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Filament Extrusion of Bioresorbable PDLGA for Additive Manufacturing utilising Diatom Biosilica to inhibit process-induced thermal degradation

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Author Statement

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Title: Filament Extrusion of Bioresorbable PDLGA for Additive Manufacturing utilising Diatom Biosilica to inhibit process-induced thermal degradation.

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Abstract:

Bone scaffolds are often fabricated by initially producing custom-made filaments by twin-screw extruder and subsequently fabricating into 3D scaffolds using fused deposition modelling. This study aims to directly compare the effect of two alternative silica-rich filler materials on the thermo-mechanical properties of such scaffolds after extrusion and printing. Poly (DL-lactide-co-glycolide) (PDLGA) was blended with either 45S5 Bioglass (5 wt %) or Biosilica (1 and 5 wt%) isolated from *Cyclotella meneghiniana* a freshwater diatom were tested. Diatom-PDLGA was found to have similar mechanical strength and ductility to pure-PDLGA, whereas Bioglass-PDLGA was found induce a more brittle behaviour. Bioglass-PDLGA was also found to have the lowest toughness in terms of energy absorption to failure. The TGA results suggested that significant thermal degradation in both the Bioglass filaments and scaffolds had occurred as a result of processing. However, diatom biosilica was found to inhibit thermal degradation of the PDLGA. Furthermore, evidence suggested the agglomeration of Bioglass particles occurred during processing the Bioglass-PDLGA filaments. Overall, diatom biosilica was found to be a promising candidate as a bone filler additive in 3D printed PDLGA scaffolds, whereas Bioglass caused some potentially detrimental effects on performance.

Key Words: Bioresorbable Scaffolds, 3D FDM Scaffolds, Bioglass, Diatoms, Additive Manufacture

1. Introduction:

Bioglass (based on the system $\text{SiO}_2\text{-CaO-Na}_2\text{O}$ and P_3O_5) has been reported to have superior performance in bone regeneration in comparison to other ceramics, however, has had issues in its clinical translation, mainly owing to difficulties with its fabrication into composite/hybrid materials for load bearing applications¹. Recently several studies, however, have reported more promising results with 3D printing of Bioglass-polymer composites²⁻⁴ suggesting that this fabrication technique may be more suitable for Bioglass. The downside of this is that there would seem to be an interaction between Bioglass and the polymer during melt-processing which results in the thermal degradation of the polymer and a detrimental lowering of mechanical properties, such as strength⁵. Interestingly natural biosilica isolated from diatoms has also started to show promise in bone repair⁶⁻⁹, which may offer an alternative silica filler option for 3D printed scaffolds. Diatom frustules (the cell walls of diatoms) are unique silica particles that host surface topographies that cannot be replicated synthetically. They range in particle size from 2 μm to 2 mm depending on the species, each with unique morphologies that vary in surface area dependent on its topographical features¹⁰. To date there have been no reports published on the influence of biosilica from diatoms on the mechanical properties of melt-processed biosilica polymer composites.

To build 3D composite scaffolds with 3D printing, bioresorbable polymers, such as poly(lactic acid) (PLA), poly(ethylene glycol) (PEG), and poly(lactic-co-glycolic acid) (PLGA) have been reinforced with ceramics including hydroxyapatite (HA) and tricalcium

phosphates^{11,12}. Other ceramic materials embedded in polymers have also been investigated for bone repair to a much lesser extent, these include strontium substituted HA, and silica¹³⁻¹⁵. Poly (DL-lactide-co-glycolide) (PDLGA) also shows good potential in 3D printing^{16,17} in terms of processing, forming and repeatability of scaffold architectures, however, it has been less studied^{18,19} in terms of thermal processing degradation when compared to other non-medical grade polymers¹⁹.

The success of 3D printing relies heavily on the quality of filaments used in the print process. For composite filaments, good homogeneity and dispersion of incorporated filler particles is required to achieve good printability²⁰. Filaments can be pre-fabricated using a range of techniques including hot melt or single/twin-screw extrusion²⁰⁻²³, or some 3D printers melt the polymers directly in a one-step process²⁴. Twin-screw extrusion has been reported to produce a more homogeneous distribution of ceramics particles within the polymer matrix in composite filaments than comparator techniques²⁵. However, caution should be taken when selecting a suitable filament/scaffold fabrication method as they can cause thermally induced degradation, which could affect their clinical performance. Little et al.,¹⁸ reported less thermal degradation in PDLGA filaments produced by twin-screw extrusion, compared to a study by Yen et al.,¹⁷ that used a single hot melt extrusion FDM process to produce PDLGA filaments. Little et al. demonstrated that the thermal degradation was a function of both exposure time and temperature during melt-processing. Important physical-chemical material properties of filaments for 3D printing that should be considered include rheological, molecular, microstructural and mechanical²².

In addition to process-induced thermal degradation, ceramics added to bioresorbable polymers, have been reported to induce a reduction in thermo-mechanical properties of the

material. A study by Simpson et al. reported significant polymer degradation in PLGA after the incorporation of Bioglass 45S5, when compared to other ceramic fillers⁵. The study attributed the decrease in thermo-mechanical properties to be a result of a reduction in molecular weight due to thermally induced degradation. Under these processing conditions, the performance of bioglass as a filler to PLDGA has shown less promise to that of biosilica.

The novelty of the study lies with the application of diatom biosilica as a potential filler material for tissue engineered scaffolds. It investigates the use of twin-screw extrusion to fabricate tailored PDLGA filaments containing either bioglass or diatom biosilica filler to establish if biosilica is suitable for 3D printing of bone tissue scaffolds, compared to bioglass, which has previously been reported to not be suitable⁵. The study investigates the effect of adding the two different filler types on the thermo-mechanical properties of PDLGA after extrusion and printing. In this study, Bioglass[®] 45S5 (trade name PerioGlas[®]) was compounded with PDLGA and compared to natural silica filler similarly compounded into PDLGA using twin-screw extrusion. This allowed direct comparison to be made with respect to the composite properties due to the filler additions. The natural silica was isolated from a fresh water diatom (*Cyclotella meneghiniana*) that was then purified to remove all organic matter. The species selected, *C. meneghiniana* is a centric diatom with a particle size in the range of 10 to 20 μm ²⁶. Biosilica isolated from diatoms was selected for this study owing to its unique morphology that creates a high surface area¹⁰ and their potential osteogenic and angiogenic attributes²⁷⁻²⁹. It was hypothesised that their unique physiochemical properties with nano-sized topographical features⁶ could introduce silica to the polymer matrix without the detrimental process-induced degradation that has been report for bioglass.

2. Materials and Methods

2.1 Materials

Medical grade PDLGA (85:15) ratio DL-LA/GA polymer granules (Boehringer Ingelheim Resomer RG 859 SR) was kept in a sealed bag and stored in the fridge at - 4 °C to prevent the degradation of polymer prior to use. This was then brought to room temperature and defrosted overnight before compounding with the ceramic fillers. After defrosting, the sealed bag was opened, and PDLGA polymer was weighed and vacuum dried at 40 °C at 10 mbar pressure for 2 days. Bioglass 45S5 (SylcTM, Research Ltd, London, UK) from Denfotex (EJR, D095325/K062502, Reg. No. 872.6030) of mean particle size of 5 to 80 µm was used in this study. It was vacuum dried as per PDLGA prior to use. Fresh water diatoms were collected from the Mississippi River, USA and cultured at St. Cloud University using a previously described culture method by Walsh *et al.*,⁶. The species of diatoms selected are known to have frustules with a mean particle of 20µm depending on the growth stage³⁰. After harvesting, the diatoms frustules were digested in by nitric acid (HNO₃) to partially remove the organic fraction, then freeze dried and sealed in a vacuum-packed bag for transport to the UK.

2.2 Methods

2.2.1 Diatom Purification

The resultant diatom frustules on arrival from St. Cloud were washed with deionised (DI) water to neutralise the residual acid. The diatom powders were placed in a Buchner funnel

and washed about 10 times with 1000 ml of DI water per wash. After that, the resultant washed diatoms were dried in an oven at 60 °C overnight to remove the moisture. The washed diatoms were then heated treated in an air atmosphere in an Elite chamber furnace (BMF 11/7) to remove the residual organic matter that was not removed during the nitric acid wash. A ramp rate of 10 °C/min was used with a 1-hour dwell at 550 °C. The samples were then cooled overnight. The purified biosilica frustules were then vacuum dried as previously described. At each stage of the treatments the samples were characterised. The sample references for each treatment are shown in Table 1. Thermogravimetric Analysis (TGA) and CHNS analysis was used to verify that the organics were removed, BET gas porosimetry was used to determine change in specific surface area after treatments and Scanning Electron Microscope (SEM) was used to verify that the frustules remained intact after purification.

Reference ID	Treatment No.	Treatments
AT	1	Acid wash (St. Cloud)
WT	2	Wash treatment
HT	3	Heat treatment

Table 1: Purification treatment references.

2.2.2 Blend preparation

The dried PDLGA polymer and ceramic filler powders (biosilica/Bioglass 45S5) were mixed homogeneously by hand in a dry blend. Blends were prepared of 1 wt % Diatom-PDLGA, 5 wt % Diatom-PDLGA and 5 wt % Bioglass-PDLGA. The filler was added as a dry weight-to-weight ratio to PDLGA. After blending the powdered blends were re-dried in a vacuum oven under the same conditions for 24 hours before extrusion.

2.2.3 Filament Fabrication

Filaments were produced by Haake twin-screw extruder (Thermo Fisher Scientific, Germany). The extrusion process used is shown in Fig. 1a. The chamber was filled with dry nitrogen to minimise hydrolysis of the PDLGA. The feed was then melted by screw barrel heater with controlled temperature in the range of 165 °C to 175 °C. A chilled air ring and two normal air rings formed the cooling process to lower the temperature (to approximately 60 °C) and extend the soft filament length while reducing its diameter to 1.75 mm (± 0.05), which was an acceptable filament size for 3D Printing. The draw-off belts were used to control the pulling speed, which in turn adjusted the diameter of filament. Four types of filaments were fabricated: Pure-PDLGA filament (Control), 1 wt % Diatom-PDLGA filament, 5 wt % Diatom-PDLGA filament and 5 wt % Bioglass-PDLGA filament.

2.2.4 Scaffold Fabrication using Fused Deposition Modelling (FDM) 3D Printing

The filaments were fabricated into a 3D scaffold using a RepRap Mendel (RepRapPro, UK) 3D printer. The method used to print the scaffolds was similar to that described by Little et al.¹⁸. Briefly, the architecture of the scaffold was created using SolidWorks 2010, then converted from STL to G-Code using opensource Slic3r 1.2.9. A standard architecture was selected that most widely represents bone scaffolds to validate the concept. The 3D printer head controlled by Printron (otherwise known as Printerface) software using G-Code. The printing parameters used are detailed in Table 2. To determine the optimum print temperature for each filament composition a trial-and-error approach was taken. The print temperature of pure PDLGA has been reported in the literature in the range of 170 to 200 °C^{18,31,32}, subject to type of PDLGA and printer used. Starting with the Pure-PDLGA filament, scaffolds were printed at different temperatures starting at 180 °C as per Little et al., study¹⁸, the print temperature was increased in increments of 5 °C until an optimum print

temperature was achieved. The scaffolds were assessed under a light microscopy in terms of pore structure and shape, and checked for signs of polymer degradation which is visible by colour change in the polymer. This process was repeated for all the filament conditions, however, in the case of 5 wt % Bioglass-PDLGA filament, lower temperature increments from 180 °C were trialled as this filament was too soft and dripped as opposed to flowed out of the nozzle at 180 °C. The printer setup is shown in Fig. 1b. The scaffolds were produced using a 90°/90° laydown pattern. The layer cycle was repeated 12 times to create the scaffold. For each scaffold approximately 150mm of filament was used.

Parameters	Temperature (°C)	Parameters	Values
Platform Temperature	60	Fan Speed	100 rpm
Print Temperature:		Print Speed	22 mm/s
Pure-PDLGA	190	Travel Speed	130 mm/s
PDLGA-Diatoms (1%)	220	Layer height	0.25 mm
PDLGA-Diatoms (5%)	225	Nozzle diameter	0.4 mm
PDLGA-Bioglass (5%)	165	Filament diameter	≈ 1.75 mm

Table 2: FDM Parameters

Figure 1: Fabrication processes of (a) filament using twin-screw extrusion and (b) 3D printing of scaffolds.

2.3 Characterisation

2.3.1 Thermogravimetric Analysis (TGA)

All TGA analysis was performed on a Mettler Toledo (DSC/TGA) 1 Star instrument. Samples were placed in an aluminium crucible and heated under a nitrogen environment. For all

studies a ramp rate of 10 °C/min was used. For the diatom purification study, 5mg of dried diatom frustules were added to the crucibles for each run. The samples were tested over a temperature range from 25 °C to 800 °C. For the filaments and 3D printed scaffolds, TGA was used to verify the mass percentage additive and to check the onset temperature to determine if any thermal degradation had taken place. In TGA, the onset temperature is defined as the temperature at which mass loss starts, and is also denoted as the upper limit of thermal stability for polymer³³. For each filament, samples were taken every 30cm. A total of 5 samples was taken along the filament length. The analysis was replicated on each sample in triplicate. For the scaffolds, samples were taken at random from different samples within the same filament type. The percentage additive was calculated as follows:

$$\% \text{ additive} = [(\text{residual mass}_{(800^{\circ}\text{C})} \text{ of PDLGA + additive}) - (\text{residual mass}_{(800^{\circ}\text{C})} \text{ of Pure-PDLGA})] * 100$$

----- Eq. 1

2.3.2 Elemental Analysis

Elemental analysis was performed on a Perkin Elmer PE2400 CHNS/O Elemental Analyser. The analyses were run by the QUB Analytical Services and Environmental Projects Division (ASEP) facility. For analysis, the diatom samples for all treatments were dried in an oven at 60 °C overnight. Prior to analysis, the instrument is purged with helium gas, then calibrated using a series of empty blank vials followed by an Acetanilide (C=71.06; H=6.71; N=10.31; O=11.82) and Cystine (C=29.95; H=5.09; N=11.59; O=26.67; S=26.67) standards. The diatom sample (1 to 2mg) was combusted in a sealed tin crucible at 975 °C, then the gas flows into a reduction furnace at 640 °C prior to collection. In the analysis, CO₂, H₂O, N₂ gases are collected in the mixing chamber, separated by frontal chromatography and measured by thermal conductivity (TC).

2.3.3 N₂ absorption-desorption analysis

Brunauer–Emmett–Teller (BET) surface area analysis was performed on a Micromeritics Tristar 3020 gas adsorption analyser. Prior to analysis the samples were dried in an oven at 60 °C overnight. For each analysis 0.2g of sample was used. The samples were degassed at 77K for 3 hours, the surface area was then measured by N₂ absorption and desorption isotherms at this temperature. This analysis was also run by QUB ASEP facility.

2.3.4 Imaging

SEM analysis was performed on diatom samples to ensure their morphology remained intact after heat treatment. It was also performed on scaffolds after 3D printing to analyse the surface of the scaffolds. A FEI Quanta FEG – Environmental SEM from Oxford EX – ACT was used in this study. Before the samples were loaded into the SEM, samples were gold coated using a sputter coater. The samples were coated for 40 seconds, and imaged in the SEM under a high vacuum at 20 kV and with a 3.0 spot. The filaments and scaffolds were also imaged under an optical microscope (Olympus Stereo Microscope).

2.3.5 Mechanical Testing

The filaments were tested using an Instron 5564 universal tensile tester. Samples were prepared by cutting segments from the filament every 30cm. These were compressed at 180 °C at a pressure of 10 bar for 90 seconds in a Collin platen press to create a polymer sheet of thickness of approximately 1mm. Dumbbells were then cut from the sheet in accordance with the British Standard ISO 527-2-1BA. An extensometer was used to measure the strain within the gauge length. A constant speed of 1mm/min was applied to deform the samples. Scaffolds were compression tested using a Zwick/Roell Z100 Instrument. A preload

of 150N was applied and loaded at a cross-head speed of 2mm/min. All samples were tested under dry conditions. The energy under the stress-strain curve was determined using the trapezoidal rule in MATLAB R2020a to determine the energy absorbed by the filaments. The calculations were confirmed in excel by fitting a high order polynomial trend and integrating the polynomial function.

2.3.6 Rheology

The viscoelastic properties of the filaments were assessed using rheology. Polymer discs with the diameter of 2.5 cm were cut from the polymer sheets that were prepared for mechanical testing (see section 2.3.5). A TA Rotational Rheometers ARG2 was used in this study. In this study, two methods were taken to study viscosity. **Method 1:** The first used a frequency sweep approach to predict the zero-rate viscosity from the change in frequency from 100 Hz to 0.1 Hz at the respective print temperatures, Table 2. The zero-rate viscosity was predicted using Carreau-Yasuda Model³⁴ (Eq. 2) that applied the Cox-Merz rule³⁵.

$$\mu_{eff}(\gamma) = \mu_{inf} + (\mu_0 - \mu_{inf})(1 + (\lambda\gamma)^2)^{\frac{n-1}{2}}$$

----- Eq 2

Where,

$\mu_0, \mu_{inf}, \lambda$ and n are the material coefficients.

μ_{eff} = viscosity at effective shear rate (Pa.S)

μ_0 = the zero-rate viscosity (Pa.S)

μ_{inf} is the viscosity at the infinite shear rate (Pa.S)

λ = relaxation time (s)

n = power index.

γ = shear rate (s^{-1})

Method 2: The second approach used a change in temperature from 130 to 250°C at a constant frequency of 1Hz to study the changes in viscosity.

2.4 Statistics Analysis

IBM SPSS version 25.0 was used for the statistical analysis to determine the variance (ANOVA) between different groups and factors in different studies. One-way ANOVA was performed on strut width, tensile, compression test data and TGA thermal data. For all statistical comparisons a Tukey post-hoc test was used. Normality was checked by Shapiro-Wilks's test ($p>0.05$).

3. Results

3.1 Diatom Silica

The removal of the organics was verified using TGA, shown in Fig 2a/Table 3. A slight mass loss was observed up to 200°C in all 3 treatments, which is a result of the dehydration of the powders. Thereafter, a significant mass loss ($\approx 20\%$) was observed between 200 and 500°C in AT and WT treatment groups, which is indicative of organic matter, which was not observed in the heat-treated sample (HT). Relatively few studies have reported on the thermal stability or pyrolysis of marine or freshwater diatoms^{36,37}. There are multiple factors that influence the organic to inorganic ratios, most importantly the availability of silica³⁸ and evolution history, as over time diatoms will become less siliceous. Also environmental factors including light which influences valve formation³⁹, and pH, salinity and temperature that effect cell division^{40–42}. These environmental stresses will impact lipid storage and metal uptake thus resulting in variation in the organic to inorganic ratio. Therefore, direct comparison of percentage mass loss to other studies is ineffective. However, the thermal degradation profile trends are comparable to other studies. Sprynskyy *et al.*, TGA profile of *Pseudostaurosira trainorii* diatoms after purification showed a similar trend to AT and WT treatment groups in this study, where the thermal decomposition of residual organics was

reported to be between 199 to 576°C³⁶. The mass loss which occurred between 576 and 800°C in their study was attributed to dehydroxylation of free hydroxyl groups within the biosilica structure. A similar mass loss was observed in our data, which was most pronounced in the AT treatment group, indicating the same reaction has taken place. In terms of the actual biosilica thermal stability, Arasuna et al., report diatom biosilica from *Chaetoceros calcitrans* was thermally stable up to 800°C using x-ray diffraction analysis, but thereafter changed from amorphous silica to a similar structure to that of quartz³⁷. They also confirmed that organics were effectively removed at temperatures below 600°C, which supports our findings. The purification method used in this study appears to have been slightly more effective than that of the study by Sprynskyy et al.,³⁶, that reported a higher residual mass loss of 11.5% after purification treatment, as opposed to 4.8% at 800°C that was observed in this study.

The reduction in organics with respect to treatments correlates directly to an increase in specific surface area (Table 3) which suggests that the pores of the diatoms are becoming unblocked as the organics are being removed, thus creating more surface area. The specific surface area of the purified frustules of 58.80m²/g was found to be within the range (16.9 to 100m²/g) of those reported in the literature^{10,36}. The same trend was observed in the CHNS analysis (Table 3), whereby a reduction in carbon and nitrogen was apparent. Since carbon is associated with the organics in the diatoms⁴³, the reduction in carbon with respect to treatments indicates the successful removal of organics, thus verifying the TGA results. The higher nitrogen levels in the AT treatment, will be a result of

the nitric acid pre-treatment by St. Cloud State. After the subsequent treatments, WT and HT, the level was reduced from 1.91 (± 0.39) % to less than 0.30%.

The SEM results shown in Fig 2c to g indicate that the frustule valves remained intact after heat treatment (Fig. 2c and g). Untreated diatoms contain two-diatom valves interlocked to form frustules and are held together with a girdle band which is bound tightly by organics as shown in the schematic in Fig. 2b. The outer surface has a nanopatterned pore structured surface around the outer edge (Fig 2c to e), whereas the inside of the valve has a recessed segmented pattern (Fig 2f). Once the organics are removed, the frustules mainly separate, although some retain their original form, as shown in Fig 2e. The outside and inside of the valves form two petri-like discs that make up the frustules as shown in Fig 2c to f. This species of diatoms also contains spikey pointed spines around the outer edge (Fig 2c and d). The remaining biosilica after the organics has been removed was a mixture of valves, girdle bands and frustules as shown in Fig 2g. The average particle size of the *C. meneghiniana* valves used in this study were found to be between 15 and 20 μm . It is noteworthy to mention, that the bulk mix also included silica girdle bands (Fig 2 f and g) which were impossible to separate from the mixture after thermal decomposition. As evident from the SEM images, the diatom particles are flat disc like particles as opposed to granules like the Bioglass powders (Fig 3). This shape and structure would give the diatom particles a greater aspect ratio to that of the Bioglass particles.

Figure 2: Profiling the removal of organics from diatoms (a) thermogravimetric analysis after each treatment (b) schematic of diatom frustule and scanning electron microscope of frustules after heat treatment (c) diatom valve (d) zoomed edge of diatom valve (e) valves and frustule intact (f) valves and gridle bands and (g) deconstructed diatom frustules. Abbreviations: (po) pore openings, (sp) spines, (gb) gridle band, (v) valve and (vgb) valve with girdle band intact.

Treatments	TGA	BET	Elemental			
	Mass Loss at 800°C (%)	Specific surface Area (m ² /g)	C (%)	H (%)	N (%)	S (%)
1	32.66	11.57	14.52 (±2.57)	2.38 (±0.28)	1.91 (±0.39)	<0.30
2	25.60	11.93	12.17(±3.16)	3.20 (±1.41)	1.86 (±0.26)	<0.30
3	4.8	58.80	<0.30	0.46 (±0.14)	<0.30	<0.30

Table 3: Diatom physiochemical profiling analysis of purification process.

Figure 3: SEM images of Bioglass 45S5 (Sylc™, Research Ltd, London, UK) from Denfotex at different magnifications

3.2 Filaments

3.2.1 Filament Processing

Fig. 4a shows optical microscopy images of the extruded filament. A slight variation of colour was observed between filament groups, with the darkest filament being 5% Diatoms-PDLGA. However, this was a result of the colour of the diatom filler which is off-white/cream in colour as opposed to decolourisation from processing. At higher concentrations the additive is more visible causing greater decolourisation. Bioglass powder is brilliant white due to light scattering so the filaments were whiter in appearance (Fig 4a). None of the filaments showed signs of polymer degradation which is typically evidenced by either discoloration of the polymer or increased brittleness.

During processing, the draw-off speed of the extruder was adjusted to achieve the desired filament diameter of 1.75mm. However, a slight variation did occur between filament groups as reported in Fig 4b. For both the groups with diatoms biosilica powder fillers, the diameter was found to be slightly under the desired diameter, in the range of 1.69 to 1.72mm, whereas the diameters of pure-PDLGA and 5 wt % Bioglass-PDLGA filaments were

found to be slightly higher, with diameters of 1.79 (+0.07/-0.12) and 1.83 (+0.1/-0.16) mm
 respectively. In addition, significant variation was observed in the 5 wt % Bioglass-PDLGA
 filament along its length. Sections of this filament were too wide for the printer and had to
 be cut out and discarded. The pure-PDLGA filament was slightly wider than that achieved by
 Little et al., of 1.75(+0.15/-0.08)¹⁸ but still within specification for the 3D printer. TGA was
 used to check the ceramic content in the filaments and its distribution along the total
 filament length. The results show (Fig 4b) that this blending method was effective for
 diatom filler but not the Bioglass filler. The samples were heated up to 800°C. The rationale
 being, that the PDLGA should melt then decompose below 800°C, whereas the ceramics
 should not decompose below 1000°C (silica usually melts at 1,200°C)^{37,44,45}. The ceramic
 fraction in the filaments was calculated as per equation 1. The filler mass of diatom-PDLGA
 filaments was found to be 1.22 (± 0.46) % and 4.56 (± 0.21) % for 1 and 5 wt % blends
 respectively. Whereas, the 5 wt % Bioglass-PDLGA filament was found to have significant
 variation along its length resulting in filler average concentration of 5.01 (± 2.02) %. The
 highest amount of Bioglass was found to be 8.91 (± 0.77) % and the lowest amount was
 found to be 3.45 (± 0.31) %. The large standard deviation denotes an uneven distribution
 along the filament length which was potentially caused by agglomeration or variation of the
 particle size (Fig. 3) of Bioglass powders used. This supports the findings from the filament
 diameter measurements, which found the greatest variation to be in the Bioglass filament.
 Rajzer *et al.*, also reported the dispersion of Bioglass in their composites to be
 heterogeneous with some agglomeration of particles⁴⁶. Better premixing, such as turbo
 blending or a more uniform particle size could alleviate particle agglomeration of Bioglass.
 However, in a twin-screw extruder, the combination of temperature and the mechanical
 rotation of the screws in the melting zone of the extruder (Fig 1a), should disperse the

agglomerated particles within the polymer²⁰. Further work is required to better understand the cause of particle agglomeration of Bioglass in PDLGA during processing.

3.2.2 Filament Mechanical Data

Figure 4: Filament profiling (a) optical microscopy images of extruded filaments, (b) filament diameter (c) ceramic additive wt. % by TGA. Error bars for 3b represent the max and min diameter, where n=10. Error bars for 3c represent SD where n=15

Figure 5: Mechanical data for filaments (a) for ultimate tensile strength and elongation at break (asterisks indicate statistically significant difference) and (b) Young's modulus. (c) Toughness. Error bars represent SD where $n = 5$, statistical significance, $p \leq 0.05$

Figure 5 shows the tensile data for the filaments. The pure-PDLGA filament had a UTS of $47.12 (\pm 1.59)$ MPa (Fig 4a), which was similar to 1 and 5% wt % diatom-PDLGA filament that had a UTS of $47.58 (\pm 0.78)$ MPa and $47.58 (\pm 1.09)$ MPa respectively. No statistically significant differences were identified between these three compositions. The strength of a composite strongly depends on stress transfer between the particles and the matrix, therefore, strength will improve with good bonding between particles⁴⁷. Therefore, additional particles will typically enhance the strength of the composite as there are more points for potential stress transfer. In this study, the bonding between particles is mainly physical (i.e. surface roughness of the particles interacting with the matrix), as no chemical linker has been used. However, some chemical interaction is also likely between the hydroxyl groups of the PDLGA and the surface chemistry of the silica. Hydrogen bonding between C=O groups in the PDLLA with the surface Si - OH groups is also likely to occur; similar interactions have been reported in the literature for composites that have been dry blended prior to processing⁴⁸. Both of these particle matrix interactions rely on surface-to-surface contact. It seems likely that stress transfer is occurring but, given that no significant difference in UTS was observed between the pure-PDLGA filament and the two diatom-

PDLGA filament groups, it can be concluded that this is minimal and likely to be negated by changes to the polymer itself, such as processed-induced degradation.

The 5wt. % Bioglass-PDLGA filament was found to have the lowest UTS at 35.71 (± 1.50) MPa, and differed statistically in the post-hoc analysis ($p < 0.05$). The addition of diatoms to PDLGA at both 1% and 5% made no significant difference to the elongation of the filaments. Whereas, the addition of Bioglass to PDLGA was found to significantly reduce the filament's ductility when compared to pure-PDLGA, $p = 0.05$ and both diatom groups, $p = 0.03$ (1%) and $p = 0.05$ (5%). Brittleness in the Bioglass filaments would suggest thermal degradation of the polymer via a chain scission mechanism¹⁸, which supports the findings of Simpson et al.,⁵

The Young's Modulus of the filaments were found to be in the range of 2,700 and 3,000MPa (Fig 5b) but no significant difference in Young's modulus between filament groups was found.

The 5wt. % Bioglass-PDLGA filament was found to have the greatest variation in modulus (2746.44 (± 314.96) MPa) compared to the other groups. This is consistent with the other data. One of the contributing factors to the high variation in Young's Modulus in the Bioglass filament is most likely to be a result of the uneven distribution of the Bioglass powder particles (Fig. 3) as previously discussed.

The toughness (defined as the energy to break of the filaments) of the 5 wt % diatom-PDLGA filament was found to be significantly higher ($p < 0.05$) than the other filament groups tested. The 5 wt % diatom-PDLGA filaments were found to absorb 45% more energy than both the pure-PDLGA and 1 wt % diatom-PDLGA filaments and just under 80% more energy

than 5wt. % Bioglass-PDLGA filaments. Both pure-PDLGA and 1 wt % diatom-PDLGA filaments were also found to absorb significantly more energy than the 5wt. % Bioglass-PDLGA filaments. However, no significant difference was observed in the energy absorbed between pure-PDLGA and 1 wt % diatom-PDLGA filaments. Although changes in energy absorption could have been influenced by the morphology of the incorporated particles (high surface to volume ratio in terms of the disc-like shape of diatoms), it is also plausible that the differences observed were influenced by the extent of thermal degradation of the matrix polymer.

Strength and fracture toughness are two of the most important properties of a composite material⁵⁰. Although the addition of the fillers did not enhance the UTS of the PDLGA, it did not compromise it either. However, one of the significant findings is the increase in toughness in the PDLGA with the addition of 5% diatoms. High toughness is important to reduce microdamage crack formation and development that can occur in implanted scaffolds through load-bearing activities⁵¹ or potential manufacturing defects. Ceramic-polymer composite materials have been well studied in several fields, and it is well known that several factors can affect the tensile strength of a composite material. These include particle concentration, distribution of particles within the matrix, physical attributes of the particles (size, morphology profile) and the matrix-particle interfacial properties^{11,52}. It was hypothesised that the unique topographical features of diatom frustules could potentially enhance the interfacial bond strength between the matrix and the filler thus enhancing the mechanical properties of the composite. However, the results did not support the hypothesis at the concentrations tested. The concentrations of the fillers were kept deliberately low as the addition of ceramics particles in higher concentrations (>10%) have

previously been reported to reduce the accuracy of 3D printed scaffolds¹¹. In addition, the diatom frustules were considered to have exceptional mechanical properties with a Young's modulus of 22.5GPa which is similar to cortical bone (≈ 20 GPa), owing to their unique morphologies and complex hierarchical structures^{10,53}. A further point to consider is the possibility of fragmentation of the particles during processing due to the high shearing forces of the twin-screw extrusion. Such damage would have reduced the aspect ratio of the diatoms, a factor known to play a role in mechanical properties of clay-filler polymers⁵⁴. The effect of twin-screw extrusion on the particle size of ceramic particles, therefore, could be investigated in future work in this area.

In this study, the mechanical properties were measured in a dry state to determine an understanding of the fundamental mechanical properties of these composite materials. To validate the scaffolds for their application in bone tissue, further degradation studies are required to assess their temporal mechanical behaviour in a wet environment.

3.2.2 Rheology of Filaments

Figure 6: Rheology data, **Method 1:** (a) viscosity to shear rate curves at fixed temperature of 190°C and (b) average zero-rate viscosity ($n=3$) calculated from data from Fig 5a and b. **Method 2:** (c) Viscosity - temperature curves for each filament blend.

The viscoelastic properties of the filaments were studied using oscillatory rheology. The results are shown in Fig. 6. The frequency sweep on the second oscillation experiment (Fig 6 a) was selected to study the viscosity changes in the polymers from 100 Hz to 0.1 Hz, which is equal to shear rates from 628.3 s^{-1} to 0.628 s^{-1} . The experiment (Fig 6a) was performed at a fixed temperature of 190 °C. The slow shear rate setting replicates the 3D printing process

without applying any shear strength on filament. The optimum temperature to print Pure PDLGA filaments was found to be 190 °C, however, at this temperature both 1 wt % and 5 wt % diatom-PDLGA filaments were difficult to print and blocked the printer nozzle. They required much higher print temperatures of 220 and 225 °C respectively. This is confirmed by the results from the rheology in Fig 6a showing that both 1 wt % and 5 wt % diatom-PDLGA had significantly higher viscosities of magnitudes 25 (1 wt %) and 15 (5 wt %) times higher than pure-PDLGA. This explains the printing difficulties experienced at 190 °C for both diatom filaments. High viscosities would have resulted in pressure increase in the flow of the melted polymer as it meets the constrained geometry of the print nozzle. Fig. 6b summarises the zero-rate viscosity from the filaments at different temperatures correlating to the actual temperatures used in the printing trials. The viscosities at 1 and 5 wt % diatom-PDLGA were found to be 4729.3 ($\pm$ 1345.0) Pa.s and 3438.7 ($\pm$ 722.0) Pa.s respectively at 190°C, which dropped to 703.3 ($\pm$ 180.7) Pa.s and 300.2 ($\pm$ 27.9) Pa.s at 220°C and 225°C respectively. The zero-rate viscosity of 5wt% Bioglass-PDLGA was found to be 42.8 ($\pm$ 19.6) Pa.s at 165 °C, which was the lowest viscosity found within the groups. The final rheology experiment investigated the viscosity changes with respect to temperature (Method 2). The results are shown in Fig 6c. From the results an inverse relationship was observed with respect to temperature and viscosity, as the temperature increased the viscosity was found to decrease. According to this graph, the ideal print temperatures for printing 1 and 5 wt % diatom-PDLGA and 5 wt % Bioglass-PDLGA is 235, 220 and 140 °C respectively. For 1 wt % diatom-PDLGA this was 15 °C higher than the final print temperature used, whereas for 5 wt % diatom-PDLGA and Bioglass-PDLGA it was lower (See Table 2). According to the literature, the optimal print temperature of PDLGA has been reported to be 170³¹, 180¹⁸ and 200 °C³², all of which were tested in the study but found unsuitable. The pure-PDLGA filaments in this

study were found to print best at 190 °C. The discrepancies in temperature could be a result of a number of factors, including degradation to the polymer during filament fabrication, different printers or batch variation in the raw PDLGA. This 190 °C print temperature for the pure-PDLGA filaments was used as a reference temperature to print the other filaments. Step-by-step intervals of 5 °C from 190 °C were used to determine their optimum print temperature, which were found to be slightly outside of the range of the optimum print temperature determined by the rheology results. Overall the rheology results show that the composites can be melt processed successfully providing the print temperature is adjusted to their melt point, which varies with respect to filler added.

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693 **3.3 Scaffolds**

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695 **3.3.1 Scaffold Processing**

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Figure 7: 3D printed scaffolds (a) optical microscopy and SEM images of top view surface of scaffolds and (b) the width of the struts in the scaffold determine from SEM images. Error bars represent SD where $n = 3$, statistical significance 1-way ANOVA, $p \leq 0.005$ exist between α and β columns.

3D printing of all four filament groups proved successful as shown in Fig 7. A trial-and-error approach was taken to determine the optimum print temperature for each filament. The final temperatures used to print the filaments are shown in Table 1. The print temperature required for PDLGA with diatoms silica powder was significantly higher at 220 and 225°C for 1 and 5% diatom filler respectively, as opposed to the print temperature of pure-PDLGA of 190°C. However, for the 5 wt. % Bioglass-PDLGA filaments temperatures above 165°C were found to result in scaffolds that sagged and lost their shape.

After printing, striation marks were visible on all scaffold groups (visible on SEM images) of the scaffolds in Fig 7a. This is a result of the extrusion of the polymers from the nozzle of the 3D printer. The surface roughness as a result of these striation is likely to be a positive attribute for cell adhesion⁵⁵. The width of the struts was found to vary across all groups.

Where the struts in the scaffold overlapped due to layering, they were found to be slightly wider than in pore sections, as evident from the SEM images in Fig 7a. A significant difference in strut width was observed between 5 wt % diatom-PDLGA and both pure-PDLGA and 5 wt % Bioglass-PDLGA groups (Fig 7b).

Thermal processing is known to affect the molecular weight of polymers however, it is unavoidable in these types of extrusion-based technologies that are used to fabricate scaffolds. Processing time in the twin-screw extruder and 3D printer and the temperature in both will affect the molecular weight of PDLGA as demonstrated by Little et al.,¹⁸. In this study, TGA analysis was used to assess the thermal stability of the scaffolds. In other words, a material with a higher onset temperature is relatively more thermally stable than a material with a low onset temperature. There is a linear relationship between the onset temperature and the average molecular weight of the polymer⁵⁶.

3.3.2 Thermal Properties of Filaments and Scaffolds

Figure 8: Thermal degradation of PDLGA polymer by TGA (a) after filament and scaffold fabrication (asterisks indicate statistically significant difference) and (b) additive versus onset temperature of Bioglass filament. Error bars represent SD where $n = 3$, statistical significance, $p \leq 0.05$.

The results for the thermal degradation study are shown in Fig 8. The onset temperature for pure-PDLGA for filaments, $350.13 (\pm 0.69)^{\circ}\text{C}$ and scaffolds, $345.24 (\pm 0.25)^{\circ}\text{C}$ was found to be lower than both diatom filler groups. For the filaments this difference was not found to be statistically significant for either the 1% ($p = 0.095$) or 5% ($p = 0.819$) diatom filler groups, however, for the scaffolds the onset temperature was found to be significantly higher ($p \leq 0.05$) than PDLGA. In pure-PDLGA printing the scaffolds resulted in further thermal degradation, which was found to be a significant reduction ($p = 0.096$) in temperature. However, in both diatom groups no further reduction in thermal degradation was observed after printing the scaffolds. This would suggest that the diatoms are somehow inhibiting thermal degradation. A similar phenomenon has been reported for inhibition of thermal degradation other polymers, such as PMMA by silica, where the surface area is high⁵⁷. This is likely to be due to an interaction of the hydroxyl groups of the silica diatom surface with the carbonyl groups of the PLGA polyester. This causes PLGA molecules to absorb on the surface of the diatoms locally, thus reducing their molecular mobility, thereby rendering the molecules more stable. This effect is likely to be more pronounced the more surface area that is available for absorption. This unexpected advantageous property could be of significant benefit in 3D printing to minimise polymer degradation during processing while adding potential osteogenic benefit to the PDLGA. This phenomenon was not observed in Bioglass. There was a significant reduction ($P = 0.0001$) of $306.20 (\pm 5.50)$ and $304.69 (\pm 2.86)^{\circ}\text{C}$ in the onset temperature for 5 wt. % Bioglass-PDLGA for the filaments and scaffolds respectively. These results indicate that the addition of Bioglass significantly increases thermal degradation of PDLGA during processing, which supports the findings of other

studies^{4,5,49}. Other studies investigating the addition of Bioglass into 3D composite scaffolds using 3D printing, did not investigate the effect of Bioglass on the thermal stability of the polymers^{2,3}. The effect of Bioglass on the onset temperature was studied further in the Bioglass filaments. In addition, the diatom biosilica filaments required a much higher temperature (220 to 225°C) to print compared to the Bioglass filaments (165°C), however, the higher temperature did not cause any additional polymer degradation (Fig 8a). A study of the ceramic content with respect to onset temperature was performed on the filament in different sections. The results (Fig 8b) found that as the percentage of Bioglass increased, the onset temperature decreased, confirming that Bioglass reduced the thermal stability of the PDLGA with respect to the weight percentage added.

4 Conclusion

The study has demonstrated that both Bioglass and natural biosilica isolated from freshwater diatoms can be successfully 3D printed into a composite scaffold. The results found that natural diatom Biosilica had less impact on the PDLGA composites as indicated by thermo-mechanical properties, in comparison to those obtained from the Bioglass PDLGA composites. The addition of higher concentrations of diatoms was found to have significant impact on the toughness of the filaments. One of the most promising results was the ability of diatom Biosilica to inhibit thermal degradation of PDLGA. This holds significant promise for bone scaffolds as thermal degradation of polymers during FDM can compromise the mechanical integrity of the scaffold. Further analysis of the osteogenic potential of these diatom-PDLGA in both *in vitro* and *in vivo* systems is required to further assess their potential as a bone scaffold.

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: