characterized for the degree of conversion using Raman technique. All spectra were obtained with a Perkin-Elmer FT-Raman spectrometer system 2000R supplied with radiation of 1064 nm from Nd^{3+}: YAG laser and InGaAs detector. The power was set at 400 mW and the spectral resolution was 4 cm^{-1}. The degree of conversion (DC) of light-cured resin was calculated by the following equation:

$$DC = 100* [1-(R_{polymerized}/R_{unpolymerized})]$$

where R = peak height at 1640 cm^{-1}/peak height at 1610 cm^{-1}

10. Cytotoxicity Test

Three pieces of infiltrated samples after curing and post-heated at 100ºC for 0.5 hour were tested for toxicity by direct contact method using L-929 mouse fibroblasts following ISO 10993-02. The sample was washed by alcohol and placed in the center of the culture disc and the cultured cells were then seeded on. The incubation period was 48 hours. The morphology of cells was then observed using an inverted light microscope after staining the cells with solution of 0.01% neutral red in phosphate buffered saline.

Results and Discussion

1. Preliminary observation

All the materials used as a component in the materials mixture system were selected based on their potentially biocompatible nature and wetting property by water. Starch and gelatin were recently studied by many researchers as biomaterials whereas maltodextrin and cellulose are found in many food and pharmaceutical products (Ten Huisen *et al*., 1994; Mendes *et al*., 2001; Gomes *et al*., 2002; Marquesa *et al*., 2002; Nazzal *et al*., 2002; Usta *et al*., 2003; Levy *et al*., 2004). Hence, a combination of these materials would be a useful biomaterial. However, the mixture of these materials must have properties that are suitable to be operated on well within the parameters and constraints of the 3DP technology, which is based on the bonding of selected area in each layer of powder by an interaction between powder and binder. Therefore, the powder should absorb binder and harden sufficiently fast to stabilize the structure before the next layer of powder is spread over. Prior to formulating the material system, the interaction between each material and the binder, which was water in this investigation, was then studied. It was seen that starch, maltodextrin and gelatin could absorb water relatively faster than cellulose fiber. Gelatin was the only material that could gel upon contact with water. After water was absorbed by the bed, the wetted areas of maltodextrin and gelatin shrank away from the surrounding dry powder whereas cellulose fiber and starch showed limited shrinkage. After drying, starch was very brittle and only low force could break it easily. In contrast, cellulose fiber, maltodextrin and gelatin changed from packed powder into a homogeneous hard solid structure. Therefore, starch should help in part stability and the fineness of the mixture. Maltodextrin and gelatin deliver strength whereas cellulose fiber helped in both part stability and strength. Although cellulose fiber aids both part stability and strength, it cannot be used in large percentage due to its slow water absorption. From this observation, various material systems with different weight percentages were formulated as shown in Table 1.

2. Part characteristics and properties

The printing characteristics of tested samples and ordinary 3DP material (ZP 15E) supplied with

the supplier of the machine were compared. It was observed that all tested samples could be printed in the machine successfully. However, samples A1-A3 warped upward in the lengthwise direction during the drying period in the machine. This is thought to be due to too high an amount of the components that were observed to shrink when contacting with water, maltodextrin and gelatin. In addition, during the drying by evaporation process, the top surface normally dries relatively faster than the bottom surface. This could induce a compressive force on the top surface. When this phenomenon is coupled with a fast drying and shrinking property of the powder while the structure is still not hardened sufficiently to counter the force, the specimen tends to warp. Samples A4 and A5 were devised attempting to solve the warping by decreasing the percentage of shrinkable component while increasing the percentage of the low shrinkage component. It could be observed that the specimen appeared straight during the drying period in the machine, but warped later after being taken out and left to dry further on the bench. Sample A6 was modified from A5 in that the percentage of shrinkable component was decreased further. The A6 specimen had a low degree of warping even after drying outside the machine and was comparable to ZP 15E specimen.

A comparison of dimensional accuracy between printed specimens of all formulations and the original computer image is shown in Table 2. It can be seen that the dimension control of all experimental materials was close. The maximum deviation from the original computer image of all formulated materials was not more than 5% in all directions. In the case of samples A1-A5, the length was not measured due to the warpage along this direction. The dimension accuracies of samples in this study are better than sample fabricated from commercial ZP 15E material especially in length and width direction. Figures 2 and 3 showed the flexural modulus and strength of all materials. In the case of flexural strength, sample A1 and A2 had the greatest strength. Although the strength of

Table 1. Materials formulations.

Sample	Weight Percentage			
	Potato starch	Cellulose fiber	Maltodextrin	Gelatin
A1	40	-	40	20
A2	40	-	20	40
A3	40	20	40	-
A4	40	20	20	20
A5	60	-	30	10
A6	60	15	5	20

Table 2. Dimension accuracy of samples.

Sample	Deviation of dimension [%]		
	Length	Width	Thickness
A1	N.A.	+2.7	+1.1
A2	N.A.	+3.4	+1.0
A3	N.A.	+2.9	+0.4
A4	N.A.	-3.8	+0.4
A5	N.A.	+0.5	+0.4
A6	-0.4	-4.4	-0.5
ZP 15E	+2.8	+11.0	+0.9

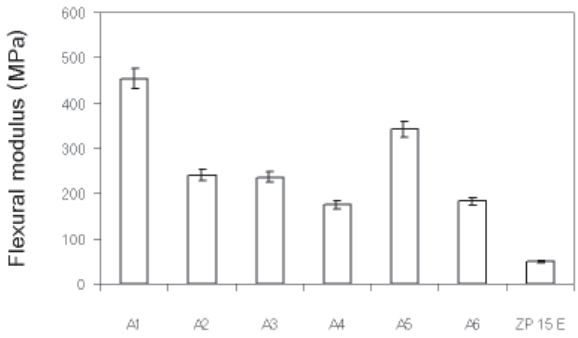

Figure 2. Flexural modulus of uninfiltrated samples.

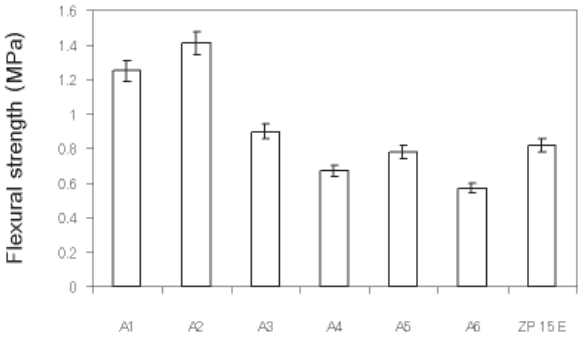

Figure 3. Flexural strength of uninfiltrated samples.

A6 was approximately half the maximum value and slightly lower than ZP 15E, its modulus was greater than ZP 15E.

3. Effect of infiltration

Although the part that was built by 3DP machine is sufficiently strong for general handling purposes, it is still not suitable for use as an implant because the strength is too low. Generally, the method to enhance the strength of a 3DP part is infiltration by a strengthening material, for example cyanoacrylate adhesive, wax or epoxy resin (Z Corporation, 2004). However, these infiltrants are not biocompatible for use in medical applications. Various solutions of biocompatible polymers such as polylactic acid and polycaprolactone were used to infiltrate the starch-based scaffold part (Lam *et al*., 2002). Although the solution can fully infiltrate the part, only the re-solidified polymer remains in the part after solvent evaporation. Since the viscosity of the polymer solution should be low to aid the penetration into 3DP part, the percentage of the polymer in the solution cannot be high resulting in the limitation in degree of strengthening. In addition, using a polymer solution will contradict the claim of using water binder to avoid the residual solvent in the part since the use of polymer solution infiltration will unavoidably result in residual solvent within the part.

In indirect fabrication process of implant by rapid prototyping, heat-cured or self-curing poly(methyl methacrylate) are frequently used as casting materials. However, due to their high viscosity during preparation, they cannot be used for infiltration purpose. Therefore, in this study, light-cured dental sealant was selected as an infiltrant due to its nontoxicity, low viscosity and high strength compared to poly(methyl methacrylate) (Ratner *et al*., 1996; Moszner *et al*., 2001; Fujimura *et al*., 2003). In the study, it was observed

that the sealant could fully penetrate samples easily without the aid of vacuum or pressure. After curing by light, no dimension changes could be detected and samples were then flexural tested. The infiltrated samples were compared with poly (methyl methacrylate) and light-cured sealant specimens in terms of Shore D hardness, flexural modulus and strength (Table 3 and Figures 4-5).

Table 3. Comparison of Shore D hardness of pure sealant, dental acrylic and infiltrated samples

Sample	Shore D Hardness
Sealant	85
PMMA	86
Infiltrated A1	74
Infiltrated A2	75
Infiltrated A3	75
Infiltrated A4	75
Infiltrated A5	74
Infiltrated A6	75

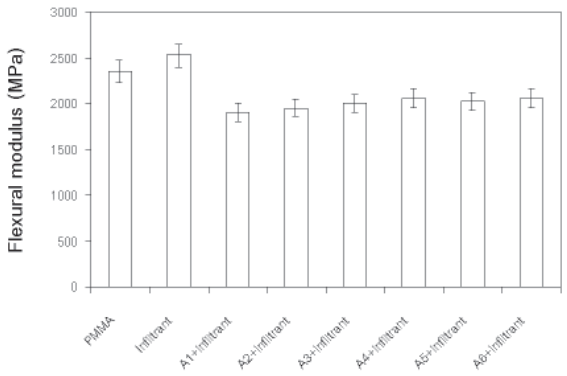

Figure 4. Effect of infiltration on flexural modulus of samples.

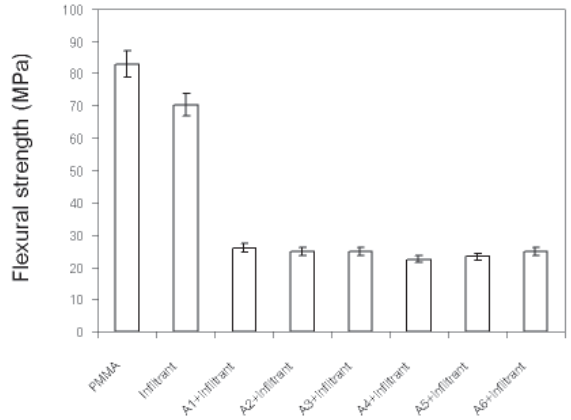

Figure 5. Effect of infiltration on flexural strength of samples.

No significant difference of mechanical properties amongst all infiltrated samples (A1-A6) was noticed. This is probably due to the fact that the infiltrant is much stronger than the formulated powder. Therefore, once they are infiltrated, the majority of exhibited properties would mainly come from the infiltrant part.

In comparison to control samples, dental acrylic and sealant, it can be observed that infiltration greatly enhanced the modulus experimental 3DP samples well over 10 times, to be close to the values of dental acrylic and sealant. In the case of strength, infiltration cannot increase the strength as effectively as modulus. The strength of the infiltrated specimen can reach approximately one third of the values of dental acrylic and sealant. Hardness of all infiltrated samples were slightly lower than pure sealant and acrylic resin.

In general, modulus results from the strongest part in the material while strength results from the weakest part. Therefore, the modulus of the infiltrated sample mainly results from the resistance to deformation of sealant portion which had a modulus similar to pure sealant or acrylic resin. The presence of low modulus natural polymers in the system did not have a significant effect. In the case of strength, since the natural polymers were much weaker than the resin, they would be a starting point of rupture upon loading. Once the cracks occurred, the load was transferred from natural polymers to the infiltrant resin in the composite. The resin would then be under greater load and finally failed. Figure 6 displays the typical fracture surface of pure sealant and infiltrated sample. The fracture surface of sealant was smooth while the infiltrated sample was rough, which was resulted from the crack propagation through debonding of natural polymer particles and infiltrant matrix. In addition, the difference in the flexural properties of infiltrated samples and pure sealant could be further explained by the degree of conversion of the infiltrant after curing by light. From Raman studies, it was shown that pure sealant had a greater degree of conversion than infiltrated sample (Figure 7 and Table 4). This is due to the fact that pure sealant is more transparent than infiltrated samples. Thus, the intensity of light that can pass through the material during curing is greater for initiating polymerization and results in a stronger resin. Although the sealant is reported to be non-toxic, the unreacted monomer which remains in the sample after curing steps can migrate out over a period of time. This can cause a toxic reaction in the surrounding tissue. Generally, alcohol is used to wipe off the surface of sealant after curing in dental practice to remove an uncured monomer. Therefore, washing an infiltrated sample with alcohol can be done to decrease the possibility of toxicity in long-term use.

Although infiltrated specimens are still weaker than sealant or poly(methyl methacrylate) resin, which are biomaterials that are generally used in surgical applications, they are stronger than previously reported compressive and tensile properties of biomedical 3DP parts, modulus of ~ 60-600 MPa and strength of ~ 1.7-14 MPa (Giordano *et al*., 1996; Lam *et al*., 2002). Moreover, the strength of materials in this study is still

Table 4. Comparison of degree of conversion of pure sealant and infiltrated samples.

Sample	Degree of conversion (%)
Sealant	77
Infiltrated A1	58
Infiltrated A2	62
Infiltrated A3	58
Infiltrated A4	59
Infiltrated A5	60
Infiltrated A6	61

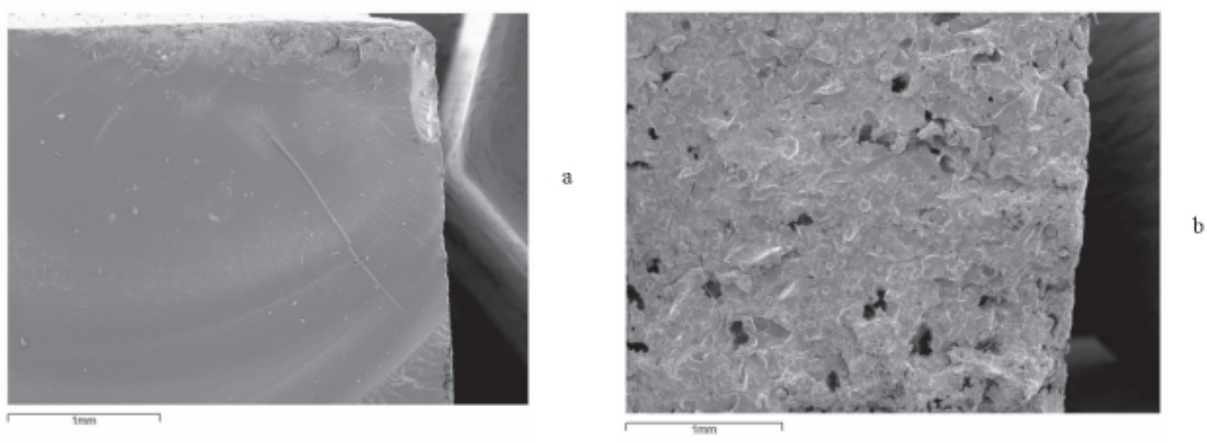

Figure 6. SEM micrographs of typical fracture surfaces of pure sealant (a) and infiltrated sample A6 (b).

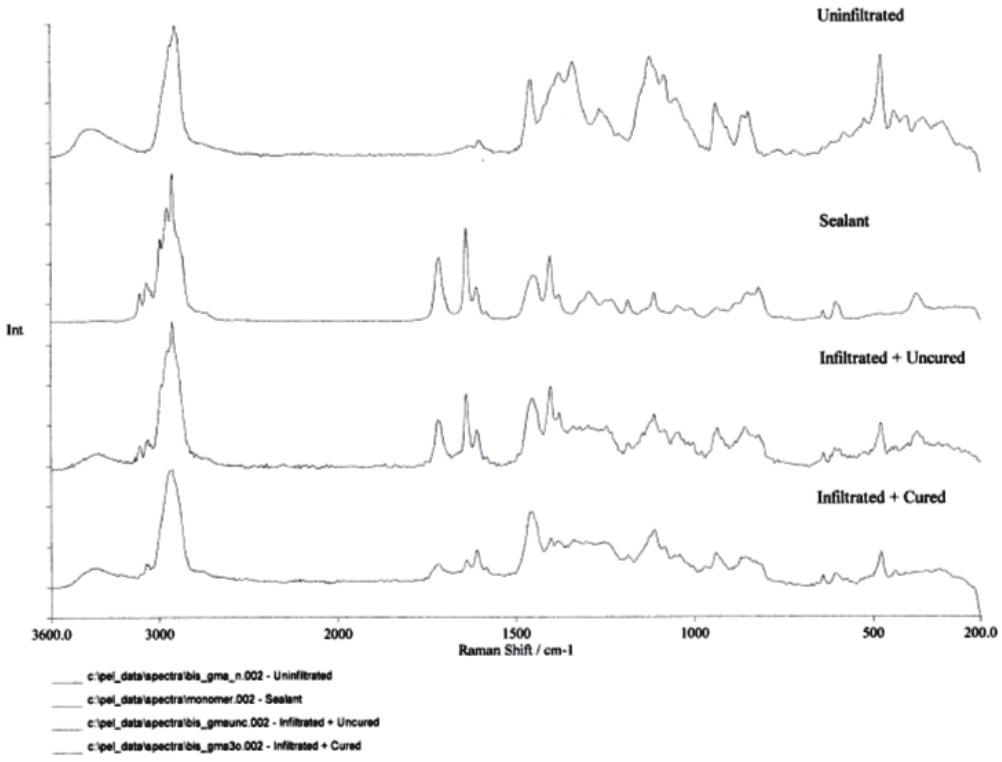

Figure 7. Raman spectra of pure sealant, uninfiltrated sample, uncured infiltrated sample and cured infiltrated sample.

in the range of trabecular bone and some synthetic materials that are normally employed as an implant for reconstructive surgery, for example polyethylene and silicone (Black *et al*., 1998; ASTM, 1999; Landuyt *et al*., 1999). The ability of infiltrated samples to withstand a drilling force by an electrical drill without breakage was also observed. This is impossible for uninfiltrated

sample to tolerate such force without breakage. In case of toxicity of the infiltrated 3DP materials, preliminary *in vitro* toxicity test using L-929 cells showed that the cells which were in contact with samples were red stained and healthy. No inhibition zone was observed.

Conclusions

In this study, the influence of mixture composition of natural polymers and post processing reinforcement by solvent-free infiltrant resin which is potentially suitable for rapidly direct fabrication of implants in reconstructive applications on physical and mechanical properties was investigated. It was observed that the amount of individual component influenced the properties and characteristics of the samples. Physical and mechanical results demonstrate an alternative technique to possibly achieve a sufficiently strong and dimensionally accurate complex-shaped implant based on natural polymers by freeform 3DP technology.

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