Synthesis of Bioactive Glass using Cellulose Nano Fibre Template

M Sarmast, Sh1, S George1, C. AB Dayang Radiah1,2*, N Abdullah1, S Kamarudin1
1Department of Chemical and Environmental Engineering
2Safety Engineering Interest Group
Faculty of Engineering, Universiti Putra Malaysia, 43400, Serdang, Selangor

*e-mail: dradiah@upm.edu.my

Abstract. Bioactive glass is one of the biomaterials that is used as a bone graft. The important property desired for a bone graft material is to have well suited porosity to enable cell penetration and enhance oxygen and nutrient exchanges. The common methods to produce bioactive glass are melting and sol-gel methods. Melting method is operated at a temperature higher than 1300 °C; the sol-gel method, on the other hand, is usually operated at a much lower temperature, i.e. in the range of 600–800 °C. The objective of this study is to evaluate the effect of using cellulose nano-fiber (CNF) template on the properties of the synthesized bioactive glass. Hypothetically, the templating process will create channels within the bioactive glass structures, which improves both its porosity and the interstitial network. In this study, SiO2–CaO–P2O5–Na2O bioactive glass (BG) was prepared via sol-gel method. The effects of the manipulated parameters on the morphology, chemical properties, porosity and crystallinity of BG were assessed by scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), Brunauer–Emmett–Teller (BET) analysis, Energy dispersive X-ray (EDX) analysis and X-ray diffraction (XRD). It was found that the sintering temperatures significantly affect the structure and performance of samples. The best property was obtained by sintering bioglass with 10 wt% of CNF at 750 °C.

Keywords: Bioactive glass; Sol-gel; Bio-templating, Cellulose nano-fiber, Sintering temperature

1. Introduction:
In the increased in bone related diseases that occurred in both developing and developed countries are quite alarming, repairing the damaged bone is a challenging field in biomedical engineering and regeneration medicine [1,2]. The use of bioglass had been in placed since 1969. The most common material used is the 45S5 Bioglass® (Hench et al., 1971) that contains SiO2–CaO–P2O5–Na2O. Bioactive glass can be prepared via melt or sol-gel methods. The main difference between the two methods are their synthesis temperature and synthesis mechanism. To produce bioactive glass via melt-quenching technique, the compounds including oxides, carbonates, and phosphates are mixed and melted together at a temperature between 1100 to 1450 °C (Jones, 2015). In the sol-gel method, small and colloidal particles suspension are formed and then interconnected to produce a 3D network gel (Li, 1991; Jones, 2015). This mechanism involves the hydrolysis of phosphorous containing alkoxides and calcium salt and subsequent polycondensation; aging, drying and thermal stabilization, respectively (Owens et al., 2016). The sol-gel technique, on the other hand, requires much lower sintering temperature (600-800°C). The produced bioactive glass was described to have better and faster reaction with body tissue (Owens et al., 2016) as well as higher purity and has more homogenous structure (Jones, 2015). In some works, it has been identified that bioglass produced via sol-gel method may have bioactive content, i.e. Silica of up to 90 mol% (Jones, 2015; Owens et al., 2016), whilst that produced via melt quenched only contained silica up to 60 mol% only (Jones, 2015).

Other paragraphs are indented. Templatizing process is typically carried out to create channels in particles to increase its porosity and improve the interstitial network. The templates can be in the forms of solid or liquid. For example, Dong et al. (Dong et al., 2002) used wood cell templating of zeolitic tissue in BG fabrication. Qian et al. (Qian et al., 2009a) used sugarcane as the template for bioglass synthesis. These templates act as castings in the sol-gel method. For a liquid based template, solution containing either dissolved chitosan or PEG (Leci et al., 2012) was added into the sol mixture to improve the porosity of the produced BG. With the use of this template, microparticles with a diameter of 5 – 10 µm and with a pore size of 5–40 nm can be fabricated.
Nano Cellulose Fiber (CNF) is a natural polymer which is produced from cellulosic materials. CNF is biocompatible, biodegradable with excellent mechanical properties, has low density with high surface area, low toxicity and has low preparation costs (Jorfi and Foster, 2015; ‘A comparison of cellulose nanocrystals and cellulose nanofibres extracted from bagasse using acid and ball milling methods’, 2016; Löbmann and Svagan, 2017; Smyth et al., 2017; Souza et al., 2018). CNF has both crystalline and amorphous regions, with dimensions of 5–30 nm in width and aspect ratio (=length/width) greater than 50.

In this study, SiO\textsubscript{2}–CaO–P\textsubscript{2}O\textsubscript{5}–Na\textsubscript{2}O bioactive glass was prepared via the sol-gel method. Different amount of CNF was added into the bioglass synthesis mixture. The objective was to evaluate the effect of different CNF content on the physical and chemical properties as well as the morphology of the bioglass produced.

2. Materials and Methods

The synthesis section is consisted of two parts namely synthesis of bioglass and synthesis of bioglass with cellulose nano fiber (CNF). The intent of the work is to prepare bioglass using formulation content as follows: 45% SiO\textsubscript{2}, 24.5% CaO, 24.5% Na\textsubscript{2}O and 6% P\textsubscript{2}O\textsubscript{5} in mol %. The formulation was based on those reported in the literatures (Bahnium et al., 2012; Pirayesh and Nychka, 2013). Tetraethyl orthosilicate (TEOS 98%, Sigma Aldrich, China) were mixed with 1M nitric acid at room temperature(\textasciitilde27 ± 1°C) and were kept at ambient condition for one hour. Then, Triethyl phosphate (TEP, 99%, R&M Chemicals, Malaysia) were added dropwise into the solution. Calcium nitrate tetrahydrate (99%, R&M Chemicals, Malaysia) and Sodium nitrate (99%, R&M Chemicals, Malaysia) in powder form were added slowly to the mixture and then rigorously mixed until a transparent solution (sol) is formed. The sol was then stored in a sealed polypropylene container and stored for seven days at ambient temperature (\textasciitilde27 ± 1°C) to form gel. The formed BG gel was aged for another 24 hours at 70 ± 2 °C in a vacuum oven (ED56, Binder). Then the aged gel was dried further in the oven at 120°C for 24 hours to remove free water. The dried gel was further sintered at 750°C for 9 h in the furnace (CWF 12/13, Carbolite, UK) to obtain BG powder.

The cellulose nanofiber used in this work was purchased from Institute of Tropical Forestry and Forest Products (INTROP), Universiti Putra Malaysia. It is a 20 wt% CNF solid suspended in distilled water. The average diameter of the fibers is between 20-30 nm. The volume of CNF solution added into the sol of the bioglass was varied between 5% - 25% (a 5% interval between each sample). Similar ageing and sintering procedures were conducted to obtain bioglass powder.

3. Results and Discussion

Morphology Analysis
The morphology of the synthesized samples was analysed using Scanning Electron Microscopy (S-3400N, Thermo Scientific, Hitachi, Japan). The displayed SEM images were magnified at 3000x unless otherwise specified, i.e. Sample BG with 15% CNF was scanned at 1000x only. Figure 1 shows the morphologies of some samples produced when different amount of CNF was added into the bioglass mixture.

The pure BG particles (Figure 1a) has a uniform and well-defined shape with a compact structure. Based on the morphology shown, it can be noted that the BG had been transformed from gel to glass. Therefore, the sintering process had converted the amorphous BG particles (gel) to crystalline BG which have better mechanical strength (Bahnium et al., 2012).

The BG mixed with 10% CNF (BG-CN10) produced bioactive glass with well-defined shapes after sintered. However, the particles produced were quite porous and had adopted the structure of the mould as shown in Figure 1(b). Increasing the amount of CNF suspension into the BG mixture led to formation of larger particles, i.e rather than being covered by the bioglass particles, the large agglomerates of CNF covered the smaller BG particles leading to formation of degraded cellulose crystals when sintered at 750°C. Cellulose fibers consist of both amorphous and crystalline structures. The amorphous sections of the cellulose are more susceptible to acid hydrolysis compared to its crystalline structures. The high sintering temperature at 750°C and the acidic environment of the sol-gel synthesis reactions provide a conducive environment for acid hydrolysis to occur (Bondeson, Mathew and Oksman, 2006). In addition to that, it appears that the BG does not coat the CNF well and...
there were surplus of CNF in the sample compared to that produced from the BG+10% mixture. In Figure 1 (c) it can be observed that only degraded CNF can be observed under the SEM. The characteristic of this degraded product was not identified here. However, it is known that CNF is degraded at 300-350ºC (Borsoi et al., 2016). Therefore, it is apparent that using 20% of CNF content is too much compared to the amount of BG available and thus is not suitable to be used as BG templating.

Figure 1: SEM images of (a) Pure BG, (b) BG-CNFi0, (c) BG-CNFi20

Crystallinity Analysis
To identify the presence of crystalline phases, samples were analyzed using X-Ray Diffractometer with (X'Pert Pro PANalytical, PW 3040/60 | Netherland). The BG particles typically have amorphous structure. Wollaite, Combeite and Cristobalite are the three main silicate based minerals that appeared as main crystalline peaks in BG produced via sol-gel method. These peaks commonly appeared as broad peak at ~32.0o to 35.0o at 2θ (Chen et al., 2018). As found from the diffractogram, the main component that appeared in the produced BG are the Na2Ca2Si3O9. The XRD diffractogram obtained from the synthesized BG were found to coincide with the Na2Ca2Si3O9 peak of 45S5 sample. The FTIR spectrums of six samples of BG, BG-CNFi5, BG-CNFi10, BG-CNFi15, BG-CNFi20 and BG-CNFi25 are presented in Figure 2.

Figure 2: XRD of BG, BG-CNFi5, BG-CNFi10, BG-CNFi15, BG-CNFi20 and BG-CNFi25
Fourier transform infrared (FTIR) spectroscopy

FTIR analysis was performed to identify the nature of the chemical bonds between atoms. Spectrum 100, Perkin Elmer, was used to test the samples. The FTIR spectrums of six samples of BG, BG-CNFI5, BG-CNFI0, BG-CNFI5, BG-CNFI20 and BG-CNFI25 are presented in Figure 3.

The effect of increasing CNF content during the templating process are analyzed. Based on Figure 2, the FTIR spectrums of the BG particles have similar trend and functional groups which indicate that there is no change in the chemical properties of the BG even when the content of CNF is increased. All of the BG particles regardless of their concentration have the same functional groups as the BG.

Based on the FTIR spectra shown in Figure 2, the FTIR presented peaks at the 2306-2927 cm\(^{-1}\) region as well as the peaks that appeared at 1035- 1275 cm\(^{-1}\). Therefore, it can be deduced that the Silica and Phosphate precursors in the BG particles are present in the compound even after the sintering process.

Figure 3: FTIR spectrums of BG, BG-CNFI5, BG-CNFI0, BG-CNFI5, BG-CNFI20 and BG-CNFI25
Porosity Analysis
The specific surface area and pore size distribution of samples were measured by Micromeritics N₂ adsorption-desorption isotherms. Only one sample of BG with added CNF was selected for the BET analysis based on the previous assessment. Table 1 shows the BET results of the BG and the BG-CNF10.

Table 1. BET Analysis of Bioglass Samples

| Samples          | BET Surface Area, m²/g | Total pore volume, cm³/g | Pore Size, Å  |
|------------------|------------------------|--------------------------|--------------|
| BG               | 5.459                  | 0.217                    | 795.056      |
| BG with 10% CNF  | 4.864                  | 0.223                    | 915.536      |

The results show that BG with added CNF has increased pore size. BG with 10% CNF added to it has a 15% higher pore size compared to that of pure BG particles. Therefore, it can be deduced that BG with added CNF has higher porosity compared to pure BG. Increased porosity observed in the BG with added CNF is essential for the formation of bone scaffold since porous structures promote rapid biodegradation and enhances new bone formation (Kim et al., 2016). BG with 10% added CNF has lower total surface area compared to that of pure BG. This could be caused by the BG particles that are attached to CNF having less available surface area and forming a macromolecule compared to the mostly isolated BG particles that are present in pure BG. Usually, specific surface area of BG produced via sol-gel method should be around 100 to 300 m²/g (Chen et al., 2008). However, the results show that the specific surface area of the synthesized BG is only 5.45m²/g. Therefore, it can be concluded that BG templated with CNF content can produce more porous BG which is important for bone scaffold application.

Elemental Analysis
EDX analysis was performed to check the atomic composition of the BG. However, only one selected BG with CNF template sample was chosen for the analysis based on morphology data, FTIR and XRD analysis. Since the BG with 10% CNF was found to be the best, the EDX analysis was conducted on it to check for its composition. The results were compared with that of pure BG. Table 2 shows the EDX spectrum for pure BG and BG with 10% CNF.
EDX analysis of BG and BG with 10% CNF confirm that the four key components of bioglass are still present in the samples after the sintering process. The four key components that are essential in BG are Sodium (Na), Silica (Si), Phosphorous (P) and Calcium (Ca). These components are essential in bone scaffolds because they stimulate osteoblasts proliferation and new bone growth (Bellucci et al., 2017). The amount of SiO$_2$ present in the sample is 35.51%. The amount of SiO$_2$ present in the samples can be increased by prolonging the acid hydrolysis time of the precursor because acid hydrolysis of the precursor would break down the TEOS and separate the silica dioxide molecule from the TEOS molecules (Qian et al., 2009b; Bahniuk et al., 2012; Lacroix, Lao and Jallot, 2013).

| Element | Weight % | Formula | Compound % |
|---------|----------|---------|------------|
| Pure BG | 48.59 | NaO$_2$ | 38.61 |
| BG with 10% CNF | 48.06 | NaO$_2$ | 38.05 |
| Si | 16.60 | SiO$_2$ | 35.51 |
| P | 0.61 | P | 0.61 |
| Ca | 18.06 | CaO | 25.27 |
| Total | 100.00 | Total | 100.00 |

Comparisons between the EDX spectrum of pure BG and BG with 10% CNF reveal that there is only a slight change in the composition of the particles. Sodium, silica and phosphorous are present in higher weight percent on the pure BG sample compared to those in the BG with the 10% CNF added; however calcium presents in much higher amount on the BG sample with 10% CNF added to it. High calcium content is beneficial to the bone scaffold because calcium has osteoconductivity potential (Stratton et al., 2016).

**Conclusion**
This study has successfully synthesized BG via sol-gel method using CNF suspension as template. The amount of template used shall be controlled to ensure that the bioactive glass produced will take after the mould and no reverse effect happened. With templating, the porosity and microstructure of the bioglass was improved. There was no effect of templating on both chemical compounds that present and their respective bondings. Therefore, it can be concluded that although the addition of CNF template to BG prove to increase the porosity of BG, further study must be done before it can be implemented in bone scaffold application.

**References**
- ‘A comparison of cellulose nanocrystals and cellulose nanofibres extracted from bagasse using acid and ball milling methods’ (2016) *Advances in Natural Sciences: Nanoscience and Nanotechnology*. IOP Publishing, 7(3). doi: 10.1088/2043-6262/7/3/035004.
- Arcos, D. and Vallet-Regí, M. (2010) ‘Sol-gel silica-based biomaterials and bone tissue regeneration’, *Acta Biomaterialia*, 6(8), pp. 2874–2888. doi: 10.1016/j.actbio.2010.02.012.
- Bahniuk, M. S. et al. (2012) ‘Bioactive glass 45S5 powders: Effect of synthesis route and resultant surface chemistry and crystallinity on protein adsorption from human plasma’, *Biointerphases*, 7(1–4), pp. 1–15. doi: 10.1007/s13758-012-0041-y.
- Bellucci, D. et al. (2017) ‘A comparative in vivo evaluation of bioactive glasses and bioactive glass-based composites for bone tissue repair’, *Materials Science and Engineering C*, 79, pp. 286–295. doi: 10.1016/j.msec.2017.05.062.
- Bondeson, D., Mathew, A. and Oksman, K. (2006) ‘Optimization of the isolation of nanocrystals from microcrystalline cellulose by acid hydrolysis’, *Cellulose*, 13(2), pp. 171–180.
Borsoi, C. et al. (2016) ‘Thermal degradation behavior of cellulose nanofibers and nanowhiskers’, Journal of Thermal Analysis and Calorimetry. Springer Netherlands, 126(3), pp. 1867–1878. doi: 10.1007/s10973-016-5653-x.

Chen, J. et al. (2018) ‘Preparation and characterization of bioactive glass tablets and evaluation of bioactivity and cytotoxicity in vitro’, Bioactive Materials. doi: 10.1016/j.bioactmat.2017.11.004.

Chen, X. et al. (2008) ‘Investigation on bio-mineralization of melt and sol-gel derived bioactive glasses’, Applied Surface Science, 255(2), pp. 562–564. doi: 10.1016/j.apsusc.2008.06.101.

Dong, A. et al. (2002) ‘Zeolitic tissue through wood cell templating’, Advanced Materials, 14(12), pp. 926–929. doi: 10.1002/1521-4095(20020618)14:12<926::AID-ADMA926>3.0.CO;2-1.

Hench, L. L. et al. (1971) ‘Bonding mechanisms at the interface of ceramic prosthetic materials’, Journal of Biomedical Materials Research. Interscience Publishers, a division of John Wiley & Sons, Inc., 5(6), pp. 113–141. doi: 10.1002/jbm.820050611.

Jones, J. R. (2015) ‘Acta Biomaterialia Editor ’ s Comment on: Review of bioactive glass: From Hench to hybrids’, 23, p. 2015. doi: 10.1016/j.actbio.2015.07.005.

Jorfi, M. and Foster, E. J. (2015) ‘Recent advances in nanocellulose for biomedical applications’, Journal of Applied Polymer Science, 132(14), pp. 1–19. doi: 10.1002/app.41719.

Kim, J. A. et al. (2016) ‘Effect of the biodegradation rate controlled by pore structures in magnesium phosphate ceramic scaffolds on bone tissue regeneration in vivo’, Acta Biomaterialia. Acta Materialia Inc., 44, pp. 155–167. doi: 10.1016/j.actbio.2016.08.039.

Lacroix, J., Lao, J. and Jallot, E. (2013) ‘Green and safe in situ templating of bioactive glass scaffolds for bone tissue engineering’, Journal of Materials Chemistry B, 1(13), pp. 1782–1785. doi: 10.1039/c3tb00520h.

Lei, B. et al. (2012) ‘Versatile fabrication of nanoscale sol-gel bioactive glass particles for efficient bone tissue regeneration’, Journal of Materials Chemistry, 22(33), pp. 16906–16913. doi: 10.1039/c2jm31384g.

Li, R. (1991) Sol-gel processing of bioactive glass powders: Li, Rounan, 1945- : Free Download & Streaming : Internet Archive. University of Florida. Available at: https://archive.org/details/solgelprocessing00liro (Accessed: 10 November 2017).

Lian, H. and Meng, Z. (2018) ‘Materials Science & Engineering C Fabrication, characterization and osteoblast responses of poly (octanediol citrate)/bioglass nanofiber composites’, Materials Science & Engineering C. Elsevier, 84(November 2017), pp. 123–129. doi: 10.1016/j.msec.2017.11.042.

Löbmann, K. and Svagan, A. J. (2017) ‘Cellulose nanofibers as excipient for the delivery of poorly soluble drugs’, International Journal of Pharmaceutics, 533(1), pp. 285–297. doi: 10.1016/j.ijpharm.2017.09.064.

Owens, G. J. et al. (2016) ‘Sol–gel based materials for biomedical applications’, Progress in Materials Science. Pergamon, 77, pp. 1–79. doi: 10.1016/J.PMATSCI.2015.12.001.

Pirayesh, H. and Nychka, J. A. (2013) ‘Sol-gel synthesis of bioactive glass-ceramic 45S5 and its in vitro dissolution and mineralization behavior’, Journal of the American Ceramic Society, 96(5), pp. 1643–1650. doi: 10.1111/jace.12190.

Qian, J. et al. (2009a) ‘Fabrication and characterization of biomorphic 45S5 bioglass scaffold from sugarcane’, Materials Science and Engineering C, 29(4), pp. 1361–1364. doi: 10.1016/j.msec.2008.11.004.

Qian, J. et al. (2009b) ‘Fabrication and characterization of biomorphic 45S5 bioglass scaffold from sugarcane’, Materials Science and Engineering C. Elsevier B.V., 29(4), pp. 1361–1364. doi: 10.1016/j.msec.2008.11.004.

Smyth, M. et al. (2017) ‘Tunable Structural and Mechanical Properties of Cellulose Nanofiber Substrates in Aqueous Conditions for Stem Cell Culture’, Biomacromolecules, 18(7), pp. 2034–2044. doi: 10.1021/acs.biomac.7b00209.

Souza, S. F. et al. (2018) ‘Cell interactions and cytotoxic studies of cellulose nanofibers from Curauá natural fibers’, Carbohydrate Polymers, 201(May), pp. 87–95. doi: 10.1016/j.carbpol.2018.08.056.

Stratton, S. et al. (2016) ‘Bioactive polymeric scaffolds for tissue engineering’, Bioactive
Materials. Elsevier Ltd, 1(2), pp. 93-108. doi: 10.1016/j.bioactmat.2016.11.001.