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Improved biocomposite development of poly(vinyl alcohol) and hydroxyapatite for tissue engineering scaffold fabrication using selective laser sintering

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Abstract In scaffold guided tissue engineering (TE), temporary three-dimensional scaffolds are essential to guide and support cell proliferation. Selective Laser Sintering (SLS) is studied for the development of such scaffolds by eliminating pore spatial control problems faced in conventional scaffolds fabrication methods. SLS offers good user control over the scaffold's microstructures by adjusting its main processing parameters, namely the laser power, scan speed and part bed temperature.

This research focuses on the improvements in the fabrication of TE scaffolds using SLS with powder biomaterials, namely hydroxyapatite (HA) and poly(vinyl alcohol) (PVA). Grinding of as-received PVA powder to varying particle sizes and two methods of mixing are investigated as the preparation process to determine a better mixing method that would enhance the mixture homogeneity. Suitable sintering conditions for the improved biocomposite are then achieved by varying the important process parameters such as laser power, scan speed and part bed temperature.

SLS fabricated samples are characterized using Fourier Transform Infrared Spectrometer (FTIR) and Scanning Electron Microscope (SEM). FTIR results show that the grinding and sintering processes neither compromise the chemical composition of the PVA nor cause undue degradation. Visual analysis of the grinding, powder mixing and sintering effect are carried out with SEM. The SEM observations show improvements in the sintering effects. The favorable outcome ascertains PVA/HA biocomposite as a suitable material to be processed by SLS for TE scaffolds.

Introduction

Tissue regeneration process or tissue engineering (TE) combines the knowledge of cell biology and engineering, to allow the body to repair itself by delivering the necessary cells and biological signals as directed by the scaffolds to damaged or diseased tissues and organs [1]. Many rapid achievements in this field have arisen in part from significant advances in cell and molecular biology. One major advantage of TE is that the bio-compatibility issues of cell transplants and shortage of organ donors for transplantation can be overcome as the cells can now be harvested from the patient's own body and tissues regenerated [2, 3].

One of the engineering challenges in this field is the provision of good TE scaffolds [4]. Tissue scaffolds have been developed as means of proliferating anchorage-dependent cells. They are built according to the required tissue shape in a cellular matrix using biodegradable material. When implanted in the body, this scaffold will provide temporary mechanical support for the cells to grow into and multiply. The scaffold must be carefully manufactured to ensure that the microstructure allows the proliferation of cells and growth.

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The materials used should not provoke inflammation or toxicity and can be removed from the body via normal metabolic pathways while its degradation rate and the concentration must be at a tolerable level. Suitable materials include ceramics, natural or synthetic polymers as well as their composites [5, 6].

Several conventional scaffold fabrication methods have been investigated, such as solvent casting-particulate leaching [7–9], melt molding-particulate leaching [10], fiber bonding [11, 12], phase separation [13–16] and gas foaming [17]. However, these techniques lack precise control of the pore shape, pore geometry and spatial distribution. Some methods require the use of organic solvents that will leave undesirable residues in the finished products. This may create host reactions due to inflammation or toxicity [18].

One of the technologies being explored to improve the manufacturing process of scaffolds is rapid prototyping (RP). The process consists of first designing 3D objects in a computer environment and the slicing of the designed object into many thin layers in the computer. Afterwards, the RP machine would build the objects layer-by-layer. By doing so, RP is able to build detailed intricate objects. Consistently, RP techniques are fast becoming important tools for the fabrication of scaffolds, as it can provide the consistency, the flexibility in geometry and reproducibility in physical properties without any usage of organic solvents [19, 20].

Selective Laser Sintering (SLS) is a versatile powder-based RP system, as it can process various powder materials while providing good part stability. 3D objects are fabricated by selectively sintering the cross-sectional area of each layer of powder material in a powder bed using a CO_2 laser. The powder is brought to its glass transition temperature (T_g) by heaters on the machine prior to fusing by the laser. After each layer is successfully sintered, the building platform lowers down while the powder cartridge platform rises to supply the next layer. The powder bed is then leveled with the help of a roller [21]. Figure 1 illustrates the SLS process. Fabrication parameters are optimized through experiments to achieve suitable mechanical properties of the TE scaffolds.

Commercially available SLS modeling materials are non-biocompatible, hence suitable biomaterials need to be identified for scaffold fabrication on the SLS. Polymers are naturally highly elastic and low in stiffness while ceramics are highly rigid and brittle. Therefore, by combining them to make a composite, the mechanical properties will be greatly enhanced, as the problem of brittleness and the difficulty of shaping hard ceramics can be overcome [18].

Hydroxyapatite (HA) or $Ca_{10}(PO_4)_6(OH)_2$, is widely used in TE especially in bone and cartilage regeneration due to its excellent bioactivity and chemical similarities to the inorganic component of hard tissues [22]. However it is brittle and more importantly it is not possible to heat HA to its processing temperature using the laser on the SLS machine. Therefore sintering HA on the SLS has to be achieved by introducing polymeric binders to coat the HA [23–26].

Poly(vinyl alcohol) (PVA) is one such polymer investigated to form a biocomposite with HA as the biomaterial to build the scaffolds on the SLS. PVA, being biocompatible and biodegradable, is employed in several biomedical applications including making contact lenses, as ophthalmic materials, for tendon repair and as drug delivery devices [27]. Furthermore, PVA can be sintered at a temperature attainable on the SLS [24, 28–31].

This work presents two improvements as compared to the earlier publication [24], namely the selection of mixing method and the incorporation of grinding as-received PVA powder, so as not to compromise the homogeneity of the PVA/HA biocomposite. The feasibility of using such biocomposite powders and the influence of SLS process parameters on the sintering effect of the specimens are investigated. Specimens are characterized and analyzed

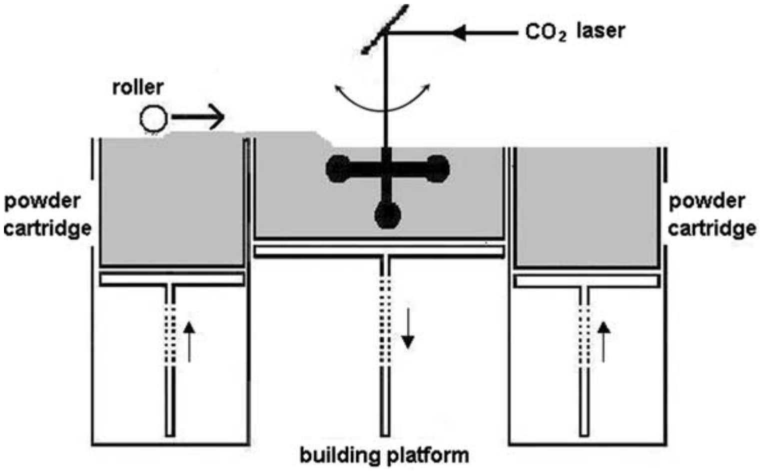

Fig. 1 Schematic diagram of SLS process

using Scanning Electron Microscope (SEM) and Fourier transform infrared spectroscopy.

Materials and methods

Poly (vinyl alcohol) (PVA) and hydroxyapatite (HA) powder

PVA is a semi-crystalline polymer, with its melting temperature, T_m, ranging between 220 °C and 240 °C, depending on its cross linkage and glass transition temperature, T_g, of 58–85 °C, depending on the degree of hydrolyze of the PVA. Pure PVA powder (99% + hydrolyzed, average Mw 89,000–98,000) is obtained from Aldrich Chemical Company, Inc. HA is a complex crystal of calcium phosphate, with calcium to phosphate ratio of 1.67. The HA powder for the PVA/HA biocomposite (branded as Camceram II HA Coating Powder), is supplied by Cam Implants BV, Netherlands.

Preparation of poly (vinyl alcohol)/hydroxyapatite biocomposite

PVA serves as a polymer binder to bond the substrate particles of HA into a contiguous object during SLS processing. As-received PVA particles are found to be in the range of 42–372 μm in size. HA particles have smooth surface texture, with particle size distribution of 56 μm (D_{90}), 37 μm (D_{50}) and 18 μm (D_{10}), according to Coulter Multisizer II. The HA powder has a tendency to agglomerate. Figure 2a and b show the micrographs of as-received PVA and HA powders respectively.

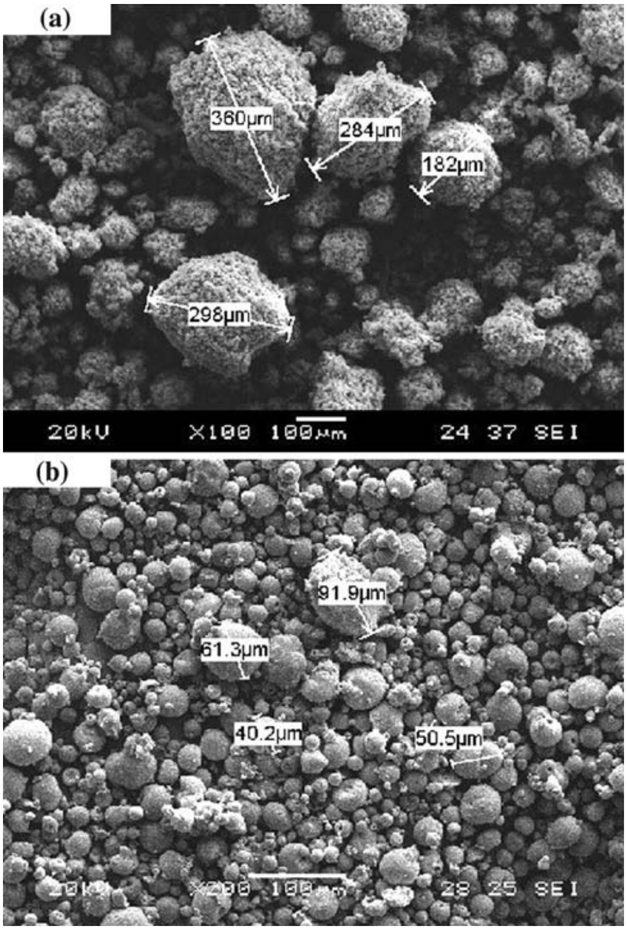

Fig. 2 Micrographs of (**a**) as-received PVA, (**b**) as-received HA

The difference in the particle size of the PVA and HA can result in HA agglomeration as there is not enough exposed PVA surface to attach to, especially when there is a higher percentage of HA. This can lead to HA particles not being uniformly distributed in the composite. To avoid such a problem of uneven mixing, PVA powder is ground and sieved to obtain a more even particle size distribution and mixed with HA by different types of blending methods. By grinding, the exposed PVA surface is also increased.

To prepare the powder mixture, two alternative mixing methods are tried and observations are done to see which of the methods is more effective in yielding a more homogenous biocomposite. The composition used is 10 volumetric (vol.)% HA and 90 vol.% PVA (or 21.5 weight (wt.)% HA and 78.5 wt.% PVA), similar to the successful PVA/HA composition reported by Chua, et al. [24].

Dry powder mixing with ball mill mixer

The first mixing method is by using a ball mill mixer, where a 12 cm-diameter glass bottle is coupled with glass ball chargers. The volume of the balls is approximately 50% of the glass bottle volume and the amount of material makes up 25% volume of the glass bottle [32]. The glass bottle is then placed on the mixing roller (U.S. Stoneware. East Palastine, OH 44413. Serial no. CZ98174).

Optimal mixing rate can be obtained at approximately 76% of critical rotation rate [33]. At the critical rotation speed, centrifugal force equals gravitational force, thus $2\,mV^2/d = mg$, where m is the mass of the loaded bottle, V is the velocity of the rotating bottle, d is the diameter of the bottle and g is the gravitational acceleration, 9.81 m/s^2. Simplifying this, the optimal rotational rate can now be calculated by Eq. (1).

$$\text{Optimal rotational Speed (RPM)}, \ \omega_{optimal} = \frac{32}{\sqrt{Outer\ diameter\ of\ bottle}} \tag{1}$$

Powder mixing on ball milling is performed in a 12 cm-diameter bottle with a rotational speed of 92 rpm, as determined from Eq. (1).

Dry powder mixing with tumbler-mixer

The second alternative method of powder mixing is by using a tumbler-mixer (Inversina, Bio Engineering AG). The powder mixed is placed in an 8.5 cm diameter polypropylene (PP) container, filled with 30% volume of powder. The mixing speed is set at 110 rpm.

Powder grinding

PVA particles are ground using a domestic grinder (Kenwood, Domestic Grinder). As heat is generated during grinding due to interparticle friction, the powders are kept frozen in the freezer at –20 °C, to enable prolonged grinding of the powder and reduce the risk of PVA decomposition during grinding. Grinding is done in batches of 100 mL for 5 min for each batch. Prior to mixing with HA, the ground PVA powder is sieved to below 75 μm, so as to keep the size close to the particle size of the HA, which is at 50–100 μm. These size ranges are chosen as they produce relatively high yield for SLS test sintering.

Design and fabrication of test specimens

The PVA/HA test specimen is of a rectangular strip of size 10 mm × 25 mm, with thickness of 0.762 mm, prepared using Pro-ENGINEER Wildfire 2.0. The specimen size is designed in such a way so that the SLS sample is sufficiently large for SEM observation but small enough to be produced in the in-house test sintering kit, measured 63 × 51 mm. The powder layer thickness is 0.1524 mm. This thickness is chosen as it matches the SLS (DTM Corp's, Sinterstation 2500) default material setting of CastForm, which is selected to test sinter PVA. The sample thickness is estimated sufficient to get adequately stable multilayer specimen for observation. The computer-aided design (CAD) data of the model is then converted to .STL format before it is sent to the SLS machine for fabrication. The process parameters of SLS are kept at default values except part bed temperature, laser power and scan speed. The parameter settings for the optimization process are shown in Tables 1 and 2. These parameters are selected using the optimum parameters in Chua, et al. [24] as the starting point. However, the powder sizes and mixing methods used are different, therefore the parameters and settings for the improved biocomposite have to be re-evaluated. Three specimens are fabricated for each setting.

Table 1 Parameters used for PVA/HA sintering trials using composition of 90 vol.% PVA and 10 vol.% HA, with PVA particle size 10–75 μm

Laser Power (W)	Programmed part bed temperature (°C)	Scan speed (mm/s)
10	65	1,270
	75	2,540
11	65	1,270
	75	2,540
12	65	1,270
	75	2,540
13	65	1,270
	75	2,540
14	65	1,270
	75	2,540
15	65	1,270
	75	2,540
16	75	2,540
17	75	2,540
18	75	2,540
19	75	2,540
20	75	2,540

Table 2 Parameters used for PVA/HA sintering trials using composition of 90 vol.% PVA and 10 vol.% HA, with PVA particle size 50–100 μm

Laser power (W)	Programmed part bed temperature (°C)	Scan speed (mm/s)
10	80	2,032
11	80	2,032
12	80	2,032
13	80	2,032
14	80	2,032
15	80	2,032

Characterization of test specimens

As-received powder and test specimens are observed using scanning electron microscopy (SEM) (Jeol JSM-5600 L) for their surface morphology and microstructure. The SEM process is performed both using the secondary electron and back-scattered electron imaging, indicated by the signs SEI and BES, respectively, in the micrographs. Results from the secondary electron imaging are used to quantify the particle size and detect the presence of necking network of the sintering. Back-scattered imaging results are used to differentiate the PVA and HA particles due to the gray color level difference between the two materials.

Fourier transform infrared spectroscopy (FTIR) (BioRad Excalibur Series Spectrometer FTS 3000) is used to monitor possible changes in the chemical compositions of PVA after the grinding and sintering process. Approximately 5% volume of the solid sample is mixed with potassium bromide (KBr) and pressed into pellets with a press (Graseny

Specac, Die Press). The pellets are then placed into the spectrometer for examination. Three different samples are examined, namely as-received PVA powder, ground PVA powder and SLS-fabricated specimen made of sieved 50–100 μm PVA powder, sintered with parameters set at laser power 13 W, scan speed 2,032 mm/s and part bed temperature 80 °C.

Result and discussions

Result of powder mixing operation

The experiment to select the suitable powder mixing operation is performed by mixing the as-received PVA and HA powder. Two dimensional image characterizations of the SEM micrographs are used to determine the result of the powder mixing. Visual inspection of the appearance of mixture and signs of agglomeration in the mixture are also considered. The first method of mixing is using a ball-milling mixer. The mixture consists of 90 vol.% PVA and 10 vol.% HA (78.5 wt.% PVA and 21.5 wt.% HA). Powder mixture is taken for SEM observation with samples taken at three random locations. Figure 3 shows back-scattered electron SEM images that help to ascertain the mixing quality. Powder sample is taken directly from the mixing container. Particles with brighter contrast and smooth spherical structures are HA, while the ones with darker contrast and rough structures are PVA.

Upon examination, the ball mill mixture of as-received PVA and HA appears homogenous and there are no signs of HA agglomeration as shown in Fig. 3a, which shows one of the SEM micrographs taken from three random locations of the samples. The amount of HA in the composition is estimated by drawing grids on the BES images (for example shown in Fig. 3a), followed by counting the number of grid area taken by the HA. The darken area of SEM picture showing the scale and magnification degree is not included in the calculation.

Random spots show percentage area coverage of the HA is 9.95% (Standard Deviation (SD) = 0.43%, $n = 3$) in the composition. It is very close to the 10% volumetric of the intended mixture and has a good repeatability. The irregularity in the particle size of the PVA is found to affect the homogeneity of the mixture.

The next alternate mixing method investigated is using the tumbler mixer. The same ratio of 90 vol.% PVA and 10 vol.% HA is used. Visual observation of the set-up shows that there are signs of HA agglomerations mostly located at the bottom of the container. The tumbler mixer works by subjecting the powder container to a 3-dimensional movement (rotation, translation and inversion), resembling a number eight shape. This is possibly due to the HA powder, being smaller size and tends to cluster more easily, is thrown by the vigorous shaking movement and thus simply adheres at the base of the container during the mixing process. This results in poor mixing.

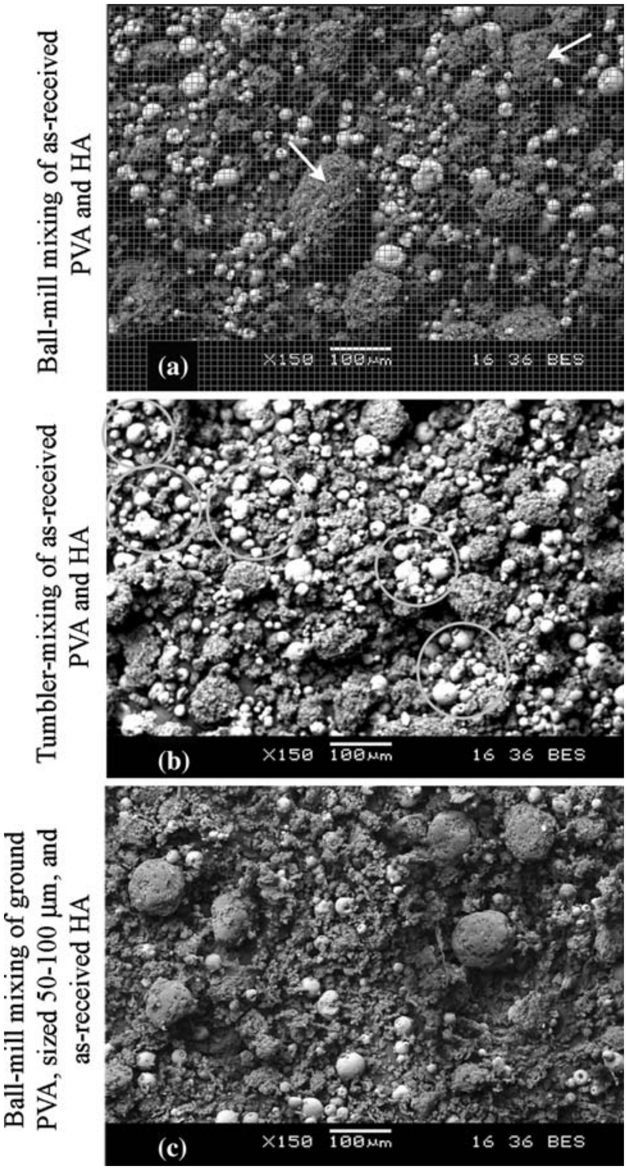

Fig. 3 Powder mixing result of various mixing methods: (**a**) Ball-mill mixing of as-received PVA and HA, (**b**) Tumbler mixing of as-received PVA and HA, (**c**) Ball-mill mixing of ground PVA, sized 50–100 μm, and as received HA. (*Note*: Darken area showing the scale and magnifying degree is not included in the calculation)

Figure 3b shows a particular area where there is a huge concentration of HA. The agglomeration signs of the HA particles in that particular area are circled on the micrograph. Measurement of tumbler-mixed as-received PVA and HA estimates that the HA particles have taken up 16.67% (SD = 3.78%, $n = 3$, taken from random areas of the specimen) of the composition which is a lot more than the 10% volumetric of the intended composition. This signifies that the HA is not uniformly distributed by this mixing method.

From these analyses, it is deduced that the ball milling mixer is a more effective mixing method to homogenously blend PVA and HA. However, the larger PVA particles, shown by the arrows in Fig. 3a, appears to have blocked the HA particles and this might prevent the HA from being distributed evenly in subsequent repeated process.

By repeating the process with PVA particles of a closer size range of 50–100 µm, a better mixing result has been achieved, as shown in Fig. 3c. The percentage area coverage of HA is 9.66% (SD = 0.38%, $n = 3$), which is also near to the 10% volumetric of the intended composition but with smaller SD than mixture of the as-received powders, thus ensuring a higher degree of repeatability. In these analyses, it is deduced that using ball mill mixing while ensuring a regular shape and size of the powder particles can achieve a favorable mixing result that promotes homogeneity.

Optimization of suitable selective laser sintering parameters

The sintering trials are performed using by mixing HA with ground pure PVA, sized 10–75 µm and 50–10 µm. Test sintering of the PVA/HA powders is performed to determine the most suitable sintering parameters for the biocomposite. Sintering parameters based on the work by Chua et al. serves as a starting point for investigation [24], as the particle size of the PVA is now reduced.

For the initial test sintering specimens, 10 vol.% HA is mixed with 90 vol.% PVA sized 75 µm and below. The laser power is set to 15 Watts (W), laser scan speed to 1,270 mm/s and part bed temperature at 65 °C. After the first layer of sintering, slight curling of the part occurred. This is due to the high thermal gradients that lead to residual stresses relieved by a shape change in the form of a curl [34]. When a second layer of sintering is carried out, it is observed that the part has shifted. This happens because the roller shifts the previous layer during powder layering as the part has curled slightly. However, bonding between the layers is good with no signs of delamination.

In Fig. 4a, the circles show long stretches of necking of PVA particles, indicating good sintering at these locations. This explains why the sample is relatively easy to handle. There are several thin necking that hold these long stretches of necking together as shown by the arrows in Fig. 4b. The HA particles indicated by the boxes are also found to be well attached within the PVA matrix. From this examination, it is observed that a good network of necking is achievable.

In the next set of test sintering, the same powder composition with 10–75 µm PVA powder is used. The laser scan speed is kept at 1,270 mm/s, part bed temperature at 65 °C and laser power varied at 10–15 W. The specimen is designed to have a thickness of 0.762 mm (5 layers). The problem of slight curling continues to all the specimens during test sintering. When a specimen curls, the roller will shift this layer inadvertently as the roller moves to level the next layer of powder. This curling problem is critical and it becomes apparent that a 5-layer test specimen cannot be fabricated. Relatively good specimens are obtained only by reducing the number of layers to 3. Apart from being curled, visual inspection indicates that all the specimens built at various laser power settings appear similar: they are

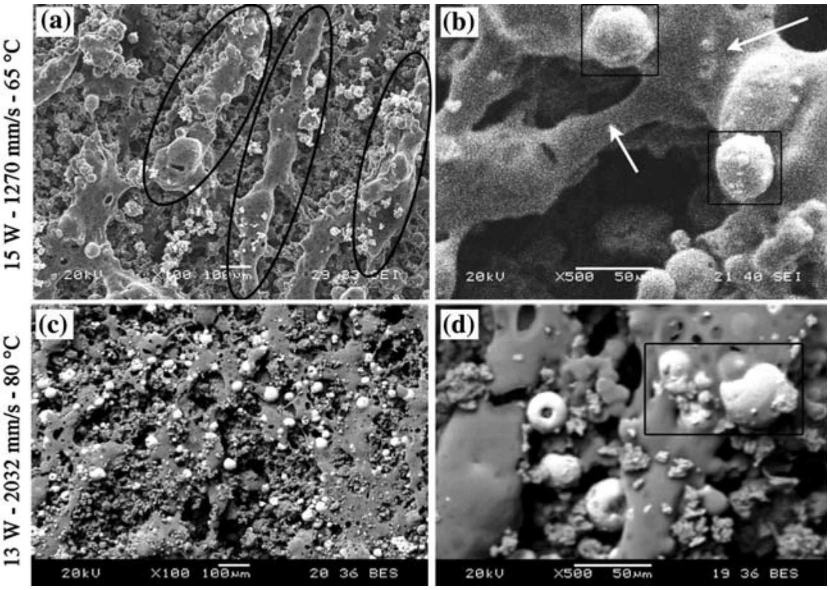

Fig. 4 Sintered PVA/HA processed with various parameters at magnification: (**a**) and (**b**) using PVA sized 10–75 µm, (**c**) and (**d**) using PVA sized 50–100 µm. (*Key*: laser power—scan speed—part bed temperature)

structurally rigid and no delamination between layers is observed.

Curling normally occurs if there is a significant temperature difference between the specimen surface and its surrounding unsintered powder and air temperature. If the hot specimen surface cools down too fast, due to intensive energy from high laser power and low scan speed, the top layer may curl. The temperature gradient during the sintering process should be lowered to reduce curling effect of the test specimens. This can be done by increasing laser scan speed or increasing part bed temperature, i.e., to narrow down the temperature gap between the sintered specimen and the surrounding unsintered powder.

In a separate independent test sintering of pure PVA sized 10–75 µm, it is observed that the powder on the part bed often sticks to the roller during the layering process. This causes uneven powder layering. Sintering PVA with particle size of 50–100 µm (i.e., incorporating powder with a slightly bigger particle size), reduces the clumping problem significantly.

Thus, the next set of sintering of PVA/HA biocomposite is performed with an increased scan speed of 2,032 mm/s. The part bed temperature is raised to 80 °C, while the laser power is varied from 10 W to 15 W. The same powder composition of 90 vol.% PVA and 10 vol.% HA is used but the particle size of PVA is increased to a range of 50–100 µm, so as to prevent the clumping problem.

The optimum result is achieved at a laser power of 13 W. The test specimen can be built to the designed thickness of 5-layer, although slight shifting is observed for the last layer of the specimen. The specimen also appears to be well bonded between the layers and is structurally rigid.

Figure 4c–d show micrographs of 50–100 µm PVA (90 vol.%) and HA (10 vol.%) sintered at 13 W, 2,032 mm/s (80 in/s) and 80 °C with magnification of 100 and 500 times respectively. A substantial amount of necking network of PVA is observed. This shows that there is good sintering effect. From Fig. 4d, the HA particles are shown to be well attached to the PVA bonds, as indicated by the box. Thus the best suitable test sintering is achieved using PVA sized 50–100 µm, with composition 90 vol.% PVA and 10 vol.% HA, sintered at laser power 13 W, scan speed 2,032 mm/s (80 in/s) and part bed temperature 80 °C. As PVA particle size of 50–100 µm is able to give more favorable results, this particle size is chosen for subsequent examinations.

Chemical composition examination

Fourier Transform Infrared Spectroscopy (FTIR) inspection is performed to check the chemical stability of the PVA powder before- and after-grinding and before- and after-sintering processes. This is to ensure that the PVA does not degrade due to exposed heat during the processes. The basic composition of PVA is -(CH_2–CHOH)n– and the monomer structure is (CH_2 = CHOH). From this structure, the following functional groups exist: C–H, C–O, O–H and C=C bonds. FTIR results of the samples are plotted and shown in Fig. 5.

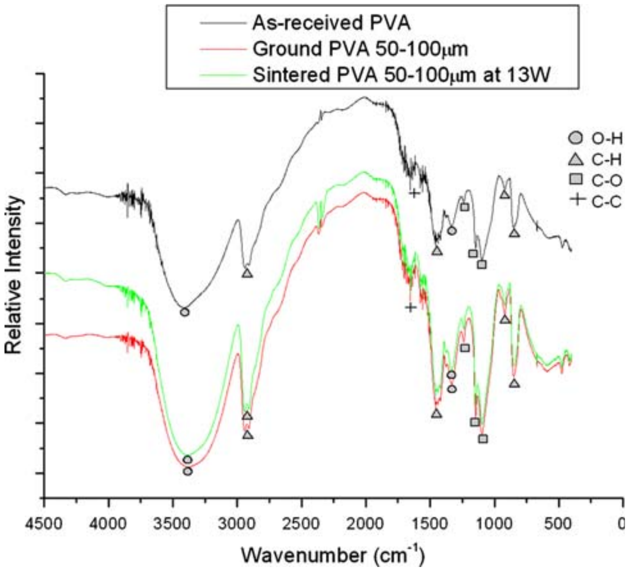

Fig. 5 FTIR comparison of as-received PVA, ground and sintered PVA

The peaks are identified according to the type of chemical bonds. Figure 5 shows that all the samples have very similar curves with the peak points appearing in the same wavenumbers for each bond. A more detailed analysis of the peak points shows that the peak occurrences of the three samples are very similar. This indicates that the important bonds of C–H, C–O, O–H and C=C, appear in a similar pattern in the analysis of the three samples. It is a clear indication that the chemical composition of the PVA has not been disturbed during the grinding and sieving process to obtain 50–100 µm particle size as well as sintering at a laser power of 13 W, scan speed 2,032 mm/s and part bed temperature of 80 °C.

Summary

This research investigates the potential of a new biomaterial powder for use on the SLS to fabricate TE scaffolds. The biomaterials involved are hydroxyapatite (HA) as the ceramic substrate for regeneration of bone tissue and polyvinyl alcohol (PVA) as the polymeric binder.

To attain a more homogenous mixture, as-received PVA powder is ground to a smaller particle size. Apart from obtaining a more homogenous mixture, the fusing among PVA powder particles improves as more surface area of the binding powder becomes available, enhancing the probability for necking to occur. Thus specimens with an overall better handling stability and rigidity are obtained.

The effects of the alternative mixing operations are investigated using a ball mill mixer and a tumbler mixer, for the composition of 90 vol.% PVA and 10% vol.% of HA. A more homogenous mixture is achieved by using the ball mill mixing method. By ensuring that particles involved in mixing have similar size, the repeatability of obtaining such a homogenous mixture can be achieved. Test sintering is performed at various SLS parameters in order to obtain samples that are structurally rigid, yet with minimal curling effect. By investigating the laser power, laser scan speed and part bed temperature, a suitable set of sintering parameters is obtained from samples produced. The suitable settings are at part bed temperature of 80 °C, scan speed of 2,032 mm/s and laser power of 13 W. There is no indication of PVA degradation, during both grinding-cum-sieving to obtain particle size of 50–100 μm and sintering processes at the suitable SLS parameters.

Conclusions

A more homogenous mixture is obtained from constituent powders with similar particle size. Sintered specimens show successful improvements being made in the fabrication process to produce a PVA/HA biocomposite as a base material for fabricating TE scaffolds with SLS.

References

1. F. GUILAK, D. L. BUTLER, S. A. GOLDSTEIN and D. MOONEY, in *Functional Tissue Engineering* (Springer, New York, 2004)
2. E. BELL, in *Principles of Tissue Engineering*, Edited by R. P. LANZA, et al. (Academic Press, San Diego, 2000) p. 181
3. D. J. MOONEY and A. G. MIKOS, *Sci. Am.* **280** (1999) 60
4. L. G. GRIFFITH and G. NAUGHTON, *Science* **295** (2002) 1009
5. C. J. KIRKPATRICK, V. KRUMP-KONVALINKOVA, R. E. UNGER, F. BITTINGER, M. OTTO and K. PETERS, *Bimol. Eng.* **19** (2002) 211
6. T. V. KUMARI, U. VASUDEV, A. KUMAR and B. MENON, *Trends Biomater. Artif. Organs.* **15** (2002) 37
7. M. KAWANISHI, T. USHIDA, T. KANEKO, H. NIWA, T. FUKUBAYASHI, K. NAKAMURA, H. ODA, S. TANAKA and T. TATEISHI, *Mat. Sci. Eng. C* **24** (2004) 431
8. S. J. PETER, M. J. MILLER, A. W. YASKO, M. J. YASZEMSKI and A. G. MIKOS, *J. Biomed. Mater. Res.-Appl. Biomater.* **43** (1998) 422
9. T. SATO, G. CHEN, T. USHIDA, T. ISHII, N. OCHIAI, T. TATEISHI and J. TANAKA, *Mat. Sci. Eng. C* **24** (2004) 365
10. H. O. SE, G. K. SOUNG, S. K. EUN, H. C. SANG and H. L. JIN, *Biomaterials* **24** (2003) 4011
11. A. G. MIKOS, Y. BAO, L. G. CIMA, D. E. INGBER, J. P. VACANTI and R. LANGER, *J. Biomed. Mater. Res.* **27** (1993) 183
12. D. J. MOONEY, C. L. MAZZONI, C. BREUER, K. MCNAMARA, D. HERN, J. P. VACANTI and R. LANGER, *Biomaterials* **17** (1996) 115
13. V. J. CHEN and P. X. MA, *Biomaterials* **25** (2004) 2065
14. J. GUAN, K. L. FUJIMOTO, M. S. SACKS and W. R. WAGNER, *Biomaterials* **26** (2005) 3961
15. G. WEI and P. X. MA, *Biomaterials* **25** (2004) 4749
16. R. ZHANG and P. X. MA, *J. Biomed. Mater. Res.* **44** (1999) 446
17. J. J. YOON and T. G. PARK, *J. Biomed. Mater. Res.* **55** (2001) 401
18. S. F. YANG, K. F. LEONG, Z. H. DU and C. K. CHUA, *Tissue Eng.* **7** (2001) 679
19. D. KOCHAN, C. K. CHUA and Z. DU, *Comput. Ind.* **39** (1999) 3
20. S. F. YANG, K. F. LEONG, Z. H. DU and C. K. CHUA, *Tissue Eng.* **8** (2002) 1
21. C. K. CHUA, K. F. LEONG and C. S. LIM, in *Rapid Prototyping: Principles and Applications* (World Scientific, Singapore, 2003)
22. R. Z. LEGEROS and J. P. LEGEROS, in *An Introduction to Bioceramics*, Edited by L. L. HENCH and J. WILSON (World Scientific, River Edge, NJ, 1993) p. 139
23. G. LEE, J. W. BARLOW, W. C. FOX and T. B. AUFDERMORTE, in *Solid Freeform Fabrication Symposium*, (Austin, TX, 12–14 August, 1996), p. 15
24. C. K. CHUA, K. F. LEONG, K. H. TAN, F. E. WIRIA and C. M. CHEAH, *J. Mater. Sci. Mater. Med.* **15** (2004) 1113
25. K. H. TAN, C. K. CHUA, K. F. LEONG, M. W. NAING and C. M. CHEAH, *Proc. Inst. Mech. Eng. Part H—J. Eng. Med.* **219** (2004) 183
26. F. E. WIRIA, K. F. LEONG, C. K. CHUA and Y. LIU, *Acta Biomaterialia* **3** (2007) 1
27. N. A. PEPPAS, in *Hydrogels in Medicine and Pharmacy*, Edited by N. A. PEPPAS (Boca Raton, CRC Press, 1987) p. 3
28. K. H. TAN, C. K. CHUA, K. F. LEONG, C. M. CHEAH, W. S. GUI, W. S. TAN and F. E. WIRIA, *Bio-Med. Mater. Eng.* **15** (2005) 113
29. C. K. CHUA, K. F. LEONG, F. E. WIRIA, K. H. TAN and M. CHANDRASEKARAN, in *International Conference on Competitive Manufacturing (COMA '04)*, Stellenbosch, South Africa, 4–6 February 2004, p. 229
30. F. E. WIRIA, C. K. CHUA, M. CHANDRASEKARAN and K. F. LEONG, in *The 3rd International Conference on Materials Processing for Properties and Performance (MP3)*, Singapore, 24–26 November 2004, Edited by K. A. KHOR, R. V. RAMANUJAN, C. P. OOI and J. H. ZHAO (Institute of Materials (East Asia), 2004) p. 373
31. F. E. WIRIA, C. K. CHUA, K. F. LEONG, M. CHANDRASEKARAN and M. W. LEE, in *The 2nd International Conference on Advanced Research in Virtual and Rapid Prototyping*, Leiria, Portugal, 28 September–1 October 2005, Edited by P. J. BÁRTOLO, et al. (Taylor & Francis, 2005) p. 509
32. R. M. GERMAN, in *Powder Metallurgy Science* (Metal Powder Industries Federation, Princeton, N.J., 1994)
33. J. ŠUNDRICA, *Int. J. Powder Metall. Powder Technol.* **17** (1981) 291
34. M. AGARWALA, D. BOURELL, J. BEAMAN, H. MARCUS and J. BARLOW, *Rapid Prototyp. J.* **1** (1995) 26